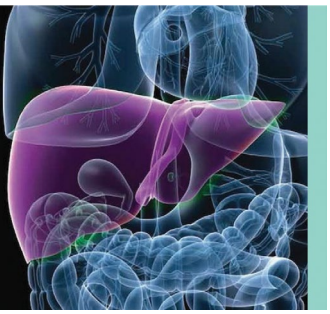

Insights Into Metabolic Mechanisms and Their Application in the Treatment of NASH

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Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic dysregulation—an alteration of fatty acid supply, production, oxidation, and storage resulting in hepatic steatosis (i.e., nonalcoholic fatty liver) with subsequent hepatocellular necroinflammatory injury (i.e., nonalcoholic steatohepatitis [NASH]) and risk for fibrotic liver injury leading to cirrhosis. In this review, we highlight key metabolic mechanisms underlying NAFLD pathogenesis and the associated targets in the emerging treatment landscape.

NAFLD: A DISORDER OF DYSREGULATED LIPID METABOLISM

Whether increased hepatic lipid exposure results in NAFLD is influenced by diet, genetics, and insulin sensitivity, and ultimately is determined by the overall balance of metabolic activities regulating lipid uptake, synthesis, export, oxidation, and retention in hepatic lipid droplets (Fig. 1). Outside of the diet, the major supply of lipids to the liver comes in the form of nonesterified fatty acids derived from the hydrolysis of adipose tissue triglyceride (TG) stores by the enzyme hormone-sensitive lipase (HSL).[1] The liver is also capable of generating lipids from other carbon sources, such as glucose or fructose, via the process of *de novo* lipogenesis (DNL). Fructose metabolism is particularly lipogenic, and thus uniquely promotes NAFLD. Fructose, absorbed via the portal vein and delivered to the liver via first-pass metabolism, increases protein levels of all DNL enzymes during its conversion into TGs by stimulating the carbohydrate

Abbreviations: ABHD5, α/β-hydrolase-domain-containing 5; ACC, acetyl coenzyme A carboxylase; ACL, ATP-citrate lyase; apoB, apolipoprotein B; ATGL, adipose triglyceride lipase; BCAA, branched-chain amino acid; BDK, branched-chain α-keto acid dehydrogenase kinase; ChREBP, carbohydrate response element binding protein; CoA, coenzyme A; DGAT, diacylglycerol acyltransferase; DNL, *de novo* lipogenesis; FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; HSL, hormone-sensitive lipase; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein 3; PPARα, peroxisome proliferator-activated receptor-α; PPm1K, protein phosphatase m1K; SCD1, steatoyl-coenzyme A desaturase 1; SGLT-2, sodium-glucose transporter 2; SREBP1c, sterol-regulatory element binding protein-1c; TCA, tricarboxylic acid; TG, triglyceride; THR β, thyroid hormone receptor-β; VLDL, very low density lipoprotein.

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PJW was supported by a Pathway to Stop Diabetes Initiator Award (#1-16-INI-17) from the American Diabetes Association.

Potential conflict of interest: Nothing to disclose.

Received May 8, 2020; accepted October 25, 2020.

