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Emerging Pharmacological Targets for the Treatment of Nonalcoholic Fatty Liver Disease, Insulin Resistance, and Type 2 Diabetes

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Abstract

Type 2 diabetes (T2D) is characterized by persistent hyperglycemia despite hyperinsulinemia, affects more than 400 million people worldwide, and is a major cause of morbidity and mortality. Insulin resistance, of which ectopic lipid accumulation in the liver [nonalcoholic fatty liver disease (NAFLD)] and skeletal muscle is the root cause, plays a major role in the development of T2D. Although lifestyle interventions and weight loss are highly effective at reversing NAFLD and T2D, weight loss is difficult to sustain, and newer approaches aimed at treating the root cause of T2D are urgently needed. In this review, we highlight emerging pharmacological strategies aimed at improving insulin sensitivity and T2D by altering hepatic energy balance or inhibiting key enzymes involved in hepatic lipid synthesis. We also summarize recent research suggesting that liver-targeted mitochondrial uncoupling may be an attractive therapeutic approach to treat NAFLD, nonalcoholic steatohepatitis, and T2D.

Keywords

type 2 diabetes; insulin resistance; ectopic lipids; liver-targeted mitochondrial uncoupling

INTRODUCTION

Diabetes mellitus is a major health issue that has reached epidemic proportions worldwide due to rapid urbanization, unhealthy eating, and a sedentary lifestyle (1). The International

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Federation Atlas (2018) estimates that over 415 million people have been diagnosed with diabetes, a figure that, by 2040, is projected to rise to more than 625 million (1). Of the three major types of diabetes, type 2 diabetes (T2D) is far more common than either type 1 diabetes or gestational diabetes, accounting for almost 90% of cases (2). The increasing prevalence of diabetes follows the spread of an epidemic of obesity, the single most important contributor to the pathogenesis of T2D. Indeed, by 2050, almost 90% of people in the United States are projected to be overweight or obese (3), and one in three are expected to have T2D (4). The tremendous costs of these related epidemics with regard to morbidity and mortality, as well as the financial costs of approximately $150–190 billion in medical spending (5, 6) and more than $4 billion in lost productivity (7) annually, demand the development of new approaches to prevent and treat T2D.

While progressive loss of pancreatic islet β-cell function is ultimately responsible for the progression from normoglycemia to hyperglycemia, insulin resistance predates β-cell dysfunction and plays a major role in the pathogenesis of T2D (8). After a carbohydrate-rich meal, glucose is primarily stored as glycogen in the muscle and liver (9, 10). Decreased insulin action in these organs leads to fasting and postprandial hyperglycemia (9, 10), the hallmarks of T2D and major risk factors for long-term microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disease) complications (11). As such, current therapy for T2D relies mainly on the following approaches intended to reduce hyperglycemia(12): lifestyle modifications, which include the adoption of a healthy diet, increased physical activity, and healthy body weight maintenance (13); metformin, which acts to reduce hepatic gluconeogenesis and hepatic glucose production (HGP) (14, 15); sulfonylureas (and related insulin secretagogues), which increase insulin secretion from pancreatic islets (16); sodium– glucose cotransporter (SGLT2) inhibitors, which block glucose reabsorption in the proximal renal tubule (17); incretin mimetics, which stimulate insulin secretion, delay gastric emptying, and suppress appetite (18); peroxisome proliferator-activated receptor-γ (PPARγ) agonists (thiazolidinediones), which enhance adipocyte lipid storage (19), decrease ectopic lipid accumulation in liver and skeletal muscle (19), and improve liver and muscle insulin sensitivity (19–21); α-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself (22), which suppresses glucose production and increases glucose utilization (23). Unfortunately, these agents have met with limited success due to reduced efficacy, limited tolerability, and significant mechanism-based side effects (Table 1). Thus, new approaches to treat the root cause of T2D are needed.

Developing better treatment strategies requires a comprehensive understanding of the pathogenesis of T2D, in which insulin resistance plays an important role. In this review, we provide a brief overview of the pathogenesis of insulin resistance and how it is related to the development of new treatments for T2D. Additionally, we highlight several therapeutic strategies that have been developed to enhance insulin sensitivity by altering energy balance or inhibiting lipid synthesis. While several diabetes medications have also been studied as potential treatments for nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of insulin resistance, we refer readers to two recent reviews that discuss this in greater detail [by Samuel & Shulman (24) and Alkhouri et al. (25)]. Lastly, we examine the potential

therapeutic utility of liver-targeted mitochondrial uncoupling, which would represent a new class of agents for the treatment of NAFLD, nonalcoholic steatohepatitis (NASH), and T2D.

