NAFLD and diabetes mellitus

Herbert Tilg[1], Alexander R. Moschen[1] and Michael Roden[2–4]

Abstract | The liver constitutes a key organ in systemic metabolism, contributing substantially to the development of insulin resistance and type 2 diabetes mellitus (T2DM). The mechanisms underlying these processes are not entirely understood, but involve hepatic fat accumulation, alterations of energy metabolism and inflammatory signals derived from various cell types including immune cells. Lipotoxins, mitochondrial function, cytokines and adipocytokines have been proposed to play a major part in both NAFLD and T2DM. Patients with NAFLD are commonly insulin resistant. On the other hand, a large number of patients with T2DM develop NAFLD with its inflammatory complication, NASH. The high incidence of NASH in patients with T2DM leads to further complications, such as liver cirrhosis and hepatocellular carcinoma, which are increasingly recognized. Therapeutic concepts such as thiazolidinediones (glitazones) for treating T2DM also show some efficacy in the treatment of NASH. This Review will describe the multifaceted and complex interactions between the liver and T2DM.

[1]*Department of Internal Medicine I, Gastroenterology, Endocrinology & Metabolism, Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria.*
[2]*Department of Endocrinology and Diabetology, Medical Faculty, Heinrich-Heine University.*
[3]*Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Heinrich-Heine University, c/o Auf'm Hennekamp 65, 40225 Düsseldorf, Germany.*
[4]*German Center of Diabetes Research, Ingolstädter Landstraße 1, 85764, München-Neuherberg, Germany.*

Correspondence to H.T. and M.R.
herbert.tilg@i-med.ac.at; michael.roden@ddz.uni-duesseldorf.de

doi:10.1038/nrgastro.2016.147
Published online 12 Oct 2016

One of the most common liver disorders worldwide[1], NAFLD covers a disease spectrum, ranging from simple steatosis in the absence of inflammation to NASH, liver cirrhosis and hepatocellular carcinoma (HCC)[2,3]. The number of patients in the general population with NAFLD who have NASH is unclear, but exceeds >10% of the overall NAFLD population, and population prevalence might be as high as 5%[4]. Definitive diagnosis of NASH is important as inflammation and/or fibrosis dictate the long-term prognosis of this disease[5]. In particular, NASH is predicted to become the leading cause of liver transplantation in many countries in the coming years[6]. Although no established treatment for NASH currently exists, several therapeutic approaches — such as the use of peroxisome proliferator-activated receptor (PPAR)γ agonists, vitamin E or liraglutide — have demonstrated some clinical efficacy in the past few years[7,8].

Insulin resistance has been characterized as the crucial pathophysiological factor in NAFLD[9]. However, the mechanistic basis of NAFLD and NASH is still incompletely understood, and besides insulin resistance per se, lipids, mitochondrial function, innate immunity, intestinal microbiota, genetic determinants, nutritional factors and lifestyle are involved in the disease process[10,11]. A strong association between NAFLD and type 2 diabetes mellitus (T2DM) has been shown, as >70% of patients with T2DM have NAFLD[12,13]. The burden of NAFLD, with clinically relevant fibrosis affecting up to 20% of those with both NAFLD and T2DM, seems to be enormous[14–16] considering the huge number of patients with T2DM worldwide[17]. Thus, not only is NAFLD highly associated with insulin resistance, but T2DM is commonly accompanied by NAFLD, with a very high rate of NASH[18–20]. This Review will highlight pathophysiological and clinical aspects of the association between NAFLD and T2DM.

Pathophysiological aspects

Hepatic lipids and energy metabolism

All forms of NAFLD tightly correlate with hepatic as well as peripheral insulin resistance[21–23], which is aggravated during NAFLD progression[24]. In the liver, insulin resistance is defined by impaired insulin-mediated suppression of glucose production, resulting from increased gluconeogenesis and decreased hepatic glycogen synthesis[25]. Insulin-stimulated hepatic glycogen synthesis in turn correlates negatively with liver fat content[25]. In patients who are obese and have T2DM, the presence of NAFLD associates with more severe hyperinsulinaemia, dyslipidaemia and insulin resistance in hepatic and adipose tissue than in patients without NAFLD[26]. Several concepts might explain the association between NAFLD and insulin resistance: liver fat accumulation could originate from insulin resistance and hyperinsulinaemia[27,28] or directly from excessive lipid availability, which then results in insulin resistance[21].

The role of hepatic lipid metabolism

Both free fatty acids (FFA) and glycerol contribute to liver triglyceride synthesis via hepatocellular long-chain fatty acids bound to coenzyme A (to form fatty acyl coenzyme A (CoA)). The hepatocellular concentration of fatty acyl CoA results from the balance between FFA formation (from circulating FFA, *de novo* lipogenesis, lipoprotein

Key points

- As a result of increased prevalence, NAFLD and type 2 diabetes mellitus (T2DM) are of increasing clinical relevance worldwide
- The liver has a key role in the pathophysiology of T2DM, as this organ contributes substantially to the development of insulin resistance
- Rates of NAFLD, NASH, liver cirrhosis and hepatocellular carcinoma in patients with T2DM are rapidly rising
- Medications used in the treatment of T2DM are also effective in the therapy of NASH

uptake and triglyceride breakdown) and utilization (lipid synthesis and oxidation)[21]. About 25% of FFA entering the liver is taken up[29], mainly via arteriovenous supply from subcutaneous adipocytes[30,31]. Overall, adipose insulin resistance is the key contributor to excess storage of hepatic triglycerides[32–34]. In fasted patients with obesity and NAFLD, circulating FFA, dietary fat supply and *de novo* lipogenesis account for ~59%, ~15% and ~26% of hepatocellular triglycerides, respectively[31]. Lambert *et al.*[35] reported that *de novo* lipogenesis is threefold higher in patients with NAFLD than in physiologically normal individuals, representing a key feature of fatty livers. *De novo* lipogenesis can be stimulated both by insulin, via sterol regulatory element binding-protein 1c (SREBP-1c) and by glucose, via carbohydrate response element-binding protein (ChREBP)[21]. Thus, hyperinsulinaemia and diets high in fat and carbohydrate will contribute to elevated *de novo* lipogenesis in obesity and NAFLD. Finally, the hepatocellular FFA pool can be further increased by impaired export of VLDL cholesterol in insulin-resistant patients with NASH[21]. Upon meal ingestion, humans with insulin resistance exhibit reduced muscle glycogen synthesis, doubling of both liver triglyceride levels and hepatic *de novo* lipogenesis without any changes in circulating adipocytokines[36]. These data indicate that muscle insulin resistance shifts postprandial energy storage from muscle glycogen to hepatic lipid storage.

Hepatic lipids and insulin sensitivity

In humans, a short-term rise in FFA availability results in hepatic insulin resistance[37]. Rodent models have shown that certain lipid intermediates (so-called 'lipotoxins') such as diacylglycerols inhibit insulin signalling and stimulate hepatic triglyceride deposition, as previously described for muscle insulin resistance[38,39]. A short-term high-fat diet induced selective hepatic steatosis, along with impaired tyrosine phosphorylation of insulin receptor substrate (IRS)2 and increased activity of protein kinase C (PKC)ε and c-Jun N-terminal kinase 1 (JNK1; also known as mitogen-activated protein kinase 8) [40]. Similarly, intraoperative liver biopsy studies found increased hepatic diacylglycerol levels in patients with obesity[41,42]. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in those with obesity[41], and correlates negatively with hepatic insulin sensitivity in patients who are obese and also have NAFLD, supporting the concept of lipotoxic hepatic insulin resistance mediated by the diacylglycerol–PKC pathway (FIG. 1).

Hepatic mitochondrial function

Hepatic lipids also undergo oxidation, mainly in mitochondria. Mitochondrial FFA entry is controlled via carnitine *O*-palmitoyltransferase 1; this enzyme, which is inhibited by insulin, malonyl-CoA and fatty acyl CoA, activates peroxisome proliferator activated receptor (PPAR)α, thereby stimulating β-oxidation of fatty acids[43]. Of note, reduced muscle mitochondrial function, as assessed from ATP synthase flux using noninvasive ^{31}P magnetic resonance spectroscopy, positively relates to liver fat content[44]. Patients with T2DM also exhibit reductions in hepatocellular ATP concentrations and ATP synthase flux[45,46]. Hepatic ATP synthesis correlates with both peripheral and hepatic insulin sensitivity, but negatively with body fat content[47].

