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Metabolic liver disease in diabetes – From mechanisms to clinical trials

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Abstract

Non-alcoholic fatty liver disease (NAFLD) comprises fatty liver (steatosis), non-alcoholic steatohepatitis (NASH) and fibrosis/cirrhosis and may lead to end-stage liver failure or hepatocellular carcinoma. NAFLD is tightly associated with the most frequent metabolic disorders, such as obesity, metabolic syndrome, and type 2 diabetes mellitus (T2DM). Both multisytem diseases share several common mechanisms. Alterations of tissue communications include excessive lipid and later cytokine release by dysfunctional adipose tissue, intestinal dysbiosis and ectopic fat deposition in skeletal muscle. On the hepatocellular level, this leads to insulin resistance due to abnormal lipid handling and mitochondrial function. Over time, cellular oxidative stress and activation of inflammatory pathways, again supported by multigene crosstalk, determine NAFLD progression. Recent studies show that particularly the severe insulin resistant diabetes (SIRD) subgroup (cluster) associates with NAFLD and its accelerated progression and increases the risk of diabetes-related cardiovascular and kidney diseases, underpinning the critical role of insulin resistance. Consequently, lifestyle modification and certain drug classes used to treat T2DM have demonstrated effectiveness for treating NAFLD, but also some novel therapeutic concepts may be beneficial for both NAFLD and T2DM. This review addresses the bidirectional relationship between mechanisms underlying T2DM and NAFLD, the relevance of novel biomarkers for improving the diagnostic modalities and the identification of subgroups at specific risk of disease progression. Also, the role of metabolism-related drugs in NAFLD is discussed in light of the recent clinical trials. Finally, this review highlights some challenges to be addressed by future studies on NAFLD in the context of T2DM.

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1. Introduction

Nonalcoholic fatty liver diseases (NAFLD) is currently defined by lipid deposition that exceeds >5% of hepatocytes, as assessed from liver biopsy, and/or by >5.6% hepatocellular fat content per liver weight, as assessed from magnetic resonance (MR) methods, in the absence of significant alcohol consumption and other causes of fatty liver [1,2]. NAFLD, which affects about 25% of the population [3], comprises a broad range of abnormalities ranging from simple fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH), characterized by inflammation, necrosis, and hepatocellular ballooning, and progression to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [2].

Some gene variants promote risk of NAFLD by altering lipid droplet formation and de novo lipogenesis (DNL), such as variants of patatin-like phospholipase domain-containing protein 3 (PNPLA3) and glucoki-

nase regulatory protein [4], or by decreasing very-low-density lipoproteins (VLDL) export as shown for a missense mutation (E167K) in transmembrane 6 superfamily member 2 (TM6SF2) [5].

Nevertheless, NAFLD is tightly associated with common acquired metabolic diseases such as obesity and type 2 diabetes (T2DM). The mutual relationship between both diseases is illustrated by several epidemiological data. The prevalence of steatosis and NASH has been estimated to be 50 and 56%, respectively, in T2DM [6]. The age-adjusted relative risk of NAFLD is about 5.36-fold higher in T2DM compared to healthy humans [7]. T2DM is also an emerging risk factor for NASH progression to advanced fibrosis, cirrhosis and HCC [8,9]. Diabetes was even a better predictor for HCC development in people with NASH and cirrhosis compared to other metabolic risk factors such as hyperlipidemia, body mass index (BMI) and hypertension [10]. Recently, a consensus panel has proposed to rename NAFLD a metabolic dysfunction-associated fatty liver disease (MAFLD) based on the presence of overweight/obesity, T2DM and evidence of so-called “metabolic dysregulation” [11]. Future will tell, if this will help to better understand the multiple relationships between NAFLD and T2DM. In this context, NAFLD per se associates with more than double risk of incident diabetes.

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pointing to specific liver-related mechanisms [12,13]. Moreover, multi-center studies in Scandinavia and Germany have recently found that diabetes can be stratified into subtypes (clusters) with different clinical and metabolic features [14,15]. The German Diabetes Study (GDS) found that only the severe insulin-resistant diabetes cluster (SIRD) had increased prevalence of NAFLD and its progression along with higher risk of diabetic-kidney disease and cardiovascular disease (CVD) [14]. Indeed, presence of T2DM in NAFLD results in a 2.3 and 2.8 fold hazard ratio for overall and CVD-related mortality, respectively [16]. Whether NAFLD is also an independent risk factor for CVD [12,17] is currently under debate [18].

Despite discussions on causality, there is evidence for a bi- or even tridirectional relationship at least between subtypes of T2DM, NAFLD, and CVD, which could be linked by insulin resistance [12,19,20]. Several mechanisms of insulin resistance and cellular metabolism can contribute to NAFLD and its progression [20], but also help to develop diagnostic tools and treatment opportunities.

