A Potential Treatment of Non-Alcoholic Fatty Liver Disease with SIRT1 Activators

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as an accumulation of fat in the liver in the absence of any significant alcohol consumption (140 g/week for women and 210 g/week for men for periods longer than two years), hereditary disease or drug consumption [1]. In cases of steatosis alone, it is defined as simple steatosis or nonalcoholic fatty liver (NAFL), while non-alcoholic steatohepatitis (NASH) is accompanied by inflammatory and/or the presence of hepatocellular damage. Nowadays, NAFLD is the most common chronic liver disease in developed countries [2]. In clinical practice, these diseases may present with a wide range of manifestations, including simple steatosis, NASH, advanced fibrosis, cirrhosis and even hepatocellular carcinoma (HCC). In most cases, metabolic syndrome (MS) parameters, such as obesity, hyperlipidemia, insulin resistance or diabetes may also be present. Therefore, this illness may be accepted as the hepatic component of MS.

The pathogenesis of NAFLD may be summarized as the accumulation of excess fat in hepatocytes, insufficient
mitochondrial capacity for beta oxidation, mitochondrial damage, increased intracellular fat, involvement of oxidative stress and inflammatory processes, cellular damage, apoptosis and triggering of fibrosis. Currently, treatment is focused on MS factors, primarily by enacting lifestyle changes, including weight loss, dietary modifications and regular exercise. There is no validated medical treatment that has been established in accordance with long-term, randomized and controlled trials.

The sirtuins (SIRTs) are proteins that act as nicotinamide adenine dinucleotide (NAD+) -dependent protein/histone deacetylases and are included in the family of silent information regulator-2 (SIR2). To date, seven different SIRTs have been reported in mammals. SIRT1 plays a role in the pathophysiology of many metabolic diseases via its capacity for protein deacetylation, thereby modulating the activation and de-activation of certain proteins. In recent years, it has been shown that SIRT1 and its activators play a key role in lipid and glucose homeostasis and in insulin sensitivity via calorie restriction mimetic effects that result from their protective effects for mitochondrial biogenesis and beta-oxidation [3, 4]. Furthermore, their anti-inflammatory activities reduce the oxidative stress and provide beneficial effects on obesity, hypertension, endothelial dysfunction, cardiovascular protection, neurodegeneration, cellular senescence, apoptosis and autophagy [5]. A potential therapeutic hypothesis was first proposed in the literature by our group, stating that the activation of SIRT1 may affect the pathogenetic molecular cascade and the therapeutic mechanisms of NAFLD [6]. This hypothesis was later confirmed by a number of studies [7-9]. In this article, the effects of SIRT1 activation on the pathophysiology and future potential treatments of NAFLD are discussed based on evidence in the literature.

SIRTUIN1 AND GLUCOSE METABOLISM

One of the major risk factors in the pathogenesis of NAFLD is insulin resistance. Insulin resistance causes dysregulation in fatty acid metabolism and leads to steatosis. With increased free fatty acid flow to the liver and increased lipogenesis, the liver is exposed to the “first hit” according to the “two hit” theory [10]. Hepatocyte mitochondria fail to oxidize this augmented free fatty acid accumulation and peroxisomes start to oxidize excess free fatty acids. As a result of peroxisome oxidation inflammatory molecules build up including reactive oxygen species [11]. These molecules damage hepatocytes causing the “second hit.” With more data on pathogenesis, nowadays other mechanisms of insults are investigated [12].

In recent years, it has been shown that SIRT1 activation has an important role in glucose homeostasis and exerts anti-diabetic effects. In these studies, SIRT1 activation in the arcuate nucleus of central hypothalamus, which regulates the nutritional behavior of central control and glucose homeostasis, was shown to reduce hepatic glucose production, thereby increasing insulin sensitivity [13].

SIRT1 over-expression also suppresses the nuclear factor kappa enhancer binding protein (NF-κB) signaling pathway, thereby protecting against cytokine-mediated damage to pancreatic β cells [14]. SIRT1 activation protects these cells against cytokine-mediated damage due to the positive effects of insulin resistance, illustrating a potential anti-diabetic activity (Fig. 1). SIRT1 activation increases glucose up-take in adipocytes, decreases the expression of inflammatory genes, and renders a protective effect against high-fat diet-induced metabolic damage [15, 16].

