Modulation of Insulin Resistance in Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) has an estimated prevalence of 25% in the general population, and cirrhosis secondary to nonalcoholic steatohepatitis (NASH) is predicted to become the leading cause of liver transplantation, yet there is a lack of effective licensed treatments for these conditions. There is a close relationship between insulin resistance (IR) and NAFLD, with prevalence of NAFLD being 5-fold higher in patients with diabetes compared to those without. IR is implicated both in pathogenesis of NAFLD and in disease progression from steatosis to NASH. Thus, modulation of IR represents a potential strategy for NAFLD treatment. This review highlights key proposed mechanisms linking IR and NAFLD, such as changes in rates of adipose tissue lipolysis and de novo lipogenesis, impaired mitochondrial fatty acid β-oxidation (FAO), changes in fat distribution, alterations in the gut microbiome, and alterations in levels of adipokines and cytokines. Furthermore, this review will discuss the main pharmacological strategies used to treat IR in patients with NAFLD and their efficacy based on recently published experimental and clinical data. These include biguanides, glucagon-like peptide 1 receptor (GLP-1) agonists, dipeptidyl peptidase 4 (DPP-4) inhibitors, peroxisome proliferator-activated receptor (PPAR-γ/α/δ) agonists, sodium glucose cotransporter 2 (SGLT2) inhibitors, and farnesoid X receptor (FXR) agonists, with further novel treatments on the horizon. Ideally, treatment would improve IR, reduce cardiovascular risk, and produce demonstrable improvements in NASH histology—this is likely to be achieved with a combinatorial approach. (Hepatology 2019;70:711-724).

Insulin resistance (IR) and nonalcoholic fatty liver disease (NAFLD) have a close relationship, such that NAFLD is present in up to two thirds of patients with type 2 diabetes mellitus (T2DM). A meta-analysis reported that risk of T2DM was more than 2-fold higher in patients with NAFLD than those without NAFLD (random-effects hazard ratio [HR], 2.22; 95% confidence interval [CI], 1.84-2.60), with the risk being highest in patients with NASH.(1)

This association has functional relevance. In patients with preexisting T2DM, the presence of NAFLD is associated with more pronounced hyperinsulinemia and increased adipose tissue/hepatic IR compared to those without NAFLD, despite the groups being of similar sex, body mass index (BMI), and total body fat. Furthermore, studies have indicated that patients with IR and NAFLD have an increased risk of progression from steatosis to NASH and to fibrosis, as well as an increase in all-cause and liver-related mortality.(2)

Notably, in glycated hemoglobin A1c (HbA1c)- and BMI-matched patients with T2DM, the presence of NAFLD confers an increased risk of cardiovascular events.(3)

This review outlines the mechanisms of IR in NAFLD and pharmacological methods of modulating this for therapeutic benefit.

Abbreviations: ACC, acetyl CoA carboxylase; ALT, alanine aminotransferase; BMI, body mass index; ChREBP, carbohydrate response element-binding protein; CI, confidence interval; CoA, coenzyme A; DAG, diacylglycerol; DPP-4, dipeptidyl peptidase 4 inhibitor; FAO, fatty acid oxidation; FFAs, free fatty acids; FGF-19, fibroblast growth factor 19; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1 receptor agonist; HbA1c, glycated hemoglobin A1c; HCB, hepatocellular ballooning; HOMA-IR, homeostasis model assessment for IR; HR, hazard ratio; HS, hepatic steatosis; IKKβ, inhibitor kappa beta kinase beta; IL-6, interleukin 6; IR, insulin resistance; IRS-1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; OCA, obeticholic acid; OD, once a day; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; RCT, randomized controlled trial; SGLT2, sodium/glucose cotransporter 2; SREBP-1c, sterol receptor-binding protein 1-c; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid cycle; TNF-α, tumor necrosis factor alpha; TZDs, thiazolidinediones.
Mechanisms of IR in NAFLD

Although the exact mechanisms of IR in NAFLD are debated, key hypothetical mechanisms are described below and summarized in Fig. 1.

ADIPOCYTES RELEASE HORMONES THAT INFLUENCE IR

Adipocytes secrete adiponectin, which is positively associated with insulin sensitivity. Serum adiponectin levels are lower in patients with NAFLD than those without NAFLD. Adiponectin-mediated signaling promotes fatty acid β-oxidation (FAO), glucose utilization, and suppression of fatty acid synthesis. Use of an oral synthetic adiponectin receptor agonist in a mouse model of diabetes was associated with improved insulin sensitivity.

ADIPOSE TISSUE INFLAMMATION IS ASSOCIATED WITH IR

Proinflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), are elevated in NAFLD. Whereas these cytokines can be released by hypertrophic adipocytes, their main source is proinflammatory (M1) macrophages in adipose tissue. In obesity, the percentage of resident macrophages in the adipose tissue is increased, with polarization toward an M1 phenotype.

TNF-α promotes adipocyte lipolysis as well as activating various stress-related protein kinases, including c-Jun N-terminal kinase (JNK), and inhibitor of kappa-B kinase beta (IKKB). These then induce serine/threonine–mediated phosphorylation of insulin receptor substrate 1 (IRS-1), which attenuates IRS-1-mediated insulin signaling. In mice, neutralization of TNF-α or its receptor was associated with improved insulin sensitivity. TNF-α antagonism has been associated with reduced IR in observational studies of nondiabetic patients with rheumatoid arthritis and psoriasis, but findings in patients with T2DM have been mixed.

