Trans-Resveratrol: A Magical Elixir of Eternal Youth?

Francisco Orallo*

Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela (La Coruña), Spain

Abstract: *Trans*-resveratrol or *(E)*-resveratrol [3,4',5 trihydroxy-*trans*-stilbene, *t*-RESV or *(E)*-RESV] is a natural component of *Vitis vinifera* L. (Vitaceae), abundant in the skin of grapes (but not in the flesh) and in the leaf epidermis and present in wines (especially red wines). In *in vitro, ex vivo* and *in vivo* experiments, *t*-RESV exhibits a number of biological activities, including anti-inflammatory, antioxidant, platelet antiaggregatory and anticarcinogenic properties, and modulation of lipoprotein metabolism. Some of these activities have been implicated in the cardiovascular protective effects attributed to *t*-RESV and to red wine.

Prior to 2002 there had been no previous studies describing the potential effects of *t*-RESV on the lifespan extension. However, in the last 5 years, several researchers have reported that *t*-RESV is a potent activator of sirtuin enzymatic activity, mimics the beneficial effects of caloric restriction (CR), retards the aging process and increases longevity in a number of organisms from different phyla such as yeasts, worms, flies and short-lived fish. In addition, *t*-RESV seems to be effective in delaying the onset of a variety of age-related diseases in mammals (e.g.: rodents).

Therefore, this review will basically focus on the possible role of *t*-RESV to extend life duration and on some of the mechanisms by which *t*-RESV may act as an anti-aging agent.

Keywords: Resveratrol isomers, wine, lifespan, caloric restriction, sirtuins, hormesis hypothesis, caloric restriction mimetics, sirtuin activating compounds, xenohormesis.

1. INTRODUCTION

Resveratrol (3,4',5-trihydroxystilbene, RESV, Fig. (**1**)) is a natural phenolic compound that exists as *cis* and *trans* isomers [the *c*-RESV or *(Z)*-RESV diasteromer, and the *t*-RESV or *(E)*-RESV diasteromer, respectively], facilitated by the double bond in its chemical structure. Both isomers are present in wines at variable concentrations, although only the *trans* isomer has been detected in grapes (for more details see, e.g., refs. [1-5]).

t-RESV, a natural component of *Vitis vinifera* L. (Vitaceae), is abundant in the skin of grapes (but not in the flesh) and in the leaf epidermis, and is present in wines, particularly red wines.

t-RESV is not unique to *Vitis* but is also present in at least 72 other plant species (distributed in 12 families and 31 genera; e.g., *Arachis, Artocarpus, Cassia, Eucalyptus, Morus, Picea, Pinus, Trifolium, Vaccinium* and *Veratrum*), some of which are components of the human diet, such as red and black mulberries (*Morus rubra* L. and *Morus nigra* L., Moraceae) or peanuts (*Arachis hypogaea* L., Fabaceae) [2,6-8].

The physiological significance of RESV in the plant kingdom (e. g.: in *Vitis*) is still unclear. However, it is thought to be a phytoalexin, i.e., an anti-infectious compound produced by some spermatophytes (*via* the induction of an enzyme called stilbene synthase) in response to injury, pathogenic attack, nutrient deprivation, temperature changes and other types of stimuli (e.g.: exposure to ozone, ultraviolet radiation, etc.) [7-9]. In this connection, it is interesting to note that *t*-RESV is present mostly as constitutive compound of the woody organs (roots, seeds, canes, stems) and as induced substance in leaves and fruits (grapes) [10-12].

t-RESV was first identified in 1940 by Michio Takaoka [13] as a constituent of the roots of white hellebore [*Veratrum grandiflorum* (Maxim. ex Baker) Loes. (Liliaceae)], and later (in 1963) found by Nonomura *et al.* [14] (together with its 3-O-*β*-D-glucoside β-glucoside, so-called *trans*-piceid or *trans*-polydatin) in the dried roots of Japanese knotweed *Polygonum cuspidatum* Sieb. et Zucc.(Polygonaceae), called Ko-jo-kon (or Itadori) in Japanese folk medicine, in which it is used for the treatment of hyperlipidemia, arterial hypertension, arteriosclerosis and a variety of other pathologies, including many inflammatory and allergic diseases.

In addition, the herb *Polygonum cuspidatum*, also called Fu-Chung and the *Rhizoma polygoni cuspidate*, also called Huzang in Chinese folk medicine, are still included in the Chinese Pharmacopoeia.

t-RESV is also an active component in herbal formulations used in traditional South African medicine [e.g.: the infusions of *Erythrophleum lasianthum* Corbishley (Caesalpinioidae, Leguminosae)] [15] and Indian Ayurvedic medicine (e.g.: the herbal preparation called Darakchasava, which contains a number of fruits including grapes and other ingredients) [16]. The above-mentioned formulations have been basically prescribed as cardiotonics in folk medicine.

In 1976, *t*-RESV was detected in grapevines by Langcake and Pryce [17], who found that it is synthesized by leaf tissues in response to fungal infection (mainly by *Botrytis cinerea*) or exposure to ultraviolet light.

Initially characterized as a phytoalexin (see above), *t*-RESV attracted little interest until 1992, when French researchers drew attention to a number of epidemiological study that showed that, in spite of a diet relatively high in saturated fat, mortality associated with coronary artery disease was lower in southern France than in other industrialized countries. This phenomenon came to be described as the "French paradox" (for a review see, e.g., ref. [12]). This term was coined in November 1991, during the CBS program "60 minutes", by Dr. Serge Renaud and the discrepancy was attributed to the prolonged and daily consumption of moderate amounts of wine, especially red wine by the southern French population [18]. Subsequently, the presence of *t*-RESV in wine was reported by Siemann and Creasy [19], who speculated that this polyphenol could be one of the active components present in wines responsible for the French paradox.

Since then and once it became known that the protective effects of wine consumption were independent of alcohol content, the pharmacological activity of *t*-RESV was extensively investigated. Several studies within the last few years have demonstrated that *t*-RESV may protect against coronary heart disease as a result of different effects, including significant antioxidant activity, modulation of lipoprotein metabolism, and vasodilatory and platelet antiaggregatory properties (see, e.g., refs. [12,20-29]).

*Address correspondence to this author at the Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, Campus Universitario Sur, E-15782 Santiago de Compostela (La Coruña), Spain; Tel: +34-981-563100; Ext: 14895; Fax: +34-981-594595; E-mail: fforallo@usc.es

0929-8673/08 $55.00+.00

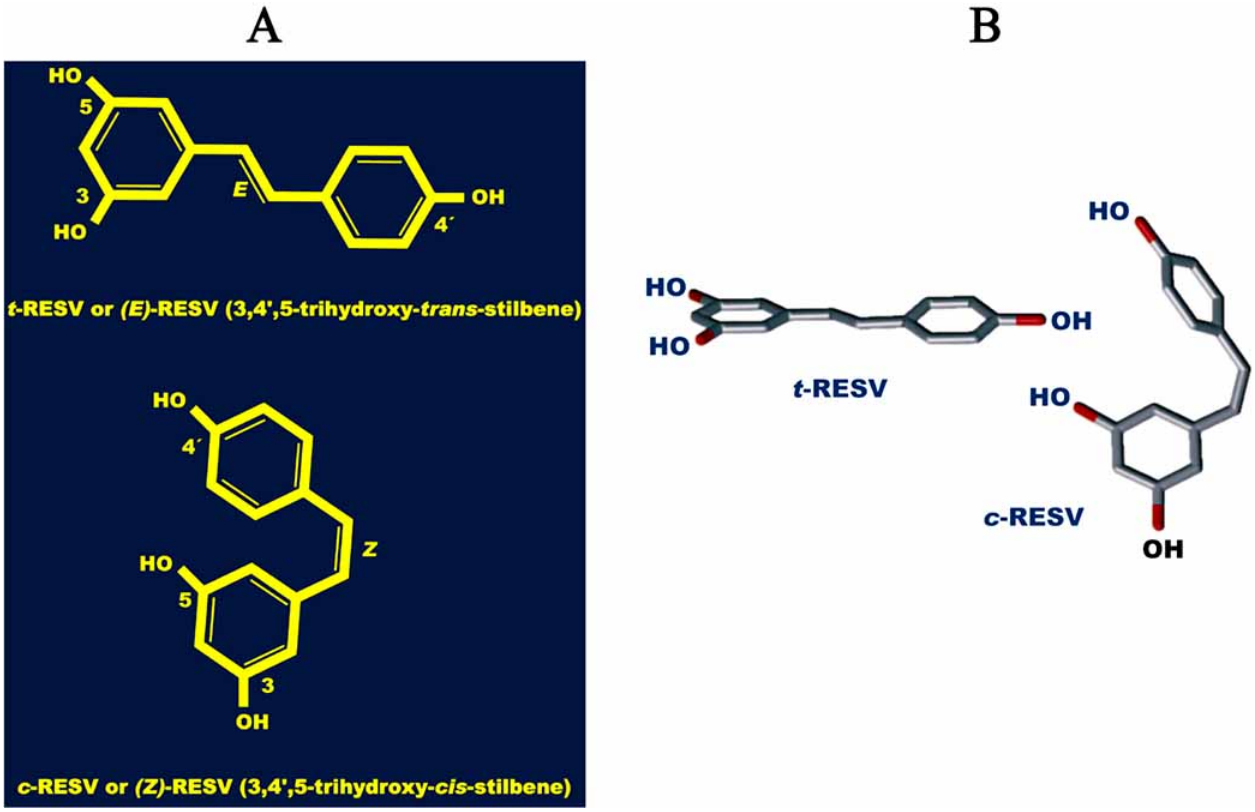


Fig. (1). Chemical structures (panel **A**) and 3D molecular configurations (panel **B**) of the *cis* and *trans* isomers of RESV.

In addition, *t*-RESV has shown a number of other biological activities including anti-inflammatory and anticarcinogenic properties (for reviews, see, e.g., refs. [1,2,6,7,12,30-37]).

As noted, RESV also exists as a *cis* isomer, which (unlike *t*-RESV) is not currently available commercially (see, e.g., refs. [2,5]); as a result, little is known about this isomer's pharmacological activity.

Most comparative studies reported in the scientific literature on the biological effects of *c*-RESV versus *t*-RESV have generally demonstrated only quantitative, not qualitative differences in the activities of the two forms, which suggests that the different spatial conformation of *c*-RESV (*versus* that of the *trans* isomer) does not seem to modify markedly its interaction with the potential cellular targets (Fig. **1**). These studies have been reviewed comprehensively elsewhere (see, e.g., refs. [3,4]) and, therefore, will not be covered here.

