Liver Research 7 (2023) 285–295

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

In-depth analysis of *de novo* lipogenesis in non-alcoholic fatty liver disease: Mechanism and pharmacological interventions☆

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ARTICLE INFO

Article history:
Received 22 June 2023
Received in revised form
28 September 2023
Accepted 15 November 2023

Keywords:
Non-alcoholic fatty liver disease (NAFLD)
De novo lipogenesis (DNL)
Therapeutics
Molecular mechanism

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is characterized by the abnormal buildup of lipids in the liver tissue. Non-alcoholic fatty liver (NAFL) may progress to non-alcoholic steatohepatitis. Triglycerides in the liver can originate from various sources, including *de novo* lipogenesis (DNL). Research indicates that DNL significantly escalates in NAFLD, worsening steatosis. However, the precise regulatory mechanism of DNL in the development of this disease is not fully understood. Therefore, the targeted reduction of DNL could be a crucial therapeutic strategy. Currently, numerous pharmaceutical agents targeting DNL have been developed, attracting significant attention. This review examines the mechanism of DNL upregulation in NAFLD, assessing its potential as a therapeutic target for hepatic steatosis. Furthermore, we thoroughly examine hepatocellular lipotoxicity and provide an extensive review of the application and limitations of relevant therapeutic drugs, with a focus on key enzymes involved in DNL. The implementation of these pharmacological strategies is expected to significantly improve the management and overall outcomes for patients with NAFLD.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD), a condition affecting a quarter of the global population, has become the most prevalent liver disorder.[1] Currently, there are limited pharmaceutical treatments available to effectively combat steatosis. Consequently, the optimal treatment for NAFLD remains the adoption of a healthy lifestyle and weight reduction, as suggested by studies.[1,2] Understanding the mechanism behind the accumulation of triglycerides (TGs) in the liver is crucial for understanding the pathogenesis and treatment of NAFLD. Fatty acids (FA), crucial substrates in TG synthesis, originate from three primary sources under physiological conditions. First, dietary TGs are transported to liver cells, known as hepatocytes, for decomposition. This process is aided by chylomicron remnants (CMR) after partial absorption. The second source of FA stems from *de novo* lipogenesis (DNL), a process that converts carbohydrates into FA. Additionally, a portion of FA is derived from the mobilization of tissues, resulting in the release of circulating free fatty acids (FFA). These FA can either oxidize or be synthesized into TGs within the liver.[3–5] Subsequently, the hepatic TGs can be excreted via very low-density lipoprotein (VLDL) or stored in the liver. In the context of NAFLD, dysregulation of endogenous lipogenesis can disrupt the balance between lipid synthesis and oxidation, leading to the accumulation of TGs in the liver. The excessive accumulation of TG deposits could contribute to NAFLD and progression to non-alcoholic steatohepatitis (NASH) (Fig. 1).

Early research into NAFLD overlooked the crucial role of DNL in its pathogenesis. This may be because DNL contributes to only 5% of FA in healthy individuals.[6–8] However, in patients with NAFLD, DNL accounts for up to 26% of liver TGs (Fig. 1).[5] Currently, there is substantial evidence highlighting the significant role of DNL upregulation in the development of NAFLD.[9–12] The combined effect of excessive tissue fat mobilization and upregulation of DNL further

☆ Edited by Peiling Zhu.

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https://doi.org/10.1016/j.livres.2023.11.003
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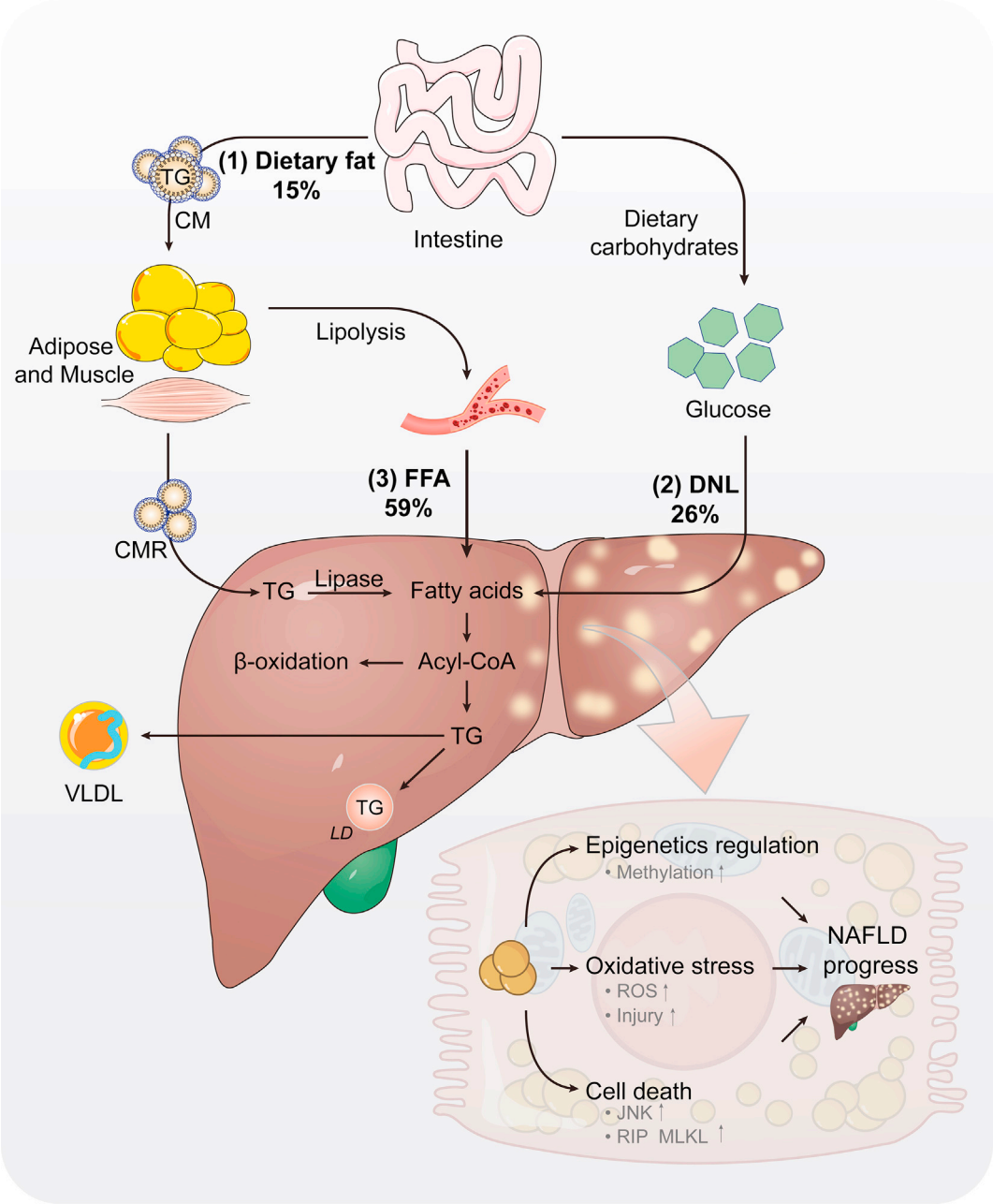

Fig. 1. Triglyceride (TG) metabolism in NAFLD. Liver TG is generated through three primary pathways: 15% of TGs come from the diet, 26% from DNL, and 59% from circulating FFA. If not sufficiently excreted through VLDL, they can be stored within hepatocytes. Excessive accumulation of TG in the liver can contribute to NAFLD and progression to non-alcoholic steatohepatitis (NASH). Abbreviations: CM, chylomicron; CMR, chylomicron remnants; DNL, *de novo* lipogenesis; FFA, free fatty acids; JNK, c-Jun N-terminal kinase; LD, lipid droplet; MLKL, mixed lineage kinase domain-like protein; NAFLD, non-alcoholic fatty liver disease; RIP, receptor-interacting protein; ROS, reactive oxygen species; VLDL, very low-density lipoprotein.

exacerbates the accumulation of liver TGs, leading to further changes in liver TG content.[13] Thus, DNL may play a crucial role in the development of NAFLD. Therefore, DNL is believed to play a vital role in the development of NAFLD. However, the mechanism responsible for the upregulation of DNL is multifaceted and not fully understood.

This review highlights the pathogenesis and treatment of NAFLD, specifically focusing on DNL. We offer a thorough review of the current knowledge on DNL in NAFLD, aiming to uncover the mechanisms that trigger its upregulation at the onset of the disease. We also present a comprehensive summary of the potential applications and limitations of therapeutic drugs targeting key enzymes involved in DNL. We anticipate that these pharmacological approaches will lead to more effective management and improved outcomes for patients.