INCREASED SERUM FREE FATTY ACIDS WORSEN IR

Patients with NAFLD have increased levels of serum free fatty acids (FFAs) compared to those

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without NAFLD,(7) attributed to a failure of insulin-mediated suppression of lipolysis, allowing release of excess FFAs into the bloodstream. In NAFLD, preferential distribution of fat into the liver rather than adipose tissue is associated with increased expression of hepatic proteins involved in triacylglycerol uptake, such as fatty acid transport proteins and fatty acid translocase/CD36. FFAs are converted to triacylglycerol by the glycerol-3-phosphate pathway. Adiponectin is an inhibitor of this pathway, but levels of adiponectin are reduced in patients with NAFLD. Diacylglycerol (DAG) and ceramides are metabolites that accumulate in this pathway and can inhibit insulin signaling, via molecules such as protein kinase C (PKC). Fatty acyl-CoA is another metabolite in the G3P pathway and serves as an entry point for mitochondrial β-oxidation. In NAFLD, there is an increase in mitochondrial β-oxidation in response to the increased lipogenesis, but there is concomitant mitochondrial dysfunction, resulting in oxidative stress, which can inhibit insulin signaling via activation of inhibitor of nuclear factor kappa-B (I-κB) kinase subunit beta (IKKβ) and c-Jun N-terminal kinase (JNK). Hyperglycemia can also increase oxidative stress. Inflammatory cytokines, such as those produced by adipocytes and macrophages can also activate (IKKβ), JNK and additionally suppressor of cytokine signaling (SOCS), which can phosphorylate IRS1 and IRS2 to inhibit insulin signaling. IKKβ and JNK can activate nuclear factor kappa-B (NF-κB), causing translocation to the nucleus. Glucose can enter cells via the GLUT-4 transporter protein. An increase in intracellular glucose causes increased activation of carbohydrate response element binding protein (ChREBP), which is involved in lipogenesis from glucose. Gut dysbiosis promotes a pro-inflammatory response in adipose tissue and is associated with impaired mitochondrial β-oxidation. The net effects of the above processes are an increase in inflammation, a reduction in glycogen synthesis, an increase in lipogenesis and an increase in blood insulin and blood glucose levels.

Abbreviations: AKT, protein kinase B; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide-3 kinase

FIG. 1. Summary of the mechanisms of insulin resistance and steatosis in NAFLD. Insulin binds its tyrosine receptor on cell membranes, which causes receptor auto-phosphorylation and then phosphorylation of its key substrates, insulin receptor substrate 1 (IRS-1) and insulin receptor substrate 2 (IRS-2). Insulin resistance selectively inhibits the hypoglycemic effects of insulin, while allowing de novo lipogenesis to continue, via activation of sterol regulatory element binding protein (SREBP1). In NAFLD, there is an increased availability of free fatty acids (FFAs) secondary to increased fatty dietary intake and release from adipose tissue. FFAs are converted to triacylglycerols (TAG) via the glycerol 3 phosphate (G3P) pathway. Adiponectin is an inhibitor of this pathway, but levels of adiponectin are reduced in patients with NAFLD. Diacylglycerol (DAG) and ceramides are metabolites that accumulate in this pathway and can inhibit insulin signaling, via molecules such as protein kinase C (PKC). Fatty acyl-CoA is another metabolite in the G3P pathway and serves as an entry point for mitochondrial β-oxidation. In NAFLD, there is an increase in mitochondrial β-oxidation in response to the increased lipogenesis, but there is concomitant mitochondrial dysfunction, resulting in oxidative stress, which can inhibit insulin signaling via activation of inhibitor of nuclear factor kappa-B (I-κB) kinase subunit beta (IKKβ) and c-Jun N-terminal kinase (JNK). Hyperglycemia can also increase oxidative stress. Inflammatory cytokines, such as those produced by adipocytes and macrophages can also activate (IKKβ), JNK and additionally suppressor of cytokine signaling (SOCS), which can phosphorylate IRS1 and IRS2 to inhibit insulin signaling. IKKβ and JNK can activate nuclear factor kappa-B (NF-κB), causing translocation to the nucleus. Glucose can enter cells via the GLUT-4 transporter protein. An increase in intracellular glucose causes increased activation of carbohydrate response element binding protein (ChREBP), which is involved in lipogenesis from glucose. Gut dysbiosis promotes a pro-inflammatory response in adipose tissue and is associated with impaired mitochondrial β-oxidation. The net effects of the above processes are an increase in inflammation, a reduction in glycogen synthesis, an increase in lipogenesis and an increase in blood insulin and blood glucose levels.
Rodents with a point mutation of this insulin receptor kinase had reduced IR, whereas those with silenced PKC-epsilon activity were protected from IR despite development of hepatic steatosis (HS). Location of DAG within the cell is also important, given that this influences its ability to interact with PKC, with DAG accumulation in the hepatic plasma membrane being closely associated with IR.