It is well established that reducing food intake [the diet known as caloric restriction (CR)] extends lifespan in a wide range of species from yeast to mammals (see below). On the other hand, it has been recently reported by several authors that *t*-RESV seems to mimic the beneficial effects of CR and to increase longevity (without an apparent cost of reproduction) in lower organisms (simple eukaryotes; e.g.: the budding yeast *Saccharomyces cerevisiae)*, in short-lived invertebrates [simple metazoans such as the nematode (roundworm) *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*] and in the short-lived vertebrates (the seasonal fish *Nothobranchius furzeri)*. In addition, *t*-RESV seems to be effective in delaying the onset of a variety of age-related diseases in mammals (e.g.: rodents) [38,39].

Bearing in mind the above considerations, in this review, I will basically focus on the possible role of *t*-RESV to prolong life duration and on some of the possible mechanisms by which *t*-RESV may act as an anti-aging agent.

The potential mechanisms by which *t*-RESV may exert beneficial effects on the aged vasculature have been exhaustively reviewed elsewhere (see, e.g., refs. [21,40]) and, therefore, will not be covered here.

2. HYPOTHESES OF CR

In 1935, after having lived on a restricted diet, Professor Maurice Gueniot (President of the Paris Medical Academy at the beginning of the 20th century) died at the age of 102. In the same year, coinciding with the death of Professor Gueniot, McCay and co-workers at Cornell University published the first widely recognized scientific study of CR and its ability to extend lifespan [41]. In this study, the above-mentioned researchers observed that rats fed 40% fewer calories tend to live longer and look younger and healthier than well-fed animals. In numerous subsequent studies in different species it has been reported that reduction of calories 30–50% below *ad libitum* levels of a nutritious diet can increase lifespan, improve stress resistance, decelerate functional decline, reduce the incidence and delay the onset of age-related diseases such as cancer, atherosclerosis, type II diabetes and even neurodegeneration [42-46]. As a result, the refinement of our understanding of the molecular mechanisms by which CR elicits its effects and whether this nutritional intervention is relevant to human aging constitutes an important and exciting focus for current and future research. Evidence emerging from studies in rhesus monkeys suggests that their response to CR parallel that observed in rodents. To assess CR effects in humans, clinical trials were initiated several years ago but conclusive results have not been obtained yet [47-49].

It is commonly believed that the antiaging effects of CR are due to the decreased intake of calories themselves (see above) rather than to decreases in specific dietary components. However, a number of recent studies have shown that variations in the proportions of some of the main dietary aminoacids [e.g.: drastic (80%) me-

thionine restriction] may be responsible for approximately one third of the CR-induced maximum lifespan extension in rodents (see, e.g., refs. [50,51]).

To explain how CR works, at least 10 plausible theories have been proposed but almost all of them have been unsuccessful because of contradicting data.

The early hypotheses of CR were the developmental delay, the reduced metabolic rate and the laboratory gluttons. The current theories of CR are the glucocorticoid cascade, the decreased fat, the reduced reactive oxygen species, the cell survival hypothesis, the protein turnover, the decreased glucose and insulin levels and other endocrinological changes. All these hypotheses have been reviewed in detail by Sinclair [52] and, therefore, will not be covered here.

The last theory of CR is the hormesis hypothesis which proposes that CR imposes a low- or mild intensity biological stress on the organism. This biological stress or the exposure to other different mild stresses (e.g.: irradation, heat, toxins, high salt, osmotic stress, etc.) elicits a defensive response that helps to protect it against the causes of aging and against subsequent stresses [42,45,52]. This hypothesis is supported by the fact that mildly stressed animals outlive their unstressed counterparts, which also possibly applies to humans.

The hormesis hypothesis is a radical departure from other CR theories since it is based on the premise that CR is due to an active defensive response of the organism, as opposed to passive mechanisms.

The hormesis hypothesis has been expanded by the group of Sinclair to include the idea that plants might synthesize a number of molecules in response to stress conditions and nutrient deprivation. Moreover, animals can pick up on chemical stress cues from plants under stress or CR, and use these stress molecules to prepare for adverse conditions (e.g.: decline in their environment and/or food supply) [52].

As reviewed by David Sinclair [52], the hormesis hypothesis of CR makes four key predictions:

1) CR induces intracellular cell-autonomous signalling pathways that respond to biological stress and nutrient limitation.

2) The pathways included in (1) contribute to protecting cells and tissues against the causes of aging.

3) The pathways in (1) regulate glucose, fat, and protein metabolism in a way that enhances the chance of survival during times of stress.

4) The pathways included in (1) are under the control of the endocrine system to ensure that the organism acts in a coordinated fashion.

In lower organisms (simple eukaryotes; e.g.: the budding yeast *Saccharomyces cerevisiae)*, in short-lived invertebrates [simple metazoans such as the nematode (worm) *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*] and in mammals, there is ample evidence that the effect of CR is due to hormesis and that the beneficial effects of CR are basically mediated by the activation of sirtuins. This evidence of hormesis in budding yeast, worms, flies and mammals has been exhaustively reviewed elsewhere (see, e.g., refs. [38,39,46,52,53]) and, therefore, will not be covered here.

3. CLASS III HISTONE DEACETYLASES (HDACs)

The NAD^+-dependent protein deacetylases have emerged as important regulators of diverse biological processes (see, e.g., refs. [39,53-59]). These enzymes, referred to as sirtuins or Sir2-like proteins, constitute the class III HDACs and are conserved from bacteria to humans. Different aspects of sirtuin biology (e.g.: classification, structure, substrates, cellular localization, physiological function and other features of the different sirtuins) has been reviewed in detail elsewhere and, consequently, will not covered here (see, e.g., refs. [60-63]).

In contrast to the class I HDACs, the class III HDACs requires the presence of the oxidized form of the cofactor nicotinamide adenine dinucleotide (β-NAD^+) as a co-substrate for deacetylation reaction. Enzymatic studies have demonstrated that class III HDACs liberates nicotinamide from β–NAD^+ and that the acetyl group of the substrate (acetyl-lysine-bearing histone) is transferred to cleaved β–NAD^+, generating a novel metabolite, 2′-*O*-acetyl-ADP-ribose (OAADPR) and the deacetylated histone lysine (Fig. **2**; for more details of the deacetylation reaction and catalytic mechanism, etc. see, e.g., refs. [61,63]). The acetyl-lysine histone may be restored again from deacetylated histone lysine by the action of a histone acetyl transferase (Fig. **2B**).

3.1. Sirtuins Implicated in Mediating Lifespan Increases in Lower Organisms

Yeast Sir2 (ySir2) is the prototype and the founding member of the large and diverse sirtuin family. The name ‘‘Sir2’’ stands for ‘‘silent information regulator 2’’ because this enzyme is essential for maintaining chromatin silencing and genomic stability since it catalyses the deacetylation of histones and, consequently, the formation of heterochromatin, the more tightly packed form of chromatin associated with deacetylated histones and gene repression.

ySir2 (basically present in the nucleolus) was originally identified as a *trans*-acting factor involved in transcriptional repression of the silent mating-type loci in yeast. Subsequently, numerous Sir2 homologues have been identified in other different lower organisms. Thus, dSir2 is the homologue of ySir2 in flies (e.g.: *Drosophila melanogaster*) whereas Sir2.1 is the most homologue of ySir2 in the roundworms (e.g.: the nematode *Caenorhabditis elegans*). ySir2, dSir2 and Sir2.1 have been implicated in mediating lifespan extension in yeast, flies and worms, respectively and also may underlie the beneficial effects of CR (see, e.g., refs. [60,61,63-65]) (although see also the ref. [66] for a review of conflicting reports).

In *S. cerevisiae* there are five sirtuins*:* ySir2 and Hst1-4. The precise cellular functions of the Hst proteins are not clear but the analysis of yeast mutants on these genes indicate that they have roles in silencing, mitochondrial metabolism and DNA repair. In addition, Hst1 and Hst2 seem to be participate in CR response [67]. Different aspects of the biology of these enzymes have been comprehensively reviewed elsewhere and, therefore, will not covered here (see, e.g., refs. [38,39,53,56,60,61,63-65,68]).

3.2. Sirtuins in Mammals

In mammals (e.g., rodents and humans), seven Sir2 homologs have been identified: SIRT1-7. Among SIRTs, SIRT1 is basically localized in the nucleus (although it has some important cytoplasmatic functions as well) and represents the probable Sir2 ortholog on the basis of the extensive sequence similarity.

SIRT1, like Sir2, deacetylates histones and, therefore, seems to play an important role in regulating the chromatin silencing and integrity of the genome, as well as the senescence of organisms.

In addition, SIRT1 deacetylates many key transcription factors and co-factors implicated in the age-related diseases, such as p53, the O subfamily of forkhead/winged helix transcriptions factors (FoxO proteins), peroxisome proliferator-activated receptor gamma (PPAR-γ) co-activator-1α (PGC-1α) and nuclear factor-kappa B (NF-κB) (see, e.g., refs. [38,39,53,56]).

Like SIRT1, SIRT6 and SIRT7 are predominantly located in the nucleus (SIR7 is basically found in the nucleolus). SIRT2 is mainly located in the cytoplasm whereas SIRT3-5 are mitochondrial and,

