Role of the AMPK/SIRT1 pathway in non-alcoholic fatty liver disease (Review)

PUTRI ANGGREINI[1,2], HADI KUNCORO[2], SRI ADI SUMIWI[3] and JUTTI LEVITA[3]

[1]Doctoral Program in Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java 46363; [2]Laboratory of Pharmaceutical Research and Development, Faculty of Pharmacy, Mulawarman University, Samarinda, East Borneo 75119; [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java 46363, Indonesia

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Abstract. Non-alcoholic fatty liver disease (NAFLD) is an increasingly prevalent ailment worldwide. Moreover, *de novo* lipogenesis (DNL) is considered a critical factor in the development of NAFLD; hence, its inhibition is a promising target for the prevention of fatty liver disease. There is evidence to indicate that AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1) may play a crucial role in DNL and are the regulatory proteins in type 2 diabetes mellitus, obesity and cardiovascular disease. Therefore, AMPK and SIRT1 may be promising targets for the treatment of NAFLD. The present review article thus aimed to summarize the findings of clinical studies published during the past decade that suggested the beneficial effects of AMPK and SIRT1, using their specific activators and their combined effects on fatty liver disease.

Contents

Correspondence to: Dr Hadi Kuncoro, Laboratory of Pharmaceutical Research and Development, Faculty of Pharmacy, Mulawarman University, Muara Muntai Street, Gunung Kelua, Samarinda, East Borneo 75119, Indonesia
E-mail: hadikuncoro@farmasi.unmul.ac.id

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition where the accumulation of lipids exceeds 5% of hepatocytes and is not generated by alcohol, drug consumption or does not damage hepatocytes (1). The global prevalence of NAFLD is increasing, with ~20-30% of patients presenting with early-stage disease (2,3). This disease is currently of great concern as it may increase the risk of developing other subsequent anomalies, as for example type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (4).

It has been revealed that *de novo* lipogenesis (DNL) may be crucial for the development of NAFLD (5). It occurs primarily in hepatocytes and is triggered mainly by a high intake of glucose or fructose. DNL turns excessive glucose or fructose into fatty acid and triglycerides (6). DNL is a normal process for the maintenance of homeostasis in the body, and its increased activation may potentially cause hepatic steatosis (7). Therefore, the inhibition of DNL is highly pursued as a therapeutic target for lipid metabolism-related disease.

The sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element-binding protein (ChREBP) are key transcription factors that play a crucial role in DNL (8). Several studies have revealed that SREBP1c and ChREBP increase the expression of lipogenic enzymes related to DNL (9-11). The simultaneous activity of SREBP1c and ChREBP is a normal process for the maintenance of cell homeostasis; however, at excessive levels, the cell has a specific mechanism to terminate the signalling activation. Several proteins are responsible for reducing DNL, including AMP-activated protein kinase (AMPK) (12).

AMPK regulates DNL through several mechanisms, phosphorylating and inactivating acetyl-CoA carboxylase (ACC), thus inhibiting fatty acid biosynthesis (13). Furthermore, AMPK also inhibits transcriptional regulators, including SREBP1c and ChREBP. The activation of AMPK has been reported to be blocked the nuclear translocation of SREBP1c and attenuates aberrant lipogenesis in diabetic mice (14). In another study on 3T3-L1 cells, AMPK was revealed to phosphorylate the precursor of SREBP1c and prevented the conversion of SREBP1c into its mature form (15). It also regulates the activity of ChREBP, as demonstrated in an

ethanol-induced fatty liver experiment, where AMPK was inhibited by ethanol, while ChREBP activity increased significantly (16). Therefore, AMPK is considered one of the proteins that can maintain cell balance, specifically concerning lipid metabolism.

Sirtuin 1 (SIRT1) is also well-known as a regulatory protein (17). Several studies have reported the activation of SIRT1 in lowering the expression of DNL enzymes (18,19). Furthermore, the increased activity of SIRT1 decreases the expression of SREBP1c, while the knockout SIRT1 has been reported to elevated the expression of ChREBP in HepG2 cells (19). This demonstrates the importance of SIRT1 in the regulation of lipid metabolism, specifically in DNL.

The effects of AMPK and SIRT1 activation on lipid metabolism are well known; however, there are still concerns as to whether the combination of their activators is beneficial for pathological lipid metabolism-related diseases, including fatty liver disease. Therefore, the present review article aimed to summarize the role of AMPK and SIRT1 in NAFLD, based on evidence obtained from randomized control studies.

