Non-alcoholic fatty liver disease in adults: Current concepts in etiology, outcomes and management

Thomas Marjot[1,2] Ahmad Moolla[2], Jeremy F Cobbold[1], Leanne Hodson[2], Jeremy W Tomlinson[2]

1. Translational Gastroenterology Unit, NIHR Oxford Biomedical Research Centre, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, UK.
2. Oxford Centre for Diabetes, Endocrinology and Metabolism, NIHR Oxford Biomedical Research Centre, University of Oxford, Churchill Hospital, Oxford, UK, OX3 7LE, UK

Address for correspondence:
Professor Jeremy Tomlinson,
Oxford Centre for Diabetes, Endocrinology and Metabolism
University of Oxford,
Oxford OX3 7LJ, UK
Email: Jeremy.tomlinson@ocdem.ox.ac.uk
Phone: +44 (0)1865857359; *Fax:* +44 (0)1865857213

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disease, extending from simple steatosis, through to inflammation and fibrosis with a significant risk for the development of cirrhosis. It is highly prevalent and is associated with significant adverse outcomes both through liver-specific morbidity and mortality, but perhaps more importantly, through adverse cardiovascular and metabolic outcomes. It is tightly associated with type 2 diabetes and obesity and both of these conditions drive progressive disease towards the more advanced stages. The mechanisms that govern hepatic lipid accumulation and the predisposition to inflammation and fibrosis are still not fully understood, but reflect a complex interplay between metabolic target tissues including adipose and skeletal muscle, and immune and inflammatory cells.

The ability to make an accurate assessment of disease stage (that relates to clinical outcome) can also be challenging. Whilst liver biopsy is still regarded as the gold-standard investigative tool, there is an extensive literature on the search for novel non-invasive biomarkers and imaging modalities that aim to accurately reflect the stage of underlying disease. Finally, although no therapies are currently licenced for the treatment of NAFLD, there are interventions that appear to have proven efficacy in randomized controlled trials as well as an extensive emerging therapeutic landscape of new agents that target many of the fundamental pathophysiological processes that drive NAFLD. It is highly likely that over the next few years, new treatments with a specific licence for the treatment of NAFLD will become available.

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1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognised as the hepatic manifestation of metabolic syndrome frequently co-existing with obesity, dyslipidaemia and insulin resistance. It is characterized by hepatic steatosis in the absence of secondary causes such as significant alcohol consumption, chronic viral hepatitis, medications that induce steatosis or other chronic liver diseases such as autoimmune hepatitis, haemochromatosis or Wilson's disease. NAFLD can be subcategorised into non-alcoholic fatty liver (NAFL) (formerly simple steatosis) and non-alcoholic steatohepatitis (NASH). NAFL represents steatosis in the absence of histological evidence of hepatocyte injury or inflammation, whilst NASH is characterized by the presence of steatosis, ballooning degeneration and lobular inflammation, with or without peri-sinusoidal fibrosis on liver histology and has a greater propensity to progress to cirrhosis and hepatocellular carcinoma (HCC).

There are currently many challenges in the diagnosis and management of patients with NAFLD; the adverse outcomes associated with the condition seem clear, yet accurate staging of disease without the use of liver biopsy is not established; in the absence of licenced pharmacotherapy, lifestyle modification remains the mainstay of treatment, but this is difficult to implement and maintain. Despite these challenges, the field is moving rapidly and in this review we will summarize the literature detailing current thoughts and concepts in pathogenesis, diagnosis, staging and clinical management. NAFLD is a condition with increasing prevalence in children, however the scope of this review will focus largely on data from adult patients.

2 Epidemiology of NAFLD

2.1 Prevalence of NAFLD

Meta-analysis of studies using imaging modalities for diagnosis has confirmed NAFLD as the commonest liver disease worldwide affecting approximately 25% of the global population (1). NAFLD is most prevalent in the Middle East (32%) and South America (30%), lowest in Africa (13%) and intermediate in Europe (24%), Asia (27%) and North America (24%). The global burden of NAFLD has rapidly increased over time with prevalence increasing from 15% to 25% between 2005 and 2010 (1). In the United States prevalence increased 2.7-fold between years 2003 and 2011 (2). However, estimates of NAFLD prevalence vary according to the modality used for diagnosis. Using liver biochemical tests (or "liver function tests", LFT), abnormalities in the absence of other causes of liver disease as the primary method of diagnosis

significantly underestimates the true prevalence of NAFLD by up to 10% (1). A summary of the major factors driving the increasing prevalence of NAFLD is presented schematically in Figure 1.

2.2 Incidence of NAFLD

There are fewer published data regarding the incidence of NAFLD. Given that the rates of obesity have increased 2-3-fold across the Americas, Europe and Asia over the past three decades, it is generally assumed that NAFLD incidence is rising proportionally. Pooled incidence of NAFLD in Asia and Israel was found to be 52 and 28 per 1000 person-years respectively (1). A recent community study in North America using coding data for case identification demonstrated NAFLD incidence increased 5-fold between 1997 and 2014 from 62 to 329 per 100,000 person years (3). The increase was found to be disproportionally higher among adults aged 18-39 years in whom the incidence of NAFLD increased 7-fold from 20 to 140 per 100,000 person years. This upward trend was also demonstrated in a second North American study in which overall incidence increased over a ten-year period particularly in the young (<45yrs) where incident cases increased by 7.5% each year (2). The rising incidence and shift in the epidemiology towards younger populations serves as a stark reminder that the global burden of NAFLD and its complications including HCC, liver failure and cardiovascular events is only set to increase.

2.3 Epidemiology of NASH, advanced fibrosis and HCC.

Six to 30% of patients with ultrasound proven NAFLD will have biopsy proven NASH, corresponding to overall population prevalence between 1.5 to 6.45% (1). Approximately 40% of NASH patients develop fibrosis progression, with an average annual fibrosis stage progression rate of 0.09. The annual HCC incident rate in NASH patients was 5.29 per 1,000 person-years. Although this HCC risk is less than that observed in chronic viral hepatitis (4), given the high prevalence of NAFLD, the global burden of NASH-related HCC will continue to rise and indeed HCC is now the fourth leading aetiology of cancer mortality worldwide (5). A UK study found that NAFLD-associated HCC had increased 10-fold over a decade (6) highlighting the clear shift in the attributable proportion of HCC from viral hepatitis to NASH.

2.4 Ethnicity and NAFLD

The contribution of ethnicity to the prevalence and severity of NAFLD is complex and remains controversial. Studies have demonstrated that Hispanic patients have a disproportionately higher, and African-Americans, a lower prevalence of NAFLD

compared with Caucasian populations and whilst these observations have been confirmed in recent meta-analysis of epidemiological studies from the United States, the interpretation of detailed aspects of the data needs to be considered. Importantly, the ethnicity-related differences were smaller in high-risk groups (obesity and type 2 diabetes mellitus (T2D)) compared with population-based cohorts (7); the prevalence of NAFLD (and NASH) in high-risk Hispanic patients was not different to that of Caucasians. Further studies have suggested that when Hispanic and Caucasian patients are matched for obesity, the severity of NASH and advanced fibrosis are not different between the 2 groups (8). The data with regards to African-Americans is a little clearer in that they do have a lower prevalence of NAFLD, however NASH can develop as frequently as is seen in Caucasian patients and, there is no evidence to suggest any difference in the rates of advanced fibrosis with differing ethnicity (9). Overall, these data would suggest that ethnicity might have a relatively greater influence on NAFLD prevalence rather than its severity.

The causes for ethnicity related differences in NAFLD prevalence and severity are complex, with contributions from genetic and environmental factors, socioeconomic status and differential access to health care. Although obesity and T2D are well established risk factors for NAFLD these factors cannot entirely explain for the observed racial differences considering that metabolic syndrome is highly prevalent in Black compared with White populations (10), yet the latter group has far higher rates of NAFLD. Gene variants may have a greater influence over susceptibility to NAFLD prevalence; for example the prevalence the polymorphism in PNPLA3 in different racial groups was found in 49% of Hispanics, 23% in White persons and 17% in black persons (11).

The epidemiology of NAFLD in south Asian populations has been extensively reviewed. A recent systematic review and meta-analysis (that included data from 237 studies and >13 million participants), concluded that in Asia the prevalence of NAFLD was 29·6% and that the annual incidence rate was 50 cases per 1000 person-years (12). Furthermore, in Asian patients with biopsy-proven NAFLD, NASH was identified in approximately 63.5% of cases (1). Within south Asian populations living in western countries, NAFLD was most prevalent in those patients originating from Bangladesh (13). Whilst the absolute prevalence and incidence rates of NAFLD and NASH are not markedly different compared with other populations (in fact in absolute terms may actually be slightly lower) (14), for a given BMI, prevalence and severity of NAFLD are likely to be higher mirroring observations with respect to T2D.

The reasons for this are not fully understood, but genetic variability, alterations in adipose tissue biology as well as lifestyle differences (diet and physical activity) are likely to be important (15).

2.5 NAFLD, obesity and T2D

The prevalence of NAFLD increases in high-risk groups particularly in those with obesity and T2D. In an unselected Italian population sample 91% of patients who were obese (BMI ≥30 kg/m^2) and 67% of those who were overweight (BMI 25–30 kg/m^2) had ultrasound evidence of NAFLD (16). Similarly, the pooled prevalence of biopsy-proven NAFLD in morbidly obese patients undergoing bariatric surgery was found to be 91% (17). There has been a global increase in obesity in the last 30 years, which has largely been accounted for by new cases arising in Asia as a result of urbanisation, lifestyle changes, westernised diet and over-nutrition. The number of obese people in China was below 0.1 million in 1975 and rose to 43.2 million in 2014, accounting for 16.3% of global obesity (18). The number of obese people in India rose from 0.4 to 9.8 million in the same timeframe (19). This has correlated with year-on-year increases in NAFLD prevalence (20) even in traditionally rural areas (21,22). In patients with T2D, two large stuies from Italy reported NAFLD prevalence as 60–70% (23,24) and data from the UK suggested a prevalence of 42.6% (25). Recent meta-analysis of studies in T2D demonstrated a pooled NAFLD prevalence estimate of 60% although there was a high degree of heterogeneity among eligible studies with proportions ranging from 29.6% to 87.1% (26). In a global meta-analysis of studies in unselected general populations, obesity and T2D were present in 51% and 22.5% of those with NAFLD respectively (27). Taken together these data indicate the close association of obesity and T2D with NAFLD and in these high-risk groups NAFLD prevalence is roughly double that found in the general population (7).

2.6 NAFLD in patients with BMI <25kg/m^2

Although NAFLD is usually associated with obesity it can occur in those with a BMI <25kg/m^2 and this has been extensively and recently reviewed (28,29). The prevalence of NAFLD ranges from 7% in the United States (30) to 19% in Asia (14) although ethnicity–specific cut-offs for BMI need to be considered when interpreting published epidemiological which can make direct comparisons challenging. Despite patients with lean NAFLD having a more favourable cardiac and metabolic profile than obese patients with NAFLD, they have worse metabolic parameters (dyslipidaemia, hypertension, insulin resistance) relative to lean controls without NAFLD (31). This cardio-metabolic risk is in part attributable to alterations in body fat

distribution particularly increased visceral adiposity (32). Polymorphisms in palatin-like phospholipase domain-containing protein 3 (PNPLA3), and transmembrane 6 superfamily member 2 (TM6SF2) may also have a relatively greater contribution in the pathogenesis of 'non-obese' *vs.* 'obesity-associated' NAFLD (see section 3.6) (33–35). Data are conflicting regarding the histological correlates of lean NAFLD with studies suggesting both a reduced (35–37) and equivalent (38,39) prevalence of NASH and/or fibrosis compared with NAFLD in overweight patients. Those with lean NAFLD may however have accelerated progression of fibrosis with one study showing a higher rate of developing cirrhosis or HCC over 20-years compared with overweight patients with NAFLD (39) however this remains controversial. This suggests that once an individual develops NASH, obesity is unlikely to be the primary driver in fibrosis progression. The effect of lean NAFLD on overall prognosis is similarly controversial. Whilst a retrospective study of 646 NAFLD patients (19% with lean NAFLD) found no increase in overall mortality (39) a prospective study of >1090 biopsy-proven NAFLD patients (11.5% with lean NAFLD), published in abstract form only, did demonstrate a higher overall mortality compared with overweight patients with NAFLD despite presenting with a healthier metabolic profile and less advanced fibrosis (40). Further studies are clearly required to better establish the pathogenesis, progression and prognosis in this condition. Disentangling obesity from insulin resistance is important. Non-obese individuals who have NAFLD are more insulin resistant than those without NAFLD and indeed may be as insulin resistant as some obese individuals and this may be the most important driver to disease progression (rather than obesity *per se*) (41). However consensus remains that NAFLD in non-obese patients is not benign and represents an important adverse risk factor for cardiovascular and liver health.

2.7 Associations with other endocrine disease

NAFLD is also associated with other endocrine conditions and this has been reviewed previously (42). Hypogonadism in men and androgen excess in women are both associated with hepatic steatosis (43). Testosterone treatment has been shown to reduce liver fat in men (44) and we have shown that limiting androgen availability using 5α-reductase inhibition can increase hepatic steatosis (45). In patients with polycystic ovary syndrome (PCOS), there is continued debate as to the relative contributions of androgen excess and systemic insulin resistance as the main driver of the increased prevalence of NAFLD (46). There are currently no published data that have examined the impact of limiting androgen action in patients with PCOS as a strategy to improve NAFLD. Glucocorticoid excess is associated with NAFLD (47)

and furthermore, limiting endogenous glucocorticoid availability through the pre-receptor inhibition of the cortisol-regenerating enzyme, 11β-hydroxysteroid dehydrogenase type 1, deceases liver fat content (48). Hypothyroidism is also associated with NAFLD (49) and it is interesting to note that liver-specific thyroid receptor agonism is being developed as a possible therapeutic strategy (see section 7.3.8) (50).

NALFD has a sexually dimorphic prevalence (see section 2.8) and estrogens are likely to play a crucial role. Estradiol is believed to be protective for the development of NAFLD (51) and its prevalence increases in post-menopausal women. There is also evidence to suggest that post-menopausal hormone replacement therapy can protect against NAFLD (52)(53). Growth hormone (GH) deficiency is also linked to the development of NAFLD, with some evidence showing that GH replacement is associated with improvements in liver histology (54).

2.8 Gender and age in NAFLD, NASH and HCC

NAFLD is significantly more prevalent in men than women in the general population (55) although NAFLD in people with a BMI<25kg/m^2 may be more prevalent in women (30). In the Dallas Heart Study, white Caucasian men had an approximately 2-fold increased prevalence of NAFLD compared with white women (56). Several mechanisms may contribute to this observation including body fat distribution, hepatic fatty acid partitioning, lifestyle, and sex hormone metabolism (55,57,58). There also appears to be gender-specific differences in relation to age, with NAFLD prevalence differing relatively little across all age groups in men (59) but increasing significantly after the age of 50 years in women (59,60). Menopausal status is implicated in this age-sex interaction with up to double the rate of NAFLD found in post- compared with pre-menopausal women (59,61–63). The protective role of estrogens is further supported by the observation that the risk of NAFLD increases in young women having oophorectomy (64) and in those on tamoxifen (65), and that there is a risk-reduction in those receiving hormone replacement therapy (61). In those patients with NAFLD, the risk of NASH may be higher in women and data from the NASH Clinical Research Network (CRN) showed that patients with biopsy proven NASH were more likely to be female than male in a roughly 2:1 ratio. Whilst this may reflect a higher disease burden in women it may be accounted for by sex differences among those pursuing and receiving healthcare. Data regarding the impact of gender on fibrosis progression are conflicting. Whilst one systematic review of risk factors for fibrosis progression in NASH found associations with age but not with gender (66),

several cross-sectional biopsy studies have shown an increased risk of advanced fibrosis in females compared with males, independent of metabolic risk factors (67,68). Furthermore, duration of estrogen deficiency in the postmenopausal state does confer fibrosis risk among women with NAFLD (69,70). Irrespective of etiology of liver disease, HCC has a strong male preponderance, with a male to female ratio estimated to be 2–2.5:1. However the male-female-ratio may be lower in NASH-associated HCC (1.6:1) compared with hepatitis B and C virus (HBV, HCV) and alcohol related cirrhosis. Male patients with NASH also appear to develop HCC at a less advanced stage of liver fibrosis than female patients (71) and their overall survival is worse compared with women (72,73). Overall prognosis in NAFLD also seems to be effected by gender with National Health and Nutrition Examination Survey (NHANES III) data from North America showing that male gender was significantly associated with overall mortality (74).

2.9 Sleep disturbance and NAFLD

Obstructive sleep apnoea (OSA) is a highly prevalent disorder affecting 9-38% (75) of the general population and is associated with obesity in up to 60% of cases (76). The disease has become increasingly clinically relevant due to its association with cardiovascular morbidity and mortality (77–79). A recent meta-analysis found OSA to be independently associated with the presence of NAFLD and with degree of hepatic steatosis, lobular inflammation, ballooning degeneration and fibrosis (80). OSA is characterised by the repetitive occurrence of partial or complete pharyngeal collapse during sleep leading over time to chronic intermittent hypoxia (CIH). CIH has been implicated in the pathogenesis and progression of NAFLD (81) through mitochondrial dysfunction (82–84) and increased hepatic *de novo* lipogenesis (85,86), reactive oxygen species (84,85,87), gut permeability (88) and liver inflammation (83,89). Nocturnal continuous positive airway pressure (CPAP) is the gold-standard treatment for OSA and improves cognitive function, daytime somnolence, and quality of life. Despite observational data suggesting CPAP may improve LFTs in those with OSA and suspected NAFLD (90) and improve cardiovascular disease (91), randomised control trials in adults have failed to demonstrate a significant benefit on metabolic syndrome (92) and non-invasive markers of steatosis, hepatic inflammation and fibrosis (93,94).

2.10 Diet and NAFLD

2.10.1 Westernised versus Mediterranean diet in NAFLD

NAFLD prevalence has mirrored the global epidemic of obesity and T2D and is associated with consumption of a Westernised diet (95–102) characterised by high intake of fast foods, confectionary, refined grains, red and processed meats, full-fat dairy products, and soft drinks. NAFLD patients have a higher median daily total energy intake compared with age- and sex-matched healthy controls (100,103). By contrast, the Mediterranean diet, characterised by low consumption of saturated fat and cholesterol, high consumption of monounsaturated fatty acids, a balanced omega-6 to omega-3 fatty acid ratio and high content of complex carbohydrates and fibre, has been associated with lower rates of NAFLD (98,104–106), NASH (106) and fibrosis (107) as well as cardiovascular events and cancer (108–110). Randomized trials have shown the Mediterranean diet to reduce plasma ALT levels in obese patients with T2D (111) and improve insulin sensitivity and hepatic steatosis measured by magnetic resonance spectroscopy in patients with NAFLD (112,113), independent of weight loss. The biological mechanisms involved in these improvements are likely to include the anti-inflammatory and lipid lowering properties of the Mediterranean diet and its impact on gut microbiota composition (114). The Mediterranean dietary pattern is therefore the current recommended macronutrient composition in NAFLD and is recommended in the joint EASL-EASD-EASO clinical practice guidelines (115).

2.10.2 Fructose in NAFLD

Fructose is a major component of the two most widely used sweeteners; sucrose and high-fructose corn syrup (HFCS). Its consumption, predominantly in the form of soft drinks, has increased significantly over the last hundred years and now makes up around 15% of the energy consumed as part of a Westernised diet (116). Patients with NAFLD consume nearly 3-times more fructose compared with age, sex and BMI matched controls (117) and daily intakes of sucrose sweetened drinks significantly increases hepatic and visceral fat accumulation compared with milk, diet cola, and water (118). Fructose consumption was associated with higher fibrosis stage in 427 adults enrolled in the NASH Clinical Research Network (119). Furthermore, low fructose intake may protect against future development of NAFLD in obese individuals (120). Mechanistically, fructose appears to have a major role in inducing hepatic steatosis both by stimulating hepatic *de novo* lipogenesis (DNL) and attenuating β-fatty acid oxidation (121–125). In contrast to glucose, fructose is rapidly phosphorylated by fructokinase leading to a reduction in adenosine triphosphate

(ATP). This fall in ATP induces a cascade of impaired protein synthesis (126), oxidative stress and mitochondrial dysfunction (127,128); features well established in the pathogenesis of NASH. The drop in intracellular phosphate also drives rapid purine nucleotide turnover culminating in the formation of uric acid (124,129). Fructose is the only common carbohydrate to generate uric acid during its metabolism which has also been implicated in the pathogenesis of NAFLD through increased oxidative stress and impaired β-oxidation (127,128). Epidemiological studies have demonstrated an association between hyperuricemia and NAFLD (130) with meta-analysis showing a dose-dependent rise in incidence of NAFLD by 3% for every 1 mg/dL increase in serum uric acid (131).