2. DNL in the liver

DNL is a biological process that enables the production of endogenous TGs from dietary substrates.[8] A high intake of carbohydrates has been shown to significantly enhance DNL in the liver.[14] This complex process can be divided into three sequential steps: fatty acid synthesis, elongation/desaturation, and assembly into TGs.[8]

2.1. Basic raw materials and enzymes for DNL

The synthesis of TGs requires two primary components: glycerol and FA. These building blocks are primarily obtained via carbohydrate metabolism. Glycerol-3 phosphate, specifically, serves as the initial material esterified with FA to form TGs.[15] The liver uses carbohydrate metabolism to generate endogenous FA. The enzyme acetyl-CoA carboxylase (ACC), which catalyzes the conversion of acetyl-CoA into malonyl-CoA, is central to lipogenesis. Fatty acid synthase (FAS or FASN), an enzyme responsible for producing palmitic acid, is another crucial component. Fatty acid elongases and desaturases catalyze the transformation of palmitic acid into various FA.

2.2. DNL pathway

The first stage of DNL involves the production of endogenous FA. Carbohydrates are crucial for producing substantial amounts of acetyl-CoA within the mitochondria.[16] Citrate-isocitrate carrier (CIC) is essential for facilitating the transport of acetyl-CoA into the cytoplasm by converting it into citric acid. ATP-citrate lyase (ACLY) subsequently cleaves citric acid to produce acetyl-CoA.[17] This acetyl-CoA is then transformed into malonyl-CoA by ACC, which acts as the precursor for FA synthesis. FA is synthesized in a stepwise process, incorporating two-carbon units from malonyl-CoA, ultimately producing palmitic acid.[15] This palmitic acid can then be further elongated and modified in the endoplasmic reticulum and mitochondria. Ultimately, the resulting FA and glycerol 3-phosphate are converted into TG. TG synthesis relies on the coordinated action of various enzymes. Modulating the activity or expression of these enzymes can significantly affect DNL (Fig. 2).

3. Molecular mechanisms of DNL upregulation in NAFLD

DNL regulation primarily occurs at the transcriptional level.[18,19] This is especially noticeable during postprandial periods, characterized by a significant increase in glucose uptake and insulin secretion, which induces the upregulation of lipogenic genes.[20,21] Following this, glucose and insulin activate two key transcription factors: carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein-1c (SREBP-1c). These proteins are crucial in regulating lipogenic gene expression. Several genes, including FAS, ACC, and stearoyl-CoA desaturase 1 (SCD1), responsible for lipogenesis, are upregulated.[18,22] Modifications to these factors could potentially serve as key determinants of DNL upregulation.

3.1. Overconsumption of fructose

Excessive fructose intake significantly contributes to the development of NAFLD.[23] Recent research indicates a positive correlation between increased consumption of fructose-based beverages and the onset of fatty liver.[24] A specific study revealed that NAFLD patients consumed 2–3 times more fructose than controls.[25] Fructose can stimulate DNL in two ways: through long-term induction effects and by directly providing a carbon source for DNL.

Fructose primarily activates ChREBP. High fructose intake can activate low-activity ChREBP-α, subsequently inducing high-activity ChREBP-β.[26] Experimental studies have shown that such dietary interventions in animal models increase the expression of ChREBP target genes. Fructose is associated with the activation of SREBP-1c and can induce its expression in both insulin-dependent and insulin-independent manners.[27,28] Additionally, fructose can activate other transcription factors associated with TG metabolism, such as liver X receptor (LXR),[29] and peroxisome proliferator-activated receptor (PPAR)-gamma coactivator-1 beta (PGC-1β),[30] which can further enhance DNL reactions.

Fructose is a significant carbohydrate that provides a substantial carbon source. In the conventional DNL pathway, ACLY converts citric acid to acetyl-CoA. When fructose intake exceeds the small intestine's capacity, gut microbiota metabolizes fructose into acetate.[31] Recent studies have revealed that acyl-CoA synthetase short-chain family member 2 (ACSS2) can convert acetate to acetyl-CoA, establishing an alternative pathway for TG production independent of ACLY (Fig. 2).[32]

In conclusion, the increased intake of fructose stimulates the transcriptional activation of DNL and provides essential substrates, both of which significantly contribute to the development of hepatic steatosis. This emphasizes the complex interaction between dietary factors, gut microbiota, and host organs in regulating liver metabolism. Controlling fructose intake long-term through dietary intervention is challenging. Therefore, directly targeting fructose metabolism or DNL could potentially improve the efficacy of pharmacological treatments for NAFLD.

3.2. Insulin resistance

Insulin resistance, a key feature of NAFLD,[23] promotes DNL in a high insulin environment.[33] Recent studies have found a positive correlation between hepatic DNL and 24-h plasma glucose and insulin levels, and a negative correlation with the hepatic insulin sensitivity index.[33] NAFLD is typically associated with selective insulin resistance,[15,23] which maintains the sensitivity of the SREBP-1c pathway, leading to increased SREBP-1c levels and enhanced DNL.[34] SREBP-1c can also be activated by noninsulin-dependent mechanisms such as the mechanistic target of rapamycin complex 1 (mTORC1),[35,36] and endoplasmic reticulum stress in insulin-resistant conditions.[37] This state is often marked by blood glucose levels exceeding normal physiological levels. The surge in glucose levels stimulates liver DNL by enhancing ChREBP and SREBP-1c, activating nearly all associated genes.[33] DNL may produce toxic metabolites like diacylglycerol and ceramides, potentially leading to insulin resistance.[38,39] This creates a positive feedback loop with insulin resistance, worsening disease progression.[33,34] Both NAFLD and type 2 diabetes mellitus (T2DM) are characterized by insulin resistance, with a significant epidemiological and pathological link between them.[40,41] Therefore, drugs targeting insulin resistance for T2DM could be highly effective in NAFLD treatment.

3.3. MicroRNA (miRNA)

Recently, there has been a surge in interest regarding the implications of dysregulated miRNA expression in the context of NAFLD. Certain miRNA markers, namely miRNA-34a, miRNA-122, and miRNA-192,[42] have been identified as potential diagnostic indicators for NAFLD. These miRNAs act as vital epigenetic mechanisms, reprogramming the entire liver metabolism. They primarily suppress gene expression and bind to complementary mRNA sequences to inhibit their translation, demonstrating a form of post-transcriptional regulation. Some miRNAs can enhance gene expression, adding to their functional complexity. For example, miRNA-122, which makes up 70% of the total hepatic miRNA pool, is one of the most abundant miRNAs in the liver.[43] Research indicates a significant elevation in serum miRNA-122 content in NAFLD patients.[43–45] Furthermore, a study by Long *et al.*,[44] revealed that miRNA-122 directly targets silent information regulator 1 (SIRT1). MiRNA-122 binds to the mRNA of SIRT1, inhibiting its function and reducing its expression. This triggers an upregulation of the downstream SIRT1/liver kinase B1 (LKB1)/AMP-activated