PATHOPHYSIOLOGY OF TYPE 2 DIABETES

Although the pathophysiological mechanisms of T2D are not fully understood, there is compelling evidence that insulin resistance plays a major role in its development. Indeed, cross-sectional and longitudinal studies demonstrate that insulin resistance occurs 10–20 years before the onset of T2D and that it is the best predictor of whether an individual will become diabetic (26, 27). Insulin resistance arises when nutrient availability and demands can no longer be balanced and is present in multiple tissues, including skeletal muscle, liver, adipose tissue, kidney, gastrointestinal tract, vasculature, and brain (8). In the muscle, insulin resistance is manifested by impaired glucose uptake following ingestion of a carbohydrate-rich meal and results in postprandial hyperglycemia (28), which in turn can be attributed to decreased insulin-stimulated muscle glycogen synthesis (29) due to decreased insulin-stimulated glucose transport (30). Hepatic insulin resistance is characterized by an inability of insulin to stimulate hepatic glycogen synthesis and suppress HGP under postprandial conditions. In T2D patients, increased rates of HGP, due to increased rates of hepatic gluconeogenesis, contribute to both fasting and postprandial hyperglycemia (31). Recent studies have implicated increased rates of white adipose tissue lipolysis, due to macrophage-induced white adipocyte inflammation, as a major factor responsible for the increased rates of hepatic gluconeogenesis in T2D via increased hepatic acetyl-CoA content (derived from increased fatty acid delivery), allosteric activation of pyruvate carboxylase, and increased conversion of glycerol to glucose by a substrate push mechanism (32). Collectively, alterations in insulin responsiveness in these tissues place a major stress on the pancreatic β-cells to increase insulin secretion in an attempt to offset the defect in insulin action (33). As the disease progresses, however, β-cells begin to fail, and a vicious cycle ensues involving gluco- and lipotoxicity and, possibly, factors other than the β-cells, causing a further decline in insulin secretion, worsening of hyperglycemia, and overt T2D (Figure 1).

Ectopic lipid accumulation in the liver (NAFLD) and muscle (intramyocellular lipid) has been identified as a root cause of insulin resistance in these tissues (29, 34–39). Under conditions of overnutrition, bioactive lipid metabolites accumulate and cause cellular dysfunction and insulin resistance. While several lipid metabolites have been proposed to play a causal role in insulin resistance (40), a substantial body of work supports the role of diacylglycerol (DAG) accumulation and novel protein kinase C (PKC) activation in impairing insulin action in liver and skeletal muscle (Figure 2). Specifically, intrahepatic and intramyocellular DAG promote increased PKCε translocation in liver and PKCθ translocation in skeletal muscle, increasing the phosphorylation of insulin receptor threonine 1160 in liver (41) and serine 1101 in skeletal muscle (42, 43) and impairing insulin signaling and action in liver (41, 44–88) and muscle (42, 43, 50, 51, 60, 68, 69, 78, 80, 88–109).

Evidence demonstrating a causal role for ectopic lipids in driving insulin resistance and T2D comes from studies showing that short-term caloric restriction, yielding minimal changes in body weight, reverses NAFLD and normalizes hepatic insulin sensitivity in obese humans (110, 111) and rodents (112). Although modest weight loss is highly effective at reversing

T2D, with diabetes remission rates comparable to those of bariatric surgery (110, 113–119), weight loss is difficult to sustain, with approximately 70–95% of those who lose significant weight subsequently regaining it (120–123).

NOVEL AND EMERGING TARGETS TO IMPROVE INSULIN SENSITIVITY

Novel therapeutics aimed at reducing ectopic lipids and improving insulin sensitivity may be of great benefit for the treatment of T2D. In particular, several strategies have been developed to influence ectopic lipid deposition by broadly altering energy balance or inhibiting key enzymes involved in lipid synthesis (24). In this section, we highlight one nonpharmacological approach (bariatric surgery) and two pharmacological targets currently under clinical development [fibro-blast growth factor 21 (FGF21) and acetyl-CoA carboxylase (ACC)] for the treatment of T2D. We also discuss the potential use of liver-targeted mitochondrial uncoupling agents to treat NAFLD, NASH, and T2D and present our view on what will be needed to generate liver-targeted mitochondrial uncouplers that are appropriate for clinical use.

Bariatric Surgery

Recently, bariatric surgery has emerged as an effective treatment option for obesity, NAFLD, and T2D (124, 125), resulting in long-term sustained weight loss with significant improvements in glycemic parameters (30% decrease in endogenous glucose production), histological features in NAFLD, insulin resistance, and cardiovascular disease risk factors [40% decrease in very low-density lipoprotein (VLDL) production] (126, 127). These beneficial effects likely relate, at least in part, to weight loss, although an improved incretin effect (115) and forced reduction in caloric intake [leading to a reduction in hepatic acetyl-CoA content, glycogenolysis, and DAG–PKCε activation (128)], may contribute to the improvements in glycemic control. Nonetheless, longer-term studies have demonstrated that the metabolic effects of bariatric surgery persist for an extended period; Schauer et al. showed that surgery improved diabetes control over a 5-year period (129). However, a study of a larger cohort of patients undergoing sleeve gastrectomy, the most common bariatric procedure in the United States (130), found weight regain at 3–5 years after surgery and relapse of diabetes in a significant number of patients (131). As such, while bariatric surgery can greatly reduce weight and resolve T2D, it may not be appropriate for all patients.

FGF21

FGF21, a distinctive member of the FGF family that functions as an endocrine hormone, is a key mediator of energy homeostasis and lipid and glucose metabolism (132). Circulating FGF21 is predominantly derived from the liver but is also found in the gut, brain, adipose tissue, muscle, and pancreas (133). FGF21 may coordinate the metabolic shift from the fed to fasted states and regulates hepatic ketogenesis, gluconeogenesis, and adipose lipolysis (133, 134). Liver-specific knockout of FGF21 causes glucose intolerance in high-fat diet (HFD)-fed mice (135), whereas mice overexpressing FGF21 are resistant to diet-induced obesity and have enhanced glucose tolerance (136). While FGF21 appears to exert its antidiabetic effects through multiple tissues (132), recent pharmacological studies in rodents have shown that exogenous FGF21 normalizes glucose and lipid homeostasis by increasing

cellular energy expenditure independently of brown adipose tissue activation (69, 137). In particular, these improvements were associated with a reduction in liver and muscle triglycerides, DAGs, and novel PKC (nPKC) translocation in the liver and muscle (69). FGF21 has also been shown to improve β-cell function (possibly secondary to the reversal of systemic insulin resistance) and survival in T2D mice (138).

