

Conference on ‘Malnutrition matters’

Symposium 5 (Joint Nutrition Society and BAPEN Symposium): Too many pies: metabolic competencies in obesity The importance of fatty liver disease in clinical practice

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The worldwide obesity epidemic over the last 20 years has led to a dramatic increase in the prevalence of non-alcoholic fatty liver disease, the hepatic manifestation of the metabolic syndrome. Estimates of prevalence vary depending on the population studied and the methods used to assess hepatic fat content, but are commonly quoted as between 10 and 30% of the adults in the Western hemisphere. Fatty liver develops when fatty acid uptake and synthesis in the liver exceeds fatty acid oxidation and export as TAG. Studies of pathogenesis point to insulin resistance, lipotoxicity, oxidative stress and chronic inflammation being central to the development and progression of the disease. A proportion of individuals with fatty liver develop progressive disease, though large prospective longitudinal studies are lacking. Nevertheless, fatty liver is associated with increased all-cause and liver-related mortality compared with the general population. Management of fatty liver centres around lifestyle and dietary measures to induce controlled and sustained weight loss. Management of cardiovascular risk factors aims to reduce mortality, while certain dietary interventions have been shown to reduce steatosis and inflammation. Specific pharmacological treatments also show promise, but their use is not widespread. A multi-system and multi-disciplinary approach to the management of this disorder is proposed.

**Hepatic steatosis: Non-alcoholic fatty liver disease: Non-alcoholic steatohepatitis:
Insulin resistance: Hepatitis: Diabetes mellitus**

The mounting clinical and research interest in fatty liver disease is a consequence of the worldwide epidemic of obesity. Fatty liver is one of the most common liver disorders in the western world. In the absence of specific causes of hepatic fat accumulation such as high alcohol consumption, this condition is termed non-alcoholic fatty liver disease (NAFLD).

NAFLD represents a spectrum of liver disease of increasing severity that encompasses steatosis (fatty change), non-alcoholic steatohepatitis (NASH) with or without fibrosis and cirrhosis⁽¹⁾. Most frequently, NAFLD is associated with obesity, insulin resistance (IR), dyslipidaemia and hypertension and should be considered the hepatic manifestation of the metabolic syndrome⁽¹⁾. Alternative

aetiologies of fatty liver include high alcohol consumption, rapid weight loss, total parenteral nutrition and drugs (Table 1)⁽²⁾.

Although highly prevalent, NAFLD/NASH is frequently asymptomatic. Until recently it was considered to be an innocent bystander in the multi-system metabolic syndrome with few clinical sequelae. It is now generally accepted that NAFLD is not a benign condition and poses a major challenge to health care provision worldwide. This review will consider the importance of NAFLD/NASH in terms of (1) the prevalence and natural history; (2) associated morbidity and mortality and (3) potential for successful treatment, with an emphasis on nutritional factors.

Abbreviations: ¹H MRS, proton magnetic resonance spectroscopy; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

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Table 1. Aetiology of non-alcoholic fatty liver disease and non alcoholic steatohepatitis*

Category	Specific examples
Metabolic syndrome	Obesity Insulin resistance/type 2 diabetes mellitus Dyslipidaemia
Obesity related surgical procedures/severe weight loss	Jejunioileal bypass/jejunocolic bypass Massive intestinal resection
Factors altering nutritional state	Fasting/eating disorders Total parenteral nutrition Jejunal diverticulosis and small bowel bacterial overgrowth
Drug-induced liver injury	Cardiovascular (amiodarone, diltiazem and nifedipine) Hormonal (glucocorticoids, tamoxifen and synthetic oestrogens) Antiretroviral medication (HAART)
Alcohol consumption (alcoholic steatohepatitis)	
Metabolic disorders	Lipodystrophy (generalised/partial) Genetic insulin resistance
Viral hepatitis	Hepatitis C (genotype 3)

HAART, highly active antiretroviral therapy.
*Table includes data from reviews^(1,3).

How prevalent is fatty liver?

Estimates of prevalence rely on both an accurate case definition (i.e. how a case of fatty liver is defined) and sample frame (i.e. the population from which estimates of prevalence are derived).

Case definition

There is no universally applicable diagnostic test for hepatic steatosis. A number of techniques with varying degrees of accuracy and applicability have been used. Generally, those tests that supply most information are less acceptable to patients (such as liver biopsy) or prohibitively expensive for population-wide screening (for example, proton magnetic resonance spectroscopy (¹H MRS)), while acceptable tests such as waist-to-hip measurements or simple blood variables may lack precision and/or accuracy. Elevated liver enzymes in the absence of other causes may reveal only a subset of individuals with hepatic steatosis. The choice of test will therefore influence the estimate of prevalence.