2.10.3 Coffee, caffeine and NAFLD

Coffee is one of the most commonly consumed beverages worldwide with 85% of the United States population drinking caffeine daily, 98% of which is accounted for by coffee (132). An association between coffee consumption and a decreased risk of liver disease has been documented in a range of aetiologies including NAFLD (133). Using dietary intake data from four continuous cycles of the National Health and Nutrition Examination Surveys (NHANES), Bierdinc *et al.* demonstrated caffeine intake, mostly as coffee, to be independently associated with a lower risk of NAFLD (OR 0.931, CI 0.900–0.964) (134). Similarly, two case-control studies have shown a beneficial effect of coffee on the risk of NAFLD; Catalano *et al.* and Gutiérrez-Grobe *et al.* both found that coffee drinking was associated with less severe steatosis on ultrasound in Italian and Mexican patients with NAFLD respectively (135,136). In contrast, a recent large Italian population study including 2800 participants found that coffee drinking was not associated with decreased odds for hepatic steatosis detected on ultrasound (137). Coffee has also been linked with a significant reduction in the risk of fibrosis among NASH patients (138) and those with morbid obesity but only with filter coffee and not espresso coffee (139). This may be due to the confounding fibrogenic effect of sucrose (glucose + fructose), which may be added in greater quantity to espresso coffee as compared to filter coffee. Recent meta-analysis of observational studies found that regular coffee consumption was significantly associated with reduced hepatic fibrosis in patients with NAFLD, but not with NAFLD prevalence. This anti-fibrotic effect seems to be related to coffee and not to caffeine specifically and further work is needed to identify which of the >100 compounds found within coffee confers hepato-protection. More broadly, amongst coffee drinkers, a lower mortality and HCC rate has been well documented in patients with cirrhosis across a range of chronic liver disease aetiologies (133). As a

result, coffee consumption is often recommended for patients with chronic liver disease whilst recognising the need for randomised controlled trials and further investigation into the threshold levels of coffee consumption required for benefit.

3 Pathogenesis of NAFLD

3.1 Development of hepatic steatosis – a multiorgan disease

3.1.1 Intrahepatic fat accumulation

Intrahepatic free fatty acids (FFA) can be esterified with glycerol and stored in the form triglyceride (TAG), the predominant lipid accumulating in patients with NAFLD. Alternatively, FFA can undergo β-oxidation or TAG can be exported from the liver in very low-density lipoprotein (VLDL). Steatosis in NAFLD represents an inherent imbalance in these processes of lipid influx and synthesis *vs.* disposal. There are three sources of FFA that contribute to liver TAG in NAFLD; 59% from circulating FFA; 26% from the generation of FFA from non-lipid precursors (including glucose and fructose), through DNL; and 14% from the diet (140).

3.1.2 Adipose tissue dysfunction

Under normal conditions, adipose tissue is extremely insulin responsive; storing lipid and inhibiting TAG lipolysis. Conversely, with developing insulin resistance, increased circulating FFA are made available for hepatic uptake and storage, at least in part through the impaired ability of insulin to supress lipolysis (141). Adipose dysfunction has a major role in modulating severity of liver injury and cardio-metabolic risk in NASH (142–144). Targeting adipose insulin resistance with weight loss (145) or thiazolidinediones (146) is likely therefore to be of therapeutic benefit (see section 7.1.2). In turn, NASH itself drives hepatic and peripheral insulin resistance thereby setting up a vicious cycle of insulin resistance, hepatic FFA flux, steatosis and inflammation (147,148) (Figure 1).

Whilst obesity is a major determinant of the high global prevalence of NAFLD, the precise connection between adiposity and NAFLD remains unclear. Expansion of the peripheral adipose depot may provide a buffering capacity, protecting the liver from excessive FFA flux. This is exemplified in those patients with lipodystrophy, a group of syndromes characterised by partial or complete absence of adipose tissue but severe insulin resistance, ectopic fat accumulation, NAFL and NASH (149). However, even metabolically healthy obese patients (MHO), who may be expected to be relatively protected from steatosis, are still at significant increased risk of NAFLD (150) suggesting that obesity *per se* is a risk factor for NAFLD independent of insulin

sensitivity. Dysfunctional adipose tissue also leads to reduced secretion of adiponectin, an insulin-sensitizing adipokine paradoxically reduced in obesity. Adiponectin has wide-ranging hepato-protective effects increasing FFA oxidation and decreasing FFA influx, gluconeogenesis and DNL (151–153). It also has hepatic anti-inflammatory and anti-fibrotic properties through the suppression of pro-inflammatory cytokines (e.g. TNF-α and IL-6) (154,155) and reduced activation and proliferation of hepatic stellate cells (HSC) respectively (156–158). Levels of circulating adiponectin are significantly reduced in patients with NAFLD and NASH and correlate with the degree of hepatic steatosis, necro-inflammation, and fibrosis (159–161). Furthermore, plasma levels have been found to be the best indicator of histological improvement of NASH with pioglitazone (162). Adiponectin treatment has been shown to ameliorate NASH in rodent models (163) but human data are currently lacking.

3.1.3 Intrahepatic de novo lipogenesis (DNL)

The second major source of intrahepatic FFA is synthesised from non-lipid precursors, predominantly glucose and fructose, through DNL. Up-regulation of DNL is a hallmark of NAFLD with stable isotope infusion studies showing up to a 3.5-fold increase in patients with NAFLD compared with healthy controls (164,165). Furthermore, whilst adipose-derived FFA accounts for the majority of liver TAG, the contribution from this source is less up-regulated in NAFLD compared with marked increase in DNL that is seen in NAFLD (164). DNL is a multi-step, highly regulated process during which acetyl-CoA, derived from carbohydrate glycolysis, is converted to malonyl-CoA and ultimately palmitic acid under the control of several key enzymes and transcription factors (including SREBP1c, FAS and ACC1) all of which are overexpressed in NAFLD (166,167). Insulin promotes lipogenesis through the transcription and activation of SREBP1c, a master regulator of lipogenesis (168) and even in insulin resistant conditions such as T2D, obesity and NAFLD, insulin continues to selectively support DNL whilst failing to reduce hepatic gluconeogenesis (169,170). Deciphering the precise mechanisms for this differential regulation of hepatic lipid and glucose metabolism has proven difficult with studies implicating a range of insulin-dependent (171–173) and independent molecular pathways (174). Dietary sugars form a major source of substrate for hepatic DNL (170,175) and even short-term hypercaloric diet, overfeeding with simple carbohydrate markedly increases liver fat through DNL (175). The post-prandial surge of intrahepatic carbohydrate *via* the portal circulation contrasts with dietary lipid, which is absorbed as chylomicrons *via* lymphatics into the systemic circulation where it is distributed to

all metabolically active tissues. This explains the relatively small direct contribution of dietary lipid to intrahepatic TAG found in patients with NAFLD (140). Dietary fructose is particularly lipogenic and a better substrate for DNL than glucose and its increasing consumption has been implicated in the rising global burden of NAFLD (see section 2.9.2). Furthermore, fructose metabolism is highly energy dependent which can compound liver injury through a cascade of impaired protein synthesis (126), oxidative stress and mitochondrial dysfunction (127,128). Skeletal muscle insulin resistance may also contribute to steatosis by causing a redistribution of post prandial glucose away from peripheral glycogen storage and towards hepatic DNL (176,177) (Figure 1). A large population study has also demonstrated skeletal muscle mass to be inversely correlated with NAFLD incidence and positively associated with the resolution of baseline NAFLD (178).

3.1.4 Triglyceride as an epiphenomenon alongside liver injury

Although TAG is the most conspicuous intrahepatic lipid in NAFLD, there are widespread changes in liver lipid composition including diacylglycerol (DAG), ceramides, TAG/DAG ratio, free cholesterol and phospholipids which are likely to be of greater pathogenic significance and these have been reviewed previously (179–181). Indeed, TAG *per se* is not hepatotoxic, despite its accumulation traditionally being the basis for the clinical grading of NAFLD 'severity'. For example, inhibition of TAG incorporation into VLDL leads to increased triglyceride accumulation but without liver injury and inhibition of diacylglycercol acyltransferase 2 (DGAT2), an enzyme involved in TAG formation, results in a reduction of intrahepatic TAG and worsening of steatohepatitis in mouse models (182). TAG accumulation can therefore be seen as a protective response to the increased burden of hepatotoxic free fatty acids and an epiphenomenon occurring alongside liver injury (183).

3.2 The transition to NASH and the development of fibrosis

3.2.1 Hepatic inflammation, immune cells and inflammasome activation.

In NAFLD, the convergence of multiple toxic insults on the liver including FFAs, insulin resistance, gut derived endotoxins and adipose tissue dysfunction culminates in a profound pro-inflammatory state ultimately propagating NASH and fibrosis. As an example, patients with NASH having increased hepatic and adipose tissue concentrations of tumour necrosis factor-alpha (TNF-alpha) compared with obese controls, which positively correlates with fibrosis severity (184). Furthermore, the persistent activation or overexpression of transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), a crucial regulator of the acute

inflammatory response has been found in animal models of NAFLD and insulin resistance (185) as well as patients with NASH (186) (Figure 1).

The immune response plays a crucial role in triggering and amplifying hepatic inflammation (187). With hepatocyte injury, host biomolecules called damage-associated molecular patterns (DAMPs) are released. These are able to drive inflammation by activating resident macrophages, Kupffer cells (KC) acting *via* pattern recognition receptors (PRRs), the best characterised of which are the toll-like receptor (TLR) family. Activated KC then drive the production of pro-inflammatory cytokines including TNF-alpha, interleukin1b (IL-1b), interleukin-6 (IL-6) and C–C motif ligand 2 and 5 (CCL2 and CCL5), which exacerbate hepatocyte injury and cell death, predominantly through apoptosis (186). KC also secrete tumour growth factor-beta (TGF-beta) and platelet-derived growth factor (PDGF) providing additional stimulation to hepatic stellate cells (HSC) which promote fibrosis through up-regulation of smooth muscle actin, desmin and type I collagen. KC and HSC can also be activated directly by gut-derived bacterial products, including lipopolysaccharide (LPS), collectively known as pathogen-associated molecular patterns (PAMPs). Suppression of TLR4, the receptor for LPS, and TLR-9, which binds bacterial DNA, have been shown to reduce liver inflammation in experimental rodent models of NASH (188,189).

DAMPs, PAMPs and KC also have an important role in the activation of inflammasomes, which are multi-protein intracellular complexes assembled in response to cell damage or pathogens to produce pro-inflammatory cytokines IL-1β and IL-18. Inflammasomes have a pivotal role in fine tuning the host inflammatory response and have been implicated in a range of acute and chronic liver diseases including NASH (190). Expression of inflammasome-associated proteins are significantly elevated in patients with NASH compared to those with simple steatosis (191) and FFA were found to directly up-regulate inflammasome activation and sensitise hepatocytes to the effects of LPS in rodent models of NASH (192). Conversely, inflammasome deficient mice are relatively protected from diet induced NASH and fibrosis (191).

Neutrophil infiltration is often seen in the livers of patients with NASH and contributes to macrophage recruitment and cell damage through the release of reactive oxygen species (ROS) and inflammatory mediators such as myeloperoxidase and elastase (193). Deficiency of these enzymes in knockout mice attenuates the development of

NASH (194) and insulin resistance (195) and diminishes adipose tissue inflammation in response to a high fat diet (194). Neutrophil activation may also contribute to NASH progression with secreted extracellular hepatic neutrophil peptides (HNPs) enhancing liver fibrosis *via* HSC activation in steatotic mice (196). Beyond KC and neutrophils however, the role of other innate immune cells is less clear. Natural killer (NK) cells have a complex role in cell damage detection having the ability to both enhance and limit the magnitude of inflammation (197). As such, disentangling their involvement in NASH has proven difficult with discrepant data showing both pathogenic and protective influences (198,199). The role of dendritic cells (DC) is similarly controversial; despite these cells assuming an activated immune phenotype in NASH with increased expression of TNF-alpha and IL-6, ablation of DC populations is paradoxically associated with worse hepatic inflammation and fibrosis (200). The adaptive immune response is also mobilised in parallel with the innate response with lymphocytes, along with macrophages representing the most abundant inflammatory cells in the lobular and peri-portal infiltrates of NASH. Experiments in mice demonstrate that activated $CD4^+$ and $CD8^+$ T-lymphocytes are recruited within the liver through DC maturation (200) and up-regulated vascular adhesion protein-1 (VAP-1) (201) and their prevalence parallels worsening parenchymal injury and lobular inflammation (202). This is analogous to findings in obesity where adipose tissue CD4+/CD8+ T lymphocytes have an important role in supporting macrophages and generating pro-inflammatory mediators in the cascade towards insulin resistance (203). Data regarding the role of B-lymphocytes is relatively lacking. Whilst studies have shown elevated titres of IgG to antigens originating from oxidative stress in methionine-choline deficient mice (202), further investigation is needed to better clarify the role of humoral immunity in NASH.

3.3 Mitochondrial dysfunction

Mitochondria are responsible for the intrahepatic β-oxidation of FFA as well as co-ordinating the tricarboxylic acid (TCA) cycle, adenosine triphosphate (ATP) synthesis through oxidative phosphorylation, and the generation of reactive oxygen species (ROS) (204). In patients with NAFLD, mitochondrial biogenesis, mitochondrial mass and maximal respiratory rates are all up-regulated in order to cope with the increased lipid burden compared with healthy controls (205). Over time, this 'mitochondrial flexibility' can become exhausted leading to uncoupling, enhanced ROS formation and oxidative stress culminating in NASH and impaired hepatic insulin resistance (206–208). Mitochondrial derived ROS have a central role in propagating hepatocyte injury by generating both lipid peroxidation products (209) and TNF-alpha, both of

which induce further mitochondrial damage, permeability and uncoupling (205). Enhanced hepatocellular sensitivity to TNF-alpha conferred by mitochondrial accumulation of cholesterol and subsequent glutathione depletion may then help the precipitation of NASH (210). Impaired mitophagy, the selective autophagic removal of damaged mitochondria, may also have a pathogenic role in NASH. Under physiological conditions mitophagy helps prevent cell death by reducing oxidative stress and preserving mitochondrial bioenergetics whereas this function is diminished by most features of the metabolic syndrome including obesity, insulin resistance and dyslipidemia (211,212). Autophagy has been shown to be significantly inhibited in patients with NASH as compared with steatosis alone and correlates with severity of liver disease and markers of oxidative stress (213).

3.4 Bile acids and the pathogenesis of NAFLD

There is an increasing recognition that bile acids are potent signalling molecules with important cell functions over and above their role to facilitate lipid absorption. Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesised within the liver, secreted into the bile and delivered to the intestine where the actions of the gut microbiota generate secondary bile acids that are reabsorbed (>90%), largely in the small intestine into the portal circulation. A smaller proportion enters the systemic circulation.

Bile acids act *via* a variety of receptors including the farnesoid X receptor, takeda G-protein-coupled (TGR5), pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). Activation of these receptors is dependent upon the individual bile acid and therefore the impact of alteration of the total bile acid pool depends upon both on changes in quantity and composition.

There is an extensive literature that has examined the impact of genetic manipulation of bile acid receptors (most commonly FXR using rodent models) in the development of NAFLD. Global FXR deletion appears to worsen metabolic phenotype (214), however, alterations in tissue-specific expression are important. Intestinal FXR activation is able to promote NAFLD (215) and activation of these receptors leads to fibroblast factor 19 secretion (FGF-19) secretion, which, in addition to being a potent negative regulator of bile acid synthesis, has also been linked to the development of an adverse metabolic phenotype. FGF-19 analogues are currently being trialled in the context of NASH (see section 7.3.6).

Data are conflicting with regards to total fasting and post-prandial bile acid levels in obese patients and those with T2D; studies have shown unchanged, decreased or elevated levels (recently reviewed in (216)). Many small studies have also

specifically looked at bile acid levels in the context of NAFLD (using serum, plasma, fecal and liver samples) (216). In the majority of studies, bile acid levels are elevated in NAFLD and there are some specific associations of individual bile acids with hepatic inflammation (for example, high levels of circulating glycholic acid were associated with lobular inflammation on liver biopsy (217)

The mechanisms driving the changes in the bile acid pool are not fully determined. Changes in hepatic expression of key enzymes involved in bile acid synthesis have been described (217–219) and importantly the changes observed in the gut microbiome will have implications for the generation of secondary bile acids (see section 3.5).

The relevance of bile acids and their receptor activation in the pathogenesis of NALFD is reflected in the fact that several pharmaceutical agents currently under investigation are targeting these signalling pathways and it is likely that the first therapies licenced for NALFD will be specific FXR agonists (see section 7.3.1)

3.5 The gut microbiome

The gut microbiome is increasingly recognised in the pathogenesis and progression of a range of diseases including NAFLD/NASH, through the so-called gut-liver axis. Seminal findings initially demonstrated the existence of an 'obese microbiota' with an increased capacity to harvest energy from the diet (220). Furthermore, this trait was transmissible, with germfree mice transplanted with faeces from an obese donor accumulating more total body fat as compared to germfree mice receiving faeces from a lean donor (220). A similar study of microbiota transplantation from human lean donors increased insulin sensitivity in obese individuals with metabolic syndrome (221). This ability of the microbiota to transmit a particular phenotype has also been demonstrated in rodent models of NASH with hepatic steatosis and inflammation being exacerbated in wild-type mice co-housed with mice with inflammasome-mediated liver injury (222). Developing gut dysbiosis (an unhealthy gut microbiome) is complex and highly variable between individuals with multiple environmental influences including diet, lifestyle, antibiotic exposure and perinatal conditions (223). Due to difficulties in controlling for these variables, alongside small sample sizes and variation in laboratory techniques, microbiota composition studies in NAFLD/NASH are sparse and inconclusive (224). Nevertheless, several studies support an inverse correlation between *Bacteroidetes* and presence of NAFLD, NASH and obesity (219,220,225). *Bacteroidetes* are relatively inefficient at dietary energy extraction and their reduction may allow more efficient species to dominate (e.g. *Firmiculates*). Indeed a 20% increase in *Firmicutes* and corresponding decrease

in *Bacteroidetes* was associated with an approximate 150kcal increase in energy nutrient absorption (226). Specific patterns of microbiome composition have also been implicated in fibrosis progression (227,228) and the pathogenesis of HCC (229,230). Not only do patients with NAFLD/NASH have compositional differences in microbiota compared to controls but they also have a greater microbiota volume with the prevalence of small intestinal bacterial overgrowth (SIBO) ranging from 50-77% (231,232). SIBO is associated with several of the pathogenic mechanisms thought to implicate the microbiota in NASH including increased gut permeability through loss of tight-junction integrity (233), enhanced toll like receptor expression and a greater burden of circulating pro-inflammatory cytokines (e.g. IL-8 and TNF-alpha) (231,232). Additional pathways by which dysbiosis and SIBO may adversely impact the liver in NASH include endogenous ethanol production (234) and disruption of both choline (235) and bile acid metabolism (236). The microbiome has also become a potential therapeutic target in the pharmacological treatment of NAFLD. Only data from small studies have been published and studies are currently actively recruiting (see section 7.5).