However, a study published in 2015 employing high-resolution respirometry in mitochondria isolated from liver biopsy tissue found that hepatic mitochondrial function is not uniformly impaired in obesity and NAFLD[48]. Individuals who are obese with or without steatosis have up to fivefold higher maximal mitochondrial respiration rates than lean individuals, whereas these rates are up to 40% lower in patients with NASH[48]. Patients with NASH also have increased mitochondrial uncoupling and proton leakage compared with lean individuals, as well as augmented hepatic oxidative stress paralleled by reduced antioxidant defence capacity and increased inflammatory responses[48]. After meal ingestion, insulin-resistant individuals who are obese with simple hepatic steatosis also show a sixfold greater rise in liver ATP than lean insulin-sensitive humans[49], whereas patients with NASH demonstrate impaired hepatic ATP repletion after transient fructose-induced ATP depletion[50]. In rat and mouse models, elevation of FFA delivery not only induces oxidative metabolism but also amplifies anaplerosis and cataplerosis, which is necessary for gluconeogenesis, as well as causing oxidative stress and inflammation[51]. These functional *in vivo* and *ex vivo* findings support previous studies on liver biopsy tissue, which show that patients with NASH have hepatic mitochondrial alterations, possibly correlating with fibrosis[32,52], including increased proton leak across the electron transport chain owing to upregulated expression of mitochondrial uncoupling protein 2 (REF. 53), mitochondrial DNA depletion and abnormal mitochondrial redox homeostasis with increased reactive oxygen species generation[54,55].

Collectively, these data suggest that hepatic mitochondria transiently adapt to increased lipid availability by upregulating their oxidative capacity at the expense of decreased coupling efficiency[48]. Loss of mitochondrial adaptation will favour lipid deposition and insulin resistance. Finally, excessive lipid overloading will impair antioxidant capacity and accelerate oxidative stress with mitochondrial leakage, resulting in NASH and aggravated insulin resistance (FIG. 1).

Gluconeogenesis and lipolysis

Although lipid-induced insulin resistance and hepatic fat storage can occur independently of inflammatory pathways, data suggest that alternative mechanisms can lead to fasting hyperglycaemia during T2DM development[38,56].

Current evidence suggests that impaired insulin secretion reduces hepatic activation of RAC-α serine/threonine-protein kinase (also known as AKT1 or PKB) and excludes forkhead box protein O1 (FOXO1) from the nucleus of the hepatocyte, leading to transcription-mediated gluconeogenesis[57]. In addition, IL-6 activates NF-κB–JNK–ceramide pathways, which inhibit insulin signalling and in turn increase gluconeogenic protein transcription. Studies in mice with hepatic AKT and FOXO1 depletion, which adapt their glucose fluxes properly to both fasting and feeding[58], have revealed that IL-6, released from adipose tissue macrophages, inhibits insulin-mediated lipolysis in white adipose tissue and leads to increased delivery of FFA and glycerol to the liver[59]. As a result, hepatic acetyl-CoA concentrations are raised, due to increased fatty acid β-oxidation, which then stimulates

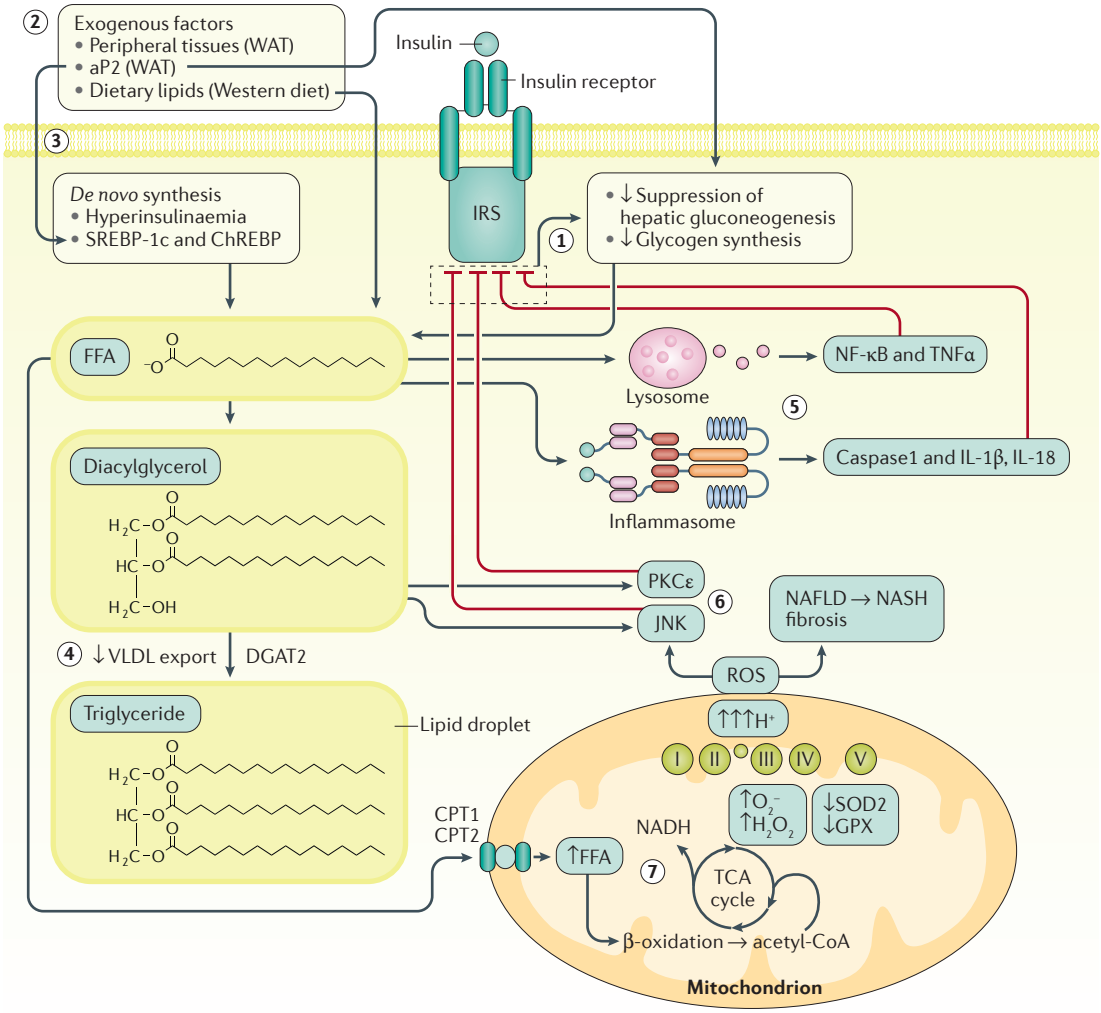

Figure 1 | **Pathophysiological aspects of insulin resistance in NAFLD: role of lipids and energy metabolism.** NAFLD is associated with hepatic and peripheral insulin resistance, resulting in an insufficient suppression of hepatic gluconeogenesis, decreased glycogen synthesis and increased lipid accumulation (1). High amounts of free fatty acids (FFA) are attributed to an increased delivery from white adipose tissue (WAT), which releases additional soluble factors such as the lipid chaperone aP2, thereby further promoting hepatic glucose metabolism. Levels of FFAs are further augmented by the availability of dietary lipids (2). *De novo* synthesis of FFA is driven by sterol regulatory element binding-protein 1c (SREBP-1c) and carbohydrate response element binding-protein (ChREBP) and is catalysed by hyperinsulinaemia and hyperglycaemia (3). Aggravatingly, lipid export through VLDL is decreased (4). Whereas hepatic triglycerides stored in lipid droplets can be relatively inert or even protective, FFA might substantiate insulin resistance, causing lysosomal instability with leakage of cathepsin B and induction of the NF-κB–TNFα pathway, or by activating the caspase-1–IL-1β/IL-18 pathways through the NALP3 inflammasome (5). Diacylglycerol promotes insulin resistance through the activation of protein kinase C (PKC)ε and c-Jun N-terminal kinase (JNK) (6). The hepatocyte attempts to limit FFA by increasing mitochondrial β-oxidation. Coenzyme A (CoA)-linked FFAs are shuttled into the mitochondrial matrix via carnitine *O*-palmitoyltransferase (CPT)1 and CPT2. Excessive acetyl-CoA is channelled into the tricarboxylic acid (TCA) cycle and NADH into the electron transport chain. Exhaustion of the antioxidant capacities of superoxide dismutase (SOD)2 and glutathione peroxidase (GPX) ultimately results in increased oxidative stress mediated by superoxide (O_2^-) anions, H_2O_2 and mitochondrial leakage, leading to aggravation of insulin resistance and progression to NASH and fibrosis (7). DGAT2, diacylglycerol O-acyltransferase 2; IRS, insulin receptor substrate.

hepatic gluconeogenesis through allosteric activation of mitochondrial pyruvate carboxylase. Similarly, adolescents who are obese and insulin-resistant show higher adipose tissue IL-6 concentrations than lean individuals, along with adipose and hepatic insulin resistance[59]. The rapid insulin-mediated reduction in hepatic glucose production (occurring within minutes)[25] and lack of any relationship between hepatic expression of gluconeogenic proteins and fasting hyperglycaemia in individuals who are obese, with and without T2DM[41], supports the clinical relevance of this mechanism (FIG. 1).

