

Rho-Kinase as a Therapeutic Target for Nonalcoholic Fatty Liver Diseases

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Nonalcoholic fatty liver disease (NAFLD) is a major public health problem and the most common form of chronic liver disease, affecting 25% of the global population. Although NAFLD is closely linked with obesity, insulin resistance, and type 2 diabetes mellitus, knowledge on its pathogenesis remains incomplete. Emerging data have underscored the importance of Rho-kinase (Rho-associated coiled-coil-containing kinase [ROCK]) action in the maintenance of normal hepatic lipid homeostasis. In particular, pharmacological blockade of ROCK in hepatocytes or hepatic stellate cells prevents the progression of liver diseases such as NAFLD and fibrosis. Moreover, mice lacking hepatic ROCK1 are protected against obesity-induced fatty liver diseases by suppressing hepatic *de novo* lipogenesis. Here we review the roles of ROCK as an indispensable regulator of obesity-induced fatty liver disease and highlight the key cellular pathway governing hepatic lipid accumulation, with focus on *de novo* lipogenesis and its impact on therapeutic potential. Consequently, a comprehensive understanding of the metabolic milieu linking to liver dysfunction triggered by ROCK activation may help identify new targets for treating fatty liver diseases such as NAFLD.


Keywords: Rho-kinase; AMP-activated protein kinase; Diet, high-fat; Lipogenesis; Nonalcoholic fatty liver disease

The Sulwon Award for Scientific Achievement is the Korean Diabetes Association's highest scientific award and honors an individual who has excellently contributed to the progress in the field of diabetes and metabolism. The Sulwon Award is named after an emeritus professor, Eung Jin Kim, who founded Korean Diabetes Association. Prof. Young-Bum Kim received the 12th Sulwon Award at the 2020 International Congress of Diabetes and Metabolism which was held as a virtual congress from September 18 to 19 in 2020.

INTRODUCTION

During the 20th century, dramatic changes in lifestyle and eating habits led to a remarkable increase in obesity, which is a major risk factor for the development of nonalcoholic fatty liver

er disease (NAFLD) [1,2]. NAFLD, known as the hepatic manifestation of the metabolic syndrome, is the most common form of chronic liver disease. This condition is closely associated with insulin resistance, type 2 diabetes mellitus (T2DM), and obesity [3]. NAFLD is an umbrella term covering a broad spectrum of liver diseases characterized by different grades of severity in fat accumulation, inflammation, injury, and fibrosis [4]. The pathological manifestation of NAFLD ranges from simple steatosis or nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and fibrosis/cirrhosis that can ultimately lead to hepatocellular carcinoma and liver failure [1,2,4]. NAFL or fatty liver refers to simple steatosis characterized by storage of excess macrovesicular fat in more than 5% of hepatocytes. NASH is considered the most severe disease grade which includes inflammation, hepatocyte damage and

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pericellular fibrosis, and ultimately can progress to cirrhosis and hepatocellular carcinoma [5].

The worldwide prevalence of NAFLD is 6% to 35% (median 20%) [6], whereas the estimated prevalence in the United States varies from 18% in the overall population to 28% in overweight individuals [7]. Individuals with NAFLD are generally obese and often present other pathophysiologic features of the metabolic syndrome, including insulin resistance, hypertension, dyslipidemia, and diabetes [8,9]. In fact, NAFLD prevalence amongst individuals with T2DM is 55.5% and 37.3% for NASH [10]. Thus, identifying new insights into the biological foundations of obesity-related fatty liver is highly crucial given the substantial rise in NAFLD prevalence worldwide. In this review, we discuss the emerging roles of Rho-kinase in the development of fatty liver disease and the consequences on systemic glucose homeostasis, with a particular focus on a molecular link with hepatic *de novo* lipogenesis (DNL).

PATHOLOGY OF NONALCOHOLIC FATTY LIVER DISEASES

The liver is an important metabolic organ that is essential for maintaining whole-body glucose and lipid homeostasis [1]. Impaired liver functions lead to insulin resistance causing T2DM and is often found in obesity-associated metabolic diseases such as NAFLD [1,11]. The pathophysiological mechanism(s) underlying NAFLD onset and progression appears to be a 'multiple-hit' process that includes insulin resistance, hormones secreted from the adipose tissue, nutritional factors, gut microbiota as well as genetic and epigenetic factors [12]. Initially, an increase in liver fat accumulation through a disruption of the homeostatic balance between triglyceride synthesis and clearance is detected. However, hepatic lipogenic pathways and concomitant inflammation are activated when obesity develops [12]. Specifically, pro-inflammatory cytokines secreted by adipose tissue is involved in the pathological process of ectopic fat accumulation within the hepatocyte and overall steatosis [13]. This development implies that the liver is not necessarily responsible for the initial development of metabolic inflammation but, with the gradual progression of obesity, is an important contributor to perpetuating this overall state of inflammation. Additionally, mitochondrial dysfunction in the liver is crucial in NAFLD development in metabolic disorders such as metabolic syndrome, obesity, and T2DM [14,15]. The

delicate metabolic homeostasis between lipid accumulation and fuel utilization in liver cells is regulated by mitochondrial activity, including β -oxidation of free fatty acids, electron transfer and adenosine triphosphate production, as well as reactive oxygen species (ROS). Any imbalance between pro- and anti-oxidant activities leads to a decrease in β -oxidation and an increase in ROS production [14], which could ultimately contribute to NAFLD's pathogenesis.

Several types of the cells were found in the liver, including hepatic parenchymal cells, sinusoidal endothelial cells, hepatic stellate cells (HSCs), Kupffer cells, and dendritic cells [16]. HSCs comprise approximately 10% of all liver cells. These cells are typically found in the space of Disse, lying between liver sinusoidal endothelial cells and hepatocytes [17]. In a non-pathological state, HSCs are dormant, displaying a quiescent, non-proliferative phenotype (qHSCs). This cell stores vitamin A, cholesteryl esters and triglycerides in lipid vacuoles in the cytosol [18]. HSCs, in physiological conditions, regulate extracellular matrix (ECM) homeostasis through the release of ECM proteins, degrading enzymes (matrix metalloproteinases), and their tissue inhibitors. qHSCs are also known to produce a range of growth factors and other metabolic mediators such as the hepatocyte growth factor and vascular endothelial growth factor [19]. Following liver injury, HSCs are activated into myofibroblasts that drive expressing fibrogenic genes, including collagen type 1, α -smooth muscle actin, and the tissue inhibitor of metalloproteinase. They proliferate and migrate to the liver injury site, where ECM secretion is stimulated [20]. Several studies have shown that fibrosis can be reversed upon the injury's removal, specifically, myofibroblasts will undergo apoptosis and inactivation, promoting fibrosis regression [21,22].

Kupffer cells are liver resident macrophages located within the lumen of the sinusoids and are adherent to the endothelial cells composing the blood vessel walls [18]. These cells play a key role in preventing undesired immune response under normal conditions, whereas they are activated under the pathophysiological conditions such as NAFLD. Activated Kupffer cells induce numerous inflammatory chemokines and cytokines secretion, resulting in detrimental effects on hepatic function, inflammation, and insulin resistance [18,23]. In fact, chemical-mediated ablation of Kupffer cells protects hepatic insulin resistance and hepatic steatosis in response to a high-fat diet (HFD) [24,25], highlighting the vital role for Kupffer cells in the progression of hepatic lipid accumulation. Notably, Kupffer cells also induce HSCs from a quiescent state to an ac-

tivated state, leading to the production of collages and initiation of liver fibrogenesis [26]. The regulatory mechanisms underlying this phenomenon remain to be elucidated.