Nevertheless, there are some data regarding hyperglycemic effects of SIRT1. Some studies have shown that gluconeogenesis in hepatocytes is induced by the activation of SIRT1, and in animal studies, SIRT1 knockdowns led to a reduction in basal hepatic glucose production in the liver [17-20]. In order to clarify the hyperglycemic effects of SIRT1, more research is required.

SIRTUIN1 AND LIPID METABOLISM

Hyperlipidemia and lipid metabolism disorders are the other two important predisposing factors in the pathogenesis of NAFLD. As discussed previously, increased free fatty acid accumulation and molecules generated after oxidation cause hepatocyte damage and lead to NAFLD. Recent studies have revealed an important role of SIRT1 in lipid homeostasis. These effects are achieved not only within the adipose tissue but also through skeletal muscle and liver lipid metabolism.

SIRT1 inhibits lipogenesis, increases lipolysis and fatty acid production in adipose tissue as a result of the stimulation of adipose triglyceride lipase (ATGL) gene transcription, which is related to the activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and the lipase-mediated deacytlation of the Forkhead box, group O1 (FOXO1) [21, 22]. SIRT1 activation, when stimulated by resveratrol, inhibits the proliferation and differentiation of pig adipocytes [23]. Additionally, SIRT1 overexpression in the adipose tissue inhibits macrophage accumulation after a high fat diet, indicating a positive effect on the inflammatory process in adipose tissues [24].

In the skeletal muscle, SIRT1 activation leads to the deacytlation of PPAR-γ coactivator 1-alpha (PGC-1α), which induces the activation of mitochondrial fatty acid oxidation genes, especially when there are low glucose levels due to nutrient deprivation. As a result, the energy demand switches from glycolysis to mitochondrial fatty acid beta-oxidation, and the adaptation of skeleton muscle to hunger occurs [25, 26]. SIRT1 regulates mitochondrial protein expression in skeletal muscles via uncoupling protein 3 (UCP3), followed by a decrease in the membrane potential and a reduction of fatty acid accumulation [27, 28].

The liver is a central metabolic organ that regulates the nutritional status and lipid metabolism as a result of hormonal signals, such as triglyceride uptake, lipoprotein uptake and secretion, fatty acid beta-oxidation and lipogenesis [29]. The effects of the liver on lipid metabolism are exerted through many different pathways. The liver X receptors (LXRs) act as nuclear receptors, detecting cholesterol and initiating a reverse cholesterol transport from the peripheral tissues to the liver [30, 31]. Some of the effects of LXRs include the inhibition of intestinal cholesterol absorption, stimulation of the cholesterol efflux to high-density lipoprotein (HDL) from cells via ATP-binding cassette transporters (ABCTs), transformation of cholesterol to bile acids in the liver and...
activation of cholesterol and bile acid excretion [32]. Therefore, the LXR have been suggested to be a potential therapeutic target for the treatment of NAFLD and atherosclerosis [33, 34]. SIRT1 leads to an increased expression of LXR due to deacylation [35]. Sterol regulatory element binding proteins (SREBPs) control the expression of genes associated with the synthesis of lipids, cholesterol, fatty acids, phospholipids and triglycerides in tissues, such as the liver and adipose tissues [36]. Therefore, the inhibition of the activation of SREBP-1c deacetylation by SIRT1 reduces lipogenic gene expression and has been shown to contribute to the regulation of the hepatic lipid metabolism [37]. In vivo models of leptin-deficient, SRT1720, a synthetic SIRT1 activator that leads to the inhibition of SREBP expression, had a positive effect on the healing process of hepatosteatosis [38].

In a study by Purushotham et al, it is shown that in mice, hepatocyte-specific deletion of SIRT1 causes PPARα signal failure and results in reduced fatty acid beta-oxidation [39]. On the other hand over-expression of SIRT1 induces the expression of PPARα’s targets leading to increased hepatic beta-oxidation. Findings suggest SIRT1 regulates hepatic energy homeostasis mainly via fatty acid metabolism.

As a result, the activation of SIRT1 positively affects the liver, skeletal muscles and adipose tissues and leads to lipolysis, enhances fatty acid mobilization from peripheral tissues, reduces lipogenesis, increases hepatic beta-oxidation activity and shows favorable effects on hepatic steatosis (Fig. 1).

