**Mechanisms, screening modalities and treatment options for individuals with non-alcoholic fatty liver disease and type 2 diabetes**

**Short title:** Non-alcoholic fatty liver disease and type 2 diabetes

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**Novelty statement (What’s new?):**

* Individuals with type 2 diabetes have a near 3 fold higher risk of dying from chronic liver disease.
* The presence of non-alcoholic fatty liver disease has implications for liver disease progression, microvascular and macrovascular complications of diabetes and additional extra-hepatic manifestations.
* Screening for non-alcoholic fatty liver disease, followed by risk stratification to identify individuals with liver fibrosis, should be adopted into routine diabetes care.
* Significant weight loss of 7-10% can cause regression of histological abnormalities including of fibrosis, in non-alcoholic steatohepatitis, even at advanced stages.
* Thiazolidinediones, glucagon like peptide-1 receptor agonists and sodium-glucose co-transporter 2 inhibitors have all been shown to improve hepatic steatosis. The only currently available data on histological improvement relate to the use of thiazolidinediones and glucagon like peptide-1 receptor agonists but is lacking in the case of sodium-glucose co-transporter 2 inhibitors.

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**Abstract**

Non-alcoholic fatty liver disease (NAFLD) exists as a spectrum of disease ranging from excessive accumulation of fat within the liver (simple steatosis), inflammation (non-alcoholic steatohepatitis, NASH) through to fibrosis, cirrhosis and end-stage liver disease. There is also an increased risk of hepatocellular carcinoma. The principal risk factor for NAFLD is overweight or obesity, along with type 2 diabetes, and NAFLD itself is also a risk factor for incident type 2 diabetes. Overweight/obesity is synergistic with alcohol consumption in causing progressive and insidious liver damage. Recent consensus advocates a change in nomenclature from NAFLD to *‘*metabolic associated fatty liver disease’ (MAFLD), reflective of the associated metabolic abnormalities (insulin resistance/type 2 diabetes and metabolic syndrome components). Additional extra-hepatic manifestations of NAFLD include cardiovascular disease, chronic kidney disease and certain cancers.

Unlike other micro-and macrovascular complications of type 2 diabetes, systematic screening or surveillance protocols have not been widely adopted in routine diabetes care to assess for presence/severity of NAFLD. Various screening tools are available (non-invasive tests and biochemical indices) combined with imaging techniques (e.g. transient elastography) to detect steatosis and more importantly advanced fibrosis/cirrhosis to facilitate appropriate surveillance. Liver biopsy may be sometimes necessary.

Treatment options for type 2 diabetes, including lifestyle interventions (dietary change and physical activity), glucose-lowering therapies and metabolic surgery, can modulate hepatic steatosis and to a lesser extent fibrosis. Awareness of the impact of liver disease on the choice of glucose-lowering medications in individuals with type 2 diabetes is also critical.

**Key words:** liver disease, novel agents, nutrition and diet, insulin sensitivity, insulin sensitisers

**Introduction**

The terms NAFLD (non-alcoholic fatty liver disease) and non-alcoholic steatohepatitis (NASH) were first used in 1980 to describe a condition characterised by the histological features of alcoholic liver disease in the absence of clinically significant alcohol consumption or other liver disease. The first series from the Mayo clinic described 20 people, with minimal/no alcohol consumption, who were moderately obese, with associated components of the metabolic syndrome or type 2 diabetes [S1]. Since this original description, the prevalence, and in parallel the clinical and research relevance of NAFLD, has increased exponentially as rates of obesity have dramatically increased. It has been suggested to represent the hepatic manifestation of the metabolic syndrome [S2]. NAFLD is the most common cause of chronic liver disease worldwide, affecting 25% of adults globally and in Europe [1].

NAFLD exists as a spectrum of disease ranging from an excessive accumulation of fat within the liver (simple steatosis), associated inflammation and cellular injury (non-alcoholic steatohepatitis, NASH) through to fibrosis, cirrhosis and end-stage liver disease. There are variable rates of progression, but type 2 diabetes is an important risk factor for its development, histological progression and outcome. Without intervention and left untreated, 30% of individuals with NAFLD develop the next phase of hepatic damage: NASH where the hepatocytes become inflamed. Of those who develop NASH there is a high risk of progression to cirrhosis, with an increased risk of liver cancer, which can develop even in the absence of cirrhosis [S3-5].

More recently, consensus opinion has highlighted concerns about the nomenclature of NAFLD and an alternate term of ‘metabolic associated fatty liver disease’ (MAFLD) has been proposed, although this is yet to be formally adopted by the European Association for the Study of the Liver (EASL) or the American Association for the Study of Liver Diseases (AASLD) [2]. This shifts the focus towards inclusionary diagnostic criteria, the presence of metabolic abnormalities, rather than the absence of excessive alcohol intake, is less stigmatising and avoids the competition between a diagnosis of alcoholic liver disease and MAFLD, recognising their synergistic effects on the liver [S6].

This review will describe the aetiology, epidemiology, assessment and management of NAFLD with reference to type 2 diabetes. It will also consider the impact of treatment options and how these maybe influenced by hepatic dysfunction.

**A. The liver as a metabolic organ**

***Central metabolic role of liver in carbohydrate and fatty acid metabolism* (Fig. 1)**The liver plays a major role in the regulation of carbohydrate and fat metabolism, handling influxes of non-esterified fatty acids (NEFAs) from adipose tissue lipolysis during fasting, and of dietary sugars and fat from the digestive tract post-prandially. Dietary sugars that reach the liver maybe oxidised or may be converted to fatty acids by *de novo* lipogenesis. Fatty acids that reach the liver may originate from different sources including i) dietary carbohydrate and stimulation of hepatic *de novo* lipogenesis, ii) dietary fatty acids, through spillover into the plasma non-esterified fatty acid (NEFA) pool, or through uptake of intestinally derived chylomicron remnants, and iii) from adipose tissue lipolysis liberating NEFAs into the plasma NEFA pool [S7]. Fatty acids within the liver may be oxidised, stored as intrahepatic triglyceride (contributing to fatty liver) or secreted as very low-density lipoproteins, the principal transporter of triacylglycerol, giving rise to the characteristic hypertriglyceridemia of NAFLD. In obesity, these processes are upregulated with increased dietary carbohydrate and fat intake, and adipose tissue insulin resistance, resulting in excessive delivery of NEFAs to the liver [3]. Insulin resistance drives greater hepatic *de novo* lipogenesis further increasing liver fat accumulation [S8]. These metabolic fluxes are under hormonal regulation by insulin and glucagon respectively depending on nutrient availability

***Metabolic impact of excess liver fat accumulation***Application of magnetic resonance (MR) and/or stable isotope-based methodologies have facilitated parallel quantification of liver fat and non-invasive measurement of metabolic fluxes through various hepatic and extra-hepatic metabolic (oxidative, gluconeogenic and lipogenic) pathways [S7, 9].NAFLD refers to excessive fat accumulation in the liver affecting more than 5% of hepatocytes [S2]. During progression from normal liver fat to excess liver fat content (NAFLD), elevated flux through hepatic carbohydrate and lipid biosynthesis pathways leads to excessive systemic glucose and lipid levels, contributing to transition from normal glucose tolerance/preserved metabolic health through to the metabolic syndrome and type 2 diabetes [S10, 11].