INCREASED LIPOGENESIS

Isotopic tracer studies have shown that patients with NAFLD have an increase in de novo lipogenesis compared to those without NAFLD. This is a paradoxical finding given that insulin normally promotes lipogenesis, and that downstream from the insulin receptor, the pathway by which insulin modulates glucose levels hypothetically must diverge from the pathway that mediates lipogenesis. Two transcription factors play key roles in de novo lipogenesis: sterol receptor-binding protein 1-c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP). SREBP-1c is considered a master regulator of fatty acid synthesis, and in mice with hepatic SREBP-1c overexpression, there was an increase in DAG content and PKC-epsilon translocation in association with IR. High levels of postprandial glucose arriving at the liver stimulate activation of ChREBP, which increases transcription of genes involved in conversion of glucose to fatty acids. ChREBP knockdown in obese mice was associated with an improvement in HS and systemic IR (SIR), although in wild-type mice, ChREBP deficiency was associated with a worsening of IR. Furthermore, patients with NASH and SIR had lower levels of ChREBP on liver biopsy. These apparently contradictory effects may be because this transcription factor is important in modulating both glycolysis and lipogenesis, and its predominant effect depends on the degree of steatosis in the surrounding environment.

THE ROLE OF GUT MICROBIOTA

Pederson et al. demonstrated that insulin-resistant individuals had higher levels of branched-chain amino acids in the serum metabolome, which were linked to an increased prevalence of *Prevotella copri* and *Bacteroides vulgatus* in the microbiome. *P. copri* transfer to mice could induce IR and increase branched-chain amino acids. Patients with NAFLD have distinctive microbiomes from those without NAFLD, with the magnitude of differences correlating with disease severity. In murine studies, modulation of gut microbiota with antibiotics and probiotics has been associated with improvements in IR and reductions in visceral fat. Notably, transfer of microbiota from lean to obese human donors was associated with enhanced insulin sensitivity.

INCREASED MITOCHONDRIAL FAO

As detailed earlier, there is an increase in de novo lipogenesis and adipose tissue lipolysis in NAFLD. It is hypothesized that the increased lipid load arriving in the liver promotes increased FAO, by the mitochondrial tricarboxylic acid (TCA) cycle. Satapati et al. demonstrated that in both mice and humans, NAFLD was associated with increased lipolysis (~50%) and gluconeogenesis (~30%), which correlated with increased mitochondrial TCA activity and mitochondrial anapleurosis (nonoxidative TCA flux).

Koliaki et al. demonstrated that patients with NAFLD had increased rates of oxygen consumption in liver tissue and isolated hepatic mitochondria, but these decreased once patients developed NASH. A possible explanation is that hepatic mitochondria may compensate for chronic nutritional overload with increased activity at early stages of NAFLD (i.e., show mitochondrial “flexibility”), while at later stages the mitochondria are unable to compensate (i.e., mitochondrial “inflexibility”). Impaired mitochondrial energetics can result in incomplete fat oxidation and generation of toxic lipid intermediates, which impair insulin signaling, trigger reactive oxygen species, and lead to inflammation and hepatocyte necrosis.

Modulation of IR in NAFLD

Weight loss is an important element in treatment of NAFLD, and in overweight adults, a 10% reduction in weight results in a 44%-58% reduction in hepatic triglyceride content, with significant improvements in insulin sensitivity. This review will focus on the impact of pharmacological agents modulating IR
In NAFLD, each of which has different mechanisms of action (Fig. 2). Table 1 summarizes key clinical studies of these drugs in NAFLD.

**INSULIN SENSITIZERS**  
(BIGUANIDES/GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONISTS/ DIPEPTIDYL PEPTIDASE 4 INHIBITORS)

The biguanide, metformin, is the first-line agent for treatment of T2DM because of its association with improved insulin sensitivity and weight loss, without the risk of hypoglycemia. The exact mechanism of action of metformin is unclear, but it appears to involve an increased activation of adenosine monophosphate–activated protein kinase (AMPK) and/or an alteration of the hepatocellular cytosolic/mitochondrial redox state, consequently reducing hepatic gluconeogenesis. Although earlier open-label studies suggest positive effects of metformin on HS and necroinflammation, excess weight loss was an obvious confounding factor. In two meta-analyses, the researchers concluded that metformin does not improve NASH histology or biochemical outcomes. Thus, in patients with established diabetes, metformin is safe, but cannot be recommended solely for treatment of NAFLD.

Glucagon-like peptide 1 receptor agonist (GLP-1) is an incretin hormone secreted by intestinal L-cells following meal ingestion. GLP-1 receptors exist on both pancreatic and extrapancreatic tissues, although their existence on hepatocytes is controversial. GLP-1 regulates plasma glucose levels by stimulating insulin secretion from the pancreas, while inhibiting glucagon secretion in a glucose-dependent manner. In addition, GLP-1 can influence IR through promotion of...
| Study Design | Histology | Serum ALT/AST | Steatosis | IR |
|--------------|-----------|---------------|-----------|----|
| Meta-analysis of nine (n = 417) RCTs | No change | Improvement in ALT, but not AST | No change | Improved |
| Meta-analysis of 11 RCTs (n = 671), including six RCTs in NASH with posttreatment histology | No change | n/a | No change | Improved |

