REVIEW

Available from: URL: http://www.jgld.ro/2014/3/16.html
DOI: http://dx.doi.org/10.1543/jgld.2014.1121.233.yck

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

Yasar Colak[1], Atakan Yesil[2], Hasan Huseyin Mutlu[3], Ozge Telci Caklili[4], Celal Ulasoglu[1], Ebubekir Senates[1], Mumtaz Takir[5], Osman Kostek[4], Yusuf Yilmaz[6], Feruze Yilmaz Enc[1], Guralp Tasan[1], Ilyas Tuncer[1]

1) Department of Gastroenterology, Istanbul Medeniyet University, School of Medicine;
2) Department of Gastroenterology, Haydarpasa Training and Research Hospital;
3) Department of Family Medicine, Istanbul Medeniyet University, School of Medicine;
4) Department of Internal Medicine, Istanbul Medeniyet University, School of Medicine;
5) Department of Endocrinology, Istanbul Medeniyet University, School of Medicine;
6) Department of Gastroenterology, Marmara University, School of Medicine,
Istanbul, Turkey

Address for correspondence:
Yasar Colak
Istanbul Medeniyet University School of Medicine, Goztepe Training and Research Hospital, Gastroenterology
Istanbul, Turkey
dryasarcolak@yahoo.com

Received: 25.05.2014
Accepted: 25.08.2014

ABSTRACT

Sirtuins (SIRTs) are members of the silent information regulator-2 family and act as nicotinamide adenine dinucleotide (NAD+)-dependent histone/protein deacetylases. The de-acetylation of proteins and histones results in an up- or down-regulation of gene transcription and protein function. In recent years, the regulatory action of the deacetylation activity of SIRT1 has been shown to have a positive impact on the pathophysiological mechanisms of nonalcoholic fatty liver disease (NAFLD). Among the effects of SIRT1 are: its healing activity on insulin sensitivity, thereby ameliorating glycemic regulation; its mimetic activity on calorie restriction; its antihyperlipidemic activity on lipid homeostasis via the liver, adipose tissues and skeletal muscles; its anti-inflammatory activities; its protective effects against cardiovascular events and endothelial dysfunction; its positive influence on autophagy, apoptosis and cancer; and finally, its anti-aging activity.
The current approach for the treatment of NAFLD involves the treatment of etiological factors and recommendation of life-style changes including more physical activity and a low-calorie diet. However, there is no specific medical treatments for NAFLD. The therapeutic potential of SIRT1 activity in the treatment of NAFLD discovered in humans has been presented in this article. In this review, the potential effects of SIRT1 activation on NAFLD-related pathophysiological mechanisms and on the treatment of NAFLD are discussed.

Key words: nonalcoholic fatty liver disease – resveratrol – sirtuin 1 – therapy.

Abbreviations: ACC: acetyl-coa carboxylase; AP-1: activator protein-1; ATGL: adipose triglyceride lipase; AT1R: angiotensin II with type I receptor; abcts: ATP-binding cassette transporters; COX-2: cyclooxygenase-2; enos: endothelial nitric oxide synthetase; FOXO1: forkhead box, group O1; HCC: hepatocellular carcinoma; HDL: high-density lipoprotein; IL-6: interleukin 6; LOX-1: lectin-like oxidized low-density lipoprotein receptor-1; LKB/AMPK: liver kinase B/AMP-activated protein kinase; LXrs: liver X receptors; MS: metabolic syndrome; NAD+: nicotinamide adenine dinucleotide; NASH non-alcoholic steatohepatitis; NAFLD: nonalcoholic fatty liver disease; NF-κb: nuclear factor kappa enhancer binding protein; Nrf1: nuclear respiratory factor 1; PPAR-γ: peroxisome proliferator- activated receptor-gamma; PGC-1α: PPAR-γ coactivator 1-alpha; ROS: reactive oxygen species; SIR2: silent information regulator-2; SIRT: sirtuin; srebps: sterol regulatory element binding proteins; SOD: superoxide dismutase; TNF-α: tumor necrosis factor alpha; UCP3: uncoupling protein 3.

