

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

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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.

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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

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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 expression 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  $\alpha$  subunit and two regulatory subunits, namely  $\beta$  and  $\gamma$ . The  $\alpha$  subunit contains a kinase domain and an important residue (Thr172), which is phosphorylated by upstream kinases. The  $\beta$  subunit contains a binding site for carbohydrates that causes AMPK to associate with glycogen. Additionally, the  $\gamma$  subunit acts as a sensor for changes in the AMP/ADP ratio (29). When AMP increases and ADP decreases, AMP binds to the  $\gamma$  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  $\alpha$  sub-unit (30,31). The major upstream kinase of AMPK is liver kinase B1 (LKB1) and  $\text{Ca}^{2+}$ /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 LXR $\alpha$  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).

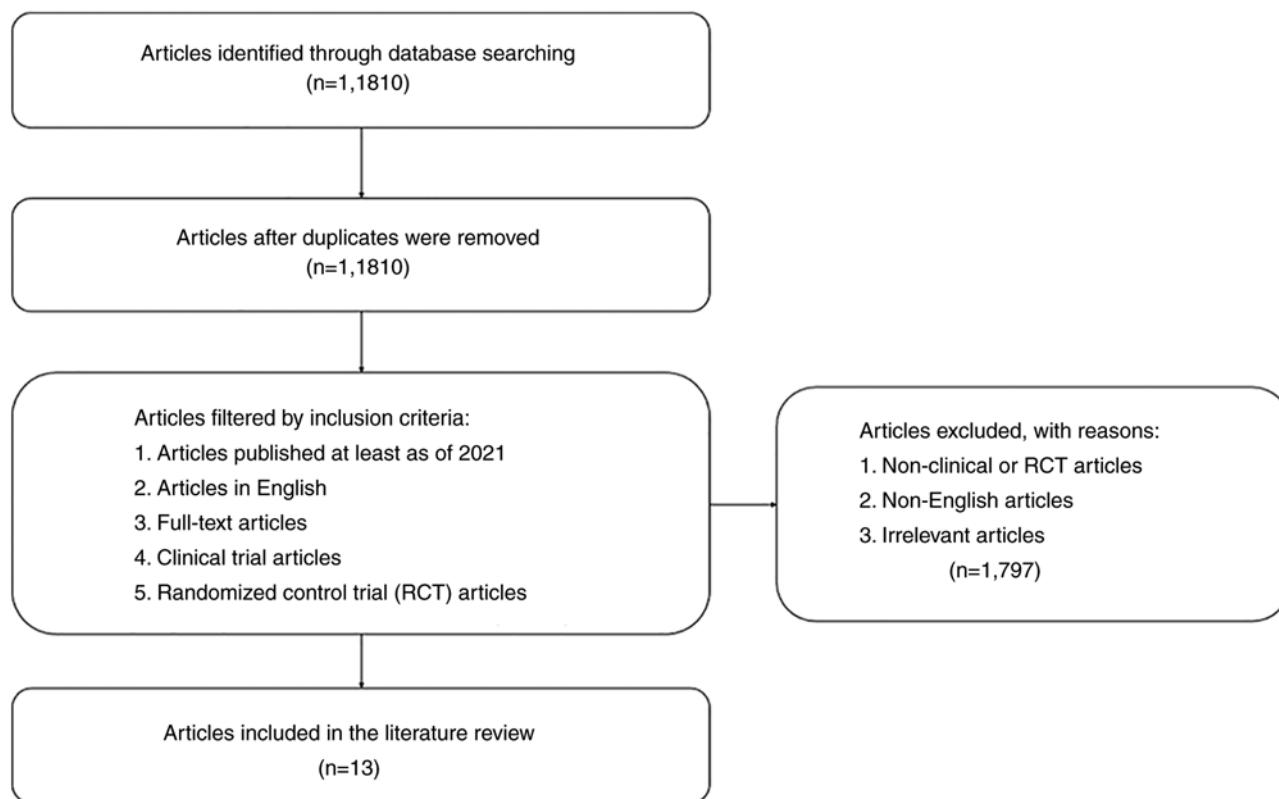


Figure 1. Flowchart for the literature search.