2. Mechanisms and consequences of metabolic alterations

The two-hit hypothesis, which is based on exposure of the liver to a first hit “liver steatosis” followed by a second hit such as oxidative stress does not serve to explain the complexity of NAFLD. The current “multiple- or continuous-hit model” suggests the existence of different and continuous insults, which might be generated in individuals with T2DM or metabolic syndrome due to altered inter-organ crosstalk between liver, adipose tissue, intestine, and skeletal muscle (Fig. 1) [21]. These multiple insults would cooperatively and synergistically drive the development and progression of NAFLD, particularly in genetically predisposed individuals [22]. The earliest events initiating NAFLD may reside in hypercaloric energy-dense dietary habits [23]. A single oral intake of saturated fat has been shown to rapidly induce skeletal muscle, adipose tissue and hepatic insulin resistance along with 70% increased gluconeogenesis and upregulated inflammatory pathways [24]. Chronically positive energy balance will enlarge adipose tissue compartments and lead to dysfunctional adipose tissue with excessive lipolysis, overflow of free fatty acids (FFA) elevated gluconeogenesis and again insulin resistance [25].

2.1. Tissue-crosstalk

2.1.1. Adipose tissue

Early studies in rodents suggested that inflammation in adipose tissue is dispensable for local and systemic insulin resistance [26].
Inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 were several-fold higher in subcutaneous and visceral tissue, as compared to liver in severely obese humans [27]. Mechanistically, inflammatory cytokines trigger insulin resistance through activation of cellular kinases such as c-Jun N-terminal kinase (JNK) and inhibitor kappa B kinase (IKKβ), which phosphorylate serine residues in insulin receptor substrate (IRS)-1 [28], resulting in increased lipolysis and FFA release into the circulation. On the contrary, a recent study demonstrated that obesity-induced insulin resistance preceded inflammation in adipose tissues of mice [29]. Indeed, adipose tissue inflammation might be a protective feedback mechanism as its local inhibition in mice induced ectopic lipid accumulation in liver, glucose intolerance, and systemic inflammation [30].

Besides released inflammatory cytokines and FFA, adipose tissue could still communicate with the liver and muscle through secretion of different adipokines such as adiponectin and leptin. Persons with NASH have lower serum adiponectin compared to those with NAFLD with or without normal liver enzymes [31]. By contrast, the circulating levels of leptin were higher in people with NAFLD and T2DM, probably due to increased leptin resistance, and were associated with disease severity [32].

2.1.2. Skeletal muscle

Increased FFA influx to skeletal muscle promotes accumulation of intramyocellular lipid (IMCL). Reduced mitochondrial oxidation contributes as well to IMCL as shown in aging and insulin-resistant humans [33]. Consequently, skeletal muscle exhibits insulin resistance, which often precedes the onset of T2DM and insulin resistance in the liver [34]. Lipid intermediate metabolites, in particular 1,2 diacylglycerols (DAG), link IMCL to skeletal muscle insulin resistance through activation of protein kinase C-theta (PKCθ) resulting in its translocation from cytoplasm to the plasma membrane [35]. Muscles of insulin-resistant humans with obesity and T2DM showed increased DAG content and PKCθ activity as compared to healthy humans [35]. Mutation studies highlighted serine amino acid residue (Ser1101) of IRS1 to be a substrate for activated PKCθ [36]. As a consequence of skeletal muscle insulin resistance, postprandial energy storage shifts from glycogen synthesis in the muscle into triacylglycerol (TAG) in the liver, promoting NAFLD development [25].

2.1.3. Intestine

Gut microbiome is increasingly recognized as a modulator of liver pathogenesis through what is called the “gut-liver axis” [37]. Distinctive alterations of gut microbiome were reported in humans having NASH and T2DM [38]. The intestinal microbiome alters host metabolism by modulating the production of short-chain fatty acids (SCFA) e.g. butyrate, acetate, and propionate, which have beneficial effects on insulin sensitivity, lipid and glucose metabolism [38]. Also, intestinal dysbiosis could associate with increased intestinal permeability permitting translocation of bacterial lipopolysaccharide (LPS) into the systemic circulation, which could induce fat deposition in the liver, NASH progression, weight gain, and diabetes [39]. Moreover, intestinal microbiome could suppress the expression of fasting-induced adipocyte factor (FIAF) in intestinal epithelium, which functions as an inhibitor of circulating lipoprotein lipase, resulting in increased TAG storage in the peripheral tissues [22]. Also, ethanol-producing microbiome could increase blood alcohol concentration in NASH, which is metabolized in the liver generating high levels of reactive oxygen species (ROS). The last mechanism could explain the histological similarity between NASH and alcoholic steatohepatitis [22]. Furthermore, microbiota metabolize liver-derived primary bile acids into secondary bile acids. The latter are reabsorbed into bloodstream and may act as signaling molecules via a variety of receptors including farnesoid X receptor (FXR), which regulates the transcription of different metabolic genes involved in bile acid synthesis, transport, lipogenesis, and glucose homeostasis [40].

2.2. Hepatocellular mechanisms

2.2.1. Insulin resistance and increased DNL

Insulin resistance in both skeletal muscle and adipose tissues initiates liver steatosis by providing precursors and substrates for DNL and mitochondrial β-oxidation e.g. glucose, FFA and glycerol [25]. Although reesterification of FFA derived from diet and adipose tissue is the dominant contributor to TAG pool in the liver (59%), it did not increase in people with NAFLD. On the other side, DNL-derived FFA contribute by about 26%, which is several-fold higher as compared to individuals without NAFLD (10%) [41].