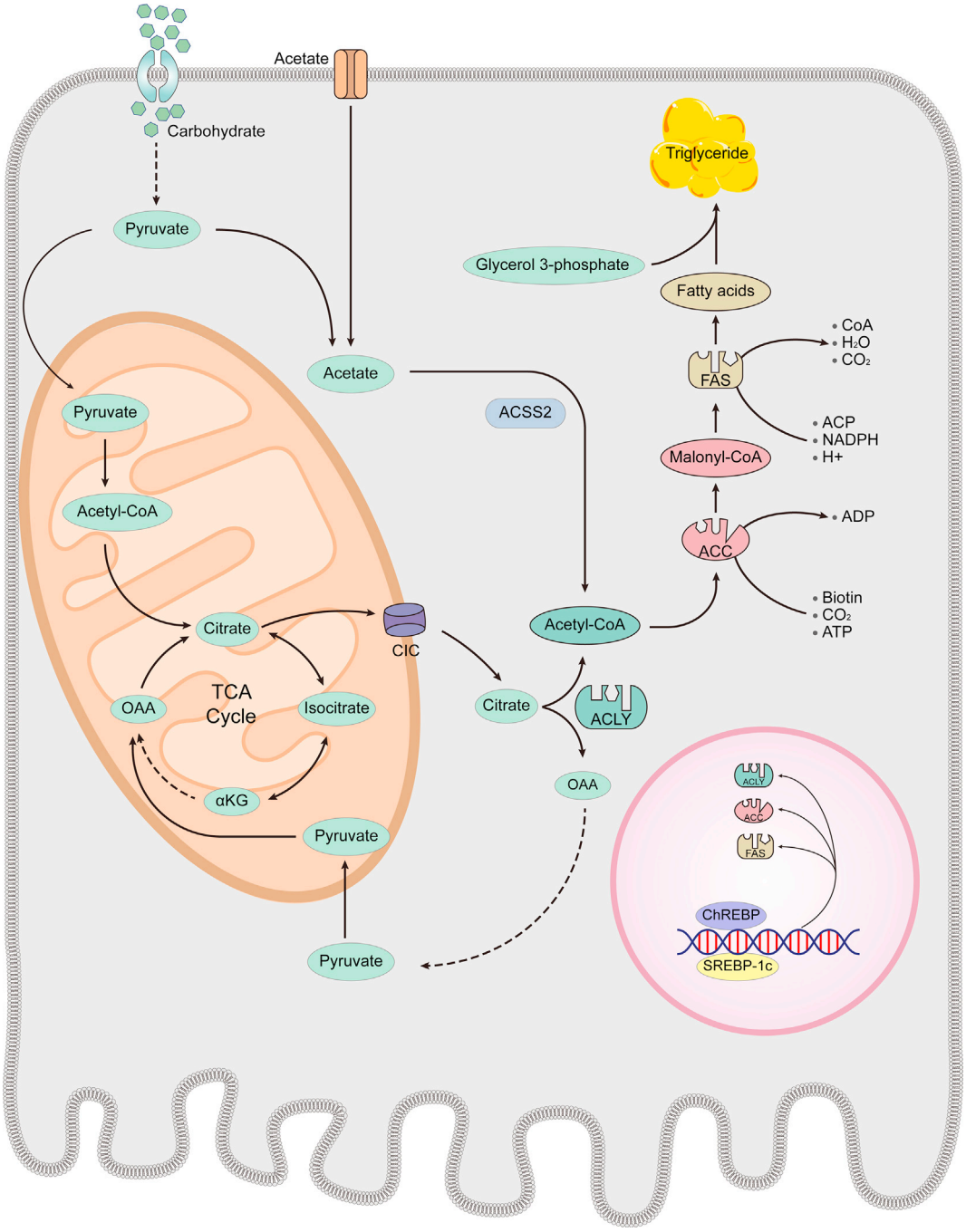

Fig. 2. *De novo* lipogenesis (DNL). DNL relies on the orchestration of numerous enzymatic reactions. The regulation of enzyme catalytic activity and expression may play a significant role in controlling intrahepatic lipogenesis. Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP-citrate lyase; ACSS, acetyl-CoA synthetase 2; ChREBP, carbohydrate response element-binding protein; CIC, citrate-isocitrate carrier; FAS, fatty acid synthase; αKG, alpha-ketoglutarate; OAA, oxaloacetic acid; SREBP-1c sterol regulatory element-binding protein-1c; TCA, tricarboxylic acid.

protein kinase (AMPK) pathway, increasing the expression of lipogenic enzymes such as SREBP-1, FASN, SCD1, ACC1, and apolipoprotein A5 (ApoA5). Specific miRNAs, such as miRNA-24, significantly enhance hepatic TG accumulation by effectively suppressing insulin-induced gene 1 (INSIG1) expression.[46] Recent studies also indicate that miRNAs such as miRNA-17, miRNA-20a, and miRNA-20b are closely associated with NAFLD development and progression.[47] While further investigation of the relevant pathways is needed, these findings underscore the crucial role of miRNAs in hepatic TG metabolism. Currently, clinical research on miRNA-related transformations is receiving significant attention. Besides serving as biomarkers, miRNA drugs also hold substantial potential for therapeutic effects. Despite the significant challenges in this field, advancements in related preclinical work indicate that solutions are imminent.[48–50]

3.4. Inflammation

Inflammation plays a significant role in the progression of NAFLD. This process involves various cells and molecules, with the

immune system playing a pivotal role. Evidence suggests that inflammation is associated with DNL. For instance, the anti-inflammatory factor interleukin (IL)-22 has been demonstrated to alleviate fatty liver degeneration induced by a high-fat diet.[51] However, the molecular mechanism linking inflammation and DNL remains largely unexplored.[52] During an inflammatory response, inflammatory factors may promote DNL through various pathways.[53] Nuclear factor-kappaB (NF-κB) is a significant signaling pathway that can be activated by numerous inflammatory factors, such as tumor necrosis factor-alpha (TNF-α) and IL-1β. NF-κB activation may stimulate DNL through a rare post-translational regulatory mechanism.[54] DNL upregulation could be due to increased protein stability of ACLY and FAS,[55] and altered phosphorylation of ACC1 protein,[54] leading to increased FA production. Other inflammation-related pathways, like mTORC1 and PDE3,[52,56] may also contribute to DNL upregulation.

3.5. Oxidative stress

Patients with NAFLD frequently experience oxidative stress. Reactive oxygen species (ROS), such as superoxide anion radicals and hydrogen peroxide (H_2O_2), consistently accumulate within the body.[57] Extensive research indicates that ROS regularly influence intracellular nuclear receptors, altering downstream metabolism.[58] H_2O_2 can increase the expression of SREBP-1c and promote DNL.[59] Hyperuricemia, closely linked to NAFLD,[60] can also promote DNL through ROS-dependent c-Jun N-terminal kinase (JNK)/activator protein-1 (AP-1) signaling pathways. Certain antioxidants can reduce SREBP-1c expression and mitigate hepatic steatosis.[61] Therefore, ROS signaling plays a crucial role in regulating liver lipid homeostasis.

4. Excessive lipids damage hepatocytes

Overactivation of DNL can result in excessive fat accumulation in liver cells, potentially leading to NAFLD.[62] This process is governed by intricate mechanisms involving various cellular signaling pathways and molecular regulatory systems. We summarize the toxicity of excessive lipids to hepatocytes, focusing on aspects of epigenetic regulation, oxidative stress, and cell death.

4.1. Epigenetics regulation

Initially, dysfunctions in lipid metabolism can lead to changes in the epigenetic state, including DNA methylation and histone modification. These changes may affect various cellular physiological processes, leading to accelerated liver cell deterioration and death.[63] Numerous studies have highlighted the impact of high-fat diet on the transcriptional profiles of liver mRNA, including ApoB and dipeptidyl peptidase-4 (DPP-4). Research findings suggest that the suppression of essential genes is triggered by an increase in DNA methylation frequency.[64,65] These epigenetic mechanisms may contribute to the development of NAFLD by modifying the cellular cycle, apoptosis, and inflammation signaling pathways.[66] Furthermore, the accumulation of ROS will intensify TG accumulation, leading to a vicious cycle.

4.2. Oxidative stress

Excess lipids lead to heightened mitochondrial stress and ROS production, initiating an oxidative stress response that disrupts the structure and function of hepatocytes. A study found that treatment with palmitic acid, a key fat metabolite, significantly increased ROS levels in cells, leading to mitophagy and endothelial cell damage.[67] Scientists have long recognized various forms of cell death, including apoptosis, autophagy, and necrosis, in the development of liver disease. Notably, oxidative stress critically regulates these processes.[68,69]

4.3. Cell death

Liver diseases primarily originate from the death of numerous hepatocytes, often triggered by a high-fat diet or other lipid metabolic disorders, through various mechanisms. In the NASH model, hepatocytes showed increased expression of apoptosis-related proteins, such as caspase and JNK.[70] Simultaneously, there was activation of phosphorylation of necrosis-associated receptor-interacting protein (RIP)1, RIP3, and mixed lineage kinase domain-like (MLKL) proteins.[71,72] Lipid accumulation concurrently promotes necrosis and apoptosis in hepatocytes.[70]

5. DNL-targeted NAFLD therapy

5.1. Traditional NAFLD treatment

National guidelines for diagnosing and treating NAFLD recommend lifestyle-based interventions, primarily exercise and dietary modifications, as the most effective approach to manage the disease.[1,73] Reducing total caloric intake and increasing the consumption of soy protein,[74,75] omega-3 FA,[76,77] and monounsaturated fatty acids (MUFA) are recognized for their preventive and therapeutic benefits. Sport is equally important, with a 5%–10% body mass reduction significantly improving histological alterations in NAFLD.[78] Research shows that daily 60-min treadmill walks, along with improved sedentary habits could improve NAFLD cell apoptosis markers and insulin sensitivity.[79] Weight loss is also crucial in NAFLD treatment. Bariatric surgery, in addition to lifestyle changes, is the most effective treatment for obesity and NAFLD.[80,81] A study showed that after one year, 85% of the 109 recipients did not develop NASH, and 33% experienced remission of fibrosis (Fig. 3).[82] However, achieving sustained weight loss through lifestyle modifications alone can be challenging for most people with obesity, including those with NAFLD. Moreover, bariatric surgery may not be feasible for all NASH patients and should only be considered for those with concurrent obesity-related comorbidities. The 2018 American Association for the Study of Liver Diseases (AASLD) guidelines assert, "It is premature to consider foregut bariatric surgery as an established option to specifically treat NASH."[83] As a result, patients frequently need medication for additional treatment.

