Resveratrol and novel potent activators of SIRT1: effects on aging and age-related diseases

Mitchell D Knutson and Christiaan Leeuwenburgh

Studies show that the plant polyphenol resveratrol can extend the life span of yeast, worms, flies, and fish. It also mitigates the metabolic dysfunction of mice fed high-fat diets. Resveratrol appears to mediate these effects partly by activating SIRT1, a deacetylase enzyme that regulates the activity of several transcriptional factors and enzymes responsive to nutrient availability. However, few foods contain resveratrol and humans metabolize it extensively, resulting in very low systemic bioavailability. Substantial research effort now focuses on identifying and testing more bioavailable and potent activators of SIRT1 for use as pharmacologic interventions in aging and age-related disorders.

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenol that belongs to the stilbene family of phytoalexins, which are antibiotic compounds produced by plants in response to infection. Resveratrol has been detected in at least 72 plant species[1] but is present in only a limited number of common foods. Grapes, grape juice, red wine, and peanuts represent the richest dietary sources of resveratrol.[2] Cranberries, blueberries,[3] and tomato skin[4] also contain this polyphenol, though at levels <10% of those reported for grapes. Dietary resveratrol exists as the free *trans*- or *cis*-isomer or conjugated with glucose (known as resveratrol glucoside or piceid). Although *trans*-resveratrol is absorbed efficiently by humans, the gut and liver metabolize it extensively, resulting in exceedingly low systemic bioavailability.[5]

Resveratrol has generated intense scientific and public interest in recent years, mainly because of its widely reported ability to delay aging and prevent age-related diseases. As a result, a multitude of different resveratrol supplements have now appeared on the market. A cross- sectional study found that supplemental resveratrol is taken by two-thirds of individuals who routinely consume multiple dietary supplements.[6] The salutary effects of resveratrol were originally thought to derive from its antioxidant properties.[7] Indeed, the high concentration of resveratrol in red wine is frequently cited to account for the "French paradox," the observation that the French have relatively low rates of cardiovascular disease despite consuming diets rich in saturated fat.[8] Recent research, however, is converging on a different molecular mechanism that underlies the pleiotropic effects of this compound. Studies in a variety of species indicate that resveratrol seems to exert benefit by activating SIRT1, a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases. Much research effort is now directed at identifying more potent, and more bioavailable, activators of SIRT1 in an effort to combat aging and associated diseases.

SIRT1, AGING, AND THE ROAD TO RESVERATROL

Sirtuin 1 (SIRT1) is an enzyme that removes acetyl groups from specific proteins (Figure 1). Acetylation of lysine residues is a frequent post-translational modification affecting protein activity and stability. Deacetylation of nuclear proteins, such as histones, plays a major role in regulating gene expression. SIRT1 was first identified as the human orthologue of yeast Sir2p (silent information

Affiliations: *MD Knutson* is Assistant Professor with the Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida, USA. *C Leeuwenburgh* is Professor and Chief, Biology of Aging, with the Department of Aging and Geriatrics, College of Medicine, University of Florida, Gainesville, Florida, USA.

Correspondence: *MD Knutson*, Food Science and Human Nutrition Department, PO Box 110370, University of Florida, Gainesville, FL, USA. E-mail: mdknutson@ifas.ufl.edu, Phone: +1-352-392-1991 ext. 204; Fax: +1-352-392-9467.