Later, insulin resistance of the liver develops, resulting in increased gluconeogenesis and elevation of endogenous glucose production (EGP) from the liver, which contributes to fasting hyperglycemia in individuals with T2DM [25]. Insulin signaling inhibits typically hepatic gluconeogenesis through Akt-induced phosphorylation of forhead box (FOXO1) and induces lipogenesis through activation of sterol regulatory element-binding proteins (SREBP1C) and mammalian target of rapamycin complex (mTORC1) pathways. During hepatic insulin resistance, insulin does not suppress gluconeogenesis efficiently, while DNL is preserved or even increased. To explain this discrepancy, pathway-selective hepatic insulin resistance was postulated, which means that only one arm of insulin signaling is defective i.e. Akt/FOXO1 leading to reduced insulin-mediated suppression of gluconeogenesis, whereas insulin-activated SREBP-1C/mTORC1 pathway remains intact and activates lipogenesis [28]. Actually, DNL was reduced after induction of hepatic insulin resistance by feeding mice with a high-fat diet (HFD) [42], which challenges the concept of selective hepatic insulin resistance and suggest the existence of alternate mechanisms that contribute to increased DNL and gluconeogenesis in insulin-resistant liver, for example, hyperinsulinemia, insulin-independent reesterification of adipose tissue-derived FFA, and increased acetyl CoA generation from β-oxidation of FFA, which allosterically activates pyruvate carboxylase enzyme, leading to enhanced gluconeogenesis [25]. In addition, increased blood glucose level can activate carbohydrate response element-binding protein (ChREBP) signaling pathway, thereby stimulating expression of several glycolytic genes, which provide additional metabolic precursors for DNL [43]. In line, ChREBP overexpression induced stearoyl-CoA desaturase 1 (Scd1) expression, an enzyme responsible for the biosynthesis of monounsaturated fatty acids (MUFA), resulting in increased liver fat content [44].

PKC epsilon (PKCe) is crucial in mediating hepatic insulin resistance and once activated by DAG, it translocates to the cell membrane, and phosphorylates specific threonine residue (Thr1160 in human and Thr1150 in mouse) on insulin receptor kinase and inhibition of its tyrosine kinase activity [45] (Fig. 2). In general, both hyperglycemia and toxic lipids such as ceramides, DAG, FFA, and cholesterol can induce deleterious effects on liver cells (glucolipotoxicity), which might initiate NAFLD progression from simple steatosis to NASH and fibrosis via various mechanisms, including cell death, oxidative stress, endoplasmic reticulum (ER) stress and mitochondrial disorders [46].

2.2.2. Abnormal mitochondrial function and ER stress

Alterations in the activity and abundance of oxidative phosphorylation (OXPHOS) proteins and antioxidant enzymes were described in various animal models of NAFLD [47]. Impaired hepatic mitochondrial function was evident as well in T2DM and NASH [48,49]. In a mouse model of choline-deficient diet-induced NAFLD, mitochondrial OXPHOS was increased at 12 weeks but lost at a later time point [50]. Also, the higher maximal respiration rate of liver mitochondria was several-fold higher in insulin-resistant obese individuals with fatty liver as compared to lean individuals [51]. These studies highlight the flexibility of mitochondria to adapt to increased metabolic inputs to keep energy homeostasis and to protect against the harmful effects of increased FFA and TAG in the liver. In NASH, mitochondrial flexibility was lost,
which was associated with increased ROS production and exhaustion of protective antioxidant enzymes [51] (Fig. 2). Whether loss of mitochondrial flexibility is a cause or consequence for NAFLD progression remains obscure. Depletion of ATP due to mitochondrial disorders, together with hyperglycemia and lipid overload could be inducers for another signaling pathway, termed unfolded protein response “UPR”, which is adaptive response to resolve unfolded or misfolded proteins together with hyperglycemia and lipid overload could be inducers for another signaling pathway, termed unfolded protein response “UPR”, which is adaptive response to resolve unfolded or misfolded proteins.

2.3.2. Autophagy

Autophagy is a self-degradative process which is used by the cells to remove misfolded proteins and damaged organelles [65]. Singh et al. showed that the cells can use autophagic process as lipolytic mechanism to mobilize lipids from intracellular lipid store, which is termed in this case “macrolipophagy” [66]. Various in vitro and in vivo studies showed that autophagy was decreased in fatty hepatocytes [67]. Impairment of autophagy by palmitic acid in macrophages induces in

3. Diagnostic biomarkers

The liver biopsy is considered the gold standard for NAFLD diagnosis, especially for distinguishing steatosis from inflammation and fibrosis.
Nevertheless, this technique has several limitations not only resulting from the invasive procedure and rare post-interventional complications, but also due to the small tissue volume, which might not be representative for the whole liver and may not take into account inhomogeneous distribution of NAFLD-associated alterations. Novel imaging modalities such as ultrasound- or MR-based techniques are of increasing value, as recently reviewed [71], but are still not generally available, so that there is an urgent need for noninvasive screening tools.

### 3.1. Current biomarkers and indices

Biomarker-based scores such as the fatty liver index (FLI) have been developed to diagnose steatosis using routine laboratory and anthropometric data [2], but are of limited value, also due to the increasing availability of precise imaging tools [72].