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 inflammation 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 deacetylation 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 deacetylation 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 LXRs 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 LXRs 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 mice, 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

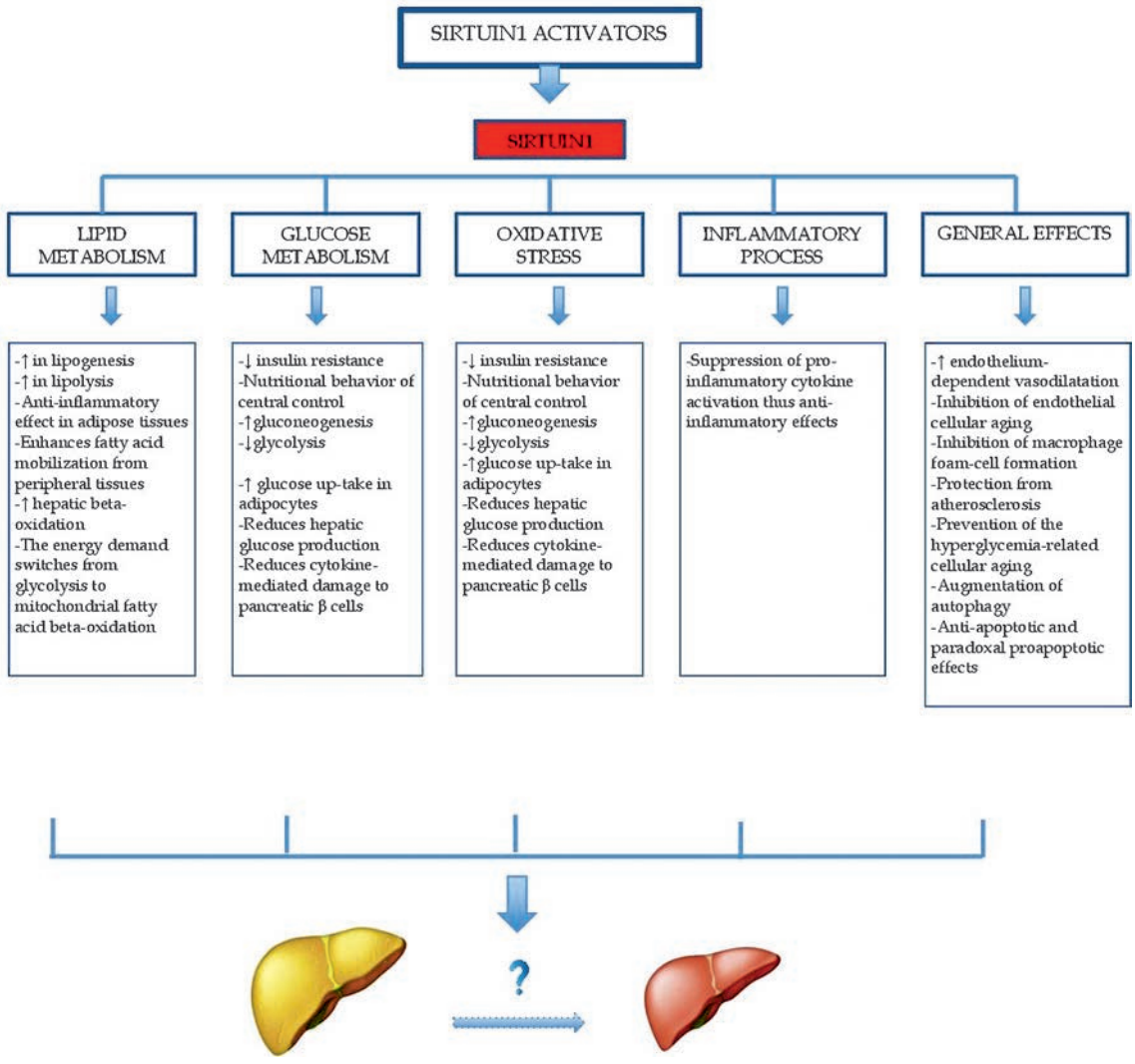

Fig. 1. The effects of activation of the SIRT1

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 mangane 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 SIRT [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 I 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, neoangiogenesis 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, deacylate histones/proteins and modulate a number of metabolic pathways. These effects may be summarized as increased insulin sensitivity and