2. Data collection methods

The present review summarizes the result of randomized control studies related to the effect of AMPK and SIRT1 activators on NAFLD. Articles were obtained from the PubMed database identified using the key words 'SIRT1 activator AND NAFLD', 'Resveratrol AND fatty liver', 'AMPK activator AND NAFLD', as well as 'Metformin AND fatty liver'. Only clinical or randomized control trial articles published over the last 10 years were included. By contrast, articles that did not include SIRT1 and AMPK activators in patients with NAFLD were excluded. The method used for data collection is summarized in Fig. 1. In total, 13 articles were collected, and the data are presented in Table I, arranged by the protein, its activator name, subject, treatment, duration, type of study, outcome and references, and the results of these studies were then discussed.

3. *De novo* lipogenesis

DNL is considered the primary factor in the development of fatty liver disease (7). In a pathological condition, such as NAFLD, DNL activation increases, generating excessive fat and culminating in intrahepatic lipid accumulation (5,20). Furthermore, DNL is a biosynthetic pathway for the productions of fatty acids and triglycerides from a non-lipid source, triggered by a high presence of carbohydrates or by insulin receptor-mediated signalling. The pathway is highly regulated by two significant factors, namely transcriptional regulation of DNL enzyme and allosteric regulation of ACC (21).

The transcriptional regulation of the DNL enzyme includes two transcription factor proteins, namely SREBP1c and ChREBP (Fig. 2). The influx of glucose and the signalling from insulin induce the activation of ChREBP and SREBP1c, respectively. Under basal conditions, the binding of SREBP1c to SREBP cleavage-activating protein (SCAP) and insulin-induced gene 1 (INSIG1) protein on the endoplasmic reticulum, prevents its translocation to the nucleus (8). Subsequently, INSIG1 is dissociated via the phosphorylation of SREBP1c and SCAP is cleaved by S1 and S2 proteases in the Golgi apparatus, and eventually, SREBP1c expresssion is released (8). Additionally, ChREBP is anchored by 14-3-3 protein and the phosphorylation of this complex permits the free ChREBP entry the nucleus (22). Furthermore, SREBP1c and ChREBP bind to the promoter gene target in the nucleus and start the transcription of lipogenic genes, including fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1) and ACC (23).

The inhibition of SREBP1c and ChREBP reduces the production of lipogenic genes as well as lipogenesis (24,25). Several proteins such as AMPK have been reported to inhibit the activity of SREBP1c and ChREBP. Another possible inhibitory mechanism of AMPK is predicted through SIRT1 which reportedly blocked both SREBP1c and ChREBP (19,23,26).

4. AMPK

The body has a system to maintain energy balance, in the form of adenosine triphosphate (ATP). When cellular ATP levels are reduced, the AMPK pathway is activated, phosphorylating the growth-regulating enzymes along with proteins, in order to generate ATP and decrease ATP consumption (27). AMPK is considered the master regulator of numerous proteins responsible for aging, inflammation, redox and the metabolism of lipids and glucose (28).

Based on the crystal structure of the protein, AMPK is a trimeric complex, consisting of a catalytic α subunit and two regulatory subunits, namely β and γ. The α subunit contains a kinase domain and an important residue (Thr172), which is phosphorylated by upstream kinases. The β subunit contains a binding site for carbohydrates that causes AMPK to associate with glycogen. Additionally, the γ subunit acts as a sensor for changes in the AMP/ADP ratio (29). When AMP increases and ADP decreases, AMP binds to the γ subunit, activating AMPK through three mechanisms, namely: i) The phosphorylation of Thr172 by stimulating the upstream proteins or stabilizing AMPK into a substrate more susceptible to phosphorylation; ii) AMP prevents the dephosphorylation by the phosphatase on Thr172; and iii) AMP causes allosteric activation of Thr172 in the α sub-unit (30,31). The major upstream kinase of AMPK is liver kinase B1 (LKB1) and Ca^{2+}/calmodulin-dependent protein (CaMKK) which phosphorylates AMPK in Thr172 (32,33). LKB1 is the main upstream activator of AMPK. It is activated by the stress signal or by the presence of activators, including aminoimidazole-4-carboxamide ribonucleoside and metformin (34). In addition, CaMKK is highly distributed in neural tissue to respond to neuronal depolarization (35).

AMPK is known to play an essential role in various metabolic-related diseases, such as NAFLD. Its activity causes the inhibition of DNL through the suppression of SREBP1c and ChREBP. AMPK inhibits the activation of SREBP1c through the phosphorylation at Ser372 residue and prevents the cleavage process by protease (14). Furthermore, a recent study demonstrated that it suppresses SREBP1c expression through the mTOR and LXRa proteins (36). ChREBP is also phosphorylated at the Ser568 residue by AMPK, causing re-binding to 14-3-3 protein and the subsequent conversion into an inactive form as well as preventing lipid synthesis (22,36).