Although FGF21 levels are positively associated with obesity and insulin resistance (139), preclinical and clinical studies suggest that pharmacological supplementation may be beneficial. Analogs in clinical trials (LY2405319, PF-052313023, and BMS-986036) have yielded mixed results. LY2405319 was tested in obese patients with T2D in a 28-day proof-of-concept trial and reduced body weight, reduced plasma insulin concentrations, and decreased the low-density lipoprotein (LDL) to high-density lipoprotein cholesterol ratio compared to baseline (140). Despite this, its effect on fasting plasma glucose concentrations was not as robust as anticipated based on prior studies in rodent and monkey models of T2D (141, 142); only modest dose-dependent reduction in plasma glucose was observed (140). In addition to LY2405319, PF-052313023, a long-acting FGF21 analog, also showed therapeutic promise. Specifically, PF-052313023 treatment decreased body weight and improved the lipid profile in rodents, monkeys, and patients with T2D. Unfortunately, PF-052313023 treatment produced dose-dependent changes in bone turnover markers (143), raising concern over the long-term use of this FGF21 analog to treat T2D patients. A recent phase 2 study with a pegylated analog of FGF21 (BMS-986036) has shown the most promise. In patients with NAFLD, NASH, and T2D, once-weekly injections decreased liver fat, serum transaminases, plasma triglycerides, and LDL cholesterol without any significant adverse events, paving the way for longer phase 3 studies (144). However, BMS-986036 did not significantly alter plasma glucose concentrations or HbA1c after 12 weeks of treatment (144). Future studies are therefore warranted to determine the long-term beneficial and potential adverse effects of FGF21 administration in T2D patients.

Acetyl-CoA Carboxylase Inhibition

Another therapeutic option to reduce ectopic lipids and improve insulin sensitivity is through inhibition of ACC. ACC catalyzes carboxylation of acetyl-CoA into malonyl-CoA, which is the rate-limiting step in fatty acid synthesis and primary regulator of mitochondrial fatty acid oxidation (145). ACC1, a primarily cytosolic isoform of ACC, is highly expressed in liver and adipose tissue and catalyzes the first committed step in de novo lipogenesis (145). The mitochondria membrane– bound ACC2 is expressed in oxidative tissues, such as the muscle and heart, and produces localized malonyl-CoA, which inhibits carnitine palmitoyltransferase I and the transfer of long-chain CoAs into the mitochondria for fatty acid oxidation (146). Thus, ACC is an intriguing therapeutic target to reduce lipid storage by simultaneously modulating fatty acid synthesis and oxidation (147).

Several rodent and human studies have shown favorable impact of ACC inhibition on NAFLD, NASH, and T2D. In a diet-induced rat model of obesity, antisense oligonucleotide (ASO)-mediated reduction of hepatic ACC1 and ACC2 resulted in marked reductions in hypertriglyceridemia, hepatic triglyceride, and DAG content and the reversal of hepatic insulin resistance, which was associated with a reduction in PKCε activation (62).

Additionally, a novel liver-specific allosteric inhibitor of ACC1 and ACC2 (ND-630) is currently being developed for the treatment of NASH and has recently been shown to reduce hepatic steatosis and improve dyslipidemia in rodent models of obesity with no adverse effects (148). Moreover, ND-630 improved glucose metabolism in high-sucrose diet–fed, HFD-fed and Zucker diabetic fatty rats (148). However, these results are not generalizable to all ACC inhibitors; Bourbeau & Bartberger demonstrated that long-term inhibition of ACC markedly increased fasting plasma glucose and worsened glucose intolerance in a diet-induced mouse model of obesity (149). In addition, we have recently observed that long-term allosteric inhibition of ACC significantly increased basal rates of glucose production, most likely due to increases in hepatic acetyl-CoA content and allosteric activation of pyruvate carboxylase (150). More concerning, allosteric inhibitors currently under development for the treatment of NAFLD and NASH (MK-4074 and GS-0976) were associated with a significant increase in fasting plasma triglyceride concentrations, despite a significant reduction in hepatic steatosis (151–153). Subsequent studies in mice and rats demonstrated that the hypertriglyceridemia was mediated by a reduction in hepatic polyunsaturated fatty acids and disequilibrium in nuclear hormone receptor signaling that increased hepatic VLDL secretion and reduced systemic triglyceride clearance (150, 151). Interestingly, cotreatment with a PPARα agonist reduced the hypertriglyceridemia associated with ACC inhibition, suggesting that ACC inhibitors may be a viable treatment option if given in conjunction with fibrates (150, 151). However, given that cardiovascular disease is the leading cause of death in T2D patients (154), additional studies are crucial to determine the therapeutic potential of ACC inhibition for the treatment of T2D.

Liver-Targeted Mitochondrial Uncouplers

Liver-targeted hepatic mitochondrial uncoupling, whereby the mitochondrial proton gradient is dissipated, thereby dissipating stored energy (fat) in the liver (155), has recently gained increasing attention as a potential therapeutic approach to burn fat and combat the life- and health-limiting consequences of T2D. The first mitochondrial uncoupler, 2,4-dinitrophenol (DNP), was originally used as a component of explosives during World War I (156, 157). After it was observed that many of the workers who handled this compound lost weight, researchers began to investigate the possibility of using DNP as a weight loss drug, and studies by multiple groups demonstrated the efficacy of this approach in obese humans (158–161). The drug was available as an over-the-counter medication and was widely taken for weight loss in the United States, but reports of toxic effects, including several deaths, led to its withdrawal from the market by the US Food and Drug Administration in 1938 (162). Despite the withdrawal from the US market, Russian soldiers continued to take DNP to stay warm on the Eastern Front during the frigid winters of World War II (163), and DNP is currently obtained illegally over the Internet and taken by body builders and individuals trying to lose weight (162, 164–175).

