Effectiveness of Resveratrol Against Cardiovascular Disease

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Abstract: In recent years, resveratrol has become a popular nutritional supplements used by humans all over the world. Much research has been conducted to determine the efficacy of its use both in preventive and therapeutic dimensions. Usage of functional foods with a history of ethno-use with no known side effects is an area of great interest in improving the overall health condition of the population. Resveratrol, with its known potency and wide variety of health benefits has shown promising results in minimizing cardiovascular complications including hypertension, hypertrophy, ischemic heart disease and atherosclerosis. However, a great deal of controversy exists regarding the use of resveratrol as an anti-aging compound. In this review, progress in the chemical and biological production of resveratrol and its derivatives, their biological targets, bioavailability as well as the mechanisms of resveratrol cardio-protection will be discussed.

Keywords: Resveratrol, heart, ischemia/reperfusion, cardioprotection, redox regulation.

1. INTRODUCTION

Resveratrol (*trans*-3, 5, 4'- trihydroxystilbene) is a phenolic phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi. Resveratrol has also been produced by chemical synthesis [1] and is sold as a nutritional supplement derived primarily from Japanese knotweed. It is found in several plants, most notably in the skin and seeds of grapes, and red wine. Resveratrol is a fat-soluble compound that occurs in a *trans*- and a *cis*- configuration (Fig. **1**). Both *cis*- and *trans*- resveratrol also occur as glucosides, which is called piceid. Resveratrol effectively scavenges free radicals and other oxidants [2] and therefore may provide benefits to health/ the heart. These effects may contribute at least in part to the "French paradox" [3], derived from the observation that cardiovascular disease is relatively low in France, despite high consumption of high fat diet because of the consumption of red wine with the meal (i.e. 2-3 glasses/day) [3,4]. At present numerous reports are available on the diverse health benefits of resveratrol cardiovascular disease [5], antitumor activity [6,7], Alzheimer's disease [8], estrogenic and anti-estrogenic activities [9], effects on biotransformation enzymes [10] and preservation of normal cell cycle regulation [11]. Recently, resveratrol has been shown to mimic caloric restriction [12], and extend the lifespan of a number of species including yeast [13] and mice [12], although this observation has been challenged by several reports [14].

Cardiovascular disease (CVD) remains the major cause of mortality and morbidity in both developed and developing countries, responsible for roughly 25% of all deaths per year. High-fat diet, abnormalities in lipoproteins, overweight, smoking, sedentary lifestyle and genetic factors contribute to the risk of CVD including atherosclerosis and stroke [15]. The main cause of these diseases is coronary arterial blockage, commonly known as atherosclerosis, resulting in an ischemic condition followed by a necrotic scar formation. Clearing of the atherosclerotic blockage can render the heart at risk by a sudden increase in oxygen and, therefore, free-radical production that result in cardiac injury through both necrosis and apoptosis [16]. Resveratrol has shown potential cardioprotective effects by its ability to inhibit platelet aggregation [17], promote vasodilation by enhancing the production of NO [18], inhibit inflammatory enzymes [19] and induce autophagy [20]. The multidimensional effects of resveratrol on pharmacology and conditions that might benefit from it administration are documented in this review.

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2. STRUCTURES, STABILITY AND SOURCES

Resveratrol is a stilbenoid, a derivate of stilbene, which is produced in plants with the help of the enzyme stilbene synthase. It exists as two geometric isomers: *cis*- (*Z*) and *trans*- (*E*), the *trans*-isomer being shown in Fig. (**1**). The *trans*- form can undergo isomerization to the *cis*- form when exposed to ultraviolet irradiation [21]. *Trans*-resveratrol in the powder form is stable under "accelerated stability" conditions of 75% humidity and 40^0C in the presence of air [22]. Resveratrol content also remains stable in the skins of grapes and pomace taken after fermentation when stored for a long period [23]. In addition, HPLC assays can distinguish the two isomers since *cis*-resveratrol exhibits a distinct λmax and retention time [24]. Therefore, resveratrol concentrations may be underestimated when only the trans-resveratrol peak is taken into account during analysis. The amount of resveratrol found in natural foods is summarized in Table **1**.

Table 1. The Amount of Resveratrol Found in Natural Foods [25,26]

Source	Resveratrol Concentration
100% Natural peanut butter	0.65 μg/g
Bilberries	16 ng/g
Blueberries	32 ng/g
Boiled peanuts	5.1 μg/g
Cranberry raw juice	0.2 mg/L
Dry grape skin	24.06 μg/g
Grapes	0.16-3.54 μg/g
Peanut butter	0.3-1.4 μg/g
Peanuts	0.02-1.92 μg/g
Pistachios	0.09-1.67 μg/g
Ports and sherries	<0.1 mg/L
Ref grape juice	0.50 mg/L
Red wines	0.1-14.3 mg/L
Roasted peanuts	0.055 μg/g
White grape juice	0.05 mg/L
White wines	<0.1-2.1 mg/L