Histology. Histological assessment is considered the reference standard for the assessment of NAFLD. Hepatic steatosis is defined pathologically as the presence of large and small vesicles of fat, predominantly TAG, accumulating within hepatocytes⁽³⁾. The National Institute of Diabetes and Digestive and Kidney Diseases NASH Research Network has developed a well-validated scoring system that facilitates uniform and reproducible diagnosis of NAFLD/NASH using H&E (haematoxylin and eosin) stained tissue⁽⁴⁾. This score measures disease severity and quantifies fat in terms of the percentage of hepatocytes containing visible lipid droplets, together with features indicative of inflammation (presence of inflammatory cells and hepatocyte ballooning degeneration) and fibrosis⁽⁴⁾. Recently, the addition of digital image analysis techniques using Oil Red O stains to improve accuracy of histological steatosis quantification, at least within clinical trials, has

been suggested⁽⁵⁾. Even so, as liver biopsy typically samples only 1/50 000th of the liver mass and fat is not always evenly distributed, sampling error may occur. Further, liver biopsy is an invasive technique and carries a small but defined morbidity and so is inappropriate for population screening⁽⁶⁾.

Ultrasound. Ultrasound is widely available, acceptable to patients and offers the prospect of providing additional morphological information. However, the assessment of the degree of steatosis relies on observing comparatively increased echogenicity of the fatty liver compared with the renal cortex. The assessment is therefore subjective and semi-quantitative at best^(7,8). The sensitivity and specificity of ultrasound is the subject of some controversy, but sensitivity for significant steatosis (equivalent to >30% on biopsy) is thought to be between 60% and 90%⁽⁷⁾.

Magnetic resonance spectroscopy and imaging. ¹H MRS and MRI offer quantitative assessment of hepatic TAG^(7,9,10) and potentially lipid composition⁽¹¹⁾. The techniques are relatively expensive, but non-invasive, and ¹H MRS has been used in population-based studies^(12,13). ¹H MRS provides a measurement of TAG and water in a region of interest, which are influenced by the tissue assessed and the sequences used. Multi-echo MRI techniques also measure fat content comparing the signal phase and relaxation time from each component (fat and water) to calculate the relative abundance⁽¹⁴⁾. This approach has the benefit of enabling fat distribution to be visualised and reduce the need for post-processing. For absolute quantification, calibration to a known standard is required.

Biometrics and blood test indices. Routine measurements such as waist circumference, waist:hip ratio, BMI, together with simple blood-derived indices such as serum TAG levels, serum gamma glutamyl transpeptidase, and raised serum alanine aminotransferase and aspartate aminotransferase enzymes measurements, without other explanation, may also be used to predict NAFLD^(15,16). Such measures are surrogate markers of hepatic steatosis and are not considered quantitative.

Table 2. Diagnostic criteria for the metabolic syndrome*

WHO proposal (1999) ⁽⁶⁸⁾	NCEP proposal (2001) ⁽⁶⁹⁾	IDF proposal (2005) ^(70,71)
<p>Altered glucose regulation (impaired fasting glucose, impaired glucose tolerance, type 2 diabetes) or insulin resistance (clamp measurement: glucose uptake below lowest quartile for background population under study)</p> <p>PLUS two or more of the following criteria:</p> <p>(a) Impaired glucose tolerance (2 h glucose during OGTT: 110–125 mg/dl)</p> <p>(b) Arterial pressure $\geq 140/90$ mmHg or drug treated</p> <p>(c) Central obesity (waist: hip ratio > 0.9 (M), > 0.85 (F) or BMI ≥ 30)</p> <p>(d) Dyslipidaemia (TAG ≥ 150 mg/dl (1.7 mmol/l) or fibrate-treated or HDL cholesterol < 35 mg/dl (0.9 mmol/l) (M), < 39 (1.0 mmol/l) (F))</p> <p>(e) Insulin resistance (see above)</p> <p>(f) Microalbuminuria (albumin excretion rate ≥ 20 g/min or albumin/creatinine ≥ 30 mg/g)</p>	<p>Three or more of the following criteria:</p> <p>(a) Blood glucose ≥ 110 mg/dl or treated for diabetes</p> <p>(b) Arterial pressure $\geq 130/85$ mmHg or drug treated</p> <p>(c) Waist circumference > 102 cm (M), > 88 (F)</p> <p>(d) TAG levels ≥ 150 mg/dl (1.7 mmol/l) or fibrate-treated</p> <p>(e) HDL cholesterol levels < 40 mg/dl (1.03 mmol/l) (M), < 50 (1.29 mmol/l) (F)</p>	<p>Abdominal obesity (waist circumference > 94 cm (M), > 80 (F) for caucasians. Values specified for other ethnic groups)</p> <p>PLUS two or more of the following criteria:</p> <p>(a) Blood glucose ≥ 100 mg/dl (5.6 mmol/l) or treated for diabetes</p> <p>(b) Arterial pressure $\geq 130/85$ mmHg or drug treated</p> <p>(c) TAG levels ≥ 150 mg/dl (1.7 mmol/l) or fibrate-treated</p> <p>(d) HDL cholesterol levels < 40 (1.03 mmol/l) mg/dl (M), < 50 mg/dl (1.29 mmol/l) (F)</p>

WHO, World Health Organization; NCEP, National Cholesterol Education Program; IDF, International Diabetes Foundation; OGTT, oral glucose tolerance test. M, male; F, female.