Rodent studies have provided ample evidence for the potential of tissue-targeted mitochondrial uncoupling to improve whole-body glucose and lipid metabolism in vivo. Overexpression of uncoupling proteins 1 or 3 in skeletal muscle has been shown to lower body weight (176–182) and improve insulin sensitivity in HFD-fed rodents (177, 178, 181). Pharmacologic mitochondrial uncoupling is similarly effective at reversing diet-induced

obesity and insulin resistance: Five days of treatment with low doses of DNP reduced weight gain, reduced hepatic steatosis, reduced DAG–PKCε activation, and improved hepatic insulin sensitivity in HFD-fed rats (183).

Like DNP, thyroid hormone has long been considered a potential therapeutic agent for the treatment of obesity because of its ability to increase mitochondrial respiration (184–186). However, studies examining the impact of thyroid hormone on glucose metabolism have yielded mixed results, with some investigators suggesting that treatment of euthyroid subjects with exogenous thyroid hormone improves glucose metabolism (187–189), but others reporting no impact (190, 191) or a deleterious effect (192) on metabolism. More problematic, treatment with supraphysiologic concentrations of thyroid hormone has been documented to cause deleterious side effects such as tachycardia, cardiomyopathy, and sarcopenia (193–196).

However, new approaches leveraging thyroid hormone action specifically in the liver have proved beneficial. In particular, the liver-selective, cytochrome P450–activated prodrug MB07811 markedly reduced hepatic steatosis and plasma lipids in rats (197) and was well tolerated and efficacious at reducing LDL cholesterol and triglycerides in patients with mild hypertriglyceridemia (198). In 2016, Finan et al. used a novel glucagon–T3 hybrid molecule to target T3 to the liver and showed that it markedly increased energy expenditure, fat mass, and plasma lipids independent of food intake (199). Importantly, this compound also reduced hepatic lipids and improved glucose tolerance without causing cardiac or bone toxicity (199), thereby suggesting that liver-specific thyroid hormone may be a therapeutic option for the treatment of obesity-associated ectopic lipid and insulin resistance.

Next-generation mitochondrial uncouplers.

Several novel mitochondrial and tissue-targeted chemical uncouplers have recently been developed (Table 2). In 2010, Skulachev and colleagues discovered that synthesized plastoquione derivatives, SkQ1 and C_{12}TPP, potentiate fatty acid–induced uncoupling of respiration and oxidative phosphorylation in mitochondria isolated from rat liver (200). While SkQ1 was further investigated as a potential treatment for Alzheimer's disease (201), mitochondria-targeted C_{12}TPP was shown to abolish diet-induced obesity in mice by reducing food intake and increasing resting metabolic rate and fatty acid oxidation (202). Similarly, Rhodamine 19 butyl ester (C4R1), a short-chain alkyl derivative of Rhodamine 19, dose-dependently reduced food intake, body weight, and fat mass in HFD-fed mice (203).

In addition to systemic mitochondrial uncouplers, novel tissue-specific uncoupling agents are also being developed, including the small molecule compounds C1 and CZ5. Acute administration of C1 increased AMPK activity and fat oxidation in chow-fed mice, while chronic C1 treatment reduced hyperglycemia and improved glucose tolerance in diabetic *db/db* mice (204). CZ5 treatment also reduced body weight and improved glucose and lipid metabolism in HFD-fed mice by increasing whole-body energy expenditure and reducing energy uptake (205). Lastly, niclosamide ethanolamine (NEN), an anthelmintic drug that uncouples the mitochondria, has recently emerged as a potential therapeutic agent for obesity-associated insulin resistance. By increasing energy expenditure, NEN reduced

fasting plasma glucose and improved glucose and insulin tolerance in mice with diet-induced obesity (206). A related compound, niclosamide piper-azine, may also hold similar promise for treatment of obesity-associated insulin resistance (207), although the weight-lowering effects of these next-generation chemical uncouplers, despite being an on-target effect of mitochondrial uncoupling, may limit their utility in clinical practice.

Liver-targeted mitochondrial uncouplers.