3. RESVERATROL SUPPLEMENTATION

As a result of extensive news coverage, sales of resveratrol supplements greatly increased in 2006, despite caution that benefits to humans are unproven [27]. Most resveratrol supplements are oral

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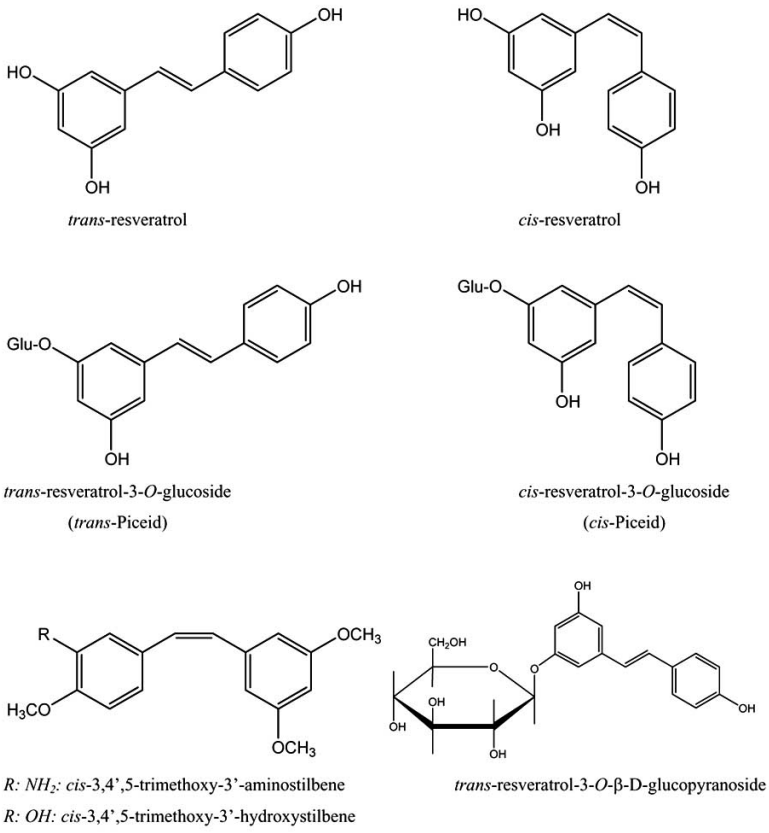

Fig. (1). Structures of Resveratrol.

pills intended to be swallowed. However, resveratrol lozenges are meant to be kept and consumed in the mouth to improve bioavailability. Red wine extracts and red grape extracts containing resveratrol and other polyphenols are also available in the U.S. as dietary supplements. Resveratrol supplements may contain anywhere from 10-50 mg of resveratrol, however, the effective dose for chronic disease prevention in humans are not known. As in many areas of sales and marketing, false advertising and free trials are often used to lure prospective customers. Consumers are strongly advised to take precautions to protect themselves.

4. PHARMACOKINETICS

Oral intake is the major route of resveratrol administration. The main dietary source of resveratrol is red wine; wine ingestion represents a way of administering resveratrol in natural conditions. Although resveratrol exists naturally as both *cis*- and *trans*- isomers, most studies have used *trans*- resveratrol for administration due to lack of stability of the *cis*- isomer, which is not commercially available. On the other hand, *trans*- resveratrol is often reported to be the major natural form, even though the *cis*- isomer is also present in wine [28]. Several resveratrol metabolites have been identified in human plasma or urine. Glucuronide and sulfate conjugates are the most frequently reported metabolites and may bind one or two hydroxyl residues or a carbon in position 2 [29]. Glucuronide-sulfate metabolites have also been described. After oral intake of 1 g resveratrol, Boocock *et al.* identified two monosulfate conjugates, one disulfate, two monoglucuronides, and, interestingly, one glucuronide–sulfate [30]. Recently, Burkon and Somoza identified up to seven *trans-resveratrol* metabolites (including two new diglucuronides) in plasma and urine from nine healthy volunteers after administration of 85.5mg of piceid (corresponding to 50mg of free resveratrol) [29]. *cis*- metabolites have also been detected in several studies. In urine samples collected from five volunteers after wine consumption, the main metabolites were *cis*-resveratrol-4'-sulfate, *cis*-resveratrol-3-*O*-glucuronide, and *cis*-resveratrol-4'-*O*-glucuronide [31]. However, no information is available to indicate whether these metabolites are the result of *cis*- resveratrol metabolism or isomerization of *trans*- sulfate or glucuronide conjugates. To determine total plasma resveratrol or metabolite concentrations, it is also necessary to take into account LDL and protein-bound fractions. Burkon and Somoza reported that, *in vitro*, more than 90% of free *trans*- resveratrol is bound to human plasma lipoproteins in a non-covalent manner [29]. In humans [32] and rats [33] less than 5% of the oral dose is observed as free resveratrol in blood plasma. The most abundant resveratrol metabolites in humans, rats, and mice are *trans*-resveratrol-3-*O*-glucuronide and trans-resveratrol-3-sulfate [34]. Walle suggested sulfate conjugates as the primary source of activity [32] while Wang *et al.* suggested the glucuronides [35] as the primary source. Boocock *et al.* emphasized the need for further study of the effects of the metabolites, including the possibility of deconjugation to free resveratrol inside cells [36]. The hypothesis that resveratrol from wine could have higher bioavailability than resveratrol from a pill [37] has been disproved by experimental data [38]. Although *trans*-resveratrol appears to be well-absorbed by humans when taken orally, its bioavailability is relatively low due to its rapid metabolism and elimination [32, 37].