Recent scientific advancements in understanding the pathogenesis of NAFLD have led to the identification of numerous therapeutic drug targets. These drugs can be classified into four categories.[84] (i) Primarily focusing on metabolic-related factors to target the metabolic stress induced by hepatic lipid deposition. (ii) Mitigating oxidative stress, inflammation, and injuries due to metabolic stress. (iii) Regulating the gut–liver axis interactions in NAFLD. (iv) Liver fibrosis therapeutics could potentially mitigate the progression of fibrosis and its associated complications (Fig. 3).

5.2. Drugs that inhibit DNL

As previously mentioned, several factors in NAFLD can collectively stimulate the upregulation of DNL, subsequently triggering lipotoxicity. Single-factor therapy may not effectively reverse the progression of NAFLD. Pharmacological inhibition of DNL may present a promising therapeutic intervention. Recently, numerous plant-derived natural products have been identified as potential DNL inhibitors,[85] setting the stage for the development of synthetic equivalents. Some DNL inhibitors are currently under clinical trial,

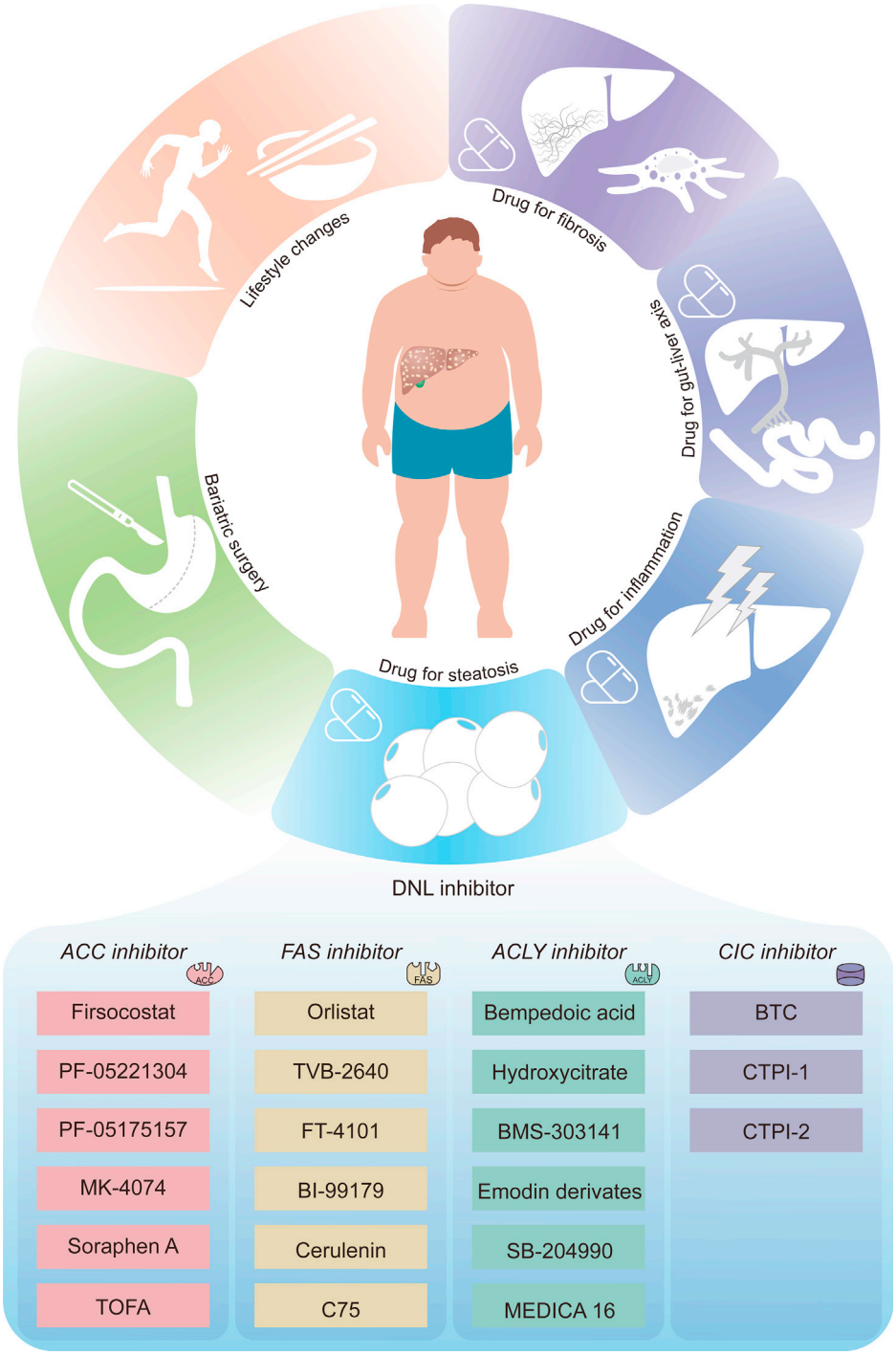

Fig. 3. NAFLD treatment. Current treatment options for NAFLD include lifestyle modifications, bariatric surgery, and drug interventions. Certain drugs that inhibit DNL show promise in managing NAFLD, with some even reaching clinical trials and gaining regulatory approval for treating other diseases. Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP-citrate lyase; BTC, benzenetricarboxylate; CIC, citrate-isocitrate carrier; DNL, *de novo* lipogenesis; FAS, fatty acid synthase; NAFLD, non-alcoholic fatty liver disease.

offering potential strategies for NAFLD treatment.[86] This review evaluates inhibitors targeting the primary enzymes of DNL, namely CIC inhibitors, ACLY inhibitors, ACC inhibitors, and FAS inhibitors (Fig. 3 and Table 1).

5.2.1. CIC inhibitor

The CIC, located within the inner mitochondrial membrane, facilitates the transport of citrate from the mitochondria into the cytoplasm.[87] Benzenetricarboxylate (BTC) has been used as a first-generation inhibitor of CIC.[87] However, its ability to bind to other proteins presents challenges for therapeutic development. The second-generation inhibitor, CTPI-1, was discovered in yeast CIC. By enhancing CTPI-1, CTPI-2 demonstrated a 20 times higher affinity than its predecessor.[88] In patients with NAFLD, there was an increase in CIC expression in the liver. Remarkably, the administration of CTPI-2 in high-fat diet-fed mice resulted in the reversal of steatohepatitis and liver injury, along with reductions in cholesterol and TG levels.[89] CTPI-2 may also influence gluconeogenesis,

Table 1
DNL inhibitors have entered clinical trials.

Target	Compound	Developer	Condition	Study phase	Clinical trial ID
ACLY	Bempedoic acid[a]	Esperion Therapeutics, Inc.	Hypercholesterolemia Atherosclerotic cardiovascular disease	Phase 3 (completed)	NCT03067441 NCT02988115 NCT02666664 NCT02993406 NCT02991118
	Hydroxycitrate	Natural product	Obesity Oxidative stress	Phase 1/2 (terminated)	NCT01238887
			Obesity Type 2 diabetes mellitus	Phase 4 (terminated)	NCT00699413
ACC	Firsocostat	Gilead Sciences	Pharmacodynamic effects of GS-0976 on fractional DNL NASH	Phase 1 (completed)	NCT02876796 NCT02891408
			NASH	Phase 2 (completed)	NCT02856555 NCT03987074
	PF-05221304	Pfizer Inc.	NAFLD NASH	Phase 2 (completed)	NCT03248882
	PF-05175157	Pfizer Inc.	Type 2 diabetes mellitus Acne vulgaris	Phase 2 (terminated) Phase 2 (withdrawn)	NCT01792635 NCT02100527
	MK-4074	Merck Sharp & Dohme LLC	NAFLD	Phase 1 (completed)	NCT01431521
FAS	Orlistat[b]	Hoffmann-La Roche	NASH Hepatitis	Phase 4 (completed)	NCT00207311
	TVB-2640	Sagimet Biosciences Inc.	NASH NASH Hepatic impairment Cirrhosis	Phase 2 (active, not recruiting) Phase 1 (recruiting)	NCT04906421 NCT05835180
	FT-4101	Forma Therapeutics, Inc.	NASH Overweight or obesity	Phase 1/2 (terminated)	NCT04004325

Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP-citrate lyase; DNL, *de novo* lipogenesis; FAS, fatty acid synthase; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.
[a] The USA Food and Drug Administration (FDA) has approved bempedoic acid for the treatment of primary hypercholesterolemia and atherosclerotic cardiovascular disease.
[b] The USA FDA has approved orlistat for weight management in cases of obesity.

leading to improved glucose tolerance and insulin sensitivity. Inhibitors of CIC hold potential for use in weight loss, anti-inflammation,[90] and anti-cancer therapies.[91,92] For instance, inhibiting CIC could hinder the proliferation of acute myeloid leukemia cells, while simultaneously increasing the rate of apoptosis.[93] CIC is widely expressed across various physiological areas, including the liver, reproductive organs, gastrointestinal tract, and adipose tissue.[86] Despite its promise for treating NAFLD, regulatory requirements necessitate that it remains in preclinical trial stages.