**SIRTUIN1 AND OXIDATIVE STRESS**

Reactive oxygen species (ROS) can be produced as natural by-products of the cellular metabolism or, occasionally, as a result of external factors, such as ionizing radiation and cytotoxic drugs [40]. Oxidative stress, which arises as an imbalance between the production of ROS and the action of antioxidant defense mechanisms, can result in cellular aging and apoptosis, depending on the surrounding oxidant environment [41]. The production of ROS-induced oxidative stress plays a role in the pathogenesis of NAFLD and also affects the pathogenesis of neurodegenerative and cardiovascular diseases [42-44]. In recent years, it has been shown that SIRT1 has a regulatory effect on oxidative stress in many tissues, reducing the ROS levels and improving cell survival via this antioxidant effect [45]. A major pathway for

![Fig. 1. The effects of activation of the SIRT1](image-url)
these activities is deacetylation of the p53 tumor suppressor protein by SIRT1, which leads to the inhibition of the oxidative stress-induced apoptotic activity of p53 [46, 47]. Furthermore, this pathway suggests a positive effect of SIRT1 activation on cancer pathogenesis [48]. Another important mechanism is the complex interaction of SIRT1 with FOXO transcription factors (FOXO1, FOXO3a, FOXO4), which leads to the production of ROS-detoxifying enzymes, including catalase, superoxide dismutase 2 (SOD2) and manganese SOD (MnSOD) [40]. Additionally, as many studies have indicated, SIRT1 has a favorable effect on the oxidative stress by increasing the levels of vascular endothelial nitric oxide via endothelial nitric oxide synthetase (eNOS) [49]. In recent years, randomized, double-blind, placebo-controlled human studies have shown that the SIRT1 activator, resveratrol, decreases the levels of ROS [50-55]. In summary, the activation of SIRT1 decreases vascular endothelial oxidative stress and offers an antioxidant activity by reducing the levels of ROS (Fig. 1).

**SIRTUIN1 AND ANTI-INFLAMMATORY ACTIVITY**

In recent years, many studies have concluded that SIRT1 activation results in a negative regulatory effect on inflammatory processes. One of the key proteins in these processes is the protein NF-κB. NF-κB plays a key role in the modulation of DNA transcription in inflammatory, infectious and apoptotic processes. The incorrect regulation of NF-κB may lead to inflammatory and autoimmune diseases, infectious processes and cancer. Increasing evidence shows that NF-κB activation contributes to the pathogenesis of NASH and the development of HCC [56-58]. The activation of SIRT1 deacylates the RelA/p65 subunit and leads to the inhibition of NF-κB signals [59, 60]. Pfluger et al showed that high-fat, diet-induced hepatic steatosis was protected by SIRT1 activation in mice. One of two proposed protective mechanisms involves MnSOD and Nuclear Respiratory Factor 1 (Nrf1) antioxidants, which are formed by PGC-1α. Especially in skeletal muscle cells PGC-1α is found to have an essential role in mitochondrial biogenesis and inflammatory pathways and increases oxidative phosphorylation [61, 62]. The activation of PGC-1α through deacylation in skeletal muscles is needed to activate fatty acid oxidation genes [25, 26].

The second proposed mechanism involves interleukin 6 (IL-6) due to the down-modulation of NF-κB and the suppression of pro-inflammatory cytokine activation via tumor necrosis factor alpha (TNF-α) [16].

In the pathogenesis of NASH, cytokine mediated inflammatory processes are an important step. Recently, activator protein-1 (AP-1), a transcription factor, has been shown to be a part of cytokine mediated processes and is involved in gene expression in response to a variety of stimuli like cell proliferation, differentiation, and inflammation due to bacterial and viral infections or stress via the actions of growth factors and cytokines [63]. One of the mediators in these inflammatory processes is the speed-limiting enzyme cyclooxygenase-2 (COX-2), the target of AP-1. Zhang et al demonstrated that SIRT1 activation suppressed AP-1 transcriptional activity and COX-2 expression in macrophages [64]. Adipose tissue derived inflammation is a key step in the pathogenesis of NAFLD. The study of Gillum and colleagues found that macrophage accumulation in response to pro-inflammatory transcription due to tissue fatty acids and the endoplasmic reticulum was blocked by SIRT2 [24]. As reported previously Purushotham et al showed that the hepatocyte-specific deletion of SIRT1 weakened PPAR-γ signals and decreased fatty acid beta-oxidation, leading to stress in the endoplasmic reticulum, hepatic inflammation and hepatic steatosis [39]. Similarly, SIRT1 null mice were found to demonstrate increased lipogenesis, decreased lipid mobilization, and increased levels of NF-κB, PPAR-γ, TNF-α, and IL-6 in fat cells and the liver, resulting in increased hepatic inflammation and a predisposition to hepatic steatosis after they were fed a high-fat diet [65]. Therefore, SIRT1 activation has anti-inflammatory effects on the inflammation mechanisms that occur within the pathways of NAFLD, especially NASH (Fig. 1).