## 5. SIRT1

SIRT1 is a class III family of histone deacetylases, and their reactions require nicotinamide adenine ( $\text{NAD}^+$ ) 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  $\text{NAD}^+$  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- $\kappa$ B, peroxisome proliferator-activated receptor  $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$  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 NAFLD (55). AMPK regulates other proteins and provides homeostasis within the cell through several mechanisms involved in lipid metabolism, glucose metabolism, protein metabolism, autophagy, 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	(57)
	Metformin combined with N-acetylcysteine	53 patients with NAFLD	850-1,500 mg/day for 48 weeks	Open-label multicenter randomized trial	- Decreased cholesterol, LDL - 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)
	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- $\alpha$	(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)
SIRT1	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). SREBP1c 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

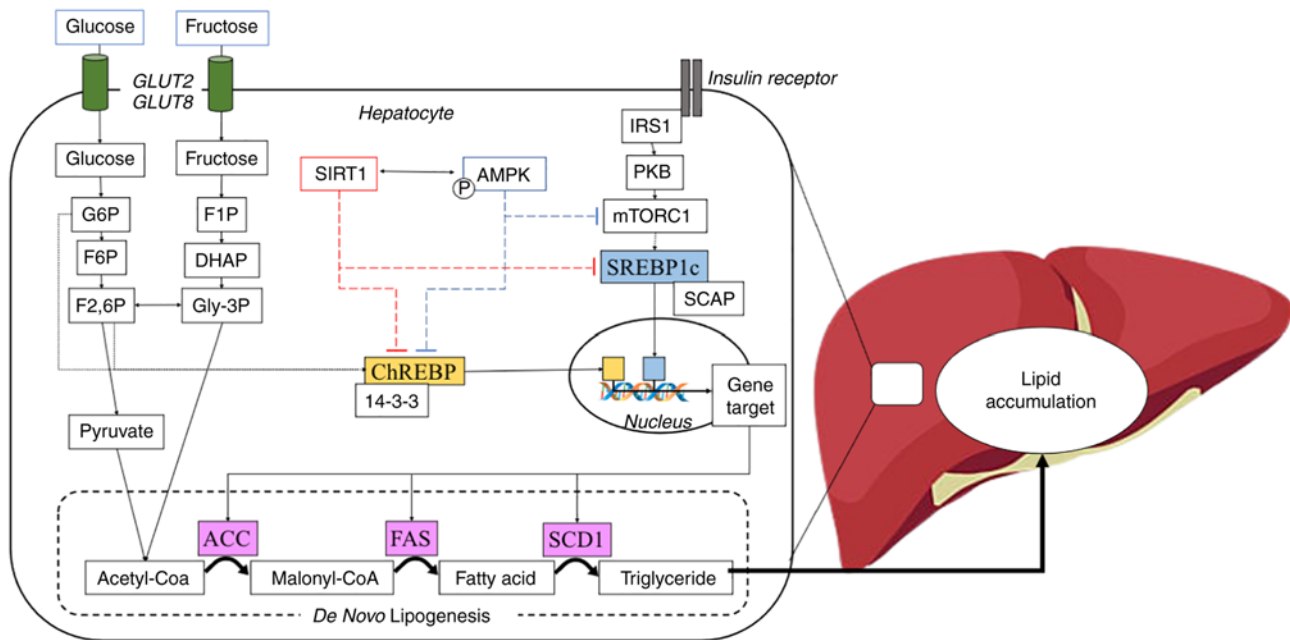


Figure 2. Schematic overview of DNL and its transcriptional regulation. Carbohydrates, including glucose or fructose enter hepatocyte cells and become a sensor for DNL activation. Glucose is converted to G6P followed by isomerization to F6P and F2,6P through the glycolysis process. By contrast, fructose 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

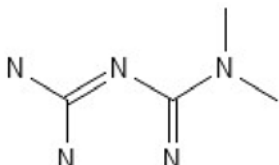
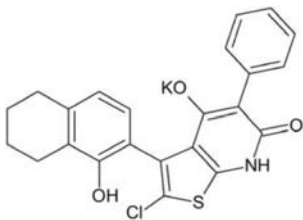
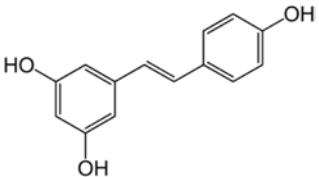
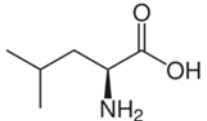
In recent years, the use of resveratrol as a therapy for certain diseases has attracted increasing attention, due to its

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- $\alpha$ , IL-6 and NF- $\kappa$ B 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- $\alpha$  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- $\kappa$ B and TNF- $\alpha$  (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 <sup>+</sup> , resulting in lower NAD <sup>+</sup> 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<sup>+</sup>, 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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## Availability of data and materials

Not applicable.