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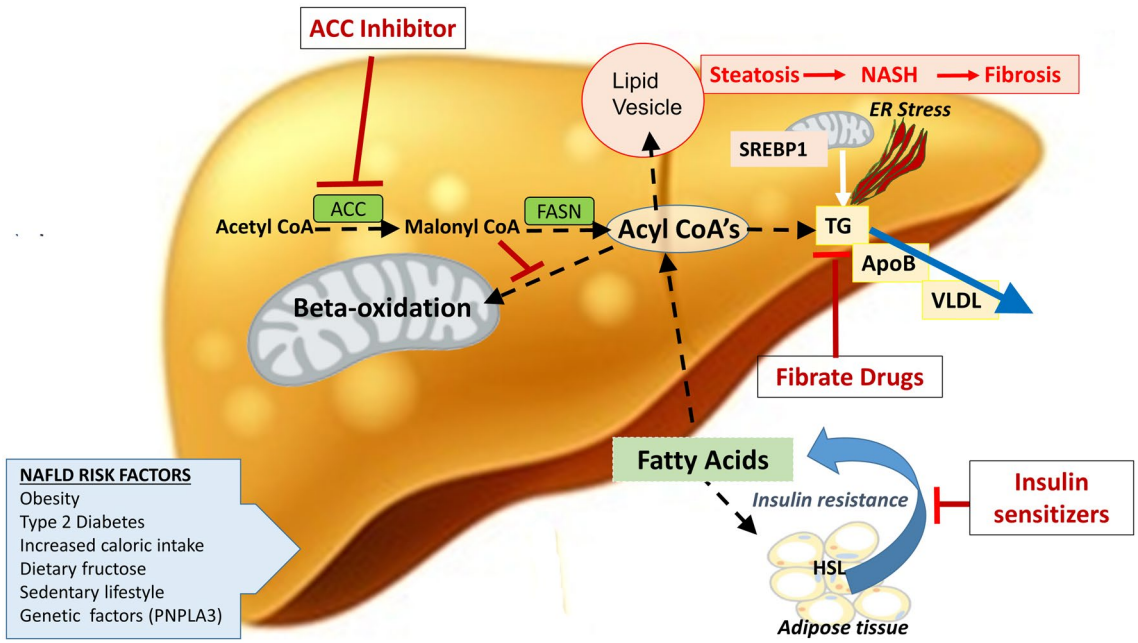

FIG 1 Hepatic lipid metabolism in NAFLD. Storage of fat in hepatic lipid vesicles is determined by balance of acyl-CoA generated by DNL, undergoing β-oxidation in the mitochondria, exported as TGs in VLDL, and arriving from adipose tissue TG hydrolysis. Inhibition of ACC enhances fatty acid β-oxidation and reduces DNL but increases VLDL excretion by activating SREBP1c. Fibrate drugs decrease TGs by enhancing fatty acid oxidation in the liver. Insulin-sensitizing agents lower lipid release from adipose tissue.

response element binding protein (ChREBP), a major transcriptional regulator of DNL. In addition, fructose also leads to ATP depletion, suppression of mitochondrial fatty acid oxidation, and increased production of reactive oxygen species, and promotes endoplasmic reticulum stress and uric acid formation, thereby inducing NAFLD/NASH. In addition to ChREBP, the DNL pathway is also induced by sterol-regulatory element binding protein-1c (SREBP1c), which also integrates both hormonal and nutritional cues. Both ChREBP and SREBP1c are more active in the liver of persons with NAFLD.[1] Insulin resistance, a primary metabolic driver of the development of NAFLD, is associated with both heightened adipose tissue lipolysis, due to loss of insulin-mediated inhibition of HSL, and increased expression of the hepatic DNL program (Fig. 1). Accordingly, therapies targeting either insulin resistance or DNL are currently undergoing testing for the prevention and treatment of NAFLD.[1] Likewise, therapies targeting nuclear receptors, such as the farnesoid X receptor (FXR) and peroxisome proliferator-activated receptor-α (PPARα), which oppose lipid accumulation by repressing lipogenic gene expression and promoting fatty acid oxidation, are also under development for NAFLD. Compounds targeting insulin resistance and/or lipid metabolism that are currently in various phases of clinical investigation are listed in Table 1.

THERAPEUTIC AVENUES IN THE DNL PATHWAY

Although insulin resistance is associated with both heightened adipose lipolysis and hepatic DNL, it is noteworthy that higher activation of the DNL pathway is the primary metabolic feature differentiating obese humans with NAFLD from those without.[2] The tight connection between DNL and NAFLD is thought to be related to the additional effect of the early lipogenic intermediate, malonyl coenzyme A (CoA), to also inhibit hepatic fatty acid ß-oxidation.[3] Acetyl CoA carboxylase (ACC) inhibitors that directly inhibit DNL by preventing the formation of malonyl CoA have been demonstrated to effectively decrease liver fat and are currently undergoing clinical testing (Table 1). However, treatment with ACC inhibitors can initiate a feedback mechanism that also promotes hypertriglyceridemia[4] (Fig. 1). Thus, further exploration of other therapeutic targets among the mechanisms regulating hepatic DNL is warranted. One emerging alternative target in this pathway is the