Rho-KINASE INHIBITOR AMELIORATES NONALCOHOLIC FATTY LIVER DISEASES

Rho-associated coiled-coil-containing kinase (ROCK) is a serine/threonine protein kinase identified as a guanosine triphosphate (GTP)-Rho-binding protein [27]. Two isoforms of ROCK, ROCK1 (also known as ROK β) [28,29] and ROCK2 (also known as ROK α) [28,30] were identified. ROCK has a kinase domain at its N-terminal domain, a coiled-coil domain in its middle portion, and a putative pleckstrin-homology domain at its C-terminal domain split by the insertion of a Cys-rich region. The Rho binding domain of ROCK is in the C-terminal portion of the coiled-coil domain [27]. ROCK activity increases when bound with (Rho-GTP) Rho-GTP through a Rho-binding domain [27]. The amino acid sequences of the two ROCK isoforms have 65% overall identity, with their kinase domains exhibiting 92% identity [27].

The initial study of the ROCK inhibitor led by the Narumiya group demonstrated that treating a hypertensive animal with a high dose of the ROCK inhibitor (Y27632, 100 mg/kg/day for 5 months) results in decreased blood pressure [31]. These data suggest the ROCK inhibitor is a potential therapeutic target for many diseases resulting from abnormally high smooth muscle contraction. Since the introduction of this work, numerous studies have consistently revealed that ROCK inhibition is beneficial for multiple cardiovascular-related illnesses [32-34]. Beyond these investigations, a pharmacotherapy with the ROCK inhibitor has also been found to be effective in treating liver diseases, including NAFLD, NASH, and fibrosis as shown in Table 1 [35-53]. Data supporting a role of ROCK on liver diseases include the fact that a ROCK chemical inhibitor ameliorated hepatic steatosis and fibrosis in rats fed a choline-deficient/L-amino acid-defined (CDAA) diet and choline-deficient diet [35-37]. These findings are also accompanied by decreased alanine aminotransferase (ALT)/aspartate aminotransferase (AST) and serum lipids levels [37]. The cellular mechanism(s) underlying this phenomenon could include involvement of the inactivation of HSCs [35-37], which have a crucial role in the development of hepatic fibrosis [54]. In support of this, the experimental evidence has demonstrated that selective delivery of the ROCK inhibitor to HSCs reduces

chronic carbon tetrachloride (CCl₄)-induced liver fibrosis or cirrhosis by inhibiting activated HSCs in mice [38,39], highlighting an essential role for ROCK action in HSCs. Similar observations have been found in various animal models, including HFD-fed mice [40], HFD and streptozotocin (STZ)-treated rats [41], STZ-induced diabetic rats [42], dimethylnitrosamine-induced hepatic fibrosis rats [43], and endotoxin-treated mice [44,45]. In animal models of CCl₄-induced liver fibrosis, the ability of a ROCK inhibitor to reduce HSCs activation is greatly enhanced, leading to the prevention of liver fibrosis development [39,46-48]. Given the fact that ROCK inhibitors, including Y27632 and fasudil (HA1077), inhibit both ROCK1 and ROCK2 kinase activity [55,56], it is conceivable that the blockage of both isoforms may play a pivotal role in the protective effects of progressive liver diseases. This limitation has been an issue in the field and appears to result from limited knowledge on the isoform specificity of ROCK inhibitor. In this respect, an orally bioavailable ROCK2 inhibitor (ANG4201), which is >200 fold selective for ROCK2 versus ROCK1, has recently been developed by Angion Biomedica Corp. (San Francisco, CA, USA) [57]. Consistent with the aforementioned findings, the treatment of NASH mice with ANG4201 for 2 months also leads to a significant improvement in fibrosis, which is associated with decreased ALT/AST and cholesterol [57]. Equally, another ROCK2 selective inhibitor (RXC007) has been found to have the antifibrotic effect [58]. However, these results will need to be confirmed by a more specific genetic approach where ROCK2 in HSCs or hepatocytes is genetically deleted selectively, providing definitive knowledge as to whether ROCK2 is an indispensable regulator of liver diseases. Nevertheless, these data highlight compelling evidence that the hyper-activation of ROCK in the liver may contribute to the etiopathogenesis of hepatic dysfunction and that ROCK is a potential therapeutic target for obesity-induced fatty liver diseases such as NAFLD.

THE IMPACT OF HEPATIC ROCK1 ON OBESITY AND INSULIN SENSITIVITY

Earlier studies demonstrated that aberrant ROCK1 expression and/or activity are associated with numerous metabolic syndrome-related disorders, including cardiovascular disease, insulin resistance, obesity, and T2DM and its complications [59-63]. These works have never explored ROCK expression and/or activity in the liver of altered metabolic states such as obesi-

Table 1. Rho-kinase inhibition improves liver diseases

Animal models with liver diseases	Treatments	Major findings	Reference
Fibrosis induced by CDAA in rats	Y-27632 (1 mg/kg) was treated for 12 weeks	Improvement of hepatic fibrosis and steatosis Decreased ALT and HA levels Reduced hepatic TNF α expression	[35]
Steatosis induced by choline-deficient diet for 6 weeks in rats and the liver was subjected to IR injury	Vitamin A-coupled liposomes carrying Y-27632 for targeted HSCs was administered before ischemia induction	Improved the survival rate after IR injury, the liver blood flow, and the portal perfusion pressure Decreased AST levels	[37]
Steatosis induced by choline-deficient diet for 6 weeks and the liver was subjected to IR injury in rats	Fasudil (10 mg/kg) was administered for 30 minutes before ischemia induction	Attenuation of HSCs activation Improvement of IR injury Decreased AST and ALT levels Reduced ET-1 serum levels	[36]
Fibrosis induced by CCl $_4$ treatment for 12 weeks in rats	Y-27635 (30 mg/kg) was treated for 6 weeks	Prevented the development of liver fibrosis Decreased α SMA and TGF β 1-positive cell Decreased total collagen Increased survival rate after hepatectomy	[46,50]
CCl $_4$ -induced acute liver injury in mice	Y-27632 (1.5 mg/kg) was injected at 24 and 48 hours after CCl $_4$ injection	Reduction of local activation of HSCs Decreased α SMA	[47]
CCl $_4$ -induced acute liver injury in mice	Single injection of HA-1077 (10 mg/kg)	Reduced hepatic apoptosis Decreased ALT levels	[51]
Fibrosis induced by CCl $_4$ treatment for 7 weeks in mice	Y27632 conjugate (45 mg/kg/day) that is taken up in activated HSCs for 2 weeks	Inhibited activation of HSCs Decreased ALT and AST level Reduced liver fibrosis	[39]
Cirrhosis induced by CCl $_4$ -intoxication and BDL in rats	Y-27635 coupled with mannose-6-phosphate targeting HSCs activation	Decreased the portal pressure Decreased the hepatic-portal resistance No extrahepatic effects	[38]
Cirrhosis induced by BDL or CCl $_4$ in rats	Y27632 coupled with human serum albumin substituted with PDGFR β -recognizing peptides (Y27pPBHSA)	Lower portal pressure, hepatic vascular resistance without effect on systemic vascular resistance Reduced intrahepatic resistance by decreased moesin and MLC	[48]
Mouse model of NAFLD induced by HFD	RKI-1447 (2 and 8 mg/kg) was treated for 3 weeks	Decreased ALT and AST levels Decreased serum cholesterol and triglyceride levels Ameliorated hepatic steatosis	[40]
Diabetic rats made by a combination of HFD and streptozotocin	Fasudil (10 mg/kg) was treated for 14 weeks	Amelioration of liver fibrosis Inhibited TGF β 1/CTGF pathway and α SMA expression	[41]
Streptozotocin-induced type 1 diabetic rats	Fasudil (2 mg/kg b.i.d., 10 mg/kg b.i.d., H-Fas group) was treated for 8 weeks	Suppression of inflammation and accumulation of the extracellular matrix Downregulated TGF β 1 and MMP9/TIMP-1 Decreased NF- κ B activation	[42]
Rat model of DMN-induced fibrosis	Y27632 (30 mg/kg) was orally administered for 4 weeks	Decrease liver fibrosis Reduced hepatic collagen and hydroxyproline Decreased hepatic α SMA expression	[43]