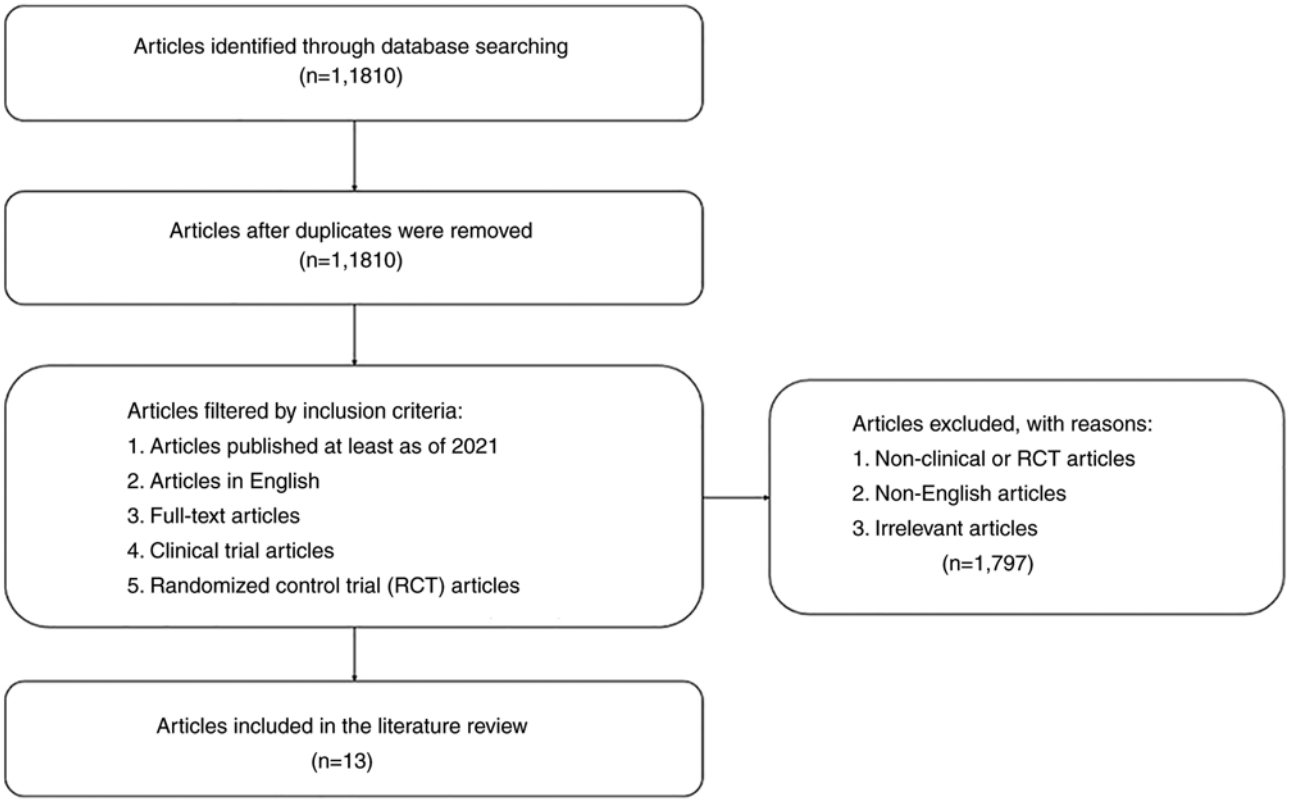


Figure 1. Flowchart for the literature search.

5. SIRT1

SIRT1 is a class III family of histone deacetylases, and their reactions require nicotinamide adenine (NAD^+) to concurrently deacetylate histones and non-histone from proteins involved in metabolic processes and stress responses (17,37). It is widely expressed in mammalian cells in a number of organs, including the brain, adipose tissue, kidneys, pancreas, endothelium, spleen, skeletal muscle and liver. Furthermore, its expression is known to be involved in several diseases, including metabolic diseases and age-related diseases, as well as CVD (38). SIRT1 is a protein that regulates metabolism, including fat cell accumulation and maturation, lipid metabolism in the liver, systemic inflammation, nutrition sensing and circadian rhythms (39). Previous studies have demonstrated that SIRT1 inhibits DNL enzymes, as well as their key regulator proteins, SREBP1c and ChREBP, culminating in abolishing perturbation of hepatic lipid metabolism (19,40).