Insulin resistance and NAFLD

Despite the tight association between NAFLD and insulin resistance, genome-wide association studies have identified genetic variants associated with NAFLD severity that do not correlate with insulin resistance. The missense mutation Ile148Met within patatin-like phospholipase domain-containing protein 3 (PNPLA3; also known as adiponutrin) modifies NAFLD progression[60] and leads to impaired hepatic triglyceride hydrolysis, but does not associate with insulin resistance[61]. Another PNPLA3 variant (PNPLA3 Glu434Lys, at single nucleotide polymorphism (SNP) rs2294918) affects PNPLA3 phenotype by decreasing its hepatic mRNA and protein levels[62]. The Glu167Lys variant (at SNP rs58542926) of the transmembrane 6 superfamily member 2 protein (TM6SF2) influences liver fat content[63]; carriers of this variant had 34% higher liver fat content than those without the variant, yet their insulin sensitivity was preserved[64]. Finally, a mutation in the gene encoding 1-acylglycerol-3-phosphate *O*-acyltransferase ABHD5 (also known as ABHD5 or CGI-58), resulting in deficiency in protein levels of ABHD5, not only leads to Chanarin–Dorfman syndrome[65] but also to excessive steatosis despite unchanged insulin sensitivity. In this case, diacylglycerol is retained within lipid droplets, thereby not allowing PKCε translocation, which is a prerequisite for diacylglycerol-mediated inhibition of insulin signalling. In other diseases such as familial hypobetalipoproteinaemia, in which a genetic defect impairs hepatic triglyceride export, steatosis and insulin resistance dissociate further, suggesting that intrahepatic triglyceride levels are more reflective of insulin resistance than directly causing insulin resistance[66].

Inflammatory pathways in insulin resistance. Inflammatory pathways have been increasingly recognized as critically involved in the development of insulin resistance[67,68]. However, the aetiology of insulin resistance is complex and involves many different pathways besides inflammation[69]. Currently, it remains unclear at which sites inflammatory processes are initiated. Aside from adipose tissue, the gastrointestinal tract with its markedly altered microbiota could reflect one of the early events in the evolution of both insulin resistance and NAFLD[11].

Insulin acts in all cells by binding to its specific receptor and activating a cascade of intracellular signalling events. Upon insulin binding, the insulin receptor phosphorylates itself and several members of the IRS family. IRS1 and IRS2 are the main mediators of insulin signalling in the liver, controlling insulin sensitivity[70]. The primary pathophysiological mechanisms of insulin resistance induced by inflammatory mediators are probably the result of interference with insulin signalling[71]. Pro-inflammatory cytokines and transcription factors are highly expressed in various tissues (such as the adipose tissue or liver) in obesity and related disorders. Various inflammatory pathways have been demonstrated to interfere with insulin signalling in preclinical models; however, clinical evidence for a major role of inflammation in insulin resistance is still in its infancy[72].

Role of the IKKβ–NF-κB pathway. Several inflammatory pathways involving pro-inflammatory cytokines, such as TNFα or IL-6, are activated in the insulin-resistant liver[73]. Substantial evidence exists that activation of the transcription factor NF-κB and downstream inflammatory signalling pathways are involved in hepatic insulin resistance. Whereas NF-κB activation is only modest in muscle, its expression is substantial in liver and adipose tissue in states of insulin resistance[74]. The inhibitor of nuclear factor-κB kinase (IKK) complex has a crucial role in the activation of NF-κB, by triggering phosphorylation of the inhibitory molecule IκBα (also known as NF-κB inhibitor α), thereby blocking its inhibitory capacity. Four different IKKs have been identified so far: IKKα, IKKβ, IKKε and TANK-binding kinase 1 (also known as TBK1). Yuan *et al.*[75] identified the IKKβ pathway as a target for TNFα-induced insulin resistance in mice and cell lines. Two other earlier studies have demonstrated a relationship between IKKβ expression in the liver and insulin resistance[76,77]. Cai *et al.*[76] induced chronic hepatic inflammation in a transgenic mouse model by constitutively expressing IKKβ specifically in hepatocytes, resulting in low-level activation of NF-κB. These mice showed a T2DM-like disease with moderate systemic insulin resistance. Arkan *et al.*[77] demonstrated similar findings in mice lacking IKKβ either in hepatocytes or myeloid cells[77]. Mice with liver-specific IKKβ deletion retained liver insulin responsiveness after consumption of a high-fat diet or when intercrossed with *ob/ob* mice, but developed insulin resistance in muscle and adipose tissue. Interestingly, mice deficient in myeloid IKKβ also retained systemic insulin sensitivity. Thus, hepatocyte-specific overexpression or activation of NF-κB is associated with insulin resistance and can mimic all features of fatty liver disease, demonstrating a key role of inflammation in insulin resistance (FIG. 2).

Receptor activator of NF-κB (RANKL; also known as tumour necrosis factor ligand superfamily member 11), a prototypic activator of NF-κB, has also been demonstrated to regulate hepatic insulin sensitivity[78]. The prospective population-based Bruneck Study identified high circulating levels of soluble RANKL as an independent risk predictor for the development of T2DM, suggesting that the NF-κB pathway could also be of relevance in humans[78]. Blockage of RANKL signalling in hepatocytes improved insulin sensitivity, normalized glucose concentrations and insulin signalling. Likewise, hepatocyte-specific blockage of RANK (also known as tumour