(Continued to the next page)

Table 1. Continued

Animal models with liver diseases	Treatments	Major findings	Reference
Endotoxin-induced mice made by a combination of LPS and D-galactosamine	Fasudil (40 mg/kg) or Y-27632 (10 mg/kg) by a single injection	Decreased ALT and AST levels Decreased hepatic apoptosis Attenuated LPS-induced liver injury Reduced LPS-induced leukocyte adhesion Decreased hepatic TNF α and CXC chemokines expression	[45]
LPS-induced acute liver injury in mice	Y-27632 (10 mg/kg) was injected prior to LPS injection	Attenuated LPS-induced liver injury Reduced the LPS-induced hepatic inflammatory response and oxidative stress Protective effects on hepatic mitochondrial function	[44]
Obstructive cholestasis induced by BDL in mice (hepatocellular damage)	Y-27632 (1 and 10 mg/kg) was injected prior to BDL	Decreased serum AST and ALT levels Reduced CXC chemokines and leukocyte recruitment Restored sinusoidal perfusion	[52]
Rats underwent intrahepatic tumor implantation followed by orthotopic liver transplantation	Y-27632 (10 mg/kg) for 28 days	Suppression of cancer cell migration Suppression of tumor recurrence Increased survival rate	[53]
Mice lacking hepatic ROCK1	HFD for 16 weeks	Prevented HFD-induced fatty liver Decreased hepatic TG and cholesterol in serum and liver Decreased <i>de novo</i> lipogenesis	[49]
Mice overexpressing a constitutively active mutant of ROCK1 in the liver	HFD for 12 weeks	Promoted HFD-induced fatty liver Increased serum TG and cholesterol levels Increased hepatic TG	[49]
Leptin-deficient mice (<i>ob/ob</i>) mice lacking hepatic ROCK1	No treatment	Prevented the development of fatty liver Decreased hepatic TG and cholesterol levels	[49]

CDAA, choline-deficient/L-amino acid-defined; ALT, alanine aminotransferase; HA, hyaluronic acid; TNF α , tumor necrosis factor alpha; IR, ischemia-reperfusion; HSC, hepatic stellate cell; AST, aspartate aminotransferase; ET-1, endothelin-1; CCl $_4$, carbon tetrachloride; α SMA, α -smooth muscle actin; TGF β , transforming growth factor- β ; BDL, bile duct ligation; PDGFR β , platelet-derived growth factor receptor β ; MLC, myosin light chain; NAFLD, nonalcoholic fatty liver disease; HFD, high-fat diet; CTGF, connective tissue growth factor; b.i.d., twice a day; MMP9, matrix metalloproteinase 9; TIMP-1, tissue inhibitors of metalloproteinases-1; NF- κ B, Nuclear factor kappa B; DMN, dimethylnitrosamine; LPS, lipopolysaccharide; ROCK1, Rho-associated coiled-coil-containing kinase; TG, triglyceride.

ty. Our study focused on investigating whether the expression or activity of ROCK1 in the liver changes in metabolic-linked diseases. In rodents, a marked increase in ROCK1 activity or expression is observed in the liver and adipose tissue of insulin-resistant, obese, or T2DM animal models [49]. In contrast, a significant decrease in ROCK1 activity is detected in the hypothalamus of these animals [63], suggesting site-specific regulation of ROCK1 in metabolically active organs. In individuals with fatty liver disease, ROCK1 expression greatly increased in the liver and strongly correlated with risk factors clustering with fatty liver diseases or liver damage [49]. These

data demonstrate that ROCK1 has a vital role in the pathogenesis of obesity-linked metabolic diseases, including NAFLD.

Given the fact that obesity is a major risk factor for developing NAFLD and drives hepatic ROCK1 expression [1,49], our laboratory has undertaken studies to establish the physiological function of ROCK1 in the liver, using animal models with genetic modifications of hepatic ROCK1. Mice lacking hepatic ROCK1 are resistant to the development of obesity during high-fat feeding, primarily due to increased energy expenditure. Hepatic ROCK1 deletion also significantly improves glucose tolerance and insulin sensitivity in mice on high-fat or

regular chow diets [49]. Support for this comes from a pharmacological study showing that 6 weeks of treatment of a ROCK inhibitor ameliorates diet-induced obesity and glucose intolerance as well as energy expenditure in mice [64]. Similarly, it was reported that 4 weeks of treatment with a ROCK inhibitor decreased body weight and improved glucose tolerance in obese Zucker animals [65]. However, it should be noted that long-term ROCK inhibition by a chemical inhibitor reduces body weight in insulin-resistant obese animals, which could possibly be secondary to insulin-sensitizing effects [64,65]. In keeping with these observations, our study further demonstrates that high-fat feeding greatly promotes obesity and insulin resistance in transgenic mice expressing a constitutively active form of hepatic ROCK1 compared with control mice. The importance of hepatic ROCK1 action on glucose metabolism is further underscored by the finding that leptin-deficient *ob/ob* mice lacking hepatic ROCK1 display improved insulin sensitivity compared with control *ob/ob* mice [49]. Collectively, these data demonstrate that hepatic ROCK1 is necessary and sufficient for regulating normal body-weight homeostasis and glucose metabolism, establishing a key role of hepatic ROCK1 in fuel metabolism.

CONTROL OF *DE NOVO* LIPOGENESIS BY ROCK1

Biochemistry and physiology of hepatic *de novo* lipogenesis

When an organism ingests an amount of food that exceeds its immediate energy needs, the excess nutrients are directed into the formation of storage products that can be later mobilized for energy generation when food is less available. Triglyceride represents an energy-dense and physiologically benign storage product and is the principal sink for the disposal of excess nutrient carbons. The biosynthesis of triglyceride can be considered a two-stage process: first, the formation of long-chain fatty acyl CoA (LCFA-CoA) by a process known as DNL whose pathway is summarized in Fig. 1, and second, the sequential esterification of glycerol-3-phosphate by three LCFA-CoA to form triacylglycerol. DNL is usually the rate-limiting step for the synthesis of triglyceride from non-lipid precursors [66]. Therefore, expression and activity of the enzymes that constitute the DNL pathway, coupled with the availability of precursor substrates, determine the rate of hepatic triglyceride synthesis [67]. DNL occurs in the cytosol and relies on the generation of cytosolic acetyl coenzyme A (acetyl-CoA) and nicotin-

amide adenine dinucleotide phosphate (NADPH). With the exception of a few substrates, such as the short-chain fatty acid acetate which can be converted to acetyl-CoA in the cytosol via acyl-coenzyme A synthetase short-chain family member 2 (ACSS2) [68], almost all other nutrients are catabolized to mitochondrial acetyl-CoA, which is then transferred to the cytosol via the citrate shuttle.