**B. Epidemiology of type 2 diabetes and liver disease**

It is estimated that 1 in 11 adults worldwide have diabetes, of which 90% is type 2 diabetes; this represents a more than three-fold increase over the past 20 years largely explained by the obesity epidemic [S12]. Current UK estimates suggest that 36% and 29% of individuals are overweight and obese respectively, with rates having more than doubled since the early 1990s [S13]. The International Diabetes Federation project the number of adults with diabetes worldwide will rise to 700 million by 2045, mostly originating from regions experiencing transitions from low-income to middle-income levels [S12].

Liver disease is the only common cause of death in UK for which mortality rates continue to rise, and within the next two years is expected to overtake coronary heart disease as the leading cause of premature death in the UK, with many affected individuals dying in working age [4]. Reflecting its high global prevalence as a cause of chronic liver disease [1], and the declining prevalence rates of viral hepatitis (with universal hepatitis B vaccination in the UK and new, highly effective anti-viral therapies for hepatitis C virus), NAFLD is expected to become the leading indication for liver transplantation in the next decade [5]. Prevalence rates of NAFLD are highest in low and middle income countries, including India (~50%), South America and the Middle East [S14]. Indeed liver disease, type 2 diabetes and obesity are all linked to health inequalities at both a global and national level. Of significant concern is that paediatric and adolescent populations are increasingly affected by NAFLD; 5-10% of UK children are thought to have NAFLD, although this may be as high as 40% for those who are obese [6]. This has implications for future projections of NAFLD incidence and prevalence.

***Interaction of alcohol with NAFLD*** The epidemiological crisis is exacerbated by synergistic hepatotoxicity of alcohol and obesity. In the UK 49% of individuals drinking over 14 units of alcohol a week (i.e. above the low risk drinking guidelines) were found to be overweight or obese [S15]. Thus alcohol and NAFLD frequently occur concomitantly and this syndrome has been referred to as “Both Alcoholic and non-alcoholic Fatty Liver Disease” (BAFLD) [S16]. Examining the relative risks (RR) of the contributions of body mass index (BMI) and alcohol to liver disease mortality, from two prospective cohort studies, the excess risk due to BMI was small compared to that due to alcohol (RR 1.29 *vs.* 3.66) but the RR due to the combination was greater than the additive effect of the two components separately (RR 9.53) [S15]. National Health and Nutrition Examination Survey data demonstrated that individuals with BAFLD (compared to NAFLD) were significantly more likely to have advanced fibrosis [S16]. Other studies have also highlighted this synergistic risk for advanced liver disease and cancer between metabolic risk factors, obesity and diabetes with alcohol, even at low levels of alcohol intake [S15-18].

**C. Pathophysiology and complications**

**Relationship between type 2 diabetes and NAFLD**

There is a clear bi-directional relationship between type 2 diabetes and NAFLD.

***NAFLD as a risk factor for type 2 diabetes*** Within the population of people with (diagnosed or undiagnosed) NAFLD there is a disproportionately higher prevalence of individuals with type 2 diabetes. A recent meta-analysis of 19 studies shows NAFLD is significantly associated with a two-fold increased risk of incident type 2 diabetes, and this risk increased to 4.7 fold in the presence of advanced liver fibrosis [7]. In studies where NAFLD was confirmed using liver biopsy, nearly 80% of participants with NAFLD had type 2 diabetes at the end of a mean follow up period of 14 years, with the risk being 3-fold greater for individuals with NASH [S3]. Global epidemiology studies recruiting >8.5 million people from 22 countries demonstrate metabolic comorbidities associated with NAFLD in 51% of cases and type 2 diabetes in 23% of cases [1].

The pathophysiology by which NAFLD promotes type 2 diabetes is yet to be fully elucidated, however it is thought to be multi-factorial including ectopic fat accumulation, poorer glycaemic control, low adiponectin levels (protects against insulin resistance, enhances fatty acid oxidation and glucose utilisation), increased pro-inflammatory cytokines, the activation of protein kinases which influence insulin signalling, gut dysbiosis and mitochondrial dysfunction [S19].

***Type 2 diabetes as a risk factor for NAFLD*** Numerous studies have highlighted the very high prevalence of NAFLD in type 2 diabetes (40-70% of individuals with type 2 diabetes) and nearly 70% of individuals who are overweight, or > 90% for those who are obese [8]. In the UK Biobank the prevalence of NAFLD was greater in individuals with type 2 diabetes *vs*. those without type 2 diabetes [S20]. Liver fat in type 2 diabetes, determined quantitatively, is positively correlated with BMI and glycaemic control (HbA1c), with a high prevalence of normal serum alanine aminotransferase (ALT) levels in those with NAFLD [S20-22], meaning hepatic transaminases are not a suitable screening test. The higher liver fat in individuals with type 2 diabetes, *vs.* age- and BMI-matched healthy controls, is explained by higher rates of hepatic *de novo* lipogenesis, driven by higher plasma glucose concentrations and hyperinsulinaemia [S7]. In another global epidemiology study of almost 50,000 individuals with type 2 diabetes from 20 countries, the prevalence of NAFLD was recently estimated to be 56% (68% in Europe), NASH 38% and advanced fibrosis 17% [9].

**Impact of type 2 diabetes on the progression of liver disease** Nearly all causes of liver injury progress through the same pathway from inflammation, to fibrosis (mild/moderate fibrosis ‘F1/F2’, advanced fibrosis, ‘F3/F4’), and cirrhosis. It should be noted however that this is a dynamic and fluid process. Individuals with cirrhosis are at risk of decompensation (the development of a sentinel event including jaundice, ascites, hepatic encephalopathy) which is associated with a 1 year survival of just 60% [S23]. The risk of both liver-related and all-cause mortality increases exponentially with fibrosis stage [10]. Cirrhosis, and advanced fibrosis in the case of some aetiologies of liver disease, can also lead to an increased risk of hepatocellular carcinoma. While prospective studies are lacking, around 23% of individuals with simple steatosis are thought to develop NASH over a 3 year period [11], and this is associated with a 25% risk of progression to cirrhosis over 10 years [S3]. While alcoholic liver disease shares a lot of histological similarities with NAFLD, disease progression is significantly more rapid, with 10% of individuals with steatohepatitis progressing to cirrhosis per year [S24].

The co-existence of type 2 diabetes with NAFLD significantly increases the prevalence of advanced fibrosis, as demonstrated by data from the community-based Rotterdam Study, and more recently in a prospective cohort using magnetic resonance elastography (MRE) to determine liver fibrosis [12–14]. Furthermore three cohort studies, looking at serial liver biopsies in individuals with NAFLD have demonstrated that type 2 diabetes is a significant predictor of fibrosis progression [S25-27]. In a multi-disciplinary clinic of 64 asymptomatic participants with type 2 diabetes, evaluation of NAFLD status using NAFLD fibrosis score (NFS), transient elastography and liver biopsy where relevant, Armstrong *et al.* identified the prevalence of advanced fibrosis to be 5-7% [S28].

**Mortality rates for individuals with liver disease and type 2 diabetes** Type 2 diabetes is associated with a substantial increase in mortality risk for all causes of cirrhosis, although this is also the case for all-cause mortality, cardiovascular, respiratory, gastro-intestinal and genitourinary related deaths, in addition to cancer [S29, 30]. It is estimated that individuals with type 2 diabetes have a 2-3 fold higher risk of dying from chronic liver disease than the general population, with the majority of being attributable to NAFLD [S31]. Diabetes has been identified as an independent predictor of liver-related morality in people with biopsy-proven NASH [S32]. NAFLD is associated with a 2 fold increase in the risk of death for individuals with type 2 diabetes, although larger studies are needed to identify whether this is due to liver disease, cardiovascular disease (CVD), cancer or other causes [15].