**Comments:**
- Short treatment duration (range 4-12 months) and variable metformin dosing (0.85-3.00 g/day)
- Incomplete histological outcomes and inconsistencies in histopathological grading systems
- No difference in any outcome in subgroup analysis of patients with NASH

GLP-1R agonists (e.g., dulaglutide, exenatide, liraglutide, albiglutide, and semaglutide)

| Study Design | Histology | Serum ALT/AST | Steatosis | IR |
|--------------|-----------|---------------|-----------|----|
| RCT (phase IIb) of patients with NASH given 1.8 g OD of liraglutide (n = 26) versus placebo (n = 26) for 48 weeks | Increased resolution of NASH, reduced progression of fibrosis | Improved | Improved | No change (but improvement in HbA1c) |
| Single-arm trial (LEAN-J), n = 19 patients with biopsy-proven NASH given 0.9 mg OD of liraglutide for 24 weeks | Improvement in NAS and fibrosis | Improved | Improved | No change (but improved HbA1c) |
| RCT: NAFLD patients given exenatide 10 µg BD + insulin glargine (n = 30) versus insulin aspart + insulin glargine (n = 30) for 12 weeks | n/a | Improved (more with exenatide) | Improved (on US, more with exenatide) | N/a (but improvement in HbA1c) |
| RCT: n=117 NAFLD patients given exenatide or metformin for 12 weeks | n/a | Improved (more with exenatide) | Improved (more with exenatide) | Improved in both groups equally |
| Meta-analysis: patient-level data from six 26-week phase III RCTs studying liraglutide in Diabetes (LEAD program) including n = 2,441 with deranged ALT at baseline | n/a | Improved | No change | n/a (but improvement in HbA1c) |
| Cohort study of n = 82 patients with NAFLD, given either liraglutide 0.9 mg OD, pioglitazone 15 mg OD, or sitagliptin 100 mg OD for 48 weeks | n/a | Improved (all groups) | n/a (but improvement in HbA1c in all groups) |

**Comments:**
- The study by Armstrong et al. (20) is the only phase II study of GLP-1 agonists in biopsy-proven NASH. It was a double-blinded study, including patients with and without diabetes.
- In four of these studies, (19,54-56) there was no histological assessment of outcomes, and in one, (53) posttreatment biopsies were only obtained in 10 of 19 patients. In several studies, NAFLD was diagnosed on ultrasound (54-56) or deranged serum transaminases (19) rather than biopsy.
- Several studies only included patients with diabetes (54-56) or had no placebo control arm (53-56).
- Treatment duration of these studies (12-48 weeks) is relatively short.
- One study (59) involved a large sample size; however, it was a retrospective analysis with power calculations based on changes in glycemic control rather than NASH. Furthermore, the beneficial effects were not maintained after controlling for weight and HbA1c.
- No RCTs have investigated use of GLP-1 agonists other than liraglutide and exenatide in NAFLD.
### TABLE 1. Continued

| Study | Study Design | Results |
|-------|--------------|---------|
| **DPP-4 inhibitor** (e.g., sitagliptin, vildagliptin) | | |
| Joy et al. (24) | RCT: patients with biopsy-proven NASH given 100 mg OD of sitagliptin (n = 6) versus placebo (n = 6) for 24 weeks | No change | No change | No change (on MRI) | No change (and no change in HbA1c) |
| Cui et al. (25) | RCT: n = 50 with NAFLD + pre-early diabetes given sitagliptin 100 mg OD versus placebo for 24 weeks | n/a | No change | No change | No change (and no change in HbA1c) |

**Comments:**
- The study by Joy et al. (24) was limited by its small sample size, and inclusion of patients with relatively mild NASH (baseline NAS score was 3.8 ± 0.8), so it is unclear whether a greater impact would have been found in patients with more-severe disease.
- The study by Cui et al. was limited by the lack of histological assessment (25).
- Both studies were placebo-controlled, double-blinded studies (24,25).
- Both studies involved a short follow-up duration (24,25).

**PPAR-γ agonists** (TZD, e.g., pioglitazone, rosiglitazone)

| Study | Study Design | Results |
|-------|--------------|---------|
| Musso et al. (57) | Meta-analysis of 8 RCTs (n = 516) assessing TZD, in patients with NAFLD with and without diabetes | Improvement in fibrosis, increased NASH resolution | n/a | n/a | n/a |
| Cusi et al. (26) | Clinical trial of n = 101 patients were randomized to either 45 mg OD of pioglitazone or placebo for 18 months, followed by an open-label phase with pioglitazone treatment for 18 months. | Increased NASH resolution and improvement in fibrosis | Improved | Improved | Improved |
| Sanyal et al. (29) | RCT Phase III Multicentre (PIVENS) trial, n = 247 patients with NASH without diabetes received either 30 mg OD of pioglitazone, 800 IU of vitamin E, or placebo for 96 weeks. | No change with pioglitazone | Improved in pioglitazone + vitamin E groups | Improved in pioglitazone + vitamin E groups | Improved in pioglitazone group only |
| Aithal et al. (27) | RCT: n = 74 patients with biopsy-proven NASH without T2DM received diet + exercise + either placebo or 30 mg OD of pioglitazone. | Improvement in features of NASH, improvement in fibrosis | Improved | Improved | Improved (but improved HbA1c) |
| Belfort et al. (28) | RCT: n = 95 patients with biopsy-proven NASH and prediabetes or T2DM had 6 months of treatment with a hypocaloric diet and 45 mg of pioglitazone or placebo. | Improvement in features of NASH, no improvement in fibrosis | Improved | Improved | Improved |
| Sanyal et al. (58) | RCT: n = 20 patients with non-diabetic patients without cirrhosis with NASH received either vitamin E 400 IU alone or in combination with pioglitazone. | Greater improvement in ballooning and fibrosis in patients receiving combination treatment | No change | Improved (both arms) | Improved in patients having combination treatment |