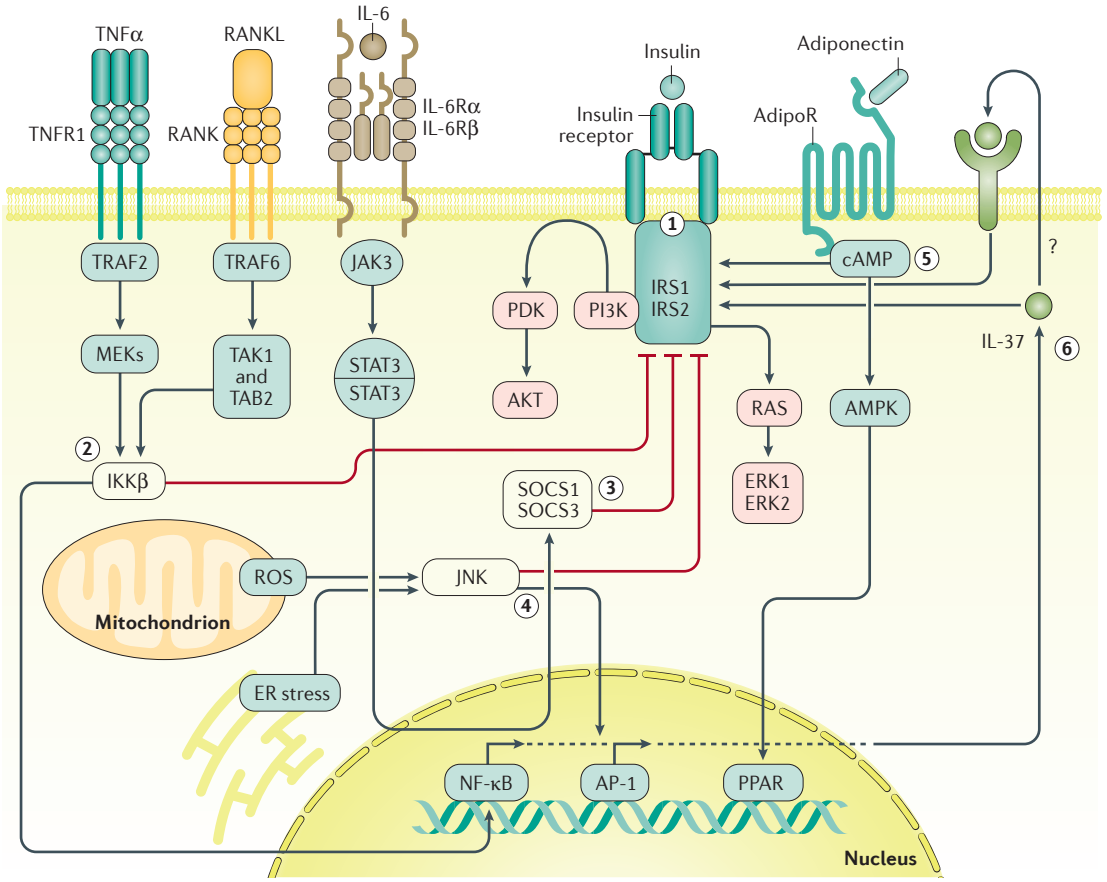

Figure 2 | **Inflammatory pathways affecting hepatic insulin resistance.** Insulin activates its receptor, which results in tyrosine phosphorylation of insulin receptor substrate (IRS) and activation of downstream effector pathways including the phosphatidylinositol 3-kinase (PI3K)–phosphoinositide-dependent kinase (PDK)–protein kinase B (AKT) and the RAS–extracellular-signal regulated kinase (ERK, also known as mitogen-activated protein kinase) pathways (1). Various pro-inflammatory pathways interfere with insulin signalling either by inhibitory phosphorylation or proteasomal degradation of IRS. High-fat diet, altered microbial composition and reduced intestinal barrier function promote local and systemic inflammation through influx of toll-like receptor ligands. The inhibitor of NF-κB kinase β (IKKβ) represents an important and potent catalyst of insulin resistance besides its role as activator of NF-κB. Numerous pro-inflammatory signalling pathways including TNFα–TNFR1, RANKL–RANK, or IL-1–IL-1R (not shown) activate IKKβ (2). Vast amounts of IL-6 originate from the adipose tissue and promote insulin resistance by inducing suppressor of cytokine signalling (SOCS)1 and 3 via the IL-6R–janus kinase (JAK)3–signal transducer and activator of transcription (STAT)3 axis. SOCS1 and SOCS3 impair insulin signalling through ubiquitin-dependent degradation of IRS (3). c-Jun N-terminal kinase (JNK, also known as mitogen activated protein kinase) represents another important inhibitory kinase of IRS that is activated in response to a variety of extracellular stimuli and cellular stressors such as oxidative and endoplasmic reticulum (ER) stress (4). Pro-inflammatory stimuli are counteracted by adipose-tissue-derived adiponectin via adiponectin receptor (AdipoR)1 and AdipoR2 (5), and by the anti-inflammatory IL-1 family member IL-37 (6) through so far unknown mechanisms. AMPK, 5′-AMP-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TAB2, TGFβ-activated kinase 1 and MAP3K-binding protein 2; TAK1, mitogen-activated protein kinase kinase kinase 7; TRAF, TNF receptor-associated factor.

necrosis factor receptor superfamily member 11A), the specific receptor of RANKL, demonstrated potent antidiabetic effects[78]. Soluble RANKL, which is produced by many tissues including skeletal muscle, several immune cell types and adipose tissue[79–81], binds to its receptor RANK in the liver and activates the NF-κB pathway, augmenting local inflammation and resulting in insulin resistance. All of these studies support the concept that immune and inflammatory mediators generated throughout the body, such as RANKL, might target the liver as the major organ of metabolism contributing to hepatic insulin resistance.

Extrahepatic drivers of liver inflammation. Adipose tissue has emerged as a major site of inflammation in obesity-related disorders. Concentrations of certain cytokines, such as IL-1β or IL-6, are 10-fold to 100-fold more highly expressed in adipose tissue than in the liver in humans[82,83]. These studies identified subcutaneous adipose tissue as a major source of pro-inflammatory cytokines, in the face of similar expression in the subcutaneous and visceral adipose tissue; therefore, high concentrations of pro-inflammatory signals from adipose tissue might under certain circumstances induce hepatic insulin resistance via systemic inflammatory

loops. Other studies have also suggested that the production of certain adipokines and lipokines in the adipose tissue regulates hepatic glucose production[84]. Cao *et al.*[84] demonstrated that circulating levels of the lipid chaperone aP2 (also known as adipocyte fatty acid-binding protein; FABP4), secreted from adipocytes, are elevated in mice and humans with obesity, and that neutralization of aP2 corrects diabetes in mice. Importantly, the use of hyperinsulinaemic–euglycaemic and pancreatic clamp experiments in mice demonstrated that aP2 acts in the liver, strongly supporting the concept that adipose-tissue-derived mediators control hepatic metabolic functions and further strengthening the concept of crosstalk between adipose tissue and the liver[85]. The evolution of adipose tissue inflammation is still not well understood. Local mechanical factors and various adipocytokines might be critically involved[86]. Adiponectin exerts glucose-lowering effects via activation of 5′-AMP-activated protein kinase (AMPK) and PPARα pathways. An oral synthetic small-molecule adiponectin receptor agonist (AdipoRon) has been shown to improve insulin resistance and diabetes in mice fed a high-fat diet[87]. As stated in the previous section, adipose tissue inflammation can also induce hepatic insulin resistance by increasing the flux of FFA from adipose tissue to the liver. Taken together, hepatic insulin resistance commonly associates with both hepatic lipid accumulation and inflammatory events.

Clinical aspects of NAFLD and T2DM

NAFLD often precedes T2DM

Obesity is the major risk factor for NAFLD, as BMI and waist circumference not only positively correlate with the presence of NAFLD but also with disease progression[88]. Patients with NAFLD almost universally exhibit hepatic insulin resistance, which substantially increases the risk of subsequent T2DM[9,27]. However, insulin resistance is not only limited to the liver but is also observed in muscle and adipose tissue[23]. Of note, liver fat content is strongly determined by muscle ATP synthase flux and muscle insulin sensitivity[44]. Defining insulin resistance in nondiabetic patients with NAFLD remains a challenge. In this patient group, the homeostatic model assessment (HOMA) of insulin resistance (calculated as the product of fasting blood glucose (in mmol/L) and fasting circulating insulin (in mU/mL), divided by 22.5) is an accepted surrogate measurement of insulin resistance[89].

Presence of NAFLD promotes the consecutive development of T2DM. Many large population-based studies have convincingly demonstrated that elevated serum levels of alanine aminotransferase (ALT) and/or γ-glutamyltransferase (GGT), as surrogate markers of NAFLD, are independently associated with an increased incidence of T2DM, even after adjustment for several risk factors[90–93]. Despite the availability of many studies highlighting these associations, the predictive value of various laboratory parameters remains limited, particularly because a substantial number of patients with NAFLD exhibit normal transaminase levels[94]. Ultrasonography-defined NAFLD is associated with a twofold to fivefold increased risk of developing T2DM after correction for various lifestyle and metabolic confounders[95]. Importantly, resolution of fatty liver as assessed by ultrasonography resulted in a substantial reduction in the risk of T2DM development, to a level similar to individuals without NAFLD. In this study, individuals with worsening fatty liver over 5 years showed a marked increase in T2DM[96].

McPherson and colleagues have demonstrated that, especially in patients with insulin resistance and/or diabetes, liver fibrosis might progress over the disease course, even when baseline histology described simple steatosis without hepatocellular injury[24]. This study delivers a key message that steatosis in insulin resistance states can progress to NASH and clinically relevant fibrosis. An earlier study demonstrated in follow-up liver biopsy samples that a substantial proportion of patients with steatosis progress towards NASH and fibrosis, especially with worsening metabolic risk factors and increased T2DM prevalence[97]. Another cohort study evaluated 129 consecutively enrolled patients diagnosed with biopsy-proven NAFLD[98]. At mean follow-up of 13.7 years, 69 of 88 patients had T2DM or impaired glucose tolerance, and patients with progressive fibrosis were more insulin resistant[98]. Worsening of metabolic risk factors during follow-up, therefore, might allow the identification of patients with NAFLD at risk for NASH or fibrosis progression. Owing to the high prevalence and risk of insulin resistance and T2DM in patients with NAFLD[97,98], it seems mandatory in our opinion to screen patients with NAFLD for diabetes by established diagnostic tests (fasting blood glucose levels, haemoglobin A_{1c} (HbA_{1c}) level or — if available — the standardized 75 g oral glucose tolerance test (OGTT)). Prevalence of NAFLD is increased in subjects at risk of T2DM, defined as an HbA_{1c} of 5.7–6.4%, impaired fasting glucose level (5.55–6.94 mmol/l) and/or impaired glucose tolerance (7.77–11.04 mmol/l at 2 h during the standardized 75 g OGTT)[99,100].

NAFLD in patients with T2DM

NAFLD and the metabolic syndrome. NAFLD is highly prevalent in patients with the metabolic syndrome, defined as the presence of any three the following clinical features: increased fasting blood glucose concentration, hypertriglyceridaemia, low plasma HDL-cholesterol levels, increased waist circumference and high blood pressure[18]). All components of the metabolic syndrome correlate with the degree of liver fat content, resulting in the important clinical recommendation to evaluate the risk of NAFLD in all persons with any component of metabolic syndrome, and vice versa (FIG. 3)[99,100].

Steatosis in patients with T2DM and normal ALT levels. Patients who have T2DM and normal serum ALT levels exhibit a high prevalence of steatosis as assessed by magnetic resonance spectroscopy (^{1}H-MRS)[101]. Presence of insulin resistance and diabetes is considered a risk factor for more severe liver disease in NAFLD even in patients with normal levels of serum ALT[94].