Non-invasive detection of NASH and fibrosis remains challenging today. Biomarker-based panels such as aspartate aminotransferase (AST)/platelets count ratio index, fibrosis-4 (FIB-4) index, FibroTest, FibroSpect II, and NAFLD fibrosis score (NFS) offer variable diagnostic efficacy for assessing different stages of liver fibrosis [73,74]. Although combination of these panels could enhance their predictive values [73], they still suffer from limited accuracy even compared to the alanine aminotransferase (ALT) and AST, particularly in T2DM [75]. In this regard, transient elastography looks like a promising alternative in diabetes clinics for detection of liver fibrosis in NAFLD [74].

Numerous biomarkers have been developed to specifically track features of NASH progression and fibrosis. Cytokeratin (CK) 18, an intermediate filament protein that is cleaved during cell death to CK18 M30 and CK18 M65, has been intensively investigated as a surrogate of NASH-associated liver cell damage, but a recent meta-analysis of 25 studies reported a maximum sensitivity of 0.75 for NASH diagnosis [76] and suboptimal diagnostic value was shown in T2DM [75]. The ECM turnover marker, type III procollagen, can offer a diagnostic efficacy of 0.81 and 0.88 to detect moderate and advanced liver fibrosis in T2DM, respectively [77].

In 2016, the European Associations for the Study of the Liver, Obesity and Diabetes (EASL-EASO-EASD) jointly released recommendations for diagnosis and monitoring of disease severity in persons with suspected NAFLD and metabolic risk factors [78]. People with metabolic risk factors, such as T2DM, should undergo assessment by abdominal ultrasound, serum transaminases and fibrosis markers (e.g. FIB-4, NFS).
Elevated transaminases or steatosis plus abnormal fibrosis test shall require referral to a specialist. This strategy has raised the question of a possible overreferral when adhering to these guidelines [79]. However, recent analyses show that a refined strategy of specific combining indices such as FIB-4 and NAFLD-Fibrosis score (NAF) could reduce the number of people with T2DM for further diagnostic work up to a reasonable size [80]. This retrospective analysis also found that certain non-invasive biomarkers are consistently associated with different patterns of diabetes-related complications.

### 3.2. Novel biomarkers

Analyses of the plasma metabolomic of insulin-resistant individuals with NAFLD suggested that bile salts, e.g. glycocholate, taurocholate, and glycinecholate allow to detect NASH in one study [81], while another study reported an association with insulin resistance but not with liver necroinflammation [82]. Also amino acids, specifically a glutamate-serine-glycine index, was associated with hepatic insulin resistance and transaminases and discriminated individuals with fibrosis grade 3–4 from those with grade 0–2 independently of BMI [83]. The diagnostic performance of a serum-based lipidomic analysis was substantially lower for NAFLD detection in T2DM compared to healthy individuals [84]. Nevertheless, certain sphingolipid species were recently found to be increased in insulin-resistant NASH and to correlate with hepatic oxidative stress and inflammation [85].

One circulating small noncoding RNA, miRNA-122, was found to be >5.7fold in steatosis and further doubled in NASH [86]. This miRNA was also higher in T2DM with NAFLD than in those without NAFLD and provided better prediction of NAFLD when combined with LDL and waist-to-hip ratio [87]. Moreover, extracellular vesicles (EV), which among other cargo also transport miRNA, may be promising biomarkers for NAFLD as shown by higher serum levels in people with NAFLD, obesity and diabetes [180].

Recently, metagenomics data derived from gut microbiota alterations allowed to detect advanced fibrosis in 86 NAFLD patients, of whom 23% had T2DM, with a robust diagnostic accuracy (AUROC: 0.936) [88].

### 4. Treatment of NAFLD in T2DM

The current guidelines recommend only lifestyle modification and - for certain groups - bariatric or metabolic surgery to treat NAFLD [1,2]. Although the numerous activities in this field, no pharmacological treatment is currently approved or expecting approval for the use in NAFLD with or without concomitant T2DM (Tables 1 and 2), except for obeticholic acid (OCA). Marketing authorization application for OCA has been submitted to the U.S. Food and Drug Administration (F DA) and European Medicines Agency (EMA), awaiting FDA decision by June 2020 [89]. Against this background, current guidelines and recommendations primarily advise lifestyle modification (healthy nutrition and exercise) and weight loss in overweight/obese persons. The EASL-EASD guidelines recommend to consider pharmacological treatment in people with NASH when combined with fibrosis and in those with less severe disease, but high risk conditions for disease progression such as T2DM [78]. The American Association for the Study of Liver Diseases (AASLD) recommends to limit pharmacological treatment to those with biopsy-proven NASH and fibrosis [1].