Key words: life span, polyphenol, red wine, sirtuin

doi:10.1111/j.1753-4887.2008.00109.x

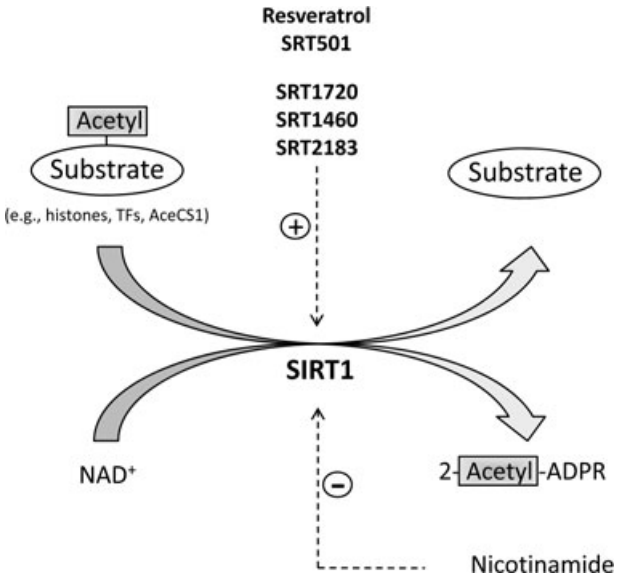

Figure 1 **Activation of SIRT1 activity by resveratrol and novel small molecules.** SIRT1 removes acetyl groups from substrates including histones H4K16 and H1K26, a number of transcription factors (TFs), and cytosolic acetyl CoA synthetase 1 (AceCS1). The reaction requires nicotine adenine dinucleotide (NAD^+) and yields deacetylated substrate, 2-acetyl-ADP-ribose (ADPR), and nicotinamide. Deacetylase activity of SIRT1 can be activated by resveratrol and SRT501, a more bioavailable reformulation of resveratrol. SRT1720, 1460, and 2183 are small-molecule activators of SIRT1 that are structurally unrelated to resveratrol, but are up to 1000 times more potent. Nicotinamide is an end-product inhibitor of SIRT1.

regulator 2 protein), an NAD^+-dependent histone deacetylase involved in chromatin silencing. Structural similarities to yeast Sir2p led to the initial characterization of human SIRT1 as a histone deacetylase, but a number of nonhistone targets of SIRT1 have been identified as well. Nonhistone SIRT1 substrates include cytosolic acetyl-CoA synthetase,[9] involved in fatty acid synthesis, and a variety of transcription factors that mediate cellular responses to fasting,[10] insulin,[11] and inflammation.[12]

Studies in the yeast *Saccharomyces cerevisiae* established a link between *SIR2* (the gene encoding Sir2p) and aging by demonstrating that *SIR2* mutants had shorter life spans and that increasing *SIR2* dosage extended life span.[13] In a follow-up study, the decreased longevity of the *SIR2* mutants could not be increased by caloric restriction,[14] the only dietary intervention that consistently extends life and health span in all organisms studied to date. This observation directly implicated Sir2p – and by extension SIRT1 – in mediating the positive biological effects of calorie restriction. Efforts were next directed at identifying compounds that activated SIRT1. By screening a number of small molecule libraries, Howitz et al.[15] identified a structurally related group of polyphenolic compounds effective at stimulating recombinant SIRT1 activity. The group included the plant polyphenols resveratrol, butein, piceatannol, and quercetin. Of these compounds, resveratrol proved most potent, increasing the catalytic rate of SIRT1 in vitro by 13-fold. Resveratrol also increased the activity of recombinant yeast Sir2p. Importantly, treatment of *S. cerevisiae* with resveratrol increased average life span by 70% and significantly increased maximum life span. Since these studies in yeast, resveratrol has proven effective at increasing the life span of nematode worms (*Caenorhabditis elegans*), flies (*Drosophila melanogaster*),[16] and a short-lived species of fish (*Nothobranchius furzeri*).[17]