The primary function of SIRT1 is to deacetylate the acetyl-lysine residue of histone substrate or non-histone proteins, including transcription factors, co-regulators and enzymes (41,42). Therefore, SIRT1 has multiple physiological functions, particularly in metabolism. It has been characterized as the 'master of metabolic regulators', due to its pivotal role in maintaining the homeostasis of lipid metabolism by affecting several proteins involved. SREBP1c is a critical transcription factor that initiates several lipogenic genes, inducing lipogenesis within the cell. SIRT1 inhibits SREBP1c activity and decreases lipogenesis in mouse liver (19). Another lipogenesis inducer aside SREBP1c and ChREBP is SIRT1 (43). Furthermore, AMPK, which is the natural regulator of ChREBP and SREBP1c, is also affected by SIRT1 activity through an indirect mechanism by deacetylating the upstream kinase of AMPK, LKB1 (18,44). This demonstrates that SIRT1 plays a prominent role in the development of lipid-related diseases, including non-alcoholic liver disease. This is in line with several studies demonstrating that SIRT1 activator alleviates fatty liver in rodent models and NAFLD patients (45-49).

A previous *in silico* study revealed that the crystal structure of SIRT1 is composed of the following three major domains: the catalytic, N-terminal, and C-terminal (50). The catalytic region consists of the binding site of substrate and NAD^+ that promotes the deacetylation of lysine, whereas the N- and C-terminals bind to several compounds such as resveratrol, suramin, or EX-527 and regulate SIRT1 deacetylase activity (51). Inside the cell, SIRT1 is localized in the cytoplasm and affects other proteins, including NF-κB, peroxisome proliferator-activated receptor γ, peroxisome proliferator-activated receptor-γ coactivator, AMPK and p-53 (52,53), while in the nucleus, it affects the translocation of proteins, including FOXO3a and several antioxidant genes such as SOD2/3, HO-1, and NQO-1 (54).

6. AMPK activators in NAFLD clinical studies

Evidence supports the role of AMPK in metabolism-related diseases, such as NALFD (55). AMPK regulates other proteins and provides homeostasis within the cell through several mechanisms involved in lipid metabolism, glucose metabolism, protein metabolism, autopaghy, and mitochondrial biogenesis (27,55,56). It has been well-established that AMPK is involved in the prevention of hepatic steatosis.

Table I. Clinical trials of AMPK and SIRT1 activators on patients with NAFLD.

Protein	Activator	Subjects	Treatment and duration of study	Type of study	Outcome	(Refs.)
AMPK	Metformin	173 children with NAFLD	500 mg twice a day for 96 weeks	Randomized, placebo-controlled, double blind	- Decreased NAS - Decreased cholesterol, LDL	(57)
	Metformin combined with N-acetylcysteine	53 patients with NAFLD	850-1,500 mg/day for 48 weeks	Open-label multicenter randomized trial	- Decreased NAS	(58)
	PXL770	12 patients with NAFLD	- 250 mg once a day - 250 mg twice a day - 500 mg once a day For 12 weeks	Randomized, double-blind, placebo	- Decreased ALT, AST - Decreased triglycerides and VLDL - Increased ApoB, HDL-c - Decreased DNL percentage - Increase dHOMA-IR	(65)
	Metformin	63 patients with NAFLD	500 mg metformin once a day for 4-12 month	Randomized, placebo-controlled	- Decreased ALP and ALT - Decreased triglycerides - Increased HDL-c	(68)
	Metformin	10 patients at a risk of developing NAFLD	500 mg once a day for 12 weeks	Single center, open label trial	- Decreased VLDL and triglycerides	(69)
	Metformin	35 patients with NAFLD	850 mg daily for 24 weeks	Prospective controlled trial	- Decreased ALT, AST - Decreased total cholesterol, triglyceride - Increased HDL	(70)
	Metformin	29 patients with type 2 diabetes and NAFLD	- 250 mg twice a day - 500 mg three times a day - 1,000 mg twice a day For 24 weeks	Single center, open-label, prospective, randomized trial	- Decreased ALT, AST - Decreased triglycerides	(71)
SIRT1	Resveratrol	50 patients with NAFLD	500 mg once a day for 12 weeks	Randomized, placebo-controlled, double blind	- Reduced steatosis grade - Reduce level of ALT and AST - Reduced inflammatory markers	(75)
	Resveratrol	60 patients with NAFLD	150 mg Twice a day for 3 months	Randomized, placebo-controlled, double blind	- Decreased ALT, AST, LDL-c, glucose level - Reduction of adiponectin level and TNF-α	(76)
	Resveratrol	25 patients with NAFLD	500 mg once a day for 12 weeks	Randomized, placebo-controlled, double blind	- Reduced hepatic steatosis - Reduced level of ALT	(48)
	Resveratrol	28 patients with NAFLD	1,5 g daily for 6 months	Randomized, placebo-controlled, double blind	- Decreased 3.8% lipid content	(49)
	Resveratrol	44 patients with NAFLD	50 mg and 200 mg once a day for 6 months	Randomized	- Reduced triglycerides, LDL	(86)

Table I. Continued.