Systemic mitochondrial uncoupling agents (e.g., DNP) have a narrow therapeutic window due to the on-target effects of these agents to promote hyperthermia. Our group examined whether the therapeutic index could be significantly increased by targeting a mitochondrial uncoupler to the liver. In this regard, we developed a liver-targeted mitochondrial uncoupling agent, DNP–methyl ether (DNPME), which both prevented and reversed diet-induced hepatic insulin resistance without affecting body weight (51). Surprisingly, despite its liver specificity, DNPME also decreased intramyocellular ectopic lipid content and reversed muscle insulin resistance in HFD-fed rats due to reduced hepatic VLDL export. Targeting DNP to the liver improved its toxic to effective dose ratio 50-fold, in association with marked reductions in peak plasma DNP concentrations relative to standard DNP administration. Based on these data, we hypothesized that the toxicity of DNP is related to its peak (C_{max}) concentrations, whereas its efficacy is related to the area under the curve of DNP exposure throughout the day. Consistent with that hypothesis, adding an extended-release coating to DNP to generate a controlled-release mitochondrial protonophore (CRMP) increased the toxic to effective dose ratio even further, with a ratio of toxic to effective dose 200-fold higher than that of nontargeted DNP(50). We demonstrated that, akin to DNPME, CRMP (by virtue of its first pass uptake by the liver following ingestion) is a liver-targeted mitochondrial uncoupler (208) that is able to reverse insulin resistance, hepatic inflammation, and hepatic fibrosis in rodent models of T2D, NASH, and lipodystrophy (50, 51, 60). The reversal of hyperglycemia and hepatic insulin resistance by CRMP was attributed to increased fat oxidation exclusively in the liver, with reductions in hepatic triglycerides, DAGs, and PKCε translocation as well as reductions in hepatic acetyl-CoA content and pyruvate carboxylase activity (50). Moreover, CRMP treatment also lowered hepatic VLDL export, thereby reducing intramyocellular ectopic lipid (DAG) content, reducing PKCθ activity, and reversing muscle insulin resistance. Overall, these improvements in liver and muscle insulin resistance, caused by reductions in ectopic lipid in liver and skeletal muscle, as well as in hepatic acetyl-CoA leading to reductions in pyruvate carboxylase activity and gluconeogenesis, produced a reversal of liver inflammation, fibrosis, and diabetes in rodent models of NASH and T2D (50) (Figure 3).

Similarly, via the same mechanisms, CRMP administration in a mouse model of severe lipodystrophy (fatless A-ZIP/F1 mice) reversed hepatic insulin resistance, hepatic inflammation, and diabetes, lowering fasting plasma glucose by approximately 75 mg/dl and fasting plasma insulin by approximately 75% (60). Taken together, these data suggest that CRMP or other liver-targeted mitochondrial protonophores with a similarly high ratio of toxic to effective dose represent an attractive class of agents for potential use to resolve NAFLD and its downstream consequences, including NASH, liver fibrosis, insulin resistance, and T2D.

CONCLUSIONS

T2D and its downstream sequelae, including an increased risk of end-stage vascular dysfunction and cardiovascular disease, are well known to reduce the quality and duration of life. Unfortunately, current treatments have had limited success and do not address a major contributor to T2D: ectopic lipid-related insulin resistance. Over the past years, our understanding of the pathogenesis of insulin resistance and T2D has improved, and accordingly, new pharmacological targets have emerged. Unfortunately, the development and successful use of new treatments have proved to be challenging due to the complexity of insulin resistance and the presence of multiple feedback loops that make it difficult to predict the consequences of a particular intervention (209). For example, liver-specific inhibition of ACC increased plasma triglycerides via the derepression of nuclear receptor signaling as a means of compensating for reduced hepatic lipids (151). Moving forward, identifying therapeutic targets for blocking biochemical pathways involved in glucose and lipid metabolism may prove to be difficult in practice. Therefore, we propose that promoting increased hepatic cellular energy expenditure through the use of liver-targeted mitochondrial uncoupling agents may hold more promise. Indeed, animal studies suggest that liver-targeted mitochondrial uncoupling has a wide therapeutic index and can safely reverse NAFLD, NASH, liver fibrosis, and diabetes in rodent models of NASH, cirrhosis, and T2D (50, 51, 60, 206, 207). Liver-targeted mitochondrial agents are now being developed by several pharmaceutical companies, and studies in humans will be required to determine whether this approach can safely reverse the related epidemics of NAFLD, NASH, and T2D.

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SUMMARY POINTS

1. Ectopic lipid (DAG) accumulation in skeletal muscle promotes muscle insulin resistance by activation of PKCθ, leading to impaired insulin signaling.
2. Ectopic lipid (DAG) accumulation in liver promotes hepatic insulin resistance by activation of PKCε, leading to increased insulin receptor threonine 1160 phosphorylation, which leads to decreased insulin receptor tyrosine kinase activity.
3. Increased hepatic acetyl-CoA content promotes increased rates of hepatic gluconeogenesis and fasting hyperglycemia in T2D by allosteric activation of pyruvate carboxylase.
4. Several agents aimed at reducing ectopic lipid accumulation in the liver by promoting increased hepatic fat oxidation, inhibiting hepatic lipid synthesis, or increasing hepatic mitochondrial energy expenditure are currently under development for the treatment of NAFLD, NASH, and T2D.
5. Liver-targeted mitochondrial uncoupling has been shown to reverse liver and muscle insulin resistance and diabetes in rodent models of NAFLD and T2D by decreasing hepatic acetyl-CoA content and DAG–nPKC activation in liver and skeletal muscle.
6. Liver-targeted mitochondrial uncoupling has been shown to safely reverse NAFLD, NASH, liver fibrosis, and T2D in rodent models of these diseases.