#### 4.1. Strategies to induce weight loss

##### 4.1.1. Lifestyle modification

Weight management and physical exercise are key to the treatment of both NAFLD and T2DM. A proof-of-concept study showed that a hypocaloric diet with weight loss of −8 kg within 12 weeks not only normalized fasting EGP and thereby hyperglycemia, but decreased hepatic TAG content down to normal concentrations in obese T2DM humans with NAFLD [90]. Subsequent studies in larger cohorts extended these results by demonstrating that a very low-caloric diet with weight loss of about 15% rapidly normalized liver fat content in 90 individuals with T2DM, which persisted for one year if weight loss was maintained [91]. Interestingly, Mediterranean, low-saturated fat and high plant-based protein diets also improve steatosis in NAFLD combined with T2DM despite minor or no relevant weight loss [92,93]. In addition, physical activity and exercise training interventions can also decrease liver fat content, which may not be exclusively depending on concomitant weight loss [94]. Although the beneficial effects of structured behavioral treatment to improve histological endpoints, likely extend beyond reduction of hepatic fat content to ameliorating the grade and stage of inflammation and fibrosis [95], only a minority of the people manages to adhere to dietary weight loss and exercise programs, which raises the issue of other therapeutic approaches [94].

##### 4.1.2. Antiobesity drugs

Weight-loss inducing drugs could be an attractive option for persons with NAFLD with a BMI >30 kg/m² or >27 kg/m² in the presence of at least one metabolic comorbidity, as an adjunct treatment to lifestyle modifications [96,97]. Currently, the most-popular weight-loss inducing medications associated with at least 5% body weight decrease in one year as compared to placebo are orlistat, the fixed combination of phentermine and topiramate or naltrexone and bupropion, and the glargonan-like peptide-1 (GLP-1) receptor agonist (GLP-1ra), liraglutide [97]. Of these drugs, orlistat treatment failed to improve liver histology when compared to placebo [98], while no reports are available for topiramate, naltrexone, bupropion and phentermine regarding hepatic endpoints in humans with NAFLD [99]. Only liraglutide treatment associated with the resolution of NASH without worsening of fibrosis in overweight/obese persons (mean values; liraglutide 34.2 g vs. placebo 37.7 kg/m²) [100]. In addition, novel GLP-1ra and dual agonists with superior efficacy for weight loss are being tested in preclinical and early clinical trials.

##### 4.1.3. Bariatric surgery

Bariatric or metabolic surgery is a therapeutic option to induce sustained weight loss particularly in people with combined NAFLD and T2DM. In obese humans, bariatric surgery resulted in 85% resolution of NASH within one year [101] and in 77% complete remission of T2DM [102]. Surgical weight loss improves glucose metabolism and insulin sensitivity by different mechanisms such as increased GLP-1 secretion [103] and epigenetic modification [104,105].

##### 4.2. Antihyperglycemic drugs

Several antihyperglycemic drug classes were or are currently being investigated in clinical trials and preclinical models to evaluate their efficacy for people with NAFLD and with or without T2DM.

##### 4.2.1. Metformin

Metformin reduces body weight, hepatic gluconeogenesis - by yet unclear mechanisms [106], and the risk of macrovascular complications, which is still controversially discussed due to lack of optimally designed clinical trials [107,108]. Despite its action on hepatic metabolism, former small-scale studies reported conflicting results [109,110] (Table 1) and recent metaanalysis failed to demonstrate any effect of metformin on liver histology [111]. Nevertheless, epidemiological, observational and preclinical studies suggest that metformin may reduce the risk of HCC and also in T2DM, possibly by promoting apoptosis, but controlled clinical trials are not available [112].

##### 4.2.2. Dipeptidyl peptidase-4 (DPP-4) inhibitors (DPP-4i)

DPP4 degrades incretins such as GLP-1 so that DPP-4i treatment increases the postprandial levels of endogenous GLP-1, which leads to
Table 1
Placebo-controlled double-blind RCTs investigating the effects of drugs approved for T2DM on NAFLD by MR-based methods or liver histology.