3.6 Genetic predisposition to NAFLD and disease progression

Despite shared environmental risk factors, there remains considerable inter-individual variation across the spectrum of NAFLD. For example, normal liver histology can be found even in those with morbid obesity (237) and only a minority of those with NAFLD will progress to NASH, fibrosis and HCC. These phenotypic inconsistencies suggest a genetic contribution to the disease. Compared with the general population, first degree relatives of patients with NAFLD have up to 3 times the risk of developing the disease themselves (238). Prospective studies have also demonstrated strong NAFLD concordance between monozygotic versus dizygotic twins and that heritability (the degree of phenotypic variation accounted for by genetics) of steatosis and fibrosis are both ~50% after controlling for age, sex and ethnicity (239). Several common gene variants have emerged out of genome-wide association studies (GWAS), which show an association with the development and progression of NAFLD. The most significant and reproducible of which is the PNPLA3 isoleucine to methionine substitution at position 148 (rs738409 C>G encoding for PNPLA3 I148M). Through screening ethnically diverse adults from the Dallas Heart Study, Romeo and colleagues were the first of many to describe a significant association between PNPLA3 I148M and hepatic fat without impacting on components of the metabolic syndrome (11). Since then the association has extended to histological severity, including NASH (odds ratio (OR) 3.24 [2.79–3.76])

(240), fibrosis (OR 3.11 [2.66, 3.65]) (241), cirrhosis (OR 1.86 [1.64, 2.12]) (242) and HCC (OR 2.32 [1.76, 3.06]) (243). The mechanistic basis of PNPLA3's relationship with NAFLD is yet to be fully characterised. Whilst wild-type PNPLA3 seems to mediate TAG turnover, deletion of PNPLA3 in knockout mice confers no obvious hepatic phenotype (244). The loss-of-function 148M variant protein however manages to escape degradation and its accumulation appears to result in entrapment of TAG in lipid droplets (245) and increased fibrosis through hepatic stellate cell (HSC) activation (246). Furthermore, reduced expression of PNPLA3 148M due to the co-presence of the 434K variant ameliorates liver damage (247) highlighting 148M down-regulation as a potential treatment target in NAFLD (248). The second most important gene variant is the TM6SF2 guanine to adenine substitution at position 167 (rs58542926 E>K encoding for TM6SF2 E167K) which promotes steatosis by interrupting the enrichment of TAG in secreted VLDL (249) whilst simultaneously conferring protection against circulating dyslipidaemia and cardiovascular disease (250). Other variants with moderate effect size include loss-of-function polymorphisms in membrane bound O-acyltransferase domain containing 7 (MBOAT7) rs641738 and glucokinase regulator (GCKR) rs1260326. Down-regulated MBOAT7 appears to mediate steatosis (251,252), inflammation (252), fibrosis (251,252) and carcinogenesis (253) through the toxic accumulation of polyunsaturated fatty acids following impaired incorporation into hepatocyte phospholipids whilst reduced GCKR causes hepatic fat accumulation through constitutive activation of hepatic glucose uptake and increased DNL (254,255). Finally, it is important to note that NAFLD is a complex condition with a high degree of gene-environment interaction. For example, adiposity has been shown to significantly amplify the genetic risk conferred by common gene variants across the full spectrum of NAFLD (256). There is also evidence that dietary factors, particularly carbohydrate and fructose intake augment the impact of PNPLA3 I148M (257,258).

More recently, polymorphisms within 17β-hydroxysteroid dehydrogenase type 13 (HSD17B13) have been identified that are associated with NAFLD (although not when adjusted for BMI), as well as with NASH, fibrosis and cirrhosis (259,260). HSD17B13 is a lipid associated droplet protein that appears to possess retinol dehydrogenase enzyme activity (261), although its true functional role is yet to be determined. Most recently, a loss of function variant has been shown to protect from the development of HCC in patients with alcohol related liver disease (262).

Further work is needed to interrogate the precise molecular mechanisms underpinning the association between gene variants and progressive liver disease in order to identify novel and personalised therapeutic targets.

3.7 Clinical determinants of NAFLD progression and prognosis

There are many putative factors that may drive NAFLD progression to the more advanced stages of disease, many of which (e.g. genotype, microbiome, mitochondrial function, immune response) are not easily or routinely assessed in clinical practice. As a result, we must look to natural history studies to help provide clinical, biochemical, and histological variables that can be used to help predict which patients will develop severe disease with poorer outcomes. Regarding clinical features, in a paired biopsy study McPherson *et al.* highlight the impact of insulin resistance on NAFL with 80% of those with fibrosis progression developing T2D at the time of follow-up biopsy compared with 25% of non-progressors (263). Angulo *et al.* also showed that diabetes at baseline was associated with overall mortality in NAFLD alongside smoking, age, and absence of statin use (264). Data for biochemical predictors are relatively lacking although in patients with biopsy-proven NAFLD and compensated cirrhosis, lower levels of serum cholesterol, ALT, and platelets were shown to be independently associated with liver-related complications (development of varices, ascites, encephalopathy and HCC) and higher AST/ALT ratio with overall mortality (265). A serial biopsy study of 320 NAFLD patients presented in abstract form showed that Fib-4 score (comprising ALT, AST, platelet count and age) was the only baseline non-histological factor associated with fibrosis progression but had inadequate discriminative precision for use in clinical practice with an AUROC of 0.62 (266). Baseline histology provides good prognostic value in NAFLD. Systematic review and meta-analysis of paired biopsy studies has shown that a third of NAFLD patients will have fibrosis progression with overall annual fibrosis progression rate of 0.07 stages for NAFL and 0.14 stages for NASH, corresponding to one stage of fibrosis progression over a median of 14.3 and 7.1 years, respectively (267). Furthermore, several epidemiological studies have now confirmed the presence and severity of fibrosis as the most robust marker for future mortality and liver specific morbidity and have de-emphasised the presence of NASH, which adds very little prognostic value (264,268,269).

4 Clinical consequences and outcomes

4.1 Cardiovascular risk and mortality in NAFLD

Cardiovascular disease (CVD) and NAFLD have shared risk factors including insulin resistance, T2D, obesity, hypertension and dyslipidaemia (270). Even after adjusting

for conventional risk factors, NAFLD remains an independent risk factor for the development of CVD (271)(27)(272). In their global epidemiology study of NAFLD, Younossi *et al*. estimated the association between NAFLD and coexisting CVD risk factors including obesity (51%), T2D (23%), hyperlipidemia (69%), hypertension (39%), and metabolic syndrome (43%) (1).

Once CVD is established, there is also strong evidence suggesting that those patients with NAFLD have an increased severity of CVD burden (273). This may be related to the observation that those patients with NASH have additional risk and have a greater degree of endothelial dysfunction, impaired left and right ventricular function and carotid artery disease increasing the risk of cerebrovascular disease and stroke (274). NASH, through its pro-inflammatory state has systemic effects on multiple organ systems and appears to exacerbate insulin resistance and promote dyslipidaemia, thus exacerbating the development of CVD (275).

Body fat distribution (over and above obesity and measurements of BMI) is also an important consideration; those patients with greater visceral adipose tissue, which has higher metabolic activity, are more likely to have NAFLD and have a higher risk of developing cardio-metabolic complications (271).

A recent systematic review and meta-analysis has demonstrated that patients with NAFLD have a greater prevalence of coronary artery disease 7.5% *vs.* 1.4% and stroke 0.9% *vs.* 0.2% when compared to those without NAFLD. The risk of cardiovascular morbidity was 77% higher compared to controls (defined as those patients without NAFLD) and cardiovascular mortality 46% higher (276). Similarly, a further meta-analysis has shown that the presence of NAFLD is associated with a 64% increase in the risk of both fatal and non-fatal cardiovascular events compared to patients without NAFLD (275). These figures are similar to a recent retrospective study of patients with both NAFLD and T2DM in which the risk of cardiovascular events was 70% higher, and all-cause mortality 60% higher when compared to those without liver disease (277). In addition to these associations, patients with more advanced NAFLD have worse 10-year cardiovascular risk profiles (272) with poorer cardiovascular outcomes, and are approximately 4-times more likely to suffer a fatal CVD event and twice as likely to have a non-fatal CVD event (268)(275).

NAFLD has a bidirectional relationship with T2D; it is a risk factor for the development of T2D with studies showing that those with NAFLD have a 1.5 to 2-fold

increased risk of developing T2D. Similarly, those patients with T2D are more likely to develop NAFLD (270,278). Indeed, the strongest predictor of fibrosis progression in those with NAFLD is the development of incident T2D (263). The presence of NASH is also an important factor with these individuals having an additional up to 3-fold risk of developing T2D (279). In turn, the presence of T2D worsens the course of liver fibrosis, is also more likely to lead to the development of NASH, is a predictor of the development of HCC and is associated with increased all-cause and cardiovascular mortality (273). Finally, the combination of both T2D and NASH together leads to even worse outcomes, both with respect to CVD and liver-related outcomes.

Patients with NAFLD are also at increased risk of chronic kidney disease (CKD) where the prevalence has been shown to be as high as 55% in some studies even after adjustment for other causal factors including T2D and hypertension. Patients with NASH and advanced fibrosis both have a higher prevalence and incidence of CKD (270,280,281). Understanding the precise mechanisms that link CKD and NAFLD remains challenging, but several mechanisms have been postulated including the role of inflamed adipose tissue, circulating pro-inflammatory cytokines and free fatty acids that may comprise renal endothelial cell function and induce oxidative stress leading to end-organ damage (282).

4.2 All cause morbidity and mortality in NAFLD

After cardiovascular disease, cancer is the second clinical entity through which NAFLD exerts its morbidity and mortality and this is manifested *via* both non-hepatic and hepatic malignancies. Cancers of the gastrointestinal tract are most closely associated with NAFLD; the strongest association being increased colorectal cancer. Studies have shown a 2-3 fold increase in the occurrence of adenomatous polyps or adenocarcinoma of the colon (283) and the risk of colorectal cancer is also influenced by the presence of NASH with this group having both an increased absolute risk as well as relatively higher grade of dysplasia. As such, mathematical modelling has suggested that targeted colorectal cancer screening in NAFLD patients may be cost-effective although further studies regarding patient acceptability and feasibility are required. (284).

Though the evidence is less well established, other gastrointestinal cancers are associated with NAFLD including oesophageal, stomach and pancreatic cancer. For non-gastrointestinal cancers, those associated with NAFLD include kidney and

breast cancer as well as melanoma (283). Of additional note, the development of some cancers may also be gender specific, for example a preponderance of breast cancer in females and of colorectal cancer in males (283,285).

4.3 Liver–specific morbidity and mortality

Liver failure, including end-stage liver disease is the third clinical scenario through which NAFLD exerts its morbidity and mortality. Consequentially, accurate staging of liver disease is crucially important, allowing for both risk stratification and appropriate monitoring for disease progression and complication development. In this regard, liver biopsy remains the gold standard diagnostic tool allowing for both assessment of liver inflammation and liver fibrosis. Scoring systems used to grade liver biopsy tissue include the NAFLD Activity Score (NAS) to quantify histological features associated with a diagnosis of NASH (286) and the NASH CRN system used to stage fibrosis and to determine early fibrosis (F1-2) from advanced (clinically significant) fibrosis (F3-F4), with F4 fibrosis being cirrhosis (287) (see sections 5.1.3 and 5.2).

Fibrosis progression in NAFLD has been estimated in 30-40% of patients with NAFLD (1,267). Traditionally, it has been assumed that NAFL was an indolent disease with relatively slow progression compared to NASH which progresses faster to advanced fibrosis. Indeed, it is NASH which is the predominant form of NAFLD that is thought to progress to advanced fibrotic disease with estimates suggesting that 10-15% of those with NASH progress on to cirrhosis with higher rates of decompensated liver disease and HCC development (27,288). Furthermore, the presence of T2D in patients with NASH is an independent predictor of liver-related mortality and similarly, advanced fibrosis in those with NASH is an independent risk factor for predicting mortality (27).

Nevertheless, recent studies have shown that a significant proportion of those with simple NAFL do progress to advanced fibrosis. *McPherson et al.* reported that 44% of patients progressed to NASH, 37% progressed to fibrosis and 22% to advanced fibrosis (263,267,289). Although this means that the majority of those with NAFLD have stable disease and do not progress, *Singh et al*. in their meta-analysis showed rate of progression in those with NAFL at 1 stage of fibrosis progression approximately every 14 years and double this rate for those with NASH at 1 fibrosis stage every 7 years (267).

Previously it had been thought that only those with advanced liver fibrosis were at risk of increased all-cause mortality (268,290). However, there is now convincing data indicating that even those patients with early (F1) fibrosis have an increased all-cause mortality compared to reference populations without fibrosis. Furthermore, liver-specific mortality rises exponentially with each increase of fibrosis stage (264,291). Though still a keenly debated issue, this more recent data also suggests that fibrosis stage, irrespective of the presence of NASH, appears to be the most important determinant of outcomes in patients (268,269)(292) and that the presence of other risk factors, including but not limited to age, T2D and smoking appear to accelerate fibrosis progression and the potential development of liver failure. As expected, patients with NASH cirrhosis have worse liver-related and cardiovascular outcomes compared to those patients without cirrhosis; liver-related mortality is estimated at 24% in F3 compared with 59% in those with cirrhosis (F4) (291).

4.4 NAFLD and HCC

Between 2004 and 2009, there was an approximate 10% increase in NAFLD associated HCC incidence in the USA (1). NAFLD HCC patients were older, had a reduced life expectancy with more cardiovascular complications, and were more likely to die from their liver malignancy compared with other HCC aetiologies. Similarly, in Europe, *Dyson et al.* (6) reported a 10-fold increase in HCC associated with NAFLD between 2000-2010 with patients again being older on average and experiencing greater cardio-metabolic complications, though survival in this cohort was similar to other etiologies of HCC (see also section 2.7).

The incidence of HCC is significantly higher in those with NASH compared to those without NASH (1). This is in agreement with data demonstrating that the incidence of HCC in patients with NAFLD cirrhosis is higher than those without cirrhosis. In patients with NAFLD cirrhosis, the incidence of HCC is estimated at 6.7-15% over a 5-10 year period (293). Studies estimate the incidence of HCC in patients without cirrhosis to be much lower; one large study showing a rate of 2.7% at 10 years, with the highest risk in older patients aged >55 years (294). Cardio-metabolic disease appears to be important in driving the development of HCC in patients both with and without cirrhosis (295,296). A recent study has shown that patients with NAFLD and T2D were >19-times more likely to develop HCC and >6 times more likely to die from HCC compared to people without liver disease (277).

Current clinical consensus remains that screening for HCC in patients with NAFLD is only advocated for patients with established liver cirrhosis (293).

4.5 NAFLD and liver transplantation

NAFLD principally leads to the need for liver transplantation through hepatic decompensation as a consequence of cirrhosis, which affects 15-25% of those with NASH, and or the development of HCC in those with established cirrhosis (1).

The first liver transplant worldwide was performed in 1963 at a time when NAFLD / NASH was not a fully appreciated or recognised clinical entity. Indeed, NAFLD / NASH remained an insignificant etiology for liver transplantation until the mid-2000s. Since that time its prominence and prevalence have risen to mirror the rise of the global obesity and T2D epidemic. NAFLD / NASH is currently the fastest growing etiology for liver failure. It is currently the second leading cause of liver transplantation worldwide, and is predicted to be the commonest cause for liver transplantation within the next few years (297,298). Indeed, the waiting list of entrants with NAFLD on the USA transplant register increased by 170% between 2004 and 2013 (299). Importantly, the prevalence of 'cryptogenic cirrhosis' was historically up to 30% in those with 'all-cause cirrhosis', of which a substantial number would have undergone liver transplantation. A large number of studies have subsequently demonstrated strong associations between the prevalence of metabolic conditions such as obesity and T2D in those with cryptogenic cirrhosis which makes it likely that NAFLD accounted for a significant proportion, if not the majority etiology of these cases (300). As diagnostic advances in hepatology have improved, the proportion of cryptogenic cirrhosis diagnoses made has reduced to around 5% (299,301).

The age of those undergoing liver transplantation for NASH has fallen significantly over the last 15 years. Analysis of transplant registrants in the United Network for Organ Sharing (1995-2015), which has profiled over 180,000 individuals, has shown a marked upward trend in NASH with, and without HCC since 2005. Those aged between 35-55 years have shown the greatest increase in registration rates for liver transplantation (302). The authors termed this the 'adipose wave effect' as it is line with data that shows obesity and metabolic diseases continue to affect people at increasingly younger ages. Data from both the USA and Europe demonstrate the prevalence of childhood obesity to be approximately 20% or higher (303) with this trend continuing to increase. Children who are obese tend to remain obese into adulthood and this prolonged 'exposure to obesity' is likely to be an important factor in the development of liver failure and HCC that require transplantation.

As the need and rate of transplantation for patients with NAFLD rises, the need for clear guidance as to how to manage and select suitable candidates has grown. The United Kingdom was one of the first countries to publish detailed guidance on NAFLD and liver transplantation in 2012, a significant proportion of which was based on consensus statements (304). As a large number of questions concerning transplantation in NAFLD and NASH continue to remain unanswered, such as natural history and management of co-morbidities in those undergoing transplantation, the International Liver Transplantation Society (ILTS) published the first International Consensus Statement on NAFLD and NASH in the setting of liver transplantation in early 2019 (305). This reviewed the available evidence with the aim of answering both important practical considerations and questions regarding NASH outcomes and natural history. Important aspects in this regard include risk assessment for patients listed for transplant, cardiovascular risk and management of medical co-morbidities including cardio-metabolic diseases and obesity. Other considerations include the impact on the donor pool (299) and whether there is a need for a specific approach to donor steatosis in those donating organs.

Analysis of outcomes for patients with NASH undergoing liver transplantation is important as numbers of procedures performed continue to rise. Recently reported data from Europe has shown that survival for patients with NASH is similar to those transplanted for other liver diseases (305,306). These results are consistent with other studies including data from the USA, some of which have suggested a survival advantage for those transplanted for NAFLD compared, for example, to HCV (298,300,307,308). As may be expected, infection and sepsis were the commonest causes of death (~24%) in those with NASH without HCC, followed by cardio-metabolic complications (~5%), with higher age, severity of liver disease and extremes of BMI (low or high) being predictors of death (306).

Data from a meta-analysis by *Wang et al*., confirmed that patients with NASH had greater risk of death from sepsis and cardiovascular complications (308), with additional evidence suggesting a lower risk of graft failure post-transplant compared to other liver failure aetiologies (305). Additional studies have also demonstrated that patients with extremes of BMI are more at risk from post-operative complications (309), as well as increased cardio-metabolic complications pre and post transplantation compared with other non-NAFLD indications for transplantation.

There is evidence demonstrating that complications of cardio-metabolic diseases, for example, mild renal dysfunction around the time of transplant, is linked with adverse all-cause and cardiovascular outcomes (310). Immunosuppressive therapy is used in patients undergoing transplantation. Their role to prevent organ rejection is essential, however, it is well recognised that these agents also have adverse cardio-metabolic effects and potentially could contribute to the *de novo* development NAFLD or cardio-metabolic disease.

NAFLD recurrence post-transplantation is common and affects at least one third of patients though the true number is likely to be higher (299). *De novo* NAFLD post-transplantation is thought to affect around 18-33% of patients (311). This condition remains poorly understood and longer-term studies are required to fully understand and characterise differences from recurrent NAFLD, for example if it is a more aggressive in nature. Nevertheless, regular surveillance and aggressive management of NAFLD recurrence*, de novo* NAFLD and cardio-metabolic disease is required post transplantation. In this regard, work is on-going to assess how patients with NAFLD in need of liver transplantation may be better managed in the future with additional targeted interventions, for example bariatric surgery, where work is underway to establish the optimal timing and nature of such procedures (305).

5 Clinical Evaluation and Approach to Management

5.1 Establishing the diagnosis

5.1.1 Clinical

A diagnosis of NAFLD may be suspected in patients with mildly elevated liver biochemistry and/or echogenic liver on ultrasound scanning in the presence of one or more cardio-metabolic risk factors, such as (abdominal) obesity, T2D or impaired glucose tolerance, hypertension and dyslipidemia, and in the absence of other causes of hepatic steatosis and chronic liver diseases (312)(288). Typically, patients with NAFLD are asymptomatic, although fatigue and dull ache or discomfort in the right upper quadrant of the abdomen are not uncommon. More usually, abnormalities in imaging or liver biochemistry are discovered incidentally, such as the appearance of fatty liver on ultrasound scanning or computed tomography (CT) imaging performed because of abdominal symptoms, or the finding of elevated liver biochemistry in patients undergoing blood test monitoring for other conditions (313).