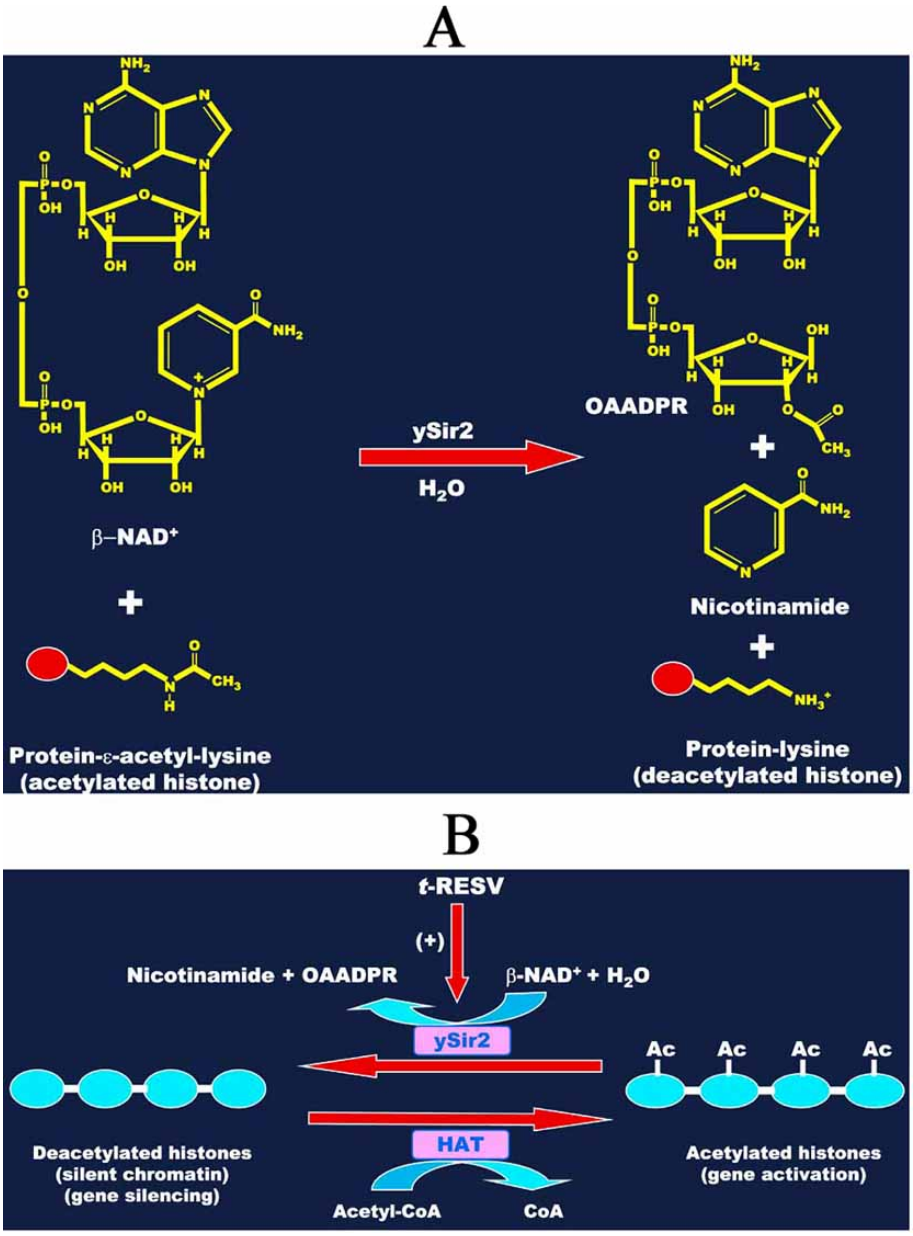


Fig. (2). Scheme of the overall reaction catalysed by class III HADCs (e.g.; ySir2; panel **A**) and of the histone acetylation/deacetylation pathways (panel **B**). For details see text. As noted, *t*-RESV is an activator of ySir2 enzymatic activity. –Ac = acetyl group (–$COCH_3$). HAT = histone acetyl transferase. OAADPR = 2′-*O*-acetyl-ADP-ribose.

thus, have the potential to exert broad effects on stress resistance and metabolism in cells.

Unlike other SIRT family members, SIRT4 seems not to have deacetylase activity. Furthermore, no robust activity has been found for SIRT7 as yet. Different features of the SIRT2-7 biology have been reviewed in detail elsewhere and, consequently, will not covered here (see, e.g., refs. [38,39,53,56]).

3.3. Regulation of Sirtuin Activity

To explain the regulation of ySir2 activity, a number of theories have been proposed (for review see, e.g., refs.[53,69,70]).

An early theory was that levels of the co-substrate, β–NAD^+, regulates the enzyme [71]. Another theory is that Sir2 activity is mainly regulated by alterations in the NAD^+/NADH ratio [72]. When, for example, yeast cells are deprived of food (CR), stress pathways are activated and the cells are forced to derive energy from alternative substrates. This produces alterations in oxygen consumption which, in turn, increases the ratio of oxidized to reduced forms of nicotinamide adenine dinucleotide (NAD^+/NADH) and stimulates the activity of Sir2.

A different but not mutually exclusive hypothesis is that Sir2 is regulated by nicotinamide concentrations. Free nicotinamide is a product of the Sir2 reaction (Fig. **2**) and an inhibitor of Sir2 enzymatic activity *in vitro* and *in vivo*. When cells are subjected to CR or any of the other treatments known to extend yeast lifespan, a single “master regulatory” gene called pyrazinamidasenicotinamidase 1 (PNC1) is highly upregulated (Fig. **3**). This up-regulation depletes nicotinamide from the cell since PNC1 gene encodes the expression of the corresponding PNC1 protein, a nicotinamidase 1 that is an essential component of the β-NAD^+ salvage pathway and that converts nicotinamide to nicotinic acid (vitamin B_3) [73,74]. The increase of nicotinic acid intracellular levels generates, in turn, high concentrations of β–NAD^+ through the yeast β-NAD^+ salvage

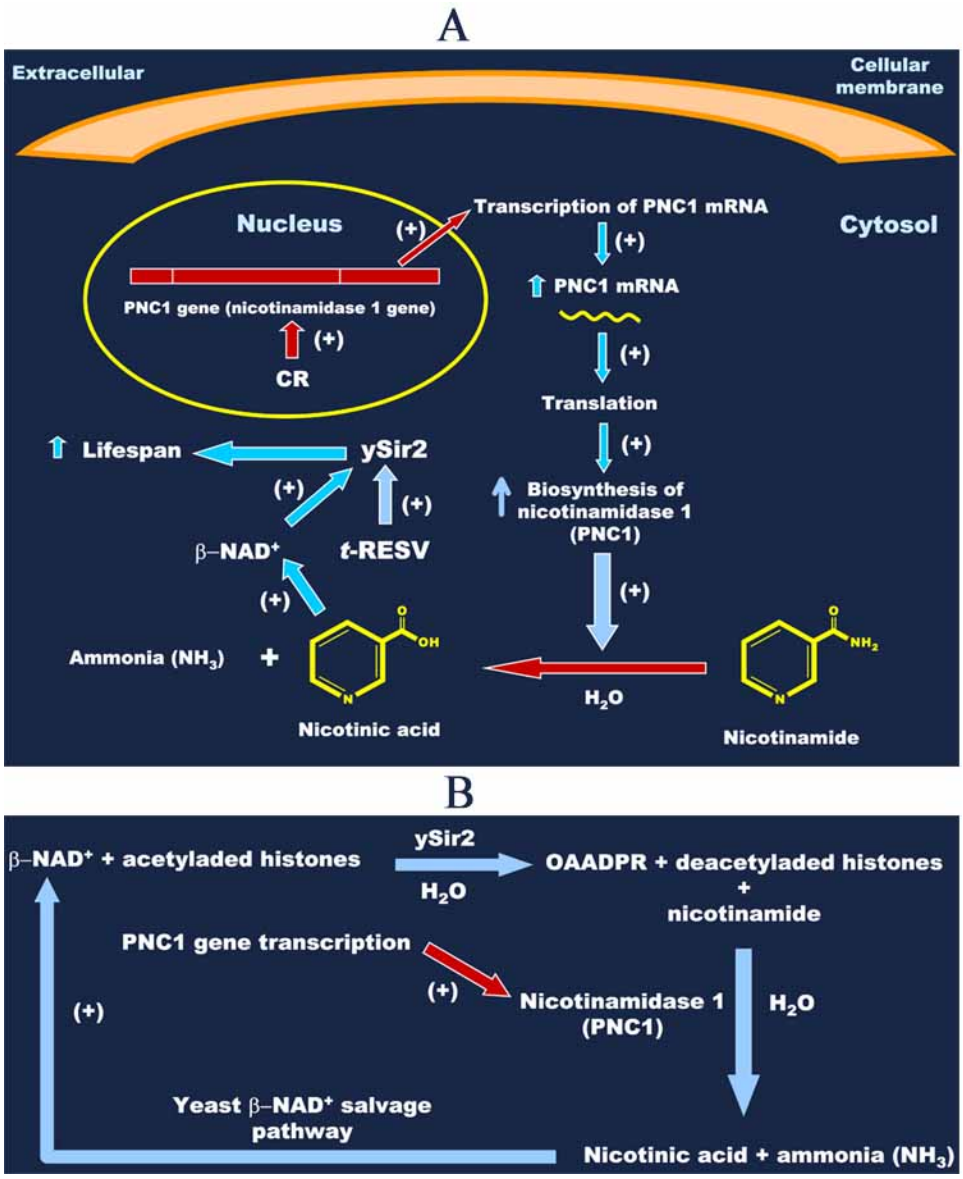


Fig. (3). Schematic representation of the nicotinamidase 1 (PNC1 protein) biosynthesis in yeasts (*via* transcription of PNC1 gene, panel **A**) and of the regulation of ySir2 activity by PNC1 (panel **B**). For details see text. As noted, *t*-RESV is an activator of ySir2 enzymatic activity. OAADPR = 2′-*O*-acetyl-ADP-ribose.

pathway, which notably stimulates Sir2 activity (Fig. (**3**); for more details see, e.g., refs. [53,63,64,70]). Whether PNC1 or NAD^+/NADH plays the major role in regulating ySir2 during CR is an ongoing debate.

It is likely that the functional equivalent of PNC1 in mammals is a nicotinamide phosphoribosyl transferase called Nampt, which catalyses intracellularly the first step in the conversion of nicotinamide to β-NAD^+ as part of the mammal β-NAD^+ salvage pathway (for more details see, e.g., refs. [53,63,64,70]). This hypothesis is supported by the recent finding that Nampt is a stress- and diet-responsive regulator of mitochondrial NAD^+ in mammalian cells [75].

Nampt is also known by two other names: pre-B-cell colony enhancing factor (PBEF) and visfatin. PBEF is a cytokine released by a variety of cells that enhances the effect of interleukin-7 on pre-B-cell colony formation [76,77]**.** In addition, PBEF stimulates SIRT1 when over-expressed [78,79]. On the other hand, visfatin is an adipokine secreted by adipocytes that binds the insulin receptor and increases glucose uptake from peripheral tissues [70,80,81]. Both PBEF and visfatin have been identified as Nampt.

4. IMPLICATIONS OF THE HORMESIS HYPOTHESIS: CR MIMETICS

As indicated above (see section 2), it is well established that reducing food intake (CR) extends lifespan in a wide range of species from very different taxa. It is possible that these observations are also true in primates (e.g.: rhesus monkeys and humans). But given the current epidemic of obesity in industrialized countries, it is unlikely that many of the people would be willing to eat less and maintain a CR diet for the sake of longevity. More palatable would be a drug that could mimic the effects of CR, i.e., a chemical that would allow the individual to maintain their normal eating habits while tricking the body to respond as though food were in short supply but without requiring a draconian diet. Such mimetics might be thought of as the pharmaceutical equivalent of "eating your cake *without* having it" [82]. Among others (see section 2), this is one of the probable predictions of the hormesis hypothesis.

4.1. Modulators of Energy Metabolism

Until recently, the search for potential CR mimetics has focused solely on those that could modulate energy metabolism. Two typical examples include 2-deoxyglucose and biguanides from the French lilac [*Galega officinalis* L. (Papilionaceae)]. 2-Deoxyglucose is a synthetic glucose analog that inhibits the enzymatic activity of phosphohexose isomerase. Unfortunately, although this strategy has some success in short-term treatments, chronic administration of 2-deoxyglucose may produce a number of side effects since this drug exhibits an apparent narrow therapeutic window between efficacy and toxicity [48,52].