**Extra-hepatic manifestations of NAFLD and associated diseases (Fig. 2)**

NAFLD should be considered a multi-system disease, as it carries a number of extra-hepatic manifestations, relating to an increased risk of cardiovascular and chronic kidney disease (CKD), retinopathy in the context of diabetes and an increased risk of extra-hepatic cancers [16]. These extra-hepatic manifestations of NAFLD commonly share insulin resistance and many other traditional and non-traditional risk factors, but the association is evident in many studies regardless of the confounding effects of obesity, hypertension or type 2 diabetes.

***Cardiovascular disease*** Although NAFLDis very clearly associated with CVD (reviewed in [S33]), there remains debate regarding whether this is independent of the confounding effects of shared cardiovascular risk factors and metabolic abnormalities. In some studies NAFLD is an independent risk factor for major cardiovascular events [17], with prospective data from a 5 year follow up study estimating that NAFLD was significantly associated with an increased risk of CVD (non-fatal coronary heart disease, ischemic stroke, cardiovascular death) in individuals with type 2 diabetes independent of diabetes duration and HbA1c, even following additional adjustment for metabolic risk factors [S34]. Multiple pathophysiological mechanisms contribute to this CVD risk including systemic inflammation, endothelial dysfunction (an early defect), hepatic insulin resistance, oxidative stress and lipid abnormalities [S33]. The spectrum of CVD extends beyond atherosclerotic CVD but also involves an increased risk of cardiomyopathy (mainly left ventricular diastolic dysfunction and hypertrophy, leading to congestive heart failure), cardiac valvular calcification (mainly aortic-valve sclerosis), cardiac arrhythmias (mainly atrial fibrillation) and some cardiac conduction defects [S35].

***Microvascular complications*** In terms of micro-vascular complications, a prospective cohort study (Valpolicella Diabetes Heart Study) followed 1,760 outpatients with type 2 diabetes (and normal/near-normal renal function and without proteinuria) over 6.5 years and demonstrated that the presence and severity of NAFLD was independently associated with CKD incidence in this cohort (hazard ratio, HR 1.49; CI 95% 1.1-2.2) [S36]. Further cross-sectional data from 2,103 people with type 2 diabetes demonstrated a higher age- and sex-adjusted prevalence of non-proliferative and proliferative retinopathy and CKD in those with NAFLD, independent of confounders [18]. A subsequent meta-analysis revealed an association between NAFLD and incidence and prevalence of CKD in a population of individuals with and without diabetes [S37]. Furthermore there was a higher prevalence of CKD in people with NASH *vs.* simple steatosis and those with advanced fibrosis *vs.* those with non-advanced fibrosis. Of clinical relevance, a liver stiffness measurement (LSM) of ≥ 7.0/6.2 kPa (medium / extra-large probes) determined by FibroScan® is independently associated with prior CVD (odds ratio, OR 3.3), microvascular complications (OR 4.2), CKD (OR 3.6) and retinopathy (OR 3.7) [S38]. This convincing relationship between NAFLD and CKD has been recently reviewed [S39]. Emerging evidence suggests that a polymorphism of the PNPLA3 gene, encoding an Ile148Met change (a major common genetic variant associated with a greater predisposition to NASH and progressive liver fibrosis) is significantly associated with decreased estimated glomerular filtration rate values, irrespective of established renal risk factors and the presence of NAFLD [S40].

***Extra-hepatic cancers*** In addition to obesity, NAFLD and type 2 diabetes have both been linked to an increased risk of cancer [19]. In the case of NAFLD the strongest evidence is for malignancies involving the gastrointestinal tract [20]. In the largest longitudinal cohort studies to date linking NAFLD to extra-hepatic cancer incidence, a sensitivity analysis adjusting for diabetes attenuated the association, but did not change the overall findings [20]. Within a cohort of individuals with type 2 diabetes, NAFLD was found to be an independent predictor of cancer deaths [15]. It is not known whether there is any synergism between diabetes and NAFLD in terms of cancer incidence, nor whether NAFLD is a mediator of causal pathways or merely a predictor of cancer risk.

**Other conditionsassociated with NAFLD** There is a recognised association between NAFLD and other endocrine conditions, which are at least partly mediated by obesity, including hypothyroidism, polycystic ovary syndrome and growth hormone deficiency [S41]. There is also a link with obstructive sleep apnoea [S42] and psoriasis [S43].

**D. Screening**

We have suggested an algorithm based on the current guidelines and latest evidence for the assessment and management of NAFLD in people with type 2 diabetes (**Fig. 3**). Liver disease is frequently clinically and biochemically silent until there is end-stage liver disease. It is estimated that 75% of individuals with liver disease present for the first time with an emergency hospital admission [4]. Furthermore NASH has been proven on biopsy in up to 20% of asymptomatic individuals with type 2 diabetes and normal liver function tests [S21]. For clinical practice, the degree of liver fibrosis is the main prognostic factor as this correlates with the risk of cirrhosis and liver-related and CVD-related mortality in people with NAFLD. Thus screening is predominantly aimed at evaluating the likely extent of fibrosis.

The diagnosis of NAFLD is made after exclusion of other causes including alcohol and after hepatic steatosis is confirmed by non-invasive methods. Althoughbiochemical indices may be used to detect steatosis, most commonly the fatty liver index, their use is predominantly reserved for epidemiological studies rather than routine clinical practice [21]. Hepatic steatosis is detected by liver ultrasound, which is widely available and offers high levels of sensitivity and specificity (85% and 94% respectively); the optimum sensitivity for ultrasound is achieved at a liver fat content of 12.5% (cut-off point using magnetic resonance (MR) spectroscopy is 5.5%).

**Non-invasive tests** There is much interest in reliable, non-invasive tests for the diagnosis of advanced fibrosis. Clinical parameters such as age, BMI, liver transaminases, platelet count and glycaemic status are used to generate these scores. These include the Enhanced Liver Fibrosis (ELF) test, NAFLD fibrosis score (NFS), fibrosis-4 index (FIB-4) and Aspartate Aminotransferase (AST) to Platelet Ratio Index (APRI) score (**Table 1**). They all have their own specific cut-off points, above and below which fibrosis needs to be further evaluated or can be excluded. However, all of the non-invasive tests have only a modest positive predictive value for fibrosis but a much higher negative predictive value, i.e*.* they perform better at excluding advanced fibrosis than diagnosing it. Their utility is also limited for intermediate stages of fibrosis. Thus their role is only as part of a staged diagnostic approach to exclude people who are unlikely to have advanced fibrosis and to identify those individuals who necessitate more detailed evaluation of potential fibroinflammatory changes with imaging-based approaches plus/minus liver biopsy [S44].

The choice of non-invasive test will often be dictated by local availability [S44]. If available, the ELF test is recommended by NICE [22], but is expensive and not universally available; if this is not available another non-invasive test may be employed such as the NFS or FIB-4 score, as recommended in other guidelines [23]. These scores need further validation in populations with type 2 diabetes based on the significant variability of the percentage of people with advanced fibrosis depending on which of these scores were used [24,25]. This was demonstrated in a cohort of 121,513 individuals with type 2 diabetes in whom the prevalence of hepatic steatosis and advanced fibrosis was determined using non-invasive tests, and was further highlighted in another study by Bril *et al.* involving a smaller cohort of people with type 2 diabetes who underwent a more comprehensive assessment with plasma biomarkers, diagnostic panels and liver biopsy [24,26]. These observations have significant implications for which (and how many) individuals undergo more detailed diagnostic evaluation.