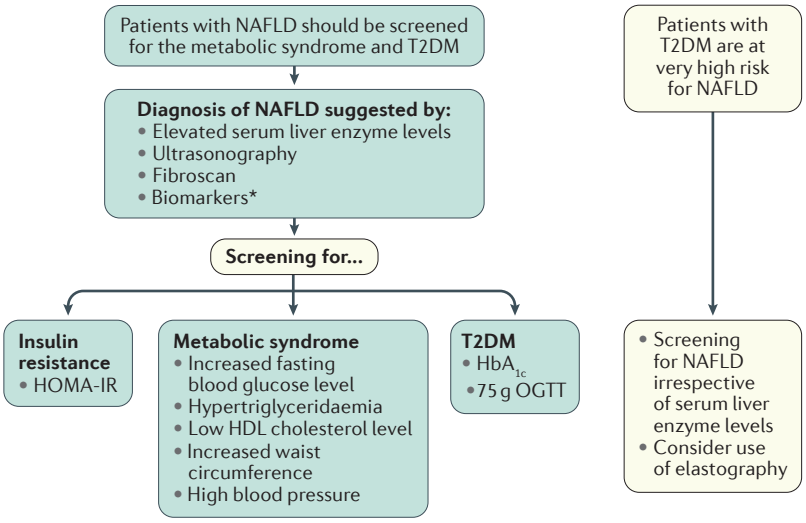

Figure 3 | **Clinical algorithms in the management of NASH and diabetes mellitus.** These statements are in part based on the reported European NAFLD guidelines[99,100]. *Biomarkers have been well-studied to detect steatosis and fibrosis in NAFLD and have value in guiding clinical management. HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

NASH and fibrosis in T2DM. Cases of cryptogenic cirrhosis have long been recognized to show a high rate of both obesity and T2DM[102]. Patients with T2DM also have a high prevalence of raised serum transaminase levels and liver steatosis as assessed by ultrasonography[103,104]. Studies performing liver stiffness measurements by means of transient elastography have demonstrated high rates of advanced fibrosis, up to 17.7%, in patients with diabetes[15,16]. A 2016 study using MRI to assess the liver proton-density fat fraction and magnetic resonance elastography (MRE) to estimate hepatic stiffness demonstrated high prevalence of both NAFLD and advanced fibrosis in patients with T2DM[105]. Early studies using liver histology from biopsy tissue suggested that patients with T2DM could have an ~40% risk of NASH[106]. Several other studies reported that T2DM strongly associates with NASH including mild and advanced fibrosis[12,13]. Further investigations based on liver histology observed that patients with T2DM have more severe NAFLD than those without T2DM, with NASH rates up to 80% and advanced fibrosis in ~30–40% of subjects[107,108]. Overall, liver histology remains the gold standard for diagnosing liver fibrosis, as various biomarkers were not able to accurately assess fibrosis in a large cohort of patients with T2DM[109]. The high fibrosis rate in those with T2DM is clinically relevant as liver fibrosis is the key feature associated with long-term outcomes in patients affected by NAFLD[110]. Bazick and colleagues developed a clinical model predicting NASH and advanced fibrosis in adult patients with NAFLD and concomitant T2DM that detected advanced fibrosis better than the NAFLD fibrosis score[108]. Incorporating BMI, waist circumference, HbA_{1c} concentration, insulin resistance by HOMA and serum levels of ALT, aspartate aminotransferase, albumin and ferritin, the model diagnosed NASH presence in 1,249 patients with histology-proven NASH with a specificity of 90.0% and a sensitivity of 56.8% (REF. 108). In addition, the presence of diabetes in individuals with NAFLD is an independent predictor of moderate-to-severe fibrosis[111,112]. Presence of NAFLD in patients with T2DM has not only been associated with poor glycaemic control but also with microvascular and macrovascular complications, such as retinopathy, nephropathy and cardiovascular disease, in addition to overall mortality[113–116].

Liver cirrhosis and HCC. Epidemiological studies have repeatedly demonstrated that patients with obesity and diabetes have a high risk of HCC development, and a high rate of HCC was reported in those with NAFLD and/or cryptogenic cirrhosis[117–119]. Mortality associated with HCC is increasing in many Western countries, typically affecting elderly patients with the metabolic syndrome or T2DM[120]. Dyson *et al.*[120] observed a >10-fold increase in HCC associated with NAFLD between 2000–2010 in the area around Newcastle, UK. In their cancer cohort, the authors observed a remarkable increase in the prevalence of the metabolic syndrome and T2DM, and importantly over a third of patients developed HCC on a background of NAFLD[120]. Interestingly, HCC associated with NAFLD is increasingly seen and reported in patients without cirrhosis. Many patients with NASH developing HCC exhibit T2DM and have high rates of hypertension and obesity[121,122]. Indeed, T2DM and obesity reflect the greatest population-attributable fraction affecting HCC risk in the USA[123]. A large case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study convincingly showed that increased levels of inflammatory biomarkers and hyperinsulinaemia are associated with raised risk of HCC, thereby further strengthening the pathophysiological link between T2DM and HCC[124]. Furthermore, smoking and alcohol might have a synergistic effect on HCC risk in patients with T2DM[125,126].

Treatment of NASH

As NAFLD and T2DM share some common pathophysiologic features, the fact that several therapies used in the treatment of T2DM also demonstrate efficacy in treating NAFLD is not surprising. Lifestyle modification aimed at inducing weight loss and/or reduction of intake of saturated fat and increased physical activity in those who are overweight not only improves insulin sensitivity but also leads to a reduction in liver fat and signs of NASH. A meta-analysis identified 38 randomized trials of treatments for NASH. Overall, eight randomized controlled trials studied the effect of lifestyle or drug-induced weight loss in NAFLD. In four of the eight trials in which post-treatment histology was available, weight loss of at least 7% of baseline body weight improved histological activity, but this amount of weight loss was achieved by <50% of patients[127] (TABLE 1).

Metformin. The first-line blood-glucose-lowering drug in T2DM, metformin effectively improves both hepatic and peripheral insulin resistance and decreases endogenous glucose production by various mechanisms resulting from primary inhibition of complex I

Table 1 | **Medical treatment modalities in NASH and T2DM**

Intervention	Metformin	GLP-1	Thiazolidinediones	SGLT2 inhibitors	DPP4 inhibitors	Sulphonylurea	Insulin
Glucose lowering efficacy	++	++	+ or ++	+ or ++	+	+++	+++
Hypoglycaemia risk	Low	Low	Low	Low	Low	High	High
Effect on body weight	Loss	Loss	Gain	Loss	Neutral	Gain	Gain
Adverse effects	Gastrointestinal	Gastrointestinal	• Oedema • Heart failure • Fractures	• Genitourinary infections • Dehydration	Pancreatic	Hypoglycaemia	Hypoglycaemia
Liver-specific effects							
Steatosis	NE	↓	↓	?	?	NE	↑
Inflammation	NE	↓	↓	?	?	?	?
Hepatocyte ballooning	NE	↓	↓	?	?	?	?
Fibrosis	NE	NE	?	?	?	?	?
RCTs showing effectiveness in NAFLD	NE	Liraglutide	Pioglitazone (Rosiglitazone)	ND	ND	ND	ND

Diet and exercise should be advised for all patients, and continued throughout medical treatments. DPP4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide 1; ND, not done; NE, no effect; RCT, randomized controlled trial; SGLT2, sodium glucose co-transporter 2.

of the mitochondrial respiratory chain[128]. Nevertheless, metformin treatment does not consistently reduce fat content or inflammatory markers in NASH[129–133].

Metformin treatment in patients with T2DM resulted in an odds ratio of 0.34 for HCC in a review of 41 observational studies, but no statistically significant effect on HCC risk was observed in 12 prospective trials[134].