*Table modified from Bugianesi *et al.* 2005⁽⁷²⁾.

Furthermore, biometric and blood test indices have been applied to discriminate between patients with NAFLD and mild or no fibrosis from those with severe fibrosis, at greater risk of progression to cirrhosis and hepatocellular carcinoma. The NAFLD Fibrosis Score is an algorithm based on age, BMI, presence of hyperglycaemia, aspartate aminotransferase, alanine aminotransferase, platelet count and albumin. The model, built using a cohort of 480 patients with biopsy-proven NAFLD and validated in an independent cohort of 253, had an overall diagnostic accuracy of 0.88 and 0.82, respectively, in the two cohorts⁽¹⁷⁾. While this marker panel uses widely available clinical data, other markers aim to measure the intermediates of fibrogenesis, such as the Enhanced Liver Fibrosis panel, validated for the detection of moderate to severe fibrosis in NAFLD⁽¹⁸⁾. Scoring systems such as these are being used as surrogate markers in clinical studies and may reduce the requirement for liver biopsy in a subset of patients.

Sample frame. The sample frame must be considered while estimating the prevalence of NAFLD in the general population. Differences in race, geography and socio-economic grouping may contribute different risk factors and thus they may impact on the results of population-based assessments of hepatic steatosis, while the prevalence of hepatic steatosis in at risk groups such as those with type-2 diabetes and obesity, is higher than the general population, making extrapolation difficult. This variation has similarly been recognised within the diagnostic criteria for the wider metabolic syndrome, reflected in the range of diagnostic criteria that have been produced. Thus, the International Diabetes Foundation (Table 2) criteria have been established as a single internationally adopted definition for future studies⁽¹⁹⁾.

Prevalence assessed by population. Angulo estimated the prevalence of NAFLD in the US population to be about 30 million (or 21% of adults) in 2000 on the basis of the prevalence of obesity in the US population, multiplied by the proportion of those found to have hepatic steatosis in studies of obese people⁽¹⁾. Clark and coworkers, estimated the prevalence to be 31 million (or 23% of adults) in the US on the basis that 79% of patients with mildly abnormal liver function tests and no other cause described were likely to have NAFLD⁽³⁾. In at-risk groups, the estimates of prevalence are markedly higher with obese and morbidly obese subjects having a prevalence of 60–90%, respectively, while half of those with type-2 diabetes have steatosis. In obese diabetic patients, the presence of hepatic steatosis is universal⁽¹⁾.

Analysis performed as part of the Dallas Heart Study examined 2349 subjects. Of these, 345 had no risk factors for fatty liver. Hepatic TAG content was measured by ¹H MRS and a threshold, quoted as 5.56% TAG, found at the 95th centile, used to define abnormality. When applied back to the larger population, 34% of the individuals were found to have hepatic fat in excess of this level and thus hepatic steatosis⁽¹³⁾. Still using ¹H MRS, the prevalence of hepatic steatosis was studied in different races: prevalence in black people was 24%, 33% in the white population (42% in males and 24% in females), while in the Hispanic group, the prevalence was estimated at 45%, regardless of

