Sirtuins and nonalcoholic fatty liver disease

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Abstract

Mammalian sirtuins are seven members belonging to the silent information regulator 2 family, a group of Class III histone/protein deacetylases. Sirtuins (SIRT 1-7) have different subcellular localization and function and they regulate cellular protein function through various posttranslational modifications. SIRT1 and 3, the most studied sirtuins, use the product of cellular metabolism nicotinamide adenine dinucleotide as a cofactor to post-translationally deacetylate cellular proteins and consequently link the metabolic status of the cell to protein function. Sirtuins have been shown to play a key role in the development and rescue of various metabolic diseases including non-alcoholic fatty liver disease (NAFLD). NAFLD is currently the most chronic liver disease due mainly to high-calorie consumption and lower physical activity. No pharmacological approach is available to treat NAFLD, the current recommended treatment are lifestyle modification such as weight loss through calorie restriction and exercise. Recent studies have shown downregulation of sirtuins in human as well as animal models of NAFLD indicating an important role of sirtuins in the dynamic pathophysiology of NAFLD. In this review, we highlight the recent knowledge on sirtuins, their role in NAFLD and their unique potential role as novel therapeutic target for NAFLD treatment.

Key words: SIRT1; SIRT3; Sirtuins; Non-alcoholic fatty liver disease

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Core tip: Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease with no effective pharmacological therapy. The discovery of treatment is hindered by the insufficient understanding of the pathophysiology of the disease. Sirtuins are key players in hepatic carbohydrate and lipid metabolism, insulin signaling, and inflammation and hence may represent a novel therapeutic target for NAFLD. However, the particular role for each sirtuin, the cross talk between sirtuins in different cell compartments or within a given organelle, and the development of selective sirtuins...
activators/inhibitors still need further investigation.

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INTRODUCTION

Non-alcoholic fatty liver diseases (NAFLD) is emerging as the leading cause of chronic liver diseases affecting one billion of people in the world. The current model for NAFLD pathophysiology, “the multiple-hit hypothesis”, characterizes NAFLD as the manifestation of both genetic and environmental factors, dysfunction of various organs and organelles, as well as the complex interaction between hepatocytes and other cells (e.g., Kupffer and stellate cells) in the liver[1]. Moreover, the liver is a hub for many metabolic pathways making NAFLD a multistage, progressive disease with systemic consequences. NAFLD is commonly associated with obesity, insulin resistance and enhanced risk of cardiovascular disease and mortality[2-4]. Importantly, cardiovascular diseases are the main cause of morbidity in NAFLD patients. High-calorie consumption and lower physical activity have contributed to the rise in the prevalence of NAFLD. To date, no approved pharmacological approaches are available to treat NAFLD, the current confirmed recommendations for NAFLD are lifestyle modifications such as weight loss through caloric restriction (CR) and increased physical activity[7-9]. Therefore, a pressing need for developing new novel pharmacological treatments, is still remaining. An inclusive pharmacological approach would be one that addresses the pathogenic complexity of NAFLD. Currently, sirtuins have been under intense investigation as a novel therapeutic target for the treatment of NAFLD. In this review, we summarize the current knowledge on the pathophysiology of NAFLD and on the sirtuins as a potential target for the treatment of NAFLD.

NAFLD PATHOPHYSIOLOGY

NAFLD is a spectrum of liver diseases that occurs in the absence of excessive alcohol intake or viral infection. It includes hepatic steatosis (> 5% of fat in the liver), nonalcoholic steatohepatitis (NASH, fat deposit with inflammation), cirrhosis and hepatocellular carcinoma[9-12]. NAFLD is currently the most widespread form of liver disease affecting 10%-30% of all ages from childhood to adult population, and is predicted to be the leading cause of liver pathology and liver failure in the coming years[13,14]. NAFLD is more prominent in obese and insulin resistant individuals affecting 70%-90% in these populations[15,16]. NAFLD is also present in 10%-20% of the general pediatric population; this proportion increases to 50% in obese children in western society[13,17-22]. A more recent study suggests that metabolic derangements may start early in life, even in utero. Exposure to excess fuel in fetal life may result in NAFLD in the offspring[23,24]. Our understanding of the mechanisms involved in the pathophysiology of NAFLD are insufficient to pinpoint the major determinants involved in the development and progression of the disease and to develop therapeutic strategies for NAFLD. Studies on genetic and molecular factors involved in NAFLD clearly implicate lipid and glucose metabolism in the development of the disease. Moreover, functional studies implicates the different cell population in the liver as well as interaction between the liver, adipose tissue, gut and the muscle in the pathogenesis of NAFLD. In contrast to the “two-hit hypothesis” proposed by Day[25] in which hepatic accumulation of triglyceride (TG) (“1st hit”) sensitizes the liver to additional insults such as oxidative stress and pro-inflammatory cytokines (“2nd hit”) resulting in NASH. The current understanding, “the multiple parallel hypothesis”, refers to NAFLD as a systemic, multifactorial disease involving multiple organs, such as adipose tissue, muscle and the intestine, and organelles such the endoplasmic reticulum and the mitochondria.