EFFECTS OF RESVERATROL AND SIRT1 ACTIVATION IN ANIMAL STUDIES

The first indication that resveratrol could extend life span in mammals, as it did in lower organisms, was provided by Bauer et al.,[18] who studied middle-aged (1-year-old) mice fed high-calorie diets (60% of calories from fat). High-fat diets are well known to induce obesity, triggering inflammatory states and comorbidities, such as diabetes and atherosclerosis, which decrease life span. To determine if resveratrol exhibited positive effects on life span under this dietary condition, mice were fed either a standard diet or a high-fat diet with or without resveratrol. The resveratrol diet provided an average of 22.4 mg resveratrol/kg/day, which is a feasible daily dose for humans. Although both groups of mice consuming the high-fat diet became obese, the ones receiving resveratrol lived longer, having survival curves similar to control animals. Moreover, the resveratrol-treated mice displayed enhanced insulin sensitivity and increased hepatic mitochondrial numbers similar to calorie-restricted animals with greater SIRT1 expression.[19]

An important remaining question is whether resveratrol can extend life span in animals fed a standard diet. One recent study[20] found that mice consuming a standard diet did not live longer when supplemented with resveratrol starting at 1 year of age, although it did increase the average life span of mice fed a high-fat diet, as observed previously.[18]

Evidence is now rapidly emerging showing positive effects of resveratrol and SIRT1 activation on several age-related disorders including type 2 diabetes, cardiovascular disease, neurodegeneration, and inflammation. Table 1 provides a summary of selected studies published within the last 2 years.[18,20–29] SIRT1 activation most likely mediates the therapeutic effect of resveratrol, at least in diet-induced obesity, because transgenic overexpression of Sirt1 in mice recapitulates many of the positive metabolic effects of dietary resveratrol.[29] As with resveratrol, future

Table 1 **Effects of SIRT1 modulation on age-related disorders.**

Disorder	Animal model	SIRT1 modulation	Effects
Type 2 diabetes	DIO mouse Baur et al. (2006)[18]	Dietary resveratrol	Increased insulin sensitivity, decreased IGF-1
	DIO mouse Lagouge et al. (2006)[21]	Dietary resveratrol	Increased insulin sensitivity, increased aerobic capacity
	DIO, *ob/ob* mouse, *fa/fa* rat Milne et al. (2007)[22]	Gavage resveratrol, SRT1720, SRT501	Increased insulin sensitivity, decreased blood glucose
Cardiovascular disease	Transgenic mouse Alcendor et al. (2007)[23]	Heart-specific Sirt1 overexpression	Decreased cardiac hypertrophy, apoptosis and dysfunction
	Conditional knockout mouse Potente et al. (2007)[24]	Sirt1 deletion in vascular endothelial cells	Blunting of ischemia-induced neovascularization
	apo $E^{-/-}$ mouse Do et al. (2008)[25]	Dietary resveratrol	Increased plasma HDL-C, decreased plasma LDL-C
Neurodegeneration	p25 transgenic mouse Kim et al. (2007)[26]	ICV resveratrol injection, Sirt1 lentivirus	Protection against neurodegeneration
	EAE mouse Shindler et al. (2007)[27]	Intravitreal injection of SRT647, SRT501	Reduced neuronal damage in optic neuritis
	MPTP-treated mouse Lu et al. (2008)[28]	Intravenous injection of resveratrol	Attenuated neuronal damage in substantia nigra
Inflammation	Transgenic mouse Pfluger et al. (2006)[29]	Whole-body Sirt1 overexpression	Reduced hepatic inflammatory cytokines
	Mouse Pearson et al. (2008)[20]	Dietary resveratrol	Decreased expression of inflammatory cytokines

Abbreviations: *apo* $E^{-/-}$, apolipoprotein E-deficient; DIO, diet-induced obesity; EAE, experimental autoimmune encephalitis model of multiple sclerosis; *fa/fa*, leptin receptor-deficient obese; IGF-1, insulin-like growth factor 1; *ob/ob*, leptin-deficient obese; SRT1720, SRT501, SRT647, small-molecule SIRT1 activators; MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), neurotoxin causing permanent symptoms of Parkinson's disease; p25 (activator of the protein kinase cdk 5) transgenic, mouse model of Alzheimer's disease.

studies need to determine if SIRT1 overexpression increases life span in mammals. Overexpression of heart-specific SIRT1, though cardioprotective, did not extend the life span of mice fed a standard diet.[23]