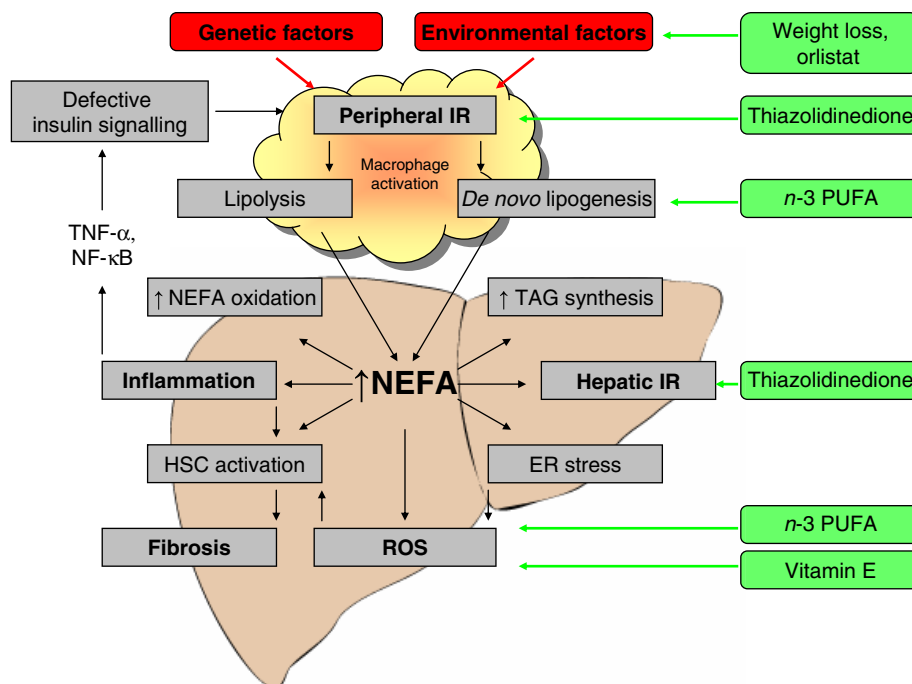


Fig. 1. Key pathways in the pathogenesis of non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH). Development of insulin resistance (IR) through genetic and environmental means, leads to increased *de novo* lipogenesis and TAG storage, with decreased oxidation and export, leading to net lipid accumulation. Increases in NEFA lead to endoplasmic reticulum (ER) stress and the production of reactive oxygen species, which cause hepatocellular inflammation and apoptosis. Pro-inflammatory cytokines, such as TNF α via NF- κ B, further contribute to IR. These factors also lead to the activation of hepatic stellate cells (HSC) and net deposition of pathological extracellular matrix (fibrosis). Beneficial therapies act at multiple points, including those indicated on the right hand side of the Figure, leading to decreased IR and further beneficial downstream effects. Adapted from Cheung and Sanyal⁽²³⁾.

gender⁽¹²⁾. The Dionysos study in northern Italy estimated a prevalence of 20–25% using ultrasound in a population of over 3000 individuals with and without suspected liver disease. It is estimated that between 43% and 55% of NAFLD patients with raised aminotransferases have histological evidence of NASH, while necroinflammation on histology from initial liver biopsy is the strongest predictor of fibrosis progression⁽²⁰⁾.

Pathogenesis

When discussing whether fatty liver is important, the pathogenesis of the condition should be considered to determine whether fat *per se* is pathogenic in progressive steatohepatitis, or whether it is the metabolism, utilisation and indeed the type of fat that contributes to disease. A foundation for research in the field has been the ‘Two-Hit Hypothesis’ proposed by Day and James⁽²¹⁾, where an initiating event promotes fat deposition which in turn sensitises the liver to hepatocellular damage and inflammation. Although our understanding of the complexity of NAFLD/NASH pathogenesis has expanded, there has been a move away from the linear model of pathogenesis; it remains a useful schema for clinical practice. A full discussion of the pathogenesis of NAFLD/NASH is outside

the scope of this article, but has recently been comprehensively reviewed⁽²²⁾, while key pathways are summarised in Fig. 1⁽²³⁾.

NAFLD/NASH is best considered a complex disease trait where host genetic factors and the environment interact to produce disease phenotype and is therefore subject to subtle inter-patient variations beyond those that may be addressed by lifestyle modification⁽²⁴⁾. Central to the pathogenesis of NAFLD/NASH are the combined effects of IR, both hepatic and peripheral and intracellular NEFA accumulation. In general, the development of hepatic steatosis occurs when the rate of synthesis or import of NEFA by hepatocytes exceeds the rate of export or catabolism⁽²⁴⁾. In the presence of IR, insulin-mediated suppression of hormone sensitive lipase falls, increasing the release of NEFA from adipose stores into the circulation and subsequent uptake into hepatocytes⁽²⁵⁾. Hyperinsulinaemia generated by compensatory pancreatic beta-cell hyper-secretion, mediates increased *de novo* lipogenesis through the transcription factor, sterol regulatory element-binding protein-1c and once beta-cell failure develops, hyperglycaemia acts via carbohydrate response element-binding protein to further promote lipogenesis. In parallel, mitochondrial fatty acid β -oxidation oxidative capacity becomes overwhelmed and alternative peroxisomal β -oxidation and microsomal ω -oxidation pathways

assume greater importance, both of which lead to the increased production of reactive oxygen species⁽²⁶⁾.

Does the presence of hepatic steatosis lead to inflammation?