The pentose phosphate pathway (PPP) is the primary source of NADPH generation. Recent studies indicate that oxidative pentose pathway flux is highly coupled to that of DNL, at least during high-sugar feeding [69]. The generation of two NADPH equivalents via the PPP requires oxidation of one carbohydrate carbon to CO₂, therefore synthesis of one equivalent of palmitoyl-CoA involves the oxidation of 1.17 equivalents of glucose-6-P for the generation of NADPH alone. In part, because of its strong dependence on PPP activity, DNL is more responsive to the availability of carbohydrates compared to other nutrients. DNL activity is highly modulated by insulin through positive- and negative-feedback loops which are conditional on carbohydrate availability and feeding state [70,71] and stimulated by sugar-sensing factors such as carbohydrate-responsive element-binding protein (ChREBP) [72]. Thus, diets high in simple sugars such as glucose and fructose promote high hepatic DNL rates [73,74]. In addition to contributing directly to the hepatic lipid burden, DNL also attenuates hepatic fatty acid oxidation by generating malonyl-CoA, a potent inhibitor of long-chain fatty acid transported into the mitochondria via the carnitine shuttle. Normally, DNL is acutely suppressed following intake of high fat/low carbohydrate diets. However, the development of insulin resistance can result in maintaining DNL activity in this setting in part because of the increased availability of hepatic glucose-6-phosphate resulting from chronic hyperglycemia and/or uncontrolled hepatic gluconeogenesis. This is associated with increased ChREBP activity that upregulates DNL independently of any impairments of hepatic insulin signaling [75].

Measurement of hepatic DNL with deuterated water (²H₂O)

Tracers that directly measure DNL provide estimates of fractional synthetic rates (i.e., the percent contribution of DNL to the total triglyceride pool over a defined interval). These estimates are derived from the precursor enrichment analysis, measured either directly or inferred from mass isotopomer distribution analysis (MIDA) of the product [76]. Although earlier studies of DNL were performed with infused [1-¹³C]- or

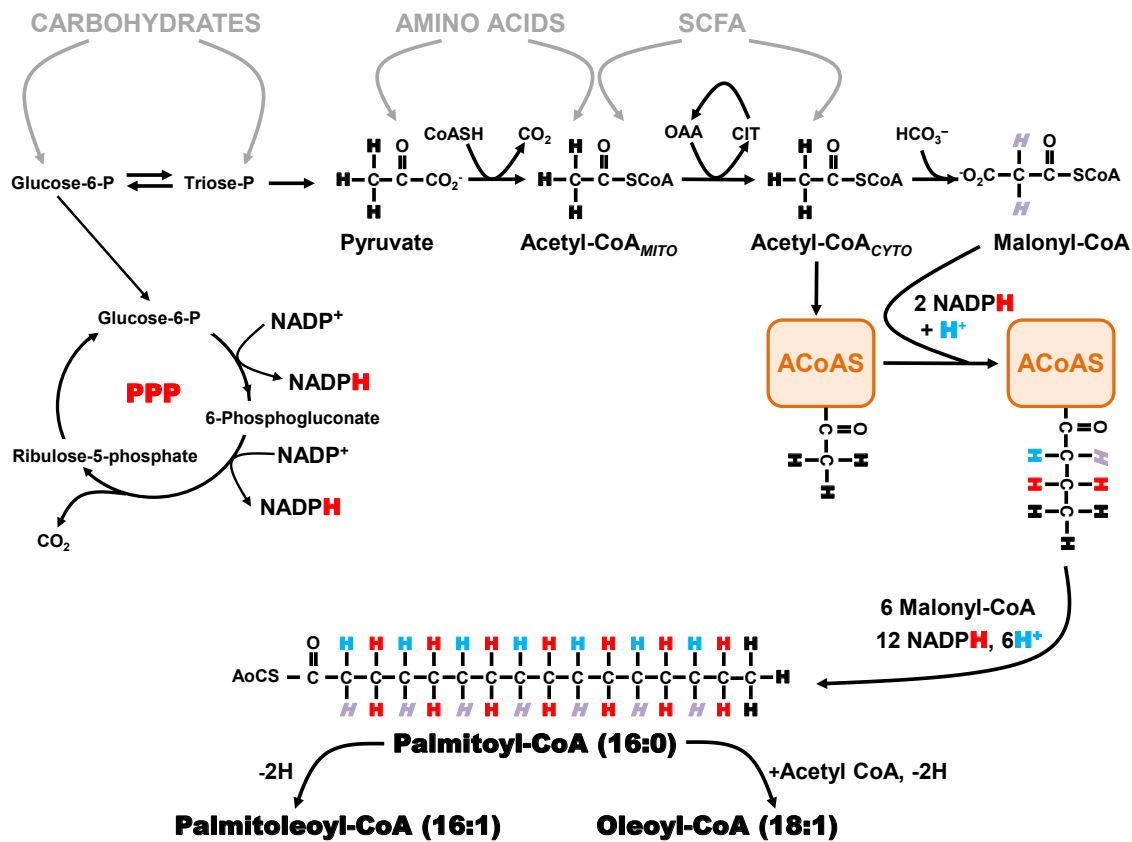


Fig. 1. Schematic of *de novo* lipogenesis. Entry points for the main nutrient precursors are shown in gray and the sources of hydrogens of the fatty acyl coenzyme A (acyl-CoA) product are represented by different colors: ‘H’ hydrogens derived from body water, black ‘H’ for the methyl hydrogens of the initial acetyl-CoA that binds to acyl-CoA synthase, red ‘H’ for the hydrogens derived from nicotinamide adenine dinucleotide phosphate (NADPH) via the pentose phosphate pathway (PPP), and ‘H’ for the hydrogens derived from malonyl-CoA. Also represented is the acyl-CoA synthase complex (ACoAS) following the initial acetyl-CoA and malonyl-CoA additions. Intracellular acetyl-CoA is represented as distinct mitochondrial (Acetyl-CoA_{MITO}) and cytosolic (acetyl-CoA_{CYTO}) pools. CoASH, free acetyl CoA; SCoA, CoA moiety; SCFA, short chain fatty acids; OAA, oxaloacetate; CIT, citrate; AoCS, Co moiety.

[1-¹⁴C] acetate, there has been a widespread shift to the use of ²H₂O, in large part because it is less costly than the acetate tracer and can be administered orally over an unlimited period of time. This latter aspect is important for the study of DNL contributions to lipid pools because of their relatively slow turnover rates, particularly for humans. In addition, ²H₂O can be administered to humans on an outpatient basis under “real life” conditions [77] or to animals without disrupting their normal feeding routines [78].