| Study | Patient characteristics | Intervention (n) | Dosage/duration | Metabolic effects | Liver effects |
|-------|-------------------------|-----------------|-----------------|-------------------|--------------|
|       |                         | MET+             | MET 2 g, VIT E 800 IU daily/12 months | ↓ With MET and diet | MET (but not VIT E and diet changes) ↓ liver fat (assessed histologically), necroinflammation, and fibrosis |
| Bugianesi et al., 2005 Ref. [110] | Biopsy-proven NASH, 4–12% had T2DM | MET (55) vs VIT E (28) and WT-reducing diet (27) |  | ↓ | ↓ |
| Haukeland et al., 2009 Ref. [109] | Biopsy-proven NAFLD, ~50% with prediabetes or T2DM, HbA1c = -5.7 ± 0.6% | MET (24) vs PL (24) | Initial 0.5 mg and titrated to 2.5 or 3 g daily/6 months | ↓ | ↓ |
| Cui et al., 2016 Ref. [115] | MRI-diagnosed NAFLD with prediabetes or controlled diabetes, HbA1c = -5.7–8% | SITA (25) vs PL (25) | 100 mg daily/24 weeks | ↔ | ↔ |
| Joy et al., 2017 Ref. [116] | T2DM with liver biopsy, NAFLD, HbA1c = -7.9 ± 1% | SITA (6) vs PL (6) | 100 mg daily/24 weeks | ↔ | ↓ |
| Macauley et al., 2015 Ref. [117] | T2DM on stable MET therapy with HbA1c ≤ 7.6% | VILDA (22) vs PL (22) | 50 mg twice daily/6 months | ↓ (p = 0.08) | ↓ |
| Armstrong et al., 2016 Ref. [120] | Biopsy-confirmed NASH, 31–35% had T2DM, HbA1c = -5.9 ± 0.7% | LIRA (26) vs PL (26) | 1.8 mg daily/48 weeks | ↓ | ↓ |
| Bizino et al., 2020 Ref. [121] | Controlled T2DM with short known disease duration, HbA1c = -6.6 ± 0.5% | LIRA (24) vs PL (26) | 1.8 mg daily/26 weeks | ↓ | ↓ |
| Kahl et al., 2020 Ref. [122] | Inadequately controlled T2DM, HbA1c = 7.7 ± 0.7% | EMP (42) vs PL (42) | 25 mg daily/24 weeks | ↓ | ↓ |
| Cusi et al., 2019 Ref. [123] | Biopsy-proven NAFLD, 35–45% had T2DM, HbA1c = -5.9 ± 0.7% | CANA (26) vs PL (30) | Initial 100 mg and titrated to 300 mg daily/6 months | ↓ | ↓ |
| Latva-Rasku et al., 2019 Ref. [124] | T2DM with HbA1c ≥ 6.5–10.5% and ≥3 months of stable treatment with MET and/or DPP4i | DAP (15) vs PL (16) | 10 mg daily/8 weeks | ↓ | ↓ |
| Eriksson et al., 2018 Ref. [125] | T2DM with MRI-diagnosed NAFLD, HbA1c = ~7.38 ± 0.56% | DAP (21) and OM-3CA (20) as monotherapy or combined (22) vs PL (21) | DAP 10 mg, OM-3CA 4 g daily/12 weeks | Only in DAP monotherapy and combined | DAP, OM-3CA, and combined therapy ↓ liver fat (assessed by MRI-PDFF) by 13, 15, and 21% respectively compared to BL. Only combined therapy-mediated PDFF changes was significant compared to PL. Only DAP monotherapy ↓ hepatocytes injury biomarkers. |
lower glucose peaks after meals [113,114]. In individuals with NAFLD with prediabetes or recent onset diabetes, sitagliptin did not improve liver steatosis or liver fibrosis compared to placebo as assessed by MR-based techniques [115]. In line, another recent trial reported no benefits for sitagliptin versus placebo on liver fibrosis or steatohepatitis in T2DM [116] (Table 1). In contrast, a moderate reduction in liver fat content was observed with vildagliptin in individuals with T2DM [117].

### 4.2.3. Incretin mimetics

The actions of endogenous GLP-1 are mimicked by GLP-1 receptor agonists (GLP-1ra), which effectively reduce blood glucose levels and also reduce the risk for CVD in T2DM [118]. In individuals with NAFLD and T2DM, liraglutide in combination with metformin reduced liver, subcutaneous, and visceral fat [119]. Also, liraglutide improved hepatic steatosis measured by MR-based methods as well as resolved biopsy-proven NASH without worsening of fibrosis [100,120] (Table 1). However, a recent subanalysis did not detect an effect of liraglutide on liver fat content quantified by “H-MRS [121] (Table 1). Respective trials with semaglutide and dulaglutide are still waiting for results (NCT02970942, NCT03648554). In an animal model of T2DM, exendinate also counteracted HCC development by suppression of STAT3-regulated genes [122].

Glucagon-like peptide-1 (GLP-1) receptors are a family of nuclear receptors composed of multiple isoforms with wide tissue distribution [137]. The PPAR-γ agonist pioglitazone had convincing efficacy on NASH resolution in individuals with and without T2DM, but with conflicting results on fibrosis [138–140] (Table 1). Of note, pioglitazone has been withdrawn in many European countries because of its disadvantageous safety profile [181]. However, pioglitazone exerts beneficial effects on cardiovascular outcomes in persons with T2DM and a history of CVD [141].

### 4.2.4. Sodium glucose cotransporter-2 inhibitors (SGLT2i)

SGLT2i inhibit reabsorption of glucose in the proximal renal tubule resulting in glucosuria and calory loss, thus effectively reducing blood glucose level and body weight in T2DM. Moreover, SGLT2is show beneficial effects on cardiovascular risk and progression of nephropathy [118,126]. Most, but not all recent randomized controlled trials (RCTs) showed that SGLT2i treatment resulted in reduction in liver fat content compared to placebo [127–131] (Table 1). In an open-label pilot study in liver-biopsy proven cohort of NASH with T2DM, treatment with empagliflozin for 6 months reduced liver steatosis, ballooning and fibrosis and induced NASH resolution in approximately half of those persons [132]. Animal models further suggested anti-inflammatory, anti-oxidant and pro-apoptotic actions of SGLT2i with cardio-protective effects as well as inhibition of NASH progression and tumor growth of HCC [133,134]. The dual SGLT1/2i, liscigliflozin, is expected to block both intestinal and renal glucose (re)absorption [135] and currently investigated to evaluate its efficacy on resolution of NASH as monotherapy and as combination therapy with the FXR agonist, tropifexor, in people with NASH and fibrosis (NCT04065841). First results hint at improvement of transaminases and reduction of steatosis by liscigliflozin in NASH [136].

