REVIEW ARTICLE

Resveratrol: A Sirtuin Activator and The Fountain of Youth

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Abstract

BACKGROUND: An organism's lifespan is inevitably accompanied by the aging process, which involves functional decline, a steady increase of a plethora of chronic diseases, and ultimately death. Thus, it has been an ongoing dream of mankind to improve healthspan and extend life.

CONTENT: There are only a few proposed aging interventions: caloric restriction, exercise, and the use of low-molecular-weight compounds, including spermidine, metformin, resveratrol, and rapamycin. Resveratrol, a constituent of red wine, has long been suspected to have cardioprotective effects. Interest in this compound has been renewed in recent years, first from its identification as a chemopreventive agent for skin cancer, and subsequently from reports that it activates sirtuin deacetylases and extends the lifespans of lower organisms. Resveratrol have been shown to prevent and reduce the severity of age-related diseases such as atherosclerosis, stroke, myocardial infarct, diabetes, neurodegenerative diseases, osteoarthritis, tumors and metabolic syndrome, along with their ability to extend lifespan.

SUMMARY: The purpose of aging research is the identification of interventions that may avoid or ameliorate the ravages of time. In other words, the quest is for healthy aging, where improved longevity is coupled to a corresponding healthspan extension. It is only by extending the healthy human lifespan that we will truly meet the premise of the Roman poet Cicero: "No one is so old as to think that he may not live a year."

KEYWORDS: aging, caloric restriction, mimetic, healthspan, sirtuin activator

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Introduction

For thousands of years humanity has searched for the fountain of youth. In the 16th century the Spanish explorer Juan Ponce de León, first Governor of Puerto Rico, searched for the fountain of youth in the legendary land of Florida, specifically in the waters of Bimini, but without success. This quest continues, although all living species on our planet are designed to age. A fountain-of-youth strategy has been proposed by several investigators during recent decades. This strategy to slow down the aging processes is based on caloric restriction (CR) and increased physical activity.(1)

The reduction in the intake of calories without malnutrition is defined as CR. Such reduction ideally corresponds to a decrease of approximately 30% of calories per day, at least in mice. In humans, there is some indication that a CR of around 15% may be most favorable against mortality during aging.(2) One of the best correlations between CR and improvement in healthspan and prolonged life in humans is the long-lived population in Okinawa, Japan.(3) In comparison to the rest of the Japanese population, Okinawan people usually combine an above-average amount of daily exercise with a below-average food intake.(2) However, when Okinawan families moved to Brazil, they adopted a Western lifestyle that impacted both their diet and physical activity, resulting in increased weight gain and a drop in life expectancy of 17 years.(3) CR changes many parameters in the aging human body, including the transcriptome, the hormonal status (in particular the serum concentration of Insulin-like Growth Factor (IGF)-

1 and thyroid hormones), oxidative stress, inflammation, mitochondrial function, and glucose homeostasis.(4,5)

Recent discoveries, however, have focused attention towards interesting molecules able to increase lifespan and prevent age-related diseases. These molecules are called sirtuin (SIRT). The mammalian SIRT 1–7 belong to a family of histone deacetylases, named for their homology to the *Saccharomyces cerevisiae* gene silent information regulator (Sir)2. SIRT require nicotinamide adenine dinucleotide (NAD^+) as a cofactor to deacetylate substrates ranging from histones to transcriptional regulators.(6) An increasing number of studies clearly reported that activation of SIRT, especially SIRT1 and SIRT3, increases longevity by mimicking the beneficial effects of CR. Moreover, recent findings have suggested that moderate prolonged exercise training is able to increase SIRT1 activity in aged animals, counteracting age-related dysfunctions.(7) Does taking SIRT's activators produce the same effect as drinking water from the legendary fountain of youth? This is the main question that several scientists worldwide are trying to answer.(1)

Resveratrol is a polyphenol that is found in grapes and in red wine. Its potential to promote lifespan was first identified in yeast (8), and it has since gained fame because it was suggested to be responsible for the so-called French paradox (French winemakers do not suffer from cardiovascular diseases though enjoying a high-fat diet) (5). Overall, it is clear that resveratrol treatment or SIRT1 overexpression prevents several age-associated diseases and pathogenic conditions, including oxidative stress in the aging heart, neurodegeneration, or diabetes.(9) Importantly, activation of autophagy by resveratrol is required for lifespan extension in *Caenorhabditis elegans* (*C. elegans*). (5,10)

Aging and Age-related Diseases

The more complex a problem, the more important it may be to ask simple questions. So, why do we age? Actually, we do hold the capacity for immortality. The molecular clock in our germline stem cells, which sustain gamete production, is kept at zero as evidenced by the fact that our offspring are not born with the father's or mother's age. Then why do somatic cells age? First, the maintenance of repair activity in all our cells represents a vast energetic demand and, second, the pressure of recombination and dying of generations allows organisms to adapt to changing environments (*e.g.*, ice ages). This implies that aging may be an atavistic, adaptive and altruistic program, by which single cells or organisms eventually die for the benefit of the whole population in a highly coordinated (programmatic) fashion.(5)