A clinical history is required to exclude excessive alcohol consumption, which is defined by *European Association for the Study of the Liver* (EASL) as ≥20g/day for a woman or ≥30g/day for a man (312) and medications that may cause hepatic steatosis, such as corticosteroids, amiodarone and tamoxifen. Physical examination is required to determine the presence of abdominal obesity (waist circumference ≥94cm for Europoid men and ≥80cm for Europoid women), hypertension (≥130/85mmHg or treated for hypertension) and any signs of chronic liver disease that might indicate advanced disease or portal hypertension.

Blood testing should include a full/complete blood count, liver biochemistry (including alanine aminotransferase (ALT) and aspartate aminotransferase (AST)); renal function; fasting blood glucose and/or glycosylated haemoglobin (HbA1c); blood lipid profile; serum ferritin and transferrin saturation; thyroid function; viral hepatitis B and C serology; liver autoantibodies (anti-nuclear antibody, anti-mitochondrial antibody, anti-smooth muscle antibody, anti-liver kidney microsomal antibody); immunoglobulins (A, G, M); α-1 antitrypsin levels. Other tests that may be required include serum caeruloplasmin and coeliac serology in selected cases. Normal liver enzymes and normal liver ultrasound do not exclude NAFLD; indeed, of 103 patients with T2D and normal ALT, 51 had NAFLD as defined by proton magnetic resonance spectroscopy (^{1}H MRS) (314).

5.1.2 Non-invasive assessments for diagnosis of hepatic steatosis

Imaging, particularly B-mode ultrasound, is often used for the diagnosis of NAFLD in order to look for evidence of hepatic steatosis (312). Typically this is a subjective assessment of increased hepatic echogenicity compared with the parenchyma of the adjacent right kidney. In a meta-analysis of studies including 4720 patients comparing ultrasound to liver histology, ultrasound was sensitive for the detection of moderate to severe steatosis (approximating to steatosis in >33% of hepatocytes) with a sensitivity of 84.8% (95% confidence interval: 79.5-88.9) and specificity of 93.6% (87.2-97.0) (255)(315) but did not reliably detect steatosis of <20% (316). Ultrasound is unable to quantify hepatic steatosis, although the severity may be assessed and scored subjectively. Ultrasound is also used to exclude structural hepato-biliary comorbidity such as gallstones and to look for evidence of macro-nodular cirrhosis and signs of portal hypertension including splenomegaly and the presence of ascites.

No single blood test can diagnose NAFLD, but there are a number of indices based on simple clinical assessments and blood tests that can predict the presence of significant hepatic steatosis. One such test is the Fatty Liver Index which incorporates BMI with waist circumference, gamma-glutamyl transferase and plasma TAG levels (317). Is was validated against hepatic steatosis as defined by ultrasonography, itself a surrogate reference marker and may be used in patients with metabolic risk factors to assess for the presence of NAFLD in the absence of ultrasound scanning (312). While other simple indices to diagnose hepatic steatosis have been evaluated against reference standards, including histology, ^{1}H-MRS (318) and ultrasound, the diagnostic accuracy of three indices, the Fatty Liver Index, NAFLD Liver Fat Score and Hepatic Steatosis Index were validated retrospectively with a cohort of patients with histologically defined NAFLD and the area under the receiver operator curve (AUROC) varied from 80% to 83% (319). The utility of such markers is unclear given the widespread availability of ultrasound, the high prevalence of steatosis in the general population (1) and that hepatic steatosis is frequently detected through other means. Such indices may be helpful in identifying cohorts of patients at high risk of NAFLD in whom further investigation would be indicated, or in epidemiological studies.

5.1.3 Role of liver histology in diagnosis

Liver histology obtained by liver biopsy can be helpful diagnostically where the cause of abnormal liver biochemistry is unclear despite having performed a non-invasive liver disease screen. In such cases, in the absence of excessive alcohol intake or steatogenic drugs, the presence of intrahepatocellular lipid droplets in >5% of hepatocytes would be indicative of NAFL. NASH is diagnosed by global histological assessment. Characteristic features include the presence of lobular inflammation and ballooning of hepatocytes with or without fibrosis. Scoring systems for NASH including the NASH-CRN (NASH Clinical Research Network) score and the SAF (Steatosis, Activity, Fibrosis) score have been derived and are widely used (287)(320). These scoring systems have been developed to standardize assessment of histological lesions seen in NASH and are not intended to be diagnostic, rather to provide a semi-quantitative scoring system to improve agreement between histopathologists and assessment of change over time, predominantly in the context of clinical trials. Other histological features that are frequently seen in NASH include portal inflammatory infiltrate, Mallory-Denk bodies (Mallory's hyaline) and megamitochondria (312). These features may also be seen in alcohol-related liver

disease and alcoholic hepatitis so an accurate history of alcohol consumption is crucial to the interpretation of the histology.

5.2 Risk stratification

Severity of hepatic fibrosis is the strongest predictor of overall and liver-related outcome in patients with NAFLD (see section 3.7) (264,268,291). Establishing the severity of fibrosis in patients with NAFLD is therefore central to risk stratification. Diagnosis of NASH *per se* is not important for risk stratification (see section 4.3), but remains an endpoint in phase 3 clinical trials, given the current understanding of the pathogenesis of the condition (321,322). Histological fibrosis staging remains the reference standard for gauging the severity of fibrosis in clinical practice and as a clinical trial endpoint. The NASH CRN system for fibrosis staging is widely used and employs a scale of 0-4 (287,323). These stages correspond descriptively to mild fibrosis (stage 1a, 1b, 1c), significant fibrosis (presence of pericellular fibrosis, stage 2), advanced fibrosis (presence of bridging fibrosis, stage 3) and cirrhosis (bridging fibrosis with nodule formation, stage 4). The stages are descriptive of the pattern of fibrosis and the extent of fibrosis can vary substantially within each stage. Quantitation of fibrosis in histological liver samples has been developed by assessment of the collagen proportionate area (CPA) by digital image analysis of liver sections stained with Sirius red for collagen. In a cohort of 437 patients with NAFLD with retrospective follow-up over a median of 103 months, the CPA was an independent predictor of hepatic decompensation and, being a continuous variable, may be a useful surrogate marker for disease severity, particularly in the context of clinical trials (324).

5.2.1 Clinical, routine imaging and laboratory measurements

Routine liver biochemical tests include measurement of ALT and/or AST, alkaline phosphatase, serum bilirubin and gamma glutamyl transferase values. Elevations in ALT values are a common finding in patients with NAFLD, but they do not indicate the presence or absence of advanced disease. Importantly, a normal ALT value does not always indicate the absence of NAFLD. A study of 222 patients with NAFLD of whom 23% had elevated ALT and 77% had T2D found that the proportions of those with advanced fibrosis did not differ according to whether or not they had elevated ALT (325). There was a significantly lower proportion of people with NASH in the group with normal ALT compared to the group with elevated ALT making it more likely that NASH is present in the context of elevated ALT.

5.2.2 Non-invasive biomarkers of liver disease severity

Histological assessment through liver biopsy is associated with established risks to the patient (326), sampling variability, as well as inter- and intra- observer variation (327). Considerable progress has been made in the development of non-invasive markers of liver disease severity applied to NAFLD in the last 20 years with a number that are now routinely used in clinical practice. Markers can broadly be divided into "biological" markers (typically blood, but also other biological material) and "physical" markers (typically imaging-based technologies) (328). The performance of these modalities to accurately stage liver disease and therefore provide accurate risk stratification has been reviewed in detail (329) and a summary of their key features is provided below.

5.2.2.1 Biological markers

Blood markers can be categorised into simple, usually inexpensive, blood tests, markers or indices based on routine biochemical and haematological tests, or more complex, usually proprietary, panels of tests including intermediates of fibrogenesis. These have been reviewed recently and extensively (329–331).

Simple blood marker indices of disease severity are based on routine blood tests and many have been derived from cohorts of patients with other chronic liver diseases, notably HCV. Central to these is the ratio of AST to ALT to which other components are appended. Examples include the AST/ALT ratio (332), the Fib-4 score which also includes age and platelet count (333), the NAFLD Fibrosis Score which includes platelet count, albumin concentration, BMI and the presence or absence of T2D or impaired fasting glucose (334), the BARD score which includes BMI and presence or absence of T2D or impaired fasting glucose (335). A number of these indices employ two thresholds or cut-off values; a lower value below which there is a high negative predictive value for the exclusion of advanced fibrosis and a higher value above which the specificity and positive predictive value is maximised (336). The choice of the thresholds to be used depends on how the test is to be employed in clinical practice. These indices were independently validated in a cohorts of patients with biopsy-proven NAFLD and found to have similar performance characteristics, notably for their high negative predictive value excluding advanced fibrosis/cirrhosis in 92-95% of cases (336). However, any clinical study validated against liver biopsy is prone to selection bias with a higher proportion of more severe disease stages than is represented in most primary care populations, which would be predicted to improve the negative predictive value of the test at the risk of including many false positive results (lower positive predictive value).

More complex proprietary panel markers have been developed using a number of biochemical tests in combination. The Enhanced Liver Fibrosis (ELF) score is designed to model matrix (including collagen) turnover, combining assays for hyaluronic acid, the amino terminal of procollagenase 3 and tissue inhibitor of metalloproteinase 1 and the diagnostic accuracy (AUROC) for advanced fibrosis in NAFLD was 93% (337). Other proprietary panel tests include FibroTest, which includes serum α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin and gamma-glutamyl transpeptidase, adjusted for age and gender (338), which performed similarly to, but not better than, the NAFLD Fibrosis Score compared to histological fibrosis staging for the diagnostic accuracy across all fibrosis stages in an analysis of 574 patients (339).

5.2.2.2 Physical markers

Physical markers are based on assessment of the physical properties of the liver parenchyma and include imaging-based technologies. Elastography is based on the measurement of vibration-induced shear waves through the liver tissue and include a means of generating a pulse or vibration and a means of detecting the velocity of the shear wave generated by the pulse. Broadly these are divided into ultrasound-based and magnetic resonance elastography. Most widely validated and used of these techniques is vibration-controlled transient elastography (TE), marketed as FibroScan® which employs a probe placed against the skin between the ribs overlying the right lobe of the liver and generates a liver stiffness measurement (LSM) (340). As previously reported (328), there is published evidence in 25 studies and approximately 4000 patients with NAFLD using TE and a review of 10 recent studies comparing TE to histology, using either medium (M) or large (XL) probes indicated AUCs for prediction of advanced fibrosis or cirrhosis (F0-2 *vs.* F3-4) of between 0.83 and 0.93. The sensitivity and specificity vary according to the cut-off values used, which may be set according to the disease state being sought, the tolerance of the local service for false positive and false negative results and setting in which the technique is used. LSM by TE has a significant failure rate, either through an inability to obtain a reading or if the reading is technically inadequate. It is contraindicated in patients with cardiac pacemakers, implantable defibrillators and in pregnancy. A review of recent studies of TE in patients with NAFLD described inadequate readings (failure to obtain or unreliable) in between 5% and 23% of cases (328). However, a prospective, multi-centre study of TE in more than 400 patients

with NAFLD found that, when an appropriate probe size was used for each patient, only 3% of readings were invalid (341).

Other ultrasound techniques that allow elastography measurement include Acoustic Radiation Force Imaging (ARFI) and 2-dimensional shear wave elastography which are applications integrated on ultrasound scanning machines. These techniques have increasingly been validated with similar performance characteristics, but on fewer patients than TE (328).

Magnetic resonance technologies have been applied in the context of NAFLD. Whilst ^{1}H-MRS (342) and the proton density fat fraction (PDFF) (343,344) can sensitively quantify hepatic steatosis, their use in risk stratification of disease severity is limited, although a recent study suggested that increased liver fat as assessed by PDFF was associated with increased risk of disease progression (345). Magnetic resonance elastography (MRE) applies the same concept of liver stiffness or elasticity measurement as ultrasound-based techniques and has been validated in patients with NAFLD (346) with high diagnostic accuracy for advanced fibrosis; pooled sensitivity 86%, specificity 91%, in a recent comprehensive meta-analysis (347). MRE requires dedicated hardware and standardization of protocols between centres. A multi-parametric MR imaging technique has been developed that incorporates an iron-corrected T1 (longitudinal relaxation time) sequence which correlates inversely with disease severity in NAFLD (348). As it is a software analysis of predefined sequences, it has the benefits of not requiring specific hardware, is available across different scanner makes, models and field strengths, and provides reproducible and standardised assessments. MR scanning applications are contraindicated in patients whose bodies contain ferrous material (penetrating eye injuries, shrapnel injuries, and certain implants), cardiac devices such as pacemakers and implantable defibrillators, but also may not be acceptable in patients with anxiety and claustrophobia.

The place of MR techniques in practice is, as yet, unclear. They are unlikely to form standard of care as first line assessments in resource-constrained environments, but the detail and reproducibility of the data obtained lend them to use in clinical trials and longitudinal observational studies.

5.3 Putting risk stratification into practice

With the plethora of options now available for non-invasive risk stratification, attention has turned to development of pragmatic strategies for their use in practice. The low cost and performance characteristics of simple non-proprietary indices such as the

Fib-4 and NFS make them an attractive first line for community-based risk stratification to enrich the population for whom more involved second line tests can be employed.

A study of a UK primary care population of 1118 patients in Birmingham, UK, with elevated liver biochemistry looked at the prevalence of chronic liver diseases, and, after exclusion of other chronic liver diseases, a diagnosis of NAFLD was made in 295 (26.4%) (349). The NAFLD Fibrosis Score was used retrospectively to risk stratify these patients. 57% fell into the low risk category for advanced fibrosis, nearly 7.6% in the high-risk category, while the remaining 35% were classified as indeterminate, in whom additional risk stratification by other means was indicated. This study is notable in being one of the first to risk stratify patients in the community by non-invasive means and to establish the prevalence of advanced NAFLD in the population. However, the cause of abnormal liver tests was unclear in 45% despite use of a chronic liver disease screen of blood tests and ultrasound scanning and risk stratification was not performed in these patients. 30.5% of these patients were obese, 19% had diabetes and 41% were hypertensive, so a proportion are likely to have had NAFLD given that ultrasound scanning is not sensitive for mild hepatic steatosis, when the patient is obese, or in advanced fibrosis where the degree of steatosis can decrease. Conversely, Koehler *et al*. conducted a population study of older people (mean age 76 years) in the Netherlands. 3205 people underwent abdominal US scanning, of whom 2811 did not have risk factors for secondary steatosis (350). Of these, 35.1% had evidence of NAFLD, but importantly, 88% had normal ALT value. This suggests that the prevalence of NAFLD and NAFLD with advanced fibrosis in the Birmingham study may have been underestimated.

In Nottingham, UK, TE was used in primary care for patients at risk of chronic liver disease (351). Risk factors for chronic liver disease were examined in the primary electronic medical record of over 20,000 patients. Those with hazardous alcohol intake or T2D were invited for TE and those without exclusion criteria (n=2022) were invited for TE, of whom 919 underwent the test. 669 patients had normal liver stiffness (<8kPa) and follow-up was arranged in primary care. 230 patients had elevated liver stiffness (≥8kPa) and were seen in the hepatology clinic, of whom, 27 were diagnosed with cirrhosis (and of whom 16 had NAFLD as the underlying etiology, alcohol-related liver disease in 3 and a combination of risk factors in 8 individuals). A total of 23 patients with cirrhosis had been identified prior to the use of TE for those at increased risk, indicating a doubling of the pick-up of cirrhosis using this strategy. In another study, among 705 patients with T2D (352), the estimated

prevalence of significant fibrosis, advanced fibrosis and cirrhosis was estimated at 12.7%, 7.3% and 2.1% respectively using pre-defined liver stiffness thresholds.

Despite extensive validation of simple markers in secondary care, a primary care based study in Edinburgh, UK, examined a cohort of 831 patients with T2D using US scanning followed by a number of simple blood markers (353). The correlation between different classes of biomarker was poor for the prediction of advanced fibrosis, including amongst those with NAFLD. However, in keeping with the good negative predictive value for advanced fibrosis seen in other studies, there was good agreement for the absence of advanced fibrosis among those patients with values in the lower 95 centiles for each test. This study illustrates the potential pitfalls of rolling out risk stratification in primary care by extrapolating biomarker data from secondary care, particularly as the "true" disease state of those identified as 'low-risk' in a primary care cohort cannot be established easily as liver biopsy cannot be justified in such patients. Nevertheless, on a population basis, these tests serve to enrich the population for those at risk of advanced liver disease and the negative predictive value should improve as the population prevalence of advanced disease decreases from secondary care to primary care populations.

The Birmingham study indicated that when using NFS, 35% of patients with NAFLD in the primary care cohort were classed neither in the high- or low-risk groups and so required further testing (349). This concept underlines the British Society of Gastroenterology's guidance that, in patients with presumed NAFLD, a simple panel test, for example Fib-4 or NFS, should, in those with indeterminate values, be followed by a second line test such as the ELF score or LSM (354). The lack of correlation between ELF or LSM and Fib4 as determined by Morling *et al.* may thus be harnessed in sequential testing (353). This was tested retrospectively by Petta *et al*. in a cohort of 741 patients with biopsy-defined NAFLD (355). Simple biological panel markers including NFS and Fib4, and LSM with FibroScan were tested against histology using published cut-off values. The markers were evaluated singly and in combination. Combination of Fib4 or NFS with LSM in all cases led to very low false positive results (1.5% to 2.1%) and false negative rates (3.8% to 5.5%) for inclusion or exclusion of advanced fibrosis. There was however a large range of uncertainty (indeterminate) results comprising more than half of cases, leading to low accuracy in the region of 39% to 43% overall. If tests were employed serially (Fib4 or NFS first, then LSM only in those with indeterminate scores) the number of cases in the "uncertainty area" reduced to 6.4-8% and the overall accuracy increased to 76-

77.8%, but at the expense of a higher number of false positive and false negative results. Care should be taken when extrapolating these results to a primary care setting as it was a retrospective study against liver biopsy adding selection bias and making it likely that the study population was enriched for advanced fibrosis and with less mild disease. Such a strategy in primary care where the prevalence of advanced disease is lower might be expected to have fewer false negative results, but more false positive results.

A sequential testing strategy was used by in London, UK, by the Camden and Islington care commissioning group and the Royal Free hospital and evaluated from March 2014 to May 2016 (356). The pathway consisted firstly of Fib-4 testing by general practitioners, then those considered low-risk (Fib-4 <1.3) continued to be managed in primary care. Those with a high-risk Fib-4 test (Fib-4 >3.25) were referred to the hepatology clinic, while those with an indeterminate result (Fib-4 >1.3 <3.25) were advised to undergo second line ELF testing. Of 1452 patients entering this pathway, 1022 fell into the low-risk category, 43 into the high-risk category, while 387 at indeterminate risk underwent ELF testing, of whom 155 had ELF <9.5 and were considered low risk and remained in primary care, while 232 had ELF >9.5 and referral to the liver clinic was recommended. The authors compared referrals before and after the initiative and from practices that had adopted the pathway with those that had not and reported a five-fold increase in detection of advanced fibrosis, a three-fold increase in detection of cirrhosis and an 81% reduction in unnecessary referrals.

5.4 Assessment of cardio-metabolic risk

While the focus of this section has been on the risk assessment of the liver disease, CVD remains the chief cause of death among patients with NAFLD. We advocate the use of cardiovascular risk scores in the clinic setting for objective assessment of cardiovascular mortality risk so as to guide optimisation of cardio-metabolic risk factors. Examples of cardiovascular risk scores include QRisk3 (357) with an online calculator (https://qrisk.org/three), the closely related JBS3 calculator (http://www.jbs3risk.com) and the Framingham risk score (358). QRisk2 and Framingham risk score were compared retrospectively in a cohort of patients with NAFLD and a further score was derived which included the mean platelet volume and was validated prospectively (359). AUROCs for the prediction of major acute cardiovascular events in the subsequent year were 0.83 for the NAFLD CV score, 0.73 for QRisk2 and 0.64 for Framingham.

5.5 Practical considerations in the assessment of NAFLD

Any strategy for risk stratification of NAFLD needs to include consideration of the practicalities of delivering the service beyond the absolute performance characteristics of the tests.