Table I. The effects of sirtuin1 activators in human studies

References	Sample population	SIRT1 activator	Duration, Dose	Effect
Venkatasubramanian et al (2013)	24 healthy cigarette smokers	SRT2104	28 days, 2 g/day	Improved lipid profile
Hoffmann et al (2013)	65 healthy volunteers	SRT2104	7 days, 0.3-3 g/day	Good tolerability and safety
Bo et al (2013)	50 healthy cigarette smokers	Resveratrol	30 days, 0.5 g/day	Anti-inflammatory, anti-oxidant and hypotriglyceridemic effects
Amiot et al (2013)	16 healthy volunteers	Resveratrol	Single dose, 40 mg	Increased bioavailability for lipid caplets form
Libri et al (2012)	17 elderly volunteers	SRT2104	28 days, 0.5 or 2 g/day	Improved lipid profile, no serious adverse events and good tolerability
Timmers et al (2011)	11 healthy, obese men	Resveratrol	30 days, 150 mg/day	Decreased blood pressure and hepatic lipid content, and improved in insulin resistance and mitochondrial functions. No adverse events and good tolerability
Brasynó et al (2011)	10 type 2 diabetic patients	Resveratrol	28 days, 10 mg/day	Improved insulin resistance and decreased in oxidative stress
Ghanim et al (2010)	10 healthy volunteers	Resveratrol	42 days, 40 mg /day	Reduction in oxygen species and anti-inflammatory effects
Poulsen et al (2013)	12 male, obese otherwise healthy volunteers	Trans-resveratrol	4 weeks, 500 mg/day	No significant effect
Yoshino et al (2012)	15 postmenopausal women	Resveratrol	12 weeks, 75 mg/day	No significant effect
Chachay et al (2014)	10 overweight or obese men diagnosed with NAFLD	Resveratrol	8 weeks, 3 g/day	No beneficial effect

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 derives 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.

Authors' contributions: C.Y, Y.A, U.C, and C.O.T, designed the review; M.H.H, S.E and Y.Y, analyzed data; C.Y, C.O.T, and U.C wrote the paper; E.F.Y, K.O, T.G and T.M revised the paper and provided important suggestions; T.I and Y.Y supervised the manuscript; all authors have read and approved the final manuscript.

REFERENCES

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005-2023.
2. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274-285.
3. Lam YY, Peterson CM, Ravussin E. Resveratrol vs. calorie restriction: data from rodents to humans. Exp Gerontol 2013;48:1018-1024.
4. Silva JP, Wahlestedt C. Role of Sirtuin 1 in metabolic regulation. Drug Discov Today 2010;15:781-791.
5. Xu Q, Si LY. Resveratrol role in cardiovascular and metabolic health and potential mechanisms of action. Nutr Res 2012;32:648-658.
6. Colak Y, Ozturk O, Senates E, et al. SIRT1 as a potential therapeutic target for treatment of nonalcoholic fatty liver disease. Med Sci Monit 2011;17:HY5– HY 9.
7. Li L, Hai J, Li Z, et al. Resveratrol modulates autophagy and NF-κB activity in a murine model for treating non-alcoholic fatty liver disease. Food Chem Toxicol 2014;63:166-173.
8. Castro RE, Ferreira DM, Afonso MB, et al. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. J Hepatol 2013;58:119-125.
9. Choi Y, Yanagawa Y, Kim S, Park T. Involvement of SIRT1-AMPK signaling in the protective action of indole-3-carbinol against hepatic steatosis in mice fed a high-fat diet. J Nutr Biochem 2013;24:1393-1400.
10. Day CP. Pathogenesis of steatohepatitis. Best Pract Res Clin Gastroenterol 2002;16:663-678.
11. Başaranoğlu M, Ormeci N. Nonalcoholic fatty liver disease: diagnosis, pathogenesis, and management. Turk J Gastroenterol 2014;25:127-132.
12. Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). Int J Mol Sci 2013;14:20704-20728.
13. Knight CM, Gutierrez-Juarez R, Lam TK, et al. Mediobasal hypothalamic SIRT1 is essential for resveratrol's effects on insulin action in rats. Diabetes 2011;60:2691-2700.
14. Lee JH, Song MY, Song EK, et al. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. Diabetes 2009;58:344-351.
15. Yoshizaki T, Milne JC, Imamura T, et al. SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. Mol Cell Biol 2009;29:1363–1374.
16. Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschöp MH. Sirt1 protects against high-fat diet induced metabolic damage. Proc Natl Acad Sci U S A 2008;105: 9793–9798.
17. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 2005;434:113–118.
18. Liu Y, Dentin R, Chen D, et al. A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature 2008;456:269–273.
19. Nie Y, Erion DM, Yuan Z, et al. STAT3 inhibition of gluconeogenesis is downregulated by SirT1. Nat Cell Biol 2009;11:492–500.
20. Erion DM, Yonemitsu S, Nie Y, et al. SirT1 knockdown in liver decreases basal hepatic glucose production and increases hepatic insulin responsiveness in diabetic rats. Proc Natl Acad Sci U S A 2009;106:11288–11293.
21. Picard F, Kurtev M, Chung N, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004;429:771–776.
22. Chakrabarti P, English T, Karki S, et al. SIRT1 controls lipolysis in adipocytes via FOXO1-mediated expression of ATGL. J Lipid Res 2011;52:1693–1701.
23. Bai L, Pang WJ, Yang YJ, Yang GS. Modulation of Sirt1 by resveratrol and nicotinamide alters proliferation and differentiation of pig preadipocytes. Mol Cell Biochem 2008;307:129–140.
24. Gillum MP, Kotas ME, Erion DM, et al. SirT1 regulates adipose tissue inflammation. Diabetes 2011;60:3235–3245.