As shown in Fig. 1, the hydrogens of the fatty acid product of DNL can be traced to four specific sources: (1) body water; (2) the methyl hydrogens of the first acetyl-CoA molecule that binds to fatty acyl-CoA synthase; (3) malonyl-CoA; and (4)

NADPH. The PPP NADPH hydrogens are in turn derived from hydrogens 1 and 3 of glucose-6-phosphate. When ²H₂O is administered, the ²H rapidly mixes with the hydrogens of body water and is directly incorporated into the nascent fatty acid chain during the second hydrogenation step. In addition, the ²H present in body water can be incorporated into acetyl-CoA and malonyl-CoA precursors via specific exchange processes that occur during the formation of these metabolites. The NADPH hydrogens become enriched in part through exchange as well as by acquisition of ²H from glucose-6-P that was itself enriched from body water ²H via metabolic exchange processes associated with gluconeogenesis and Cori cycle activity. As a result, all the fatty acyl product hydrogens become

enriched with ^2H with the level of enrichment of a particular hydrogen relying on that of the body water precursor and the extent to which the exchange processes are complete. Although early studies suggested that ^2H enrichment of newly-synthesized fatty acids was only ~50% to 70% of the theoretical value, thereby requiring a correction factor for determining DNL rates [79], a more recent analysis suggests that under some conditions, $^2\text{H}/^1\text{H}$ exchanges approach 100% [69]. Fatty acids that undergo elongation also incorporate ^2H by the same exchange mechanisms, but only into the elongated front portion. Positional analysis of fatty acid enrichment by ^2H nuclear magnetic resonance (NMR) allows elongation and DNL rates to be resolved [80] and for transfer of hydrogen from glucose via NADPH to be followed [69].

DNL measurements in animal models and humans

The deuterated water method has been widely applied to study the involvement of DNL in NAFLD pathogenesis in both humans and animal models. The main constraint of this measurement in humans is that direct sampling of liver triglyceride via needle biopsy is typically not performed *per se* because of the unacceptable risk/benefit. As a less invasive alternative, triglycerides from circulating very low-density lipoprotein (VLDL) are sampled, with the assumption that these derived from the hepatic triglyceride pool and therefore report the same tracer enrichment levels as hepatic triglyceride [81]. Conversion of fractional synthetic rates to absolute DNL rates requires an independent measurement of triglyceride turnover. In the liver, this is complicated by the appearance of fatty acids from sources other than DNL, such as from chylomicron remnants and circulating non-esterified fatty acids, and a disposal of fatty acids by several processes other than VLDL export, such as mitochondrial and peroxisomal oxidation. Finally, due to an extensive interchange of fatty acids between adipose tissues—particularly visceral adipose tissue—and the liver, a portion of hepatic fatty acid and VLDL labeling may in fact have originated from adipocyte DNL activity [77]. For humans, the liver is considered the principal site of DNL synthesis; hence, adipocyte contributions to hepatic fatty acid labeling may be minor. However, for many animals including rodents and pigs, adipocyte lipogenesis activity contributes substantially more to whole body DNL rates and could therefore have a significant influence on hepatic lipid labeling and activity attributed to hepatic DNL [82,83]. Because altered DNL is highly implicated in the pathogenesis of NAFLD [84,85], this pathway activity

has become a focus both toward enhancing our understanding of NAFLD pathogenesis and as a putative pharmacological target for NAFLD treatment. Table 2 provides an overview of selected hepatic DNL studies in human subjects with NAFLD. In summary, hepatic DNL contributes about 5% to 10% of hepatic triglyceride appearance in healthy post-absorptive humans, whereas this rate is significantly elevated to 15% to 25% for patients with NAFLD [11,81,84,86-88]. Elevated DNL activity is tightly associated with increased hepatic lipid levels. Decreases in hepatic steatosis resulting from either dietary intervention (typically, a reduction in caloric intake), and pharmacological inhibition of fatty acids synthase, are associated with decreases in fractional DNL rates.

Role of ROCK1 in hepatic DNL activity

Our research efforts first sought to identify a central role of ROCK1 in the liver mediating DNL [49]. In obese mice where hepatic steatosis was induced by high-fat feeding, liver-specific deletion of ROCK1 expression resulted in an amelioration of hepatic triglyceride levels [49]. We explored the physiologic mechanisms by which ROCK1 regulates hepatic lipid accumulation by assessing DNL activity, fatty acid uptake and oxidation, and hepatic VLDL release. A marked reduction in fractional DNL rates determined by ^2H NMR of deuterated water $^2\text{H}_2\text{O}$ in liver-specific ROCK1-deficient mice fed a HFD was observed. Because these studies were performed with body-weight matching ROCK1-deficient mice, the possible confounding effects of adiposity can be excluded. In relation to DNL measurement, differences in whole body triglyceride pools could modify hepatic fatty acid ^2H -enrichment independently of changes in absolute hepatic DNL rates. To demonstrate that this effect could not be attributed to some uncharacterized physiological aspect of liver-specific ROCK1-deficient mice beside adiposity, we further studied liver-specific ROCK1-deficient mice fed a normal chow diet that had a similar body weight to control mice. We found that DNL rates significantly reduced for this group of mice. However, deleting hepatic ROCK1 had no effects on hepatocyte fatty acid uptake or oxidation, and hepatic VLDL export rates. This suggests the decrease in hepatic lipid accumulation caused by ROCK1 deficiency is solely explained by significant reductions in fractional DNL rates. In conjunction with DNL activity, we also investigated whether decreased hepatic lipid accumulation by ROCK1 deletion is due to changes in gene expression of key enzymes involved in glucose and lipid metabolism. Consistent

Table 2. Selected studies of *de novo* lipogenesis in patients with NAFLD

Study outline	Tracer method for DNL assay	Major findings	Reference
DNL rates were measured in lean healthy subjects and in overweight patients with suspected NAFLD based on transaminase levels and hepatic ultrasound tests.	$^2\text{H}_2\text{O}$ given over 20 hours and enrichment of palmitate within TG-rich lipoprotein measured after a breakfast meal.	This was among the first published study of DNL in NAFLD subjects using the $^2\text{H}_2\text{O}$ tracer. The contribution of DNL to the appearance rate of plasma TG was ~3-fold higher for the NAFLD subjects compared to the healthy controls.	[86]
Comparison of DNL contributions to liver TG appearance in obese subjects with low or with high liver fat but matched for other metabolic factors	$^2\text{H}_2\text{O}$ given over 11 days and enrichment of palmitate within TG-rich lipoprotein measured.	Subjects with high liver fat levels had a DNL contribution to liver fat appearance that was twice that of subjects with low liver TG levels.	[84]
Determine the sources of fatty acids contributing to hepatic TG and in secreted lipoprotein of NAFLD patients during both fed and fasted states	[1- ^{13}C]acetate infused over 4 days. Hepatic TG directly sampled by liver biopsy and plasma lipoprotein TG also sampled.	Analysis of plasma lipoprotein TG yielded similar flux estimates to those of liver TG. DNL accounted for about one-quarter of liver and plasma lipoprotein TG synthesis. DNL was active during both fed and fasted states.	[81]
Determine the effect of age-induced skeletal muscle insulin resistance on postprandial hepatic lipid kinetics by studying elderly subjects and young subjects matched for body composition and physical activity	$^2\text{H}_2\text{O}$ given over 2 days and enrichment of palmitate within VLDL measured.	Hepatic DNL rates were two-fold higher in the elderly cohort. Hepatic lipid levels were three-fold higher in the elderly cohort. These were associated with increased fasting plasma glucose levels and increased muscle insulin resistance as seen by impaired muscle glycogen synthesis rates.	[87]
Determine the effects of a fatty acid synthase inhibitor (FT-4101) on hepatic DNL rates in NAFLD patients	$^2\text{H}_2\text{O}$ given over 14 days and enrichment of VLDL-palmitate measured during a period of fructose ingestion.	12 Weeks of FT-4101 administration significantly decreased fractional DNL rates over placebo in a dose-dependent manner. This was accompanied by a significant decrease in liver TG levels.	[88]
Compare DNL rates in lean subjects without NAFLD, obese subjects without NAFLD and obese subjects with NAFLD before and after placement on a weight-loss diet	$^2\text{H}_2\text{O}$ given over 3–5 weeks and enrichment of palmitate within TG-rich lipoprotein measured.	DNL rates were highest in obese-NAFLD subjects and were directly correlated with plasma insulin and glucose concentrations. Moderate weight loss resulted in significant decreases in both DNL rates and liver TG levels that was linked to significant decreases in plasma glucose and insulin levels.	[11]