Metformin is a biguanide developed from the French lilac (also known as Goat's rue, Spanish sainfoin or Italian fitch) that is used (alone or in combination with sulfonylureas) to treat type II diabetes. Although the actual mechanism of action of metformin is unknown, recent findings indicate that it acts, at least in part, by activating AMP-activated protein kinase (AMPK) in liver cells, a cellular energy sensor that modulates appetite, glucose, and insulin metabolism. Other biguanides derived from the French lilac are buformin, and phenformin. Questions arise about the long-term safety of these compounds in humans, since phenformin was removed from the market due to the severe lactic acidosis observed in a number of patients [48,52,83]. Taken together, these findings indicate that mimicking CR in mammals by modifying glucose/insulin metabolism might be possible, although the use of this strategy in humans will never be completely safe.

Taking into account the above considerations, the development as CR mimetics of other different molecules such as enhancers of fatty acid oxidation and autophagy, antioxidants, metal chelators, etc. may be probably an alternative to the use of the above energy metabolism modulators [48,84].

There is, however, a new class of CR mimetics with a promising future to prevent age-related diseases and extend lifespan in mammals, and relatively non-toxic (i.e., practically devoid of negative side effects). These molecules do not directly target metabolic enzymes but instead increase sirtuin activity.

Since the discovery that these enzymes are regulators of cell survival and longevity in a number of organisms from different phyla, there has been a great interest in finding small molecules that can alter the activity of sirtuins. Nicotinamide, a product of the reaction catalysed by class III HADCs (see section 3) is a remarkable physiological inhibitor of these enzymes. In addition, a number of synthetic sirtuin inhibitors including splitomicin, dihydrocoumarin and sirtinol were identified several years ago [52,56,61,63-65]. Finally, several researchers have recently reported the synthesis and characterization of a series of new compounds as class III HDACs effective inhibitors (see, e.g., refs. [85-89]).

4.2. Activators of Sirtuin Activity

Although sirtuin inhibitors may be useful in treating some diseases, sirtuin activating compounds (STACs) have more promising therapeutic potential than sirtuin inhibitors since the use of STACs could be one of the keys to extending lifespan in mammals (in the absence of CR or genetic manipulation) and a possible pathway to get a long life. Therefore, the screening of novel natural and synthetic STACs will be, without a shadow of a doubt, an important and exciting area for future research.

To date, a significant number of STACs have been identified. In fact, at least 45 small molecules from plants were tested in 2003 by Howitz and co-workers as putative STACs in a high-throughput screening [90]. These molecules are natural polyphenols such as stilbenes (non-flavonoids) and flavonoids.

The stilbenes studied in this screening led by David Sinclair include *t*-RESV and a number of RESV derivatives (e.g.: piceatannol or astringenin 3,3',4,5'-tetrahydroxy-*trans*-stilbene; Fig. (**4**), for more details see the supplementary information of the ref. [90]).

Besides *t*-RESV, other polyphenolic compounds (called flavonoids) have been also tested by Howitz *et al.* [90]. Flavonoids are a large group of polyphenols present in plants, regularly consumed foods (e.g. vegetables and fruits), and beverages like tea and wine. These low molecular weight substances are phenylbenzo-γ-pyrones (phenyl-γ-chromones) with an assortment of structures based on a common three-ring nucleus. They are usually subdivided according to their substituents into several subclasses including anthocyanidins, flavanones, flavones, flavonols, flavanonols (or dihydroflavonols), chalcones, isoflavones and flavanols (flavan-3-ols) (also

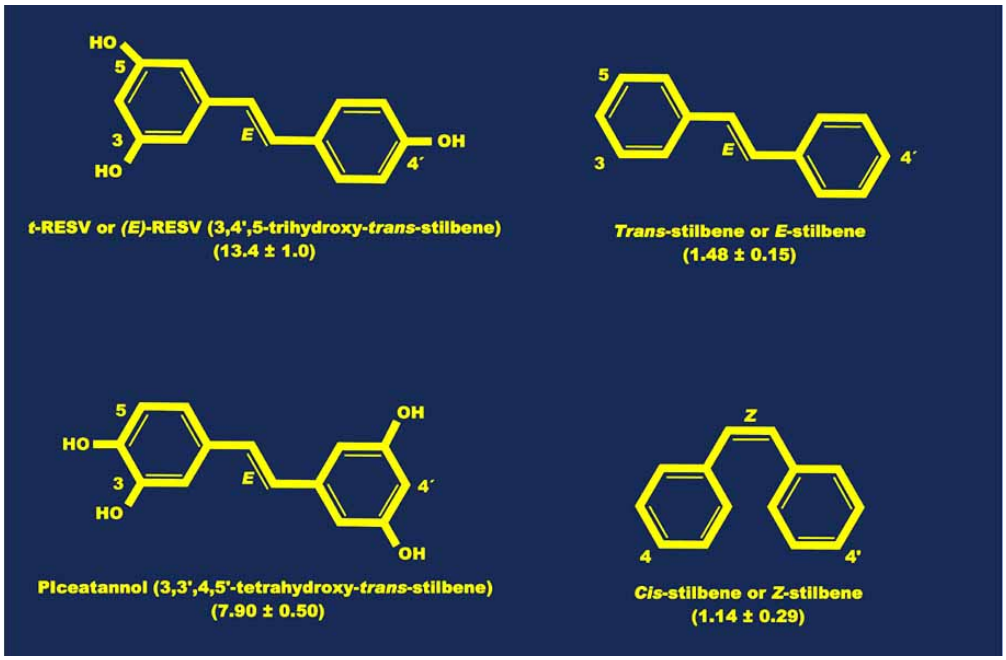
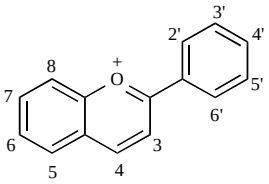
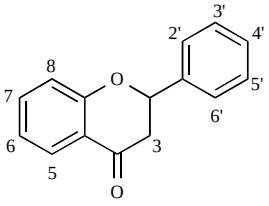
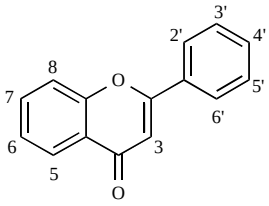


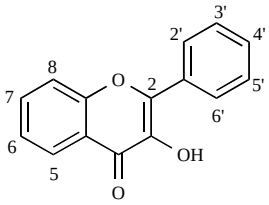
Fig. (4). Chemical structures of the most common stilbenes tested as human SIRT1 activators. Effects on SIRT1 catalytic rate. The corresponding values of fold activation of SIRT1 (at 100 μM) are shown in brackets.

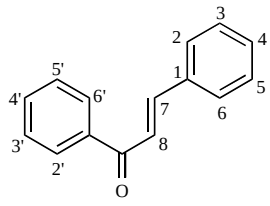
Table 1. Chemical Structures of the Most Common Flavonoids Tested as Human SIRT1 Activators. Effects on SIRT1 Catalytic Rate. The Corresponding Values of Fold Activation of SIRT1 (at 100 μM) are Shown in Brackets

ANTHOCYANIDINS	Flavonoid	3	5	6	7	3´	4´	5´
	Cyanidin (0.45 ± 0.015)	OH	OH	—	OH	OH	OH	—
	Delphinidin (0.45 ± 0.007)	OH	OH	—	OH	OH	OH	OH
	Pelargonidin (1.59 ± 0.037)	OH	OH	—	OH	—	OH	—

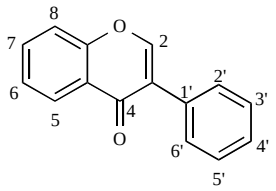
FLAVANONES	Flavonoid	3	5	7	3´	4´
	Flavanone (1.92 ± 0.24)	—	—	—	—	—
	Naringenin (2.10 ± 0.23)	—	OH	OH	—	OH
	Taxifolin (1.97 ± 0.22)	OH	OH	OH	OH	OH

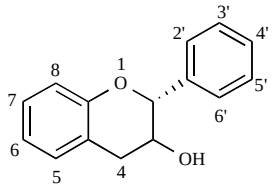
FLAVONES	Flavonoid	5	6	7	8	3´	4´
	Apigenin (2.77 ± 0.40)	OH	—	OH	—	—	OH
	Luteolin (5.66 ± 0.80)	OH	—	OH	—	OH	OH
	Scutellarein (3.06 ± 0.29)	OH	OH	OH	—	—	OH

FLAVONOLS	Flavonoid	5	7	2´	3´	4´	5´
	Fisetin (6.58 ± 0.69)	—	OH	—	OH	OH	—
	Kaempferol (3.55 ± 0.56)	OH	OH	—	—	OH	—
	Myricetin (0.90 ± 0.07)	OH	OH	—	OH	OH	OH
	Morin (1.46 ± 0.071)	OH	OH	OH	—	OH	—
	Quercetin (4.59 ± 0.47)	OH	OH	—	OH	OH	—

CHALCONES	Flavonoid	2´	4´	6´	3	4
	Butein (8.53 ± 0.89)	OH	OH	—	OH	OH
	Chalcone (1.34 ± 0.17)	—	—	—	—	—
	Isoliquiritigenin (7.57 ± 0.84)	OH	OH	—	—	OH

(Table 1). Contd…..

ISOFLAVONES	Flavonoid	5	7	4´	5´
	Daidzein (2.28 ± 0.74)	—	OH	OH	—
	Genistein (1.11 ± 0.026)	OH	OH	OH	—

FLAVANOLS (CATECHINS)	Flavonoid	3	5	7	3´	4´	5´
	(-)-Catechin (1.41 ± 0.21)	αOH(R)	OH	OH	OH	OH	—
	(-)-Epicatechin (1.53 ± 0.31)	αOH(R)	OH	OH	OH	OH	—
	(-)-Epigalocatechin (0.41 ± 0.11)	αOH(R)	OH	OH	OH	OH	OH

called catechins) (Table **1**). Some of the most representative flavonoids tested by Howitz *et al.* [90] and included in the different above-mentioned subclasses are shown in Table **1**.

The biological activities of flavonoids (including their antioxidant properties) and other features of these polyphenols have been reviewed comprehensively elsewhere and, therefore, will not be covered here. (see, e.g., refs. [91-97]).

Among the polyphenols studied as sirtuin activators in the screening led by David Sinclair [90], non-flavonoids such as hydroxylated stilbenes *(t*-RESV, piceatannol 3,3',4,5'-tetrahydroxy-*trans*-stilbene) and flavonoids such as chalcones (e.g.: butein, isoliquiritigenin), flavonols (e.g.: fisetin, quercetin, kaempferol) and flavones (e.g.: luteolin, scutelarein) are more powerful activators than non-hydroxylated stilbenes (*cis*- and *trans*-stilbene) and flavonoids such as isoflavones (e.g.: daidzein, genistein), flavanones (e.g.: naringenin, taxifolin), catechins [e.g.: (-)-catechin, (-)-epicatechin] and anthocyanidins (e.g.: pelargonidin, cyanidin, delphinidin) (Fig. (**4**), Table **1**; for more details on a possible structure-activity relationship see ref. [90]).