Protein	Activator	Subjects	Treatment and duration of study	Type of study	Outcome	(Refs.)
AMPK and SIRT1	Metformin + leucine + sildenafil (NS-0200)	91 patients with NAFLD	- Low dose NS-0200 (1.1 g leucine + 0.5 g metformin + 0.5 mg sildenafil) - High dose NS-0200 (1.1 g leucine +0.5 g metformin + 1.0 mg sildenafil) Twice daily for 16 weeks	Randomized, placebo-controlled, double blind	- Reduced 15,7% intra hepatic fat - Increased fatty acid oxidation	(103)

AMPK, AMP-activated protein kinase; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; DNL, *de novo* lipogenesis; HOMA-IR, homeostatic model assessment for insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL-c, high-density lipoprotein cholesterol.

Metformin, an indirect activator of AMPK, has been widely studied for its effects on NAFLD. Several clinical trials have reported the beneficial effects of metformin on certain features of NAFLD. A previous randomized control trial on children diagnosed with NAFLD and treated with metformin at 500 mg twice per day for 24 months, reported an improvement in steatosis grade and lipid profiles (57). Moreover, an open-label, multi-centred, randomized trial, reported that metformin in combination with acetylcysteine administered for 12 months led to the significant improvement in the NAFLD Activity Score measured by liver biopsies of adult patients with NAFLD (58). Acetylcysteine provides a potent antioxidant effect on the liver, thereby protecting the liver from oxidative stress (59,60). AMPK activity also affects the antioxidant defense system in cells (61,62). A combination of AMPK activator and antioxidant such as acetylcysteine yielded a positive impact against hepatic steatosis (58). AMPK activation through metformin exerts a beneficial effect by reducing hepatic steatosis in patients with NAFLD (57,58). Another study with a direct AMPK activator, PXL770, supports this statement. The mechanisms of action of metformin and PXL770 as activators of AMPK are summarized in Table II (58-67). A randomized, double-blind, placebo-controlled trial reported that treatment using PXL770 for 12 weeks decreased DNL percentage and improved glucose metabolism. Lipid profiles concerning triglycerides and very-low-density lipoprotein (VLDL) decreased in the PXL770 group compared to the placebo group (65). Furthermore, AMPK activation, direct or indirect, has a beneficial effect by reducing steatosis in patients with NAFLD.

Metformin also has a beneficial effect on the lipid profiles of patients with NAFLD, according to a previous trial, where 500 mg metformin administered for 4 months significantly decreased liver enzyme and triglyceride levels, and increased high-density lipoprotein (HDL)-cholesterol levels in patients (68). This is in line with another study which revealed that 500 mg metformin administered for 3 months decreased VLDL and triglyceride levels in 10 patients who were at a risk of developing NAFLD (69). Another study similarly reported that the daily administration of 850 mg metformin for 6 months reduced liver enzyme, total cholesterol and triglyceride levels, and increased HDL-cholesterol levels (70). Furthermore, in children diagnosed with NAFLD, treatment with metformin 500 mg twice per day, for 24 months, led to a beneficial effect in the form of improvement in lipid profiles (57). Different doses of metformin, including 250 mg three times per day, 500 mg three times per day, and 1,000 mg twice per day, administered for 6 months, have been shown to produce similar results, namely an improvement in liver enzyme levels and lipid profiles in patients with T2DM and NAFLD (71). Lipid profiles are greatly influenced by metformin at various doses in children and adult patients.

AMPK activation is involved in several mechanisms in lipid metabolism. A previous study revealed that the activation of AMPK decreased SREBP1c activity in mice fed a high-fat diet, thereby attenuating hepatic steatosis (14). SREB1c regulates the protein that is crucial for lipid and glucose metabolism. AMPK activation has been reported to inhibit fat-forming enzymes, including ACC, FAS and SCD1 through SREBP1 inhibition, leading to decreased intracellular