Information about the bioavailability of resveratrol in humans is important because much of the basic research on resveratrol has been conducted in cultured cells exposed to unmetabolized resveratrol at concentrations that are often 10-100 times greater than peak concentrations observed in human plasma after oral consumption [39]. Although cells that line the digestive tract are exposed to unmetabolized resveratrol, research in humans suggests that other tissues are exposed primarily to resveratrol metabolites. Little is known about the biological activity of resveratrol

metabolites, and it is not known whether some tissues are capable of converting resveratrol metabolites back to resveratrol [32].

5. BIOLOGICAL ACTIVITIES RELATED TO CARDIOVASCULAR DISEASE PREVENTION

5.1. Free Radicals Mediated Disease Prevention

Reactive oxygen species (ROS) and/or oxidative stress play a significant role in the pathogenesis of a wide variety of human cardiovascular diseases including congestive heart failure, vascular heart disease, cardiomyopathy, hypertrophy, atherosclerosis and ischemic heart disease [40]. NADPH oxidase, a key enzyme responsible for the generation of ROS that is expressed by many cell types found both in vessel wall and in blood (leukocytes), has been implicated in the pathogenesis of hypercholesterolemia [41]. It is suggested that excessive ROS generation induces atherosclerosis [42]. Manifestation of atherosclerotic plaque formation is materialized when there is an alliance of intracellular lipid accumulation or lipidoses with accumulation of extracellular connective tissue matrix or fibrosis [42]. Sato *et al*. [43] suggested that resveratrol can inhibit ischemia/reperfusion induced free radicals mediated myocardium stress. The results demonstrated that both red wine extract and resveratrol were equally cardioprotective, as evidenced by their abilities to improve postischemic ventricular functions including developed pressure and aortic flow. The amount of malonaldehyde formation in the postischemic myocardium was reduced by both wine and resveratrol, indicating a reduction of oxidative stress developed in the ischemic reperfused myocardium. *In vitro* studies revealed that red wine extract is a potent antioxidant as evidenced by its ability to scavenge peroxyl radical *in vitro*. eNOS is an enzyme that catalyzes the formation of nitric oxide (NO) by vascular endothelial cells. NO is needed to maintain arterial relaxation (vasodilation), and impaired NO-dependent vasodilation is associated with increased risk of cardiovascular disease [44]. Several studies also demonstrated a direct role of NO in resveratrol-mediated cardioprotection [45, 46]. For example, resveratrol induced an expression of eNOS in the human umbilical vein endothelial cells (HUVEC) [46]. In addition to its long-term effects on eNOS expression, resveratrol also enhanced the production of bioactive NO in short term basis (within 2 min), suggesting a role of iNOS. Our results support these previous observations as we also observed iNOS expression within 24 h, while eNOS expression did not become apparent until after 3 days [47]. In another study, resveratrol induced the expression of iNOS in cultured bovine pulmonary artery endothelial cells [48]. This result was further supported by the finding that resveratrol protected isolated working rat hearts through the up-regulation of iNOS [47]. Resveratrol failed to provide cardioprotection in iNOS knockout mice devoid of any copy of iNOS gene, further supporting the role of NO [49]. In a more recent study, resveratrol reduced myocardial ischemia/reperfusion injury through both an iNOS-dependent and an iNOS-independent manner [45]. Similar to NO, resveratrol significantly reduced the amount of pro-adhesive molecules including sICAM-1, sVCAM-1 and E-selectin in the ischemic reperfused myocardium [50].

ANTI-INFLAMMATORY

There is increasing evidence that resveratrol exerts multifaceted anti-inflammatory effects in various disease models [51]. The ability of resveratrol to protect cardiovscular disorders mostly comes from the anti inflammatory property of resveratrol. Resveratrol significantly improveed postischemic ventricular function and reduced myocardial infract size compared to the non-treated control group. Suppressed aberrant expression of tissue factor and cytokines in vascular cells achieved by resveratrol is due to the anti inflammatrory role of resveratrol [52]. During the last decade, a growing number of experimental studies have demonstrated beneficial effects of both poly (ADP-ribose) polymerase (PARP) inhibitors and the genetic deletion of the PARP-1 enzyme in various animal models of increased cardiovascular oxidative stress and inflammation [53]. The proven cardioprotective effects of PARP inhibitors in diabetes are of particular interest, because diabetes is known to be associated with accelerated vascular aging. There are also reports that chronic administration of PARP inhibitors improved cardiac and vascular dysfunction in aged rats (reviewed in references [54]. It is thought that inhibition of PARP decreases NAD^+ consumption and raises nuclear NAD^+ levels, which in turn is likely to activate the sirtuins. Additionally, the mechanism behind the protective effects of PARP-1 inhibitors may involve prevention of the upregulation of various proinflammatory pathways (cytokines, adhesion receptors, mononuclear cell infiltration [55].