Hepatic steatosis

Hepatic steatosis, which is previously considered as the benign form of NAFLD, results from an imbalance between influx of fatty acids to the liver from the diet, adipose tissue lipolysis or de novo lipogenesis; and their oxidation or export in the circulation as very low density lipoproteins (VLDL)[9]. Failure of insulin to suppress lipolysis in insulin resistant adipose tissue is commonly associated with NAFLD[26,27]. Moreover, it is estimated that in NAFLD patients, roughly 60% of fatty acids in the liver originate from adipose tissue, 25% from de novo lipogenesis, and 15% from the diet[28]. Interestingly, both β-oxidation of fatty acids in the liver and VLDL secretion, are initially upregulated in non-alcoholic fatty liver in an attempt to compensate for the rise in fatty acids in the liver[29-32]. However, this short term compensatory mechanism is insufficient to sustain the ongoing influx of fatty acid to the liver leading to liver injury[30-32]. NASH patients have lower VLDL secretion and lower fatty acid oxidation (FAO) than patients with fatty liver[30,31].

Non-alcoholic steatohepatitis and fibrosis

Non-alcoholic steatohepatitis (NASH) is a more severe form of NAFLD that is generally defined by the presence of steatosis with inflammation and cellular damage. Fibrosis is commonly described as an irreversible scarring of liver tissue with excessive presence of

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extracellular matrix. The presence of fibrosis is one of the most important predictors of NAFLD related mortality[10,33]. The current understanding of NASH pathogenesis follows a multiple hits mode[34,35] that implicate multiple stressors. Lipotoxicity, endoplasmic reticulum stress, adipose tissue derived adipokines (TNFs and IL6), gut endotoxins and LPS produced by gut microbiota that drift into the liver through the portal vein due to changes in the intestinal permeability in NAFLD, and oxidative stress trigger inflammatory response and progressive liver damage. Inflammation can sometimes precede steatosis, and patients with NASH can present without much steatosis suggesting that inflammation can sometimes occur first. Recent studies have also shown that individuals with hepatic steatosis may progress to fibrosis in a relatively short period of time (3-7 years)[36,37]. NAFLD patients may be classified into two categories, slow and fast progressors. The slow progressors may develop NASH but no fibrosis while the fast progressors may develop fibrosis and sometimes skip NASH stage of the disease[38]. Changes in mitochondrial function is an important mechanism that may drive the switch from hepatic steatosis to NASH. Several reports indicate that mitochondrial respiration is elevated in NAFLD patients[29,30]. However, in humans with NASH, respiration may be uncoupled from ATP production, causing significant increases in reactive oxygen species (ROS)[30]. Importantly, elevated ROS production was associated with an increase in detoxification and antioxidant capacity in hepatic steatosis, but not in NASH, indicating that mechanisms to cope with excess ROS generation may be insufficient in NASH[30].

### ROLE OF SIRTUINS IN NAFLD

Sirtuins are a group of proteins that belong to the family of silent information regulator 2. Sirtuins have been shown, in recent years, to play an important role in the pathophysiology of various metabolic diseases including NAFLD[39]. Sirtuins are implicated in many cellular and physiological functions including hepatic glucose and fatty acid metabolism, mitochondrial function, hepatic gluconeogenesis, insulin secretion and the maturation of fat cells[40,41] as illustrated in Figure 1. Sirtuins regulate protein function through a growing list of posttranslational modification including deacetylation, succinylation and malonylation[42,43]. Seven mammalians sirtuins (SIRT1-SIRT7) have been identified and shown to share the same conserved NAD binding site and catalytic core domain but with different N and C termini[44]. The different sirtuins have various subcellular localization and expression[44]. SIRT 1, 6, and 7 are localized mainly in nucleus while SIRT 3, 4 and 5 are localized to the mitochondrial matrix and SIRT2 predominantly cytoplasmic[44]. Recent studies have shown reduced levels of most sirtuins in NAFLD. Direct evidence came from Wu et al[45] who demonstrated decreased expression of SIRT1, SIRT3, SIRT5, and SIRT6 in NAFLD patients compared to the control group. This was associated with increased expression of lipogenic genes including sterol regulatory element binding protein-1, fatty acid synthase, and acetyl-CoA carboxylase. In contrast to the other sirtuins, the expression of SIRT4 was upregulated in NAFLD patients[45]. Interestingly, in a recent study, Bruce et al[46] indicated that exposure to excess dietary fat during early and post-natal life increases the susceptibility to develop NASH in adulthood and this was associated with reduced sirtuin abundance. Offspring fed a high fat diet (HFD) developed NAFLD while HFD-fed offspring of mothers fed a HFD diet developed NASH in combination of reduced NAD+/NADH, SIRT1, SIRT3 and increased expression of genes involved in lipid metabolism[46]. SIRT1 and SIRT3 are the most studied sirtuins; we will focus mainly on these two sirtuins, their mode of action and their role in NAFLD.