5.2.2. ACLY inhibitor

ACLY, exhibiting the highest expression levels in fat and liver tissue and the lowest in skeletal muscle, is a compelling drug target. Several ACLY inhibitors have been identified that effectively inhibit the synthesis of both FA and cholesterol, while simultaneously promoting the vital process of FA β-oxidation. The initial ACLY inhibitors discovered and studied were natural compounds: (−)-hydroxycitric acid (HCA),[94] purpurone,[95] radicicol,[96] and cucurbitacin b.[97] Despite their potent activity in inhibiting ACLY, their action mechanisms and specificity remain uninvestigated. The USA Food and Drug Administration (FDA) recently authorized the use of bempedoic acid for patients with heterozygous familial hypercholesterolemia and arteriosclerotic cardiovascular disease (ASCVD). The drug, being the first oral lipid-lowering medication approved in the USA in two decades, specifically targets ACLY. Bempedoic acid has shown its competitive potential in producing a CoA conjugate. The conversion of this substance to CoA conjugates involves the long-chain acyl-CoA synthetase (ACSVL1/FATP2/SCL27A2). This enzyme is predominantly expressed in liver tissue and rarely in other areas. Bempedoic acid's characteristics make it liver-specific.[98] Research has shown that administering bempedoic acid to NASH model mice increases FA oxidation. This intervention concurrently suppressed lipogenesis, alleviated steatosis, and reduced serum TG. Interestingly, bempedoic acid participates in the collagen production pathway, lessening liver fibrosis and encouraging hepatic stellate cells to adopt an anti-fibrotic role.[99] While it is currently only approved as a prescription drug for hypercholesterolemia and ASCVD, the therapeutic potential and liver-specific characteristics of bempedoic acid indicate a possible future use in NAFLD treatment.

5.2.3. ACC inhibitor

In mammals, ACC comprises two subtypes, termed ACC1 (also known as ACC α) and ACC2 (also known as ACC β). ACC1 is ubiquitously expressed, while ACC2 is primarily found in skeletal muscle, breast, fat, and liver.[86] As a potential therapeutic target, ACC inhibition offers a dual benefit: it both suppresses DNL and enhances FA oxidation. Significant advancements in developing ACC-targeting inhibitors have led to numerous such inhibitors entering clinical trials. PF-05221304, developed by Pfizer, is a notable inhibitor with excellent liver selectivity.[100] This drug avoids potential toxicity by not inhibiting platelet formation and preventing developmental defects.[101,102] Phase II studies of PF-05221304, used alone or combined with diacylglycerol acyltransferase (DGAT) inhibitors (PF-06865571) for NAFLD, have been completed.[103] The drug independently facilitated a 50%–65% dose-dependent reduction in liver fat for dosages ≥10 mg once daily (QD). However, complications like increased TG production arose. In contrast, when combined with PF-06865571, patients did not discontinue their medication despite some adverse effects, suggesting that co-administration of both drugs could potentially address the shortcomings of ACC inhibitors. Nimbus Therapeutics has developed a variety of ACC inhibitors, including ND-630 that targets a distinct domain. The inhibitors are designated as ND-646, ND-654, etc. ND-630 (firsocostat), currently in phase II trials for NASH, showed promising results in guinea pig models, reducing

liver DNL by 22% and liver steatosis by 21%.[104] This indicates significant potential benefits for NASH patients.[105]

5.2.4. FAS inhibitor

FAS is highly expressed in human adipose tissue and reproductive organs. Eukaryotic FAS typically falls into two categories. Type 1 FAS multifunctional complexes, expressed in the cytoplasm,[106] exclusively synthesize palmitic acid. Impressively, type 2 FAS operates independently of its counterpart, functioning exclusively within mitochondria to produce a variety of FA.[107] Successful inhibitors of FAS, such as orlistat, GSK2194069, Bi-99179, TVB-3166, and TVB-2640, have been developed. Among them, orlistat is an FDA-approved drug currently used for weight management in obese individuals. These drugs, some of which have demonstrated significant cytotoxicity against cancer cells *in vitro*, have advanced to clinical trials. This suggests potential therapeutic benefits in tumor treatment.

5.3. DNL inhibitor challenges

5.3.1. Drug safety

The safety of any drug remains a paramount concern. Various safety challenges exist regarding DNL inhibitors. One such challenge involves their potential detrimental effects on embryonic development. Mice deficient in ACLY,[108] ACC1,[109] or FAS died at different stages of embryonic development,[110] while those lacking CIC developed inflammatory neurodevelopmental disorders. Genetic defects are not the sole culprits, as pharmacological inhibition of DNL also leads to toxic effects. The ACC inhibitor PF-05175157 significantly induced developmental toxicity in both rat and rabbit models.[101] These findings suggest that DNL likely plays a vital role in embryonic neural development. It is essential to avoid systemic suppression of DNL to prevent negative effects on developing embryos. Further research is necessary to evaluate the safety of DNL inhibitors in pregnant women, and caution should be exercised until this data is available.

The differences between Glires and humans significantly influence the use of DNL inhibitors. Consider the clinical trial for the ACC inhibitor PF05175157, where no toxic effects were observed in rodents, but it led to a reduction in human platelet numbers.[102] Indeed, ACC inhibition in bone marrow can halt megakaryocyte maturation.[102] Recent studies have suggested the feasibility of selectively inhibiting key enzymes such as FAS, ACLY, and ACC within the liver.[86] However, it remains uncertain whether novel studies on other organs and cells will determine the long-term safety of inhibiting DNL. Therefore, DNL inhibitors must be as liver-selective as possible.

5.3.2. Metabolic imbalance

The use of DNL inhibitors represents a potentially innovative factor that could influence metabolic balance. For instance, their use can redistribute lipids, elevate serum TG levels, and induce hyperlipidemia.[111] These adverse effects were noted in studies on MK-4074 and ND-630.[105] It is now clear that ACC inhibition triggers FA feedback, leading to the suppression of the PPAR pathway and clinical signs of increased TG. ACC inhibitors are currently in development, and it is expected that they will be used in combination with PPAR or farnesoid X receptor (FXR) agonists to minimize potential adverse reactions. Moreover, inhibition of DNL is known to cause the accumulation of metabolic intermediates, leading to significant biological effects.[112] Optimal treatment outcomes may necessitate the integration of lifestyle modifications with the use of these inhibitors. Furthermore, ACLY inhibitors were initially anticipated to be effective due to their ability to inhibit TG production. However, excessive fructose consumption can lead to TG production independently of ACLY, potentially limiting the efficacy of these inhibitors. The activation of this compensatory pathway could potentially affect the body's metabolic profile.

6. Conclusions and future perspectives

In recent years, significant progress has been made in the investigation of the pathogenesis and pharmacotherapy of NAFLD. Nevertheless, achieving histological improvement in NASH treatment, particularly surpassing a 50% threshold, remains a significant challenge. Hepatic steatosis is the foundation of conditions associated with NAFLD. Therefore, understanding the main factors contributing to TG accumulation is of utmost importance. Among the various TG sources, DNL warrants our attention. There is substantial evidence suggesting significant alterations in DNL occur in NAFLD. Though DNL-derived TG may not form a significant part of liver fat, its production notably increases in NAFLD and is subject to complex regulation. Thus, targeting DNL is a critical research focus for the treatment of NAFLD.

Our literature review has analyzed the mechanisms of DNL upregulation, including high fructose intake, insulin resistance, microRNA dysregulation, and other factors. Despite their distinct characteristics, all these regulatory mechanisms enhance DNL gene expression at the transcriptional level. Excessive TG accumulation can lead to liver tissue damage and further disease progression. Targeting downstream DNL could effectively reduce TG accumulation, considering the complex nature of the upstream regulatory network. Some drugs have proven effective in both preclinical and clinical trials, with a few even gaining FDA approval for treating other diseases.

Nevertheless, it is crucial to acknowledge the limitations of this therapeutic strategy, including concerns about drug safety and potential metabolic imbalances. Increasing evidence suggests that the pathways implicated in NAFLD are interconnected, influencing each other as both causes and effects.[23] Therefore, targeting a single pathway or specific target may unintentionally trigger compensatory mechanisms or side effects through other pathways. To address these limitations, future strategies could explore the implementation of multi-target combination therapy. This approach aims to achieve a more comprehensive and effective intervention by simultaneously targeting multiple pathways. Clinical trials (NCT02891408, NCT03987074) are currently underway to investigate the combination of ACC inhibitors with PPAR or FXR agonists. This combination therapy has the potential to reduce side effects and potentially improve histological outcomes.