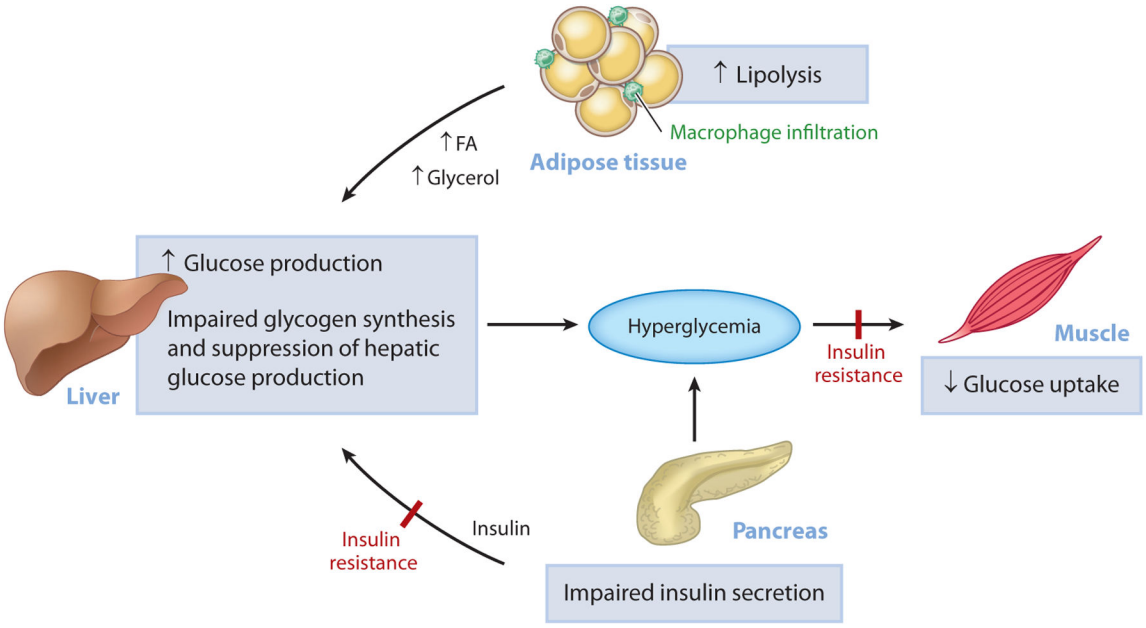

Figure 1.

Pathogenesis of hyperglycemia in T2D. Uncontrolled hyperglycemia is a hallmark of T2D and is a major risk factor for long-term microvascular and macrovascular complications. While the progressive loss of pancreatic islet β-cell function is ultimately responsible for the progression from normoglycemia to hyperglycemia, insulin resistance predates β-cell dysfunction and plays a major role in the pathogenesis of T2D. In the muscle, insulin resistance is manifested as impaired glucose uptake following ingestion of a carbohydrate-rich meal and results in postprandial hyperglycemia. In the liver, insulin resistance is characterized by the inability of insulin to stimulate hepatic glycogen synthesis and suppress hepatic glucose production under postprandial conditions. In parallel, inappropriate increases in adipose tissue lipolysis (due to increases in inflammation) can drive hepatic gluconeogenesis through increases in hepatic acetyl-CoA content, allosteric activation of PC, and increased conversion of glycerol to glucose. Initially, the β-cell compensates for alterations in tissue insulin responsiveness by increasing insulin secretion; however, over time, this compensatory mechanism fails and β-cell mass declines, causing a further reduction in insulin secretion, worsening of hyperglycemia, and overt T2D. Abbreviations: FA, fatty acid; PC, pyruvate carboxylase; T2D, type 2 diabetes.

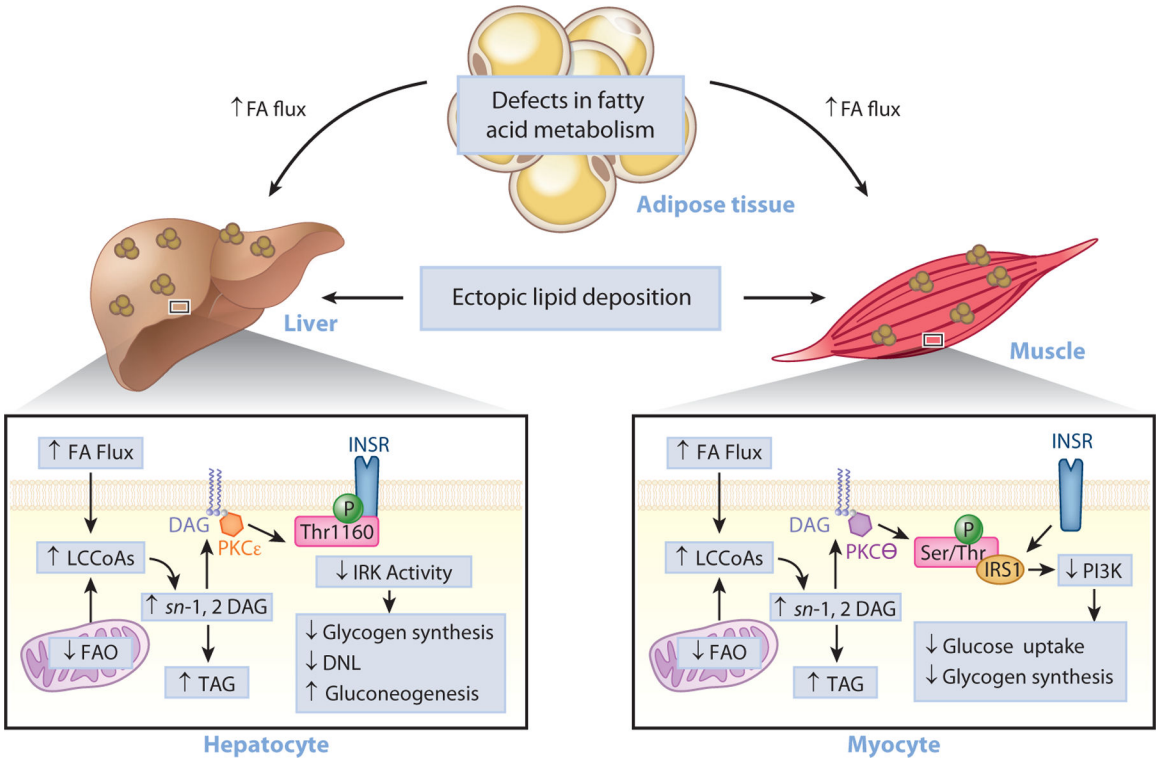

Figure 2.