The progression from simple steatosis to steatohepatitis is not simply due to the accumulation of NEFA-derived TAG within hepatocytes (whether derived from *de novo* fatty acid synthesis or flux of NEFA from peripheral adipose tissue), nor does it require a distinct second 'hit'. Visible TAG deposition within hepatocytes may be considered an innocent bystander. This was elegantly demonstrated by small-interfering RNA interference of diacylglycerol transferase 2 expression, a key enzyme in TAG synthesis, in genetically obese/diabetic leptin receptor deficient Db/Db mice fed a methionine-choline deficient diet to induce steatohepatitis⁽²⁷⁾. Although steatosis was ameliorated, histological and biochemical markers of tissue oxidative stress, hepatocellular damage and apoptosis were increased. Hepatocellular damage and inflammation are a consequence of the accumulation of NEFA and lipid peroxidation products (lipotoxicity), leading to activation of inflammatory pathways, mitochondrial dysfunction, endoplasmic reticulum stress and ultimately apoptosis⁽²⁸⁾. NEFA are produced as part of normal hepatic glucose metabolism via acetyl CoA and the citric acid cycle. NEFA are produced in excess in obesity, systemic inflammation, insulin resistant states and certain diets (such as high fructose diet). NEFA are potentially hepatotoxic⁽²⁹⁾, so experiments such as this suggest that the production and packaging of relatively inert TAG in the form of intracellular lipid droplets represents an adaptive response to the presence of NEFA. One intriguing hypothesis is that maternal nutritional factors may affect disease progression in adult offspring, possibly by the 'priming' of maternally inherited mitochondria. A study demonstrated that among mice fed a high fat diet, offspring of female mice fed a high fat diet went on to develop steatohepatitis, while offspring of those fed a control diet developed simple steatosis only. This was supported by the observation that mitochondrial electron transport chain enzymes were decreased in offspring of females fed high fat diet, while expression of genes involved in oxidative stress and inflammation were elevated⁽³⁰⁾. Case-control studies in the first instance would be required to demonstrate such an association in human subjects.

What type of fat is important?

NEFA are considered to influence progression of disease, but recent studies have established difference in the lipid profiles of patients with differing disease severity, suggesting that fat type may also be important. Murine and human studies have both demonstrated lower levels of hepatic PUFA with increasing steatosis and further, with steatohepatitis, compared to healthy controls^(31,32). Lipid profiling of human plasma showed that increased lipogenesis, desaturases and lipooxygenase activities characterised NAFLD and NASH, while impaired peroxisomal PUFA metabolism and non-enzymatic oxidation was associated

with progression to NASH⁽³³⁾. These cross-sectional studies suggest that interventions to alter hepatic lipid composition and quantity may alter the natural history of the condition.

Does hepatic steatosis increase morbidity and mortality?

Most epidemiological studies of NAFLD are cross-sectional rather than prospective longitudinal. Accordingly, while the prevalence of NAFLD-associated conditions may be estimated subject to the considerations of case definition and sample frame, the proportion of patients progressing to more severe disease is not known. In an example of selection bias, patients with biopsy-proven NAFLD have worse outcomes than those who do not undergo biopsy, reflecting the selection criteria for liver biopsy. Two longitudinal studies by Adams and coworkers warrant further description. In a population-based cohort study, 345 subjects with NAFLD diagnosed between 1980 and 2000 were followed up for a mean of 7.6 years. All-cause mortality was increased compared to the general population, while in patients with NAFLD, liver-related mortality was the third biggest cause of death, compared to the thirteenth in the general population⁽³⁴⁾. In a study of over a hundred patients with paired biopsies an average of 3.2 years apart, progression of fibrosis was seen in 37%, regression in 29% and the remainder were unchanged. Risk factors for progression were low initial fibrosis stage, diabetes and increased BMI⁽³⁵⁾. However, in a systematic review of paired biopsy studies only the presence of inflammation of any grade and increased age were independent risk factors for fibrosis progression.

In another study, patients with cirrhosis due to NAFLD were compared to a group of patients with cirrhosis due to the hepatitis C virus⁽³⁶⁾. Patients from both groups were most likely to die from complications of sepsis. However, patients with hepatitis C virus had greater all-cause and liver-related mortality, while those with NAFLD had greater cardiovascular-related mortality.

Hepatocellular carcinoma is an important cause of death in chronic liver disease, predominantly on a background of cirrhosis⁽³⁷⁾. Both obesity and diabetes are risk factors for the development of hepatocellular carcinoma, and in patients with hepatocellular carcinoma and the metabolic syndrome as the only risk factor, 65% had no significant fibrosis, demonstrating that metabolic syndrome, rather than resultant cirrhosis may predispose to hepatocellular carcinoma development⁽³⁸⁾.

What treatments are effective in fatty liver?