5.5.1 Confounding factors

Any non-invasive test or biomarker is likely to be affected by clinical confounding factors and awareness of these factors is related to how widely studied and validated a given test is. For example, well known factors that increase liver stiffness and can lead to false positive results include the presence of severe hepatocellular inflammation, high right sided heart pressures and recent meals (360). However, when controlling for the degree of fibrosis in an analysis of patients with NAFLD, the degree of steatosis or inflammation was not found to influence the liver stiffness significantly (341). The components of the ELF test can be increased in extra-hepatic fibro-inflammatory conditions, while simple panels such as Fib-4 and NFS may be affected by any condition or concomitant medication that affects the platelet count (such as chronic inflammation, iron deficiency) or the ALT to AST ratio (alcohol use, medications, muscular damage or inflammation). Future prospective studies should investigate the factors associated with false positive and false negative results, particularly in real-world and primary care cohorts where there are more likely to be comorbidities present than in the well-characterized secondary care derivation and validation cohorts reported in the initial publications.

5.5.2 Resources, Experience, Acceptability

The diagnostic performance of risk stratification pathways should be considered in the context of the patient pathway, whereby, typically, patients are detected in primary care, undergo risk stratification and then those considered to be at higher risk undergo further evaluation and treatment in secondary or tertiary care (312). Simple blood markers (Fib-4, NFS) are cheap, available routinely and can exclude advanced fibrosis with an excellent negative predictive value in the majority of patients. Using a cut-off value for Fib-4 of 1.3 in a UK primary care population, 70% of NAFLD patients were placed in the category at low risk for advanced fibrosis (356), while using a cut-off value for NFS of <1.455 in a UK primary care population, 57% of patients with NAFLD were placed in the category at low risk (349). The availability of resources in secondary care will determine the proportion of patients that can be assessed and treated in the clinic setting, the tolerance of false positive

and false negative results and thus the thresholds used for risk stratification. As awareness of NAFLD increases, referrals to secondary care increase, which increases the need for effective risk stratification tools (356). The absence of effective, discrete interventions in NAFLD, the cost-effectiveness of such pathways is unclear, although an analysis based on TE in primary care and numerous assumptions about disease progression and outcomes suggested that such a risk stratification pathway was likely to be cost-effective (361). The future availability of cost-effective treatments for NAFLD will also influence the need for risk stratification and the thresholds employed as risk stratification will be required determine eligibility for therapies.

Given the good performance characteristics of many tests for the detection or exclusion of advanced fibrosis, the decision to employ a given strategy will depend on local resources and the set-up of local services. For example, simple blood markers (Fib-4, NFS) are easily used in primary care. Second line risk stratification could be by complex panel markers (ELF, FibroTest), ultrasound based elastography (TE, SWE) or MR techniques (MRE, multiparametric MRI). Complex blood markers can be sent from primary care, while TE is available in some primary care settings, but more often in secondary care, so geographical and travel considerations are also relevant to the choice of test. Typically, MR techniques for risk stratification are still confined to tertiary centres.

As most patients with NAFLD are asymptomatic and there is widespread lack of awareness among healthcare professionals treating patients with diabetes (362), so the number of tests and visits required for risk stratification should be minimised to increase uptake. 45% of those assessed as high-risk by the Camden and Islington pathway were not seen in secondary care (356), but a sequential risk stratification pathway in Oxford, UK, used automated reflex testing of ELF in those with an indeterminate Fib4, and resulted in 80% of those patients with high risk scores being seen in secondary care (personal communication). It may be postulated that comprehensive roll-out and monitoring of a robust and acceptable system is more important than the minor differences in diagnostic accuracy of the particular technique(s) used, and recent UK guidance acknowledges the different preferences and experience of risk stratification markers among different clinicians by presenting alternative tools in the sequential testing strategy (354). Publication of further experience in clinical practice of different strategies is awaited.

5.5.3 Towards screening in high-risk groups?

NAFLD is usually asymptomatic and the prevalence of NAFLD is very high in certain groups (see section 2). Screening of high risk groups is advocated (312), but the practicalities of such initiatives have not been established. Given that the majority of patients with NAFLD have normal ALT, ALT cannot be used for screening. If the prevalence of NAFLD in high-risk groups (including those with obesity andT2D) may exceed 90%, is ultrasound scanning or use of a steatosis-detection algorithm such as the FLI necessary? Alternatively, risk stratification for advanced fibrosis could be employed directly in high-risk groups. Examples include TE in patients with obesity, T2D (or hazardous alcohol intake), (351), Fib-4 and TE in patients with T2D in secondary care (362,363). Whether a systematic approach is warranted will rely on the benefit and cost-effectiveness of interventions in NAFLD and related conditions. Novel therapies and management approaches will need to be evaluated to establish their impact (364). Similarly, the performance characteristics of tests will vary by the prevalence of advanced disease in the populations concerned. Both of these issues need to be factored in before widespread screening can be advocated in resource-constrained environments.

5.6 A multidisciplinary approach to management

Given that NAFLD is typically diagnosed in patients with abnormal liver biochemistry and/or an echogenic liver on ultrasound scanning, gastroenterologists and hepatologists have been the specialists who have managed most incident cases in secondary and tertiary care. Yet, natural history studies demonstrate a diverse range of outcomes for patients diagnosed with NAFLD.

As discussed in section 4, it is important to note that CVD is the principal cause of death affecting approximately 40% of patients, while malignant and non-malignant liver diseases account for the deaths in less than 10% of patients (264,268). Furthermore, more than 50% of patients attending secondary/tertiary care NAFLD clinics in the UK have T2D (365)(364). The role of the clinician caring for patients with NAFLD is to reduce the morbidity and mortality associated with the condition, so a “hepato-centric” management approach is insufficient and a multi-disciplinary, holistic approach is required. While the personnel required in the multi-disciplinary team may vary, the skill sets required are consistent (365):

- diagnosis and risk stratification of liver disease
- assessment and optimisation of cardio-metabolic risk factors

- therapeutic optimisation of T2D
- lifestyle and dietary assessment
- lifestyle education and intervention (including diet, exercise and smoking cessation)
- application of novel liver-directed therapies in NAFLD and recruitment into clinical trials

A suggested model for a multidisciplinary approach to management of NAFLD is shown in Figure 2. Management of NAFLD in a multidisciplinary setting is widely advocated, (366)(312)(367), but there are very few data to demonstrate the utility of such an approach in routine clinical practice.

Cobbold *et al*. analysed an ethnically diverse cohort of 180 patients with NAFLD in London, UK, of whom 92 had T2D, and who had been seen in a multidisciplinary NAFLD clinic and followed up for a median of 19.5 months (365). From baseline to latest visit, there were significant improvements in median ALT (18%), weight (3.5%) and total cholesterol (2.5%). 27% of patients achieved ≥5% weight loss and among the patients with diabetes, there was a significant 1.9% reduction in HbA1c. Another study from Birmingham, UK, analysed data from 65 patients with NAFLD seen in a multidisciplinary NAFLD clinic, of whom 32 had T2D. Over a short median follow-up of 98 days, significant improvements in weight, ALT and total cholesterol were also seen and 22% achieved ≥5% weight loss (363). Most recently, Moolla *et al.* reported experience of a multidisciplinary NAFLD clinic in Oxford, UK, where 165 patients were analysed of whom 97 had T2D over a median period of 13 months (Figure 2) (364). As in previous studies, there were significant improvements in ALT, weight, total cholesterol and HbA1c, but also in this study significant improvements were demonstrated in liver stiffness by TE as a validated marker of fibrosis severity in NAFLD (difference in median values of 1.3kPa or 14%) and also the QRisk3 relative risk (difference in median values of 0.1 or 5%). This study highlighted the benefits, particularly in the management of patients with T2D (difference in median HbA1c of 4mmol/mol or 7%), with increased use of hypoglycaemic agents associated with weight loss such as GLP-1 agonists and reduced used of agents associated with weight gain such as insulins and sulfonylureas. There was evidence of cost-effectiveness of this approach, particularly in patients with poorly-controlled T2D, but the analysis included a number of assumptions. A further UK centre has reported in conference proceedings the change in cardio-metabolic endpoints associated with attendance in a multidisciplinary NAFLD clinic (368). Of the 120 patients included, 26

had diabetes at baseline while another 13 cases of diabetes were detected and treated. They reported a median reduction in QRisk-3 score of 5.2% over the follow-up period, similar to that reported by Moolla *et al*.

None of these studies included a control intervention group and accordingly they were susceptible to bias. Moreover, the benefit of such approaches are not clear on an "intention to treat" basis. However, these studies serve as a benchmark as to what can be achieved in the clinic and to which novel interventions and methods of care delivery may be compared.

In conclusion, given the high prevalence of NAFLD, non-invasive risk stratification should be performed to determine those at increased risk of morbidity and mortality. The presence of advanced fibrosis in patients with NAFLD confers increased risk of all-cause mortality and numerous strategies are available to include or exclude this group. We advocate sequential testing strategies starting with a low cost simple blood marker such as Fib-4 or NFS, followed by a second-line test such as TE or ELF to risk stratify those in the "grey zone" from the first test. Those at higher risk should be referred to a secondary care liver clinic. Consideration should be paid to the implementation of any risk stratification pathway to ensure engagement of stakeholders so as to maximise uptake and minimise dropout. A multi-disciplinary NAFLD clinic to address liver-related and cardio-metabolic aspects of the condition, particularly diabetes, is recommended and data demonstrating the utility of such clinic models are emerging.

6 Lifestyle and surgical approaches to the management of NAFLD

Commonly, the first line recommendation in the management of patients with NAFLD is 7 – 10% weight loss, achieved through lifestyle changes (i.e. changes in diet and exercise). Whilst this may be a desirable goal, in practice implementing and maintaining such a change can be challenging.

6.1 Exercise interventions

Physical activity is defined as any body movement generated by the contraction of skeletal muscles that raises energy expenditure above metabolic rate. It is characterised by modality, frequency, intensity, duration and context of practice (369). Exercise is a sub-category of physical activity that is planned, structured, repetitive and favours physical fitness maintenance or development (369). Thus

physical inactivity represents the non-achievement of physical activity guidelines; sedentary behaviours are waking behaviours characterised by an energy expenditure ≤ 1.5 metabolic equivalent (MET) whilst in sitting, reclining or lying position, with screen time and sitting time typically being the two main indicators used to quantify sedentary behaviours (369). Physical activity and sedentary behaviours are not opposites, rather individuals are considered physically active when they achieve the guidelines but that does not preclude them from having a significant proportion of their day in sedentary behaviours (369). The effects of sedentary behaviours have been focussed on recently with increasing evidence linking excessive sedentary behaviours and adverse health outcomes (369). Observational studies have suggested a strong positive association between sedentary behaviour/decreased physical activity and prevalence of NAFLD (370). In support of these observations, *Palve et al*. reported that obese individuals classified as fit (based on their measured cardiorespiratory fitness through a peak oxygen uptake test) were at a significantly lower risk of NAFLD than participants classified as obese and unfit (371). As physical activity/exercise has the potential to lower NAFLD risk, a number of interventional studies have been undertaken using various physical activity/exercise regimens. Typically, studies have measured change in liver fat content assessed by imaging techniques such as magnetic resonance imaging (MRI), ^{1}H-MRS or ultrasound. Other studies have used the less specific and sensitive change in blood liver enzymes as their outcome measure.

The effect of aerobic exercise on liver fat content was first highlight by Johnson *et al.* (372). They reported that 4-weeks of aerobic exercise reduced liver fat content by 21% (as measured by ^{1}H-MRS), in the absence of significant weight-loss, in obese individuals. A number of other studies then followed including work by *Sullivan et al.* (373) who evaluated the weight loss-independent effects following the USA physical activity guidelines recommended by the Department of Health and Human Services on liver fat content and VLDL kinetics in sedentary, obese individuals with NAFLD (n=18). Study participants were randomised to either 16-weeks of exercise training (45%-55% $VO_{2\ peak}$, 30-60mins, 5 days/week n=12) or to continue usual behaviour (control, n=6). Overall they found exercising training significantly decreased liver fat content (on average by 10.3%), did not change body weight or body fat, and did not alter hepatic VLDL-TG or VLDL-ApoB100 secretion rates. These findings have been corroborated by a systematic review and meta-analysis investigating the effects of aerobic exercise on liver fat content. They concluded that when comparing interventions that combined exercise with diet, to diet-alone (omitting studies that

achieved substantial weight loss), 30-60 min of moderate to high-intensity exercise performed on 2-5 days/week for 1-10 months resulted in a small, but significant reduction in liver fat content despite minimal or no weight loss (374). Notably, this benefit was achieved at exercise levels below the current recommendations for obesity management.

Subsequently, a number of studies have been undertaken investigating the effect of different types of structured exercise on liver fat content. The efficacy of 8-weeks of commonly prescribed aerobic exercise on liver fat content in a group of inactive and overweight/obese adults, independent of dietary intervention or weight-loss has also been investigated (375). Participants were randomised to one of four groups i) low to moderate intensity, high volume aerobic exercise 60mins, 4 days/week (LO:HI, n=12); ii) high intensity, low volume aerobic exercise 45min, 3 days/week (HI:LO n=12); iii) low to moderate intensity, low volume aerobic exercise 45 min 3days/week (LO:LO n=12); or iv) placebo (n=12). At the end of 8 weeks there was a significant change in group x time interaction (p=0.04) in liver fat content which decreased by 2.38±0.73% (mean±sem), 2.62±1.0%, and 0.84±0.47% in the HI:LO, LO:HI and LO:LO groups respectively, and was independent of weight loss. These findings suggest that aerobic exercise reduces liver fat and this can occur with either an emphasis on intensity over volume or volume over intensity and even minimal engagement with exercise (LO:LO group) can lower liver fat in some, but not all, individuals (375). Therefore, it would seem that both the duration of exercise and its intensity are important allowing individuals to have a more personalised regimen (potentially aiding long-term compliance) to achieve similar results. A recent systematic review and meta-analysis by *Smart et al.* found that participants in interventions consisting of exercise alone typically had a liver fat content ~3.5% lower than controls subjects (376). Taken together, the evidence suggests that aerobic exercise has a beneficial effect on liver fat content, even when weight loss is not achieved.

6.2 Resistance training

Although many studies have investigated the effects of aerobic exercise on liver fat content, population-based studies have also suggested that individuals who engage in resistance training have a lower liver fat content than individuals who don't. Interventional studies support this suggestion; for example Hallsworth *et al*. (377) was one of the first to report that when sedentary adults (n=11) with NAFLD underwent 8 weeks of resistance exercise (performed 3 times / week consisting of 8

exercises with each session lasting 45-60 min) despite no change in body weight or fat mass, there was a relative reduction in liver fat content of 13%, alongside improvements in lipid oxidation, glucose control and insulin resistance. Subsequently a number of other studies investigating the effects of resistance training on liver fat content have been reported. For example, Zelber-Sagi *et al.* (378) randomized patients with NAFLD to either resistance training (3 times / week (n=31)) or the control group of stretching (n=31), for 3 months. They found the hepato-renal-ultrasound index to be significantly reduced in the resistance training group, compared to the control group; total, trunk and android fat significantly decreased whilst lean mass body mass significantly increased in the resistance training group; there was no change in the control group. Although the current data are limited it would appear that as with aerobic exercise, resistance exercise has a beneficial effect on liver fat content even when weight loss is not achieved.

6.3 Comparing aerobic and resistance exercise

Recently, a systematic review compared the effects of aerobic *vs.* resistance exercise on liver fat content and found that of the 18 studies identified as using aerobic exercise, liver fat content decreased in 17 of them whilst in 7 studies that were identified as resistance training, liver fat content was reported to decrease in 6 (379). In the study where liver fat did not change with aerobic training, a plausible explanation is that despite the intensity and duration being higher than other studies, the length of the study intervention was 7 consecutive days, which was notably shorter than other studies (379). Why liver fat content did not change in the resistance training study, remains unclear and cannot be explained by differences in study participants, intervention intensity, duration or length (379). Taken together either aerobic or resistance training, when performed regularly over a periods of longer than 7 days appear to be beneficial in decreasing liver fat content.

6.4 High-intensity interval training (HIIT)

In recent years, high-intensity interval training (HIIT) has become popular. HIIT involves high-intensity exercise divided into bouts and recover periods and has been suggested to provide comparable or greater benefits to cardiorespiratory fitness than continuous moderate-intensity training of longer duration (380). By using a modified HIIT program, that was considered realistic and safe for participants with low baseline fitness, *Hallsworth et al.* (381) demonstrated that modified HIIT training 3 times / week for 12-weeks resulted in a significant decrease (relative change ~26%) in liver fat content, along with significant decreases in body mass and fat mass, with

no change in the control group. 12-weeks of HIIT training has also been reported to significantly decrease liver fat content (39% relative reduction) in individuals with T2DM (metformin and diet controlled) compared to those randomised to standard care (382).

6.5 Mechanisms linking exercise interventions and improvements in NAFLD

The mechanisms by which exercise reduces liver fat content appear to have received little attention. It has been demonstrated that a single bout of exercise stimulation increases adipose tissue blood flow and fat mobilization, resulting in the delivery of fatty acids to other organs, such as skeletal muscle (383). It remains unclear if exercise leads to fat mobilization with in the liver, but it could be speculated that fatty acids are liberated from stored TAG during exercise and then these fatty acids, along with those liberated from adipose tissue that enter the liver are utilized in oxidation, rather than the esterification pathways (383). Indeed, the study by Hallsworth *et al.* (377) supports this concept as they found an increase in fat oxidation during a submaximal exercise test in individuals who did 8-weeks of resistance training compared to no change in the control group; there was no change in fasting fat oxidation in either group. By investigating VLDL kinetics before and after 16-weeks of moderate-intensity aerobic exercise, Shojaee-Moradie *et al.* (384) found that although exercise had no effect on VLDL production rates, the clearance rate of VLDL was significantly increased and this may have contributed to the significant decrease in liver fat that was observed. More work investigating changes in hepatic fatty acid uptake, synthesis and disposal is required to understand the effect exercise may have on these pathways.

Overall, there is clear evidence demonstrating that exercise, be it aerobic, resistance or modified HITT training, all have the ability to lower liver fat content to a similar degree, even when in the absence of weight loss, which suggests that exercise can be personalised to achieve the best results. For example, in individuals who are unable to undertake aerobic exercise (due to contraindications) then a program of resistance exercise would be of benefit. Equally combining exercise with calorie restriction, to achieve weight loss may have an additive effect.

6.6 Calorie restriction

A consistent finding in the literature is that a significant reduction in body weight is associated with a significant reduction in liver fat content, highlighting the

effectiveness of weight loss as prevention or treatment strategy for NAFLD. Therefore, weight reduction is recommended by all the scientific societies world-wide (385) and is suggested to be achieved through a calorie deficit of between 500 – 1600 kcal/day. With regards to the precise type of diet that should be consumed, there is no consensus in what is suggested; a variety of dietary regimens have been advocated including low-to-moderate fat and moderate-to-high carbohydrate intakes, low-carbohydrate, ketogenic diets and high-protein Mediterranean diets. Currently, very-low calorie diets are not recommended by the Asia-Pacific guidelines as they are considered unsustainable (385), but there is growing interest in their use as a treatment strategy in metabolic disease.

A number of studies have been undertaken investigating the effect of a hypocaloric diet on liver fat content, which we have previously reviewed (386). Briefly, studies have been undertaken with calorie restriction (between 600-1500 kcal/day) for periods between 2-weeks and two years, using a variety of dietary regimes including high-carbohydrate low-fat, low-carbohydrate and high-fat diets. Regardless of the dietary intervention, all studies found a significant decrease in liver fat content to varying degrees which will be influenced by the phenotype of the participants (these studies are typically undertaken in individuals with a BMI >25kg/m^2), the length and possibly composition of the dietary intervention and the actual calorie deficit achieved (386). With the hypocaloric diets that have been used, there are two different approaches: a relatively modest reduction in energy intake of between 600-800 kcal/day for longer periods (between 6-11months) (387–389) and a more severe reduction in energy intake so that the participant is consuming a very low calorie diet (VLDC) of 450-800kcal/day for period of 6-8 weeks (390–392). When compared the changes in body mass and liver fat content are greater on the VLCD compared to the more modest changes, however whether these changes are maintained long-term remains to be determined. In the study by *Lim et al.* (393) where individuals with T2D consumed a VLCD for 8-weeks, they identified rapid and significant changes in liver fat content. During the first week of the intervention, liver fat decreased by 30% and continued to decline to achieve a total reduction of 70% (along with a 15% reduction in body mass), with the liver fat content then being comparable to control subjects (liver fat content 2.9%) (393). These data demonstrate how rapidly a decrease in liver fat content can be achieved with aggressive calorie restriction. Following on from this observation, the Diabetes Remission Clinical Trial (DiRECT) utilised a VLCD for an average of 4-months and found that although some individuals achieved remission of their diabetes ('responders') and some did not ('non-

responders'), both groups achieved comparable decreases in body mass of 16 kg and 13 kg, respectively and in liver fat content of 13% and 12%, respectively (392). After 4-months of VLCD, participants in DiRECT went through a stepped food reintroduction program and entered a weight maintenance diet for a further 8-months. At 12 months, the decrease in liver fat content was maintained in the responders and had increased slightly in the non-responders; the authors noted that the increase in liver fat content during the weight maintenance period was related to degree of weight gain (392).