**Comments:**
- The meta-analysis (57) had low study heterogeneity and publication bias, although some of the included studies had small sample sizes. It showed that beneficial effects of TZDs were accounted for by pioglitazone rather than rosiglitazone. While pioglitazone improved advanced fibrosis in NASH, even in patients without diabetes, treated patients had a higher incidence of weight gain (+2.7%) and lower limb edema.
- Some studies included only patients with T2DM or prediabetes (26,28); others only included patients without T2DM (27,58).
- In the PIVENS study (29), 28% of patients in the pioglitazone group (vs. 17% in the placebo group) did not have HCB at baseline, which may have contributed to the failure to reach the primary endpoint.
- The study by Cusi et al. (26) reported that treatment discontinuation was associated with recurrence of aminotransferase levels of pretreatment levels.
TABLE 1. Continued

| Study | Study Design | Results |
|-------|-------------|---------|
| PPAR αδ agonists (e.g., elafibranor) | RCT Phase IIb (GOLDEN trial) in biopsy-confirmed NASH patients without cirrhosis, randomized to receive 80 mg of elafibranor (n = 93), 120 mg of elafibranor (n = 91), or placebo (n = 92) for 52 weeks | Improvement in (see comments) resolution of NASH Improved Improved Improved |

Comments:
- The primary endpoint (resolution of NASH without worsening of fibrosis) was not met according to the predefined histological endpoints, but was met when new consensus definitions were used.
- Double-blinded study, with assessments of serology and biopsies in a central location.
- Inclusion of patients with mild NASH may have contributed to the false-positive placebo response rates.
- Elafibranor treatment was associated with a mild, reversible increase in serum creatinine.

SGLT2 inhibitors (e.g., canagliflozin, ipragliflozin, and luseogliflozin)

Sattar et al. (37) Retrospective analysis of pooled data from the EMPA-REG outcome trial (n = 7,020), four 24-week placebo controlled trials (n = 2,477) and a trial of empagliflozin versus glimepiride over 104 weeks (n = 1,545) | n/a Improved n/a n/a |
Kuchay et al. (41) RCT: n = 50 patients with NAFLD and T2DM received standard diabetes treatment with or without empagliflozin. | n/a Improved in ALT, no change in AST Improved n/a (both groups had improved HbA1c) |
Ito et al. (39) RCT (open label): patients with NAFLD and T2DM received either 50 mg of ipragliflozin (n = 32) or 15-30 mg of pioglitazone (n = 34) OD for 24 weeks. | n/a Improved (both groups) n/a Improved (both groups) |
Seko et al. (36) Post-hoc analysis of canagliflozin in T2DM with elevated ALT using data from three phase II-III RCTs in Japanese patients | n/a Improved in OCA-treated groups n/a Improved |

Comments:
- The above studies only included patients with diabetes, and none of them involved histological assessment of outcomes.
- In the study by Seko et al. (36) alcohol intake was not regulated, and patients with an ALT/AST >2.5 × the upper limit of normal were excluded.
- Neither of the RCTs (39,41) was placebo-controlled, and participants were concurrently taking other medications that could have potentially impacted outcomes.

FXR agonists (e.g., OCA)

Neuschwander-Tetri et al. (44) RCT Phase IIb (FLINT): n = 273 patients received either 25 mg of OCA or placebo for 72 weeks. | Improvement in fibrosis and features or NASH, but no difference in rates of NASH resolution Improved Improved Worsened |
Mudaliar et al. (43) RCT (phase II): patients received either placebo (n = 23), 25 mg of OCA (n = 20), or 50 mg of OCA (n = 21). | n/a Improved in OCA-treated groups n/a Improved in OCA-treated groups |

Comments:
- Both studies were placebo-controlled, double-blinded RCTs.
- Treated patients had higher rates of pruritis and higher serum cholesterol.
- Mudaliar et al. (43) did not obtain histology but reported improvement in noninvasive markers of fibrosis in OCA-treated groups.

Abbreviations: AST, aspartate aminotransferase; BD, twice-daily; n/a, not applicable; US, ultrasound.
weight loss (delayed gastric emptying, appetite suppression). In patients with NAFLD, mechanistic studies demonstrated that liraglutide is associated with improvements in de novo lipogenesis, β-oxidation, and IR (systemic, adipose, and hepatic), with increased clearance of very-low-density lipoprotein (VLDL).(17,18)

Following a meta-analysis using data from over 4,000 patients with T2DM, Armstrong et al. concluded that liraglutide was associated with a dose-dependent improvement in liver enzymes.(19) However, this effect was not maintained after adjusting for changes in weight and HbA1c. These patients did not have a histological diagnosis of NAFLD, but this study paved the way for the first phase II randomized controlled trial (RCT) of GLP-1 agonists in 52 patients with biopsy-proven NASH, the LEAN (Liraglutide efficacy and action in NASH) trial.(20) Patients treated with 1.8 mg of daily liraglutide met the primary endpoint of NASH resolution without worsening of fibrosis (relative risk, 4.3 [95% CI, 1.0-17.7]; \(P = 0.019\)).