Thiazolidinediones. Sometimes referred to as 'insulin sensitizers', thiazolidinediones are PPARγ agonists and improve insulin resistance mainly by stimulating adipocyte differentiation[128]. The subsequent increase in adiponectin secretion and decrease in FFA release from adipocytes probably underlies the beneficial effect of PPARγ agonists on NAFLD. Indeed, improvement of insulin-mediated suppression of FFA seems to precede reduction of liver fat content during short-term treatment of near-normoglycaemic patients with T2DM[135]. Short-term treatment with pioglitazone showed promising results by reducing liver fat content and histologically assessed NAFLD severity in patients with[136] or without T2DM[137]. The PIVENS trial compared 2-year treatment with pioglitazone, vitamin E or placebo in patients without T2DM[7]. Pioglitazone resulted in resolution of NASH in more patients than placebo (47% versus 21%, $P<0.001$), in line with improvements in serum ALT levels and the histological features of NASH. Vitamin E resulted in resolution of NASH in 36% compared with 21% in patients treated with placebo ($P<0.05$). Both pioglitazone and vitamin E decreased steatosis and lobular inflammation. Of note, pioglitazone did not improve fibrosis and only partly decreased insulin resistance, probably owing to the low dose of 30 mg per day used. A double-blind randomized placebo-controlled study published in 2016 found that pioglitazone (45 mg daily for 18 months) ameliorated the primary endpoint, NAFLD activity score (comprising the sum of improvement of hepatic steatosis, inflammation and ballooning without worsening of fibrosis), in patients with NASH and prediabetes or T2DM[138]. Pioglitazone improved fibrosis as well as insulin sensitivity in liver, skeletal muscle and adipose tissue. All beneficial outcomes were maintained after 36 months of treatment without adverse effects, except for increased body weight gain. By contrast, another PPARγ agonist, rosiglitazone, did not cause histological improvement in patients with NASH even after 2 years of treatment[133,139,140].

Independent of the efficacy of thiazolidinediones in liver disease, several other adverse effects of this drug class warrant attention. Aside from body weight gain, fluid retention with risk of congestive heart failure and atypical bone fractures in women have been reported[128].

Incretin-based therapies. Incretin mimetics, including glucagon-like peptide 1 (GLP-1) receptor agonists, primarily stimulate glucose-dependent insulin secretion. They also lead to reductions in body weight, insulin resistance and circulating levels of liver transaminases[141]. A pilot trial of 1.8 mg liraglutide per day delivered subcutaneously showed decreases in liver fat content and histological resolution of NASH in more patients receiving treatment than those receiving placebo (39% versus 9%, respectively)[8]. This result might suggest that liraglutide exerts effects additional to simple weight loss. In another study, the dipeptidyl peptidase 4 inhibitor sitagliptin, which inhibits GLP-1 degradation, failed to reduce liver fat and fibrosis in patients with prediabetes and diabetes compared with placebo, as assessed by MRE[142].

Other treatments. At present, only nine randomized controlled trials in patients with biopsy-proven NASH have been published[143]. A Bayesian network meta-analysis revealed only moderate-quality evidence that thiazolidinediones, pentoxifylline and obeticholic acid decrease lobular inflammation, and that pentoxifylline and obeticholic acid improve fibrosis. Thus, these data do not

allow for straightforward recommendations for drug treatment of this disease[126,127]. Nevertheless, a growing number of new agents aimed at decreasing liver fat content and increasing insulin sensitivity are being tested in clinical trials, such as corticosteroid 11β-hydroxysteroid dehydrogenase 1 inhibitors[144] or the dual PPARα and PPARδ agonist elafibranor (also known as GFT505), which has been shown to exert anti-inflammatory and antifibrotic effects in mice[145]. Elafibranor administered at a dose of 120 mg per day for 1 year to patients with NASH but without cirrhosis resolved NASH without worsening of fibrosis, especially in patients with moderate or severe NASH[146].

Promising data have been also reported for an orally available controlled-release formulation of the protonophore 2,4-dinitrophenol, a historical anti-obesity drug, which improves hyperglycaemia, hepatic steatosis and even fibrosis in rodent models of diabetes and NASH[147]. This compound addresses the dynamic abnormalities of hepatic mitochondrial function in humans with NASH. Treatment of rats resulted in a 60% increase in hepatic mitochondrial tricarboxylic acid cycle flux, which could be attributed to a 65% increase in rates of fat oxidation[48].

Finally, bariatric surgery, as an effective non-pharmaceutical treatment to decrease body weight, insulin resistance and reverse T2DM, also improves hepatic necroinflammation and fibrosis[148,149]. Of note, the possible adverse effects and long-term consequences need to be considered and weighed against those of lifestyle intervention and drug treatment.

Conclusions

Although it has long been known that fatty liver is frequently observed in patients with T2DM[150], the association between NASH and diabetes has only in the past decade gained attention both in the scientific community and in clinical medicine. Here, we provide evidence that the liver is of fundamental importance for regulation of metabolism and insulin sensitivity. Because of its tight association with insulin resistance, steatosis presence requires immediate clinical investigation for features of the metabolic syndrome, insulin resistance and T2DM. In addition, established T2DM necessitates thorough clinical assessment of whether NAFLD or NASH might be present. Specifically, physicians must keep in mind that patients with T2DM have increased risk of advanced liver disease, including liver cirrhosis and HCC. Specialization in internal medicine has led to the bias that each specialist (the hepatologist and/or the diabetologist) might look only on 'one side of the coin' by focusing their clinical investigations too closely within their speciality. The epidemiology and clinical relevance of T2DM demands an integrative approach so that all physicians involved in the care of hepatological and diabetological diseases are aware of aspects reported here; only when considering these facts will the optimal treatment of this patient population become possible. Growing evidence suggests that some currently available and novel therapies for both T2DM and NASH will improve patient care and the management of these disorders.

1. Ratziu, V., Goodman, Z. & Sanyal, A. Current efforts and trends in the treatment of NASH. *J. Hepatol.* **62**, S65–75 (2015).
2. Ahmed, A., Wong, R. J. & Harrison, S. A. Nonalcoholic Fatty Liver Disease Review: Diagnosis, Treatment, and Outcomes. *Clin. Gastroenterol. Hepatol.* **13**, 2062–2070 (2015).
3. Castera, L., Vilgrain, V. & Angulo, P. Noninvasive evaluation of NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 666–675 (2013).
4. Hyysalo, J. *et al.* A population-based study on the prevalence of NASH using scores validated against liver histology. *J. Hepatol.* **60**, 839–846 (2014).
5. Yeh, M. M. & Brunt, E. M. Pathological features of fatty liver disease. *Gastroenterology* **147**, 754–764 (2014).
6. Wree, A., Broderick, L., Canbay, A., Hoffman, H. M. & Feldstein, A. E. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 627–636 (2013).
7. Sanyal, A. J. *et al.* Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* **362**, 1675–1685 (2010).
8. Armstrong, M. J. *et al.* Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* **387**, 679–690 (2016).
9. Marchesini, G. *et al.* Association of nonalcoholic fatty liver disease with insulin resistance. *Am. J. Med.* **107**, 450–455 (1999).
10. Haas, J. T., Francque, S. & Staels, B. Pathophysiology and mechanisms of nonalcoholic fatty liver disease. *Annu. Rev. Physiol.* **78**, 181–205 (2016).
11. Moschen, A. R., Kaser, S. & Tilg, H. Non-alcoholic steatohepatitis: a microbiota-driven disease. *Trends Endocrinol. Metab.* **24**, 537–545 (2013).
12. Williams, C. D. *et al.* Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* **140**, 124–131 (2011).
13. Loomba, R. *et al.* Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* **56**, 943–951 (2012).
14. Doycheva, I., Patel, N., Peterson, M. & Loomba, R. Prognostic implication of liver histology in patients with nonalcoholic fatty liver disease in diabetes. *J. Diabetes Complications* **27**, 293–300 (2013).
15. Kwok, R. *et al.* Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut* **65**, 1359–1368 (2015).
16. Koehler, E. M. *et al.* Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: the Rotterdam study. *Hepatology* **63**, 138–147 (2016).
17. Collaboration, N. C. D. R. F. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* **387**, 1513–1530 (2016).
18. Yki-Jarvinen, H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol.* **2**, 901–910 (2014).
19. Anstee, Q. M., Targher, G. & Day, C. P. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 330–344 (2013).
20. Lonardo, A., Ballestri, S., Marchesini, G., Angulo, P. & Loria, P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig. Liver Dis.* **47**, 181–190 (2015).
21. Roden, M. Mechanisms of disease: hepatic steatosis in type 2 diabetes—pathogenesis and clinical relevance. *Nat. Clin. Pract. Endocrinol. Metab.* **2**, 335–348 (2006).
22. Kotronen, A., Westerbacka, J., Bergholm, R., Pietilainen, K. H. & Yki-Jarvinen, H. Liver fat in the metabolic syndrome. *J. Clin. Endocrinol. Metab.* **92**, 3490–3497 (2007).
23. Gaggini, M. *et al.* Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients* **5**, 1544–1560 (2013).
24. McPherson, S. *et al.* Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J. Hepatol.* **62**, 1148–1155 (2015).
25. Krssak, M. *et al.* Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes* **53**, 3048–3056 (2004).
26. Lomonaco, R. *et al.* Metabolic impact of nonalcoholic steatohepatitis in obese patients with type 2 diabetes. *Diabetes Care* **39**, 632–638 (2016).
27. Marchesini, G. *et al.* Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* **50**, 1844–1850 (2001).
28. Utzschneider, K. M. *et al.* Insulin resistance is the best predictor of the metabolic syndrome in subjects with a first-degree relative with type 2 diabetes. *Obesity (Silver Spring)* **18**, 1781–1787 (2010).
29. Iozzo, P. *et al.* Defective liver disposal of free fatty acids in patients with impaired glucose tolerance. *J. Clin. Endocrinol. Metab.* **89**, 3496–3502 (2004).
30. Nielsen, S., Guo, Z., Johnson, C. M., Hensrud, D. D. & Jensen, M. D. Splanchnic lipolysis in human obesity. *J. Clin. Invest.* **113**, 1582–1588 (2004).
31. Donnelly, K. L. *et al.* Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* **115**, 1343–1351 (2005).
32. Sanyal, A. J. *et al.* Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* **120**, 1183–1192 (2001).
33. Lomonaco, R. *et al.* Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. *Hepatology* **55**, 1389–1397 (2012).
34. Fabbrini, E. *et al.* Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* **134**, 424–431 (2008).
35. Lambert, J. E., Ramos-Roman, M. A., Browning, J. D. & Parks, E. J. Increased *de novo* lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* **146**, 726–735 (2014).