## 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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Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Tiniakos DG, Anstee QM and Burt AD: Fatty liver disease. In: MacSween's Pathol Liver. 7th edition. Elsevier, Philadelphia, PA, pp308-371, 2018.
2. Iqbal U, Perumpail B, Akhtar D, Kim D and Ahmed A: The epidemiology, risk profiling and diagnostic challenges of nonalcoholic fatty liver disease. *Medicines (Basel)* 6: 41, 2019.
3. Sayiner M, Koenig A, Henry L and Younossi ZM: Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis* 20: 205-214, 2016.
4. Byrne CD and Targher G: NAFLD: A multisystem disease. *J Hepatol* 62: S47-S64, 2015.
5. Buzzetti E, Pinzani M and Tsochatzis EA: The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 65: 1038-1048, 2016.
6. Ter Horst KW and Serlie MJ: Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients* 9: 981, 2017.
7. Knebel B, Fahlbusch P, Dille M, Wahlers N, Hartwig S, Jacob S, Kettel U, Schiller M, Herebian D, Koellmer C, *et al*: Fatty liver due to increased de novo lipogenesis: Alterations in the hepatic peroxisomal proteome. *Front Cell Dev Biol* 7: 248, 2019.
8. Ferré P and Foufelle F: Hepatic steatosis: A role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab* 12: 83-92, 2010.
9. Witte N, Muenzner M, Rietscher J, Knauer M, Heidenreich S, Nuotio-Antar AM, Graef FA, Fedders R, Tolkachov A, Goehring I and Schupp M: The glucose sensor ChREBP links de novo lipogenesis to PPAR $\gamma$  activity and adipocyte differentiation. *Endocrinol* 156: 4008-4019, 2015.
10. Vijayakumar A, Aryal P, Wen J, Syed I, Vazirani RP, Moraes-Vieira PM, Camporez JP, Gallop MR, Perry RJ, Peroni OD, *et al*: Absence of carbohydrate response element binding protein in adipocytes causes systemic insulin resistance and impairs glucose transport. *Cell Rep* 21: 1021-1035, 2017.