Although *t*-RESV displays the maximal stimulatory activity on human recombinant SIRT1 (~13-fold), compared with the other polyphenolic compounds studied (see Table **1** and Fig. (**4**)), it is less efficient (~2-2.5-fold) in stimulating Sir2 of *S. cerevisiae, C. elegans* and *D. melanogaster* [90,98].

Unfortunately, *t*-RESV has limitations as a drug, which include low solubility and low stability in solution (high sensitivity to light and to oxidation). These limitations possibly decrease its efficiency in extending lifespan in yeast experiments and to produce its typical pharmacological effects.

However, Yang *et al.* [99] have recently reported the synthesis and characterization of a series of stilbene derivatives and have shown that these new derivatives are superior to *t*-RESV in terms of their stability in solution, toxicity and ability to activate SIRT1 and to extend the lifespan of yeast cells. In addition, some drugs with well-known therapeutic applications (e.g.: sodium nitroprusside) have been reported to be potent activators of human SIRT1 expression and to extend lifespan in isolated peripheral blood mononuclear cells from young and healthy volunteers [100].

Finally, a number of new compounds such as benzimidazole, oxazolopyridine, benzothiazol, 1H-imidazo(1,2-a)pyridine and thaizolopyridine derivatives (which seem to be more stable in solution and more specific for sirtuin mediated deacetylation than *t*-RESV) have been recently patented by pharmaceutical companies (mainly Sirtris Pharmaceuticals, Inc.) as sirtuins-modulating compounds, useful for promoting cell survival and for the treatment of ageing-related diseases [55].

5. ANTI-AGING EFFECTS OF *t*-RESV

5.1. Effects in Yeasts, Flies and Worms

As indicated above (see Introduction), *t*-RESV seems to mimic the beneficial effects of CR and extend longevity (without reducing fecundity) in lower organisms (simple eukaryotes, e.g.: the budding yeast *Saccharomyces cerevisiae*) [90] and in short-lived invertebrates (simple metazoans such as the nematode (worm) *Caenorhabditis elegans* [98,101] and the fruit fly *Drosophila melanogaster* [98]). Mean lifespan was extended up to 70%, 18% and 29%, respectively. These effects have been attributed to activation of Sir2 (ySir2, dSir2 and Sir.2.1) enzymes since, as indicated above (see section 3.1), they have been implicated in mediating lifespan increases in yeast (ySir2), flies (dSir2) and worms (Sir2.1).

In contrast, Kaeberlein *et al.* [102] found no significant increase in *S. cerevisiae* lifespan and ySir2 activity *in vivo* with *t*-RESV treatment in three different yeast strain backgrounds, including the PSY316 strain used in the original study of Howitz *et al.* [90]. The basis for the discrepancy between these studies has not been resolved, but may be due to variability in growth conditions. In addition, using the commercially available Fluor de Lys (FdL) assay kit (from BioMol Research Laboratories, Inc.), Kaeberlein *et al.* [102] have described that the activation of Sir2 and SIRT1 by *t*-RESV *in vitro* depends of the use of a non-physiological substrate. Specifically, *t*-RESV enhances binding and deacetylation of acetylpeptide substrates containing the non-physiological fluorophore FdL (e.g.: p53-FdL), but has no effect on binding and deacetylation of native (physiological) acetylated peptides lacking the fluorophore (e.g.: p53). Similar results have been reported by Borra *et al.* for SIRT1 *in vitro* [103] (see below).

On the other hand, it is also interesting to note that Bass *et al.* [104] were recently unable to repeat the lifespan extension in *D. melanogaster* caused by *t*-RESV treatment, despite using the same strain of flies, the same nutrient media recipe and the same source of drug as that reported in the original study of Wood and co-workers [98]. Moreover, in contrast with the results of these researchers [98], the small and intermittent lifespan extension in *C. elegans* caused by *t*-RESV and observed by Bass and co-workers in trials conducted blind does not depend on Sir.2.1 activation [104].

In view of these apparent discrepancies, the effects of *t*-RESV in lifespan extension and the precise mechanism by which *t*-RESV acts in these organisms should be re-examined. In this connection, for example, further additional experiments are required to investigate and confirm whether *t*-RESV really activates ySir2 *in vivo* (see, e.g., ref. [105]).

5.2. Effects in Short-Lived Vertebrates (Seasonal Fish *Nothobranchius Furzeri*)

Valenzano *et al.* [106] have recently demonstrated that *t*-RESV prolongs mean lifespan up to approximately 56% and retards the expression of age-dependent traits, i.e., delays the age-dependent decay of locomotor activity and cognitive performances and reduces the expression of neurofibrillary degeneration in the brain of the short lived seasonal fish (vertebrate model) *Nothobranchius furzeri*, which has a maximum recorded lifespan of 13 weeks in captivity. The possible mechanisms by which *t*-RESV produces these beneficial effects (e.g.: the sirtuin activation) is still unknown since it has not been investigated [106,107].

5.3. Effects in Mammals

Since the relatively long lifespan of mammals is a hurdle for life-long pharmaceutical trials, the effects of *t*-RESV on the lifespan of these animals have not been fully investigated yet.

Despite this, a number of studies on the effects of *t*-RESV on glucose homeostasis in rodents (mice), which live approximately 2.5 years, have been recently reported. In fact, Lagouge *et al.* [108] and Baur *et al.* [109] have described that *t*-RESV promotes longevity and improves glucose homeostasis, energy balance and increases mithochondrial function in mice fed a high-fat diet by stimulating the SIRT1-mediated deacetylation of the transcriptional coactivator PGC-1α (for comparison of both studies see ref. [110]). Similar conclusions have been subsequently obtained for *t*-RESV and other structurally unrelated SIRT1 activators by Milne *et al.* [111]. These findings may have important implications for the treatment of type II diabetes and perhaps other diseases associated with aging (e.g.: Alzheimer disease; for details see refs. [112-115]).

It is likely that the beneficial effects of *t*-RESV on these age-related diseases in mammals are only partially mediated by the deacetylation by SIRT1 of different transcription factors which play an important role in the above pathologies (see section 3.2) because *t*-RESV is implicated in the regulation of many other enzymes and molecular pathways that might contribute to lifespan extension and delay of the onset of aging-related diseases [84,116]. In this connection, for example, Zhang [117] has recently reported that *t*-RESV may additionally inhibit the insulin signalling pathway in several cell lines and rat primary hepatocytes by a mechanism independent of the SIRT1 activation.

In other short-lived mammals (rats), contradictory results on the effects of *t*-RESV on glucose homeostasis have been recently described. Thus, an insulin-like effect of *t*-RESV has been reported in diabetic rats [118,119]. In contrast, a number of recent studies have demonstrated that *t*-RESV decreases insulin secretion from pancreatic islets of normal rats [120-122] and that it is able to affect insulin concentrations [118,119,123].

To summarize, taking into account all the above reports, *t*-RESV seems to be useful for retarding the onset of a number of diseases associated with aging in rodents by SIRT1 activation and maybe by other different mechanisms.

If *t*-RESV is able to produce the above-mentioned effects in primates (e.g.: rhesus monkeys and humans) and affect the aging process in the kind of cells in the heart and brain that are particularly susceptible to degeneration with age, perhaps one day the ingestion of a pill daily of *t*-RESV as a STAC to provoke an artificial sirtuin response and promote the same remarkable health benefits currently reserved for calorie-restricted animals will not be a fairy tale. Among others (see section 6), this is one of the possible predictions that the xenohormesis hypothesis makes.

5.4. Mechanism of Sirtuin Activation by *t*-RESV

As mentioned in the above paragraphs, the beneficial effects of *t*-RESV to extend lifespan in a variety of species seem to be mainly due to the increase of sirtuin enzymatic activity. In this connection, it is interesting to note that two different studies recently reported have shown that *t*-RESV suppresses angiotensin II type 1 receptor expression in rat aorta myocytes [124] and protects coronary artery endothelial cells against the adverse effects of cigarette smoking-induced oxidative stress through SIRT1 activation [125]. This vascular activity may contribute, at leas in part, to the beneficial anti-aging and cardioprotective effects of *t*-RESV (see sections 1 and 5.3).

The detailed mechanistic basis for Sir2 activation by *t*-RESV is not well understood, although kinetic data reveal that it stimulate Sir2 activity by lowering the Michaelis constant for both β-NAD^+ and the acetyl-lysine-bearing substrate by 35- and 5-fold, respectively [90,126].

Since the β_1-α_2 loop of the Sir2 proteins undergoes significant conformational adjustments to facilitate β-NAD^+ binding and catalysis, it has been proposed that *t*-RESV may somehow more optimally reconfigure the β_1-α_2 loop for β-NAD^+ binding [127].

In addition, a comparison between the different Sir2 complexes also reveals that the small zinc-binding domain of the catalytic core also makes adjustments that appear to be important for binding to β-NAD^+ and acetyl-lysine, thus suggesting that *t*-RESV may also interact with this domain in a way that enhances binding to one or both of the substrates (for more details see ref. [127]).

On the other hand, as indicated above (see section 5.1), Kaeberlein and co-workers have recently shown in *in vitro* studies that *t*-RESV enhances the enzymatic activity of ySir2 and human SIRT1. This enhancement totally depends on the use of acetylpeptide substrates (p53-FdL) containing the non-physiological fluorescent moiety FdL since *t*-RESV has no effect on binding and deacetylation of physiological acetylated peptides lacking the fluorophore (p53) [102]. Similar results have been reported by Borra *et al. f*or SIRT1 [103]. In addition, these latter researchers have investigated *in vitro* the molecular basis for SIRT1 activation by *t*-RESV.

In contrast with the results obtained by Kaeberlein *et al.* [102], among the three enzymes (ySir2, human SIRT1 and human SIRT2) tested by Borra and co-workers [103], only SIRT1 exhibited significant enzyme activation (~8-fold) by *t*-RESV.

To examine the requirements for this activation, Borra and co-workers [103] used three p53 acetylpeptide substrates either lacking a fluorophore or containing a 7-amino-4-methylcoumarin (p53-AMC) or rhodamine 110 (p53-R110). Although SIRT1 enzymatic activity stimulation was independent of the acetylpeptide sequence, the activation of SIRT1 by *t*-RESV was completely dependent on the presence of a covalently attached fluorophore to the acetylated peptide. In addition, the presence of the fluorophore decreased the binding affinity of the peptide to SIRT1. Without *t*-RESV, the coumarin of p53-AMC peptide makes no significant contact with SIRT1. However, in the presence of *t*-RESV, fluorophore-containing substrates bound more tightly to SIRT1.