The target population for drug use needs to be further clarified. We need to improve the early detection of liver steatosis in individuals and promptly encourage them to enhance their lifestyle. The initial phases of NAFLD are typically reversible, but early drug intervention is generally not recommended. Nonetheless, it is crucial to acknowledge the potential risk of rapid progression. Once the disease enters an irreversible phase, drug interventions can effectively manage the progression of liver disease. We anticipate that implementing drug interventions for patients will yield significant benefits for a larger number of individuals.

This review emphasizes the significance of upregulating and treating DNL in NAFLD. We anticipate this will inspire further research in this area.

Authors' contributions

Jin Chai and Zhixian Zhu conceptualized the manuscript. Zhixian Zhu and Xiaoxun Zhang wrote this manuscript. Qiong Pan and Liangjun Zhang provided constructive comments and editing to the

manuscript. All authors have read and approved the final version of this manuscript.

Declaration of competing interest

The authors declare that there is no conflicts of interest.

Acknowledgements

This work was supported by grant from the National Natural Science Foundation of China (82325008).

References

1. Diehl AM, Day C. Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. *N Engl J Med*. 2017;377:2063–2072. https://doi.org/10.1056/NEJMra1503519.
2. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. *Lancet*. 2021;397:2212–2224. https://doi.org/10.1016/S0140-6736(20)32511-3.
3. Jacome-Sosa MM, Parks EJ. Fatty acid sources and their fluxes as they contribute to plasma triglyceride concentrations and fatty liver in humans. *Curr Opin Lipidol*. 2014;25:213–220. https://doi.org/10.1097/MOL.0000000000000080.
4. Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. *Compr Physiol*. 2017;8:1–8. https://doi.org/10.1002/cphy.c170012.
5. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*. 2005;115:1343–1351. https://doi.org/10.1172/JCI23621.
6. Diraison F, Beylot M. Role of human liver lipogenesis and reesterification in triglycerides secretion and in FFA reesterification. *Am J Physiol*. 1998;274:E321–E327. https://doi.org/10.1152/ajpendo.1998.274.2.E321.
7. Marques-Lopes I, Ansorena D, Astiasaran I, Forga L, Martínez JA. Postprandial de novo lipogenesis and metabolic changes induced by a high-carbohydrate, low-fat meal in lean and overweight men. *Am J Clin Nutr*. 2001;73:253–261. https://doi.org/10.1093/ajcn/73.2.253.
8. Softic S, Cohen DE, Kahn CR. Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig Dis Sci*. 2016;61:1282–1293. https://doi.org/10.1007/s10620-016-4054-0.
9. Knebel B, Haas J, Hartwig S, et al. Liver-specific expression of transcriptionally active SREBP-1c is associated with fatty liver and increased visceral fat mass. *PLoS One*. 2012;7:e31812. https://doi.org/10.1371/journal.pone.0031812.
10. Fullerton MD, Galic S, Marcinko K, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat Med*. 2013;19:1649–1654. https://doi.org/10.1038/nm.3372.
11. Cordoba-Chacon J, Majumdar N, List EO, et al. Growth hormone inhibits hepatic de novo lipogenesis in adult mice. *Diabetes*. 2015;64:3093–3103. https://doi.org/10.2337/db15-0370.
12. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. *Cell Metabol*. 2018;27:22–41. https://doi.org/10.1016/j.cmet.2017.08.002.
13. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology*. 2014;146:726–735. https://doi.org/10.1053/j.gastro.2013.11.049.
14. Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J. Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. *J Clin Invest*. 1996;97:2081–2091. https://doi.org/10.1172/JCI118645.
15. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol*. 2013;48:434–441. https://doi.org/10.1007/s00535-013-0758-5.
16. Hodson L, Gunn PJ. The regulation of hepatic fatty acid synthesis and partitioning: the effect of nutritional state. *Nat Rev Endocrinol*. 2019;15:689–700. https://doi.org/10.1038/s41574-019-0256-9.
17. Pinkosky SL, Groot PHE, Lalwani ND, Steinberg GR. Targeting ATP-citrate lyase in hyperlipidemia and metabolic disorders. *Trends Mol Med*. 2017;23:1047–1063. https://doi.org/10.1016/j.molmed.2017.09.001.
18. Csaki LS, Reue K. Lipins: multifunctional lipid metabolism proteins. *Annu Rev Nutr*. 2010;30:257–272. https://doi.org/10.1146/annurev.nutr.012809.104729.
19. Ferré P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab*. 2010;12:83–92. https://doi.org/10.1111/j.1463-1326.2010.01275.x.
20. Ferré P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res*. 2007;68:72–82. https://doi.org/10.1159/000100426.
21. Foufelle F, Ferré P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem J*. 2002;366:377–391. https://doi.org/10.1042/BJ20020430.
22. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest*. 2002;109:1125–1131. https://doi.org/10.1172/JCI15593.
23. Tilg H, Adolph TE, Dudek M, Knolle P. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. *Nat Metab*. 2021;3:1596–1607. https://doi.org/10.1038/s42255-021-00501-9.
24. Ma J, Fox CS, Jacques PF, et al. Sugar-sweetened beverage, diet soda, and fatty liver disease in the Framingham Heart Study cohorts. *J Hepatol*. 2015;63:462–469. https://doi.org/10.1016/j.jhep.2015.03.032.
25. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol*. 2008;48:993–999. https://doi.org/10.1016/j.jhep.2008.02.011.
26. Herman MA, Peroni OD, Villoria J, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature*. 2012;484:333–338. https://doi.org/10.1038/nature10986.
27. Softic S, Gupta MK, Wang GX, et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J Clin Invest*. 2017;127:4059–4074. https://doi.org/10.1172/JCI94585.
28. Matsuzaka T, Shimano H, Yahagi N, et al. Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. *Diabetes*. 2004;53:560–569. https://doi.org/10.2337/diabetes.53.3.560.
29. Bindesbøll C, Fan Q, Nørgaard RC, et al. Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity. *J Lipid Res*. 2015;56:771–785. https://doi.org/10.1194/jlr.M049130.
30. Nagai Y, Yonemitsu S, Erion DM, et al. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab*. 2009;9:252–264. https://doi.org/10.1016/j.cmet.2009.01.011.
31. Jang C, Hui S, Lu W, et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab*. 2018;27:351–361 (e3). https://doi.org/10.1016/j.cmet.2017.12.016.
32. Zhao S, Jang C, Liu J, et al. Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate. *Nature*. 2020;579:586–591. https://doi.org/10.1038/s41586-020-2101-7.
33. Smith GI, Shankaran M, Yoshino M, et al. Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. *J Clin Invest*. 2020;130:1453–1460. https://doi.org/10.1172/JCI134165.
34. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*. 2008;7:95–96. https://doi.org/10.1016/j.cmet.2007.12.009.
35. Tremblay F, Jacques H, Marette A. Modulation of insulin action by dietary proteins and amino acids: role of the mammalian target of rapamycin nutrient sensing pathway. *Curr Opin Clin Nutr Metab Care*. 2005;8:457–462. https://doi.org/10.1097/01.mco.0000172589.55434.03.
36. Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. *Cell Metab*. 2006;3:393–402. https://doi.org/10.1016/j.cmet.2006.05.003.
37. Kammoun HL, Chabanon H, Hainault I, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J Clin Invest*. 2009;119:1201–1215. https://doi.org/10.1172/JCI37007.
38. Choi CS, Savage DB, Kulkarni A, et al. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. *J Biol Chem*. 2007;282:22678–22688. https://doi.org/10.1074/jbc.M704213200.
39. Xia JY, Holland WL, Kusminski CM, et al. Targeted induction of ceramide degradation leads to improved systemic metabolism and reduced hepatic steatosis. *Cell Metab*. 2015;22:266–278. https://doi.org/10.1016/j.cmet.2015.06.007.
40. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol*. 2017;14:32–42. https://doi.org/10.1038/nrgastro.2016.147.
41. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature*. 2019;576:51–60. https://doi.org/10.1038/s41586-019-1797-8.
42. Liu CH, Ampuero J, Gil-Gómez A, et al. miRNAs in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol*. 2018;69:1335–1348. https://doi.org/10.1016/j.jhep.2018.08.008.
43. Xiao M, Li J, Li W, et al. MicroRNAs activate gene transcription epigenetically as an enhancer trigger. *RNA Biol*. 2017;14:1326–1334. https://doi.org/10.1080/15476286.2015.1112487.
44. Long JK, Dai W, Zheng YW, Zhao SP. miR-122 promotes hepatic lipogenesis via inhibiting the LKB1/AMPK pathway by targeting Sirt1 in non-alcoholic fatty liver disease. *Mol Med*. 2019;25:26. https://doi.org/10.1186/s10020-019-0085-2.
45. Alizadeh E, Akbarzadeh A, Eslaminejad MB, et al. Up regulation of liver-enriched transcription factors HNF4a and HNF6 and liver-specific microRNA (miR-122) by inhibition of let-7b in mesenchymal stem cells. *Chem Biol Drug Des*. 2015;85:268–279. https://doi.org/10.1111/cbdd.12398.
46. Ng R, Wu H, Xiao H, et al. Inhibition of microRNA-24 expression in liver prevents hepatic lipid accumulation and hyperlipidemia. *Hepatology*. 2014;60:554–564. https://doi.org/10.1002/hep.27153.
47. Ye D, Zhang T, Lou G, et al. Plasma miR-17, miR-20a, miR-20b and miR-122 as potential biomarkers for diagnosis of NAFLD in type 2 diabetes mellitus patients. *Life Sci*. 2018;208:201–207. https://doi.org/10.1016/j.lfs.2018.07.029.
48. Winkle M, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics - challenges and potential solutions. *Nat Rev Drug Discov*. 2021;20:629–651. https://doi.org/10.1038/s41573-021-00219-z.