11. Stoeckman AK and Towle HC: The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. *J Biol Chem* 277: 27029-27035, 2002.
12. von Loeffelholz C, Coldewey SM and Birkenfeld AL: A narrative review on the role of ampk on de novo lipogenesis in non-alcoholic fatty liver disease: Evidence from human studies. *Cells* 10: 1822, 2021.
13. Viollet B, Foretz M, Guigas B, Horman S, Dentin R, Bertrand L, Hue L and Andreelli F: Activation of AMP-activated protein kinase in the liver: A new strategy for the management of metabolic hepatic disorders. *J Physiol* 574: 41-53, 2006.
14. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JYJ, *et al*: AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 13: 376-388, 2011.
15. Ha JH, Jang J, Chung SI and Yoon Y: AMPK and SREBP-1c mediate the anti-adipogenic effect of  $\beta$ -hydroxyisovalerylshikonic acid. *Int J Mol Med* 37: 816-824, 2016.
16. Liangpunsakul S, Ross RA and Crabb DW: Activation of carbohydrate response element binding protein by ethanol. *J Investig Med* 61: 270-277, 2013.
17. Cantó C and Auwerx J: Targeting sirtuin 1 to improve metabolism: All you need is NAD<sup>+</sup>. *Pharmacol Rev* 64: 166-187, 2012.
18. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, Lan F, Walsh K, Wierzbicki M, Verbeuren TJ, *et al*: SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. *J Biol Chem* 283: 20015-20026, 2008.
19. Ponugoti B, Kim DH, Xiao Z, Smith Z, Miao J, Zang M, Wu SY, Chiang CM, Veenstra TD and Kemper JK: SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J Biol Chem* 285: 33959-33970, 2010.
20. Paglialunga S and Dehn CA: Clinical assessment of hepatic de novo lipogenesis in non-alcoholic fatty liver disease. *Lipids Health Dis* 15: 159, 2016.
21. Sanders FWB and Griffin JL: De novo lipogenesis in the liver in health and disease: More than just a shunting yard for glucose. *Biol Rev* 91: 452-468, 2016.
22. Sato S, Jung H, Nakagawa T, Pawlosky R, Takeshima T, Lee WR, Sakiyama H, Laxman S, Wynn RM, Tu BP, *et al*: Metabolite regulation of nuclear localization of carbohydrate-response element-binding protein (ChREBP): Role of amp as an allosteric inhibitor. *J Biol Chem* 291: 10515-10527, 2016.
23. Wang Y, Viscarra J, Kim SJ and Sul HS: Transcriptional regulation of hepatic lipogenesis. *Nat Rev Mol Cell Biol* 16: 678-689, 2015.
24. Dentin R, Benhamed F, Hainault I, Fauveau V, Foulfelle F, Dyck JRB, Girard J and Postic C: Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes* 55: 2159-2170, 2006.
25. Zhao X, Xiaoli, Zong H, Abdulla A, Yang EST, Wang Q, Ji JY, Pessin JE, Das BC and Yang F: Inhibition of SREBP transcriptional activity by a boron-containing compound improves lipid homeostasis in diet-induced obesity. *Diabetes* 63: 2464-2473, 2014.
26. Nguyen LT, Mak CH, Chen H, Zaky AA, Wong MG, Pollock CA and Saad S: SIRT1 attenuates kidney disorders in male offspring due to maternal high-fat diet. *Nutrients* 11: 146, 2019.
27. Herzig S and Shaw RJ: AMPK: Guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 19: 121-135, 2018.
28. Jeon SM: Regulation and function of AMPK in physiology and diseases. *Exp Mol Med* 48: e245, 2016.
29. Xiao B, Sanders MJ, Carmena D, Bright NJ, Haire LF, Underwood E, Patel BR, Heath RB, Walker PA, Hallen S, *et al*: Structural basis of AMPK regulation by small molecule activators. *Nat Commun* 4: 3017, 2013.
30. Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T and Neumann D: Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J Biol Chem* 281: 32207-32216, 2006.
31. Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, Tam S and Kemp BE: AMPK is a direct adenylate charge-regulated protein kinase. *Science* 332: 1433-1435, 2011.
32. Gormand A, Henriksson E, Ström K, Jensen TE, Sakamoto K and Göransson O: Regulation of AMP-activated protein kinase by LKB1 and CaMKK in adipocytes. *J Cell Biochem* 112: 1364-1375, 2011.
33. Shackelford DB and Shaw RJ: The LKB1-AMPK pathway: Metabolism and growth control in tumour suppression. *Nat Rev Cancer* 9: 563-575, 2009.
34. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Mäkelä TP, Alessi DR and Hardie DG: Complexes between the LKB1 tumor suppressor, STRAD  $\alpha/\beta$  and MO25  $\alpha/\beta$  are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2: 28, 2003.
35. Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG and Hardie DG: Calmodulin-dependent protein kinase kinase- $\beta$  is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab* 2: 9-19, 2005.
36. Lee GH, Peng C, Jeong SY, Park SA, Lee HY, Hoang TH, Kim J and Chae HJ: Ginger extract controls mTOR-SREBP1-ER stress-mitochondria dysfunction through AMPK activation in obesity model. *J Funct Foods* 87: 104628, 2021.
37. Rahman S and Islam R: Mammalian Sirt1: Insights on its biological functions. *Cell Commun Signal* 9: 11, 2011.
38. Elibol B and Kilic U: High levels of SIRT1 expression as a protective mechanism against disease-related conditions. *Front Endocrinol (Lausanne)* 9: 614, 2018.
39. Schug TT and Li X: Sirtuin 1 in lipid metabolism and obesity. *Ann Med* 43: 198-211, 2011.
40. Wang RH, Li C and Deng CX: Liver steatosis and increased ChREBP expression in mice carrying a liver specific SIRT1 null mutation under a normal feeding condition. *Int J Biol Sci* 6: 682-690, 2010.
41. Cantó C and Auwerx J: PGC-1 $\alpha$ , SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol* 20: 98-105, 2009.
42. Banks AS, Kon N, Knight C, Matsumoto M, Gutiérrez-Juárez R, Rossetti L, Gu W and Accili D: Sirt1 gain of function increases energy efficiency and prevents diabetes in mice. *Cell Metab* 8: 333-341, 2008.
43. Noriega LG, Feige JN, Canto C, Yamamoto H, Yu J, Herman MA, Matakis C, Kahn BB and Auwerx J: CREB and ChREBP oppositely regulate SIRT1 expression in response to energy availability. *EMBO Rep* 12: 1069-1076, 2011.
44. Lan F, Cacicedo JM, Ruderman N and Ido Y: SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1: Possible role in AMP-activated protein kinase activation. *J Biol Chem* 283: 27628-27635, 2008.
45. Gao M and Liu D: Resveratrol suppresses T0901317-induced hepatic fat accumulation in mice. *AAPS J* 15: 744-752, 2013.
46. Ajmo JM, Liang X, Rogers CQ, Pennock B and You M: Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol Gastrointest Liver Physiol* 295: G833-G842, 2008.
47. Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, *et al*: Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 14: 612-622, 2011.
48. Faghihzadeh F, Adibi P and Hekmatdoost A: The effects of resveratrol supplementation on cardiovascular risk factors in patients with non-alcoholic fatty liver disease: A randomised, double-blind, placebo-controlled study. *Br J Nutr* 114: 796-803, 2015.
49. Heebøll S, Kreuzfeldt M, Hamilton-Dutoit S, Poulsen MK, Stødkilde-Jørgensen H, Møller HJ, Jessen N, Thorsen K, Hellberg YK, Pedersen SB and Grønbaek H: Placebo-controlled, randomised clinical trial: High-dose resveratrol treatment for non-alcoholic fatty liver disease. *Scand J Gastroenterol* 51: 456-463, 2016.
50. Davenport AM, Huber FM and Hoelz A: Structural and functional analysis of human SIRT1. *J Mol Biol* 426: 526-541, 2014.
51. Pan M, Yuan H, Brent M, Ding EC and Marmorstein R: SIRT1 contains N- and C-terminal regions that potentiate deacetylase activity. *J Biol Chem* 287: 2468-2476, 2012.
52. McBurney MW, Clark-Knowles KV, Caron AZ and Gray DA: SIRT1 is a highly networked protein that mediates the adaptation to chronic physiological stress. *Genes Cancer* 4: 125-134, 2013.
53. Olmos Y, Brosens JJ and Lam EWF: Interplay between SIRT proteins and tumour suppressor transcription factors in chemotherapeutic resistance of cancer. *Drug Resist Updat* 14: 35-44, 2011.
54. Yanagisawa S, Baker JR, Vuppusetty C, Koga T, Colley T, Fenwick P, Donnelly LE, Barnes PJ and Ito K: The dynamic shuttling of SIRT1 between cytoplasm and nuclei in bronchial epithelial cells by single and repeated cigarette smoke exposure. *PLoS One* 13: e0193921, 2018.
55. Smith BK, Marcinko K, Desjardins EM, Lally JS, Ford RJ and Steinberg GR: Treatment of nonalcoholic fatty liver disease: Role of AMPK. *Am J Physiol Endocrinol Metab* 311: E730-E740, 2016.