**Imaging**

***Transient elastography*** Liver fibrosis can be staged using transient elastography (FibroScan®, Echosens, Paris, France) which measures liver stiffness by measuring the velocity of a low-frequency (50 Hz) elastic shear wave propagated through the liver. FibroScan is the most widely used and validated technique. This provides an LSM which can be combined with the controlled attenuation-parameters (CAP) to detect steatosis. Recently, there has been interest in combining the FibroScan derived LSM and CAP with serum transaminases to give a FibroScan-AST (FAST) score to identify those individuals with NASH, an elevated NAFLD Activity Score (NAS) and significant fibrosis [27]. MRE has also been applied successfully and can predict liver fibrosis [28].

***MRI methods*** A variety of other MR-based approaches are available. MRI assessment of liver fat using the proton density fact fraction (PDFF) [S20] is recommended in the AASLD guidelines; or Liver*MultiScan* facilitates liver tissue characterisation to quantify liver fat (using enhanced MRI-PDFF) and inflammation and fibrosis corrected for hepatic iron (cT1) [S45, 46]. This non-invasive technique has been validated against liver biopsy [S47] and is predictive of liver-related outcomes [S48]. It is now increasingly being adopted in NASH clinical trials due to low measurement failure rates, high repeatability and reproducibility (**Fig. 4**) [29,30].

**Liver biopsy** Liver biopsy remains the gold standard method for diagnosis. It is used to establish whether there is NASH, to identify stage of fibrosis or to differentiate NAFLD from other aetiologies of chronic liver disease. Limitations must also be acknowledged including cost, risk of complications (bleeding, death), sampling error (with only 1/50,000 of the whole liver tissue sampled and so potential for significant heterogeneity in the degree/stage of fibrosis of the biopsy specimen), as well as variability in interpretation [S49].

**Screening approaches** The issue of screening, and the algorithm for doing so to assess for NAFLD in individuals with obesity, metabolic syndrome and type 2 diabetes is contentious with no universal accepted approach. National and international guidelines (EASD/EASO/EASL, NICE and AASLD) are not concordant (**Table 2**) [22,23,31]. The American Diabetes Association recommends that people with type 2 diabetes/prediabetes with elevated liver enzymes or fatty liver on ultrasound should be evaluated for presence of NASH and liver fibrosis [S50].

**E. Treatment considerations**

Thus, evidence to date suggests a high burden of unrecognised NAFLD/fibrosis/cirrhosis in type 2 diabetes. This may be improved with appropriate weight loss, glycaemic improvement and appropriate glucose lowering therapies.

**Impact of lifestyle intervention including dietary and physical activity interventions**

***Weight loss*** Currently the optimal management option for NAFLD is weight loss. Three international guidelines recommend a goal of 7-10% loss of total body weight to be achieved through a structured programme of a healthy diet and physical activity [22,23,31]. These recommendations are supported by data from a prospective study of nearly 300 participants with biopsy proven NASH who were asked to consume a low fat hypocaloric diet (750 kcal/day less than their daily every requirements) and exercise (walk 200 minutes per week) [32]. After 52 weeks, 47% were reported to have reductions in their NAFLD activity score, 25% achieved complete resolution of steatohepatitis and 19% experienced fibrosis regression. While the greatest benefits were seen for individuals losing ≥ 10% body weight, favourable outcomes were also seen for weight loss of ≥ 5%.

***Exercise*** Exercise, even in the absence of weight loss has been shown to be effective at reducing liver fat [33,34]. A randomised study has shown that supervised exercise programmes for 16 weeks (starting at 30 min of moderate aerobic exercise 3 times weekly) can lead to improvements in peripheral insulin resistance and reductions in liver fat in comparison to counselling [33]. Similarly, high-intensity interval training has also been shown to lead to reductions in liver fat, whole body fat mass and aminotransferases over a 12 week period [S51]. Significantly data from two large prospective cohort studies from the United States (US) recently demonstrated that liver-related mortality declines with increasing levels of physical activity across all levels of BMI, and that just 3 hours of average paced walking a week could substantially reduce the excess liver mortality risk associated with obesity [S52]**.**

**Effects of glucose lowering therapies on NAFLD**

Many studies have addressed the impact of glucose lowering drugs in individuals with NAFLD or NASH, both with and without type 2 diabetes, examining a variety of outcomes of interest including changes in serum liver enzyme levels, liver fat, liver fibrosis and histologic resolution of NASH. The best evidence relates to thiazolidinediones, in particular pioglitazone (**Supplementary Table 1**), which has been well studied in NAFLD. Unpublished and published evidence suggests that newer type 2 diabetes drugs can reduce steatosis and potentially reverse NAFLD [35–38].

A recent systematic review was conducted by Mantovani *et al.,* examining the safety and efficacy of all glucose lowering drugs in individuals with NAFLD or NASH, both with and without type 2 diabetes, incorporating 29 RCTs and involving 2,617 people [39]. The authors concluded that although most anti-hyperglycaemic drugs improved serum liver enzyme levels and may lower liver fat, only thiazolidinediones (especially pioglitazone) and liraglutide could reverse/improve the histologic features of NAFLD, with a mild beneficial effect with pioglitazone on liver fibrosis. The important studies are described in detail below for each drug class.

***Thiazolidinediones*** The PIVENS trial compared low dose pioglitazone (30mg daily), vitamin E and placebo in participants without type 2 diabetes over 2 years. Pioglitazone was found to lead to reductions in liver enzymes, hepatic steatosis and lobular inflammation, however there was no improvement in hepatic fibrosis [40]. Smaller trials have followed reporting similar findings (**Supplementary Table 1**). An extension trial looking at prolonged (> 1 year) treatment with rosiglitazone showed that while a substantial anti-steatogenic effect was seen in the first year, no further benefit occurred after this time, with no significant change in NAS, ballooning or fibrosis. While there is some concern regarding its safety and tolerability profile, pioglitazone is currently recommended by EASL in selected individuals with NAFLD and type 2 diabetes [23].

***Glucagon like peptide-1 receptor agonists (GLP1-RA) (Supplementary Table 2)*** Both exenatide and liraglutide can reduce intrahepatic lipid content in obese individuals with type 2 diabetes, correlating with levels of HbA1c reduction [S53]. Phase 2 trial data have also shown that liraglutide can lead to resolution of NASH and reduced incidence of fibrosis progression in obese individuals over a 48 week period, and was well tolerated in this setting [37]. A phase 2 trial (NCT02970942) examining the effects of subcutaneous semaglutide *vs.* placebo in approximately 370 participants with NASH is expected to publish results mid-2020.

***Sodium-Glucose Transport Protein 2 (SGLT2) inhibitors (Supplementary Table 3)***Recent findings from both randomized controlled trials (RCT) and open-label studies have shown that SGLT2 inhibitors can improve biological markers of NAFLD, especially serum liver enzymes, in individuals with type 2 diabetes and reduce liver fat content, as assessed by different imaging techniques. The underlying mechanisms mediating this reduction involves both reductions in body weight and hyperglycaemia.