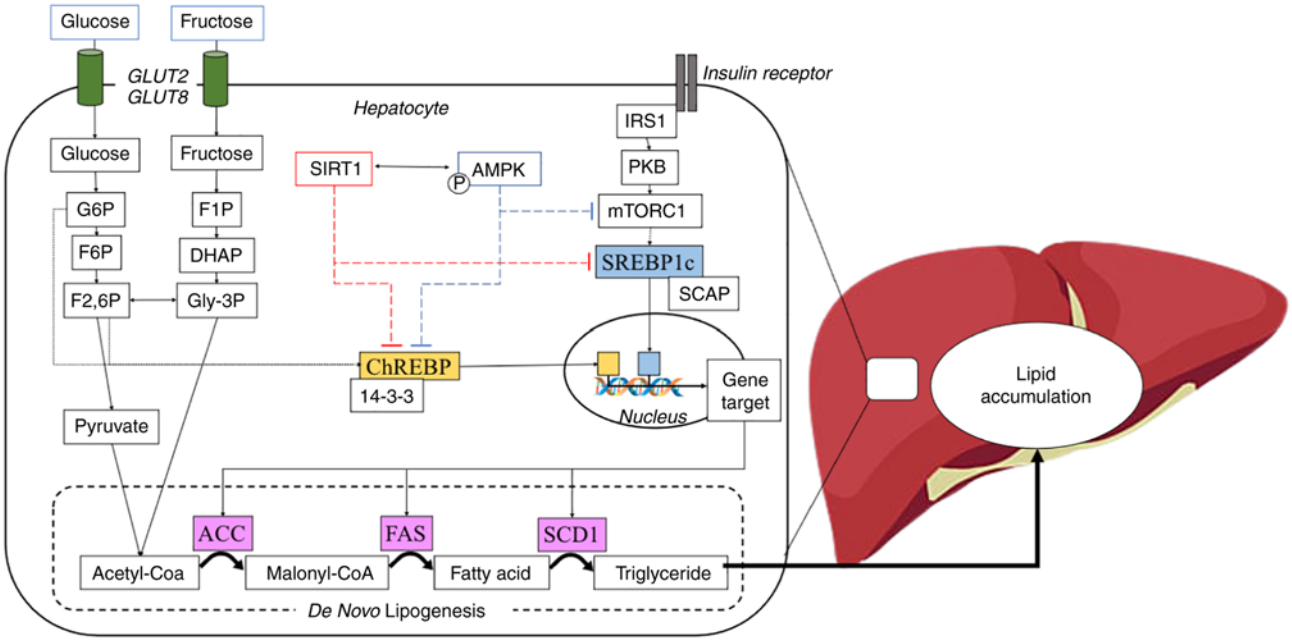


Figure 2. Schematic overview of DNL and its transcriptional regulation. Carbohydrates, including glucose or fructose enter hepatocyte cells and become a sensor for DNL activation. Glucose is converted to G6P followed by isomerization to F6P and F2,6P through the glycolysis process. By contrast, fructose also converts to Gly-3P through fructolysis and further converts to F2,6P. G6P and F2,6P induce dephosphorylation of ChREBP, and it detaches from 14-3-3 protein into an active form. Moreover, the activation of insulin receptor leads to the phosphorylation of IRS1, further activating the mTORC pathway and induces the nuclear translocation of SREBP1c. In the feedback response, SIRT1 and AMPK prevent the nuclear translocation of ChREBP and SREBP1c, resulting in the inhibition of DNL transcriptional regulation. DNL, *de novo* lipogenesis; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; Gly-3P, glycerol 3-phosphate; F2,6P, fructose 2,6-bisphosphate; ChREBP, carbohydrate response element-binding protein; IRS1, insulin receptor substrate 1; mTORC, mammalian target of rapamycin complex; SREBP1c, sterol regulatory element-binding protein 1c; SIRT1, Sirtuin 1; AMPK, AMP-activated protein kinase.

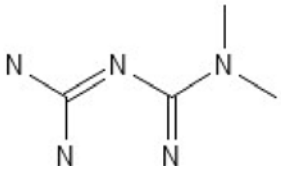
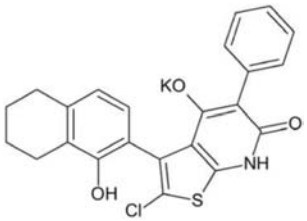
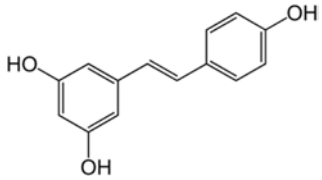
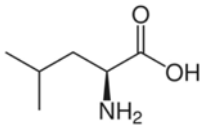
fat accumulation (15). Another possible mechanism is through the inhibition of 6-phosphogluconate dehydrogenase (6PGD), which is an enzyme involved in glycolysis. A previous *in vitro* study demonstrated that the inhibition of 6PGD activated the AMPK pathway and reduced ACC1 activity, thereby inhibiting lipid biosynthesis (72). 6PGD is the third enzyme in the pentose phosphate pathway (PPP) which is responsible for converting the 6-phosphogluconate into ribulose 5-phosphate (R-5-P). The upregulation of R-5-P frequently antagonizes the LKB1 complex, resulting in the decrease of AMPK activity. Another protein involved in this mechanism is mammalian target of rapamycin complex 1 (mTORC), which is the upstream protein target of SREBP1c. In the cancer cell, activation of mTORC may upregulate the PPP through SREBP1c (73). It is well known that AMPK activity inhibits mTORC; therefore, it may also alter the PPP, resulting in the reduction of the lipogenesis. PPP may be a critical pathway in lipogenesis. In a recent study on cancer cells, metformin was reported to interfere with several enzymes related to PPP and decreased the effect of PPP via modulation of mTORC (74). However, the information about the association between metformin and 6PGD remains unclear (74). Briefly, *in vitro*, *in vivo*, or clinical trials have provided evidence that AMPK activation may be a critical step in improving lipid metabolism.