56. Hardie DG, Ross FA and Hawley SA: AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 13: 251-262, 2012.
57. Corey KE, Vuppalanchi R, Vos M, Kohli R, Molleston JP, Wilson L, Unalp-Arida A, Cummings OW, Lavine JE, Chalasani N, *et al*: Improvement in liver histology is associated with reduction in dyslipidemia in children with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 60: 360-367, 2015.
58. de Oliveira CP, Cotrim HP, Stefano JT, Siqueira ACG, Salgado ALA and Parise ER: N-acetylcysteine and/or ursodeoxycholic acid associated with metformin in non-alcoholic steatohepatitis: An open-label multicenter randomized controlled trial. *Arq Gastroenterol* 56: 184-190, 2019.
59. Cai Z, Lou Q, Wang F, Li E, Sun J, Fang H, Xi J and Ju L: N-acetylcysteine protects against liver injury induced by carbon tetrachloride via activation of the Nrf2/HO-1 pathway. *Int J Clin Exp Pathol* 8: 8655-8662, 2015.
60. Bauerlein DK, Akbar HN, von Rosenzweig EC, Loughry ND and John PR: Benefit of N-acetylcysteine in postoperative hepatic dysfunction: Case report and review of literature. *Case Reports Hepatol* 2019: 4730381, 2019.
61. Jansen T, Kvandová M, Daiber A, Stamm P, Frenis K, Schulz E, Münzel T and Kröller-Schön S: The AMP-activated protein kinase plays a role in antioxidant defense and regulation of vascular inflammation. *Antioxidants* 9: 525, 2020.
62. Zhang M, Yang D, Gong X, Ge P, Dai J, Lin L and Zhang L: Protective benefits of AMP-activated protein kinase in hepatic ischemia-reperfusion injury. *Am J Transl Res* 9: 823-829, 2017.
63. Meng S, Cao J, He Q, Xiong L, Chang E, Radovick S, Wondisford FE and He L: Metformin activates AMP-activated protein kinase by promoting formation of the  $\alpha\beta\gamma$ -heterotrimeric complex. *J Biol Chem* 290: 3393-3802, 2015.
64. Ouyang J, Parakhia RA and Ochs RS: Metformin activates AMP kinase through inhibition of AMP deaminase. *J Biol Chem* 286: 1-11, 2011.
65. Fouqueray P, Bolze S, Dubourg J, Hallakou-Bozec S, Theurey P, Grouin JM, Chevalier C, Gluais-Dagorn P, Moller DE and Cusi K: Pharmacodynamic effects of direct AMP kinase activation in humans with insulin resistance and non-alcoholic fatty liver disease: A phase Ib study. *Cell Reports Med* 2: 100474, 2021.
66. Gluais-Dagorn P, Foretz M, Steinberg GR, Batchuluun B, Zawistowska-Deniziak A, Lambooj JM, Guigas B, Carling D, Monternier PA, Moller DE, *et al*: Direct AMPK activation corrects NASH in rodents through metabolic effects and direct action on inflammation and fibrogenesis. *Hepatol Commun* 6: 101-119, 2022.
67. Monternier PA, Parasar P, Theurey P, Dagorn PG, Kaur N, Nagaraja TN, Fouqueray P, Bolze S, Moller DE, Singh J and Hallakou-Bozec S: Beneficial effects of the direct AMP-kinase activator PXL770 in in vitro and in vivo models of X-linked adrenoleukodystrophy. *J Pharmacol Exp Ther* 382: 208-222, 2022.
68. Shargorodsky M, Omelchenko E, Matas Z, Boaz M and Gavish D: Relation between augmentation index and adiponectin during one-year metformin treatment for nonalcoholic steatohepatitis: Effects beyond glucose lowering? *Cardiovasc Diabetol* 11: 61, 2012.
69. Green CJ, Marjot T, Walsby-Tickle J, Charlton C, Cornfield T, Westcott F, Pinnick KE, Moolla A, Hazlehurst JM, McCullagh J, *et al*: Metformin maintains intrahepatic triglyceride content through increased hepatic de novo lipogenesis. *Eur J Endocrinol* 186: 367-377, 2022.
70. Resuli B, Demiraj V, Babameto A, Sema K and Malaj V: Metformin superior to low-fat diet for the treatment of patients with nonalcoholic fatty liver disease and/or steatohepatitis. *Pol Arch Med Wewn* 122: 68-71, 2012.
71. Feng WH, Bi Y, Li P, Yin TT, Gao CX, Shen SM, Gao LJ, Yang DH and Zhu DL: Effects of liraglutide, metformin and gliclazide on body composition in patients with both type 2 diabetes and non-alcoholic fatty liver disease: A randomized trial. *J Diabetes Investig* 10: 399-407, 2019.
72. Yang X, Peng X and Huang J: Inhibiting 6-phosphogluconate dehydrogenase selectively targets breast cancer through AMPK activation. *Clin Transl Oncol* 20: 1145-1152, 2018.