Two RCTs evaluated the use of exenatide in patients with NAFLD, with data suggesting that exenatide has favorable effects on HS and serum transaminases. However, both were small studies that only included patients with preestablished diabetes and neither assessed histological outcomes.

The SUSTAIN (The Semaglutide Unabated Sustainability in Treatment of Type 2 Diabetes) trials are a series of phase III clinical trials which suggest that semaglutide is more effective than other GLP-1 analogues (sitagliptin, exenatide, and dulaglutide) in reducing HbA1c in patients with T2DM. Based on these findings, a phase IIb clinical trial is underway to test the efficacy of semaglutide in patients with NASH (NCT02970942).

The safety profile of GLP-1 agonists is reassuring as is the outcome of cardiovascular event studies. In the (LEADER) Liraglutide and Cardiovascular Outcomes in type II diabetes trial, there was a reported 13% relative risk reduction with liraglutide (HR = 0.87; CI, 0.78-0.97; \(p = 0.01\)) in primary major adverse cardiac events (cardiovascular death/nonfatal myocardial infarction/nonfatal stroke), with the SUSTAIN 6 trials showing an even greater relative reduction with semaglutide (26%; HR = 0.74; CI = 0.58-0.95; \(P = 0.02\)).(21) Future research should help determine whether the effects of GLP-1 agonists on NAFLD are independent of effects on weight loss and glycemic control. Data are also required to clarify whether the beneficial effects of GLP-1 agonists are for individual drugs or whether they are a “class effect” and which drugs and doses are most effective.

GLP-1 has a short half-life attributed to rapid degradation by the dipeptidyl peptidase 4 (DPP4) enzyme. DPP4 antagonism has been explored as an alternative strategy to modulate IR, using drugs such as sitagliptin and vildagliptin. Serum/liver levels of DPP4 are higher in patients with NASH compared to controls. In patients with both NAFLD and diabetes, studies have shown mixed results for changes in serum transaminases.(22,23) In two small placebo-controlled RCTs in patients with NAFLD, sitagliptin had no beneficial effect on liver steatosis, liver enzymes, or IR.(24,25)

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA AGONISTS

Peroxisome proliferation-activated receptor gamma (PPAR-γ) is a ligand-activated nuclear receptor that forms a heterodimer with retinoid X receptor alpha and regulates gene transcription, with PPAR-γ2 being the predominant isoform expressed in adipose tissue. Thiazolidinediones (TZDs) improve IR by PPAR-γ-mediated increases in adiponectin transcription and expression.

Pioglitazone has consistently been shown to improve steatohepatitis(26,27) in contrast to rosiglitazone that only reduces steatosis. TZDs have also been shown to have non-PPAR targets, such as the mitochondrial target of thiazolidinediones (mTOT), the mitochondrial pyruvate carrier.

Belfort et al.(28) showed that pioglitazone 45 mg OD (once a day) for 6 months in patients with prediabetes or T2DM was better than placebo to improve all histological parameters, including a strong trend toward improvement in fibrosis stage. Furthermore, a 12-month study in patients without T2DM showed a significant improvement in fibrosis stage with pioglitazone 30 mg daily.(27) In a larger, 2-year study in patients without T2DM, pioglitazone was negative for a complex primary outcome,(29) but this may have been because 28% of patients randomized to that group did not have
hepatocellular ballooning (HCB) at baseline based on the central reading and therefore were unable to achieve improvement as defined by the primary outcome. Regardless, pioglitazone improved steatosis, inflammation, and HCB, and 47% of patients had resolution of nonalcoholic steatohepatitis (NASH; compared to 34% on vitamin E and 18% on placebo). More recently, a 3-year study including 101 patients with prediabetes or T2DM and biopsy-proven NASH reported that pioglitazone 45 mg daily was effective to improve insulin sensitivity in adipose tissue, liver, and skeletal muscle, reduce ~60% intrahepatic triglyceride content, and improve liver histology, including a modest, but significant, reduction in fibrosis stage.\(^{(26)}\)

Weight gain is probably the most common side effect, being in the range of 2.5–4.8 kg after 18–36 months of therapy in patients with NASH.\(^{(26,27)}\) Peripheral edema occurs in ~7%–10% of patients, but this percentage may be higher in those concomitantly taking insulin. These effects may be treatment limiting and are irreversible on treatment discontinuation. Whereas pioglitazone is associated with reduced cardiovascular risk, because undiagnosed diastolic dysfunction is more common in patients with NAFLD or T2DM, fluid retention that occurs during pioglitazone therapy may induce congestive cardiac failure and so is contraindicated in these patients. In patients without clinically evident cardiovascular disease, a small recent study found that pioglitazone was associated with improved left ventricular systolic and diastolic function.\(^{(30)}\)

Pioglitazone is associated with an increased risk of bone fractures.\(^{(31)}\) A recent meta-analysis has reported that out of 26 studies, only five showed an association between bladder cancer and pioglitazone,\(^{(32)}\) with no significant association found after exclusion of studies deemed to be of low quality.