NAFLD, nonalcoholic fatty liver disease; DNL, *de novo* lipogenesis; TG, triglyceride; VLDL, very low-density lipoprotein.

with the results of DNL rates, deficiency of ROCK1 in the liver caused a significant decrease in gene expressions of key enzymes involved in lipogenesis but not gluconeogenesis, glycolysis, fatty acid oxidation and uptake. Importantly, these effects were also observed in *ob/ob* mice lacking hepatic ROCK1 independent of changes in body weight. Together, these data suggest the major mechanism by which deletion of ROCK1 in the liver prevents hepatic steatosis could be through decreased DNL, mediated by the reduced expression of lipogenic enzymes. Thus, our studies identify hepatic ROCK1 as a principal regulator of fat metabolism and further implicate that inhi-

bition of hepatic ROCK1 could be a novel therapeutic target for the treatment of NAFLD.

ROCK1 MEDIATES ENDOCANNABINOID-INDUCED LIPOGENESIS

The endocannabinoid system is comprised of the cannabinoid receptor 1 (CB1 receptor) and 2 (CB2 receptor), their endogenous lipidic ligands (endocannabinoids), and the enzymes involved in endocannabinoid synthesis and degradation [89]. The CB1 receptor is mainly found in the brain and is responsi-

ble for the psychotropic and behavioral effects after the use of cannabinoids. The CB2 receptor is expressed in metabolically active peripheral organs such as the liver, adipose tissue, and pancreas [90,91] and functions as a key modulator of innate immunity and fuel metabolism, amongst other roles [91,92]. In particular, during the progression of liver diseases, CB1 receptor expression is induced in hepatocytes, hepatic myofibroblasts, and endothelial cells of the liver, whereas CB2 receptor expression is up-regulated in Kupffer cells [89,93]. In this regard, a significant increase in CB1 and CB2 receptor expression in the liver of humans with NAFLD was observed [94,95], suggesting that dysregulation of the endocannabinoid system may contribute to the pathogenesis of liver diseases.

Two arachidonic acid-containing endocannabinoids, arachidonoylethanolamide (anandamide [AEA]) and 2-arachidonoylglycerol (2-AG), are identified as ligands for the CB1 and CB2 receptors [96-99]. AEA and 2-AG play important roles in regulating glucose and lipid metabolism. These functions entail lipogenic enzymes activation and fat accumulation in the liver [100], modulation of insulin secretion in islets [101,102], stimulation of lipoprotein lipase activity in adipose tissue [103], and decreases in insulin-stimulated glucose uptake and fatty acid oxidation in muscle [103,104]. All these features are key hallmarks involved in the pathogenesis of metabolic syndrome.

Antagonism and agonism of the CB1 and CB2 receptors have been extensively studied in the experimental settings of liver diseases and diet-induced obesity [105]. Numerous studies have been performed with an inverse agonist/antagonist rimonabant for the CB1 receptor. These investigations have obtained consistent outcomes on the metabolic benefits of hepatic lipid metabolism in various liver diseases models [106-111]. Recent studies have also demonstrated that inverse agonist/antagonists targeting the peripheral CB1 receptor ameliorate metabolic parameters in animal models of obesity and diabetes [111-115]. For example, treating diet-induced obese mice with a pyrazole-based inverse agonist of the CB1 receptor (RTI1092769) that has minimal brain exposure decreased hepatic triglyceride contents and improved hepatic steatosis [111]. Another peripherally-restricted inverse agonist for the CB1 receptor, JD5037, is found to have a therapeutic potential in metabolic disorders of the peripheral tissues such as the liver [113,114]. In fact, JD5037 attenuated HSCs activation of liver fibrosis by inhibiting β -arrestin1/Akt signaling in humans and rodents [115]. These pharmacological observations have been

supported by the evidence from a genetic model of liver-specific CB1 receptor knockout mice [100]. Mice lacking the CB1 receptor in the liver have significantly less hepatic steatosis, hyperglycemia, and dyslipidemia during over-nutrition [100], highlighting an important role for the CB1 receptor in regulating normal hepatic metabolism. Selective studies of the endocannabinoid system in the context of liver diseases are showed in Table 3 [106-111,116-124].

In proof-of-concept studies, we made the novel observation that endocannabinoids, AEA and 2-AG, strongly increased the catalytic activity of ROCK1 in the liver and HepG2 cells [49] and DNL rate in hepatocytes. Concurrently, gene expression of key lipogenic enzymes, including fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1), acetyl-CoA carboxylase (ACC), and sterol regulatory element-binding protein 1c (SREBP1c) also markedly increased in response to 2-AG in the liver. However, the lack of endocannabinoids' response on lipogenic gene expression was noted when hepatic ROCK1 was absent, suggesting that ROCK1 activation is necessary to regulate the endocannabinoid-mediated lipogenic program. We further hypothesize that ROCK1-dependent lipogenesis is mediated through inhibition of AMP-activated protein kinase (AMPK), as evidenced from our data showing that 2-AG suppressed the activity of hepatic AMPK in control mice but not in liver-specific ROCK1-deficient mice. In addition, ROCK1 deletion stimulated AMPK activity but ROCK1 activation suppressed AMPK activity in the liver, further highlighting a negative regulation of ROCK1 for AMPK. Therefore, our work has provided mechanistic insight into the fundamental function of ROCK1 in regulating the endocannabinoid-dependent lipogenic program.