Both SIRT1 and SIRT3 are NAD⁺-deacetylase that use NAD as a cofactor to deacetylate cellular proteins. Lysine acetylation is a reversible, dynamic reaction of adding acetyl groups to lysine residues. Acetylation affects all proteins in the cell and has recently been shown to be abundant in the mitochondria where it plays a key role in the dynamic regulation of proteins and thereby cell metabolism[43,47-54]. Dysregulation of lysine acetylation plays a pathogenic role in diverse conditions such as metabolic syndrome, aging, cancer and NAFLD[55-58].

### SIRT1 and NAFLD

Studies from our group and others document strong involvement of the mitochondria in the pathogenesis of NAFLD[59-62]. SIRT3 is the most investigated mitochondrial sirtuin, while SIRT1 has been shown to be expressed in various metabolic tissues including liver, adipose tissue, skeletal muscle, pancreas and brain. SIRT1 plays a key role in the development of NAFLD through its involvement in the regulation of both lipid and carbohydrate metabolism[45,46,63-66]. Studies in mice and in cultured cells have characterized SIRT1 as a metabolic sensor that has the potential to improve NAFLD.

Inhibition of SIRT1 signaling in human fetal hepatocytes resulted in an increase in intracellular glucose and lipid levels with upregulation of de novo lipogenesis and gluconeogenesis related genes[66]. In mice, liver specific deletion of SIRT1 as well as SIRT1 downregulation using small hairpin RNA resulted in hepatic steatosis, inflammation and endoplasmic reticulum stress[52,58]. Hepatocyte-specific deletion of SIRT1 impaired PPARα signaling and decreased FAO. However, SIRT1 overexpression increased levels of PPARα and increased FAO[67].

SIRT1 is reduced by HFD while CR resulted in an increase in hepatic SIRT1 expression and improvement in NAFLD histology[69]. Overexpression
of SIRT1 in mice provided protection against HFD induced hepatic steatosis through upregulation of FAO and downregulation of lipogenesis\(^{64}\). Moreover, treatment of mice fed a HFD with resveratrol (RSV), a polyphenol found in red wine and other plants, improved lipid metabolism, and decreased NAFLD and inflammation in the liver\(^{70}\). Interestingly, it has been documented that inhibition of SIRT1 signaling in human fetal hepatocytes resulted in an increase in intracellular glucose and lipid levels\(^{46}\). SIRT1 is also modulated in obesity. Recent studies have shown a correlation between plasma SIRT1 and NAFLD in obese patients. SIRT1 was significantly lower in an obese group with severe liver steatosis compared to a group with mild steatosis, and both groups had lower SIRT1 in the plasma compared to control lean patients\(^{71}\). Phenotypic similarities exist between CR and SIRT1 overexpression. Mice overexpressing SIRT1 are leaner and resistant to hepatic steatosis and insulin resistance\(^{72}\). Together, these studies indicate a potential therapeutic use of SIRT1 in hepatic steatosis\(^{66}\).

**SIRT3 and NAFLD**

SIRT3 is a soluble protein located in the mitochondrial matrix and has been shown as a major regulator of mitochondrial protein acetylation and function\(^{44,73}\). SIRT3 regulates carbohydrate metabolism, ketogenesis, \(\beta\)-oxidation, and amino-acid metabolism and stress-related pathways\(^{73,77}\). The protein is encoded by the nuclear genome and is translated as a 45-kDa protein with an N-terminal mitochondrial targeting sequence that is cleaved to give the 28-kDa enzymatically active protein\(^{78}\). SIRT3 is expressed in many tissues including the liver, adipose tissue, heart, brain and kidney\(^{44}\).