*Impact of SGLT2 inhibitors on liver biochemistry* Several pilot studies with SGLT2 inhibitors reported a significant reduction in ALT levels, body weight and the fatty liver index in individuals with NAFLD.Data from EMPA-REG demonstrate that empagliflozin reduces aminotransferases in individuals with type 2 diabetes, in a pattern (ALT >AST reduction) that is potentially consistent with a reduction in liver fat, especially when ALT levels are high. ALT reductions were largely independent of changes in weight or HbA1c [41].

*Impact of SGLT2 inhibitors on NAFLD/NASH* In the E-LIFT study, the addition of empagliflozin to standard diabetes therapy led to a greater reduction in liver fat and ALT levels [38]*.* In the EFFECT II study the effects of dapagliflozin and omega-3 carboxylic (OM-3CA) acids were studied in individuals with type 2 diabetes and NAFLD in a double-blind randomised placebo-controlled trial [42]. Dapagliflozin led to a reduction in liver fat compared to placebo, but only when given in combination with OM-3CA, and also led to a reduction in markers of hepatocyte injury, including transaminases.

***Metformin*** While animal studies suggest metformin could be an effective therapeutic agent for NAFLD as a result of its ability to reduce hepatic fat content, RCT data have been less convincing; critically metformin has not been shown to lead to histological improvement in individuals with NASH [S56]. It is therefore not recommended by either EASL or AASLD in the management of NAFLD.

***Dipeptidyl-peptidase-4 (DPP-4) inhibitors*** Most RCTs with a DPP-4 inhibitor have used sitagliptin, and largely they have not demonstrated any benefits in the treatment of NAFLD/NASH [S55, 56]. In a small trial of 44 participants with type 2 diabetes over 6 months, vildagliptin lead to a clinically significant reduction in hepatic triglyceride levels independent of weight loss, however biopsy data are needed to prove if it can lead to resolution of NASH [S57].

***Combination therapies*** Combination of agents with proven efficacy in NAFLD is an appealing concept. In a substudy of a 52-week RCT, the SGLT2 inhibitor, dapagliflozin, co-administered with the DPP-4 inhibitor, saxagliptin significantly decreased liver fat, liver enzymes and body weight *vs*. the sulfonylurea glimepiride, with both groups also taking metformin [43]. The direct contribution of each drug in the dapagliflozin plus saxagliptin arm towards liver fat is unclear, but given that previous studies with a DPP-4 inhibitor have mostly demonstrated negative findings, would suggest that the beneficial effects on liver fat were a result of dapagliflozin.

Post-hoc analysis of the RCT DURATION-8, combining dapagliflozin and exenatide once weekly in participants with type 2 diabetes, suggests benefits in the treatment of NAFLD [S58]. Greater improvements in liver enzymes and biomarkers of steatosis were seen with combination therapy compared to monotherapy at week 28. Prospective studies examining improvements in liver enzymes, steatosis and its translation to liver histology with this combination are warranted.

**Vitamin E** Vitamin E has shown some benefit for treating NASH in people without type 2 diabetes [40]. A biopsy-based, double-blind study of 105 individuals with NASH and type 2 diabetes was conducted to assess the efficacy and safety of vitamin E alone or in combination with pioglitazone [44]. The primary endpoint was reduction in the NAFLD activity score of ≥2 points without worsening of fibrosis. The combination of pioglitazone plus vitamin E demonstrated significant improvement in NASH, but this effect was similar to that observed with pioglitazone monotherapy in this population, and vitamin E alone was ineffective. Based on these data, together with some safety concerns, vitamin E is not routinely recommended in this population.

**F. Potential treatment options for type 2 diabetes in individuals with cirrhosis**

In general, all glucose lowering medications are considered safe to prescribe for individuals with liver disease. Metformin is not considered hepatotoxic and can be safely prescribed in persons with mildly raised transaminase levels. Indeed it has been found to improve survival among individuals with diabetes at all stages of cirrhosis [S59]. Metformin can cause a metabolic acidosis in some individuals with hypoxaemia, and therefore should be avoided in cases of hepatic decompensation, particularly hepatic encephalopathy [S60].

Thiazolidinediones and SGLT2 inhibitors are thought to be safe in all stages of cirrhosis, however in the case of dapagliflozin, manufacturers suggest starting at a lower dose in severe hepatic impairment where there are a lack of clinical trial data available [S61]. In terms of GLP1-RAs, liraglutide is cautioned in individuals with an impairment in liver function because of the risk of decreased exposure to the drug, however this is not an issue with exenatide.

**G. Concluding comments**

Type 2 diabetes and liver disease are intrinsically linked. NAFLD is at the forefront of this; up to 70% of individuals with type 2 diabetes are thought to have NAFLD, and these individuals experience an accelerated course in terms of liver disease progression (over 15% may have advanced fibrosis), with consequences for mortality. NAFLD is both a risk factor for, and complication of, components of the metabolic syndrome and can accelerate progression towards micro and macro-vascular complications of diabetes. Its prevalence continues to rise in line with both the type 2 diabetes and obesity epidemics and it is predicted to become the leading indication for liver transplantation within the next decade; it will therefore be increasingly clinically encountered as an important co-morbidity. Detecting liver fibrosis (ideally non-invasively) is key to determining prognosis and the need for hepatology input. Shared lifestyle and therapeutic interventions present huge opportunities for cross-talk between specialities and emphasise the importance of multi-disciplinary approaches.