A hallmark of aging is compromised tissue maintenance.(11) Tissue-specific stem cells self-renew and persist throughout an organism's lifespan to repair and maintain tissues. The self-renewal potential and differentiation capacity of stem cells become dysregulated with age.(12,13) Stem cell aging is thought to be due to cumulative cellular and genomic damages, resulting in permanent cell-cycle arrest, apoptosis, or senescence.(12-14) A major source of cellular damage is reactive oxygen species (ROS), a natural by-product of cellular respiration. (15) ROS levels in stem cells increase dramatically with age.(16) Deficient intracellular management of ROS results in increased stem cell cycling and apoptosis, as well as compromised self-renewal and differentiation, resembling essential aspects of aged stem cells.(17-21)

The study by Brown, *et al*. provides important insights into mitochondrial metabolism in stem cell maintenance and illuminates the previously underappreciated plasticity of mitochondrial homeostasis in stem cell maintenance and tissue homeostasis during the aging process. Using oxidative stress as a readout for various mitochondrial processes regulated by SIRT3, they show that SIRT3-mediated mitochondrial homeostasis is essential for HSC maintenance under stress and that this regulatory program is downregulated with age. Together, these data suggest that suppression of SIRT3-mediated mitochondrial homeostasis contributes to increased oxidative stress in aged hematopoietic stem cell (HSC). This regulatory process complements the view that passive accumulation of damaged mitochondria with age results in increased ROS and underlies the plasticity of mitochondrial homeostasis in stem cell maintenance and tissue homeostasis.(22)

According to the World Health Organization (WHO), age itself remains the greatest risk factor for all major life-threatening disorders, and the number of people suffering from age-related diseases is anticipated to almost double over the next two decades. The fact that healthspan has not increased at the same pace as lifespan is a source of grave concern.(23) Both obesity and hypertension represent major risk factors for stroke and cardiovascular disease. Although weight loss and increase in physical activity are generally prescribed to avoid such age-associated diseases, only a small percentage of people have the discipline to change their lifestyle accordingly.(24) The prevalence of age-related pathologies represents major psychological and social impediments as well as an economic burden that urgently needs appropriate interventions.(5)

Over three quarters of deaths from cardiovascular

diseases occur among patients over 65 years of age. (25) Epidemiological studies show that even in the absence of risk factors related to lifestyle (*e.g.*, obesity, hypercholesterolemia, smoking), advanced age, per se, promotes the development of cardiovascular disease.(26) In order to develop novel therapeutic interventions to promote vascular health in older persons, it is essential to understand the mechanisms through which aging impairs homeostatic mechanisms in the vasculature.(27) Vascular oxidative stress and inflammation are thought to promote the development of atherosclerotic vascular diseases (including myocardial infarction, stroke, and vascular dementias), increasing cardiovascular mortality in elderly patients.(26)

Viewing the reality of aging from the arterial wall begins with the realization that arterial diseases, *e.g.* atherosclerosis and hypertension, are rampant in modern society, and increase exponentially with advancing age. Progressive changes occur throughout life in the structure and function of central arteries in numerous species. These changes include diffuse intimal and medial thickening, and enhanced stiffening.(28) Since the likelihood for predominantly systolic hypertension and atherosclerosis to occur increases in epidemic proportion among older persons.(28,29) it is reasonable to hypothesize that specific mechanisms that underlie alterations in the arterial substrate that accompany "aging" may be intimately linked to the age-associated exponential increase in predominantly systolic hypertension.(30)

Central arterial aging is a hallmark of systems aging, and can be viewed as the failure of key signaling pathways to execute crucial functions.(30) These aging – related changes in the molecular and cellular functions of key signaling systems facilitate adverse central arterial remodeling, such as diffuse intima – media thickening, enhanced stiffening, and endothelial dysfunction.(28,31-33) Arterial wall aging begins with chronic proinflammation, a form of 'sterile-like' inflammation that occurs in the absence of any microorganisms and with little or no white blood cell infiltration. Phenotypic shifts in arterial endothelial cell and vascular smooth muscle cell (VSMC) promote pathogenic inflammation.(28,31-33) This is why arterial aging dwarfs other risk factors for clinical manifestations and severity of hypertension and atherosclerosis.(34)

CR, Exercise and Mimetics

Nutrition is among the most important means for mitigating age-associated chronic diseases. By some estimates, 80% of coronary heart disease (CHD) and type-2 diabetes mellitus and 40% of cancers may be prevented by modifying dietary habits, engaging in regular physical activity, and avoiding tobacco use.(35-37) The Okinawans are of special interest to this topic as they, by most measures, have the world's longest-lived population, and nutritional factors appear to have played a key role.(38-40) The chronic disease profile of the older Okinawan population is especially impressive, with 80% less CHD mortality and 40% less cancer mortality than the US population.(38) Some attribute the healthy aging phenomenon in Okinawa principally to nutritional factors, and CR is thought to be one key factor.(38,39,41,42)

CR is the only regimen known to extend the life span and health span in a spectrum of organisms that include yeast, mice, and nonhuman primates.(43-45) Reducing food consumption 25-60% without undernutrition extends the life span of rodents up to 50% (45) and in different animal models delays the onset of age-related maladies, like cardiovascular disease, cancer, and diabetes (43,46). The positive effects of CR are linked to major metabolic reprogramming toward efficient fuel utilization and a reduction in oxidative damage to macromolecules.(47,48) Long-term CR in humans inhibits the IGF-1/insulin pathway in skeletal muscle, a key metabolic tissue. CR also induces dramatic changes of the skeletal muscle transcriptional profile that resemble those of younger individuals. Finally, in both rats and humans, CR evoked similar responses in the transcriptional profiles of skeletal muscle. This common signature consisted of three key pathways typically associated with longevity: IGF-1/insulin signaling, mitochondrial biogenesis, and inflammation.(49)