The binding site for *t*-RESV has not yet been determined and will likely require a co-crystal X-ray structure. One possibility is that *t*-RESV binds to SIRT1 in the region immediately surrounding the coumarin binding site and may directly interact with the coumarin ring. The fact that *t*-RESV activates the binding of the p53-R110 peptide to SIRT1, however, argues against direct interaction

of *t*-RESV with the fluorophore, since rhodamine and coumarin are structurally different. Alternatively, *t*-RESV may bind to an allosteric site separate from the coumarin binding site. *t*-RESV binding to an allosteric site would induce conformational changes in SIRT1 allowing the coumarin group of the artificial (non-physiological) acetylpeptide substrate p53-AMC to bind more tightly (for details see ref. [103]).

6. *t*-RESV AND OTHER STACS: EVIDENCE FOR XENOHORMESIS?

As indicated above (see section 4.2), a number of natural polyphenols have been identified as potent STACs. These small molecules produced by plants (e.g.: *t*-RESV) extend the lifespan of a wide range of organisms from different phyla and are effective against a variety of age-related diseases in rodents (see section 5.3). It is unlikely that these effects are mainly due to the anti-oxidant activity of *t*-RESV since oxidative stress does not decrease yeast lifespan [71], and anti-oxidants do not extend lifespan of metazoans [128] (although see ref. [107] for a review of conflicting results).

According to Sinclair [52], to explain the above-mentioned beneficial effects of *t*-RESV, there are a number of possibilities, including the hypothesis that this stilbene mimics the action of an endogenous STAC in yeast and animals (see below). Indeed, *t*-RESV is considered as a phytoestrogen and is structurally very similar to the potent synthetic estrogen diethylstilbestrol [129]. In addition, there is another intriguing possibility: *t*-RESV and possibly other sirtuin-activating polyphenols might be plant stress-signaling molecules that coordinates sirtuin-mediated defences in plants [52,69,90].

The genes that encode the biosynthesis of sirtuins are present in all eukaryotes cells (vegetables and animals) studied. Therefore, it is logical to assume that these proteins are very ancient enzymes that existed in the common ancestor of today's eukaryotes, possibly more than a billion years ago. In addition, their function in extending lifespan and stress resistance is also highly conserved. Since plants possess numerous sirtuin family members and increase their own production (biosynthesis) of *t*-RESV in response to different stresses such as pathogenic attack, nutrient limitation, dehydration, etc. (see Introduction), it is reasonable to postulate that *t*-RESV is really a signalling molecule that coordinates in plants the defensive responses mediated by sirtuin activation [52,69,90]. This idea contrasts with the general notion that the above-mentioned stilbene is basically an antioxidant or a phytoalexin (i.e., a plant antimicrobial compound; see Introduction).

According to Lamming *et al.* [69], these findings raise an important question: why do *t*-RESV and other plant polyphenols stimulate sirtuins in yeasts and animals (e.g.: humans)? It is improbable that this is simply an inherent property of these enzymes because it would be lost quickly in the absence of positive selection. So what selective force could have maintained this property of sirtuins since the major eukaryotes diverged? According to Lamming and co-workers [69], one possible explanation is that fungi (e.g.: yeasts) and animals modulate the activity of their sirtuins with endogenous molecules, which should be quite different in chemical structure from *t*-RESV because *S. cerevisiae* and metazoans do not possess genes that resemble those used for *t*-RESV biosynthesis (e.g., the genes that encode the expression of the stilbene synthase; see Introduction). In fact, Kim *et al.* [130] have very recently reported the identification of an endogenous SIRT1 activator, which they have named active regulator of SIRT1 (AROS). AROS is a 142 amino acid nuclear protein with no previously defined function or homology to other proteins (for more details see refs. [130,131].

According to Lamming *et al.* [69], another possible explanation is that fungi (e.g.: yeasts) and animals have since lost their capacity to synthesize *t*-RESV and other polyphenols but have retained the ability to be activated by these plant molecules because they provide an useful advance warning of a deteriorating environment and/or loss of food supply, allowing organisms that eat the plants to begin increasing cell defences and, in turn, to prepare for and survive adversity when they might otherwise perish. In fact, in these situations, the message of impeding crisis may be passed on from plants to animals that consume these plants since *t*-RESV accumulation in stressed plants may be sufficient to induce a hormetic response (basically by stimulation of sirtuin enzymatic activity) in animals [52,69].

This interspecies communication of stress signals has been termed xenohormesis by Howitz and Sinclair [90] and, according to these researchers, this xenohormesis hypothesis makes a number of predictions:

First, stressed plants should be an abundant reservoir for medicinal compounds (e.g.: *t*-RESV and other natural polyphenols) that exhibit conserved beneficial protective effects in humans.

Second, these compounds should interact not only with the sirtuins but also with a variety of enzymes implicated in regulating stress responses, cell survival and longevity.

Third, the "xenohormetic molecules" should be relatively safe for human consumption and relatively non-toxic (i.e., practically devoid of negative side effects) since we evolved along with them.

t-RESV fulfils all of the above qualifications.

7. CONCLUDING REMARKS

Five hundred years ago (in the spring of 1512), three wooden ships commanded by the Spanish explorer Ponce de León left the warm Caribbean waters of Puerto Rico in search of gold and of the mythical fountain of youth. In this voyage, Ponce de León discovered inadvertently the land to the North (Florida), but he did not find the gold and the fountain of youth. The quest continues today, and the modern explorers are biologists seeking ways and magical molecules to modulate aging.

As indicated above, *t*-RESV is an important natural polyphenol and a fashionable dietary component with a plethora of beneficial effects for human health, including the possible extension of the duration of life in a number of investigated organisms from different phyla, such as yeasts, worms, flies and fish. In this connection, *t*-RESV is currently in Phase I/II clinical trials for treating recurrent herpes simplex virus type 1 (HSV-1) infections and colon cancer (see, e.g., refs. [52,132]).

Taking into account the above considerations and bearing in mind that *t*-RESV exhibits relatively good pharmacokinetic properties (see below) and that the toxicity studies have shown that this remarkable and fascinating polyphenolic compound has a good safety profile in humans and is not toxic even at high doses (see, e.g., refs. [132,133]), it is likely that *t*-RESV, *t*-RESV derivatives and other SIRT1 activators may represent a novel (see sections 4.2 and 5.3) class of wonder drugs useful for combating aging and a number of age-related diseases (see section 5.3), Therefore, *t*-RESV maybe may be used in the future (when conclusive results in humans on its anti-aging effects are provided) as one of the mythical, legendary, hypothetical, miraculous, magical and long-sought "elixirs of eternal youth".

On the other hand, it is interesting to note that the *t*-RESV concentrations reached in plasma and tissues after oral administration to rats or humans or after prolonged and daily consumption of moderate amounts of red wine appear to approach the concentrations that are active *in vitro* (usually in the range 1-30 μM; see, e.g., refs. [3,4,12,25,134]). In addition to free RESV isomers (*t*-RESV and *c*-RESV) present at variable concentrations in wines (see Introduction), a number of RESV derivatives (mainly β-glucosides) are also present. These may be absorbed directly, as reported for the rat small intestine and/or hydrolysed before absorption by glucosidases

present in the human intestinal tract, with subsequent release of free RESV. These RESV derivatives may contribute to the biologically available RESV dose (for review, see, e.g., refs. [3,4]).

Moreover, *t*-RESV is a lipophilic substance which is effectively absorbed after oral administration in rats, mice and humans, and which accumulates in rat and mouse tissues including heart, liver and kidney (for review, see, e.g., refs. [3,4,135]). For this reason, Bertelli *et al.* [136] concluded that an average drinker of wine can absorb a sufficient amount of RESV, at least in the long term, to explain the beneficial effects of red wine on health.

Therefore, bearing in mind the above considerations and reports and taking into account that:

1) Although *t*-RESV has been reported to have a relative low bioavailability since it may be rapidly metabolised in humans by glucuronidation and sulfation [135,137], the corresponding sulphates and glucuronides formed seem to retain in part the pharmacological activity of the natural compound [84,138].

2) There is synergy and interactions amongst *t*-RESV and a number of polyphenol compounds present in wines, which may increase the *t*-RESV biological activity [134] and improve the *t*-RESV bioavailability (by modifications of the pharmacokinetic properties and metabolism of this stilbene) [139-141].

3) The habitual answer of the world's oldest men when they are asked for the secret of their longevity and health is: "drink at least a good glass of red wine every day",

It is likely that the long-term and daily consumption of moderate amounts of red wine and other beverages containing significant *t*-RESV concentrations can be beneficial to extend lifespan and increase the life expectancy by delaying the onset of age-related diseases such as cancer, atherosclerosis, type II diabetes, etc.

8. ACKNOWLEDGMENTS

I apologize for failing to cite many relevant primary papers because of space constraints.

This work was supported in part by grants from the *Ministerio de Sanidad y Consumo* (Spain; FISS PI061537) and the *Xunta de Galicia* (Spain; PGIDIT05BTF20302PR and INCITE07PXI 203039ES).

I am especially grateful to the *Consellería de Educación e Ordenación Universitaria de la Xunta de Galicia* (Spain) for giving me financial support to intensify my research activity and to reduce my teaching activity during the academic year 2007-2008 *[Programa de promoción de intensificación de la actividad investigadora en el sistema Universitario de Galicia (SUG)].*

ABBREVIATIONS

CR	=	Caloric restriction
c-RESV	=	Resveratrol, *cis* isomer
FdL	=	Fluor de Lys
HDACs	=	Histone deacetylases
PNC1	=	Pyrazinamidasenicotinamidase 1
RESV	=	Resveratrol
STACs	=	Sirtuin activating compounds
t-RESV	=	Resveratrol, *trans* isomer