Recently, Schwenger *et al.* (394) determined the effect of the pre-bariatric VLCD in 139 obese individuals (median BMI 47kg/m^2) and found that with a median duration on a VLCD of 3-weeks, the median weight loss was approximately 7kg and when liver histology was assessed the prevalence with normal histology, simple steatosis and NASH was 24%, 81%, and 19%, respectively. As the authors noted, prevalence was lower than predicted which may be due to the fact that individuals had followed the VLCD prior to having the liver biopsy (394). It is currently unclear as to why some individuals respond to a VLCD and others don't and how these diets influence NASH. A calorie deficit is important for weight loss as well as decreasing liver fat content and this is achieved rapidly using a VLCD; however what remains to be determined is how well weight-loss and the reduction in liver fat content are maintained longer-term.

Aside from recommending calorie restriction, there is no consensus between the difference guidelines on the precise gold standard dietary intervention (385). For example the European Association for the Study of the Liver (EASL) recommend low-to-moderate fat and moderate-to-high carbohydrate diets along with low-carbohydrate ketogenic diets and high-protein Mediterranean diets whilst the Italian Association for the Study of the Liver (AISF) recommend a Mediterranean diet and others make no specific recommendations (385). Taking the available evidence, findings are mixed, as to whether in the context of a hypocaloric diet, macronutrient composition has an effect of the decrease in liver fat content achieved. For example, *Haufe et al*. (388) observed a similar reduction in liver fat content of approximate 45% when comparing diets that achieved a calorie deficit by reducing either fat or carbohydrate. Thus, it would seem that in the context of a hypocaloric diet the macronutrient composition has little effect on the achieved decrease in liver fat content and it is likely that the key factor to achieve a decrease in liver fat content is total calorie deficit. Although the mechanisms related to these changes are not well

described, it would be reasonable to assume that changes are mediated through the following: decreased substrate for intrahepatic TAG production due to a reduction in fatty acids entering the liver either from diet or adipose tissue lipolysis, decreased intrahepatic DNL due to decreased substrate availability which would lead to a repartitioning of intrahepatocellular fatty acids away from esterification and toward oxidation pathways (395). Overall, negative energy balance leading to weight loss is associated with a reduction in liver fat content and this remains true irrespective of whether the calorie deficit is achieved by acute, very-low calorie interventions, or more modest reductions in calorie intake over an extended period.

6.7 Bariatric surgery

In 1978, bariatric surgery was defined as being metabolic surgery by *Varco and Buchwald* (396). This was because they viewed it as "the operative manipulation of a normal organ system to achieve a biological result for a potential health gain"; indeed metabolic surgery is broadly the capability of surgery to contribute to proactive healthcare of which indeed bariatric surgery is only one aspect (396). A meta-analysis undertaken by *Buchwald et al.* in 2004 (397) found that bariatric operations were effective for weight-loss in morbidly obese patients and in the majority of patients with T2D, hyperlipidemia, hypertension, and OSA there was either resolution or improvement.

Historically, the six dominant procedures in bariatric surgery, are jejunoileal bypass (JIB), Roux-en-Y gastric bypass (RYGB), vertical banded gastroplasty (VBG), biliopancreatic diversion (BPD) (and the related duodenal switch (DS)), adjustable gastric banding (AGB), and sleeve gastrectomy (SG), of which RYGB has been suggested to be the most effective treatment for obesity as it achieves greater weight loss than other procedures; SG and AGB are alternative surgical approaches that minimally alter upper gastrointestinal tract anatomy,and reduce gastric volume but still achieve weight-loss (398). *Maciejewski et al.* (399) compared the 10-year weight change in a large, multisite, clinical cohort of veterans who underwent RYGB compared with nonsurgical matches, along with the 4-year weight change in veterans who underwent RYGB, AGB, or SG and found patients who underwent RYGB lost 21% more of their baseline weight at 10-years than nonsurgical matches. At 4 years, the decrease from baseline weight was 16.9% greater in patients who underwent RYGB, than patients undergoing AGB, who lost 9.7% more than patients undergoing SG.

Due to the dramatic effects on body weight, and also the evidence for substantial improvement in obesity-related metabolic diseases, some, but not all, scientific societies world-wide, recommend considering bariatric surgery an option for NAFLD in patients unresponsive to lifestyle changes (and pharmacotherapy) for reducing weight and complications (385).In a recent systematic review and meta-analysis by *Lee et al.* (400) they concluded that bariatric surgery resulted in biopsy-confirmed resolution of steatosis in 66% of patients, inflammation in 50% of patients, ballooning degeneration in 76% of patients and fibrosis in 40% of patients. Although these findings clearly highlight the resolution of NAFLD in a large proportion of patients who undergo bariatric surgery, the authors also found that bariatric surgery was related to new or worsening features of NAFLD (e.g. fibrosis) in 12% of patients (400). It remains unclear why some patients may have worsening of conditions and it has been suggested that this may be related to the precise bariatric procedure that was performed and the extent of malnutrition and malabsorption experienced (401). Although there are a vast number of papers now investigating the effects of bariatric surgery on NAFLD and they all show, to varying degrees, that bariatric surgery leads to resolution in NAFLD in obese patients when measured histologically, using imaging methods or blood markers (e.g. fibrosis score, NAFLD activity score), it is difficult to determine if one surgical procedure has benefit over another due to a lack of randomized trials. Although a number of studies have compared the effects of different bariatric procedures, few have compared the different bariatric procedures on liver specific parameters. One such study that attempted to do this compared liver parameters (using ultrasonography and blood markers) before and then one year after individuals had undergone either RYGB or SG (402). Weight loss at one-year post operatively was significantly greater in the RYGB group when compared to those who underwent SG. In addition there were also differences in other metabolic markers including cholesterol and insulin. However, blood transaminase levels were significantly higher in the RYGB group compared the SG group. Clearly more studies robustly comparing the different bariatric procedures are required. Moreover there are no randomized clinical trials in this area nor are there studies that have assessed patients at a similar point of weight loss, rather than time after surgery, so that the effect of the procedures cannot be robustly compared. It is clear that RYGB results in a more rapid weight loss however whether the longer-term effects on liver metabolism are similar needs to be determined. In addition, the mechanisms by which bariatric surgery alters liver fat content (and presumably metabolism) need to be elucidated. Although it is assumed that the change in liver fat after bariatric surgery is due to food restriction or malabsorption or a combination, it is plausible

that changes in gut hormones or changes in other metabolic tissues, such as adipose tissue, also play a role (403).

7 Pharmacotherapy to treat NAFLD

The therapeutic landscape in NAFLD is rapidly evolving. There are still no currently licenced therapies although several treatment modalities are currently in phase 3 development and it is likely that within the next few years we will see the first therapies granted a licence specifically for the treatment of NAFLD. A summary of agents currently being evaluated for the treatment of NAFLD are presented in Tables 1, 2 and 3.

7.1 Glucose lowering agents in the treatment of NAFLD

Whilst there are many agents currently at various stages of development, there are many studies that have used established glucose lowering, anti-diabetic agents with an existing licence for glucose control as potential treatments for NAFLD (Table 1). The use of these agents can therefore be considered not only to optimize glycaemic control, but also potentially to convey a beneficial impact on the liver.

7.1.1 Biguanides

7.1.1.1 Metformin

Metformin is established as the first line pharmacotherapy therapy in the treatment of T2D. It has multiple mechanisms of action including the activation of AMP Kinase as well as altering mitochondrial function and cellular redox state (404). Rodent studies have provided a considerable body of evidence to suggest that it may have utility in the treatment of NAFLD. However, clinical studies have been less convincing (405). While there was initially evidence to suggest that it may have a benefit on lipid accumulation and inflammation, more recent meta-analysis of data has suggested a lack of convincing histological benefit and therefore as a therapy specifically for NAFLD, although safe, is not currently advocated (406,407).

7.1.2 Peroxisome proliferator-activated receptor gamma agonists (thiazolidinediones)

The thiazolidinediones are a class of glucose lowering agent that act predominantly, although not exclusively, as peroxisome proliferator-activated receptor gamma agonists. These agents act to improve insulin sensitivity; much of their activity is directed towards adipose tissue where they increase adiponectin expression and drive adipocyte differentiation. Clinical studies have consistently demonstrated clinical benefits including reductions in hepatic steatosis and resolution of NASH

(162,408,409) The land-mark PIVENS study (in patients with NASH, but without T2D) compared pioglitazone to vitamin E and placebo (410). While vitamin E appeared superior to placebo in terms of the primary end-point (although this was complicated by the lack of hepatocyte ballooning (a defined part of the composite primary end point) in many patients at baseline) pioglitazone reduced steatosis and inflammation and resolved NASH in 47% of patients (compared with 34% on vitamin E and 18% on placebo). More recent studies including patients with T2D have continued to demonstrate histological benefits of pioglitazone treatment with the additional suggestion that fibrosis may improve (162).

Despite the demonstrable benefits to liver histology, and published clinical guidance that advocates consideration of its use, it remains relatively underutilized as a therapeutic option. This may reflect concerns over its adverse effect profile, including fluid retention, weight gain (up to 5kg over 3 years) and increased risk of bone fractures (411). Concerns over cardiovascular risk have been raised. This perhaps reflects previous studies using rosiglitazone suggesting increased myocardial infarction risk, although subsequently the RECORD study showed no overall impact on cardiovascular morbidity or mortality (412,413). Specifically with regards to pioglitazone, data suggest that it may actually reduce cardiovascular and cerebrovascular risk as well as progression to T2D, all of which are important bearing in mind the adverse cardiovascular risk profile associated with NAFLD (414–416). Its use in patients with compromised cardiac function is contraindicated due to concerns that this may worsen symptoms potential due to increased fluid retention. The most recent analysis of data has been reassuring with regards to the concerns that had been raised about the risk of bladder cancer associated with pioglitazone use (417).

7.1.3 Glucagon-like peptide 1 analogues

The incretin effect describes the augmentation of the insulin secretion in response to an oral, as opposed to intravenous, glucose load. This is largely mediated by glucagon-like peptide 1 (GLP-1), which is released from the intestinal L-cells. GLP-1 agonist therapy is now established as a highly potent and efficacious glucose lowering and weight loss intervention. Retrospective analysis of data from the LEAD series of studies demonstrated that liraglutide caused a dose-dependent decrease in ALT in those individuals in whom liver chemistry was abnormal at baseline (418). There was no impact in those with normal liver blood test. Subsequently, prospective studies have demonstrated histological improvement and resolution of NASH using liraglutide; 36% of patients had resolution of NASH (compared with 9% in the

placebo treated arm) (419). Currently, this is the only study that has looked at histological outcomes, although other studies using liraglutide, exenatide and lixisenatide have shown improvements in hepatic insulin sensitivity, liver biochemistry and lipid content (420–427) .

The mechanism of action of GLP-1 analogues to improve NAFLD remains to be clarified. Controversy remains as to whether the GLP-1 receptor is expressed in human hepatocytes, although *in vitro* observations have suggested that GLP-1 agonism can decrease DNL in human hepatocyte primary cultures (428). However many studies have failed to identify the GLP-1 receptor in human and rodent hepatocytes (429,430). The confounding issue of weight loss and improvements in glycaemic control remain to be disentangled from the potential direct benefits of GLP-1 analogue therapy and therefore a mechanism of action that indirectly benefits the liver remains entirely plausible.

There is now an established body of evidence from the SUSTAIN, LEADER and REWIND trials to demonstrate deceased cardiovascular risk associated with semaglutide, liraglutide and dulagluitde use (431–433). Taking into account the increased cardiovascular risk associated with NAFLD, there is a growing body of evidence to suggest that this class of agent has the potential to offer significant clinical benefit to all patients with NAFLD irrespective of whether they do or do not have T2D. A large phase 2 study in patients with NASH using liver biopsy endpoints is currently trialling 3 different dose of semaglutide *vs.* placebo and is due to complete towards the end of 2019 (434). Long acting, once weekly, GLP-1 analogue therapy is now widely used. Although there are no histological data, weight and liver chemistry improve with treatment (435,436) and dedicated phase 2 studies are currently recruiting (437,438). More recently, novel compounds with multiple agonist properties are being developed, for example, HM1522 is a GLP-1/GIP/glucagon triple agonist and is currently be trialled in the context of NAFLD (439).

7.1.4 Dipeptidyl peptidase IV inhibitors

The enzyme dipeptidyl peptidase IV (DPP IV) is responsible to the degradation of endogenous GLP-1. Synthetic GLP-1 analogues that are currently used clinically are resistant to the actions of DPP IV and this is a crucial mechanism that prolongs their half-life and facilitates their clinical utility. Very few histological studies have been performed to examine the impact of DPP IV inhibition in NAFLD; in one study (without a placebo control arm) there was some evidence of histological

improvement in NAS after 1 year of treatment, but no benefit was found in a further study (440,441). Additional studies have looked at liver triglyceride content and biochemistry (423,442,443) and failed to demonstrate significant benefit, although a single study has reported improvement with vildagliptin (444). However, currently, there is no convincing evidence that this class of agent has any beneficial impact upon NAFLD.

7.1.5 Sodium glucose co-transporter 2 inhibitors

The most recent class of glucose lowering agents to gain approval for clinical use are the sodium glucose co-transporter 2 (SGLT2) inhibitors. Through inhibition of SGLT2, they prevent the reabsorption of the vast majority (>90%) of glucose that has been filtered. They are highly effective as glucose lowering agents and cause weight loss (as a consequence of glucose loss). In addition, studies have consistently demonstrated a significant improvement in cardiovascular outcome associated with SGLT2 use (445–447), although the mechanisms that underpin this observation are yet to be determined. Controlled clinical studies in the context of NAFLD that have histological endpoints have not been completed, however, several small studies (including open label and uncontrolled studies) have shown improvements in liver chemistry, improved glycaemic control, weight loss and reductions in liver TAG content as assessed using ^{1}H-MRS although these benefits have not been observed in all studies that have been reported (448–453) . Recently, double-blind placebo controlled studies have been reported using canagliflozin (454), and dapagliflozin (455)(456) in patients with T2D. Hepatic insulin sensitivity improved with canagliflozin, but not dapagliflozin and with both treatments and there were modest reduction in intrahepatic TAG (454,455). A single uncontrolled study has reported histological improvements in NAFLD severity after 24-weeks of treatment with canagliflozin (457). Studies in patients without T2D have not been reported.

While there is potential that this class of agents may convey significant clinical benefit (including cardiovascular risk reduction and weight loss), there is a pressing need for well-designed, prospective clinical studies with relevant clinical outcomes. These drugs are generally well tolerated; genitourinary infections are reported in up to 5% of individuals and there is the potential to develop increased urinary frequency, dehydration and postural hypotension. Concerns have also been raised about the development of diabetic ketoacidosis and while recent data seems to be reassuring, there is still a need for vigilance for potentially life-threatening adverse events (458,459). Licogliflozin is a dual SGLT1 and 2 inhibitor that has been shown to cause

weight loss in patients with and without T2D (460) and studies are currently examining its impact on hepatic steatosis (461).

7.2 Lipid lowering drugs

7.2.1 HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase inhibitors

It is well established that patients with NAFLD are at increased cardiovascular risk. Furthermore, in patients with T2D, the use of lipid lowering therapy, usually in the form of HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase inhibitors (statins) is regarded as best clinical practice. Statins are known to have multiple effects that might be beneficial to patients with NAFLD including anti-inflammatory actions as well as reducing oxidative stress. Statin therapy can be associated with elevated liver enzymes in some individuals. However, establishing a temporal relationship is important as elevations in liver enzymes may reflect underlying NAFLD and may not be related to the initiation of statin therapy.

Only a relatively small number of studies have specifically tested the hypothesis that statin therapy may alter the natural history of NAFLD and many of these have been open label and lacking appropriate controls. Short duration studies have suggested that liver chemistry may improve in those individuals with elevated liver blood tests at baseline and in those with evidence of elevated hepatic TAG content (462). In addition, there is some evidence to suggest improvements in liver chemistry as well as histological NAS assessment without alteration in fibrosis (463), but study design was compromised by the lack of a suitable placebo control group. Additional very small open label studies using pitavastatin and rosuvastatin have also shown improvements in liver chemistry, but not in liver histology (464–466). In a single, small, prospective placebo-controlled study, simvastatin (40mg) used for 12-months failed to show any histological benefit or any impact on liver chemistry (467). However, the study had very small numbers (simvastatin n=10, placebo n=6) and may well have been underpowered to detect biochemical or histological changes.

Overall, while statins appear to be safe and reasonably well tolerated (468), there is a lack of appropriately designed clinical studies that have been able to determine if they have a beneficial impact, specifically upon the natural history of NAFLD. However, they have established proven efficacy to improve cardiovascular outcome and therefore their use in patients with NAFLD should not necessarily be aimed at

disease-modification within the liver, but rather at more holistic cardiovascular reduction (469).

7.2.2 Omega-3 poly-unsaturated fatty acids

The beneficial effects of the omega-3 long chain fatty acids on circulating hypertriglyceridemia are well established. Many studies have been performed investigating whether these agents may have utility as a treatment for NAFLD, although relatively few have had histological primary end-points. The largest study included 243 patients with biopsy-proven NASH. Treatment did not impact upon liver steatosis, fibrosis or inflammation (470,471). Other studies have shown similar results (472) although a very small number of studies have shown modest improvements in NAFLD severity (473). Overall, there seems little compelling evidence that these agents significantly alter the natural history and progression of the more advanced stages of NAFLD, but may have a role to limit hepatic TAG accumulation (474).

7.2.3 Peroxisome proliferator-activated receptor alpha agonists

Fibrates are widely used as lipid lowering therapy, acting through a peroxisome proliferator-activated receptor alpha (PPARα) dependent mechanism to drive lipid oxidation and utilization. There are very few clinical data that have systematically examined the impact of fibrate therapy in the context of NAFLD. An open label study compared fibrate therapy (fenofibrate 200mg daily) to atorvastation 20mg/day or combination therapy and demonstrated improvements in liver biochemistry and ultrasonographic appearance of the liver (475). A further open label, randomized study has also shown improvements in liver biochemistry in comparison with pioglitazone treatment (476). However, detailed mechanistic studies performed as part of a randomised controlled trail (including a nicotinic acid treatment arm), failed to show any improvement in intrahepatic triglyceride content by either nicotinic acid or fenofibrate. However, both treatments decreased VLDL-TAG levels; fenofibrate through increased clearance and nicotinic acid through decreased secretion. In addition, fenofibrate had no impact on peripheral, hepatic or adipose tissue insulin sensitivity (477,478). There are currently no published histological data from clinical studies. As a result, it is hard to draw any significant conclusion as to the utility of fibrates in the treatment of NAFLD. Pemafibrate is a novel selective PPARα agonist with evidence from preclinical models to suggest that it can improve NASH (479). It is currently being used in phase 2 clinical studies in patients with NAFLD (480).

7.2.4 Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors

PCSK9 is a fundamental regulator of lipid homeostasis. It is secreted from the liver into the circulation where it binds to the LDL-receptor and enhances its lysosomal degradation and as a consequence raises plasma LDL-cholesterol levels. PCSK9 inhibitors disrupt this pathway, lower LDL cholesterol and decrease cardiovascular events (481). The relationship of PCSK9 with the pathogenesis of NAFLD and NASH is yet to be fully elucidated and some studies have suggested a positive correlation of circulating PCSK9 levels with markers of NASH severity (482), while other have found no relationship with either liver expression or circulating levels (483). The question as to whether this class of agent can improve the histological features of NASH has not been addressed in clinical studies. However, PCSK9 inhibitors seem well tolerated and there is no evidence that they cause deterioration in liver chemistry (481,484) although dedicated studies to examine the impact of NAFLD/NASH have not been performed.