7. The SIRT1 activator, resveratrol, in NAFLD clinical studies

In recent years, the use of resveratrol as a therapy for certain diseases has attracted increasing attention, due to its beneficial effects in reducing insulin resistance, the risk of CVD, hyperlipidemia, obesity and fatty liver-related diseases, such as NAFLD. Several clinical studies have demonstrated the beneficial effects of resveratrol in patients with NAFLD (Table I). A previous randomized, placebo-controlled, double-blinded study on 50 patients with NAFLD treated with 500 mg resveratrol daily for 3 months indicated an improvement in anthropometric measurements (weight, body mass index, waist circumference), liver enzyme levels, inflammatory marker levels and liver steatosis compared to the placebo group. It was proven that liver steatosis and inflammatory cytokines, including TNF-α, IL-6 and NF-κB were reduced by resveratrol for the activation of SIRT1 (75). Another randomized trial also reported that a lower dose of resveratrol (150 mg/day) for 3 months reduced the TNF-α level in patients (76). SIRT1 activation in hepatocytes in steatosis is associated with the inflammation system, preventing further hepatocyte damage (77).

Inflammation and oxidative stress have been widely reported in hepatic steatosis, due to elevated lipid peroxidation and free radical production, eventually leading to cell damage or dysfunction (78-80). A previous study on mice revealed that resveratrol inhibited the activity of NF-κB and TNF-α (81). The inhibition of SIRT1 expression can lead to an increase in inflammatory cytokine levels. Moreover, SIRT1 activation induces nuclear factor erythroid 2-related factor 2 activity, thereby providing a protective effect through the antioxidant defense system of the cell (82,83). Other pre-clinical studies have reported that resveratrol ameliorates high-fat diet induced fatty liver disease, culminating in decreased triglyceride

Table II. Chemical structure and mechanisms of action of activators.

Protein	Activator	Mechanism of action (Refs.)	(Refs.)
AMPK	Metformin 	Promotes the activation of AMPK by several mechanisms, including: i) Increasing the phosphorylation of a catalytic subunit at Thr-172; ii) increasing LKB1 action, which phosphorylates AMPK; and iii) inhibits PP2C action, which dephosphorylates AMPK.	(63,64)
	PXL770 	Activates AMPK by binding to the AdaM site and/or inhibits the dephosphorylation activity of PP2C.	(65-67)
SIRT1	Resveratrol 	Stimulates the deacetylase activity of SIRT1 by binding to the NTD site, resulting in the conformational change of SIRT1 that stabilizes or tightens the interaction between SIRT1 and the substrate.	(93,94)
	Leucine 	Activates SIRT1 by reducing the activation energy for NAD+, resulting in in lower NAD+ concentration, thus promoting SIRT1 activation.	(104,105)

AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; LKB1, liver kinase B1; PP2C, Protein phosphatase 2C; AdaM, allosteric drug and metabolism; NTD, N-terminal domain; NAD^+, nicotinamide adenine.

levels (84,85). These studies generally confirm that SIRT1 activation may inhibit fatty liver and improve the inflammation condition in hepatic steatosis both in animals and humans.