The role of ectopic lipids in insulin resistance. Under conditions of overnutrition or defective adipocyte fatty acid metabolism, lipids can be redistributed from eutopic sites (adipose tissue) to ectopic storage sites (liver and muscle) and lead to impaired insulin signaling, insulin resistance, and T2D. Lipid-induced hepatic insulin resistance may result from activation of the DAG–PKCε axis and the consequent inhibition of INSR signaling through inhibitory phosphorylation of INSR at Thr1160. This leads to impaired insulin stimulation of hepatic glycogen synthesis, impaired transcriptional upregulation of de novo lipogenic genes, and impaired transcriptional downregulation of gluconeogenic genes. Skeletal muscle insulin resistance, caused by increases in intramyocellular ectopic lipid, impairs insulin-stimulated glucose transport and glycogen synthesis through the activation of the DAG–PKCθ axis and the consequent inhibition of the PI3K pathway through inhibitory phosphorylation of IRS1. Abbreviations: DAG, diacylglycerol; FA, fatty acid; FAO, fatty acid oxidation; INSR, insulin receptor; IRK, insulin receptor kinase; IRS1, insulin receptor substrate 1; LCCoA, long-chain CoA; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; Ser/Thr, serine/threonine; T2D, type 2 diabetes; TAG, triglyceride; Thr1160, threonine 1160.

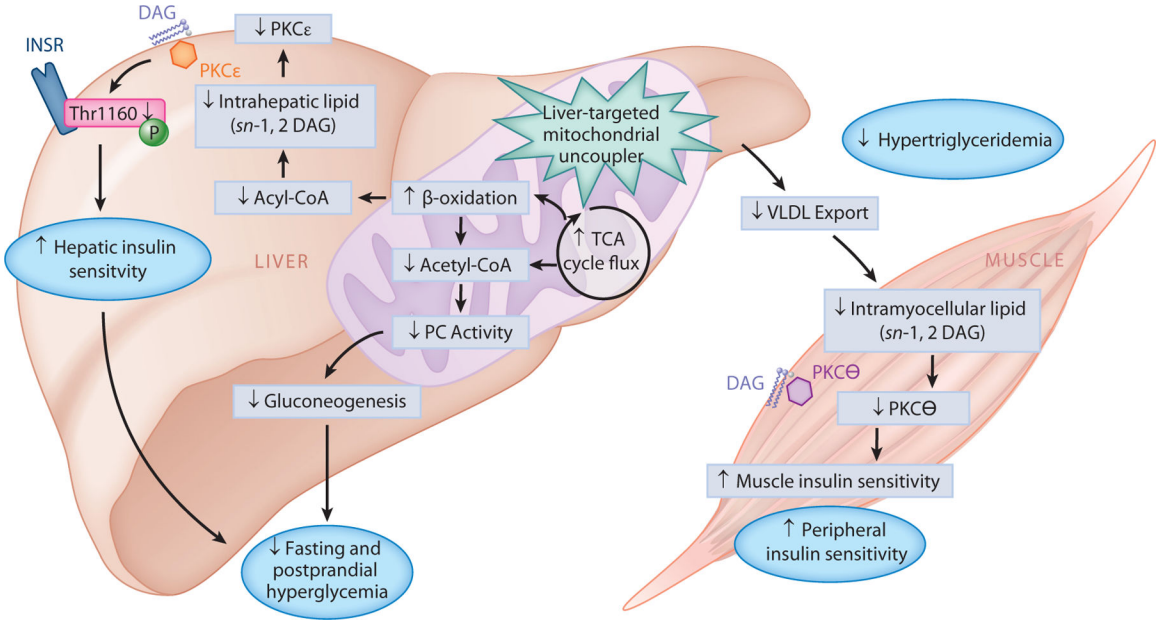

Figure 3.

Therapeutic potential of liver-targeted mitochondrial uncouplers for the treatment of T2D. Promoting increased hepatic cellular energy expenditure through the use of liver-targeted mitochondrial uncoupling agents (such as DNP analogs, DNPME, and CRMP) holds therapeutic promise for the treatment of NAFLD, NASH, and T2D. By increasing fat oxidation exclusively in the liver, DNPME and CRMP lower hepatic triglycerides, DAGs, and PKCε translocation, which increases hepatic insulin sensitivity. Liver-targeted mitochondrial uncoupling also increases TCA cycle flux, which reduces hepatic acetyl-CoA content, PC activity, and gluconeogenesis; collectively, this leads to reduced fasting and postprandial hyperglycemia. Moreover, DNPME and CRMP also lower hepatic VLDL export, thereby reducing muscle DAG content, reducing PKCθ activity, and reversing muscle insulin resistance. Overall, these improvements in liver and muscle insulin resistance can reverse diabetes in rodent models of NASH and T2D and suggest that liver-targeted mitochondrial uncoupling agents may be a therapy for the treatment of T2D in humans. Abbreviations: CRMP, controlled-release mitochondrial protonophore; DAG, diacylglycerol; DNP, 2,4-dinitrophenol; DNPME, DNP–methyl ether; INSR, insulin receptor; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PC, pyruvate carboxylase; PKC, protein kinase C; T2D, type 2 diabetes; TCA, tricarboxylic acid; Thr1160, threonine 1160; VLDL, very low-density lipoprotein.