Given that pioglitazone has shown greater impact on NASH than rosiglitazone despite being a weaker PPAR-\(\gamma\) agonist, there has been interest in PPAR-\(\gamma\)-independent mechanisms of pioglitazone action, such as mTOT activation, with phase IIB clinical trials underway (NCT02784444). PPAR-\(\alpha/\delta\) AGONIST: ELAFIBRANOR

Elafibranor is a dual PPAR-\(\alpha\) and PPAR-\(\delta\) agonist. PPAR-\(\alpha\) is highly expressed in the liver and plays a key role in hepatic lipid and lipoprotein metabolism. PPAR-\(\delta\) is broadly distributed, with predominance in skeletal muscle, kidneys, macrophages, and the gastrointestinal tract. Among its key functions, PPAR-\(\delta\) regulates peroxisomal \(\beta\)-oxidation of FFA, improves insulin sensitivity, contributes to improvements in lipid and glucose homeostasis, and has anti-inflammatory effects.

In a large clinical trial (GOLDEN trial), adults with NASH (n = 247) were treated with elafibranor (80 or 120 mg) or placebo for 52 weeks.\(^{(33)}\) The study did not meet its predefined primary endpoint (histological resolution of NASH) for elafibranor, although post-hoc analyses in a subgroup with more-pronounced inflammation (NAFLD Activity Score [NAS], >4) demonstrated a positive effect with 120 mg daily (19% with elafibranor compared to 12% on placebo). Treatment with elafibranor was also associated with an improvement in HbA1c, fasting plasma glucose, homeostasis model assessment for IR (HOMA-IR), plasma triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, and FFA levels.\(^{(33)}\) Treated patients also had a slight rise in creatinine. A phase III study focusing on patients with more-severe NASH is underway (ClinicalTrials.gov, NCT02704403). It is unclear whether elafibranor would be beneficial in children and whether it would be safe to use in patients with renal impairment.

SODIUM GLUCOSE COTRANSPORTER 2 INHIBITORS

This new class of agents exert their glucose-lowering effects by inhibition of the sodium glucose cotransporter 2 (SGLT2) that accounts for around 90% of the glucose reabsorbed by the kidney.\(^{(34)}\) SGLT2 inhibitors (SGLT2i) promote weight loss (around 3%-4%) and can prevent kidney disease and cardiovascular events in patients with T2DM.\(^{(35)}\) The most common side effects of SGLT2i include urinary frequency, thirst, orthostatic hypotension, dizziness, genital mycotic and urinary tract infections, and, more rarely, diabetic ketoacidosis and risk of bone loss and lower extremity amputations (canagliflozin).

Most clinical studies have reported a reduction in plasma alanine aminotransferase (ALT) concentration,\(^{(36,37)}\) with effect size correlating with changes
in body weight and glycemic control. In two recent open-label studies,\(^\text{(38,39)}\) investigators reported a significant decrease in the liver-to-spleen attenuation ratio in patients with T2DM treated for 6 months with luseogliflozin or ipragliflozin, respectively. In another 6-month uncontrolled study in 20 Japanese patients with T2DM and NAFLD, ipragliflozin decreased liver fat measured by proton magnetic resonance spectroscopy, as well as abdominal subcutaneous and visceral fat volume. In contrast, Bolinder et al. did not observe improvements in liver fat with dapagliflozin, despite a reduction in subcutaneous and visceral fat.\(^\text{(40)}\)

Kuchay et al. recently published outcomes from the first RCT investigating use of SGLT2 inhibitors in patients with T2DM and NAFLD,\(^\text{(41)}\) reporting that empagliflozin was associated with improvement in HS (on magnetic resonance imaging [MRI]) and serum ALT. Unfortunately, none of the aforementioned studies evaluated histological outcomes.

**FARNESOID X RECEPTOR AGONISTS**

The farnesoid X receptor (FXR) is a nuclear receptor that is activated by bile acids. FXR is highly expressed in the intestine, liver, kidney, and adrenal glands. The FXR can bind DNA response elements to regulate transcription of genes involved in a number of metabolic pathways. First, FXR is involved in pathways that suppress bile synthesis, thereby providing a negative feedback loop. Second, FXR activation helps to reduce lipid levels by inducing repression of genes involved in lipogenesis (SREBP-1) and gluconeogenesis (ChREBP). FXR activation also increases HDL clearance through increased expression of hepatic scavenger receptors and promotes peripheral VLDL clearance.\(^\text{(42)}\) Third, FXR activation increases hepatic regeneration, which has raised concerns about its neoplastic potential.