THE REGULATORY MECHANISMS UNDERLYING ROCK1-MEDIATED LIPOGENESIS

Experimental evidence has demonstrated that AMPK plays a pivotal role in regulating glucose and fatty acid metabolism. AMPK, a highly conserved serine/threonine protein kinase, was viewed as a cellular sensor that responds to depletion in cellular energy reserves by switching off anabolic pathways and enhancing catabolic processes [125]. Activation of the AMPK and its downstream signaling pathways was found to suppress hepatic lipid synthesis [126], increase muscle fatty acid oxidation [127], and enhance insulin action [128]. In particular, ac-

Table 3. Blockade of endocannabinoid signaling ameliorates liver diseases

Animal model of metabolic dysfunction	Treatments	Major findings	Reference
Obese Zucker <i>fa/fa</i> rats	Rimonabant (30 mg/kg/day) was orally administered for 8 weeks	Decreased plasma ALT, GGT, and ALP levels Decreased hepatic TNF α levels Reduced plasma TG and cholesterol levels Improved hepatic steatosis	[106]
OLETF rats	Rimonabant (10 mg/kg/day) was orally administered for 6 weeks	Decreased serum ALT and AST levels Decreased hepatic TG levels Improved hepatic steatosis	[107]
C56BL/6J mice fed a HFD for 20 weeks	Rimonabant (10 mg/kg) was orally administered from 18th to 20th week	Decreased serum AST and ALT levels Reduced hepatic palmitic, stearic, and oleic acid proportion Ameliorated hepatic steatosis	[108]
Sprague-Dawley rats fed a choline-deficient diet for 12 weeks	Rimonabant (10 mg/kg) orally administered from 10th week to 12th week	Decreased serum AST and ALT levels Decreased hepatic malondialdehyde levels Increased hepatic glutathione peroxidase activity Reduced hepatic TGF β immunoreactive area	[109]
Male C57BL/6 mice fed a HFD for 20 weeks	Rimonabant (10 mg/kg) was orally administered from 18th week to 20th week	Decreased serum AST and ALT levels Decreased adipokines levels (leptin, visfatin, IL-6, IFN γ levels in adipose tissue) Reduced hepatic-6, IFN γ levels Improved hepatic steatosis	[110]
C57Bl/6J male mice fed a HFD underwent hypoxia exposure for 10 weeks	CB1 receptor agonist (WIN55212-2, 1 mg/kg) was treated for 4 weeks	Hypoxia-induced improvement in hepatic steatosis was abolished by CB1 receptor agonist Increased hepatic CB1 and SREBP1 expression	[116]
Male Sprague-Dawley and WKY rats injected with LPS/D-galactosamine (acute liver injury model)	CB2 agonist (JWH-133, 2.5 mg/kg) or CB1 antagonist (AM6545, 10 mg/kg) was acutely injected	CB1 antagonist or CB2 agonist had no effect on LPS/D-galactosamine-induced liver injury	[117]
NAFLD was induced by a high-fat cholesterol diet for 6 weeks	Cannabis extracts (5 mg/kg) were administered orally every 3 days for 6 weeks	No change in hepatic lipid accumulation CBD-rich extracts increased hepatic iNOS and TNF α expression THC-rich extracts decreased liver enzymes	[118]
Mice lacking CHOP fed a HFD for 14 weeks	CB1R blocker (JD5037, 3 mg/kg) was administered for 7 days	Failed to reverse DIO-induced reduction of sOB-R levels and hepatic steatosis	[119]
Liver fibrosis induced by thioacetamide (200 mg/kg) treatment for 6 weeks in rats	AM1241 (CB2 receptor activator, 3 and 6 mg/kg) was injected for 3 weeks	Suppressed hepatic TNF α , IL-1 β , and IL-6 levels Decreased hepatic TLR4, TGF β 1, α SMA, and miR-155 gene expression Inhibited the development of fibrosis	[120]
APOE*3-Leiden.CETP transgenic mice fed Western-type diet for 20 weeks	Rimonabant (20 mg/kg/day) for 4 weeks	Decreased plasma TG and non-HDL levels Increased plasma HDL-C levels Reduced VLDL-TG production Increased VLDL-TG turnover Prevented atherosclerosis	[121]

(Continued to the next page)

Table 3. Continued

Animal model of metabolic dysfunction	Treatments	Major findings	Reference
Obese mice fed on a HFD	RTI1092769 (inverse agonist/ antagonist of CB1)	Decreased hepatic TG contents Decreased plasma AST and ALT levels Improved hepatic steatosis	[111]
C57BL/6J and fat-1 transgenic mice fed a LFD for 10 weeks +10 weeks of a HFD	Fatty acids SAF oil or LIN oil were supplemented to a LFD for 10 weeks, prior to HFD exposure	Decreased hepatic AEA and 2-AG contents in LIN-treated mice Improved glucose tolerance and insulin tolerance in LIN-treated mice	[122]
Global CB1 receptor knockout mice	SR161716A (10 mg/kg) was injected before and after CCl ₄	Reduced fibrosis associated with chronic liver injury Decreased hepatic TGFβ1 expression	[123]
Global CB1 receptor knockout mice	No treatment	Decreased hepatic TG levels Reduced hepatic PLIN2 expression Suppressed lipogenesis	[124]

ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; ALP, alkaline phosphatase; TNF α , tumor necrosis factor alpha; TG, triglyceride; OLETF, Otsuka Long-Evans Tokushima Fatty; AST, aspartate aminotransferase; HFD, high-fat diet; TGF β , transforming growth factor- β ; IL-6, interleukin 6; IFN γ , interferon gamma; CB1, cannabinoid receptor type 1; SREBP1, sterol regulatory element-binding protein 1; WKY, Wistar Kyoto; LPS, lipopolysaccharide; CB2, cannabinoid receptor type 2; NAFLD, nonalcoholic fatty liver disease; CBD, cannabidiol; iNOS, inducible nitric oxide synthase; THC, tetrahydrocannabinol; CHOP, C/EBP homologous protein; CB1R, cannabinoid receptor type 1 receptor; DIO, diet-induced obesity; sOb-R, soluble leptin receptor; IL-1b, interleukin 1b; TLR4, toll like receptor 4; α SMA, α -smooth muscle actin; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; VLDL, very low-density lipoprotein; LFD, low-fat diet; SAF, safflower oil; LIN, linseed oil; AEA, arachidonoyl ethanolamide; 2-AG, 2-arachidonoylglycerol; CCl₄, carbon tetrachloride; PLIN2, perilipin 2.

tivated AMPK decreases the hepatic gene expression of the lipogenic transcription factor SREBP1c and its downstream targets ACC, SCD1, and FAS [126]. Although ROCK1 has not been suspected to be involved in AMPK signaling, our work reveals that AMPK acts as a downstream regulator of ROCK1 in the context of hepatic DNL [49]. In fact, ROCK1 negatively regulates AMPK activity, as demonstrated by the evidence that AMPK activity increased due to ROCK1 deletion but decreased by ROCK1 activation in the liver. Correspondingly, reciprocal changes in hepatic lipogenesis are seen (i.e., decreased or enhanced fatty acid synthesis caused by AMPK activation or suppression due to ROCK1 deletion or hyperactivation, respectively). Pharmacological modulations of AMPK activity in hepatocytes are enabled to regulate gene expression of key lipogenic enzymes despite the metabolic conditions where ROCK1 is either absent or activated, further confirming that ROCK1 is an upstream component of AMPK signaling. Thus, our data suggest the hepatic ROCK1 \rightarrow AMPK signaling cascade is a crucial determinant of hepatic lipid synthesis (Fig. 2) [49]. This novel pathway appears to be functional in response to an insulin sensitizer metformin [129], which further

implicates clinical significance and relevance of ROCK1 signaling. Of note, our recent work further identified G $_{\alpha 12}$ or G $_{\alpha s}$ as an upstream component of ROCK1 \rightarrow AMPK signaling during melanocortin stimulation in the hypothalamus [130]. Thus, it is important to know that G $_{\alpha 12}$ or G $_{\alpha s}$ plays a role in the development of NAFLD in the context of ROCK1 action (Fig. 2).