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**Table 1** Comparison of recommendations for screening via biochemical indices or imaging based approaches for NAFLD (steatosis and fibrosis)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Score name | Variables included | Pros/cons | Cut-off values | Evidence | Management plan |
| Steatosis | | | | | |
| Fatty Liver Index (FLI) | BMI, waist circumference, GGT, triglycerides | * Predict liver fat with moderate accuracy * Does not reflect changes in liver fat when altered by low carbohydrate/fat diet | * > 60 high risk * 30-60 indeterminate * < 30 low risk |  | * USS followed by assessment for fibrosis for indeterminate/high risk scores |
| NAFLD Liver Fat Score | Metabolic syndrome, type 2 diabetes, fasting serum insulin, AST, ALT | * Predict liver fat with moderate accuracy, does not reflect changes in liver fat when altered by low carbohydrate diet (moderate precision with low fat diet) | * > -0.640 positive |  | * USS followed by assessment for fibrosis if positive |
| MRI-PDFF | Ratio, expressed as a percentage, of the fraction of the MRI-visible protons attributable to fat divided by all MRI-visible protons in that region of the liver attributable to fat and water | * Measures liver fat with high accuracy without requiring expertise of 1H MR spectroscopy * Included in AASLD guidelines for steatosis assessment for NAFLD | * > 5.5% diagnostic of NAFLD | * Correlation to biopsy-based steatosis grades [S62] * General population studies [S20] | * Requires referral to hepatology for assessment |
| Fibrosis | | | | | |
| Enhanced Liver Fibrosis (ELF) | Serum hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) | * Recommended by NICE * Expensive * Not processed in all laboratories | * ELF ≥ 10.51 suggests F3/F4 * ELF < 10.51 excludes advanced fibrosis | * AUC 0.90 for distinguishing severe fibrosis, 0.82 for moderate fibrosis, 0.76 for no fibrosis [45] | * Repeat test in 3 years (adult) / 2 years (child) (NICE) * Hepatology review / fibroscan if ≥ 10.51 |
| Fibrosis-4 Index (FIB-4) | Age, AST, platelets, ALT | * Recommended by EASL-EASD-EASO * Originally for Hepatitis C +/- HIV co-infection * Validated for Hepatitis B/C & NAFLD/NASH. | * FIB-4 ≥ 3.25 suggests F3/F4 * FIB-4 ≤ 1.3 (≤ 65 years) / ≤ 2.0 (> 65 years) to exclude F3/F4 * Not accurate if < 35 years | * ≥ 3.25: 26% sensitivity, 98% specificity, 75% PPV, 85% NPV for F3/F4 * ≥ 1.3: 85% sensitivity, 65% specificity, 36% PPV, 95% NPV for F3/F4 [46] | * Hepatology review / fibroscan if ≥ 3.25 * Fibroscan/ARFI/ELF if intermediate result |
| NAFLD Fibrosis Score (NFS) | Age, BMI, hyperglycaemia, platelet, albumin, AST, ALT | * Longer consultation time as need to check height and weight to calculate BMI | * NFS ≥ 0.676 suggests F3/F4 * NFS < -1.455 (≤ 65 years) / < 1.2 (> 65 years) to exclude F3/F4 (90% NPV) * Not accurate if < 35y | * ≥ 0.676: PPV 90% for F3/F4 * < -1.455: NPV 93% for F3/F4 [47] | * Hepatology review / fibroscan if ≥ 0.676 * Fibroscan/ARFI/ELF if intermediate result |
| AST to Platelet Ratio Index (APRI) | AST, platelets | * Originally for Hepatitis C +/- HIV co-infection. * Lower performance; recommended to perform another score | * ≥ 1.5 suggests F3/F4 * < 0.5 excludes significant fibrosis * Intermediate results inaccurate | * ≥ 1.5: PPV 56% for F3/F4 * < 0.5: NPV 90% for F3/F4 [48] | * Hepatology review / fibroscan if ≥ 1.5 * Alternative non-invasive marker if intermediate result |
| Transient Elastography (e.g. FibroScan®) | Shear wave velocity measurement using ultrasound probe. Velocity measured in metres/second which is then converted into “liver stiffness” expressed in kilopascals (kPa) | * Avoids sampling error; liver biopsy examines multiple liver sections * Non-invasive and quick bedside test * Cheaper than liver biopsy * Cannot be performed in individuals with ascites, pregnancy or pacemaker * Unreliable in morbidly obese/those with small rib spaces * Overestimates fibrosis in: mass lesions (e.g. tumours), hepatic congestions (e.g. heart failure), liver inflammation (e.g. active hepatitis), cholestasis (e.g. biliary obstruction) | * ≥ 7.9 kPa suggests F3/F4 * A valid result requires 8-10 measurements with a 60% success rate and an interquartile range <0.3 | * ≥ 7.9 kPa: 91% sensitivity, 75% specificity, 52% PPV, 97% NPV 97% for F3/4 [S63] * Results range from 2.5kPa-75kPa * Validation studies comparing with liver biopsy as the “gold standard” suggest an optimal cut-off for cirrhosis at 14kPa [49,50] | * Hepatology review if ≥ 7.9 kPa * Due to the low PPV of TE, a biopsy is needed to definitively diagnose cirrhosis and is required for the approval of drugs & diagnostic modalities |
| FibroScan-AST (FAST) Score | Transient elastography (e.g. FibroScan®), AST | * Algorithm developed to identify participants who could be recruited to clinical trials and for optimal prescription of therapies without having to undergo liver biopsy | * Rule-in zone ≥ 0.67 * Grey zone 0.35-0.67 * Rule-out zone ≤ 0.35 | * ≥ 0.67: ≥ 90% specificity, 0.85 NPV * >0.35: ≥ 90% sensitivity, 0.83 PPV * Inclusion of other MetS parameters in the score did not improve performance [27] | * Used in secondary care to identify individuals most likely to benefit from treatment or to be included in clinical trials * For those in “grey zone” repeat score in 2-3 months/proceed to liver biopsy |
| Corrected T1 (cT1) | MRI parameter corrected for iron content that can quantify extracellular water content or interstitial space, which rises with fibrosis and inflammation | * Correlates with ballooning and histological features of NASH * Non-invasive detection of disease feature change in clinical trials * Standardised across scanners in clinical use * Less accurate at stratification of high fibrosis | PDFF   * ≥ 5.6% suggests NAFLD   cT1   * >825 ms predicts liver-related events * 795-825 ms grey zone * 794 ms upper limit of the reference range | * Predicts event-free survival with HR 10.6 (95% CI: 1.40-81.3, p=0.004) * Predicts all-cause mortality with HR 7.74 (95% CI: 0.98-61.2, p=0.02) | * Requires referral to hepatology for assessment |

BMI: body mass index; GGT, gamma-glutamyl transferase; USS, ultrasound scan; NAFLD, non-alcoholic fatty liver disease; AST, aspartate transaminase; ALT, alanine aminotransferase; NICE national institute for health and care excellence; F3/F4, fibrosis stages 3 or 4, i.e. advanced fibrosis; AUC, area under the receiver operator curve; EASL, european association for the study of the liver; EASD, european association for the study of diabetes; EASO, european association for the study of obesity; HIV, human immunodeficiency virus; NASH, non-alcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value; ARFI, Acoustic Radiation Force Impulse; MetS, metabolic syndrome; MRI, magnetic resonance imaging; PDFF, proton density fat fraction; HR, hazard ratio

**Table 2** Summary of guideline recommendations for investigation and management of NAFLD

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Guidelines | Who should undergo screening? | Investigations | Recommended treatment | |
| EASL/EASD/  EASO 2015 [23] | * Individuals with **insulin resistance and/or metabolic risk factors** (obesity or MetS) (regardless of liver biochemistry) * Those with persistently elevated liver enzymes (NAFLD is the most common cause) * Individuals with steatosis should be screened for MetS and other secondary causes of NAFLD, including assessment of alcohol intake * Case finding of advanced disease (i.e. NASH with fibrosis) in high risk individuals (age>50 years, type 2 diabetes, MetS) | *Non-invasive techniques:*  **Abdominal ultrasound**   * First line diagnostic test: limited sensitivity when steatosis <20% or in individuals with BMI>40 kg/m2 * USS also provides additional hepatobiliary information   **Serum biomarkers and scores of steatosis**   * If imaging unavailable or for large epidemiological studies, biomarkers and scores acceptable * **Recommended scoring systems:** fatty liver index, Steatotest® and NAFLD liver fat score (not for more advanced stages of NAFLD)   **Serum biomarkers and scores of fibrosis/transient elastography**   * Useful for identification of cases at low risk of advanced fibrosis/cirrhosis * If imaging or scores confirm high risk of fibrosis a liver biopsy should be performed for confirmation of advanced fibrosis/cirrhosis/NASH * Genotyping and **1H-MRS** (quantitative assessment of liver fat): for clinical trials and experimental studies | **Lifestyle**   * 7-10% weight loss * Exercise: aerobic and resistance training decrease liver fat; choice according to individual preference/functional ability   **Medical**   * Statins can confidently be used to lower LDL cholesterol and CV risk with no benefit or harm on liver disease * Drugs reserved for NASH – F2 or higher. Pioglitazone and/or vitamin E could be used for NASH * Bariatric surgery improves type 2 diabetes and obesity and is likely to reduce NASH progression   **Monitoring**   * For those with increased ALT at baseline, liver transaminases should be assessed at 6 months to assess for improvement * Follow up is mandatory in obesity, and in lean people with NAFLD due to potential disease progression * If an individual is at high risk of disease progression (e.g. ongoing risk factors such as type 2 diabetes) a repeat biopsy should be performed after 5 years |
| NICE 2016 [22] | * Individuals with **type 2 diabetes** or **metabolic syndrome** * Take a detailed alcohol history * Do not use routine blood tests to rule out NAFLD | * For those with fatty liver on USS offer testing for advanced fibrosis using ELF test * Repeat ELF test every 3 years in adults/2 years in children * Refer those with positive ELF test to specialist | * Lifestyle advice (physical activity and diet) regardless of BMI * Recommend alcohol within national limits * Continue statins unless liver enzymes double within 3 months of starting * Consider pioglitazone or vitamin E whether they have diabetes or not (unless contraindications – heart failure, bladder cancer or uninvestigated macroscopic haematuria) * Insufficient evidence for omega 3   **Monitoring:** Consider using ELF test to assess response to pharmacological therapy. If ELF increases, stop treatment and consider other agents. |
| AASLD 2018 [31] | * Routine screening not recommended in high-risk populations * High index of suspicion in individuals with type 2 diabetes: clinical decision aids such as NFS or FIB-4 or transient elastography can be used to identify those at low or high risk * Screen for other liver disease and co-existing disease such as obesity, IR or diabetes, PCOS, sleep apnoea or hypothyroidism |  | **Recommended treatments if biopsy proven NASH:**   * Exercise and weight loss of 7-10% * Pioglitazone (with or without type 2 diabetes) * Vitamin E (individuals without diabetes only)   **Not recommended:**   * Metformin or GLP-1 receptor agonists (insufficient evidence) * Bariatric surgery (although it can be considered in otherwise eligible individuals) |