7.3 Liver-targeted therapies

7.3.1 Farnesoid X Receptor (FXR) Agonists

7.3.1.1 Obeticholic Acid

Signalling through FXR regulates multiple processes within the liver including carbohydrate and lipid metabolisms as well as regeneration and repair. There is an extensive body of pre-clinical literature suggesting that FXR agonism may have beneficial effects in human NAFLD. Obeticholic acid (OCA) is a synthetic FXR agonist that is already licenced for the treatment of primary biliary cholangitis (PBC) where it has been shown to decrease bilirubin and alkaline phosphatase levels (485) .

Proof-of-concept and phase 2b studies using OCA have been completed and shown improvements in liver histology in 45% of individuals (compared to 21% in the placebo arm) and resolution of NASH in 22% (*vs.* 13% in the placebo arm). 35% of patents had improvements in liver fibrosis (*vs.* 19% in the placebo arm) (486). A *post hoc* analysis suggested that most benefit of OCA was derived in those patients who also lost weight (487). These encouraging data led to the initiation of a global, multicentre phase 3 study that is currently recruiting (REGENERATE) (488). A recent planned interim analysis after 18-months of therapy including the 931 patients recruited so far has suggested that the study has met the primary end point of improvement in fibrosis and the adverse effect profile has not identified anything

unexpected. Cardiovascular events reported to date are no difference across the 2 active treatment arms or placebo (489).

The REGERATE study is recruiting patients with mild to moderate fibrosis (F2-F3). A further phase 3 study (REVERSE) has also been initiated in which OCA is being trialled in patients with established (but fully compensated) NAFLD cirrhosis (490).

7.3.1.2 Other FXR agonists

Other FXR agonists are currently in development although at a much earlier stage. GS-9674 has passed through phase 1 studies and currently a phase 2 study in the context of NASH is on-going (491) . Recently published data have shown biochemical improvements in liver chemistry in patients with PSC (492). Tropifexor (LJN452) is a non-bile acid derived FXR agonist and is currently being trialled in phase 2 studies in patients with NASH (493).

7.3.2 Dual and pan-Peroxisome Proliferator-Activated Receptor (PPAR) agonists

7.3.2.1 Elafibranor (PPAR α/δ dual agonist)

PPARα and PPARδ are nuclear transcription factors that are potent regulators of lipid metabolism and inflammation in hepatocytes as well as other tissues. A substantial body of evidence has implicated their role in the pathogenesis of NAFLD, but also highlighted their potential role as a therapeutic target. The dual PPARα and PPARδ agonist, elafibranor, has been trialled in phase 2 studies. In a 52-week randomized, double-blind, placebo-controlled study, 19% of patients in the active treatment arm resolved NASH compared to 13% in the placebo arm without worsening of fibrosis (494). The primary end point was not met in this study, but in a *post hoc* analysis in those patients with more advanced disease, there did appear to be significant benefit. In addition, there were metabolic benefits in patients with diabetes (at the highest doses tested) that included reduction in fasting plasma glucose, circulating FFAS, C-peptide and glycated hemoglobin. A large phase 3 study (RESOLVE-IT) is currently recruiting (495).

7.3.2.2 Lanifibranor (IVA337, pan-PPAR agonist)

Lanifibranor is able to activate all three (α, δ and γ) PPARs. In preclinical studies, it has been reported to decrease tissue fibrosis in non-liver (496,497) as well as liver models (498). A phase 2, randomised, double-blind, placebo-controlled study in patients with NASH with histological end-points is currently recruiting (499).

7.3.2.3 Saroglitazar (dual α/γ agonist)

Dual PPARα/γ agonism using saroglitazar has been shown to improve liver histology in preclinical rodent models (500). Clinical studies have shown benefits on circulating lipid profiles in those patients inadequately treated on atorvastatin therapy (501). Currently, an early phase 2 study in patients with NAFLD looking at changes in liver biochemistry with 3 doses of saroglitazar *vs.* placebo is actively recruiting (502)**.**

7.3.3 C-C chemokine receptor type 2 (CCR2) and type 5 (CCR5) dual antagonist

7.3.3.1 Cenicriviroc

Targeting the inflammatory response within the liver has been proposed as a strategy to limit cellular damage that may ultimately drive fibrosis. In response to hepatocyte injury or insult, Kupffer cells within in the liver secrete C-C chemokine ligand 2 (also known as monocyte chemo-attractant protein 1, MCP1) which can lead to monocyte recruitment driving the inflammatory response and as a consequence causing hepatic stellate cell activation and the resulting fibrotic response. The key signalling receptors mediating these actions appear to be CCR2 and 5 and dual antagonism in preclinical rodent models validated this strategy as a therapeutic target. In a phase 2b study including 289 patients with biopsy-proven NASH (51% with T2DM), after 48-weeks of treatment with the dual CCR2/5 antagonist, Cenicriviroc (150mg once daily), the primary end point was not met (2 point improvement in NAS score without worsening of fibrosis), however, there was a significant increase in the proportion of patients who demonstrated improvements in liver fibrosis on biopsy (20.0% vs. 10.4%, p=0.023) (503). There were no changes in steatosis, liver biochemistry, or metabolic variables, but systemic markers of inflammation (including CRP and IL6) were decreased. The drug was well tolerated with few significant side effects.

Despite the modest benefits that were observed in the phase 2b study, a phase 3 study (AURORA) is currently underway with the aim of recruiting 2000 patients with a primary completion date during 2021 (504).

7.3.4 Vitamin E

Vitamin E is a potent anti-oxidant with the potential to exert anti-inflammatory actions. In the PIVENS trial (patients without T2D or cirrhosis), vitamin E, at a dose of 800mg/ day, was superior to pioglitazone (at the intermediate dose of 30mg/day) in terms of the pre-defined primary end-point of the study leading to an improvement in NASH in

43% *vs.* 19% in the placebo arm) (505). Using endpoints that have adopted in recent studies (NASH resolution), vitamin E achieved borderline significance over placebo (36% *vs.* 21%, p=0.05), but was inferior to pioglitazone, There was no impact on fibrosis. In the TONIC trial, in which children and adolescents were treated with 300mg /day, there was no overall benefit in terms of improvements in liver chemistry, steatosis, inflammation or fibrosis (506). Concerns have been raised with respect to the long-term safety of higher doses of vitamin E (507), although this remains a contentious area with continued debate in the published literature with regards to the precise methodologies that have been used in the analyses (508). There is some evidence to suggest increased all-cause mortality, increased risk of haemorrhagic stroke although the data on the relationship to prostate cancer risk are less clear (509,510).

7.3.5 Galectin-3 Protein Inhibitors

7.3.5.1 GR-MD-02

Galectin-3 protein is believed to play a crucial role in orchestrating the fibrotic response to inflammation with in the liver. The evidence base for its role as a therapeutic target is relatively limited; rodent models with genetic deletion have shown increased lipid accumulation, but either increased or decreased inflammation and fibrosis dependent upon the precise model used. The GT-020 study was a first-in-class, phase 1 study using the galectin-3 inhibitor, GR-MD-02 (511); the drug was well tolerated with no major treatment emergent adverse effects. An early phase 2 study has been completed (NASH-CX) in patients with NASH cirrhosis. The primary endpoint was a reduction in portal pressure, but liver biopsies were taken. The data are not formally published in the peer-reviewed literature (512), but there was no significant change in NAS assessment (although decreased hepatocyte ballooning was observed) and no change in fibrosis score or collagen content on morphometric analysis.

7.3.6 Fibroblast Growth Factor 19 (FGF19) analogues

7.3.6.1 NGM282

FGF19 has been implicated in the pathogenesis of NAFLD. It has a crucial role in bile acid synthesis as well as having important metabolic actions and in rodents administration has been shown to enhance energy expenditure, promote weight loss and improve lipid profiles (513). Transgenic over expression limits lipid synthesis (514).

NGM282 is a potent FGF19 analogue and in a phase 2 study including 166 patients with biopsy-proven NASH, the impact of 2 different doses of NGM282 were examined (3mg or 6mg administered subcutaneously) (515). Over the 12-week duration of the study, there was a significant reduction in liver fat (48% reduction with 3mg and 60% reduction with 6mg) as well as improvements in liver biochemistry. Adverse events were relatively common with injection site reactions as well as gastro-intestinal disturbance being reported. Serum LDL-cholesterol also increased (with a parallel decrease in HDL-cholesterol), but recent data has shown that this can be lowered by co-administration with rosuvastatin without significant additional adverse effects (516). More recently, liver biopsy findings from an open label, 12-week study (doses 1mg and 3mg) have been reported demonstrating significant reductions in steatosis, inflammation and fibrosis (517) . A larger phase 2 study aiming to recruit 250 participants with biopsy-proven NASH is currently actively recruiting (518).

7.3.7 FGF21 analogues

Fibroblast growth factor 21 is highly expressed in the liver and it has an established role in the regulation of many metabolic features including weight, energy expenditure and insulin sensitivity. The relationship between circulating levels of FGF21 and metabolic phenotype are complex and there is evidence to suggest that levels rise with worsening metabolic phenotype that is consistent to resistance to the effects of FGF21. Specifically in the context of NAFLD, recently published data suggest that FGF21 levels correlated most closely with adipose and skeletal muscle insulin resistance (but not hepatic) and that levels were positively associated with inflammation and ballooning (but not steatosis) as well as fibrosis stage (519). In a proof-of-concept study, 4 weeks of treatment with LY2405319 (LY), a variant of FGF21, in patients with obesity and T2D, improved circuiting lipid profiles (decreased LDL-cholesterol and TAG and increased in HDL-cholesterol) (520).

More recently, Pegbelfermin (BMS-986036), a PEGylated human FGF21 analogue has been trialled in 80 overweight / obese individuals with NASH. Patients were recruited into a double-blind, randomised, placebo-controlled study investigating 2 doses of BMS-986036 (10 and 20mg). The study was terminated early due to a larger than anticipated impact on the primary endpoint at the planned interim analysis after 8-weeks of treatment. There was a significant reduction in hepatic steatosis as measured by MRI PDFF and the drug was well tolerated with no serious adverse

events or treatment related drug withdrawals (521). A series of phase 2 studies are currently actively recruiting (522–529).

7.3.8 Thyroid Hormone Receptor-β (THR-β) Agonists

7.3.8.1 MGL-3196 (Resmetirom)

MGL-3196 was developed as a liver-specific agonist of the thyroid hormone receptor-β (THR-β). Original studies aimed to explore its role in the treatment of hyperlipidemia and demonstrated improvements in LDL-cholesterol, non-HDL cholesterol, lipoprotein(a) and a trend to improved circulating TAG levels importantly, without alteration in circulating thyroid hormone levels (530,531). A randomised double blind, placebo-controlled study with 36-weeks of treatment in 116 patients with biopsy-proven NASH has been reported in abstract form only. The primary end point was reduction in hepatic fat as measured by MRI PDFF and this was achieved with a relative reduction of 36.3% *vs.* 9.6% in the placebo arm (532). Further data have now been released although have not been peer-reviewed and suggest that after 36-weeks of treatment there were significant improvements in the resolution of NASH alongside a sustained improvement in steatosis (50,533). The drug was well tolerated in this cohort with no serious adverse events. On the basis of these results, a programme of phase 3 studies is now being initiated.

7.3.9 Apoptosis Signal-Regulating Kinase 1 (ASK1) inhibitors

7.3.9.1 Selonsertib

ASK1 is induced by cellular stress and is an important regulator of both inflammation and fibrosis. Preclinical data in rodent models has suggesting that inhibition of ASK1 has the potential in rodent models to modify the natural history and progression of NASH. Selonsertib (GS-4997) is a selective ASK1 inhibitor and has been used in an open label phase 2 study in 72 patients treated with 6 or 18mg orally for 24-weeks (alone or in combination with simtuzumab – a monoclonal antibody directed against lysl oxidase-like-2 monoclonal which subsequently has been shown to have no efficacy as monotherapy) (534). In the final analysis, liver histology comparison was made with all selonsertib treated patients (with or without simtuzumab) vs. simtuzumab alone. At the highest doses (18mg), fibrosis decreased in 43% or patients (30% in the 6mg dose arm and 20% in the simtuzmab alone treatment arm) (535). Changes in non-invasive markers, including imaging and serum tests paralleled the changes in liver histology. A further analysis of the data from this study aimed to look at quality of life; where there was histological improvement in

NAS assessment (14 out of 68 patients), there was also improvement in quality of life scores (536).

7.3.10 Pentoxifyline

The mechanisms by which pentoxifylline may improve NASH are not fully understood, although it has an inhibitory action on pro-inflammatory cytokines as well as reducing the generation of free oxygen radicals. Treatment with pentoxifylline for 1-year resulted in histological improvement in NAS assessment as well as fibrosis (537). A further study also showed histological improvements, but without significant changes in liver chemistry (538). Currently there are no actively recruiting studies registered on clinicaltrials.gov investigating the role of pentoxifylline in the treatment of NASH.

7.3.11 Caspase inhibition

7.3.11.1 Emricasan

Emricasan is a pan-caspase inhibitor that has been reported to decrease apoptosis and inflammation in liver disease. In patients with cirrhosis (due to NASH and HCV) there was improved liver function after 3-months of treatment that was observed not only in the whole cohort, but in a subgroup analysis, the improvement was statistically significant in those patients with NASH cirrhosis, although the numbers treated were very small (placebo n=9; emricasan n=11) (539). Specifically in NAFLD patients without cirrhosis, but with elevated ALT, emricasan decreased ALT and AST as well as reducing circulating cleaved and full-length cytokeratin-18. The most dramatic effects were seen within 7-days of treatment and some, but not all of the effects persisted throughout the full 28-days of treatment (540). Further studies in NASH are planned although currently not actively recruiting (541).

7.4 Targeting hepatic lipid metabolism

7.4.1 Acetyl-Coenzyme A Carboxylase (ACC) Inhibition

7.4.1.1 GS-0976

ACC is the rate-limiting step in DNL and has therefore become a target to limit hepatic TAG accumulation and potentially enhance lipid oxidation. GS-0976 is a small molecular inhibitor of ACC and has been investigated in a small number of clinical studies. In an open label, uncontrolled, prospective study in 10 individuals treated for 12-weeks, DNL (measured by deuterated water incorporation into palmitate) was reduced by 22%. Hepatic steatosis measured by MRI-PDFF decreased as did ALT and liver stiffness (542). In a much larger, randomized,

placebo-controlled study (n=126), GS-0976 at the highest doses only (20mg) decreased hepatic steatosis, but there was no change in liver stiffness although tissue inhibitor of metalloproteinase 1 (TIMP1) levels decreased (543). The drug was safe and well tolerated, although serum TAG levels increased in all groups treated with GS-0976

7.4.2 Stearoyl coenzyme A desaturase 1 inhibition

7.4.2.1 Aramchol

Aramchol is a novel fatty acid-bile acid conjugate molecule (3β-arachidyl-amido, 7α-12α-dihydroxy, 5β-cholan-24-oic acid). Its mechanism of action is likely to be through inhibition of stearoyl coenzyme A desaturase 1 which is an important regulatory step in lipid synthesis within the liver. In an early phase 2 trial for 3-months, there was a significant reduction in liver fat as measured by ^{1}H-MRS when compared to placebo (only in the higher 300mg/day dose). There were no significant changes in liver chemistry when compared to placebo (544)**.** The drug was safe and well tolerated and a larger phase 2b study has been completed although data are not formally reported in the peer-reviewed literature. There were significant improvement in liver chemistry as well as resolution of NASH, but no significant change in fibrosis (545)

7.5 Modulation of the gut microbiome

It is now established that the gut microbiome has the potential to have a profound regulatory impact upon metabolic phenotype (see section 3.4). Therefore various strategies have been employed in order to try and modify the microbiome composition in such a way as to promote a beneficial metabolic phenotype. A small number of randomized, controlled trials have been performed that have administered differing probiotics. Improvements in liver chemistry as well as markers of inflammation and insulin sensitivity have improved, however additional studies are clearly required with demonstrable histological benefit before their widespread use can be recommended in patients with NAFLD (546).

A very small (n=6) proof-of-concept study has been performed using solithromycin, a macrolide antibiotic. After 13-weeks of treatment, there was histological improvement in NAS in all patients as well as improvements in liver chemistry in almost all individuals (547). However, treatment of patients with biopsy-proven NASH and elevated aminotransferases (n=15) with rifaximin (400mg twice daily for 6-weeks) had no impact on ALT levels, hepatic insulin sensitivity or liver TAG content (548).

On-going studies are also looking at the potential of fecal microbiome transplantation as a therapeutic strategy in NAFLD (549–553) .

7.6 Other treatments in early development

Several other treatment targets are at the very early stages of development and have yet to be formally trialled with dedicated histological or robust and meaningful clinical end points. SGM-1019 is an inflammasome inhibitor and has been hypothesised to limit hepatocyte injury. Early phase clinical studies have been performed and it is safe and well tolerated and phase 2 studies are currently in progress (554)(555).

Additional modulation and limitation of the inflammatory response could be mediated through antagonism of the Toll-like receptor 4 (TLR4). JKB-121 is a weak molecular antagonist of TLR4 receptor and whilst preclinical data suggest promise in its ability to prevent the development of NASH, an early phase 2 study has shown no difference when compared to placebo in reductions in liver fat content and relatively high (dose-dependent) adverse events that lead to drug withdrawal (556).

IMM-124E is a product derived from bovine colostrum that contains high levels of anti-E.coli-LPS IgG. It has been suggested that this may limit exposure to bacterial endotoxins and has been shown to limit acute colitis in preclinical models (557). A phase 2 study in 133 patients with NASH has been completed using an oral preparation at 2 different doses although results have not yet been reported (558).
Amlexanox is an inhibitor of IKKb and TANK-binding kinase 1 both of which are important signalling molecules that co-ordinate the inflammatory response. In a small proof-of-concept study in patients with type 2 diabetes, there was improvement in glycaemic control as well as evidence for decreased hepatic steatosis (559).

Tipelukast (MN-001) is an anti-inflammatory and anti-fibrotic agent that inhibits phosphodiesterase and 5-lipoxygenase activity as well as being a leukotriene receptor antagonist. It has potential as a lipid-lowering agent and is currently being trialled in patients with NASH/NAFLD and hypertriglyceridemia (560). The stated primary outcomes relates to cholesterol metabolism and circulating TAG levels as opposed to direct assessments of liver (561). DS102 is a bioactive lipid that also inhibits 5-lipoxygenase activity. Phase 1 studies have been completed (562) and a phase 2 study in patients with NAFLD is actively recruiting (561).

Vascular adhesion protein-1 (VAP-1) is a membrane-bound amine oxidase that is thought to have a key role in recruiting inflammatory cells into the liver. Circulating levels of the soluble form of VAP are elevated in patients with NASH (201) and an early phase 2 study trialling a novel VAP inhibitor (BI 1467335) is currently recruiting (563).

Inhibition the mitochondrial pyruvate carrier has been proposed as a potential treatment strategy for NAFLD. This limits pyruvate flux into the TCA and is associated with metabolically beneficial effects including enhanced insulin sensitivity and decreased DNL alongside decreased stellate cell activation (564) . A large phase 2 study (EMMINENCE) is currently recruiting using the MPC inhibitor MSDC-0602K (565). An interim analysis has shown improvement in metabolic parameters as well as surrogate markers of inflammation and fibrosis (566).

DGAT2 catalyses the final step in hepatic TAG synthesis. Anti-sense mediated inhibition of DGAT2 may have potential to limit hepatic steatosis although data have currently been presented in abstract form only (567).

CF102 is an adenosine A3 receptor agonist that in pre-clinical models has shown anti-inflammatory and pro-apoptotic actions in the liver (568). It has been trailed in small numbers of patients with HCC (569) and is currently under evaluation as a potential treatment for NASH (570).