5.2. Cardioprotection and Anti-Aging Mechanism

Cardioprotection was supported by the generation of survival signal as evidenced by increased phosphorylation of Akt and activation of Bcl-2. In contrast, there was increased activation of Ref-1 and the transcription factor IκB [56]. Resveratrol generated a survival signal by inducing the activation of p38MAP kinase β and Akt, and inhibition of p38 MAP kinase α as well as to increase DNA binding of NFκB. High doses of resveratrol completely reversed the survival signal into a death signal by reducing Bcl-2 expression, Akt phosphorylation and NFκB activation [57]. The longevity mechanism of resveratrol are not fully understood, some studies indicate that resveratrol activates SIRT1 [58] and PGC-1α and improve functioning of the mitochondria [59]. Further research is required to connect the mechanism of resveratrol's action on SIRT1 and calorie restriction [60]. In addition resveratrol's ability to directly activate SIRT1 has been called into question [61]. Resveratrol has been shown to cause SIRT1 activation and to cause migration of FOXO transcription factors to the nucleus [62] which stimulates FOXO3a transcriptional activity [63]. The enhancement of the sirtuin-catalyzed deacetylation (activity) of FOXO3a. MnSOD is known to be a target of FOXO3a, and MnSOD expression is strongly induced in cells overexpressing FOXO3a [64]. MnSOD reduces superoxide and thereby confers resistance to mitochondrial dysfunction, permeability transition, and apoptotic death in various diseases [65].

5.3. Redox Regulated Heart Diseases

The cellular changes associated with ischemic heart diseases are redox regulated. Ischemia and reperfusion render the heart in an oxidized environment maintained by the stabilizing disulfides present in the extracellular surface, while the intracellular environment is maintained in the reduced state with the help of free sulfhydryl groups. The principal disulfide reductase responsible for maintaining the inside of the cell in the reduced state is thioredoxin [66]. Thioredoxins and other members of the thioredoxin superfamily such as glutaredoxins and peroxiredoxins are ubiquitously present in mammalian cells including the heart [66] and play an important role in maintaining the redox environment of the cell. Our recent studies demonstrate that transgenic mice overexpressing Grx1 reduced the number of apoptotic cardiomyocytes in the ischemic reperfused heart [66, 67]. Dudley *et al.* compared the effects of low and high doses of resveratrol on redox cycling of several redox proteins and redox-sensitive transcription factors. The results indicated down-regulation of the transcripts of Grx1 and Grx2 as well as of Trx1 and Trx2 in the ischemic reperfused hearts with high doses of resveratrol. Lower doses of resveratrol increased the transcripts of Trx1 and Trx2 as well as of Grx1 and Grx2 compared to control [57]. Malik *et al.* showed potentiation of a survival signal through the redox activation of Ref-1, a major protein of the DNA base excision repair pathway, following myocardial ischemia reperfusion injury [56]. Further, a recent study showed that ischemia/reperfusion could potentiate a rapid translocation of TRx-1 into

the nucleus, which then interacts with Ref-1, leading to the generation of a survival signal [68]. Consistent with these reports, Dudley *et al.* summarized that low doses of resveratrol generated a survival signal by activating both Trx and Ref-1, while high doses of resveratrol produced a death signal by reducing both Ref-1 and Trx expression [58].