REFERENCES

[1] Aggarwal, B.B.; Bhardwaj, A.; Aggarwal, R.S.; Seeram, N.P.; Shishodia, S.; Takada, Y. *Anticancer Res.*, **2004**, *24*, 2783.
[2] Frémont, L. *Life Sci.*, **2000**, *66*, 663.
[3] Orallo, F. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 577-600.
[4] Orallo, F. *Curr. Med. Chem.*, **2006**, *13*, 87.
[5] Pervaiz, S. *FASEB J.*, **2003**, *17*, 1975.
[6] Alarcón de la Lastra, C.; Villegas, I. *Mol. Nutr. Food Res.*, **2005**, *49*, 405.
[7] Soleas, G.J.; Diamandis, E.P.; Goldberg, D.M. *Clin. Biochem.*, **1997**, *30*, 91.
[8] Soleas, G.J.; Diamandis, E.P.; Goldberg, D.M. *Adv. Exp. Med. Biol.*, **2001**, *492*, 159.
[9] Halls, C.; Yu, O. *Trends Biotechnol.*, **2008**, *26*, 77.
[10] Hain, R.; Bieseler, B.; Kindl, H.; Schroder, G.; Stocker, R. *Plant Mol. Biol.*, **1990**, *15*, 325.
[11] Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. *Phytochemistry*, **2000**, *55*, 77.
[12] Wu, J.M.; Wang, Z.R.; Hsieh, T.C.; Bruder, J.L.; Zou, J.G.; Huang, Y.Z. *Int. J. Mol. Med.*, **2001**, *8*, 3.
[13] Takaoka, M.J. *Fac. Sci. Hokkaido Imp. Univ.*, **1940**, *3*, 1.
[14] Nonomura, S.; Kanagawa, H.; Makimoto, A. *Yakugaku Zasshi*, **1963**, *83*, 988.
[15] Orsini, F.; Pelizzoni, F.; Verotta, L.; Aburjai, T.; Rogers, C.B. *J. Nat. Prod.*, **1997**, *60*, 1082.
[16] Paul, B.; Masih, I.; Deopujari, J.; Charpentier, C. *J. Ethnopharmacol.*, **1999**, *68*, 71.
[17] Langcake, P.; Pryce, R.J. *Experientia*, **1977**, *33*, 151.
[18] Renaud, S.; de Lorgeril, M. *Lancet*, **1992**, *339*, 1523.
[19] Siemann, E.H.; Creasy, L.L. *Am. J. Enol. Vitic.*, **1992**, *43*, 49.
[20] Bradamante, S.; Barenghi, L.; Villa, A. *Cardiovasc. Drug Rev.*, **2004**, *22*, 169.
[21] Delmas, D.; Jannin, B.; Latruffe, N. *Mol. Nutr. Food Res.*, **2005**, *49*, 377.
[22] Hao, H.D.; He, L.R. *J. Med. Food*, **2004**, *7*, 290.
[23] Olas, B.; Wachowicz, B. *Platelets*, **2005**, *16*, 251.
[24] Olas, B.; Wachowicz, B.; Tomczak, A.; Erler, J.; Stochmal, A.; Oleszek, W. *Platelets*, **2008**, *19*, 70.
[25] Orallo, F.; Álvarez, E.; Camiña, M.; Leiro, J.M.; Gómez, E.; Fernández, P. *Mol. Pharmacol.*, **2002**, *61*, 294.
[26] de la Lastra, C.A.; Villegas, I. *Biochem. Soc. Trans.*, **2007**, *35*, 1156.
[27] Baxter, R.A. *J. Cosmet. Dermatol.*, **2008**, *7*, 2.
[28] Saiko, P.; Szakmary, A.; Jaeger, W.; Szekeres, T. *Mutat. Res.*, **2008**, *658*, 68.
[29] Campos-Toimil, M.; Elíes, J.; Orallo, F. In *Recent Progress in Medicinal Plants. Vol. 21: Phytopharmacology and Therapeutic Values III.* Singh, V.K.; Govil, J.N., Eds.; Studium Press LLC: Houston, USA, **2008**, pp. 263-294.
[30] Granados-Soto, V. *Drug News Perspect.*, **2003**, *16*, 299.
[31] Ulrich, S.; Wolter, F.; Stein, J.M. *Mol. Nutr. Food Res.*, **2005**, *49*, 452.
[32] Pezzuto, J. M. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 233-383.
[33] Young-Joon, S.; Kundu, J. K. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 601-617.
[34] Das, S.; Das, D.K. *Inflamm. Allergy Drug Targets*, **2007**, *6*, 168.
[35] Gescher, A.J. *Planta Med.*, **2008**, in press.
[36] Harikumar, K.B.; Aggarwal, B.B. *Cell Cycle*, **2008**, in press.
[37] Pirola, L.; Frojdo, S. *IUBMB Life*, **2008**, *60*, 323.
[38] Chen, D.; Guarente, L. *Trends Mol. Med.*, **2007**, *13*, 64.
[39] Michan, S.; Sinclair, D. *Biochem. J.*, **2007**, *404*, 1.
[40] Labinskyy, N.; Csiszar, A.; Veress, G.; Stef, G.; Pacher, P.; Oroszi, G.; Wu, J.; Ungvari, Z. *Curr. Med. Chem.*, **2006**, *13*, 989.
[41] Mcay, C.M.; Crowell, M.F.; Maynard, L.A. *J. Nutr.*, **1935**, *10*, 63.
[42] Calabrese, E.J. *Ageing Res. Rev.*, **2008**, *7*, 8.
[43] Dilova, I.; Easlon, E.; Lin, S.J. *Cell Mol. Life Sci.*, **2007**, *64*, 752.
[44] Fontana, L.; Klein, S. *JAMA*, **2007**, *297*, 986.
[45] Masoro, E.J. *Interdiscip. Top. Gerontol.*, **2007**, *35*, 1.
[46] Guarente, L. *Mech. Ageing Dev.*, **2005**, *126*, 923.
[47] Anderson, R.M.; Weindruch, R. *Exp. Gerontol.*, **2006**, *41*, 1247.
[48] Ingram, D.K.; Zhu, M.; Mamczarz, J.; Zou, S.; Lane, M.A.; Roth, G.S.; deCabo, R. *Aging Cell*, **2006**, *5*, 97.
[49] Yu, B.P. *Biogerontology*, **2006**, *7*, 179.
[50] López-Torres, M.; Barja, G. *Biochim. Biophys. Acta*, **2008**, in press.
[51] Pamplona, R.; Barja, G. *Ageing Res. Rev.*, **2007**, *6*, 189.
[52] Sinclair, D.A. *Mech. Ageing Dev.*, **2005**, *126*, 987.
[53] Dali-Youcef, N.; Lagouge, M.; Froelich, S.; Koehl, C.; Schoonjans, K.; Auwerx, J. *Ann. Med.*, **2007**, *39*, 335.
[54] Longo, V.D.; Kennedy, B.K. *Cell*, **2006**, *126*, 257.
[55] Pallàs, M.; Verdaguer, E.; Tajes, M.; Gutiérrez-Cuesta, J.; Camins, A. *Recent Patents. CNS. Drug Discov.*, **2008**, *3*, 61.
[56] Westphal, C.H.; Dipp, M.A.; Guarente, L. *Trends Biochem. Sci.*, **2007**, *32*, 555.
[57] Yamamoto, H.; Schoonjans, K.; Auwerx, J. *Mol. Endocrinol.*, **2007**, *21*, 1745.
[58] Guarente, L. *Cell*, **2008**, *132*, 171.
[59] Neugebauer, R.C.; Sippl, W.; Jung, M. *Curr. Pharm. Des.*, **2008**, *14*, 562.
[60] Blander, G.; Guarente, L. *Annu. Rev. Biochem.*, **2004**, *73*, 417.
[61] Denu, J.M. *Curr. Opin. Chem. Biol.*, **2005**, *9*, 431.
[62] Holbert, M.A.; Marmorstein, R. *Curr. Opin. Struct. Biol.*, **2005**, *15*, 673.
[63] Sauve, A.A.; Wolberger, C.; Schramm, V.L.; Boeke, J.D. *Annu. Rev. Biochem.*, **2006**, *75*, 435.