Although SIRT3-KO mice are metabolically indistinguishable from WT controls under basal conditions, they show increased hyperacetylation of mitochondrial proteins in the liver and the heart\(^{54,74,75,79}\). About 65% of all mitochondrial proteins have at least one acetylated lysine\(^{48,54,73}\). SIRT4 and SIRT5 are also localized to the mitochondria and unlike SIRT3-KO mice, SIRT4 and SIRT5-KO mice did not display the global increase in hepatic mitochondrial acetylation observed in SIRT3-deficient animals.

Mitochondria play a key role in the adaptation to CR and SIRT3 has been identified as an important regulator in CR-associated metabolic changes\(^{44}\). The expression of SIRT3 is considerably increased in response to CR or prolonged fasting\(^{77,80,81}\). SIRT3 regulates the function of several mitochondrial proteins involved in oxidative phosphorylation, FAO, the urea cycle, and the antioxidant response system\(^{73,75,82-85}\). Unlike wild-type mice where FAO is upregulated with fasting, fasted SIRT3 deficient mice display reduced FAO and ATP production with increased hepatic TG.

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**Figure 1** An illustration representing various sirtuins with summary findings for SIRT1, SIRT3, and SIRT4. NAFLD: Non-alcoholic fatty liver diseases; FAO: Fatty acid oxidation; HFD: High fat diet.
expression of SIRT3 resulted in opposite effects suggesting that SIRT3 ablation aggravates MCD induced NAS while SIRT3 overexpression alleviates the MCD induced phenotype\[106\].

Palmitate modulated oxygen consumption and enhanced ROS levels and apoptosis in SIRT3 deficient mouse primary hepatocytes and SIRT3 siRNA-depleted hepatocytes\[96\]. Recent studies using HFD induced NAFLD in mice identified a differentially expressed microRNA (miRNA) in livers of NAFLD mice compared with controls. The expression of miRNA-421 was significantly upregulated in mice with NAFLD and SIRT3 was identified as target for this micro-RNA. Overexpression of miRNA-421 in hepatocytes decreased SIRT3 and FOXO3 protein levels, and reduced oxidative damage while suppression of this miRNA had opposite effects\[97\]. Interestingly, exposure of fetuses to maternal obesity contributes to early perturbations in whole body and liver energy metabolism, and this was associated with reduced SIRT3 and reduced hepatic FAO. These findings suggest that changes in SIRT3 activity precedes the development of obesity associated insulin resistance and NAFLD in the offspring\[98\].

**Sirtuins activators and inhibitors**

Weight loss through CR and exercise have been shown to improve insulin resistance and inflammation. Based on the beneficial effect of CR on NAFLD and other diseases and the associated increase in sirtuins levels or activity, the development of molecules that activate or inhibit sirtuins is of great interest\[99\].

The discovery of selective and potent sirtuins activators and inhibitors is still in its early stages. A list of Sirt1 activators that were tested in human and animal NAFLD is shown in Table 1\[100,104\]. RSV, a natural polyphenol found in grapes and other plants, mimicks CR and enhances sirtuins activity\[102,109\]. However, due to its poor bioavailability, reformulated forms of RSV-related compounds have been developed such as resVida, Longevinex\[98\], SRT50 along with other RSV unrelated molecules such as SRT1720, SRT2104, and SRT2379. The formulated form of RSV resVida (150 mg/d RSV) showed beneficial effects, similar to CR effect, in healthy obese men including reduced intrahepatic lipid, plasma glucose, TG, alanineaminotransferase and inflammation markers\[104\].

SIRT1720 was the most potent SIRT1 activator; it enhanced SIRT1 activity by 750% at 10 μmol/L although other studies by Pacholec et al\[106\] concluded that neither SRT1720 nor RSV are direct activators or activity, the development of molecules that activate or inhibit sirtuins is still in its early stages. A list of Sirt1 activators that were tested in human and animal NAFLD is shown in Table 1\[100-104\]. RSV, a natural polyphenol found in grapes and other plants, mimicks CR and enhances sirtuins activity\[102,109\]. However, due to its poor bioavailability, reformulated forms of RSV-related compounds have been developed such as resVida, Longevinex\[98\], SRT50 along with other RSV unrelated molecules such as SRT1720, SRT2104, and SRT2379. The formulated form of RSV resVida (150 mg/d RSV) showed beneficial effects, similar to CR effect, in healthy obese men including reduced intrahepatic lipid, plasma glucose, TG, alanineaminotransferase and inflammation markers\[104\].