Mitochondrial dysfunction plays an important role in cellular aging (50) and reduces fuel utilization plasticity. As metabolic centers for fuel utilization and primary producers of cellular ROS, mitochondria are poised to act as mediators of reprogramming under CR. However, the molecular basis for global metabolic adaptation induced by CR remains unknown.(51)

Fasting has been practiced for millennia, but, only recently, studies have shed light on its role in adaptive cellular responses that reduce oxidative damage and inflammation, optimize energy metabolism, and bolster cellular protection. In lower eukaryotes, chronic fasting extends longevity, in part, by reprogramming metabolic and stress resistance pathways. In rodents intermittent or periodic fasting protects against diabetes, cancers, heart disease, and neurodegeneration, while in humans it helps reduce obesity, hypertension, asthma, and rheumatoid arthritis. Thus, fasting has the potential to delay aging and help prevent and treat diseases while minimizing the side effects caused by chronic dietary interventions.(52)

The traditional Okinawan diet is a rich source for potential CR mimetics. In the Okinawan language, the term "nuchi gusui", a term in common use, literally means 'food is medicine' as commonly consumed dietary items, including foods, herbs, and spices are also used as folk medicines.(53) Popular items that play dual roles as foods and traditional medicines are sweet potatoes (pulp, skin, and leaves), bitter melon, turmeric, ginger, mugwort (*Artemisia vulgaris*), peppers (*Piper hancei*), and carotenoid-rich marine foods, among others.(2) Compounds that have potential CR mimetic properties, such as carotenoids, flavonoids, and other phytochemicals, are synthesized (mostly by plants) to help scavenge-free radicals formed due to stress from extremes of heat, cold, or ultraviolet light. As the sun in Okinawa is particularly strong many locally grown plants contain high quantities of these phytochemicals.(54)

While CR increases lifespan, studies support more of a beneficial role for exercise on healthspan.(23) Although CR and exercise have similar effects, clearly disparities exist between these two interventions, with potential molecular mechanisms excellently reviewed by Huffman.(55) Despite not altering lifespan, regularly performed moderate exercise will delay certain age-associated changes and protect against several metabolic disorders.(56) Previous research has shown that exercise is associated with greater benefits than CR, or vice versa. The most noticeable health benefits of exercise over CR are maintenance of aerobic capacity, muscle mass and muscle strength, and an improved bone health.(57,58) As a consequence, regularly performed exercise has a stronger impact particularly in cardiovascular disease, diabetes and osteoporosis. Therefore, as pointed out by Huffman, *et al*., the effect of exercise may be more pronounced in humans, who perish more from cardiovascular disease, than in animal models that primarily die of renal disease or cancers.(59) Additionally, as aging is associated with a decline in physical activity, regular physical activity plays an essential role in the elderly by lessening disability and prolonging independent living.(60) Taken together, these findings provide compelling evidence that regularly performed exercise is associated with an improved quality of life, without slowing the aging process.(23)

SIRT in Aging and Regeneration Medicine

Sir2 family proteins, now called SIRT, have been demonstrated to coordinate metabolic responses to changes in nutritional availability and maintain physiological homeostasis in mammals.(61) These functions of SIRT are ascribed to their unique NAD-dependent enzymatic activities (62), placing SIRT at the perfect position to integrate energy metabolism information into many other biological regulations. In particular, the mammalian Sir2 ortholog SIRT1 plays a critical role for the regulation of metabolic responses in multiple tissues, including the liver, skeletal muscle, adipose tissue, and brain, through its deacetylase activity.(61) SIRT1 also plays a role in the regulation of phenotypes induced by CR, a diet regimen

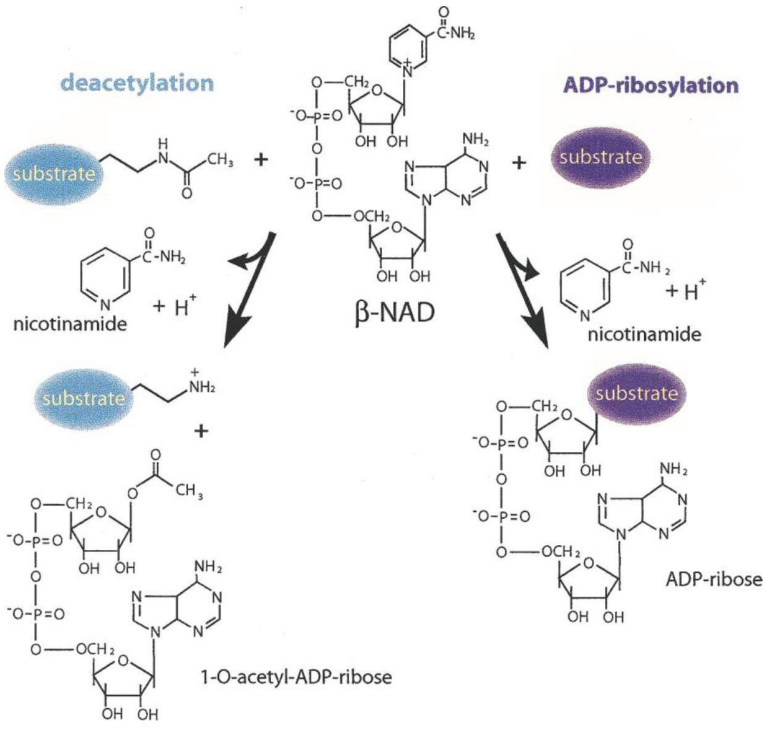

Figure 1. SIRT deacetylation and ADP-ribosylation reactions. Both deacetylation and ADP-ribosylation occur via cleavage of NAD to release nicotinamide. (9) (Adapted with permission from Cold Spring Harbor Laboratory Press).