**GENERAL EFFECTS OF SIRTUIN1**

**Cardiovascular effects**

The most common cause of morbidity and mortality in patients with NAFLD are the cardiovascular events [1]. The possible causes of increased cardiovascular events in patients with NAFLD are endothelial dysfunction, hyperlipidemia, insulin resistance and oxidative stress with underlying atherosclerosis, coronary heart disease and left atrial and ventricular dysfunction [66-70]. In recent years, studies have shown that SIRT1 plays a significant role in the pathogenesis of cardiovascular events through different protective mechanisms [71].

The majority of SIRT1’s beneficial effects on atherosclerosis and endothelial dysfunction are due to the anti-inflammatory and antioxidant effects mentioned above [72]. SIRT1 deficiency decreases endothelium-dependent vasodilatation, while SIRT1 over-expression leads to increased endothelium-dependent vasodilatation, leading to protection from atherosclerosis [73, 74]. Resveratrol-induced SIRT1 activation suppresses angiotensin II with type 1 receptor (AT1R) expression in vascular smooth muscle cells, preventing increases in blood vessel contraction and blood pressure [75]. In a recent study, SIRT1 was shown to suppress the expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which is a scavenger receptor in macrophages, and thus suppresses the oxidized LDL that is uploaded by macrophages, inhibiting macrophage foam-cell formation [76].

Potente et al reported that SIRT1 was expressed in great amounts during blood vessel growth, which implies a role for SIRT1 in angiogenesis neovascularization. In cases of disabled SIRT1 function, neangiogenesis was blocked due to the down-regulation of genes related to blood vessel development and vascular remodeling [77]. Aside from the effects of SIRT1 activation at the molecular and cellular levels, its cardiac benefits have been demonstrated in recent years. Studies have revealed that SIRT1 in the heart protects against ischemia and reperfusion injury and myocardial infarction and is able to reduce the infarct area through NO-dependent and NO-independent mechanisms [73, 78, 79]. As a result, many SIRT1
pathways prevent endothelial dysfunction and atherosclerosis, providing cardioprotection (Fig. 1).

**The effects on autophagy and apoptosis**

In recent years, the failure of autophagic mechanisms has been suggested to occur in the pathogenesis of hepatic steatosis and NASH, and the augmentation of autophagy has been suggested as a potential therapeutic approach in NASH [80]. The activation of SIRT1 by resveratrol was shown to increase autophagy in cardiomyocytes, achieving a possible cardioprotective effect [81, 82]. Furthermore, it has been shown that SIRT1 activation increases autophagy in cancer cells [5].

Resveratrol has also demonstrated an anti-apoptotic activity in intact cardiomyocytes [83]. However, in states of metabolic stress, especially in the presence of oxidative stress and cancer, a paradoxical proapoptotic effect has been demonstrated, limiting the existing process in this form [84]. Therefore it is too early to have a hypothesis about the effects of SIRT-1 activation on both autophagy and apoptosis.

**Anti-aging effects**

In recent years, due to the growing evidence of the effects of SIRT1 activation on multisystem protection, its potential anti-aging activity has become a topic of discussion [85]. One of the most important mechanisms of this activity is the inhibitory activity of SIRT1 on endothelial aging. In a recent study, Ota et al showed that two down-regulators of SIRT1, sirolimus and everolimus, led to endothelial cellular aging and SIRT1 overexpression reversed this effect [86]. Ota et al showed that SIRT1 had premature senescence-like effects on human umbilical vein endothelial cells, whereas the overexpression of SIRT1 down-regulated premature senescence [87]. They also reported that the up-regulation of SIRT1 inhibited premature aging in human endothelial cells [88]. Similarly, Zu et al found a progressive decrease in SIRT1 expression in aging endothelial cells, while the overexpression of SIRT1 inhibited endothelial cellular aging via signals from the liver kinase B/AMP-activated protein kinase (LKB/AMPK) [89]. Similar SIRT1 activation in the liver has been shown to lead to a regulatory effect via the LKB/AMPK pathway [90].