73. Sarfraz I, Rasul A, Hussain G, Shah MA, Zahoor AF, Asrar M, Selamoglu Z, Ji XY, Adem S and Sarker SD: 6-Phosphogluconate dehydrogenase fuels multiple aspects of cancer cells: From cancer initiation to metastasis and chemoresistance. *Biofactors* 46: 550-562, 2020.
74. Marini C, Cossu V, Bauckneht M, Lanfranchi F, Raffa S, Orenco AM, Ravera S, Bruno S and Sambucetti G: Metformin and cancer glucose metabolism: At the bench or at the bedside? *Biomolecules* 11: 1231, 2021.
75. Faghihzadeh F, Adibi P, Rafiei R and Hekmatdoost A: Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. *Nutr Res* 34: 837-843, 2014.
76. Chen S, Zhao X, Ran L, Wan J, Wang X, Qin Y, Shu F, Gao Y, Yuan L, Zhang Q and Mi M: Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. *Dig Liver Dis* 47: 226-232, 2015.
77. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X and Li X: Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab* 9: 327-338, 2009.
78. Rezzani R and Franco C: Liver, oxidative stress and metabolic syndromes. *Nutrients* 13: 301, 2021.
79. Cichoz-Lach H and Michalak A: Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol* 20: 8082-8091, 2014.
80. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, *et al*: Chronic inflammation in the etiology of disease across the life span. *Nat Med* 25: 1822-1832, 2019.
81. Andrade JMO, Paraíso AF, de Oliveira MVM, Martins AME, Neto JF, Guimarães ALS, de Paula AM, Qureshi M and Santos SHS: Resveratrol attenuates hepatic steatosis in high-fat fed mice by decreasing lipogenesis and inflammation. *Nutrition* 30: 915-919, 2014.
82. Chai D, Zhang L, Xi S, Cheng Y, Jiang H and Hu R: Nrf2 activation induced by Sirt1 ameliorates acute lung injury after intestinal ischemia/reperfusion through NOX4-mediated gene regulation. *Cell Physiol Biochem* 46: 781-792, 2018.
83. Ren Z, He H, Zuo Z, Xu Z, Wei Z and Deng J: The role of different SIRT1-mediated signaling pathways in toxic injury. *Cell Mol Biol Lett* 24: 36, 2019.
84. Du F, Huang R, Lin D, Wang Y, Yang X, Huang X, Zheng B, Chen Z, Huang Y, Wang X and Chen F: Resveratrol improves liver steatosis and insulin resistance in non-alcoholic fatty liver disease in association with the gut microbiota. *Front Microbiol* 12: 611323, 2021.
85. Wardani HA, Rahmadi M, Ardianto C, Balan SS, Kamaruddin NS and Khotib J: Development of nonalcoholic fatty liver disease model by high-fat diet in rats. *J Basic Clin Physiol Pharmacol* 30: 1-7, 2020.
86. Theodotou M, Fokianos K, Moniatis D, Kadlenic R, Chrysikou A, Aristotelous A, Mouzouridou A, Diakides J and Stavrou E: Effect of resveratrol on non-alcoholic fatty liver disease. *Exp Ther Med* 559-565, 2019.
87. Zhou Q, Wang Y, Han X, Fu S, Zhu C and Chen Q: Efficacy of resveratrol supplementation on glucose and lipid metabolism: A meta-analysis and systematic review. *Front Physiol* 13: 795980, 2022.
88. Zhao H, Zhang Y, Shu L, Song G and Ma H: Resveratrol reduces liver endoplasmic reticulum stress and improves insulin sensitivity in vivo and in vitro. *Drug Des Devel Ther* 13: 1473-1485, 2019.
89. León D, Uribe E, Zambrano A and Salas M: Implications of resveratrol on glucose uptake and metabolism. *Molecules* 22: 398, 2017.
90. Abd El-Haleim EA, Bahgat AK and Saleh S: Resveratrol and fenofibrate ameliorate fructose-induced nonalcoholic steatohepatitis by modulation of genes expression. *World J Gastroenterol* 22: 2931-2948, 2016.
91. Ding S, Jiang J, Zhang G, Bu Y, Zhang G and Zhao X: Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats. *PLoS One* 12: e0183541, 2017.
92. Yang H, Liu Y, Wang Y and Xu S and Su D: Knockdown of Sirt1 gene in mice results in lipid accumulation in the liver mediated via PGC-1 $\alpha$ -induced mitochondrial dysfunction and oxidative stress. *Bull Exp Biol Med* 172: 180-186, 2021.
93. Hou X, Rooklin D, Fang H and Zhang Y: Resveratrol serves as a protein-substrate interaction stabilizer in human SIRT1 activation. *Sci Rep* 6: 38186, 2016.
94. Cao D, Wang M, Qiu X, Liu D, Jiang H, Yang N and Xu RM: Structural basis for allosteric, substratedependent stimulation of SIRT1 activity by resveratrol. *Genes Dev* 29: 1316-1325, 2015.