that delays aging and extends life span in a wide variety of organisms.(61)

SIRT1 is the closest to yeast Sir2 in terms of sequence and enzymatic activity, and is also the most extensively studied mammalian SIRT having numerous known substrates, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), p53 and forkhead box O (FOXO)1.(63-66) SIRT1 is an important regulator of metabolism. SIRT1 upregulates mitochondrial biogenesis in several tissues, stimulates fat and cholesterol catabolism in liver, skeletal muscle and adipose tissue.(67) In addition, liver glucose metabolism is regulated by SIRT1 inducing the gluconeogenic genes, phosphoenolpyruvate kinase and glucose-6-phosphatase by forming a protein complex with PGC-1α and hepatocyte nuclear factor (HNF)4α. Moreover, SIRT1 modulates the effects of PGC-1α repression of glycolytic genes, glucokinase and pyruvate kinase, acting the adenosine monophosphate (AMP)-activated protein kinase (AMPK) as the prime sensor that translates this information into SIRT1-dependent deacetylation (68-70), and also seems to activate fatty acid oxidation systemically by promoting adiponectin synthesis (71). SIRT1 deficiency in mice results in hyperglycemia, oxidative damage and insulin resistance and deficient animals become obese and insulin resistant when chronically challenged with a 40% fat diet, developing hepatomegaly.(71-73)

Over the past decade, a number of evolutionarily conserved regulators and signaling pathways have been identified for the control of aging and longevity. These regulators and signaling pathways, including insulin/IGF-1 signaling (IIS) (74), mammalian target of rapamycin (mTOR) signaling (75), and NAD-dependent SIRT (61), provide excellent probes to dissect complex hierarchical mechanisms that affect the aging process and longevity in each model organism. Recent studies in worms and flies have also suggested that systemic interplay between multiple tissues regulates aging and longevity.(76,77) In mammals, however, the complexity of tissue interplay is multiplied, and a blueprint for a systemic network regulating aging and longevity still remains elusive.

Alterations in NAD^+ levels have a powerful metabolic impact because it serves as an obligatory substrate for the deacetylase activity of the SIRT proteins.(78-80) The best-characterized mammalian SIRT is SIRT1, which controls mitochondrial function through the deacetylation of targets that include PGC-1α and FOXO.(81,82) The administration of NAD^+ precursors, such as nicotinamide mononucleotide (83) or nicotinamide riboside (NR) (84), has proven to be an efficient way to increase NAD^+ levels and SIRT1 activity, improving metabolic homeostasis in mice.(85) Considering the intimate link between metabolism and longevity (78,86), it was hypothesized that increasing NAD^+ levels may be sufficient to increase mitochondrial activity and extend lifespan (82).

Sir2 is an NAD-dependent deacetylase that connects metabolism with longevity in yeast, worms and flies. (9) Sir2 is required for lifespan extension by CR in yeast, worms, and flies.(87-89) In yeast, CR (0.5% glucose), was previously shown to increase mitochondrial function and to up-regulate Sir2 activity.(90,91) However, in this case, the mitochondrial activation is SIR2-independent, suggesting that it lies upstream of Sir2. A more severe CR regimen (0.05% glucose) extends yeast replicative lifespan by a different mechanism that is apparently independent of both Sir2 and mitochondrial respiration.(92,93)

Stem cells, through their regenerative ability, maintain tissue homeostasis during an individual's lifespan. However, stem cell-associated mechanisms of tissue repair become impaired with aging. Very interestingly, a recent study demonstrated that SIRT3, which is highly expressed in hematopoietic stem cells, is not essential for tissue repair at a young age under physiological conditions; however, it is crucial at an old age.(1) Moreover, it is important to highlight that induced SIRT3 overexpression, which is suppressed with aging, significantly improves aged hematopoietic stem cells' regenerative power.(22) The plasticity of mitochondrial homeostasis controlling stem cell and tissue maintenance during the aging process and shows that aging-associated degeneration can be reversed by a SIRT.(22)

Therapeutic Potential of Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) was first isolated from the roots of white hellebore (*Veratum grandiflorum O. Loes*) in 1940 (94), and later, in 1963 from the roots of *Polygonum Cupsidatum*, a plant used in traditional Chinese and Japanese medicine (95). However, the first real interest in this compound came when in 1992 resveratrol was postulated to explain some of the cardioprotective effects of red wine (96) and was suggested to be an important factor in the French Paradox, a term coined to describe the observation that the French population has a very low incidence of cardiovascular disease, despite a diet high in saturated fat (97). Five years later, in 1997, Jang

and colleagues reported resveratrol to work as a chemo-preventive agent, by the ability to inhibit carcinogenesis at multiple stages.(98) Meanwhile, also anti-inflammatory and anti-oxidant properties were identified for resveratrol. (99,100) Interest in resveratrol peaked after 2003, when Howitz and colleagues (8) identified resveratrol as a potent SIRT1 activator capable of mimicking the effects of calorie restriction (101,102) and regulating longevity in lower organisms, by extending lifespan in yeast (8), worms (103), flies (104,105) and in short-lived fish (106). Although there are a considerable amount of data supporting the role for resveratrol in SIRT1-mediated lifespan extension.(107)