NOVEL POTENT ACTIVATORS OF SIRT1

A recent study by Milne et al.[22] used a high-throughput screening method to identify novel potent activators of human SIRT1. The chemical screen was performed by Sirtris Pharmaceuticals, a biotechnology company focused on discovering and developing drugs to treat diseases of aging. In total, they screened 290,000 small molecules and identified 127 candidate compounds. Three of these small molecule activators – named SRT1460, SRT1720, and SRT2183 – were synthesized for further study. The compounds are structurally related to each other, but notably distinct from resveratrol. Potency of each compound was initially assessed in vitro by determining the maximum activation as well as the concentration at which the compounds increased SIRT1 enzyme activity by 50% ($EC_{1.5}$). All compounds were compared to resveratrol, which displayed an $EC_{1.5}$ of 46 μM and maximum activation of 201%. SRT1460, SRT2183, and SRT1720 were effective at much lower concentrations than resveratrol, exhibiting $EC_{1.5}$ values of 2.9, 0.36, and 0.16 μM, respectively. SRT1720 maximally activated SIRT1 activity by 780%, nearly four times more than resveratrol, whereas SRT1460 and SRT2183 achieved a maximum activation of 447% and 296%. The ability of these compounds to activate endogenous SIRT1 function was investigated by using an osteosarcoma cell line (U2OS cells) and an in-cell Western assay to measure the extent of deacetylation of p53, a known substrate of SIRT1.[30] All compounds induced cellular SIRT1 activity, which could be abrogated by a selective SIRT1 inhibitor. Importantly, the novel SIRT1 activators were effective at concentrations 10- to 1000-fold lower than resveratrol. The most potent activator, SRT1720, was then tested in mice and rats to determine its pharmacokinetic profile. After administration of the compound via oral gavage (100 mg/kg body weight), serum concentrations of SRT1720 reached nearly 1 μM within 1 hour, with a terminal plasma half-life of about 5–8 hours, an amount of time usually adequate to deliver sufficient amounts of a drug to target tissues. Its oral bioavailability was calculated to be 25% in rats and 50% in mice.

In light of previous studies showing beneficial effects of resveratrol on insulin resistance in diet-induced obesity (DIO) mice,[18,31] Milne et al.[22] tested the therapeutic potential of the more potent and bioavailable SRT1720 to treat this metabolic disorder. In DIO mice, daily oral gavage of SRT1720 (100 mg per kg body weight) decreased blood

glucose levels within 2 weeks of dosing, with the effect persisting over 10 weeks of dosing. After 10 weeks of treatment, plasma insulin levels decreased by 50% compared to vehicle-treated controls. Glucose and insulin tolerance tests revealed positive effects of SRT1720, similar to those achieved when DIO mice were treated with rosiglitazone, a drug prescribed widely to lower blood glucose levels in patients with type 2 diabetes. SRT1720 equally improved glucose homeostasis in genetically obese, insulin-resistant *Lep*$^{ob/ob}$ mice, decreasing plasma glucose levels to near normal levels after 1 week of daily oral dosing. Therapeutic efficacy of SRT1720 was additionally examined by using genetically obese Zucker *fa/fa* rats, one of the most commonly used models of insulin resistance. Similar to the obese mice, treatment of *fa/fa* rats with SRT1720 (100 mg/kg) favorably affected blood glucose and insulin levels. Additional studies using the gold-standard hyperinsulinemic-euglycemic clamp technique confirmed that SRT1720 improved insulin sensitivity in obese *fa/fa* rats. Milne et al.[22] also showed limited data regarding the effectiveness of SRT501, a reformulated version of resveratrol with improved bioavailability (11%) but a shorter half-life than SRT1720 (1 hour versus 5 hours). Oral dosing with SRT501 (500–1000 mg/kg) for 2–4 weeks decreased plasma fasting glucose levels to near normal levels in DIO and *Lep*$^{ob/ob}$ mice.