25. Amat R, Planavila A, Chen SL, Iglesias R, Giralt M, Villarroya F. SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD. J Biol Chem 2009;284:21872-21880.
26. Gerhart-Hines Z, Rodgers JT, Bare O, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J 2007;26:1913-1923.
27. Nogueiras R, Habegger KM, Chaudhary N, et al. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. Physiol Rev 2012;92:1479-1514.
28. Amat R, Solanes G, Giralt M, Villarroya F. SIRT1 is involved in glucocorticoid-mediated control of uncoupling protein-3 gene transcription. J Biol Chem 2007;282:34066–34076.
29. Nguyen P, Leray V, Diez M, et al. Liver lipid metabolism. J Anim Physiol Anim Nutr (Berl) 2008;92:272-283.
30. Zhao C, Dahlman-Wright K. Liver X receptor in cholesterol metabolism. J Endocrinol 2010;204:233-240.
31. Ducheix S, Lobaccaro JM, Martin PG, et al. Liver X Receptor: an oxysterol sensor and a major player in the control of lipogenesis. Chem Phys Lipids 2011;164:500-514.
32. Wójcicka G, Jamroz-Wiśniewska A, Horoszewicz K, Bełtowski J. Liver X receptors (LXRs). Part I: structure, function, regulation of activity, and role in lipid metabolism. Postepy Hig Med Dosw (Online) 2007;61:736-759.
33. Ducheix S, Montagner A, Theodorou V, Ferrier L, Guillou H. The liver X receptor: a master regulator of the gut-liver axis and a target for non alcoholic fatty liver disease. Biochem Pharmacol 2013;86:96-105.
34. Fiévet C, Staels B. Liver X receptor modulators: effects on lipid metabolism and potential use in the treatment of atherosclerosis. Biochem Pharmacol 2009;77:1316-1327.
35. Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Mol Cell 2007;28:91–106.
36. Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F. SREBP transcription factors: Master regulators of lipid homeostasis. Biochimie 2004;86:839-848
37. Ponugoti B, Kim DH, Xiao Z, et al. SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. J Biol Chem 2010;285:33959-33970.
38. Walker AK, Yang F, Jiang K, et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. Genes Dev 2010;24:1403–1417.
39. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab 2009;9:327-338.
40. Hori YS, Kuno A, Hosoda R, Horio Y. Regulation of FOXOs and p53 by SIRT1 Modulators under Oxidative Stress. PLOS ONE 2013;8:e73875.
41. Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. Free Radic Biol Med 2013;61C:95-110.
42. Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med 2012;52:59-69.
43. Konstantinidis K, Whelan RS, Kitsis RN. Mechanisms of cell death in heart disease. Arterioscler Thromb Vasc Biol 2012;32:1552-1562.
44. Mattson MP. Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 2000;1:120-129.
45. Horio Y, Hayashi T, Kuno A, Kunimoto R. Cellular and molecular effects of sirtuins in health and disease. Clin Sci (Lond) 2011;121:191-203.
46. Luo J, Nikolaev AY, Imai S, et al. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 2001;107:137-148.
47. van Leeuwen I, Lain S. Sirtuins and p53. Adv Cancer Res 2009;102:171-195.
48. Lin Z, Fang D. The roles of SIRT1 in cancer. Genes Cancer 2013;4:97-104.
49. Nakata R, Takahashi S, Inoue H. Recent advances in the study on resveratrol. Biol Pharm Bull 2012;35:273-279.
50. Carrizzo A, Forte M, Damato A, et al. Antioxidant effects of resveratrol in cardiovascular, cerebral and metabolic diseases. Food Chem Toxicol 2013;61:215-226.
51. Ghanim H, Sia CL, Abuaysheh S, et al. An antiinflammatory and reactive oxygen species suppressive effects of an extract of Polygonum cuspidatum containing resveratrol. J Clin Endocrinol Metab 2010;95:E1-E8.
52. Ghanim H, Sia CL, Korzeniewski K, et al. A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. J Clin Endocrinol Metab 2011;96:1409-1414.
53. Timmers S, Konings E, Bilet L et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 2011;14:612-622.
54. Brasnyó P, Molnár GA, Mohás M, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. Br J Nutr 2011;106:383-389.
55. Bo S, Ciccone G, Castiglione A, et al. Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial. Curr Med Chem 2013;20:1323-1331.
56. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11:183-190.
57. Pikarsky E, Porat RM, Stein I, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461-466.
58. Day CP. From fat to inflammation. Gastroenterology 2006;130:207-210.
59. Yeung F, Hoberg JE, Ramsey CS, et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004;23:2369-2380.
60. Schug TT, Xu Q, Gao H, et al. Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress. Mol Cell Biol 2010;30:4712-4721.
61. Sparks LM, Xie H, Koza RA, et al. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 2005;54:1926-1933.
62. Scarpulla RC. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. Biochim Biophys Acta 2002;1576 1-14.
63. Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. J Cell Sci 2004;117:5965-5973.
64. Zhang R, Chen HZ, Liu JJ, et al. SIRT1 suppresses activator protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages. J Biol Chem 2010;285:7097-7110.
65. Xu F, Gao Z, Zhang J, et al. Lack of SIRT1 (Mammalian Sirtuin 1) activity leads to liver steatosis in the SIRT1+/- mice: A role of lipid mobilization and inflammation. Endocrinology 2010;151:2504-2514.
66. Colak Y, Senates E, Yesil A, et al. Assessment of endothelial function in patients with nonalcoholic fatty liver disease. Endocrine 2013;43:100-107.
67. Oni ET, Agatston AS, Blaha MJ, et al. A systematic review: Burden and severity of subclinical cardiovascular disease among those with