5.4. Autophagosome and Resveratrol

Autophagy is a self-clearing process to remove the damaged proteins or organelles, an alternate mechanism for proteasomal degradation, which can generate a survival signal, as in the case of myocardial ischemia [69]. In order to study the mechanism of resveratrol-induced autophagy, we examined the activation of mTOR, a molecule known to be repressed during autophagic induction. Resveratrol at lower doses (0.1 and 1 mM in H9c2 cardiac myoblast cells and 2.5 mg/kg/day in rats) induced cardiac autophagy shown by enhanced formation of autophagosomes and its marker LC3-II after hypoxia-reoxygenation or ischaemia-reperfusion [20]. The autophagy was attenuated with the higher dose of resveratrol. The induction of autophagy was correlated with enhanced cell survival and decreased apoptosis. Treatment with rapamycin (100 nM), a known inducer of autophagy, did not further increase autophagy compared with resveratrol alone. Autophagic inhibitors, Wortmannin (2 mM) and 3-methyladenine (10 mM), significantly attenuated the resveratrol induced autophagy and induced cell death. The activation of mammalian target of rapamycin (mTOR) was differentially regulated by low-dose resveratrol, i.e. the phosphorylation of mTOR at serine 2448 was inhibited, whereas the phosphorylation of mTOR at serine 2481 was increased, which was attenuated with a higher dose of resveratrol. Although resveratrol attenuated the activation of mTOR complex 1, low-dose resveratrol significantly induced the expression of Rictor, a component of mTOR complex 2, and activated its downstream survival kinase Akt (Ser 473). Resveratrol-induced Rictor was found to bind with mTOR. Furthermore, treatment with Rictor SiRNA attenuated the resveratrol-induced autophagy. Our results indicate that at lower dose, resveratrol-mediated cell survival is, in part, mediated through the induction of autophagy involving the mTOR-Rictor survival pathway. These results are in accordance with a recent study, where resveratrol has been shown to inhibit oxidized LDL-induced PI3K/Akt-mediated phosphorylation of mTOR (Ser2448) and its downstream molecule p70s6k in rabbit femoral smooth muscle cells [70].

6. ADVERSE EFFECTS

The adverse effects of high dose resveratrol intake in humans have been investigated in several studies resveratrol intake [36,71,72], representing a total of 104 patients (including placebo). The highest doses were 5g/70 kg for a single intake and 0.9g/day for iterative administration, corresponding, respectively, to approximately 1/40 and 1/200 of the dose reported to cause nephrotoxicity and 1/4 and 1/20 of the highest dose reported to be safe in rats [73]. No serious adverse event was detected in any of these studies. Adverse events were mild and lasted a few days. After a single administration of 400 mg of resveratrol, Vaz-da-Silva *et al*. [71] reported three events (blood electrolyte changes, nasopharyngitis and erythematous rash) in 3/24 patients, possibly related to treatment. In the other single-dose administration pharmacokinetic study [36], 2/40 patients receiving 1 g resveratrol exhibited one or more minor biological adverse event, consisting of a small increase in blood bilirubin [44] or alanine amino transferase level. In the multiple-dose study, 40 volunteers received one dose of resveratrol (25, 50 100, 150 mg, or placebo) every 4 h for 48 h. The most frequent adverse event was frontal headache (three cases). The other adverse events appeared only once: headache, myalgia of the lower extremities, somnolence (25mg group), epididymitis (100mg group), and dizziness and occipital headache (150mg group), without any clear relation to the administered dose [72]. In a study in rats, Crowell *et al.* [73] administered 0.3, 1 and 3 g/kg/day *trans*-resveratrol for 4 wk (corresponding to 21, 70, and 210 g/day, respectively, in a human weighing 70 kg). Only two of the 40 rats receiving the highest doses died due to the treatment. Most of the adverse events occurred with the higher dose and consisted mainly of nephrotoxicity. No histological effect on the liver was observed and no adverse event was observed in animals treated with 0.3 g/kg/day. In addition, the results of this study did not confirm previous observations of a mild increase in serum liver aspartate aminotransferase enzyme levels and brain and testicular weight after a 20 mg/kg/day intake for 4 weeks [74]. In a recent study conducted on high-purity *trans*-resveratrol, Williams *et al.* have studied numerous toxicity models *in vivo* and *in vitro*. Low and high doses of resveratrol, up to 750 mg/kg/day for 3 months, were investigated *in vivo* in rabbits and rats. The authors concluded that resveratrol is well tolerated and non-toxic and has no effect on reproductive capacity in male or female rats and no embryofetal toxicity [75].

CONCLUSION

The present review consolidates the findings of resveratrol intake, especially on metabolism, bioavailability and cardioprotection, which in a way reveals inspiring outcomes though there are some questions of its adverse effect at higher doses. The main affects generally come *via* an antioxidant defense mechanism and interacting with several factors which are responsible for cardioprotection. Further, it is speculated that the cardioprotective ability at low doses of resveratrol is related to the current popular proposition about the health benefits of "moderate" wine drinking. The combination of the indirect and direct actions along with no-known side-effects, confirmed by the multi-millennial consumption, makes the resveratrol highly attractive for the patients of ischemic heart disease both in preventive and therapeutic point of view.

The bioavailability and use of proper doses remain the problem of use of resveratrol. The future research should emphasize on these two issues. A number of methods are being used to increase the bioavailability of resveratrol. Some manufacturers are micronizing the resveratrol to increase the bioavailability. Resveratrol displays hormesis [76]. As a phytoalexin, like many plant-derived toxins, resveratrol is also beneficial at lower doses and detrimental at higher doses resulting a J-shape or inverted U-shape curve suggesting howmetic action. Before clinical use and epidemiological studies should be altered regarding the hormetic action of resveratrolol.