49. Liu M, Cho WC, Flynn RJ, Jin X, Song H, Zheng Y. microRNAs in parasite-induced liver fibrosis: from mechanisms to diagnostics and therapeutics. *Trends Parasitol*. 2023;39:859–872. https://doi.org/10.1016/j.pt.2023.07.001.
50. Biscans A, Coles A, Haraszti R, et al. Diverse lipid conjugates for functional extra-hepatic siRNA delivery in vivo. *Nucleic Acids Res*. 2019;47:1082–1096. https://doi.org/10.1093/nar/gky1239.
51. Yang L, Zhang Y, Wang L, et al. Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. *J Hepatol*. 2010;53:339–347. https://doi.org/10.1016/j.jhep.2010.03.004.
52. Gao Y, Hua R, Hu K, Wang Z. Carbohydrates deteriorate fatty liver by activating the inflammatory response. *Nutr Res Rev*. 2022;35:252–267. https://doi.org/10.1017/S0954422421000202.
53. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology*. 2003;38:413–419. https://doi.org/10.1053/jhep.2003.50316.
54. Heida A, Gruben N, Catrysse L, et al. The hepatocyte IKK:NF-κB axis promotes liver steatosis by stimulating de novo lipogenesis and cholesterol synthesis. *Mol Metab*. 2021;54:101349. https://doi.org/10.1016/j.molmet.2021.101349.
55. Gu L, Zhu Y, Lin X, et al. The IKKβ-USP30-ACLY axis controls lipogenesis and tumorigenesis. *Hepatology*. 2021;73:160–174. https://doi.org/10.1002/hep.31249.
56. Zmuda-Trzebiatowska E, Oknianska A, Manganiello V, Degerman E. Role of PDE3B in insulin-induced glucose uptake, GLUT-4 translocation and lipogenesis in primary rat adipocytes. *Cell Signal*. 2006;18:382–390. https://doi.org/10.1016/j.cellsig.2005.05.007.
57. Arroyave-Ospina JC, Wu Z, Geng Y, Moshage H. Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy. *Antioxidants (Basel)*. 2021;10:174. https://doi.org/10.3390/antiox10020174.
58. Quijano C, Trujillo M, Castro L, Trostchansky A. Interplay between oxidant species and energy metabolism. *Redox Biol*. 2016;8:28–42. https://doi.org/10.1016/j.redox.2015.11.010.
59. Sekiya M, Hiraishi A, Touyama M, Sakamoto K. Oxidative stress induced lipid accumulation via SREBP1c activation in HepG2 cells. *Biochem Biophys Res Commun*. 2008;375:602–607. https://doi.org/10.1016/j.bbrc.2008.08.068.
60. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med*. 2018;24:908–922. https://doi.org/10.1038/s41591-018-0104-9.
61. Murase T, Misawa K, Minegishi Y, et al. Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice. *Am J Physiol Endocrinol Metab*. 2011;300:E122–E133. https://doi.org/10.1152/ajpendo.00441.2010.
62. Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J Hepatol*. 2018;68:280–295. https://doi.org/10.1016/j.jhep.2017.11.014.
63. Lee JH, Friso S, Choi SW. Epigenetic mechanisms underlying the link between non-alcoholic fatty liver diseases and nutrition. *Nutrients*. 2014;6:3303–3325. https://doi.org/10.3390/nu6083303.
64. Chen HC, Chen YZ, Wang CH, Lin FJ. The nonalcoholic fatty liver disease-like phenotype and lowered serum VLDL are associated with decreased expression and DNA hypermethylation of hepatic ApoB in male offspring of ApoE deficient mothers fed a with Western diet. *J Nutr Biochem*. 2020;77:108319. https://doi.org/10.1016/j.jnutbio.2019.108319.
65. Baumeier C, Schlüter L, Saussenthaler S, et al. Elevated hepatic DPP4 activity promotes insulin resistance and non-alcoholic fatty liver disease. *Mol Metab*. 2017;6:1254–1263. https://doi.org/10.1016/j.molmet.2017.07.016.
66. Li J, Yuan S, Norgard RJ, et al. Epigenetic and transcriptional control of the epidermal growth factor receptor regulates the tumor immune microenvironment in pancreatic cancer. *Cancer Discov*. 2021;11:736–753. https://doi.org/10.1158/2159-8290.CD-20-0519.
67. Wu W, Xu H, Wang Z, et al. PINK1-Parkin-mediated mitophagy protects mitochondrial integrity and prevents metabolic stress-induced endothelial injury. *PLoS One*. 2015;10:e0132499. https://doi.org/10.1371/journal.pone.0132499.
68. Fulop T, Pawelec G, Dupuis G, et al. Immunosenescence, Oxidative Stress, and Cancers. In: Rezaei N, ed. *Cancer Immunology: A Translational Medicine Context*. Springer International Publishing; 2020:513–531.
69. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat Rev Drug Discov*. 2021;20:689–709. https://doi.org/10.1038/s41573-021-00233-1.
70. Akazawa Y, Nakao K. To die or not to die: death signaling in nonalcoholic fatty liver disease. *J Gastroenterol*. 2018;53:893–906. https://doi.org/10.1007/s00535-018-1451-5.
71. Christofferson DE, Yuan J. Necroptosis as an alternative form of programmed cell death. *Curr Opin Cell Biol*. 2010;22:263–268. https://doi.org/10.1016/j.ceb.2009.12.003.
72. Zhang DW, Shao J, Lin J, et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science*. 2009;325:332–336. https://doi.org/10.1126/science.1172308.
73. Mantovani A, Byrne CD, Targher G. Efficacy of peroxisome proliferator-activated receptor agonists, glucagon-like peptide-1 receptor agonists, or sodium-glucose cotransporter-2 inhibitors for treatment of non-alcoholic fatty liver disease: a systematic review. *Lancet Gastroenterol Hepatol*. 2022;7:367–378. https://doi.org/10.1016/S2468-1253(21)00261-2.
74. Oliveira LP, de Jesus RP, Boulhosa RS, et al. Effect of soy protein supplementation in patients with chronic hepatitis C: a randomized clinical trial. *World J Gastroenterol*. 2012;18:2203–2211. https://doi.org/10.3748/wjg.v18.i18.2203.
75. Bortolotti M, Kreis R, Debard C, et al. High protein intake reduces intrahepatocellular lipid deposition in humans. *Am J Clin Nutr*. 2009;90:1002–1010. https://doi.org/10.3945/ajcn.2008.27296.
76. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol*. 2012;56:944–951. https://doi.org/10.1016/j.jhep.2011.08.018.
77. Thérien A, Cieślak A, Verreault M, et al. Omega-3 polyunsaturated fatty acid: a pharmaco-nutraceutical approach to improve the responsiveness to ursodeoxycholic acid. *Nutrients*. 2021;13:2617. https://doi.org/10.3390/nu13082617.
78. Goodpaster BH, Delany JP, Otto AD, et al. Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults: a randomized trial. *JAMA*. 2010;304:1795–1802. https://doi.org/10.1001/jama.2010.1505.
79. Fealy CE, Haus JM, Solomon TP, et al. Short-term exercise reduces markers of hepatocyte apoptosis in nonalcoholic fatty liver disease. *J Appl Physiol (1985)*. 2012;113:1–6. https://doi.org/10.1152/japplphysiol.00127.2012.
80. Courcoulas AP, Christian NJ, Belle SH, et al. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. *JAMA*. 2013;310:2416–2425. https://doi.org/10.1001/jama.2013.280928.
81. Ikramuddin S, Korner J, Lee WJ, et al. Lifestyle intervention and medical management with vs without Roux-en-Y gastric bypass and control of hemoglobin A1c, LDL cholesterol, and systolic blood pressure at 5 years in the diabetes surgery study. *JAMA*. 2018;319:266–278. https://doi.org/10.1001/jama.2017.20813.
82. Lassailly G, Caiazzo R, Buob D, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology*. 2015;149:379–388. https://doi.org/10.1053/j.gastro.2015.04.014.
83. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018;67:328–357. https://doi.org/10.1002/hep.29367.
84. Rotman Y, Sanyal AJ. Current and upcoming pharmacotherapy for non-alcoholic fatty liver disease. *Gut*. 2017;66:180–190. https://doi.org/10.1136/gutjnl-2016-312431.
85. Chen Q, Wang T, Li J, et al. Effects of natural products on fructose-induced nonalcoholic fatty liver disease (NAFLD). *Nutrients*. 2017;9:96. https://doi.org/10.3390/nu9020096.
86. Batchuluun B, Pinkosky SL, Steinberg GR. Lipogenesis inhibitors: therapeutic opportunities and challenges. *Nat Rev Drug Discov*. 2022;21:283–305. https://doi.org/10.1038/s41573-021-00367-2.
87. Klingenberg M. Kinetic study of the tricarboxylate carrier in rat liver mitochondria. *Eur J Biochem*. 1972;26:587–594. https://doi.org/10.1111/j.1432-1033.1972.tb01801.x.
88. Fernandez HR, Gadre SM, Tan M, et al. The mitochondrial citrate carrier, SLC25A1, drives stemness and therapy resistance in non-small cell lung cancer. *Cell Death Differ*. 2018;25:1239–1258. https://doi.org/10.1038/s41418-018-0101-z.
89. Tan M, Mosaoa R, Graham GT, et al. Inhibition of the mitochondrial citrate carrier, Slc25a1, reverts steatosis, glucose intolerance, and inflammation in preclinical models of NAFLD/NASH. *Cell Death Differ*. 2020;27:2143–2157. https://doi.org/10.1038/s41418-020-0491-6.
90. Infantino V, Iacobazzi V, Menga A, Avantaggiati ML, Palmieri F. A key role of the mitochondrial citrate carrier (SLC25A1) in TNFα- and IFNγ-triggered inflammation. *Biochim Biophys Acta*. 2014;1839:1217–1225. https://doi.org/10.1016/j.bbagrm.2014.07.013.
91. Catalina-Rodriguez O, Kolukula VK, Tomita Y, et al. The mitochondrial citrate transporter, CIC, is essential for mitochondrial homeostasis. *Oncotarget*. 2012;3:1220–1235. https://doi.org/10.18632/oncotarget.714.
92. Poolsri WA, Phokrai P, Suwankulanan S, et al. Combination of mitochondrial and plasma membrane citrate transporter inhibitors inhibits de novo lipogenesis pathway and triggers apoptosis in hepatocellular carcinoma cells. *Biomed Res Int*. 2018;2018:3683026. https://doi.org/10.1155/2018/3683026.
93. Liu F, Deng S, Li Y, Du J, Zeng H. SLC25A1-associated prognostic signature predicts poor survival in acute myeloid leukemia patients. *Front Genet*. 2023;13:1081262. https://doi.org/10.3389/fgene.2022.1081262.
94. Watson JA, Fang M, Lowenstein JM. Tricarballylate and hydroxycitrate: substrate and inhibitor of ATP: citrate oxaloacetate lyase. *Arch Biochem Biophys*. 1969;135:209–217. https://doi.org/10.1016/0003-9861(69)90532-3.
95. Chan GW, Francis TA, Thureen DR, et al. Purpurone, an inhibitor of ATP-citrate lyase: a novel alkaloid from the marine sponge Iotrochota sp. *J Org Chem*. 1993;58:2544–2546. https://doi.org/10.1021/JO00061A031.
96. Ki SW, Ishigami K, Kitahara T, Kasahara K, Yoshida M, Horinouchi S. Radicicol binds and inhibits mammalian ATP citrate lyase. *J Biol Chem*. 2000;275:39231–39236. https://doi.org/10.1074/jbc.M006192200.
97. Gao Y, Islam MS, Tian J, Lui VW, Xiao D. Inactivation of ATP citrate lyase by Cucurbitacin B: a bioactive compound from cucumber, inhibits prostate cancer growth. *Cancer Lett*. 2014;349:15–25. https://doi.org/10.1016/j.canlet.2014.03.015.
98. Pinkosky SL, Newton RS, Day EA, et al. Liver-specific ATP-citrate lyase inhibition by bempedoic acid decreases LDL-C and attenuates atherosclerosis. *Nat Commun*. 2016;7:13457. https://doi.org/10.1038/ncomms13457.
99. Morrow MR, Batchuluun B, Wu J, et al. Inhibition of ATP-citrate lyase improves NASH, liver fibrosis, and dyslipidemia. *Cell Metab*. 2022;34:919–936 (e8). https://doi.org/10.1016/j.cmet.2022.05.004.