nonalcoholic fatty liver; Should we care? Atherosclerosis 2013;230:258-267.
68. Kocabay G, Karabay CY, Colak Y, et al. Left atrial deformation parameters in patients with non-alcoholic fatty liver disease: A 2D speckle tracking imaging study. Clin Sci (Lond) 2014;126:297-304.
69. Karabay CY, Kocabay G, Kalayci A, et al. Impaired left ventricular mechanics in nonalcoholic fatty liver disease: a speckle-tracking echocardiography study. Eur J Gastroenterol Hepatol 2013;26:325-331.
70. Colak Y, Karabay CY, Tuncer I, et al. Relation of epicardial adipose tissue and carotid intima-media thickness in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2012;24:613-618.
71. Chong ZZ, Wang S, Shang YC, Maiese K. Targeting cardiovascular disease with novel SIRT1 pathways. Future Cardiol 2012;8:89-100.
72. Stein S, Matter CM. Protective roles of SIRT1 in atherosclerosis. Cell Cycle 2011;10:640-664.
73. Mattagajasingh I, Kim CS, Naqvi A, et al. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 2007;104:14855-14860.
74. Zhang QJ, Wang Z, Chen HZ, et al. Endothelium-specific overexpression of class III deacetylase SIRT1 decreases atherosclerosis in apolipoprotein E-deficient mice. Cardiovasc Res 2008;80:191-199.
75. Miyazaki R, Ichiki T, Hashimoto T, et al. SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2008;28:1263-1269.
76. Stein S, Lohmann C, Schäfer N, et al. SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis. Eur Heart J 2010;31:2301-2309.
77. Potente M, Ghaeni L, Baldessari D, et al. SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev 2007;21:2644-2658.
78. Hsu CP, Zhai P, Yamamoto T, et al. Silent information regulator 1 protects the heart from ischemia/reperfusion. Circulation 2010;122:2170-2182.
79. Hung LM, Su MJ, Chen JK. Resveratrol protects myocardial ischemia-reperfusion injury through both NO-dependent and NO-independent mechanisms. Free Radic Biol Med 2004;36:774-781.
80. Liu K, Czaja MJ. Regulation of lipid stores and metabolism by lipophagy. Cell Death Differ 2013;20:3-11.
81. Lekli I, Ray D, Mukherjee S, et al. Co-ordinated autophagy with resveratrol and γ-tocotrienol confers synergetic cardioprotection. J Cell Mol Med 2010;14:2506-2518.
82. Gurusamy N, Lekli I, Mukherjee S, et al. Cardioprotection by resveratrol: A novel mechanism via autophagy involving the mTORC2 pathway. Cardiovasc Res 2010;86:103-112.
83. Usta E, Mustafi M, Walker T, Ziemer G. Resveratrol suppresses apoptosis in intact human cardiac tissue - in vitro model simulating extracorporeal circulation. J Cardiovasc Surg 2011;52:399-409.
84. Raynes R, Brunquell J, Westerheide SD. Stress inducibility of SIRT1 and its role in cytoprotection and cancer. Genes Cancer 2013;4:172-182.
85. Tang BL. Sirt1's systemic protective roles and its promise as a target in antiaging medicine. Transl Res 2011;157:276-284.
86. Ota H, Eto M, Ako J, et al. Sirolimus and everolimus induce endothelial cellular senescence via sirtuin 1 down-regulation: Therapeutic implication of cilostazol after drug-eluting stent implantation. J Am Coll Cardiol 2009;53:2298-2305.
87. Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 2007;43:571-579.
88. Ota H, Eto M, Kano MR, et al. Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. Arterioscler Thromb Vasc Biol 2008;28:1634-1639.