8 Conclusions

The magnitude of the clinical burden associated with NAFLD has heightened the need to understand the natural history and risks associated with the condition. Whilst in many individuals, in the absence of significant fibrosis, this may be a relatively benign condition, there is no doubt that with advancing fibrosis, there is significant morbidity and mortality. A significant challenge continues to be the development of non-invasive biomarkers (to replace liver biopsy) that can accurately detect and stage disease (and therefore risk), but that can also be used to track changes over time and the impact of specific interventions.

Our understanding of the determinants of disease progression is developing rapidly, but we are still some way from being able to categorically identify those patients who will progress to advanced disease and those who will not. This is clearly important if we are to try to target appropriate therapies to individuals who are most likely to

benefit. The future therapeutic landscape is relatively rich, with an impressive array of compounds with mechanisms of action targeting differing elements of the pathogenesis of NAFLD. The future of NAFLD pharmacotherapy will undoubtedly include combination therapies (paralleling the current treatment strategies for hypertension and T2D) for example, combining limitation of hepatic TAG accumulation alongside therapies to reduce fibrosis and those to reduce cardiovascular risk. NAFLD epitomizes the multi-system disease and necessitates a holistic approach to its management. While we wait for the development of novel drug therapies, adopting a multidisciplinary approach to its management, combining the skills and expertise of multiple healthcare professions would appear to set the current gold-standard of care.

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Figure legends

Figure 1.

Multiple mechanisms and risk factors contribute to the development of hepatic steatosis and NASH. Obesity, insulin resistance and type 2 diabetes (T2D) remain the key drivers to disease progression (BMI; body mass index, GC; glucocorticoids, PCOS; polycystic ovary syndrome, *↓T4; hypothyroidism, PNPLA3;* phospholipase domain-containing protein 3, *TM6SF2; t*ransmembrane 6 superfamily 2, MBOAT7; membrane bound O-acyltransferase domain containing 7, HSD17B13; 17β-hydroxysteroid dehydrogenase type 13, FFA; free fatty acids, DNL; *de novo* lipogenesis, IR; insulin resistance, ROS; reactive oxygen species, DAMPs; damage associated molecular patterns, PAMPs; pathogen associated molecular patterns, CVD; cardiovascular disease).

Figure 2.

Schematic representation of the structure of a metabolic hepatology clinic. Adopting a multidisciplinary approach to NAFLD has the potential to improve cardiovascular health and reduce liver stiffness (b), improve glycemic control in those patients with type 2 diabetes (T2D) (c) and promote significant weight loss (d). From Moolla A, Motohashi K, Marjot T, Shard A, Ainsworth M, Gray A, Holman R, Pavlides M, Ryan JD, Tomlinson JW, Cobbold JF. A multidisciplinary approach to the management of NAFLD is associated with improvement in markers of liver and cardio-metabolic health. Frontline Gastroenterol. 2019. doi:10.1136/flgastro-2018-101155.

Table 1. The impact of anti-diabetic, glucose lowering agents as pharmacotherapy to treat NAFLD.

Agent	***Mechanism of action***	***Impact on liver biochemistry and / or non-invasive assessments of NAFLD disease status***	***Impact on liver histology***	***Comments***	***References***
Metformin	Multiple mechanisms including AMPKinase activation	Some but not all studies, have shown improvements in liver chemistry and hepatic steatosis	Meta-analyses have shown no significant benefit on liver histology	Remains the first line pharmacotherapy for patients with T2D. Its use specifically for the treatment of NAFLD is not recommended.	(406,571)
Pioglitazone	PPARγ agonist	Consistent improvements in liver chemistry and reductions in hepatic steatosis.	Decreased steatosis and inflammation, with resolution of NASH. Some evidence to suggest that fibrosis may improve	Despite the histological benefits, widespread use is currently limited, potentially by concerns over adverse effects including weight gain, bone fracture risk and fluid retention.	(408–411,452,572)
Liraglutide	GLP-1 analogue	Improved liver chemistry and decreased hepatic steatosis.	Decreased steatosis, inflammation and resolution of NASH without worsening of fibrosis	To date, only data from small studies have been published and the relative contribution of weight loss and improvement in glycaemic control to the observed benefits in NASH are yet to be determined.	(419–425,573)
Exenatide	GLP-1 analogue	Improvements in liver chemistry and fatty liver index	n/a		(426,574,575)
Lixisenatide	GLP-1 analogue	Improvement in ALT in obese and overweight individuals	n/a		(427)
Semaglutide	GLP-1 analogue	n/a	n/a	Phase 2 studies currently recruiting	(434)

Dulaglutide	Long acting GLP-1 analogue	Improved liver chemistry	Histological report of improvement in a single case	No dedicated histological studies performed to date. Phase 2 studies are recruiting.	(435,436)
HM1522	GLP-1/GIP/Glucagon triple agonist	n/a	n/a	Early phase 2 studies currently recruiting.	(439)
Sitagliptin	DPPIV inhibitor	Inconsistent results and some studies have failed to show improvements in liver chemistry or liver TAG content.	Limited histological evidence of benefit with inconsistent results; improvement in NAS in some studies but not all.	Overall the data do not suggest that there is significant benefit when used as a treatment for NAFLD.	(423,442,443)
Vildagliptin	DPPIV inhibitor	Improved liver chemistry and decreased liver triglyceride content	n/a		(444)
Empagliflozin	SGLT2 inhibitor	Improvements in liver chemistry and decreased hepatic TAG content	Some evidence for histological improvement in an open label study.		(448,453,576)
Canagliflozin	SGLT2 inhibitor	Some evidence of improvements in liver chemistry and surrogate markers of fibrosis in very small numbers of patients. Reduction in liver TAG content and increased hepatic insulin sensitivity	Improvement in inflammation and steatosis in a single biopsy study.	Uncontrolled liver biopsy study, with very small numbers (n=9) with no placebo or other comparator arm	(449,454)
Ipragliflozin	SGLT2 inhibitor	Some evidence for improvement in liver chemistry with variable changes in markers of fibrosis.	Single case report of histological improvement in steatosis, inflammation and ballooning		(452,577,578)
Dapagliflozin	SGLT2 inhibitor	Improvements in liver chemistry and reduction in liver TAG content. No impact on increased hepatic insulin sensitivity	n/a		(455,456)

Licogliflozin	Dual SGLT1 and 2 inhibitor	n/a	n/a	Early phase 2 studies currently recruiting.	(461)

Table 2. Liver-targeted therapies currently in development for the treatment of NAFLD

Agent	***Mechanism of action***	***Impact on liver biochemistry and / or non-invasive assessments of NAFLD disease status***	***Impact on liver histology***	***Comments***	***References***
Liver-targeted therapies (anti-inflammatory and anti-fibrotic)					
Obeticholic acid	FXR agonist	Improvements in liver chemistry	Resolution of NASH and improvements in fibrosis	Phase 3 studies are currently recruiting (including patients with NASH cirrhosis).	(486)
GS-9674	FXR agonist	n/a	n/a	Biochemical improvements in patients with PSC, dedicated studies in patients with NAFLD are currently recruiting.	(492)
Tropifexor (LJN452)	Non-bile acid FXR agonist	n/a	n/a	Phase 2 studies currently recruiting	(493)
Elafibranor	Dual PPAR α/δ agonist	Improvements in liver chemistry as well as lipid profiles and glucose homeostasis in patients with T2D.	Resolution of NASH without worsening in fibrosis	Phase 3 studies are currently recruiting	(579)
Lanifibranor (IVA337)	Pan-PPAR agonist	n/a	n/a	Phase 2 studies are currently actively recruiting	(499)

Saroglitazar	Dual PPARα/ γ agonist	n/a	n/a	Phase 2 studies are currently actively recruiting	(501)
Cenicriviroc	Dual CCR2/5 antagonist	No changes in liver chemistry or steatosis	Primary end point for resolution of NASH was not met in the phase 2 studies, but improvements in fibrosis were observed.	Some improvement in systemic markers of inflammation. A large phase 3 is currently recruiting	(503)
Vitamin E	Multiple mechanisms including anti-oxidant actions	Inconsistent improvements in liver chemistry and steatosis	Resolution of NASH in some studies, but not all; no impact on fibrosis		(505,506)
GR-MD-02	Glactin-3 protein inhibition	No changes in liver chemistry in the early phase studies published to date.	No significant change in NAS, although ballooning decreased. No change in fibrosis	Liver biopsy studies performed in patients with NASH cirrhosis.	(511,512)
NGM282	FGF 19 analogue	Improvements in liver chemistry and steatosis	Early phase 2 study has demonstrated improvements in steatosis, inflammation and fibrosis	A larger phase 2 study is actively recruiting.	(515,517)
LY2405319	FGF 21 analogue	n/a	n/a	Evidence for lipid lowering in patients with type 2 diabetes	(520)
Pegbelfermin (BMS-986036)	FGF 21 analogue	Decreased hepatic steatosis	n/a	A series of phase 2 studies are currently recruiting.	(521)

Resmetirom, MGL-3196	Thyroid Hormone Receptor-β agonist	Decreased hepatic steatosis	Resolution of NASH and decreased steatosis	A phase 3 study is actively recruiting	(50,532)
Selonsertib	Apoptosis Signal-Regulating Kinase 1 Inhibitor	Improvements in liver chemistry.	Improved fibrosis at the highest doses.		(580)
Simtuzumab	lysl oxidase-like-2 inhibition	No significant changes in liver biochemistry.	No improvement in NAS or fibrosis.	No benefit on histological analysis or on clinical outcomes	(534)
Pentoxifylline	Multiple mechanisms including anti-inflammatory actions and reduction in free oxygen radial generation	No change in liver chemistry	Evidence for improvement in NAS and fibrosis		(537,538)
Emricasan	Pan-caspase inhibition	Improved liver chemistry	n/a	Short duration early phase studies only have been reported	(539,540)
SGM-1019	Inflammasome inhibition	n/a	n/a	Safe and well tolerated in a phase 1 study. A phase 2 study is actively recruiting	(554,555)
JKB-121	Toll-like receptor 4 antagonism	No impact on hepatic steatosis	n/a	Relatively high adverse event rate leading to drug withdrawal	(556)
IMM-124E	Limitation of endotoxin exposure	n/a	n/a	Phase 2 study completed, but results not yet reported	(558)

Amlexanox	IKKb and TANK-binding kinase 1 inhibition	Some evidence for decreased hepatic steatosis in patients with diabetes	n/a		(559)
Tipelukast (MN-001)	Phosphodiesterase and 5-lipoxygenase inhibition and leukotriene receptor antagonism	n/a	n/a	Phase 2 studies are currently recruiting	(561)
DS102	5-lipoxygenase inhibition	n/a	n/a	Phase 2 studies are currently recruiting	(562,581)
BI 1467335	Vascular adhesion protein-1 inhibition	n/a	n/a	Early phase 2 studies are currently recruiting	(563)
MSDC-0602K	Mitochondrial pyruvate carrier inhibition	Evidence for improved metabolic as well as surrogates of hepatic inflammation and fibrosis on interim analysis	n/a	Large phase 2 study (EMMINENCE) currently recruiting	(565,566)
Hepatic lipid metabolism					
GS-0976	Acetyl-Coenzyme A Carboxylase Inhibition	Improved liver chemistry and hepatic steatosis	n/a		(542,543)

Aramchol	Stearoyl coenzyme A desaturase 1 inhibition	No change in liver chemistry, but decreased hepatic steatosis	n/a	Phase 2b completed although not reported.	(544)
Gut microbiome					
Probiotics	Alteration in gut microbiome	Improvements in liver chemistry and steatosis	n/a		(546)
Solithromycin	Macrolide antibiotic	n/a	Improved NAS in small proof-of-concept study		(547)
Fecal microbiome transplantation		n/a	n/a	Several phase 2 studies currently recruiting	(549,550,552)

Table 3. Lipid lowering drugs as pharmacotherapy to treat NAFLD.

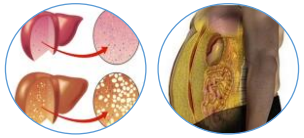
Agent	***Mechanism of action***	***Impact on liver biochemistry and / or non-invasive assessments of NAFLD disease status***	***Impact on liver histology***	***Comments***	***References***
Simvastatin	HMG CoA reductase inhibitor	Some evidence for improved liver chemistry	No evidence of benefit in a small (n=16) study	Study may have been underpowered to detect histological improvement.	(465,467)
Atorvastatin	HMG CoA reductase inhibitor	Some evidence for improved liver chemistry	No improvement on inflammation or fibrosis on liver histology		(463,466,582)
Pitavastatin	HMG CoA reductase inhibitor	Some evidence for improved liver chemistry	No improvement on liver histology		(462,464)
Omega3-fatty acids		Some evidence for improved liver chemistry and decreased steatosis	Little evidence to suggest significant improvements in inflammation or fibrosis		(470–474)
Fenofibrate	PPARα agonist	Some evidence for improved liver chemistry and steatosis on ultrasound scanning, but no change in intra-hepatic TAG in detailed mechanistic studies	n/a	Some conflicting data, unlikely to have any significant impact on liver TAG content.	(475–478)
Pemafibrate	PPARα agonist	n/a	n/a	Phase 2 studies are currently actively recruiting.	(480)

Non-alcoholic fatty liver disease (NAFLD)

High prevalence and adverse outcomes

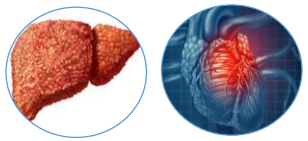
Global disease burden

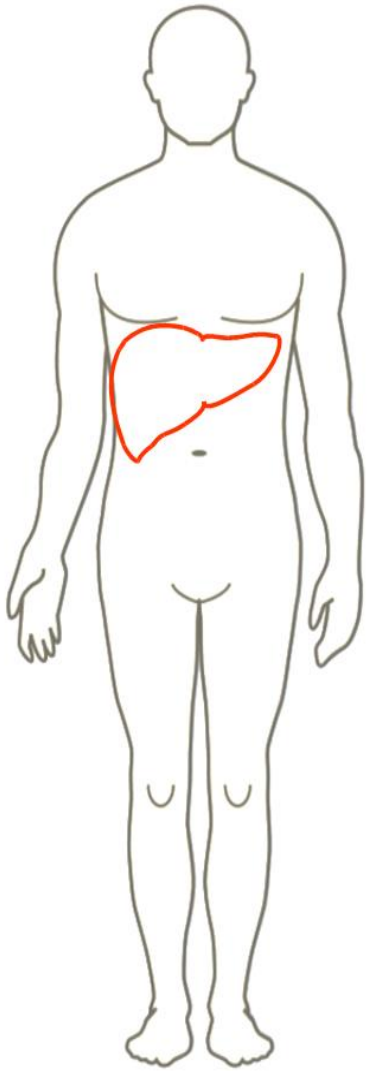
Complex pathophysiology



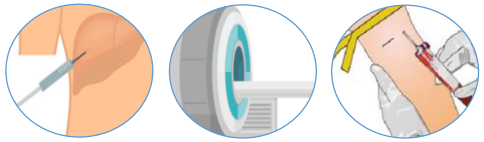
Multiple risk factors

Morbidity & mortality





Diagnosis and staging



Exercise, diet & weight loss

Cardiovascular risk reduction

Developing therapeutics

Holistic and multidisciplinary approach

Figure 1

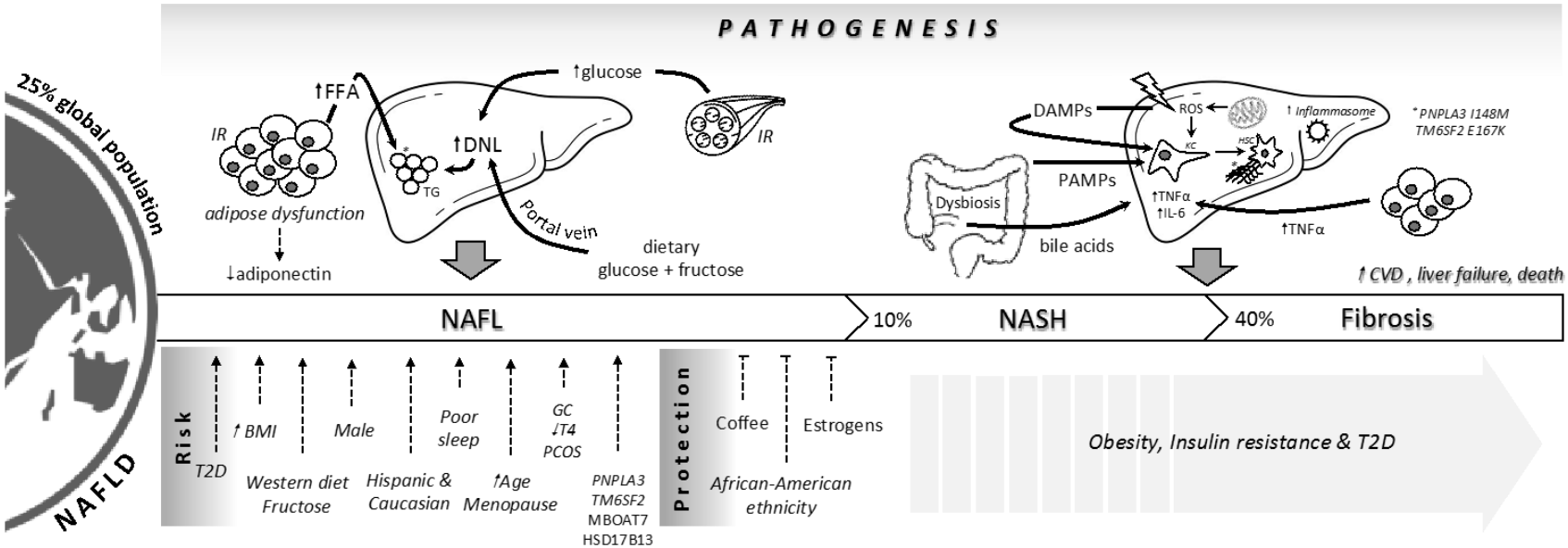
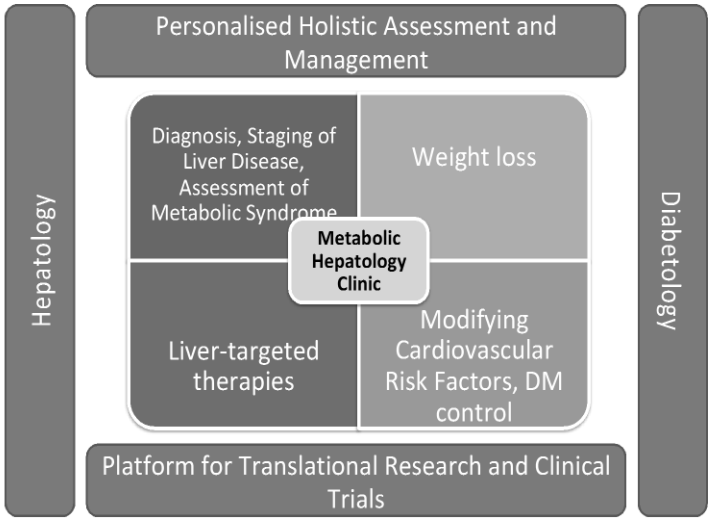


Figure 2

a



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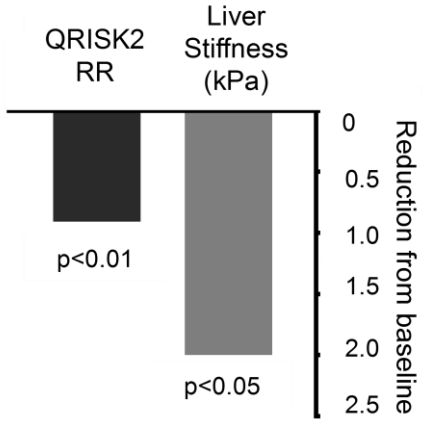

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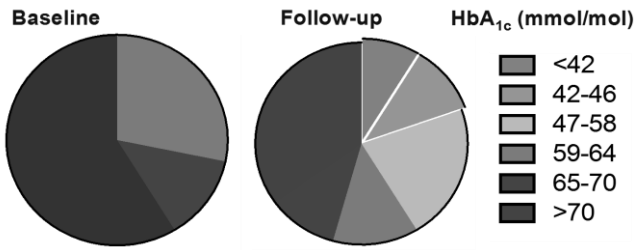

- HbA1C >58mmol/mol at baseline, n=46
- HbA1C >58mmol/mol at follow-up, n=34

d