TABLE 1. SUMMARY OF ANTIMETABOLIC DRUGS UNDER INVESTIGATION FOR TREATMENT OF NAFLD/NASH

Compound	Mechanism	Drugs in Class	Drug Manufacturer	ClinicalTrials.gov
PPARs	Modulate adipose tissue distribution, decreasing visceral fat; play key role in glucose, lipid metabolisms, and inflammation; improve insulin resistance	Pioglitazone (PPAR-γ)	Takeda	NCT00013598
	Regulate insulin sensitivity by activation of PPARγ	Elafibranor (PPARα/δ)	Genfit	NCT02704403
		Saroglitazar (PPARα/γ	Zydus Cadila	NCT03061721
		Lanifibranor (PPARα/δ/γ)	Inventiva	NCT03008070
FXR agonists	Increase the metabolic rate and reverse high-fat-induced diabetes, decreasing adiposity	Obeticholic acid	Intercept	NCT02548351
		Cilofexor	Gilead Sciences	NCT03987074
		Tropifexor	Novartis	NCT02855164
		EDP-305	Enanta	NCT04378010
FGF-19 analogue	Nontumorigenic, engineered variant of FGF-19 that binds FGF receptors 1c and 4 to reduce lipotoxicity caused by lipid accumulation	Aldafermin	NGM Bio	NCT03912532
FGF-21 analogue	Pegylated recombinant analogue of FGF-21 that binds FGF receptors to reduce lipotoxicity caused by lipid accumulation	Pegbelfermin	Bristol-Myers Squibb	NCT03486899
SGLT-2 inhibitors	Decrease renal glucose reabsorption and increase renal glucose excretion, thereby promoting energy loss	Ipragliflozin	Astellas Pharma	NCT02875821
		Dapagliflozin	Astra Zeneca	NCT03723252
		Empagliflozin	Medanta	NCT02964715
SCD1 inhibitor	Inhibitor of DNL Inhibition of SCD	Aramchol	Galmed	NCT04104321
GLP-1 analogues	Naturally occurring incretin is released subsequent to food intake and stimulates the secretion of insulin, inhibits the release of glucagon, delays gastric emptying, and decreases food intake through increased satiety	Liraglutide	Novo Nordisk	NCT01237119
		Semaglutide	Novo Nordisk	NCT02970942
		Exenatide	Eli Lilly	NCT03648554
THR β agonists	Highly selective THR β agonists that reduce low-density lipoprotein cholesterol and TGs	Resmetirom	Madrigal	NCT03900429
		VK-2809	Viking	NCT04173065
DGAT inhibitors	Inhibits, DGAT, a microsomal enzyme that assists in the synthesis of fatty acids into TGs	PF-0686557	Pfizer	NCT04321031
ACC inhibitors	Inhibitor of DNL	Firsocostat	Gilead Sciences	NCT03449446
		PF-05221304	Pfizer	NCT04321031

activating phosphorylation of ATP-citrate lyase (ACL), the enzyme immediately upstream of ACC (Fig. 2). We recently determined that the branched-chain keto acid dehydrogenase kinase (BDK) and phosphatase (protein phosphatase m1K [PPm1K]), better known for their regulation of the rate-limiting step in branched-chain amino acid (BCAA) catabolism, control the phosphorylation state of ACL and thereby exert an important influence over hepatic DNL[5] (Fig. 2). Overexpression of BDK in liver was sufficient to increase hepatic DNL by greater than 2.5-fold in healthy rats. Moreover, small-molecule-based inhibition of BDK increased fatty acid oxidation and decreased hepatic TG content by 40% in liver of genetically obese Zucker rats without affecting circulating TGs.[5] In addition to providing a new target for NAFLD therapeutics, our discovery of the BDK/PPm1K-ACL axis provides a mechanism connecting dysregulated lipid metabolism to BCAA utilization and potentially explains observations of an association between NAFLD and circulating BCAA in human cohorts.[6] However, whether targeting BDK can yield the benefits of ACC inhibition for NAFLD in humans without the off-target effects on circulating TGs remains to be determined.