[64] Grubisha, O.; Smith, B.C.; Denu, J.M. *FEBS J.*, **2005**, *272*, 4607.
[65] Porcu, M.; Chiarugi, A. *Trends Pharmacol. Sci.*, **2005**, *26*, 94.
[66] Kaeberlein, M.; Powers III, R.W. *Ageing Res. Rev.*, **2007**, *6*, 128.
[67] Lamming, D.W.; Latorre-Esteves, M.; Medvedik, O.; Wong, S.N.; Tsang, F.A.; Wang, C.; Lin, S.J.; Sinclair, D.A. *Science*, **2005**, *309*, 1861.
[68] Marmorstein, R. *Biochem. Soc. Trans.*, **2004**, *32*, 904.
[69] Lamming, D.W.; Wood, J.G.; Sinclair, D.A. *Mol. Microbiol.*, **2004**, *53*, 1003.
[70] Yang, H.; Lavu, S.; Sinclair, D.A. *Exp. Gerontol.*, **2006**, *41*, 718.
[71] Lin, S.J.; Kaeberlein, M.; Andalis, A.A.; Sturtz, L.A.; Defossez, P.A.; Culotta, V.C.; Fink, G.R.; Guarente, L. *Nature*, **2002**, *418*, 344.
[72] Lin, S.J.; Ford, E.; Haigis, M.; Liszt, G.; Guarente, L. *Genes Dev.*, **2004**, *18*, 12.
[73] Anderson, R.M.; Bitterman, K.J.; Wood, J.G.; Medvedik, O.; Sinclair, D.A. *Nature*, **2003**, *423*, 181.
[74] Gallo, C.M.; Smith, D.L., Jr.; Smith, J.S. *Mol. Cell Biol.*, **2004**, *24*, 1301.
[75] Yang, H.; Yang, T.; Baur, J.A.; Pérez, E.; Matsui, T.; Carmona, J.J.; Lamming, D.W.; Souza-Pinto, N.C.; Bohr, V.A.; Rosenzweig, A.; de Cabo, R.; Sauve, A.A.; Sinclair, D.A. *Cell*, **2007**, *130*, 1095.
[76] Ognjanovic, S.; Bao, S.; Yamamoto, S.Y.; Garibay-Tupas, J.; Samal, B.; Bryant-Greenwood, G.D. *J. Mol. Endocrinol.*, **2001**, *26*, 107.
[77] Samal, B.; Sun, Y.; Stearns, G.; Xie, C.; Suggs, S.; McNiece, I. *Mol. Cell Biol.*, **1994**, *14*, 1431.
[78] Revollo, J.R.; Grimm, A.A.; Imai, S. *J. Biol. Chem.*, **2004**, *279*, 50754.
[79] Revollo, J.R.; Grimm, A.A.; Imai, S. *Curr. Opin. Gastroenterol.*, **2007**, *23*, 164.
[80] Fukuhara, A.; Matsuda, M.; Nishizawa, M.; Segawa, K.; Tanaka, M.; Kishimoto, K.; Matsuki, Y.; Murakami, M.; Ichisaka, T.; Murakami, H.; Watanabe, E.; Takagi, T.; Akiyoshi, M.; Ohtsubo, T.; Kihara, S.; Yamashita, S.; Makishima, M.; Funahashi, T.; Yamanaka, S.; Hiramatsu, R.; Matsuzawa, Y.; Shimomura, I. *Science*, **2005**, *307*, 426.
[81] Tanaka, M.; Nozaki, M.; Fukuhara, A.; Segawa, K.; Aoki, N.; Matsuda, M.; Komuro, R.; Shimomura, I. *Biochem. Biophys. Res. Commun.*, **2007**, *359*, 194.
[82] Finkel, T. *Nature*, **2003**, *425*, 132.
[83] Blagosklonny, M.V. *Drug Discov. Today*, **2007**, *12*, 218.
[84] Baur, J.A.; Sinclair, D.A. *Nat. Rev. Drug Discov.*, **2006**, *5*, 493.
[85] Kiviranta, P.H.; Leppanen, J.; Kyrylenko, S.; Salo, H.S.; Lahtela-Kakkonen, M.; Tervo, A.J.; Wittekindt, C.; Suuronen, T.; Kuusisto, E.; Jarvinen, T.; Salminen, A.; Poso, A.; Wallen, E.A. *J. Med. Chem.*, **2006**, *49*, 7907.
[86] Mai, A.; Massa, S.; Lavu, S.; Pezzi, R.; Simeoni, S.; Ragno, R.; Mariotti, F.R.; Chiani, F.; Camilloni, G.; Sinclair, D.A. *J. Med. Chem.*, **2005**, *48*, 7789.
[87] Napper, A.D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J.F.; Barker, J.; Yau, W.T.; Amouzegh, P.; Flegg, A.; Hamelin, E.; Thomas, R.J.; Kates, M.; Jones, S.; Navia, M.A.; Saunders, J.O.; DiStefano, P.S.; Curtis, R. *J. Med. Chem.*, **2005**, *48*, 8045.
[88] Neugebauer, R.C.; Uchiechowska, U.; Meier, R.; Hruby, H.; Valkov, V.; Verdin, E.; Sippl, W.; Jung, M. *J. Med. Chem.*, **2008**, *51*, 1203.
[89] Trapp, J.; Meier, R.; Hongwiset, D.; Kassack, M.U.; Sippl, W.; Jung, M. *Chem. Med. Chem.*, **2007**, *2*, 1419.
[90] Howitz, K.T.; Bitterman, K.J.; Cohen, H.Y.; Lamming, D.W.; Lavu, S.; Wood, J.G.; Zipkin, R.E.; Chung, P.; Kisielewski, A.; Zhang, L.L.; Scherer, B.; Sinclair, D.A. *Nature*, **2003**, *425*, 191.
[91] Arts, I.C.; Hollman, P.C. *Am. J. Clin. Nutr.*, **2005**, *81*, 317S.
[92] Manach, C.; Mazur, A.; Scalbert, A. *Curr. Opin. Lipidol.*, **2005**, *16*, 77.
[93] Middleton, E.; Kandaswami, C.; Theoharides, T.C. *Pharmacol. Rev.*, **2000**, *52*, 673.
[94] Zern, T.L.; Fernández, M.L. *J. Nutr.*, **2005**, *135*, 2291.
[95] Li, Y.; Fang, H.; Xu, W. *Mini. Rev. Med. Chem.*, **2007**, *7*, 663.
[96] Lotito, S.B.; Frei, B. *Free Radic. Biol. Med.*, **2006**, *41*, 1727.
[97] Shankar, S.; Ganapathy, S.; Srivastava, R.K. *Front Biosci.*, **2007**, *12*, 4881.
[98] Wood, J.G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S.L.; Tatar, M.; Sinclair, D. *Nature*, **2004**, *430*, 686.
[99] Yang, H.; Baur, J.A.; Chen, A.; Miller, C.; Adams, J.K.; Kisielewski, A.; Howitz, K.T.; Zipkin, R.E.; Sinclair, D.A. *Aging Cell*, **2007**, *6*, 35.
[100] Engel, N.; Mahlknecht, U. *Int. J. Mol. Med.*, **2008**, *21*, 223.
[101] Viswanathan, M.; Kim, S.K.; Berdichevsky, A.; Guarente, L. *Dev. Cell*, **2005**, *9*, 605.
[102] Kaeberlein, M.; McDonagh, T.; Heltweg, B.; Hixon, J.; Westman, E.A.; Caldwell, S.D.; Napper, A.; Curtis, R.; DiStefano, P.S.; Fields, S.; Bedalov, A.; Kennedy, B.K. *J. Biol. Chem.*, **2005**, *280*, 17038.
[103] Borra, M.T.; Smith, B.C.; Denu, J.M. *J. Biol. Chem.*, **2005**, *280*, 17187.
[104] Bass, T.M.; Weinkove, D.; Houthoofd, K.; Gems, D.; Partridge, L. *Mech. Ageing Dev.*, **2007**, *128*, 546.
[105] Kaeberlein, M.; Kennedy, B.K. *Aging Cell*, **2007**, *6*, 415.
[106] Valenzano, D.R.; Terzibasi, E.; Genade, T.; Cattaneo, A.; Domenici, L.; Cellerino, A. *Curr. Biol.*, **2006**, *16*, 296.
[107] Valenzano, D.R.; Cellerino, A. *Cell Cycle*, **2006**, *5*, 1027.
[108] Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. *Cell*, **2006**, *127*, 1109.
[109] Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; López-Lluch, G.; Lewis, K.; Pistell, P.J.; Poosala, S.; Becker, K.G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K.W.; Spencer, R.G.; Lakatta, E.G.; Le Couteur, D.; Shaw, R.J.; Navas, P.; Puigserver, P.; Ingram, D.K.; de Cabo, R.; Sinclair, D.A. *Nature*, **2006**, *444*, 337.
[110] Koo, S.H.; Montminy, M. *Cell*, **2006**, *127*, 1091.
[111] Milne, J.C.; Lambert, P.D.; Schenk, S.; Carney, D.P.; Smith, J.J.; Gagne, D.J.; Jin, L.; Boss, O.; Perni, R.B.; Vu, C.B.; Bemis, J.E.; Xie, R.; Disch, J.S.; Ng, P.Y.; Nunes, J.J.; Lynch, A.V.; Yang, H.; Galonek, H.; Israelian, K.; Choy, W.; Iffland, A.; Lavu, S.; Medvedik, O.; Sinclair, D.A.; Olefsky, J.M.; Jirousek, M.R.; Elliott, P.J.; Westphal, C.H. *Nature*, **2007**, *450*, 712.
[112] Anekonda, T.S. *Brain Res. Rev.*, **2006**, *52*, 316.
[113] Chen, J.; Zhou, Y.; Mueller-Steiner, S.; Chen, L.F.; Kwon, H.; Yi, S.; Mucke, L.; Gan, L. *J. Biol. Chem.*, **2005**, *280*, 40364.
[114] Kim, D.; Nguyen, M.D.; Dobbin, M.M.; Fischer, A.; Sananbenesi, F.; Rodgers, J.T.; Delalle, I.; Baur, J.A.; Sui, G.; Armour, S.M.; Puigserver, P.; Sinclair, D.A.; Tsai, L.H. *EMBO J.*, **2007**, *26*, 3169.
[115] Rasouri, S.; Lagouge, M.; Auwerx, J. *Med. Sci. (Paris)*, **2007**, *23*, 840.
[116] O'Brian, C. A.; Chu, F. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 133-147.
[117] Zhang, J. *Biochem. J.*, **2006**, *397*, 519.
[118] Chi, T.C.; Chen, W.P.; Chi, T.L.; Kuo, T.F.; Lee, S.S.; Cheng, J.T.; Su, M.J. *Life Sci.*, **2007**, *80*, 1713.
[119] Su, H.C.; Hung, L.M.; Chen, J.K. *Amer. J. Physiol. Endocrinol. Metab.*, **2006**, *290*, E1339.
[120] Szkudelski, T. *Eur. J. Pharmacol.*, **2006**, *552*, 176.
[121] Szkudelski, T. *Am. J. Physiol. Endocrinol. Metab.*, **2007**, *293*, E901.
[122] Szkudelski, T. *Life Sci.*, **2008**, *13*, 430.
[123] Chen, W.P.; Chi, T.C.; Chuang, L.M.; Su, M.J. *Eur. J. Pharmacol.*, **2007**, *568*, 269.
[124] Miyazaki, R.; Ichiki, T.; Hashimoto, T.; Inanaga, K.; Imayama, I.; Sadoshima, J.; Sunagawa, K. *Arterioscler. Thromb. Vasc. Biol.*, **2008**, in press.
[125] Csiszar, A.; Labinskyy, N.; Podlutsky, A.; Kaminski, P.M.; Wolin, M.S.; Zhang, C.; Mukhopadhyay, P.; Pacher, P.; Hu, F.; de Cabo, R.; Ballabh, P.; Ungvari, Z.I. *Am. J. Physiol. Heart Circ. Physiol.*, **2008**, in press.
[126] Hall, S.S. *Science*, **2003**, *301*, 1165.
[127] Zhao, K.; Harshaw, R.; Chai, X.; Marmorstein, R. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 8563.
[128] Bauer, J.H.; Goupil, S.; Garber, G.B.; Helfand, S.L. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 12980.
[129] Gehm, B. D.; Levenson, A. S. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 439-464.
[130] Kim, E.J.; Kho, J.H.; Kang, M.R.; Um, S.J. *Molecular Cell*, **2007**, *28*, 277.
[131] Verdin, E. *Molecular Cell*, **2007**, *28*, 354.
[132] Espín, J.C.; García-Conesa, M.T.; Tomás-Barberán, F.A. *Phytochemistry*, **2007**, *68*, 2986.
[133] Boocock, D.J.; Faust, G.E.; Patel, K.R.; Schinas, A.M.; Brown, V.A.; Ducharme, M.P.; Booth, T.D.; Crowell, J.A.; Perloff, M.; Gescher, A.J.; Steward, W.P.; Brenner, D.E. *Cancer Epidemiol. Biomarkers Prev.*, **2007**, *16*, 1246.
[134] Pignatelli, P.; Ghiselli, A.; Buchetti, B.; Carnevale, R.; Natella, F.; Germano, G.; Fimognari, F.; Di Santo, S.; Lenti, L.; Violi, F. *Atherosclerosis*, **2006**, *188*, 77.
[135] Bertelli, A. A. E. In *Resveratrol in Health and Disease*; Aggarwal, B.B.; Shishodia, S., Eds.; CRC Press: Boca Raton, USA, **2006**, pp. 631-642.
[136] Bertelli, A.; Bertelli, A.A.; Gozzini, A.; Giovannini, L. *Drugs Exp. Clin. Res.*, **1998**, *24*, 133.
[137] Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E., Jr.; Walle, U.K. *Drug Metab. Dispos.*, **2004**, *32*, 1377.
[138] Wang, L.X.; Heredia, A.; Song, H.; Zhang, Z.; Yu, B.; Davis, C.; Redfield, R. *J. Pharm. Sci.*, **2004**, *93*, 2448.
[139] de Santi, C.; Pietrabissa, A.; Mosca, F.; Pacifici, G.M. *Xenobiotica*, **2000**, *30*, 1047.
[140] de Santi, C.; Pietrabissa, A.; Spisni, R.; Mosca, F.; Pacifici, G.M. *Xenobiotica*, **2000**, *30*, 857.
[141] de Santi, C.; Pietrabissa, A.; Spisni, R.; Mosca, F.; Pacifici, G.M. *Xenobiotica*, **2000**, *30*, 609.

Received: March 12, 2008 **Revised: May 09, 2008** **Accepted: May 15, 2008**