The exact mechanisms through which resveratrol exerts a wide range of beneficial effects across species and disease models is currently still unclear.(99) Similar to most other polyphenols, resveratrol is suggested to possess intrinsic anti-oxidant capacity, but it is also implicated to induce the expression of a number of antioxidant enzymes, with probably both mechanisms contributing to an overall reduction in oxidative stress.(108) Resveratrol further interacts with a large number of receptors, kinases, and other enzymes that could plausibly make a major contribution to its biological effects.(107)

In mammals, there is growing evidence that resveratrol can prevent or delay the onset of cancer, heart disease, ischaemic and chemically induced injuries, diabetes, pathological inflammation and viral infection. These effects are observed despite extremely low bioavailability and rapid clearance from the circulation.(99)

Resveratrol provides diverse health benefits including cardioprotection, inhibition of low-density lipoprotein, activation of nitric oxide (NO) production, hindering of platelet aggregation and promotion of anti-inflammatory effects. Studies have shown that at a lower dose, resveratrol acts as an anti-apoptotic agent, providing cardioprotection as evidenced by increased expression in cell survival proteins, improved post-ischemic ventricular recovery and reduction of myocardial infarct size and cardiomyocyte apoptosis and maintains a stable redox environment compared to control. (109)

In 1997, Jang and colleagues published a seminal paper reporting the ability of resveratrol to inhibit carcinogenesis at multiple stages. Their finding that topical application of resveratrol reduced the number of skin tumours per mouse by up to 98% triggered research on resveratrol around the world.(98) Systemic administration of resveratrol has since been shown to inhibit the initiation and growth of tumours in a wide variety of rodent cancer models.(99) Jang and colleagues originally proposed that resveratrol might be an effective chemo-preventive agent because it inhibits the enzymatic activity of both forms of cyclooxygenase (COX). (98)

The *in vitro* studies indicate that transcriptional inhibition of COX2, as well as another important player in carcinogenesis, ornithine decarboxylase (ODC) could be accomplished through inhibition of protein kinase C (PKC). (110,111) Resveratrol does not directly inhibit ODC activity (112), but reduces its expression in vivo and prevents its induction by carcinogens (113-115).

Angiogenesis is required to support the growth of most solid tumours beyond a diameter of 2–3 mm. When delivered systemically at a dose of 2.5–100 mg per kg (body weight), resveratrol inhibits tumour-induced neovascularization. (116,117) Resveratrol modulates the expression and activity of multiple drug-metabolizing enzymes. In vitro, resveratrol inhibits the enzymatic activity of various cytochrome P450 (CYP) (118-121) and blocks their transcription through antagonism of the aryl hydrocarbon receptor (AHR) (122,123), suggesting that resveratrol could cause a reduction in the exposure of cells to carcinogens. Another mechanism by which resveratrol could combat tumour formation is induction of cell cycle arrest and apoptosis. The anti-proliferative and pro-apoptotic effects of resveratrol in tumour cell lines have been extensively documented in vitro (124) and are supported by downregulation of cell cycle proteins (125-127) and increases in apoptosis (128-130) in tumour models *in vivo*.

ROS have been shown to have a role in the initiation and progression of cancer through directly damaging DNA and other macromolecules.(131,132) In addition to its possible modulation of antioxidant enzymes involved in the Phase II response, resveratrol has an intrinsic antioxidant capacity that could be related to its chemopreventive effects.

In addition to its anticancer activity, resveratrol has displayed beneficial activity against inflammatory responses via inhibition of COX1 and COX2 expression. (133) Resveratrol was reported to reduce the production of prostaglandin E2 (PGE2) and the formation of ROS in lipopolysaccharide (LPS)-activated microglial cells. (134,135) Moreover, resveratrol was reported to suppress the activity of T- and B-cells, and macrophages.(136) Singh, *et al*., showed that resveratrol induced both caspase-dependent and caspase-independent apoptosis in activated T-cells in experimental allergic encephalomyelitis- induced mice.(137) One study showed that resveratrol possesses analgesic property by inhibition of COX1 and COX 2.(138)

Resveratrol also possesses neuroprotective properties. It has been reported that resveratrol could protect against

Huntington's disease (139), Alzheimer's disease (140) and Parkinson's disease (141).

In rodent models of diet-induced obesity, a high dose of resveratrol (400 mg/kg/d) improves insulin sensitivity and lowers body weight (102), which has increased the interest and the speculation about its potential use as an anti-diabetic agent in humans. Nevertheless, recent work has shown that a one-year intervention with resveratrol at a dose of 200 mg/kg/d seems to cause an increase in basal metabolic rate and total daily energy expenditure in the non-human primate *Microcebus murinus* (142,143); indicating that resveratrol might have the potency to enhance energy expenditure thereby promoting weight loss.