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REFERENCES

[1] Farina, A.; Ferranti, C.; Marra, C. An improved synthesis of resveratrol. *Nat. Prod. Res.*, **2006**, *20*(3), 247-52.
[2] Stojanovic, S.; Sprinz, H.; Brede, O. Efficiency and mechanism of the antioxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. *Arch. Biochem. Biophys.*, **2001**, *391*(1), 79-89.
[3] Constant, J. Alcohol, ischemic heart disease, and the French paradox. *Coron. Artery Dis.*, **1997**, *8*(10), 645-649.
[4] Cottart, C.H.; Nivet-Antoine, V.; Laguillier-Morizot, C.; Beaudeux, J.L. Resveratrol bioavailability and toxicity in humans. *Mol. Nutr. Food Res.*, **2010**, *54*(1), 7-16.
[5] Renaud, S.C.; Gueguen, R.; Schenker, J.; d'Houtaud, A. Alcohol and mortality in middle-aged men from eastern France. *Epidemiology*, **1998**, *9*(2), 184-188.
[6] Kundu, J.K.; Surh, Y.J. Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. *Cancer Lett.*, **2008**, *269*(2), 243-261.
[7] Bishayee, A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev. Res.*, **2009**, *2*(5), 409-418.

[8] Marambaud, P.; Zhao, H.; Davies, P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. *J. Biol. Chem.*, **2005**, *280*(45), 37377-37382.

[9] Tangkeangsirisin, W.; Serrero, G. *Resveratrol in the Chemoprevention and Chemotherapy of Breast Cancer*. Bagchi, D.; Preuss, H.G. Eds. Phytopharmaceuticals in Cancer Chemoprevention. Boca Raton: CRC Press. **2005**, 449-463.

[10] Chen, Z.H.; Hurh, Y.J.; Na, H.K. Resveratrol inhibits TCDD-induced expression of CYP1A1 and CYP1B1 and catechol estrogen-mediated oxidative DNA damage in cultured human mammary epithelial cells. *Carcinogenesis*, **2004**, *25*(10), 2005-2013.

[11] Joe, A.K.; Liu, H.; Suzui, M.; Vural, M.E.; Xiao, D.; Weinstein, I.B. Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. *Clin. Cancer Res.*, **2002**, *8*(3), 893-903.

[12] Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-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. Resveratrol improves health and survival of mice on a highcalorie diet. *Nature*, **2006**, *444*(7117), 337-342.

[13] 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.; Sinclairm D.A. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*, **2003**, *425*(6954), 191-196.

[14] Bass, T.M.; Weinkove, D.; Houthoofd, K.; Gems, D.; Partridge, L. Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*. *Mech. Ageing Dev.*, **2007**, *128*(10), 546-52.

[15] Khot, U.N.; Khot, M.B.; Bajzer, C.T.; Sapp, S.K., Ohman, E.M.; Brener, S.J.; Ellis, S.G.; Lincoff, A.M.; Topol, E.J. Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA*, **2003**, *290*(7), 898-904.

[16] Buja, L.M.; Eigenbrodt, M.L.; Eigenbrodt, E.H. Apoptosis and necrosis. Basic types and mechanisms of cell death. *Arch. Pathol. Lab Med.*, **1993**, *117*(12), 1208-14.

[17] Kirk, R.I.; Deitch, J.A.; Wu, J.M.; Lerea, K.M. Resveratrol decreases early signaling events in washed platelets but has little effect on platelet in whole blood. *Blood Cells Mol. Dis.*, **2000**, *26*(2), 144-150.

[18] Wallerath, T.; Deckert, G.; Ternes, T.; Anderson, H.; Li, H.; Witte, K.; Förstermann, U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation*, **2002**, *106*(13), 1652-1658.

[19] Szewczuk, L.M.; Forti, L.; Stivala, L.A.; Penning, T.M. Resveratrol is a peroxidase-mediated inactivator of COX-1 but not COX-2: a mechanistic approach to the design of COX-1 selective agents. *J. Biol. Chem.*, **2004**, *279*(21), 22727-22737.

[20] Gurusamy, N.; Lekli, I.; Mukherjee, S.; Ray, D.; Ahsan, K.M.; Gherghiceanu, M.; Popescu, M.L.; Das, K.D. Cardioprotection by resveratrol: a novel mechanism *via* autophagy involving the mTORC2 pathway. *Cardiovasc. Res.*, **2010**, *86*(1), 103-112.

[21] Rosa, M.L.; Ana, I.; Romero-Perez, Andrew, L.; Waterhouse, M.; Carmen de la Torre-Boronat. Direct HPLC Analysis of cis- and trans-Resveratrol and Piceid Isomers in Spanish Red Vitis vinifera Wines. *J. Agric. Food Chem.*, **1995**, *43*(2), 281-283.

[22] Prokop, J.; Abrman, P.; Seligson, A.L.; Sovak, M. Resveratrol and its glycon piceid are stable polyphenols. *J. Med. Food*, **2006**, *9*(1), 11-14.