### 4.2.5. Peroxisome proliferator-activated receptor (PPAR) agonists

PPARs are a family of nuclear receptors composed of multiple isoforms with wide tissue distribution [137]. The PPAR-γ agonist pioglitazone had convincing efficacy on NASH resolution in individuals with and without T2DM, but with conflicting results on fibrosis [138–140] (Table 1). Of note, pioglitazone has been withdrawn in many European countries because of its disadvantageous safety profile [181]. However, pioglitazone exerts beneficial effects on cardiovascular outcomes in persons with T2DM and a history of CVD [141].
It has been proposed that non-PPAR-γ dependent mechanisms, as the inhibition of the mitochondrial pyruvate carrier (MPC), might mediate the beneficial effects of pioglitazone on NAFLD [142]. However, a novel drug with PPAR-γ sparing effects and MPC binding activity did not improve histological components of NASH in individuals with T2DM compared to placebo [143].

Elafibranor is a dual agonist for PPAR-α and PPAR-β without PPAR-γ stimulation [144]. Based on a post-hoc analysis, NASH was resolved without worsening of fibrosis in a higher percentage of people with and without T2DM in the elafibranor group as compared to the placebo [145] and a follow-up study on these findings has been initiated (Table 2). Recent announcements on an interim analysis state that elafibranor treatment failed to resolve NASH and improve fibrosis when compared to placebo [146].

Saroglitazar is another dual PPAR-α/γ agonist with higher affinity for PPAR-α. In a mouse model of NASH, saroglitazar reduced liver steatosis, inflammation and prevented fibrosis development and a respective clinical trial is ongoing [147] (Table 2). Lanifluran is a pan-PPAR agonist for PPAR-α, β, and γ receptors focused on treatment of liver fibrosis and NASH (NCT03008070). In an animal model of liver fibrosis, lanifluranor decreased hepatic collagen deposition [148].

4.3. Antiinflammatory drugs

Several other strategies for pharmacological targeting of NASH have emerged over the last few years; however, these strategies are not specifically designed for T2DM, but for the whole NAFLD collective. They comprise antiinflammatory drugs, but also modulators of other pathways, which are briefly summarized in the following sections.

4.3.1. Farnesoid X receptor (FXR) agonists

FXR can be activated by bile acids in a negative feedback mechanism to suppress bile acid synthesis [40]. OCA is a potent semisynthetic and selective FXR agonist approved for treatment of primary biliary cholangitis [149]. OCA treatment resulted in histologic improvement of NASH in people with and without T2DM compared to placebo [150,151]; however, concerns were raised about OCA-induced changes in plasma lipoprotein profile [152]. Tropifexor, another potent FXR agonist [153], is currently being tested in combinatorial approaches of NASH treatment (cenicriviroc and licoglibozone; NCT03517540).

4.3.2. Liver X receptor alpha (LXR-α) inhibitors

Oltipraz is a synthetic dithiolethione with antisteatotic effects by inhibiting the activity of LXR-α. It activates adenosine monophosphate activated protein kinase (AMPK) and inactivates S6K1, affecting LXR-α thus reducing lipogenesis and increasing lipid oxidation [154]. A recent 24-week phase 2 clinical trial found decreased steatosis measured by 1H-MRS with oltipraz compared to placebo treatment [154] and a respective phase 3 clinical trial is ongoing (Table 2).

4.3.3. Chemokine receptors (CCR) 2/5 antagonists

Chemokine receptors type 2 (CCR2) and type 5 (CCR5) are expressed on various inflammatory and fibrogenic cells [155]. Cenicriviroc is a CCR2/CCR5 dual antagonist that reduced insulin resistance, liver inflammation and fibrosis in diet-induced models of NASH [62]. In recent RCTs in a NASH cohort with fibrosis, cenicriviroc treatment did not improve NAS but may reduce liver fibrosis [156,157]. Currently, there is an ongoing clinical trial to evaluate the effects of cenicriviroc on fibrosis in NASH (Table 2).

4.4. Modulation of lipid metabolism

4.4.1. Lipid lowering drugs

Both people with obesity, metabolic syndrome or T2DM as well as those with NAFLD are at increased risk for dyslipidemia. Statins, inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, are generally safe and have unmet efficacy to decreased serum LDL and prevent cardiovascular outcomes, despite slightly increasing the risk of T2DM [2]. Use of statins associated with a 46% lower relative risk of hepatic decompensation and mortality in cirrhosis and a trend towards lower fibrosis progression in non-cirrhotic liver diseases [158]. However, the data on liver histology is limited [159] so that statins are not currently recommended for the management of NAFLD [2]. The inhibitor of intestinal cholesterol absorption, ezetimibe, decreased the histological NAS, but not consistently liver fat content in an analysis of the few available studies [160]. Fenofoitate, a PPAR-α agonist, does not affect or even increase liver fat content or volume [161,162]. Nevertheless, the combination of statins with certain anti-NASH drugs, such as OCA, could be beneficial to counteract drug-related increases in LDL during long-term treatment.

4.4.2. Acetyl CoA carboxylase (ACC) 1/2 inhibitors

Inhibition of DNL in NAFLD may be achieved by inhibition of ACC as this enzyme catalyzes the conversion of acetyl CoA into malonyl CoA, which acts as a substrate for fatty acid synthesis and inhibitor for fatty acid β-oxidation [163]. In a recent clinical trial in individuals with NASH and without T2DM, treatment with the dual ACC1 and ACC2 inhibitor GS-0976 decreased liver steatosis without improvement of fibrosis [164]. The major concern of this strategy is that decreased DNL in NAFLD might channel lipids towards other harmful directions, e.g. increased blood lipids [163].