The liver X receptors (LXRs), LXR α and LXR β , play a pivotal role in regulating lipid and glucose metabolism in insulin-target tissues by controlling its downstream targets' gene expression [131,132]. Insulin induces LXR α in hepatocytes, resulting in an increased expression of key enzymes in lipogenesis, including SREBP1c, the master regulator of hepatic lipogenesis, and its several downstream genes such as FAS and SCD1 [133-135]. A central role for LXRs in the regulation of lipogenesis is further established by the study showing that deleting LXRs from *ob/ob* mice reduces hepatic triglyceride amounts and lipogenic gene expression [136]. Conversely, treating mice with synthetic LXR agonists promotes triglyceride synthesis in the liver by inducing lipogenic gene expression [137,138], thereby leading to hypertriglyceridemia, a risk factor for developing insulin resistance and obesity [133]. The fact

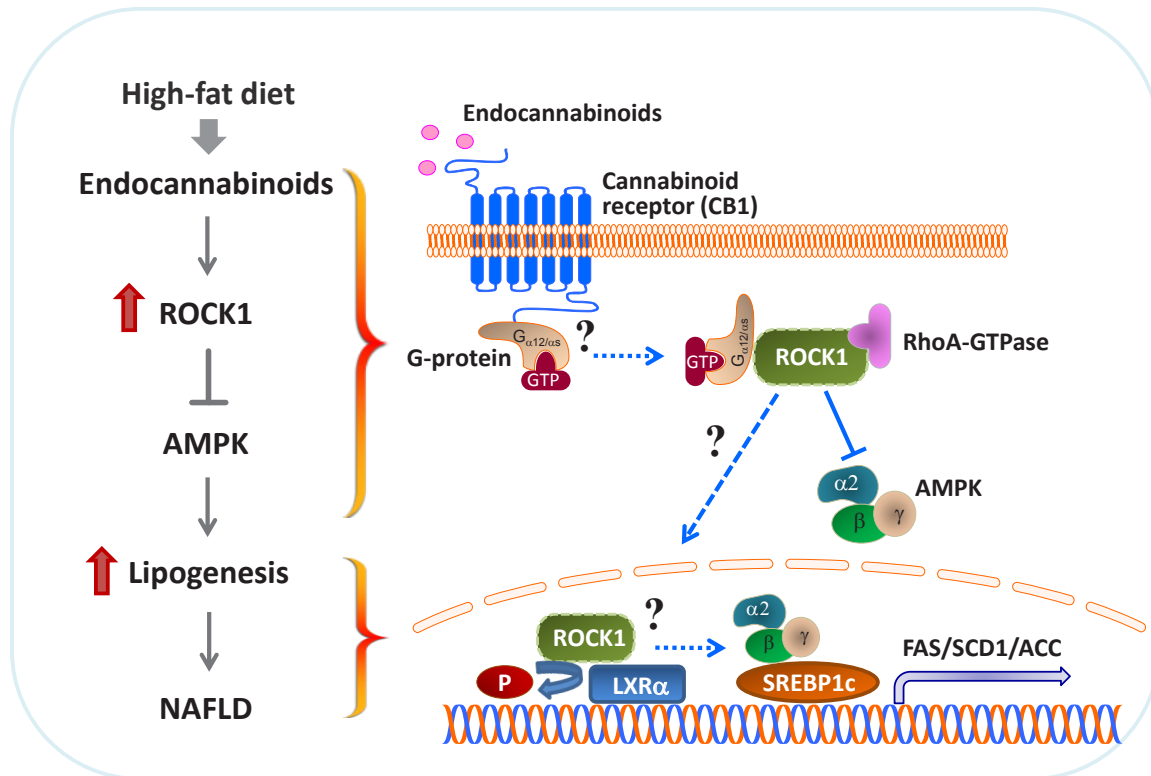


Fig. 2. Role of Rho-associated coiled-coil-containing kinase (ROCK1) in the development of nonalcoholic fatty liver disease (NAFLD). High-fat feeding increases endocannabinoids 2-arachidonoylglycerol and anandamide levels produced from hepatic stellate cells of the liver. Endocannabinoids stimulate ROCK1 activity via cannabinoid receptor type 1 (CB1), which inhibits AMP-activated protein kinase (AMPK) activity. Suppressed AMPK activity then drives the sterol regulatory element-binding protein 1c (SREBP1c)-mediated lipogenic program, leading to a rise of lipid accumulation. NAFLD ultimately develops with the progression of obesity. On the other hand, it is hypothesized that activated ROCK1 binds to liver X receptor (LXR α) and may prompt the lipogenic pathway through SREBP1c. As an early event of ROCK1 signaling, CB1-mediated G $_{\alpha 12/13}$ (or G $_{\alpha s}$) activation could be an initial step for ROCK1-dependent *de novo* lipogenesis. GTP, guanosine triphosphate; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase-1; ACC, acetyl-CoA carboxylase.

that deficiency of hepatic ROCK1 selectively suppresses gene expression of lipogenic key enzymes in the liver [49] allowed this research to raise the possibility that LXR α is involved in ROCK1 action in the context of lipogenesis. Interestingly, co-immunoprecipitation assay indicates that ROCK1 physically binds to LXR α in the liver and possibly could phosphorylate LXR α serine residues *in vitro* (not shown). Whether LXR α is a critical determinant for the metabolic regulation of ROCK1-mediated DNL by controlling its target gene expression is an intriguing question that future studies should address. These investigations will establish the biological significance of ROCK1-dependent LXR α action in hepatic metabolism and provide a novel mechanism for the ROCK1 \rightarrow LXR α signaling axis that is linked to lipogenesis (Fig. 2).

SUMMARY

Significant progress in comprehending the critical signaling pathways that regulate normal glucose and lipid homeostasis has been made in the field. In particular, much attention has focused on hepatic fatty acid metabolism in the context of obesity-related NAFLD. Growing evidence from pharmacological and genetic studies demonstrated that ROCK1 plays an essential role in the homeostatic regulation of DNL in the liver and its activation is the key defect that underlies the etiology of obesity-linked NAFLD. In addition, the ROCK1 \rightarrow AMPK signaling cascade is required to mediate hepatic DNL provoked by endocannabinoid generated from HSCs. Thus, the emergence of ROCK1 as a pivotal player of hepatic lipid me-

tabolism provides new insight into the etiopathogenesis of NAFLD and may lead to new treatment approaches for obesity-related metabolic disorders such as NAFLD.

Outstanding questions are as follows:

- What are the specific roles of ROCK1 or ROCK2 in HSCs in the development of liver diseases?
- What are the functions of ROCK1 or ROCK2 in Kupper cells?
- Is ROCK1 involved in the regulation of LXR α -mediated lipogenic programs?
- What are the key downstream pathways of ROCK1 \rightarrow AMPK signaling?
- What is the physiological role of the ROCK1 \rightarrow AMPK signaling pathways in other metabolic organs?
- Does G $_{\alpha 12}$ or G $_{\alpha s}$ act as an upstream signal of ROCK1 in the liver?

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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