100. Bergman A, Carvajal-Gonzalez S, Tarabar S, Saxena AR, Esler WP, Amin NB. Safety, tolerability, pharmacokinetics, and pharmacodynamics of a liver-targeting Acetyl-CoA carboxylase inhibitor (PF-05221304): a three-part randomized phase 1 study. *Clin Pharmacol Drug Dev*. 2020;9:514–526. https://doi.org/10.1002/cpdd.782.
101. Catlin NR, Bowman CJ, Campion SN, et al. Inhibition of Acetyl-CoA carboxylase causes malformations in rats and rabbits: comparison of mammalian findings and alternative assays. *Toxicol Sci*. 2021;179:183–194. https://doi.org/10.1093/toxsci/kfaa169.
102. Kelly KL, Reagan WJ, Sonnenberg GE, et al. De novo lipogenesis is essential for platelet production in humans. *Nat Metab*. 2020;2:1163–1178. https://doi.org/10.1038/s42255-020-00272-9.
103. Calle RA, Amin NB, Carvajal-Gonzalez S, et al. ACC inhibitor alone or co-administered with a DGAT2 inhibitor in patients with non-alcoholic fatty liver disease: two parallel, placebo-controlled, randomized phase 2a trials. *Nat Med*. 2021;27:1836–1848. https://doi.org/10.1038/s41591-021-01489-1.
104. Lawitz EJ, Coste A, Poordad F, et al. Acetyl-CoA carboxylase inhibitor GS-0976 for 12 weeks reduces hepatic de novo lipogenesis and steatosis in patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2018;16:1983–1991 (e3). https://doi.org/10.1016/j.cgh.2018.04.042.
105. Loomba R, Kayali Z, Noureddin M, et al. GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. *Gastroenterology*. 2018;155:1463–1473 (e6). https://doi.org/10.1053/j.gastro.2018.07.027.
106. Smith S, Witkowski A, Joshi AK. Structural and functional organization of the animal fatty acid synthase. *Prog Lipid Res*. 2003;42:289–317. https://doi.org/10.1016/s0163-7827(02)00067-x.
107. Hiltunen JK, Schonauer MS, Autio KJ, Mittelmeier TM, Kastaniotis AJ, Dieckmann CL. Mitochondrial fatty acid synthesis type II: more than just fatty acids. *J Biol Chem*. 2009;284:9011–9015. https://doi.org/10.1074/jbc.R800068200.
108. Beigneux AP, Kosinski C, Gavino B, Horton JD, Skarnes WC, Young SG. ATP-citrate lyase deficiency in the mouse. *J Biol Chem*. 2004;279:9557–9564. https://doi.org/10.1074/jbc.M310512200.
109. Abu-Elheiga L, Matzuk MM, Kordari P, et al. Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. *Proc Natl Acad Sci U S A*. 2005;102:12011–12016. https://doi.org/10.1073/pnas.0505714102.
110. Chirala SS, Chang H, Matzuk M, et al. Fatty acid synthesis is essential in embryonic development: fatty acid synthase null mutants and most of the heterozygotes die in utero. *Proc Natl Acad Sci U S A*. 2003;100:6358–6363. https://doi.org/10.1073/pnas.0931394100.
111. Kim CW, Addy C, Kusunoki J, et al. Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: a bedside to bench investigation. *Cell Metab*. 2017;26:576. https://doi.org/10.1016/j.cmet.2017.08.011.
112. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science*. 2009;324:1076–1080. https://doi.org/10.1126/science.1164097.