36. Petersen, K. F. *et al.* The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc. Natl. Acad. Sci. USA* **104**, 12587–12594 (2007).
37. Roden, M. *et al.* Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. *Diabetes* **49**, 701–707 (2000).
38. Shulman, G. I. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N. Engl. J. Med.* **371**, 2237–2238 (2014).
39. Szendroedi, J. *et al.* Role of diacylglycerol activation of PKCθ in lipid-induced muscle insulin resistance in humans. *Proc. Natl. Acad. Sci. USA* **111**, 9597–9602 (2014).
40. Samuel, V. T. *et al.* Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J. Biol. Chem.* **279**, 32345–32353 (2004).
41. Kumashiro, N. *et al.* Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc. Natl. Acad. Sci. USA* **108**, 16381–16385 (2011).
42. Magkos, F. *et al.* Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology* **142**, 1444–1446.e2 (2012).
43. Unger, R. H. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends Endocrinol. Metab.* **14**, 398–403 (2003).
44. Szendroedi, J. *et al.* Lower fasting muscle mitochondrial activity relates to hepatic steatosis in humans. *Diabetes Care* **37**, 468–474 (2014).
45. Szendroedi, J. *et al.* Abnormal hepatic energy homeostasis in type 2 diabetes. *Hepatology* **50**, 1079–1086 (2009).
46. Schmid, A. I. *et al.* Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. *Diabetes Care* **34**, 448–453 (2011).
47. Schmid, A. I. *et al.* Quantitative ATP synthesis in human liver measured by localized ^{31}P spectroscopy using the magnetization transfer experiment. *NMR Biomed.* **21**, 437–443 (2008).
48. Koliaki, C. *et al.* Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab.* **21**, 739–746 (2015).
49. Fritsch, M. *et al.* Time course of postprandial hepatic phosphorus metabolites in lean, obese, and type 2 diabetes patients. *Am. J. Clin. Nutr.* **102**, 1051–1058 (2015).
50. Cortez-Pinto, H. *et al.* Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* **282**, 1659–1664 (1999).
51. Satapati, S. *et al.* Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. *J. Clin. Invest.* **125**, 4447–4462 (2015).
52. Perez-Carreras, M. *et al.* Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* **38**, 999–1007 (2003).
53. Serviddio, G. *et al.* Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia-reperfusion injury. *Gut* **57**, 957–965 (2008).
54. Morris, E. M., Rector, R. S., Thyfault, J. P. & Ibdah, J. A. Mitochondria and redox signaling in steatohepatitis. *Antioxid. Redox Signal.* **15**, 485–504 (2011).
55. Romestaing, C. *et al.* Mitochondrial adaptations to steatohepatitis induced by a methionine- and choline-deficient diet. *Am. J. Physiol. Endocrinol. Metab.* **294**, E110–E119 (2008).
56. Titchenell, P. M., Chu, Q., Monks, B. R. & Birnbaum, M. J. Hepatic insulin signalling is dispensable for suppression of glucose output by insulin *in vivo*. *Nat. Commun.* **6**, 7078 (2015).
57. Haeusler, R. A. *et al.* Integrated control of hepatic lipogenesis versus glucose production requires FoxO transcription factors. *Nat. Commun.* **5**, 5190 (2014).
58. Lu, M. *et al.* Insulin regulates liver metabolism *in vivo* in the absence of hepatic Akt and Foxo1. *Nat. Med.* **18**, 388–395 (2012).
59. Perry, R. J. *et al.* Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell* **160**, 745–758 (2015).
60. Romeo, S. *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **40**, 1461–1465 (2008).
61. He, S. *et al.* A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J. Biol. Chem.* **285**, 6706–6715 (2010).
62. Donati, B. *et al.* The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. *Hepatology* **63**, 787–798 (2016).
63. Kozlitina, J. *et al.* Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **46**, 352–356 (2014).
64. Zhou, Y. *et al.* Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J. Hepatol.* **62**, 657–663 (2015).
65. Lee, H. Y. *et al.* Apolipoprotein CIII overexpressing mice are predisposed to diet-induced hepatic steatosis and hepatic insulin resistance. *Hepatology* **54**, 1650–1660 (2011).
66. Amaro, A. *et al.* Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. *Gastroenterology* **139**, 149–153 (2010).
67. Tilg, H. & Moschen, A. R. Inflammatory mechanisms in the regulation of insulin resistance. *Mol. Med.* **14**, 222–231 (2008).
68. Johnson, A. M. & Olefsky, J. M. The origins and drivers of insulin resistance. *Cell* **152**, 673–684 (2013).
69. Moller, D. E. & Kaufman, K. D. Metabolic syndrome: a clinical and molecular perspective. *Annu. Rev. Med.* **56**, 45–62 (2005).
70. Morris, E. M., Rector, R. S., Thyfault, J. P. & Ibdah, J. A. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J. Clin Invest.* **94**, 1543–1549 (1994).
71. Samuel, V. T. & Shulman, G. I. Mechanisms for insulin resistance: common threads and missing links. *Cell* **148**, 852–871 (2012).
72. Wieser, V., Moschen, A. R. & Tilg, H. Inflammation, cytokines and insulin resistance: a clinical perspective. *Arch. Immunol. Ther. Exp. (Warsz)* **61**, 119–125 (2013).
73. Tilg, H. & Diehl, A. M. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N. Engl. J. Med.* **343**, 1467–1476 (2000).
74. Le, K. A. *et al.* Subcutaneous adipose tissue macrophage infiltration is associated with hepatic and visceral fat deposition, hyperinsulinemia, and stimulation of NF-κB stress pathway. *Diabetes* **60**, 2802–2809 (2011).
75. Yuan, M. *et al.* Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ. *Science* **293**, 1673–1677 (2001).
76. Cai, D. *et al.* Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. *Nat. Med.* **11**, 183–190 (2005).
77. Arkan, M. C. *et al.* IKK-β links inflammation to obesity-induced insulin resistance. *Nat. Med.* **11**, 191–198 (2005).
78. Kiechl, S. *et al.* Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat. Med.* **19**, 358–363 (2013).
79. Kong, Y. Y. *et al.* Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* **402**, 304–309 (1999).
80. Goto, H. *et al.* Primary human bone marrow adipocytes support TNF-α-induced osteoclast differentiation and function through RANKL expression. *Cytokine* **56**, 662–668 (2011).
81. Lee, N. K. *et al.* Endocrine regulation of energy metabolism by the skeleton. *Cell* **130**, 456–469 (2007).
82. Moschen, A. R. *et al.* Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor α expression. *Gut* **59**, 1259–1264 (2010).
83. Moschen, A. R. *et al.* Adipose and liver expression of interleukin (IL)-1 family members in morbid obesity and effects of weight loss. *Mol. Med.* **17**, 840–845 (2011).
84. Cao, H. *et al.* Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* **17**, 768–778 (2013).
85. Tilg, H. & Moschen, A. R. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* **52**, 1836–1846 (2010).
86. Mathis, D. Immunological goings-on in visceral adipose tissue. *Cell Metab.* **17**, 851–859 (2013).
87. Okada-Iwabu, M. *et al.* A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. *Nature* **503**, 493–499 (2013).
88. Angulo, P. Nonalcoholic fatty liver disease. *N. Engl. J. Med.* **346**, 1221–1231 (2002).
89. Matthews, D. R. *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419 (1985).
90. Lee, D. H. *et al.* Gamma-glutamyltransferase and diabetes—a 4 year follow-up study. *Diabetologia* **46**, 359–364 (2003).
91. Hanley, A. J. *et al.* Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes* **54**, 3140–3147 (2005).
92. Goessling, W. *et al.* Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology* **135**, 1935–1944.e1 (2008).
93. Fraser, A. *et al.* Alanine aminotransferase, γ-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. *Diabetes Care* **32**, 741–750 (2009).
94. Fracanzani, A. L. *et al.* Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* **48**, 792–798 (2008).
95. Armstrong, M. J., Adams, L. A., Canbay, A. & Syn, W. K. Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology* **59**, 1174–1197 (2014).
96. Sung, K. C., Wild, S. H. & Byrne, C. D. Resolution of fatty liver and risk of incident diabetes. *J. Clin. Endocrinol. Metab.* **98**, 3637–3643 (2013).
97. Pais, R. *et al.* A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J. Hepatol.* **59**, 550–556 (2013).
98. Ekstedt, M. *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* **44**, 865–873 (2006).
99. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) & European Association for the Study of Obesity (EASO) EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J. Hepatol.* **64**, 1388–1402 (2016).
100. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) & European Association for the Study of Obesity (EASO) EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* **59**, 1121–1240 (2016).
101. Portillo-Sanchez, P. *et al.* High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J. Clin. Endocrinol. Metab.* **100**, 2231–2238 (2015).
102. Poonawala, A., Nair, S. P. & Thuluvath, P. J. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* **32**, 689–692 (2000).
103. Ghouri, N., Preiss, D. & Sattar, N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology* **52**, 1156–1161 (2010).
104. Leite, N. C., Salles, G. F., Araujo, A. L., Villela-Nogueira, C. A. & Cardoso, C. R. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int.* **29**, 113–119 (2009).
105. Doycheva, I. *et al.* Non-invasive screening of diabetics in primary care for NAFLD and advanced fibrosis by MRI and MRE. *Aliment. Pharmacol. Ther.* **43**, 83–95 (2016).
106. Abrams, G. A., Kunde, S. S., Lazenby, A. J. & Clements, R. H. Portal fibrosis and hepatic steatosis in morbidly obese subjects: a spectrum of nonalcoholic fatty liver disease. *Hepatology* **40**, 475–483 (2004).
107. Goh, G. B. *et al.* Clinical spectrum of non-alcoholic fatty liver disease in diabetic and non-diabetic patients. *BBA Clin.* **3**, 141–145 (2015).
108. Bazick, J. *et al.* Clinical model for NASH and advanced fibrosis in adult patients with diabetes and NAFLD: guidelines for referral in NAFLD. *Diabetes Care* **38**, 1347–1355 (2015).
109. Morling, J. R. *et al.* Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: the Edinburgh type 2 diabetes study. *J. Hepatol.* **60**, 384–391 (2014).