EASL, european association for the study of the liver; EASD, european association for the study of diabetes; EASO, european association for the study of obesity; MetS, metabolic syndrome;NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; BMI, body mass index; USS, ultrasound scan; 1H-MRS, proton magnetic resonance spectroscopy; LDL, low density lipoprotein; CV, cardiovasular; ALT, alanine aminotransferase; NICE, national institute for health and care excellence; ELF, enhanced liver fibrosis score; AASLD, american association for the study of liver diseases; NFS, non-alcoholic fatty liver disease fibrosis score; FIB-4, fibrosis-4 index; IR, insulin resistance; PCOS, polycystic ovary syndrome; GLP-1, glucoagon-like peptide-1

**Figure Legends**

**Fig. 1: The pathophysiology of NAFLD and its relationship to diabetes**

NEFA, non-esterified fatty acids; VLDL, very low density lipoprotein; DNL, *de novo* lipogenesis; FACoA, fatty acyl co-enzyme A synthetase; TG, triglycerides; IHTG, intra-hepatic triglycerides; SREBP-1, sterol regulatory element-binding protein 1; ChREBP-1, carbohydrate-responsive element-binding protein; CM, chylomicron.



**Fig. 2. A summary of the extra-hepatic manifestations of NAFLD**



**Fig. 3. Algorithm for the identification and risk stratification of NAFLD for individuals with type 2 diabetes**



NAFLD, non-alcoholic fatty liver disease; MetS, metabolic syndrome; ARFI, acoustic radiation force impulse; NASH, non-alcoholic steatohepatitis; T2D, type 2 diabetes; GLP1-RA, glucagon-like peptide-1 receptor agonists; SGLT2i, sodium-glucose cotransporter 2 inhibitor; USS, ultrasound scan; UGI, upper gastrointestinal endoscopy

**Fig. 4. LiverMultiScan showing reduction in cT1 and PDFF values before and after bariatric surgery.**

Normal values cT1 <794 ms (>825 ms predicts liver-related events); PDFF <5.5%.

cT1, corrected T1; PDFF, proton density fat fraction

**Supplementary Material**

**Supplementary Table 1** Randomised controlled trials with pioglitazone in individuals with and without type 2 diabetes and biopsy-proven NASH

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reference | Population | Intervention | Comparator | Outcome (Baseline/End of study for drug *vs* control) | | | | |
|  |  |  |  | **Body weight (kg)** | **HbA1c (%)** | **ALT (u/L)** | **Liver fat (%)** | **Liver Histology** |
| Bril *et al* (2019) [44] | Type 2 diabetes + NASH | Pioglitazone 45mg od + vitamin E 400 IU bd  18 months (n=37) | Vitamin E 400 IU bd (n=36) | -/+5.7(5.4)  *vs* -/0.5(5.6)  (p<0.05) | 7.5(1.3) *vs*  7.2(1.2)  (p<0.05) | 52(32) *vs*  53(33)  (p<0.05) | 10% reduction  *vs* 6%  (1H-MRS)  (p<0.05) | Steatosis improvement 87% *vs* 68%  (p<0.05)  NASH resolution 54% *vs* 17% (p<0.05)  Fibrosis improvement 52% *vs* 30%  (p=ns) |
| Cusi *et al* (2016) [S64] | Prediabetes or type 2 diabetes + NASH | Pioglitazone 45mg od 18 months  (n=51) | Placebo  (n=50) | 98.2(16.5)/99.4(16.6) *vs* 99.2 (17)/99.5(16.7)  (p<0.05) | 5.7(0.5)/5.6(0.3) *vs* 5.7(0.5)/5.8(0.3) prediabetes (p=ns)  7.1(0.9)/6.2(0.7) *vs* 6.8 (1.0)/6.5(0.7) type 2 diabetes (p<0.05) | 62(33)/27(12*) vs*  57(33)/44(33*)*  (p<0.05) | 19 to 7%  *vs* 15 to 11%  (1H-MRS)  (p<0.05) | Steatosis improvement 71% *vs* 26%  NASH resolution 51% *vs* 19% (p<0.05)  Fibrosis improvement 20% *vs* 13% (p=ns) |
| Sanyal *et al* (2010) [40] | NASH | Pioglitazone 30mg od 96 weeks (n=80) | Placebo (n=83) | 97(23)/+4.7  *vs* 99(21)/+0.7  (p<0.05) | - | 82(45)/-40.8 *vs* 81(48)/-20.1  (p<0.05) | - | Steatosis improvement 69% *vs* 31% (p<0.05)  NASH resolution 47% *vs* 21% (p<0.05)  Fibrosis improvement 44% *vs* 31% (p=ns) |
| Aithal *et al* (2008) [S65] | NASH | Pioglitazone 30mg od 12 months (n=37) | Placebo  (n=37) | 88.6(10.7)/91.2(12.6) *vs*  92.8(21.1)/89.3(18.6)  (p<0.05) | 5.8(0.6)/5.6(0.4)  *vs* 5.9(1.0)/6.0(1.2)  (p<0.05) | 93.6(61.3)/55.9(25.7) *vs* 84.1(37.7)/77.2(43.0) (p<0.05) | - | Steatosis improvement 48% *vs* 37%  Fibrosis improvement 29% *vs* 20% |
| Belfort *et al* (2006) [S66] | IGT or type 2 diabetes + NASH | Pioglitazone 45mg od  6 months  (n=26) | Placebo  (n=21) | 93.7(18.1)/90.2 (15.4) *vs*  96.2(19.6)/89.7 (14.8) (p<0.05) | 6.2(1.5)/5.5(0.8) v*s* 6.2(1.1)/6.1(0.9)  (p<0.05) | 67(26)/28(12)  *vs* 61(33)/40(17)  (p<0.05) | 54% reduction *vs* 0%  (1H-MRS)  (p<0.05) | Steatosis improvement 65% *vs* 38% (p<0.05)  Fibrosis improvement 46% *vs* 33% (p=ns) |