Table 1

Current pharmacological agents for T2D

Class of medication	Representative agents	Mechanism of action	Glycemic efficacy (% HbAlc reduction)	CVD risks and benefits	Side effects
Biguanide	Metformin	Insulin sensitizer; ↓. HGP and gluconeogenesis	↓ 1–2%	↓ CVD risk factors; ↓ MI and coronary deaths	GI and lactic acidosis
Sulfonylureas	Glimepiride, glipizide, glyburide	↑ Insulin secretion	↓ 1–2%	↑ CVD risk	Hypoglycemia
SGLT2 inhibitors	Canagliflozin, Dapagliflozin, Empagliflozin	↓ Glucosuria; ↓ glucotoxicity	↓ 0.5–0.7%	↓ Blood pressure; improved lipid profile	Ketoacidosis, genital mycosis, bone fractures
Incretin mimetics	GIP, GLP-1 (Liraglutide, Exenatide, Dulaglutide)	↑ Insulin secretion; ↓ glucagon secretion; delayed gastric emptying; ↑ satiety	↓ 0.5–1.5%	↓ CVD risk	Nausea, vomiting, pancreatitis
TZDs	Rosiglitazone, pioglitazone	Insulin sensitizer; ↑ adipocyte function; ↓ ectopic lipid accumulation; ↑ β-cell function	↓ 0.5–1.4%	↑ CVD risk	Weight gain, bladder cancer, bone fractures
α-Glucosidase inhibitors	Acarbose, voglibose, miglitol	↓ Carbohydrate absorption	↓ 0.8%	↓ CVD risk	Diarrhea, abdominal pain, nausea, vomiting
Insulin	Short acting: humulin R, novolin R Intermediate acting: isophane Long acting: Lantus, Levemir, Tresiba Rapid acting: Lispro, Aspart, Apidra	↓ HGP; ↑ glucose uptake	↓ 1–2.5%	Neutral	Hypoglycemia, weight gain

Abbreviations: CVD, cardiovascular disease; GI, gastrointestinal; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; HbA1c, hemoglobin A1c; HGP, hepatic glucose production; MI, myocardial infarction; SGLT2, sodium–glucose cotransporter; T2D, type 2 diabetes; TZD, thiazolidinedione.

Table 2

Next-generation mitochondrial uncouplers

Compound	Target tissue	Model	Dose	Body weight	Food intake	Whole-body energy expenditure	FA oxidation	Liver TAGs	Muscle TAGs	Plasma glucose	Plasma insulin	Insulin sensitivity	Plasma lipids	Adverse side effects	References
C_{12}TPP	Systemic	HFD-fed C57BL/6 mice (6 weeks)	50 μM/kg/day × 16 days	↓	↓	↑	↑	ND	ND	ND	ND	ND	ND	None	202
C4R1	Systemic	HFD-fed C57BL/6 mice (8 weeks)	12–14, 30 nM/kg/day × 30 days	↓	↓	↑	↑	ND	ND	ND	ND	ND	ND	Reduction in lean body mass	203
Cl	Systemic	Chow-fed C57BL/6 mice	50 ing/kg × lday	ND	ND	ND	↑	ND	ND	ND	ND	ND	ND	None	204
		db/db mice	50 mg/kg × 4 weeks	-	-	ND	ND	ND	ND	↓	-	↑	-	None	
CZ5	Systemic (in vitro uncoupling in muscle and adipose tissue)	Chow-fed C57BL/6 mice	30 mg/kg/day × 30 days	-	↑	↑	↑	ND	ND	-	-	-	ND	None	205
		HFD-fed C57BL/6 mice (8 weeks)	10 mg/kg/day × 5 weeks	↓	↓	↑	ND	-	↓	↓	↓	↑	↓ Cholesterol	None	
NEN	Liver	HFD-fed C57BL/6 mice (16 weeks)	150 mg/kg/day × 16 weeks	↓	-	↑	↑	↓	ND	↓	↓	↑	ND	None	206
		db/db mice	150 mg/kg/day	-	-	ND	ND	ND	ND	↓	-	ND	ND	None	

Compound	Target tissue	Model	Dose	Body weight	Food intake	Whole-body energy expenditure	FA oxidation	Liver TAGs	Muscle TAGs	Plasma glucose	Plasma insulin	Insulin sensitivity	Plasma lipids	Adverse side effects	References
			× 60 days												
NPP	Liver	HFD-fed C57BL/6 mice (8 weeks)	125 mg/kg/day × 8 weeks	↓	-	ND	ND	↓	ND	↓	↓	↑	ND	None	207
DNPME	Liver	HFD-fed SD rats (2 weeks)	5 mg/kg/day × 5 days	-	-	-	↑	↓	↓	↓	↓	↑	↓ TAGs	None	51
		T2D rat model	5 mg/kg/day × 14 days	-	-	ND	ND	↓	ND	↓	↓	↑	4. TAGs	None	
CRMP	Liver	HFD-fed SD rats (3 weeks)	1 mg/kg/day × 5 days	-	-	-	↑	↓	↓	↓	↓	↑	|TAGs	None	50
		ZDF rats	1 mg/kg/day × 14 days	-	ND	ND	ND	↓	↓	↓	↓	↑	|TAGs	None	
		MCD-fed rats (8 weeks)	1 mg/kg/day x× 6 weeks	-	ND	ND	ND	↓	ND	ND	ND	ND	ND	None	
		A-ZIP/F-1 mice	2 mg/kg/day × 4 weeks	-	-	-	ND	↓	↓	↓	↓	↑	|TAGs	None	60