Optimal management of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

NAFLD/NASH is only one aspect of the wider metabolic syndrome and so shares a common pathogenesis and therapeutic approach with other related conditions (e.g. dyslipidaemia, diabetes, hypertension and obesity). Optimum management may be delivered using a multi-disciplinary

approach. At our institution, a specialist NASH clinic has been established, employing a multi-disciplinary team comprising hepatologist, diabetologist, dietitian, psychologist and specialist nurse. The clinic aims to achieve sustained health improvements by simultaneously addressing the multi-factorial pathogenesis and risk factors of these patients, attempting to engender lifestyle changes and providing a platform for trialling novel therapies.

Lifestyle measures

Hepatic steatosis, as mentioned above, is closely associated with obesity, IR and diabetes. Strategies to modify these risk factors are therefore likely to improve the condition. Only recently has evidence become available to support this approach. Obese patients with NASH were randomised to lifestyle intervention (behavioural, dietary and exercise changes) or to structured education. Over one year, the intervention group lost an average of 9.3% body weight compared with 0.2% in the control group. Associated with the weight loss in the intervention group was a significant improvement in the histological indices of inflammation⁽³⁹⁾. While weight loss is an important target for intervention in the overweight and obese NAFLD/NASH population, it should also be remembered that improving skeletal muscle insulin sensitivity through aerobic exercise is also a worthwhile therapeutic goal. In a recent study, obese sedentary individuals underwent a 4-week programme of aerobic exercise. While hepatic steatosis, as measured by ¹H MRS, was shown to have decreased by 21%, body mass had not significantly altered⁽⁴⁰⁾.

Nutritional risk factors and therapies in fatty liver disease

A number of dietary factors have been identified, which may predispose to fatty liver disease either in deficiency or excess. There are relatively few studies assessing the impact of specific diets on NAFLD. Nevertheless, the close pathogenic association between NAFLD and the metabolic syndrome makes diets aimed at modifying cardiovascular risk factors in diabetics and obese patients appropriate for many patients with NAFLD. Certainly, the inclusion of *n*-3 fatty acids (for example, fish oils and walnuts) MUFA (olive oil), fruit and vegetables, low glycaemic index foods, high-fibre foods in a diet low in saturated fat, simple carbohydrates and sweetened drinks may be recommended. This has been comprehensively reviewed elsewhere⁽⁴¹⁾, but the effects of certain dietary lipids, choline-related compounds and sugars are considered further below.

Dietary lipids. Obesity and IR predispose not only to fatty liver as described above but also to CVD (the main cause of morbidity and mortality in NAFLD). The effect of dietary cholesterol intake is unclear with some studies showing high cholesterol intake associated with NAFLD⁽⁴²⁾ and others showing no difference⁽⁴³⁾. Consumption of diets high in SFA increases the risk of CVD. In a rat model, SFA led to hepatocyte injury associated with endoplasmic reticulum stress⁽⁴⁴⁾, while in human subjects, diets high in saturated fat and low in PUFA are associated with NASH, compared to controls with the same body mass⁽⁴⁵⁾.

PUFA include both *n*-6 and *n*-3 fatty acids, considered essential for the production of long-chain fatty acids, eicosanoids and PG. The *n*-3 fatty acid, α -linolenic acid, is required for synthesis of the anti-inflammatory long-chain fatty acids, DHA and EPA, which are also found in fish oils. *n*-6 fatty acids are required for the synthesis of pro-inflammatory arachidonic acid.

Increasing the ratio of dietary *n*-6:*n*-3 fatty acids in rats with sucrose-induced IR improved IR⁽⁴⁶⁾, while in human subjects, the ratio of dietary *n*-6:*n*-3 fatty acids was significantly higher in those with NAFLD compared to healthy controls, when assessed using a dietary history⁽⁴³⁾.

On the basis of animal data which showed that dietary supplementation with fish oils in rats prevented IR induced by high fat feeding⁽⁴⁷⁾ and reduced hepatic steatosis⁽⁴⁸⁾, interventional studies in human subjects have been conducted. *n*-3 PUFA supplementation (EPA and DHA at a ratio of 0.9:1.5) in 42 patients with NAFLD led to significant decreases in serum liver enzyme levels, glucose and TAG. Furthermore, there was a reduction in hepatic steatosis as assessed by ultrasound⁽⁴⁹⁾. In patients with NASH, *n*-3 PUFA (EPA) supplementation over a one-year period led to improvements in lipid indices, serum liver enzyme levels overall and in histological assessment of fibrosis, steatosis and inflammation in the seven patients who had undergone liver biopsy before and after treatment⁽⁵⁰⁾. These findings need confirmation in larger-scale randomised controlled studies, before high-dose fish oil supplementation can be recommended in clinical practice.