95. Gertz M, Nguyen GTT, Fischer F, Suenkel B, Schlicker C, Fränzel B, Tomaschewski J, Aladini F, Becker C, Wolters D and Steegborn C: A molecular mechanism for direct sirtuin activation by resveratrol. *PLoS One* 7: e49761, 2012.
96. Schug TT and Li X: Sirtuin 1 in lipid metabolism and obesity. *Ann Med* 43: 198-211, 2011.
97. Price NL, Gomes AP, Ling AJY, Duarte FV, Martin-Montalvo A, North BJ, Agarwal B, Ye L, Ramadori G, Teodoro JS, *et al*: SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab* 15: 675-690, 2012.
98. Ford RJ, Desjardins EM and Steinberg GR: Are SIRT1 activators another indirect method to increase AMPK for beneficial effects on aging and the metabolic syndrome? *EBioMedicine* 19: 16-17, 2017.
99. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F and Ido Y: AMPK and SIRT1: A long-standing partnership? *Am J Physiol Endocrinol Metab* 298: E751-E760, 2010.
100. Duarte-Vázquez MA, Gómez-Solis A, Gómez-Cansino R, Reyes-Esparza J, Luis Rosado J and Rodríguez-Fragoso L: Effect of combined resveratrol plus metformin therapy in db/db diabetic mice. *FASEB J* 31: 1001.8, 2017.
101. Li S, Qian Q, Ying N, Lai J, Feng L, Zheng S, Jiang F, Song Q, Chai H and Dou X: Activation of the AMPK-SIRT1 pathway contributes to protective effects of Salvianolic acid A against lipotoxicity in hepatocytes and NAFLD in mice. *Front Pharmacol* 11: 560905, 2020.
102. Chen XY, Cai CZ, Yu ML, Feng ZM, Zhang YW, Liu PH, Zeng H and Yu CH: LB100 ameliorates nonalcoholic fatty liver disease via the AMPK/Sirt1 pathway. *World J Gastroenterol* 25: 6607-6618, 2019.
103. Chalasani N, Vuppalanchi R, Rinella M, Middleton MS, Siddiqui MS, Barritt AS IV, Kolterman O, Flores O, Alonso C, Iruarrizaga-Lejarreta M, *et al*: Randomised clinical trial: A leucine-metformin-sildenafil combination (NS-0200) vs placebo in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 47: 1639-1651, 2018.
104. Banerjee J, Bruckbauer A and Zemel MB: Activation of the AMPK/Sirt1 pathway by a leucine-metformin combination increases insulin sensitivity in skeletal muscle, and stimulates glucose and lipid metabolism and increases life span in *Caenorhabditis elegans*. *Metabolism* 65: 1679-1691, 2016.
105. Bruckbauer A and Zemel MB: Synergistic effects of polyphenols and methylxanthines with leucine on AMPK/Sirtuin-mediated metabolism in muscle cells and adipocytes. *PLoS One* 9: e89166, 2014.
106. Liang C, Curry BJ, Brown PL and Zemel MB: Leucine modulates mitochondrial biogenesis and SIRT1-AMPK signaling in C2C12 myotubes. *J Nutr Metab* 2014: 239750, 2014.
107. Bruckbauer A and Zemel MB: Effects of dairy consumption on SIRT1 and mitochondrial biogenesis in adipocytes and muscle cells. *Nutr Metab (Lond)* 8: 91, 2011.



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