[23] Bertelli, A.A.; Gozzini, A.; Stradi, R.; Stella, S.; Bertelli, A. Stability of resveratrol over time and in the various stages of grape transformation. *Drugs Exp. Clin. Res.*, **1998**, *24*(4), 207-211.

[24] Camont, L.; Cottart, C.; Rhayem, Y.; Nivet-Antoine, V.; Djelidi, R.; Collin, F.; Beaudeux, J.L.; Bonnefont-Rousselot, D. Simple spectrophotometric assessment of the *trans*-/*cis*-resveratrol ratio in aqueous solutions. *Anal. Chim. Acta*, **2009**, *634*(1), 121-128.

[25] Burns, J.; Yokota, T.; Ashihara, H.; Lean, M.E.J.; Crozier, A. Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.*, **2002**, *50*(11), 3337-3340.

[26] Jane, H. "*Resveratrol*". Oregon State University. The Linus Pauling Institute Micronutrient Information Center, USA **2005**.

[27] Zachary, M.S. Quest for youth drives craze for 'wine' pills. *Wall Street J.* (30 November 2006). Retrieved 2008-02-02.

[28] Ribeiro de Lima, M.T.; Waffo-Teguo, P.; Teissedre, P.L.; Pujolas, A.; Vercauteren, J.; Cabanis, J.C.; Mérillon, J.M. Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid, and *cis*- and *trans*-resveratrol) in Portuguese wines. *J. Agric. Food Chem.*, **1999**, *47*(7), 2666-2670.

[29] Burkon, A.; Somoza, V. Quantification of free and proteinbound *trans*-resveratrol metabolites and identification of *trans*-resveratrol-C/O-conjugated diglucuronides - two novel resveratrol metabolites in human plasma. *Mol. Nutr. Food Res.*, **2008**, *52*(5), 549-557.

[30] Boocock, D.J.; Patel, K.R.; Faust, G.E.; Normolle, D.P.; Marczylo, T.H.; Crowell, J.A.; Brenner, D.E.; Booth, T.D.; Gescher, A.; Steward, W.P. Quantitation of trans-resveratrol and detection of its metabolites in human plasma and urine by high performance liquid chromatography. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.*, **2007**, *848*, 182-187.

[31] Urpi-Sarda, M.; Zamora-Ros, R.; Lamuela-Raventos, R.; Cherubini, A.; Jauregui, O., de la Torre, R.; Covas, M.I.; Estruch, R.; Jaeger, W.; Andres-Lacueva, C. HPLC-tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin. Chem.*, **2007**, *53*(2), 292-299.

[32] Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E.; Walle, U.K. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.*, **2004**, *32*(12), 1377-1382.

[33] Abd El-Mohsen, M.; Bayele, H.; Kuhnle, G.; Gibson, G., Debnam, E., Kaila Srai, S., Rice-Evans, C., Spencer, J.P. Distribution of [3H] *trans*-resveratrol in rat tissues following oral administration. *Br. J. Nutr.*, **2006**, *96*(1), 62-70.

[34] Yu, C.; Shin, Y.G.; Chow, A.; Li, Y.; Kosmeder, J.W.; Lee, Y.S.; Hirschelman, W.H.; Pezzuto, J.M.; Mehta, R.G.; van Breemen, R.B. Human, rat, and mouse metabolism of resveratrol. *Pharm. Res.*, **2002**, *19*(12), 1907-1914.

[35] Wang, L.X.; Heredia, A.; Song, H.; Zhang, Z.; Yu, B.; Davis, C.; Redfield, R. Resveratrol glucuronides as the metabolites of resveratrol in humans: characterization, synthesis, and anti-HIV activity. *J. Pharm. Sci.*, **2004**, *93*(10), 2448-2457.

[36] 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, DE. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol. Biomarkers Prev.*, **2007**, *16*(6), 1246-1252.

[37] Wenzel, E.; Somoza, V. Metabolism and bioavailability of trans-resveratrol. *Mol. Nutr. Food Res.*, **2005**, *49*(5), 472-481.

[38] Vitaglione, P.; Sforza, S.; Galaverna, G.; Ghidini, C.; Caporaso, N.; Vescovi, P.P.; Fogliano, V.; Marchelli, R. Bioavailability of *trans*-resveratrol from red wine in humans. *Mol. Nutr. Food Res.*, **2005**, *49*(5), 495-504.

[39] Gescher, A.J.; Steward, W.P. Relationship between mechanisms, bioavailibility, and preclinical chemopreventive efficacy of resveratrol: a conundrum. *Cancer Epidemiol. Biomarkers Prev.*, **2003**, *12*(10), 953-957.

[40] Das, D.K.; Maulik, N. Protection against free radical injury in the heart and cardiac performance. In: Sen Cen, K.; Packer, L.; Hanninen, O. Eds. *Exercise and Oxygen Toxicity*. Amsterdam: Elsevier Science, **1995**, pp. 359-388.