In a 6-week hyperinsulinemic, euglycemic clamp study of 64 patients with NAFLD and diabetes, use of an FXR agonist, obeticholic acid (OCA), was associated with improved insulin sensitivity compared to placebo (+24.5% vs. −5.5%; \(P = 0.011\)).\(^\text{(43)}\)

The FLINT (Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment) trial was a multicenter, randomized, double-blind, placebo-controlled phase IIb study in which 283 patients received either 25 mg of OCA or placebo for 72 weeks. The study met its predefined stopping criteria at interim analysis, and OCA was associated with a significant improvement in NAS. There was a significant improvement in fibrosis stage in the treated group (35% vs. 19%; \(P = 0.004\)), although there was no difference in rates of NASH resolution. Treated patients had high rates of pruritis (20%), higher HOMA-IR scores (\(P = 0.01\)), higher fasting serum insulin (\(P = 0.02\)), and higher levels of cholesterol, possibly attributed to a reduction in conversion of cholesterol to bile acids.\(^\text{(44)}\) A phase III trial (REGENERATE) is currently underway to investigate the potential utility of OCA in patients with NASH, using a lower dose to determine whether it retains its efficacy with higher tolerability (NCT02548351).

Other FXR agonists have been developed, for example, Tropifexor (LJN452), which has a different biochemical configuration than OCA, and is highly specific for FXR target genes in the liver and intestine, allowing administration of small doses with fewer systemic effects.\(^\text{(45)}\) Currently, this drug is in phase II trials for NASH (NCT02855164).

An alternative strategy to FXR agonism is to target the molecules regulated by FXR. For example, FXR activation in the terminal ileum up-regulates a hormone called fibroblast growth factor factor 19 (FGF-19), which binds hepatocytes and suppresses gluconeogenesis and promotes glycogen synthesis. There is concern that this interaction may increase the risk of hepatocellular carcinoma, and hence some nontumorigenic FGF-19 variants have been developed. In animal studies, engineered FGF-19 was associated with improvements in IR, reduction in liver enzymes and resolution of histological features of NASH. Harrison et al. have recently published the results of a phase II RCT using an FGF-19 agonist (NGM82).\(^\text{(46)}\) Patients (\(n = 82\)) with biopsy-confirmed NASH, received either 3 or 6 mg of NGM32 or placebo for 12 weeks. NGM82-treated patients had a significant reduction in liver fat (measured by MRI-proton density fraction), improvement in serum transaminases, noninvasive markers of fibrosis, and weight loss. The main limitation of this study was its short duration.

**NOVEL IR APPROACHES**

**Modulation of the Gut Microbiome**

Some researchers have investigated modulation of the gut microbiome as a potential strategy for treating
IR and NAFLD. In a meta-analysis of four RCTs including 134 patients with NAFLD/NASH, probiotics were found to significantly reduce liver amino-transferases, total cholesterol, TNF-α levels, and the HOMA-IR score (weighted mean difference, –0.46; 95% CI, –0.73 to –0.19; \( P = 0.0008 \)). However, the probiotic doses and type of microbe given varied, and histological assessments were not performed in these studies. Before manipulation of the gut microbiome can be recommended as an NAFLD treatment, it would be important to determine whether the overall bacterial abundance, bacterial distribution, or presence of specific pathogens is most important in determining clinical phenotype.

Modulation of the Immune System

Methods that reduce adipose tissue inflammation may impact on IR in NAFLD. Colostrum is rich in immunoglobulins, cytokines, and other antibacterial agents. In genetically obese mice, and in humans (n = 10), treatment with an extract of bovine colostrum rich in immunoglobulin G (IMM-124c) was associated with improved insulin sensitivity and HS, possibility mediated by an increased number of anti-inflammatory natural killer T cells, or changes in adiponectin and GLP-1 levels. Phase II RCTs to further evaluate this in NASH are now underway (NCT02316717).

IKKβ and TANK-binding kinase 1 are signaling molecules that are up-regulated in obesity. Use of an inhibitor of IKKβ and TANK-binding kinase 1 (Amlexanox) in animals was associated with weight loss, enhanced insulin sensitivity, reduced HS, and reduced expression of inflammatory genes in the liver. Phase II trials are investigating whether these effects are reproduced in obese patients with diabetes and HS (NCT01975935).

Inhibition of De Novo Lipogenesis

Acetyl coenzyme A (CoA) carboxylase (ACC) is an enzyme involved in malonyl CoA synthesis, which forms a substrate for de novo lipogenesis and inhibits mitochondrial FAO. In a recent phase I study, patients with NASH were given an ACC inhibitor (GS-0976; 20 mg OD) versus placebo for 12 weeks. Treated patients had reduced de novo lipogenesis, steatosis, and liver injury (measured by ALT); however, no significant differences were found in liver fibrosis measured using magnetic resonance elastography, and IR was not measured.

Conclusion and Further Directions

The ideal medical treatment for NAFLD would improve biochemical and histological features, reduce risk of progression to end-stage liver disease, and simultaneously reduce cardiovascular risk. Thus, modulation of IR will be a mainstay of therapeutic approaches in NAFLD given that it focuses on the upstream pathogenesis of NAFLD, yet improvements in IR do not always correlate with histological improvement.

Optimal duration of therapy is unknown, although discontinuation of rosiglitazone was associated with relapse of NASH and cardiometabolic abnormalities at 1 year. Long-term risks of drug therapy and impact on outcomes, such as development of cirrhosis need for liver transplantation and risk of HCC are also unknown.

NAFLD has a multifactorial pathogenesis; hence, combinatorial approaches targeting different mechanisms may enable synergism of beneficial effects while minimizing risks. Identification of appropriate combination therapies will depend on better understanding of disease pathogenesis and downstream effects of ligand activation.

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