Increased adventitial vasculogenesis is one of the pathological features of abdominal aortic aneurysm (AAA), and is thought to play an important role in the development of AAA, possibly through creating a conduit for inflammatory cell transport and establishing chronic inflammation in the aortic wall.(144) Resveratrol treatment resulted in downregulation of vascular endothelial growth factor (VEGF) A, a potent angiogenic and vascular permeability factor, and decreased neoangiogenesis in the aortic wall. The decreased neoangiogenesis by resveratrol treatment was associated with attenuation of macrophage infiltration and proinflammatory cytokines expression. Norata, *et al*. reported that resveratrol treatment reduced the expression of inflammatory markers and atherosclerotic plaque formation in apolipoprotein (Apo) E knockout mice.(145) Therefore, anti-angiogenic and anti-inflammatory effects of resveratrol may contribute to the prevention of AAA development. (146)

Published clinical trials have largely focused on characterizing the pharmacokinetics and metabolism of resveratrol. Recent studies have also evaluated safety and potential mechanisms of activity following multiple dosing, and have found resveratrol to be safe and reasonably well-tolerated at doses of up to 5 g/day.(147) Though limited data is available on resveratrol's efficacy in chronic metabolic diseases in humans, the clinical trials that are available show much promise that resveratrol might be applied to improve general health status and prevent chronic disease in humans.

Resveratrol: SIRT Activators

Regardless of the established benefits of a CR diet, the severity of this dietary regime has limited adoption of this approach to increasing longevity, because few people can keep to such an unappealing lifestyle. It would therefore be desirable to provide an alternative route to obtaining the benefits of CR that would avoid the need for dietary regulation and that would be amenable to widespread use. The beneficial impact of increased SIRT1 activity observed in several animal models of disease has recently been observed in humans as well, where reduced SIRT1 expression in insulin-sensitive tissues was associated with reduced energy expenditure.(148)

Reversible acetylation is a key post-translational modification of target proteins. SIRT deacetylases represent the homolog of the yeast Sir2. Although seven SIRT have been found in mammals, all SIRT activators described to

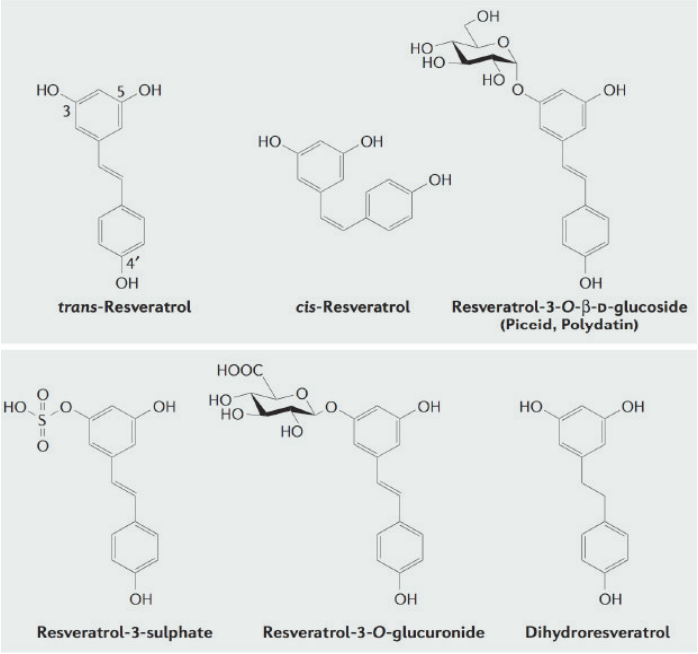

Figure 2. Trans-resveratrol and related structures. Piceid is found in grapes and other natural sources of resveratrol. Resveratrol-3-sulphate, resveratrol-3-O-glucuronide and dihydroresveratrol are metabolites of resveratrol.(99) (Adapted with permission from Nature Publishing Group).

Table 1. Dietary Sources of Resveratrol.(99) ND: not determined. (Adapted with permission from Nature Publishing Group).

Source	*trans*-Resveratrol concentration	Comments
Dietary		
Red wines	0.1–14.3 mg l^{-1}	*cis*-Resveratrol, *trans*-piceid and *cis*-piceid also present, typically at slightly lower concentrations
White wines	<0.1–2.1 mg l^{-1}	Generally resveratrol found at concentrations of <0.1 mg l^{-1}, exceptions include Swiss, Portuguese and German Riesling wines, *cis*-resveratrol, *trans*-piceid and *cis*-piceid also present
Ports and sherries	Generally <0.1 mg l^{-1}	
Grapes*	0.16–3.54 µg g^{-1}	Contents are similar for wine or table grapes, and black or white grapes. *trans*-Piceid is predominant at concentrations of 1.5–7.3 µg g^{-1}
Dry grape skins	24.06 µg g^{-1} (average)	*trans*-Piceid and *cis*-piceid found at concentrations of 42.19 µg g^{-1} and 92.33 µg g^{-1}, respectively
Red grape juices	0.50 mg l^{-1} (average)	*trans*-Piceid, *cis*-piceid and *cis*-resveratrol found at concentrations of 3.38 mg l^{-1}, 0.79 mg l^{-1} and 0.06 mg l^{-1}, respectively
White grape juices	0.05 mg l^{-1} (average)	*trans*-Piceid and *cis*-piceid found at concentrations of 0.18 mg l^{-1} and 0.26 mg l^{-1}, respectively
Cranberry raw juice	~0.2 mg l^{-1}	*cis*-Resveratrol also found at a concentration of ~0.03 mg l^{-1}
Blueberries	Up to ~32 ng g^{-1}	
Bilberries	Up to ~16 ng g^{-1}	
Other *Vaccinium* berries	7–5,900 ng g^{-1} (dry sample)	Highest concentrations in lingonberries
Peanuts	0.02–1.92 µg g^{-1}	
Roasted peanuts	0.055 µg g^{-1}	
Boiled peanuts	5.1 µg g^{-1}	
Peanut butters	0.3–0.4 µg g^{-1} (average)	*trans*-Piceid also found at a concentration of 0.13 µg g^{-1}
100% Natural peanut butters	0.65 µg g^{-1} (average)	*trans*-Piceid also found at a concentration of 0.14 µg g^{-1}
Pistachios	0.09–1.67 µg g^{-1}	
Groundnuts (*Arachis hypogaea*)	ND	
Rhubarb	ND	
Hops	0.5–1 µg g^{-1}	*trans*-Piceid and *cis*-piceid found at concentrations of 2–9 µg g^{-1} and 0.9–6 µg g^{-1}, respectively
Itadori (*Polygonum cuspidatum*) tea	0.68 mg l^{-1}	*trans*-Piceid also found at a concentration of 9.1 mg l^{-1}
Herbal		
Veratrum (Lily)	ND	
Cassia quinquangulata	ND	
Gnetum klossii	ND	
Polygonum cuspidatum	0.524 mg g^{-1}	*trans*-Piceid also found at a concentration of 1.65 mg g^{-1}
Rhubarb (*Rheum rhaponticum*) dry root	3.9 mg g^{-1}	
Yucca schidigera bark	ND	