110. Angulo, P. *et al.* Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* **149**, 389–397.e10 (2015).
111. Angulo, P., Keach, J. C., Batts, K. P. & Lindor, K. D. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* **30**, 1356–1362 (1999).
112. Hossain, N. *et al.* Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin. Gastroenterol. Hepatol.* **7**, 1224–1229, 1229.e1–1229.e2 (2009).
113. Adams, L. A. *et al.* Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. *Am. J. Gastroenterol.* **105**, 1567–1573 (2010).
114. Targher, G. *et al.* Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* **30**, 1212–1218 (2007).
115. Targher, G. *et al.* Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/ laser-treated retinopathy in type 2 diabetic patients. *Diabetologia* **51**, 444–450 (2008).
116. Byrne, C. D. & Targher, G. NAFLD: a multisystem disease. *J. Hepatol.* **62** (Suppl. 1), S47–S64 (2015).
117. Marengo, A., Rosso, C. & Bugianesi, E. Liver cancer: connections with obesity, fatty liver, and cirrhosis. *Annu. Rev. Med.* **67**, 103–117 (2016).
118. Bugianesi, E. *et al.* Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* **123**, 134–140 (2002).
119. Oberaigner, W. *et al.* Increased cancer incidence risk in type 2 diabetes mellitus: results from a cohort study in Tyrol/Austria. *BMC Public Health* **14**, 1058 (2014).
120. Dyson, J. *et al.* Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J. Hepatol.* **60**, 110–117 (2014).
121. Zheng, Z. *et al.* Diabetes mellitus is associated with hepatocellular carcinoma: a retrospective case-control study in hepatitis endemic area. *PLoS ONE* **8**, e84776 (2013).
122. Yasui, K. *et al.* Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* **9**, 428–433 (2011).
123. Welzel, T. M. *et al.* Population-attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Am. J. Gastroenterol.* **108**, 1314–1321 (2013).
124. Aleksandrova, K. *et al.* Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. *Hepatology* **60**, 858–871 (2014).
125. Chiang, C. H. *et al.* The relationship of diabetes and smoking status to hepatocellular carcinoma mortality. *Medicine (Baltimore)* **95**, e2699 (2016).
126. Raff, E. J. *et al.* Diabetes mellitus predicts occurrence of cirrhosis and hepatocellular cancer in alcoholic liver and non-alcoholic fatty liver diseases. *J. Clin. Transl Hepatol.* **3**, 9–16 (2015).
127. Musso, G., Cassader, M., Rosina, F. & Gambino, R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* **55**, 885–904 (2012).
128. Phielix, E., Szendroedi, J. & Roden, M. The role of metformin and thiazolidinediones in the regulation of hepatic glucose metabolism and its clinical impact. *Trends Pharmacol. Sci.* **32**, 607–616 (2011).
129. Bugianesi, E. *et al.* A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am. J. Gastroenterol.* **100**, 1082–1090 (2005).
130. Haukeland, J. W. *et al.* Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand. J. Gastroenterol.* **44**, 853–860 (2009).
131. Shields, W. W., Thompson, K. E., Grice, G. A., Harrison, S. A. & Coyle, W. J. The effect of metformin and standard therapy versus standard therapy alone in nondiabetic patients with insulin resistance and nonalcoholic steatohepatitis (NASH): a pilot trial. *Therap. Adv. Gastroenterol.* **2**, 157–163 (2009).
132. Lavine, J. E. *et al.* Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* **305**, 1659–1668 (2011).
133. Torres, D. M. *et al.* Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open- label trial. *Hepatology* **54**, 1631–1639 (2011).
134. Franciosi, M. *et al.* Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. *PLoS ONE* **8**, e71583 (2013).
135. Phielix, E. *et al.* Effects of pioglitazone versus glimepiride exposure on hepatocellular fat content in type 2 diabetes. *Diabetes Obes. Metab.* **15**, 915–922 (2013).
136. Belfort, R. *et al.* A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N. Engl. J. Med.* **355**, 2297–2307 (2006).
137. Aithal, G. P. *et al.* Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* **135**, 1176–1184 (2008).
138. Cusi, K. *et al.* Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. *Ann. Intern. Med.* **165**, 305–315 (2016).
139. Ratziu, V. *et al.* Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* **135**, 100–110 (2008).
140. Ratziu, V. *et al.* Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. *Hepatology* **51**, 445–453 (2010).
141. Fruci, B., Giuliano, S., Mazza, A., Malaguarnera, R. & Belfiore, A. Nonalcoholic fatty liver: a possible new target for type 2 diabetes prevention and treatment. *Int. J. Mol. Sci.* **14**, 22933–22966 (2013).
142. Cui, J. *et al.* Sitagliptin versus placebo for nonalcoholic fatty liver disease: a randomized controlled trial. *J. Hepatol.* **65**, 369–376 (2016).
143. Singh, S., Khera, R., Allen, A. M., Murad, M. H. & Loomba, R. Comparative effectiveness of pharmacological interventions for nonalcoholic steatohepatitis: a systematic review and network meta-analysis. *Hepatology* **62**, 1417–1432 (2015).
144. Stefan, N. *et al.* Inhibition of 11β-HSD1 with RO5093151 for non-alcoholic fatty liver disease: a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* **2**, 406–416 (2014).
145. Cariou, B. & Staels, B. GFT505 for the treatment of nonalcoholic steatohepatitis and type 2 diabetes. *Expert Opin. Investig. Drugs* **23**, 1441–1448 (2014).
146. Ratziu, V. *et al.* Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and -δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* **150**, 1147–1159 (2016).
147. Perry, R. J., Zhang, D., Zhang, X. M., Boyer, J. L. & Shulman, G. I. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. *Science* **347**, 1253–1256 (2015).
148. Caiazzo, R. *et al.* Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study. *Ann. Surg.* **260**, 893–898; discussion 898–899 (2014).
149. Lassailly, G. *et al.* Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology* **149**, 379–388; quiz e15–e16 (2015).
150. Thaler, H. [Fatty liver, its causes and concomitant diseases]. *Dtsch Med. Wochenschr.* **87**, 1049–1055 (in German) (1962).

Author contributions

H.T. and M.R. researched data for the article. A.R.M., H.T. and M.R. contributed to discussion of content, and wrote, reviewed and edited the manuscript before submission.

Competing interests statement

The authors declare no competing interests.