In several clinical trials, SIRT1 activation by resveratrol at different doses has been shown to lead to a decrease in lipid content. A double-blind, randomized, placebo-controlled trial with 60 participants with NAFLD treated with 150 mg resveratrol, twice per day, for 3 months, reported a significant decrease in liver enzyme, total cholesterol and low density lipoprotein (LDL)-cholesterol levels, and homeostatic model assessment for insulin resistance (HOMA-IR) compared to the placebo group (76). According to a previous study, lower doses of resveratrol, such as 150 mg reduced the intrahepatic lipid content (47). By contrast, a randomized control trial failed to show the beneficial role of resveratrol in glucose metabolism and lipid profile in higher doses, but not in the steatosis level (48). The lipid profile comprising triglycerides, LDL-cholesterol, total cholesterol and HDL, as well as HOMA-IR did not differ not significantly between the cohort treated with 500 mg resveratrol for 3 months and the placebo group. However, this trial demonstrated a significant reduction in hepatic steatosis grade and also liver enzyme, indicating the beneficial effect of resveratrol for steatosis patients (48). Another randomized control trial reported a 3.8% lipid content reduction in patients with NAFLD treated with high doses of resveratrol 1.5 g daily, for 6 months (49). Concerning lower daily doses of 50 and 200 mg for 6 months, a lower triglyceride and LDL level in patients with NAFLD has also been observed (86).

In a previous animal study, resveratrol demonstrated an undoubtedly beneficial effect on lipid metabolism (87). Lipid levels, including triglycerides, LDL-cholesterol and total cholesterol are significantly depleted in mice with hepatic steatosis treated with resveratrol (86-88). Additionally, it may also improve glucose metabolism (81,84,89) and reduce the hepatic steatosis score in high-fat/carbohydrate-induced NAFLD rats (90,91). Moreover, a previous study revealed that the overexpression of SIRT1 culminated in the alleviation of high-fat diet-induced hepatic steatosis and glucose intolerance in mice (42). Another pre-clinical study reported that mice lacking SIRT1 expression in the liver had hepatic steatosis accompanied by elevated AST levels (92). These studies prove that SIRT1 activity improves lipid and glucose metabolism in NAFLD animal models and *in vitro* study.

Several mechanisms have been proposed for resveratrol in the treatment of NAFLD. The proposed mechanisms of SIRT1 activators are summarized in Table II (93,94). As a direct SIRT1 activator, resveratrol is crucial for lipid metabolism (95,96).

The activation of SIRT1 inhibits SREBP1c activity, thereby preventing lipogenesis (19). SIRT1 also inhibits the activity of lipogenesis enzymes, including ACC and FAS (81). In an indirect mechanism, it activates the AMPK pathway to amplify the effect of AMPK on maintaining the homeostasis of lipid metabolism (97,98). In general, SIRT1 has been proven, in clinical investigations except from *in vitro* and *in vivo* studies, to possess a crucial role in improving fatty liver conditions.

8. The combination of AMPK and SIRT1 activation

AMPK and SIRT1 interact with each other, affecting lipid metabolism. It has been previously reviewed that AMPK and SIRT1 simultaneously function, in order to regulate other proteins (99). A combination of resveratrol and metformin decreased glucose and triglyceride levels as well as improved liver function in diabetic mice (100). A previous study also reported that a similar combination reduced liver weight and visceral fat in mice (100). Furthermore, the concurrent activation of AMPK and SIRT1 pathways contributes to decreasing lipogenesis, thereby alleviating hepatic steatosis in mice with NAFLD (101,102).

A previous randomized control trial of 91 participants with NAFLD reported that a combination of leucine and metformin given daily for 16 weeks culminated in decreased hepatic fat and a significantly increased fatty acid oxidation compared to the placebo group (103). L-leucine directly activates SIRT1 through allosteric interaction in an *in vitro* study. Its mechanism of action as an activator of SIRT1 is also summarized in Table II (104,105). Furthermore, leucine also affects AMPK activity (106,107). The combination of leucine and metformin produced a beneficial effect related to NAFLD features. Several studies have also demonstrated that the activation of AMPK and SIRT1 plays a principal role to improve NAFLD features (15,46-48,57,67). However, clinical trials that adopt the combination of AMPK and SIRT1 activators are still lacking; hence, further research on the combinatory use of these activators is required, in order to elucidate a strong correlation between AMPK and SIRT1 in lipid metabolism.

9. Conclusions

The existing data indicated that SIRT1 and AMPK might have a pivotal role in the pathogenesis of NAFLD. Both of Activators of SIRT1 and activators of AMPK, produce a benefit in preventing lipogenesis, thus reduce the impact of fatty liver. Several randomized control trials have proven that treatment using SIRT1 and AMPK activators in patients with NAFLD can improve hepatic steatosis, prevent inflammation, and inhibit lipogenesis. However, further studies are warranted for the confirmation of the effects of SIRT1 and AMPK activator alone or in combination for the treatment of fatty liver-related diseases. The present review demonstrates that SIRT1 and AMPK activators are promising therapeutics for treating NAFLD.

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Authors' contributions

SAS, HK and JL were involved in the conception and design of the study. PA principally collected previously published studies and wrote the original draft of the manuscript. HK and SAS were responsible for the acquisition of the collected articles. JL critically revised the article for intellectual content. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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