89. Zu Y, Liu L, Lee MY, et al. SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. Circ Res 2010;106:1384-1393.
90. Hou X, Xu S, Maitland-Toolan KA, et al. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem 2008;283:20015-20026.
91. Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 1999;277:E1–E10.
92. Ouchi N, Shibata R, Walsh K. AMP-activated protein kinase signaling stimulates VEGF expression and angiogenesis in skeletal muscle. Circ Res 2005;96:838–846.
93. Orimo M, Minamino T, Miyauchi H, et al. Protective role of SIRT1 in diabetic vascular dysfunction. Arterioscler Thromb Vasc Biol 2009;29:889-894.
94. Camins A, Sureda FX, Junyent F, et al. Sirtuin activators: Designing molecules to extend life span. Biochim Biophys Acta 2010;1799:740-749.
95. Welzel TM, Graubard BI, Quraishi S, et al. Population-attributable fractions of risk factors for hepatocellular carcinoma in the United States. Am J Gastroenterol 2013;108:1314-1321.
96. Yilmaz Y, Colak Y, Kurt R, Senates E, Eren F. Linking nonalcoholic fatty liver disease to hepatocellular carcinoma: from bedside to bench and back. Tumori 2013;99:10-16.
97. Herranz D, Muñoz-Martin M, Cañamero M, et al. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nat Commun 2010;1:3.
98. Tian Y, Wong VW, Chan HL, Cheng AS. Epigenetic regulation of hepatocellular carcinoma in non-alcoholic fatty liver disease. Semin Cancer Biol 2013;23:471-482.
99. Wang RH, Sengupta K, Li C, et al. Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell 2008;14:312-323.
100. Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased prevalence of suspected nonalcoholic fatty liver disease. Hepatology 2008;47:1947-1954.
101. Venkatasubramanian S, Noh RM, Daga S, et al. Cardiovascular effects of a novel SIRT1 activator, SRT2104, in otherwise healthy cigarette smokers. J Am Heart Assoc 2013;2:e000042.
102. Hoffmann E, Wald J, Lavu S, et al. Pharmacokinetics and tolerability of SRT2104, a first-in-class small molecule activator of SIRT1, after single and repeated oral administration in man. Br J Clin Pharmacol 2013;75:186-196.
103. Amiot MJ, Romier B, Dao TM, et al. Optimization of trans-Resveratrol bioavailability for human therapy. Biochimie 2013;95:1233-1238.
104. Libri V, Brown AP, Gambarota G, et al. A pilot randomized, placebo controlled, double blind phase I trial of the novel SIRT1 activator SRT2104 in elderly volunteers. PLoS One 2012;7:e51395.
105. Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 2011;14:612-622.
106. Ghanim H, Sia CL, Abuaysheh S, et al. An antiinflammatory and reactive oxygen species suppressive effects of an extract of Polygonum cuspidatum containing resveratrol. J Clin Endocrinol Metab 2010;95:E1-E8.

107. Poulsen MM, Vestergaard PF, Clasen BF, et al. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 2013;62:1186-1195.
108. Yoshino J, Conte C, Fontana L, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 2012;16:658-664.
109. Chachay VS, Macdonald GA, Martin JH, et al. Resveratrol Does Not Benefit Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol 2014 Feb 25.
110. Santos JA, de Carvaho GS, Oliveira V, Raposo NR, da Silva AD. Resveratrol and analogues: a review of antioxidant activity and applications to human health. Recent Pat Food Nutr Agric 2013;5:144-153.