The beneficial effects of *n*-3 PUFA are thought to be exerted by numerous inter-related mechanisms. EPA and DHA are ligands of the nuclear receptor PPAR α . Reduced PPAR α activity, seen when there are low levels of circulating *n*-3 fatty acids, may result in a number of processes including: increases in insulin sensitivity and mitochondrial oxidation of fatty acids; reduction of hepatic uptake of NEFA; reduced hepatic lipogenesis (in part by up-regulation of lipogenic transcription factors such as sterol regulatory element binding protein-1); increased lipid export in the form of VLDL, all leading to reduction in hepatic steatosis and reactive oxygen species production^(49,51) (Fig. 1).

Choline-related compounds. Choline is an essential nutrient required for numerous physiological processes including the cell membrane manufacture and function, lipid transport and methionine synthesis. Mice fed a choline deficient diet develop hepatic steatosis and steatohepatitis⁽⁵²⁾, while those fed a high fat diet were found to have decreased levels of hepatic choline-related compounds⁽³¹⁾. Phosphatidylcholine, synthesised from choline, is required for the normal hepatic secretion of VLDL⁽⁵³⁾ and decreased phosphatidylcholine leads to the accumulation of intracellular TAG⁽⁵⁴⁾. Moreover, 65% of men and women fed a diet deficient in choline developed liver dysfunction (largely steatosis as assessed by MRI)⁽⁵⁵⁾. A diet rich in choline and betaine was also associated with low levels of serum markers of inflammation, such as C-reactive protein, IL-6 and TNF (reviewed by Zeisel and da Costa⁽⁵⁶⁾). As such, choline has been considered an essential nutrient by the Institute of Medicine. The choline

Table 3. Selected randomised controlled trials of some pharmacological therapies for non-alcoholic steatohepatitis (NASH)

Agent	Mode of action	Study design	Patient group	Primary outcome measure	Outcome
Betaine ⁽⁵⁷⁾	Methyl donor, required for synthesis of phosphatidyl-choline and TAG secretion	Multi-centre, randomised double blind, placebo-controlled; 12 months treatment duration; 34 patients completed study	Histologically proven NASH	Reduction in aminotransferase levels	No change in aminotransferases, weight or plasma lipid profiles. No difference in histological activity or fibrosis scores
Orlistat ⁽⁶⁴⁾	Reversible inhibitor of gastric and pancreatic lipase (blocks absorption of approximately 30% of dietary TAG)	Dual centre, randomised, open label trial; orlistat + vitamin E + multivitamin + 1400-energy/day diet v. vitamin E + multivitamin + 1400-energy/day diet; 36 weeks duration; 41 patients completed study	Overweight, histologically proven NASH	Improvement in steatosis, NAFLD activity score and fibrosis score on follow-up liver biopsy at 36 weeks	No significant difference between Orlistat and control groups for histology, aminotransferases, IR, cholesterol or adipocytokine levels. Significant differences seen between all those who lost $\geq 9\%$ body weight, compared with those losing less
Rosiglitazone ⁽⁶²⁾	PPAR γ binding agent, increasing insulin sensitivity	Single centre, randomised, double-blind placebo-controlled study; 12 months treatment duration; 63 patients included in analysis	Histologically proven NASH	Reduction in steatosis of 30% or disappearance of steatosis on biopsy at end of treatment	>30% reduction in steatosis more frequent in Rosiglitazone, compared to placebo (47% v. 16%, $P = 0.014$). Also, significantly, more patients had normalisation of transaminases, despite significantly greater weight gain
Pioglitazone ⁽⁶³⁾	PPAR γ binding agent, increasing insulin sensitivity	Dual centre, randomised, double-blind placebo-controlled study; 12 months treatment; 61 patients included in analysis	Histologically proven NASH	Not stated	Significant reduction in steatosis in both placebo and pioglitazone groups. Pioglitazone alone associated with reduction in lobular inflammation and fibrosis
Pioglitazone or vitamin E ⁽⁶⁵⁾	PPAR γ binding agent, increasing insulin sensitivity and vitamin E, an antioxidant	Multicentre, randomised, double blind placebo-controlled study; 96 weeks treatment; 247 patients included in analysis	Histologically likely NASH	Improvement in histology at biopsy at end of treatment	Significant improvement in NASH on histology seen in vitamin E but not Pioglitazone groups. Biochemical improvement in both

NAFLD, non-alcoholic fatty liver disease.

oxidation product, betaine is also obtained through the diet. It is important as a methyl group donor used in the conversion of homocysteine to methionine and in the synthesis of phosphatidylcholine. Through varied effects on intermediary metabolism, betaine has been shown to reduce hepatic steatosis in animal models, forming the basis of trials to test its efficacy at pharmacological doses, as described later⁽⁵⁷⁾.