AMPK is an enzyme expressed from various tissues and it plays a central role in cellular energy homeostasis [91]. It is also responsible for fatty acid oxidation in the liver by inactivating acetyl-CoA carboxylase (ACC). Inactivation of ACC leads to increased fatty acid transport and oxidation [92].

Finally, SIRT1 activation prevented the hyperglycemia-related cellular aging process in human endothelial cells via p53 down regulation [90]. One of the major diseases related to metabolic syndrome, aging, and NAFLD is HCC [95, 96]. Recent studies have reported the tumor suppressor activity of SIRT1 in a number of different cancers, including HCC [32, 97, 98]. In SIRT1-mutant mice, tumors developed in multiple tissues, but tumorigenesis was reversed after resveratrol-mediated SIRT1 activation [99]. SIRT1 activation shows that its potential anti-aging activity may be due to several different molecular pathways.

**CONCLUSIONS**

There is currently no specific medical treatment modality for NAFLD; however, altering a patient's predisposing factors, such as restricting the patient to a low-calorie diet and increasing the patient's physical activity, is recommended. Sirtuins, particularly SIRT1, deacetylate histones/proteins and modulate a number of metabolic pathways. These effects may be summarized as increased insulin sensitivity and

<table>
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<th>Sample population</th>
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<th>Effect</th>
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<td>Venkatasubramaniam et al (2013)</td>
<td>24 healthy cigarette smokers</td>
<td>SRT2104</td>
<td>28 days, 2 g/day</td>
<td>Improved lipid profile</td>
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<td>Hoffmann et al (2013)</td>
<td>65 healthy volunteers</td>
<td>SRT2104</td>
<td>7 days, 0.3-3 g/day</td>
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<td>Bo et al (2013)</td>
<td>50 healthy cigarette smokers</td>
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<td>Amiot et al (2013)</td>
<td>16 healthy volunteers</td>
<td>Resveratrol</td>
<td>Single dose, 40 mg</td>
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<td>Libri et al (2012)</td>
<td>17 elderly volunteers</td>
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<td>Timmers et al (2011)</td>
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<td>Resveratrol</td>
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<td>Decreased blood pressure and hepatic lipid content, and improved in insulin resistance and mitochondrial functions. No adverse events and good tolerability</td>
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<td>Brasynó et al (2011)</td>
<td>10 type 2 diabetic patients</td>
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<td>Ghanim et al (2010)</td>
<td>10 healthy volunteers</td>
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<td>Poulsen et al (2013)</td>
<td>12 male, obese otherwise healthy volunteers</td>
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<td>4 weeks, 500 mg/day</td>
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<td>Yoshino et al (2012)</td>
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pancreas beta cell reserves; calorie restriction due to mimetic activity; improvements in the glycemic regulation of the liver; antihyperlipidemic activity on lipid homeostasis in adipose tissue and skeletal muscle; anti-inflammatory activity; protective effects on the cardiovascular events and endothelial dysfunction; and positive influences on autophagy, apoptosis, cancer and aging. Due to this evidence, SIRT1 activation may have the therapeutic potential to prevent and reduce the incidence of complications, development and progression of NAFLD. Additionally, Dunn et al [100] showed that modest wine consumption is associated with a decreased prevalence of suspected NAFLD while modest beer and liquor consumption were not. The authors suggested that this protective effect could be associated with non-alcohol components rather than alcohol in wine. These results suggest that the protective effect of wine against NAFLD may be associated with grape-sourced resveratrol [100]. In recent years, SIRT1 activation in animal studies has been shown to inhibit the development of NAFLD; however, there is not enough data to support this conclusion in humans. To date there is not enough data for optimal dose and safety and we need more potent evidence. Table I offers a summary of dose regimens and effects of SIRT1 activators in recent human studies [101-109].

In recent years, the natural and synthetic analogues of resveratrol were discovered. It was shown that these resveratrol derivatives have different action potency according to the structural variance of the molecule. Therefore, different analogues could be suggested in different molecular targets. However, large-scale randomized controlled trials are required [110].

Based on the findings discussed in this article, newly developed and more potent synthetic SIRT1 activators, including resveratrol, necessitate large-scale randomized controlled trials to assess their therapeutic efficacy and their safety profiles for human NAFLD.

Conflicts of interest: None to declare.

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REFERENCES