date act through SIRT1.(73) SIRT1 activators, with a focus on therapeutic applications, primarily related to the use of pharmaceuticals and nutraceuticals containing resveratrol, and the development of second-generation activators unrelated to resveratrol.(73)

Resveratrol offers protection in models of stress- and age-associated diseases, including chronic overfeeding, insulin resistance, type 2 diabetes, and cardiovascular dysfunction.(101,102) The mechanisms behind these effects may rely on the fact that resveratrol mimics some of the metabolic actions of CR, as a series of studies on humans have suggested.(107) Resveratrol interacts with many stress-related targets in the cell, including the mammalian NAD^+-dependent deacetylase SIRT1 (99,102), although the resveratrol-SIRT1 interaction may be indirect (149). SIRT1 is a member of a family of proteins (SIRT) that have been linked to longevity in yeast, flies, and worms.(9,85,150)

A number of subsequent studies showed that resveratrol induced SIRT1 activity in several species. (151) Furthermore, resveratrol mimics numerous aspects

of calorie restriction in all eukaryotes tested to date (8,101-103,106,152,153) and in most of them, the effect appears dependent on SIRT1 (8,102,103).

Resveratrol was also shown to increase energy expenditure in mice (101,102), through increased SIRT1 activation, and during conditions of high fat availability, resveratrol was capable of preventing diet-induced obesity and the onset of obesity-related metabolic diseases, so ultimately protecting mice against the lifespan curbing effects associated with high calorie intake. The molecular mechanism underlying these beneficial effects seem to depend on the resveratrol-induced increase in mitochondrial content, which is explained by increased signaling through the SIRT1/ PGC1α axis.(102) More recently, some studies have questioned the direct activation of SIRT1 by resveratrol.(154,155)

Several reports demonstrate that resveratrol can also activate AMPK (101,156-158), which reconciles with the positive effect on the mitochondrial respiratory chain that has been reported (159). Hawley, *et al*., reported that resveratrol-induced AMPK activation in isogenic cell lines, stably expressing AMPK complexes containing AMP-insensitive γ2 subunit variants (R531G), derives from an AMP/ATP imbalance as a consequence of interference with mitochondrial respiration.(160) Although the idea was put forward that the resveratrol-induced AMPK activation was dependent on SIRT1 (161), the use of mouse embryonic fibroblast cells from SIRT1 knock-out mice unequivocally demonstrated that SIRT1 is dispensable for resveratrol-induced AMPK activation (156,162). On the contrary, resveratrol cannot activate SIRT1 in the absence of functional AMPK.(162,163) So, the current working mechanism of resveratrol that evolved from all these reports is that SIRT1 functions as the downstream mediator of AMPK, instead of being a direct molecular target of resveratrol. Canto, *et al*. (164) have shown in that respect that the AMPK induced increase in NAD^+ levels, as a consequence of increased fatty acid oxidation, leads to SIRT1 activation.

Diminished mitochondrial oxidative phosphorylation and aerobic capacity are associated with reduced longevity. Resveratrol's effects were associated with an induction of genes for oxidative phosphorylation and mitochondrial biogenesis and were largely explained by an resveratrol-mediated decrease in PGC-1α acetylation and an increase in PGC-1α activity. This mechanism is consistent with resveratrol being a known activator of the protein deacetylase, SIRT1, and by the lack of effect of resveratrol in SIRT1-/- mouse embryonic fibroblast (MEF). Importantly, resveratrol treatment protected mice against diet-induced-obesity and insulin resistance.(102)

The mechanisms by which enhanced autophagy can improve organismal health and longevity are largely elusive. As a possibility, increased autophagy might improve cellular resistance to stress by augmenting the metabolic buffering capacity of cells. Alternatively, autophagy might enhance organellar turnover and mediate a 'cleaning effect', thereby preventing the accumulation of damaged/old (and hence potentially harmful) mitochondria and lysosomes. Irrespective of these considerations, data establish the cardinal role of SIRT-1-elicited autophagy in mediating the anti-aging effects of CR and resveratrol.(10)