LIPID DROPLET DYNAMICS, PNPLA3, AND NAFLD RISK

Understanding the mechanistic basis of genetic variants underlying disease risk can yield new therapeutic targets. A common missense mutation in the gene patatin-like phospholipase domain-containing protein 3 (PNPLA3) is associated with significantly higher incidence and severity of NAFLD in carriers.[7] Until recently, the I148M variant form of PNPLA3, found in approximately 49% of Hispanic Americans, 23% of European Americans, and 17% of African American descent,[7] had been observed to amass on the surface of lipid droplets because of its resistance to ubiquitin-mediated degradation.[8] However, the mechanism by which it promoted NAFLD was not understood. Yang et al.[9] recently revealed that PNPLA3 strongly binds to α/β-hydrolase-domain-containing 5 (ABHD5) at the surface of the lipid

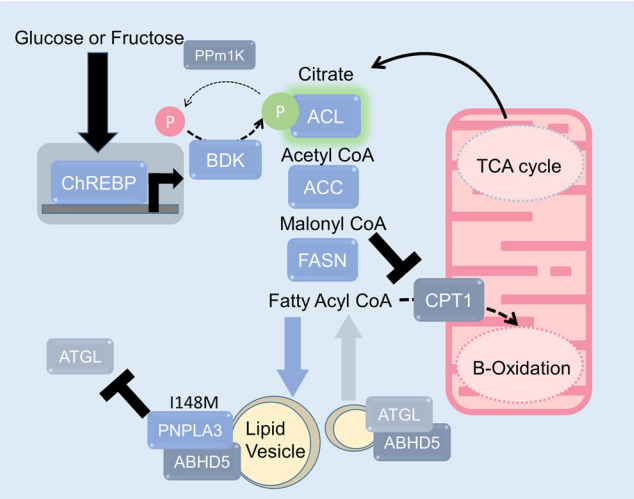

FIG 2 Schematic depicting novel mechanisms regulating NAFLD. Activation of hepatic ChREBP by glucose or fructose induces the expression of the lipogenic program and BDK, which results in activating phosphorylation of ACL, which enhances DNL. Expression of the common variant I148M form of PNPLA3 competitively inhibits ATGL activation by ABHD5 on the lipid droplet leading to lipid droplet expansion. Inhibition of PNPLA3 permits ATGL activation by ABHD5 and promotes TG hydrolysis and fatty acid oxidation.

droplet. A direct interaction with ABHD5 is required for activation of the major intracellular TG hydrolase, adipose triglyceride lipase (ATGL), which will release fatty acids for oxidation (Fig. 2). Thus, accumulation of the variant form of PNPLA3 combined with its strong affinity for binding to ABHD5 results in the competitive inhibition of TG hydrolysis and greater TG retention in lipid droplets promoting NAFLD (Fig. 2). Based on this new insight, an array of PNPLA3-lowering strategies were subsequently tested and demonstrated to be an effective means of protection against hepatic steatosis caused by the common variant form of PNPLA3. These new approaches may represent therapeutic opportunities in the future.[8]

TREATMENT OF NAFLD/NASH WITH ANTIMETABOLIC THERAPIES

Recent advances in our understanding of the mechanisms governing hepatic lipid deposition and associated lipotoxicity lend insight into the benefits of lipid-lowering therapies as a treatment for NAFLD/NASH. The transition from hepatic steatosis to NASH and hepatic fibrogenesis also requires a shift from fatty acid oxidation to glycolysis in hepatic stellate cells and macrophages.[10,11] To this end, several new therapies targeting lipid metabolism and DNL described earlier have biological plausibility for treatment for NAFLD/NASH and are currently being investigated in clinical studies.

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