4.4.3. Steroyl-CoA desaturase (SCD) 1 inhibitors

SCD1 is a key enzyme for hepatic lipogenesis that catalyzes the conversion of saturated fatty acids to MUFA and its downregulation protected mice from high carbohydrate-induced liver steatosis [165]. A clinical trial with aramchol, an inhibitor of SCD1, showed a reduction in liver fat content, resolution of NAS and improvement of liver fibrosis in persons with NAFLD with prediabetes or T2DM [166]. These results paved the way for a respective phase 3 clinical trial (Table 2).

4.5. Modulation of energy metabolism

4.5.1. Mitochondrial uncouplers (protonophores)

Mitochondrial uncoupling describes any process that uncouples the electron transport from ATP synthesis in mitochondria [167]. 2,4-Dinitrophenol (DNP) was widely used for the treatment of obesity before its discontinuation due to life-threatening serious adverse events [168]. To overcome the side effects of DNP, improved formulas of DNP were recently developed to decrease the toxic to effective dose ratio (DNP-methyl ether (DNPME) and controlled-release mitochondrial protonophore (CRMP)) [169,170]. Oral CRMP targets mainly the liver due to the first-pass effect and decreased hepatic insulin resistance, hepatic steatosis and liver fibrosis in a methionine-choline deficient rat model of NASH [170]. CRMP was also effective in nonhuman primates with diet-induced NAFLD for reduction of hepatic steatosis and EGP [171].

4.5.2. Thyroid hormone receptor (THR)-β agonists

Thyroid hormone deficiency has been associated with NAFLD development [172]. Thyroid hormone analogues decreased liver fat, liver transaminases, and inflammatory and fibrosis markers in animal models of NASH [172]. Resmetirom is a liver-targeted highly selective THR-β agonist which showed efficacy in reducing liver fat content in NASH with and without T2DM [173]. Currently, there is ongoing clinical trial for evaluation of long-term outcomes of resmetirom and its efficacy on NASH resolution (Table 2).

4.5.3. Vitamin E

Vitamin E is a potent antioxidant [174]. In NASH without T2DM, vitamin E improved NAS without worsening of fibrosis [139]. Despite concerns regarding adverse effects of long term vitamin E usage, treatment...
with vitamin E for ≥2 years reduced the risk of liver failure in a NASH cohort with advanced fibrosis and with or without T2DM [175].

4.6. Modulation of the microbiome

Therapeutic manipulation of intestinal microbiome is still in its infancy. Rodent data showed efficacy for fecal microbiota transplantation in improving NAFLD in a diet-induced NASH model [176]. Also, modulation of the intestinal microbiota by antibiotic treatment reduced liver transaminases in NAFLD [177]. The use of probiotics and prebiotics in obese NAFLD/NASH is currently not supported by high-quality clinical studies with MR- or biopsy-based endpoints [178].

5. Summary and outlook

The evidence for shared pathophysiological mechanisms between T2DM and NAFLD will help to develop strategies for detecting and treating both diseases and preventing their leading complication, CVD. Altered lipid and energy metabolism, insulin resistance, low-grade inflammation, and intestinal dysbiosis represent key targets. In addition to weight loss by lifestyle modification or bariatric surgery, GLP-1ra and SGLT2i are promising antihyperglycemic agents with beneficial effects on NAFLD and CVD. In addition, many metabolomics-based drugs are currently studied comprising PPAR agonists, endocline dual agonists to modulators of hepatic metabolism or microbiota.

Nevertheless, several roadblocks need to be overcome to reduce the burden of NAFLD in T2DM. First, there is still lack of preclinical animal models that encapsulate essential features of human NASH and diabetes. Second, available biomarkers lack diagnostic efficacy to identify NAFLD progression. Innovative strategies such as cluster analysis already enabled detection of a diabetes subtype (SIRD) with high risk of NAFLD [14]. Combination with computational integration of multiomics data shall identify specific disease signatures and pave the way to precision medicine and targeted management of T2DM and NAFLD. Finally, studies on so-called endpoints are scarce, which may be due to the need of long-term studies to evaluate liver-related mortality, to the current neglect to accept CVD morbidity and mortality as NAFLD outcome and to ongoing discussions on the relevance of surrogate markers.

CRediT authorship contribution statement

Bedair Dewidar: Conceptualization, Writing - original draft, Writing - review & editing. Sabine Kahlt: Conceptualization, Writing - original draft, Writing - review & editing. Kalliopi Papili: Writing - original draft, Writing - review & editing. Michael Roden: Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

BD, SK and KP declare no conflicts of interest. MR is on the scientific advisory boards of Allergan, Astra-Zeneca, Bristol-Myers Squibb, Eli Lilly, Gilead Sciences, Inventiva, Intercept Pharma, Novartis, Novo Nordisk, Servier Laboratories, Target Pharmasolutions, and Terra Firma and receives investigator-initiated support from Boehringer Ingelheim, Nutricia/Danone and Sanofi–Aventis.

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