SIRT1 ACTIVATION: MECHANISMS OF ACTION

Kinetic studies using recombinant SIRT1 suggest that polyphenolics such as resveratrol increase SIRT1 deacetylation activity by decreasing the Michaelis constant (K_m) of SIRT1 for acetylated substrate.[15] Studies by Milne et al.[22] reveal a similar mechanism for the novel SIRT1 activators SRT1460, SRT2183, and SRT1720. By testing each compound together with resveratrol, they determined that these structurally diverse compounds appear to bind to a single allosteric site on the SIRT1-substrate complex. Analyses of serial SIRT1 truncations mapped the putative allosteric binding region to amino acids 183-225 of the enzyme. It has been proposed that binding of compounds to SIRT1 in vivo not only activates the enzyme, it may also alter its specificity for distinct acetylated substrates.[32] Another way to alter SIRT1 activity is through changing the cellular concentration or activity of nicotinamide, an end-product inhibitor of SIRT1 (Figure 1). A reduction in nicotinamide activity by competitive inhibition with isonicotinamide has been shown to activate Sir2, the yeast orthologue of SIRT1.[33] Accordingly, in mice, supplemental nicotinamide (500 mg/kg diet) elicited some effects opposite of SIRT1 activation: it increased body weight, energy intake, fat mass, and fasting blood glucose levels.[31]

POTENTIAL TOXICITY OF SIRT1 ACTIVATORS

Limited data exist regarding the toxicity and safety of SIRT1 activators. In rats, oral administration of *trans*-resveratrol at 20 mg/kg for 28 days produced no harmful effects as assessed by growth, hematology, clinical chemistry, and histopathology.[34] This resveratrol dose is estimated to be equivalent to 1000 times the amount that would be consumed by a person drinking one glass of red wine daily. When substantially higher amounts of *trans*-resveratrol (300, 1000, and 3000 mg daily for 28 days) were tested in rats, the two highest doses caused kidney damage.[35] In mice, chronic consumption of a modest dose of resveratrol (2400 mg/kg diet), starting at 1 year of age, produced no obvious detrimental effects, as assessed by postmortem histopathological assessments.[20] However, a pilot study revealed that when mice consumed larger doses of resveratrol (18,000 mg/kg diet), five of six animals died within 3–4 months.[20] In humans, ingestion of a single dose of resveratrol (0.5, 1, 2.5, or 5 g; 10 subjects per group) was well tolerated without any severe adverse clinical, biochemical, or hematological events.[36] Studies of chronic resveratrol administration with more subjects are needed to adequately assess the safety profile of resveratrol in humans. Moreover, the optimal degree of SIRT1 activation, especially with the more potent SIRT1 activators, will need to be defined, as illustrated by a recent study of transgenic mice overexpressing heart-specific Sirt1.[23] Moderate Sirt1 overexpression (7.5-fold) made the heart more resistant to in vivo oxidative stress and apoptosis, whereas higher overexpression (12.5-fold) increased apoptosis and hypertrophy and decreased cardiac function.

CONCLUSION

In the next two decades, the projected number of individuals in the United States aged 65 years or older will double from 35 million to 71 million, increasing from 12% to roughly 20% of the population. [37] This rapid demographic shift will markedly increase the prevalence of age-related disorders including diabetes, cardiovascular disease, neurodegenerative diseases, and inflammation. Not surprisingly, the search for interventions that can alleviate age-related health problems is rapidly accelerating. Although caloric restriction remains the optimal intervention, it is not acceptable to many and is not a viable option for the old frail elderly, those with disease, or the morbidly obese. Caloric restriction mimetics, such as resveratrol and SIRT1-activating compounds, show much therapeutic promise in animal studies but will need extensive evaluation in clinical trials before they can be recommended for human use. Until then, let's continue to enjoy our wine.

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