SIRT1 activation restores bone marrow-derived early outgrowth cell (EOC) chemokine secretion and increases the *in vitro* and *in vivo* angiogenic activity of EOCs in diabetic animals. These findings suggest a pivotal role for SIRT1 in diabetes-induced EOC dysfunction and that its pharmacologic activation may provide a new strategy for the restoration of EOC-mediated repair mechanisms.(165) In fact, resveratrol treatment has been demonstrated to rescue adult stem cell decline, slow down bodyweight loss, improve trabecular bone structure and mineral density, and significantly extend the lifespan in zinc metallopeptidase STE24 $(Zmp\text{-}ste24)^{-/-}$ mice, which are deficient for Zmp-ste24, a metalloproteinase responsible for prelamin A

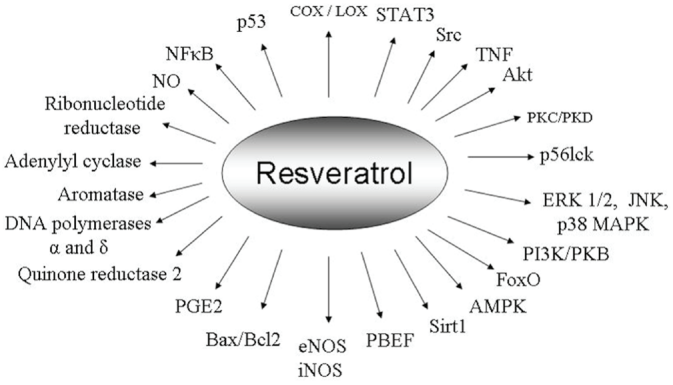

Figure 3. Molecular Targets of Resveratrol: as a pharmacological agent, resveratrol has wide spectrum of targets.(109) (Adapted with permission from University of Massachussets).

maturation.(166)

Over the past several years, chemically distinct molecules mimicking resveratrol effects were developed to activate SIRT at much lower doses.(167-169) Sirtris Pharmaceuticals identified and characterized some small molecules designed to activate SIRT1 that are structurally unrelated to resveratrol. One of them, named SRT1720, was 1000-fold more potent than resveratrol. Another strategy for SIRT activation is the use of agents that increase NAD^+ concentrations and, consequently, potentiate mammalian SIRT functions. For example, increased intracellular NAD^+ concentration has been shown to activate SIRT1 in brain, and has been proposed to help explain the protective effects of CR in a mouse model of Alzheimer's disease.(170) Sauve and collaborators developed NR and derivatives that potently stimulate NAD^+ biosynthesis in mammalian cells and thus, could provide an effective pharmacological means to increase SIRT activities.(171) Resveratrol is the best studied natural compound that is able to activate SIRT1. Synthetic drugs mimicking resveratrol and activating SIRT at much lower doses than resveratrol have been developed over the past several years.(73)

Conclusion

The natural polyphenolic compound resveratrol has received interest as several findings implicated resveratrol as a potent SIRT1 activator capable of mimicking the effects of calorie restriction, and regulating longevity in lower organisms. Given the worldwide increase in age-related metabolic diseases the beneficial effects of resveratrol on metabolism and healthy aging in humans are currently a topic of intense investigation.

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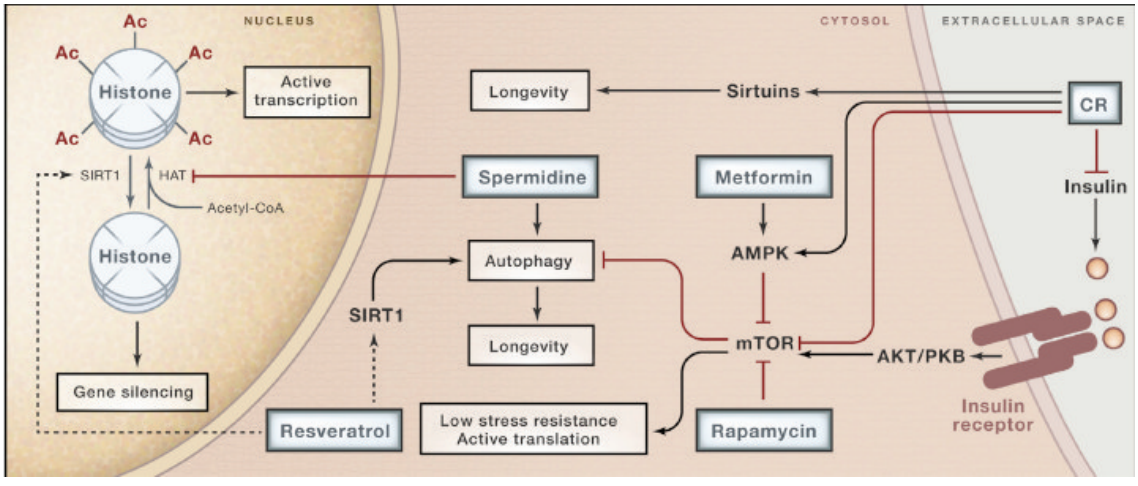

Figure 4. Molecular Targets for CR and Pharmacological Interventions against Premature Aging.(5) (Adapted with permission from Elsevier).

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