Sugars. High fructose maize starch is used as a sweetener in many processed foods and carbonated drinks. High intake of soft drinks is associated with an increased risk of NAFLD, partly through increased energy intake and in part through the consumption of advanced glycation end-products which are associated with increased IR and inflammation. Fructose intake is associated with development of NAFLD⁽⁵⁸⁾, with cases of NAFLD consuming over twice as much as the matched control group⁽⁵⁹⁾. Fructose consumption is thought to promote hepatic lipogenesis as glycolysis of fructose is not subject to the rate-limiting step of metabolism by phosphofructokinase, leading to unregulated increase in the acetyl-CoA, a substrate for lipogenesis. Fructose also up-regulates sterol regulatory element-binding protein-1c, increasing *de novo* lipogenesis⁽⁶⁰⁾. Furthermore, fructose consumption increases postprandial hypertriglycerolaemia, while reducing glucose concentrations, when subjects consumed a fructose-sweetened drink with every meal for 2 d. Fructose feeding was also observed to reduce postprandial suppression of ghrelin secretion and reducing postprandial leptin and insulin secretion, likely resulting in decreased satiety and increased energy intake⁽⁶¹⁾.

Pharmacological therapies

Those pharmacological therapies that have been applied to the treatment of NAFLD/NASH in clinical trials have provided encouraging but often mixed results at times. Table 3 highlights recent randomised controlled trials, using liver biopsy features to assess change with treatment. PPAR γ binding drugs (thiazolidinediones) show promise for the treatment of NASH, but are associated with overall weight gain^(62,63). Furthermore, there is conflicting evidence of possible increased cardiovascular morbidity and mortality associated with their use. Despite a clear rationale and promising pilot data, betaine failed to demonstrate a consistent beneficial effect⁽⁵⁷⁾, while the use of orlistat did not confer any specific benefit, though this study and that of weight loss *per se* both showed that significant weight loss is associated with histological improvement in NASH^(39,64).

An important recent study has compared the efficacy of Pioglitazone to antioxidant (vitamin E) treatment and placebo in histologically proven steatohepatitis (Table 3). While Pioglitazone was found to provide some beneficial effects, a key finding was that vitamin E given at a pharmacological dose (800 IU once daily) rather than thiazolidinediones therapy produced statistically significant improvements in histological steatohepatitis⁽⁶⁵⁾. Given the lack of targeted therapies that are proven to ameliorate steatohepatitis, it is likely that vitamin E will become part of the standard of care for NAFLD/NASH.

There remains a need to develop further targeted therapies both to address the initiation of steatohepatitis and the progression of liver fibrosis. Based on our developing understanding of disease pathogenesis, a range of agents has been tested in experimental models. One area that has provided promising results is caspase inhibition; however, the effects of long-term use are as yet unknown and to date they have not been studied in clinical trials^(66,67). There is now an emerging literature providing a foundation for evidence-based therapy in NAFLD/NASH. Epidemiological and interventional data, backed by basic science, point to certain dietary constituents (such as fructose and saturated fats) being associated with NASH, and some (*n*-3 PUFA) being potentially protective against NASH. As CVD and malignancies are the major causes of death in NAFLD/NASH, modification of risk factors for these outcomes (such as smoking, hypertension and obesity) should form a prominent part of management strategies for NAFLD. However, in patients with NAFLD/NASH, liver-related morbidity and mortality also far exceeds that of the general population, so screening for complications of chronic liver disease (hepatocellular carcinoma) and portal hypertension (oesophageal varices) is warranted, particularly where the risk of severe fibrosis or cirrhosis is high.

The importance of fatty liver

The importance of fatty liver has been discussed from the perspective of the epidemiology, pathogenesis, associated morbidity and mortality and treatment options. Hepatic steatosis is present in about a quarter to a third of the adult population in developed countries. Tools are increasingly available to screen for and stage the severity of disease. The pathogenesis is increasingly understood informing the development of treatment strategies. Our understanding of the natural history of the disorder and epidemiological studies of morbidity and mortality have demonstrated that a proportion of patients go on to develop progressive disease, which in absolute terms results in a large number of patients with significant liver disease. This is echoed by a small but increased risk of liver-related mortality and substantial all-cause mortality as a result of the multi-system consequences of the metabolic syndrome. Finally, simple dietary and lifestyle measures, when adhered to, lead to improvements in disease, while an increasing number of specific treatments are being developed. This highlights the importance of the condition and supports the case for increased clinical awareness, targeted service development and basic and clinical research.

Acknowledgements

J. F. L. C. is a Walport Clinical Lecturer at Imperial College London. Q. M. A. and S. T. R. are Imperial College/NIHR Biomedical Research Centre investigators and hold grants from the Medical Research Council and the Wellcome Trust. All authors are grateful to the NIHR Biomedical Facility at Imperial College London for infrastructure support. The authors declare no conflict of interest. J. F. L. C., Q. M. A. and S. D. T. R. wrote the manuscript and approved the final version.

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