ALT, alanine aminotransferase; IGT, impaired glucose tolerance;; NASH, non-alcoholic steatohepatitis; od, once daily; 1H-MRS, proton magnetic resonance spectroscopy; IU, international units

**Supplementary Table 2** Studies evaluating impact of GLP1 receptor agonists in individuals with type 2 diabetes and NAFLD

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reference | Population | Intervention | Comparator | Outcome ( Δ or Baseline/End of study for drug *vs* control) | | | | |
|  |  |  |  | **Body weight (kg)**  **BMI (kg/m2)** | **HbA1c (mmol/mol)** | **ALT (IU/L)** | **Liver fat (%)** | **Liver Histology** |
| Armstrong *et al* (2016) [37] | Overweight + NASH (Biopsy) | Liraglutide 1.8mg for 48 weeks (n=23) | Placebo (P) (n=22) | Body weight: Δ  -5.3 (L) *vs* -0.6 *(P)*  (L *vs* P, p<0.05)  BMI:  Δ -1.8 *vs* -0.3 (P)  (L *vs* P, p<0.05) | Δ -5.7 (L) *vs* 0.0 (P)  (L *vs* P, p<0.05) | Δ -26.6 (L) *v*s -10.2 (P)  (L *vs* P, p=ns) | - | Steatosis improvement 83% (L) *vs* 45% (P)  (L *vs* P, p<0.05)  NASH resolution  39% (L) *vs* 9% (P)  (L *vs* P, p<0.05)  Worsening fibrosis  9% (L) *vs* 36% (P)  (L *vs* P, p<0.05) |
| Cuthbertson *et al* (2012) [35] | Type 2 diabetes + NAFLD (USS) | Exenatide (E) (n=19) or Liraglutide (L) 1.2mg (n=6) 28 weeks | None | Body weight: 116.7111.3 (p<0.05)  BMI:  38.4/36.7 (p<0.05) | 9.6/7.5 (p<0.05) | 40/31 (p<0.05) | 28/21 (1H-MRS)  (p<0.05) | - |

GLP1, glucagon-like peptide receptor 1; ALT, alanine aminotransferase; BMI, body mass index; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; USS, ultrasound scan; 1H-MRS, proton magnetic resonance spectroscopy

**Supplementary Table 3** Randomised controlled trials with SGLT2 inhibitors in individuals with and without type 2 diabetes and NAFLD

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| References | Population | Intervention  (Drug/duration) | Comparator | Outcome (Baseline/20 weeks for drug *vs* control) | | |
|  |  |  |  | **ALT** | **Liver fat (%)** | **Liver fibrosis**  **(FIB-4)** |
| Johansson *et al* (2020) [43] | Type 2 diabetes | Metformin + Dapagliflozin 10mg od +  Saxagliptin 5mg od (D+S) (n=35) | Metformin + Glimepiride (G) (1-6mg) od (n=24) | Δ ALT -5.3 (D+S)  *vs* 2.1) (G) | Baseline 14.3 (6.4) (D+S)  13.7 (8.3) (G)  Week 52 9.9 (7.1) (D+S)  12.9 (8.6) (G)  (MRI-PDFF)  (D+S *vs* G, p<0.05) | Not measured |
| Sattar *et al* (2018) [41] | EMPA-REG Outcome ® trial | Empagliflozin 10/25mg  (n=4,611)  164 weeks | Placebo (P) (n=2,313) | Δ ALT -2.96 (E) *vs* - 0.73 (P)  (E *vs* P, p<0.05) | Not measured | Not measured |
| Eriksson *et al* (2018) [42] | Type 2 diabetes + NAFLD | Dapagliflozin 10mg (n=21), Dapagliflozin + OM3-CA (DO) (n=22)  12 weeks | Placebo (P) (n=21) | 67/53 (D) *vs* 57/54 (P) (D *vs* P, p<0.05) | 17.3/15.1 (D) *vs* 22.2/19.1 (DO) *vs* 15.1/14.5 (P)  (MRI-PDFF)  (D *vs* P, p=ns, DO *vs* P, p<0.05) | Not measured |
| Shibuya *et al* (2018) [S67] | Type 2 diabetes + NAFLD | Luseogliflozin 2.5 mg (L) (n=16)  26 weeks | Metformin 1500 mg (M) (n=16) | 49.5/31 (p=ns) (L) *vs* 39/39 (p=ns) (M)  (L *vs* M, p=ns) | 0.9097/1.033 (p=0.0008) (L)  0.991/0.851 (p=0.017) (M)  (CT liver/spleen ratio)  (L *vs* M, p<0.05) | Not measured |
| Kuchay *et al* (2018) [38] | Type 2 diabetes + NAFLD | Empagliflozin (E) 10 mg od (n=22)  20 weeks | Control (standard type 2 diabetes treatment) (C) (n=20) | 64(20)/50(26) (p<0.05) (E) *vs*  65(40)/62(38) (p=ns) (C)  (E *vs* C, p<0.05) | 16.2/11.3 (E) (p<0.05) *vs* 16.4/15.5 % (p=ns) (C)  (MRI-PDFF)  (E *vs* C, p<0.05) | Not measured |
| Cusi *et al* (2018) [36] | Type 2 diabetes (HbA1c = 7.7% ± 0.7%) | Canagliflozin (Cf) 300mg (n=26)  24 weeks | Placebo (P) (n=30) | 23/20 (Cf) *vs* 35/34 (P)  (Cf *vs* P, p=ns) | IHTG: -4.6% (Cf) (p<0.05) *vs* -2.4% (P) (p<0.05)  (1H-MRS)  (Cf *vs* P, p=ns)  IHTG for NAFLD patients only (n=37): -6.9% (Cf) *vs* -3.8% (P)  (Cf *vs* P, p=0.05) | Not measured |
| Ito *et al* (2017) [S68] | Type 2 diabetes + NAFLD | Ipragliflozin (I) 50mg (n=30)  24 weeks | Pioglitazone (P) 15-30mg (n=31) | 57.4/38.2 (p<0.05) (I) *v.* 53.1/46.8 (p<0.05) (P)  (I *vs* P, p=ns) | 0.78/0.98 (p<0.05) (I)  0.72/0.94 (p<0.05) (P)  (CT liver/spleen ratio)  (I *vs* P, p=ns) | 2.12/1.61 (p<0.05) (I)  2.06/1.70 (p<0.05) (P)  (I *vs* P, p=ns) |
| Bolinder *et al* (2012) [S69] | Type 2 diabetes | Dapagliflozin (D) 10 mg od + metformin (n=89)  24 weeks | Placebo (P) + metformin (n=91) | Not reported in sub-study | Change in intrahepatic lipid was -2.35% (D) *vs* -1.53% (P) (1H-MRS)  (D *vs* P, p=ns) | Not measured |

SGLT2, sodium-glucose transport protein 2; ALT, alanine aminotransferase; FIB-4; fibrosis-4 score; NAFLD, non-alcoholic fatty liver disease; OM3-CA, omega-3 carboxylic acids; MRI-PDFF, magnetic resonance imaging proton density fat fraction; 1H-MRS, proton magnetic resonance spectroscopy; CT, computed tomography; IHTG intrahepatic triglyceride

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