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**Table 1 Published SIRT1 activators**

| SIRT1 activators | Ref. |
|------------------|------|
| Resveratrol      | Howitz et al\[105\], 2003 |
|                  | Wood et al\[106\], 2004 |
|                  | Timmers et al\[107\], 2011 |
|                  | Smith et al\[108\], 2009 |
|                  | Milne et al\[109\], 2007 |
|                  | Amiot et al\[110\], 2013 |
|                  | Yoshino et al\[111\], 2012 |
| SIRT1720         | Chachay et al\[112\], 2014 |
| SRT2104          | Feige et al\[113\], 2008 |
|                  | Funk et al\[114\], 2010 |
|                  | Yamazaki et al\[115\], 2009 |
|                  | Pacholec et al\[116\], 2010 |
|                  | Libri et al\[117\], 2012 |
|                  | Venkatasubramanian et al\[118\], 2013 |
|                  | Hofmann et al\[119\], 2013 |

SIRT3 also regulates the acetylation levels of mitochondrial electron transport complex I and regulates ATP synthesis\[77\]. ATP levels were reduced by more than 50% in the heart, liver and kidney of mice lacking SIRT3\[77\]. Succinate dehydrogenase (SDH) (one of complex II subunits of the electron transport chain) has been identified as a direct target of SIRT3, suggesting a role of SIRT3 in the regulation of complex II\[86,87\]. Increased succinate concentrations is involved in hepatic stellate cells (HSCs) activation. The expression of SIRT3 and SDH activity are decreased in isolated liver and HSCs from methionine- and choline-deficient (MCD) diet-induced NAFLD. Suppression of SIRT3 using siRNA exacerbated HSC activation while SIRT3 overexpression attenuated HSC activation in vitro\[88\]. Interestingly, liver- and muscle-specific SIRT3-KO mice show no detectable changes in their metabolic phenotype in response to HFD\[89\] suggesting more studies are needed to ascertain the role of tissue specific function of SIRT3\[76,89\].

Published studies document that both obesity and chronic HFD reduce SIRT3 activity, induce hyperacetylation of various mitochondrial proteins and impair mitochondrial function\[58,76,90\]. HFD has been shown to induce SIRT3 expression and FAO early after initiation of high-fat feeding\[58\]. However, chronic HFD suppress SIRT3 expression, increase mitochondrial protein acetylation, and ultimately reduce FAO. Wild type mice fed a HFD develop obesity, hyperlipidemia, type 2 diabetes mellitus, and NASH\[91-93\]. These effects of HFD feeding are significantly accelerated in SIRT3 deficient mice\[58\]. Our unpublished data also show that overexpression of SIRT3 rescues NAFLD in mice heterozygous for the mitochondrial trifunctional protein, an animal model of mitochondrial dysfunction generated by our group\[94\].

SIRT3-KO mice subjected to MCD diet exhibit increased serum ALT levels, increased hepatic content, higher expression of inflammatory and fibrogenic genes, and reduced (SOD2) activity. However, over-
Moreover, SRT1720 reduced levels of hepatic liver content and aminotransferase and the expressions of lipogenic genes\(^\text{[10]}\). Recent studies, however, indicate that the activation of SIRT1 by RSV is indirect and is mediated by activation of AMPK\(^\text{[40,115]}\). Sirtuins are themselves regulated by the cofactor NAD\(^+\) as well as their reaction product nicotinamide (NAM) from NAD\(^+\). NAM (the amide form of vitamin B3, nicotinic acid) is a water-soluble sirtuin inhibitor. NAM binds to a conserved region in the sirtuin catalytic site and favors a reverse reaction instead of the deacetylation reaction\(^\text{[116]}\). Computational studies indicate that NAM inhibition of SIRT3 involves apparent competition between the inhibitor and the enzyme cofactor NAD\(^+\) while the inhibition of other sirtuins activity was non-competitive\(^\text{[117]}\). More detailed review on sirtuins inhibitors and activators is found in\(^\text{[99,118]}\). More studies are needed to develop more potent and specific activators and inhibitors of sirtuins activity.

**CONCLUSION**

Sirtuins represent potential targets for treatment of NAFLD due to the role they play in cellular pathways involved in hepatic lipid and carbohydrate metabolism, insulin signaling, and inflammation. Additional studies are urgently needed to further our understanding of the interaction among various sirtuins in NAFLD and to develop selective activators/inhibitors of sirtuins.

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