[41] Cave, A.C.; Breweer, A.C.; Narayanapanicker, A.; Ray, R.; Grieve, D.J.; Walker, S.; Shah, A.M. NADPH oxidases in cardiovascular health and disease. *Anitoxid. Redox Singnal.*, **2006**, *8*(5-6), 691-728.

[42] Stocker, R., Keaney, JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.*, **2004**, *84*(4), 1381-1478.

[43] Sato, M.; Ray, P.S.; Maulik, N.; Engelman, R.M.; Bertelli, A.A.E.; Bertelli, A.; Das D.K. Myocardial protection with red wine extract. *J. Cardiovasc. Pharmacol.*, **2000**, *35*(2), 263-268.

[44] Bulmer, A.C., Blanchfield, J.T., Toth, I., Fassett, R.G., Coombes, J.S. Improved resistance to serum oxidation in Gilbert's syndrome: a mechanism for cardiovascular protection. *Atherosclerosis*, **2008**, *199*(2), 390-396.

[45] Hung, L.M.; Su, M.J.; Chen, J.K. Resveratrol protects myocardial ischemia–reperfusion injury through both NO-dependent and NOindependent mechanisms. *Free Radic. Biol. Med.*, **2004**, *36*(6), 774- 781.

[46] Wallerath, T.; Deckert, G.; Ternes, T.; Anderson, H.; Li, H.; Witte, K.; Forstermann, U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation*, **2002**, *106*(13), 1652-1658.

[47] Das, S.; Alagappan, V.K.T.; Bagchi, D.; Sharma, H.S.; Maulik, N.; Das, D.K. Coordinated induction of iNOS-VEGF-KDR-eNOS after resveratrol consumption A potential mechanism for resveratrol preconditioning of the heart. *Vascul. Pharmacol.*, **2005**, *42*(5-6), 281-289.

[48] Hsieh, T.C.; Juan, G.; Darzynkiewicz, Z.; Wu, J.M. Resveratrol increasesnitric oxide synthase, induces accumulation of p53 and p21(WAF1/CIP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. *Cancer Res.*, **1999**, *59*(11), 2596-2601.

[49] Imamura, G.; Bertelli, A.A.; Bertelli, A.; Otani, H.; Maulik, N.; Das, D.K. Pharmacological preconditioning with resveratrol: an insight with iNOS knockout mice. *Am. J. Physiol.*, **2002**, *282*(6), 1996-2003.

[50] Das, S.; Falchi, M.; Bertelli, A.; Maulik, N.; Das, D.K. Attenuation of ischemia/reperfusion injury in rats by the anti-inflammatory action of resveratrol. *Arzneimittelforschung*, **2006**, *56*(10), 700-706.

[51] Delal, C.A., Astra, C.A., Villegas, I. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Mol. Nutr. Food Res.*, **2005**, *49*(5), 405-430

[52] Pendurthi, U.R., Williams, J.T., Rao, L.V. Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells : A possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. *Arterioscler. Thromb. Vasc. Biol.*, **1999**, *19*(2), 419-426.

[53] Bisht, K., Wagner, K.H., Bulmer, A.C. Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto- and DNA-protective dietary compounds. *Toxicology*, **2009**, (in press) doi:10.1016/j.tox.2009.11.008

[54] Pacher, P, Mabley, J.G., Soriano, F.G., Liaudet, L., Komjati, K., Szabó, C. Endothelial dysfunction in aging animals,the role of poly (ADP-ribose) polymerase activation. *Br. J. Pharmacol.*, **2002**, *135*(6),1347-1350.

[55] Csiszar, A., Pacher, P., Kaley G., Ungvari, Z. Role of oxidative and nitrosative stress, longevity genes and poly(ADP-ribose) polymerase in cardiovascular dysfunction associated with aging. *Curr. Vasc. Pharmacol.*, **2005**, *3*(3), 285-291.

[56] Malik, G.; Gorbounov, N.; Das, S.; Gurusamy, N.; Otani, H.; Maulik, N.; Goswami, S.; Das, D.K. Ischemic preconditioning triggers nuclear translocation of thioredoxin and its interaction with Ref-1 potentiating a survival signal through the PI-3-kinase-Akt pathway. *Antioxid. Redox Signal.,* **2006**, *8*(11-12), 2101-2109.

[57] Dudley, J.; Das, S.; Mukherjee, S.; Das, D.K. Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose. *J. Nutr. Biochem.,* **2009**, *20*(6), 443-452.

[58] Alcaín, F.J.; Villalba, J.M. Sirtuin activators. *Expert Opin. Ther. Pat.,* **2009**, *19*(4), 403-414.

[59] 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. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell*, **2006**, *127*(6), 1109-1122.

[60] 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. Substrate-specific activation of sirtuins by resveratrol. *J. Biol. Chem.,* **2005**, *280*(17), 17038-17045.

[61] Pacholec, M.; Chrunyk, B.A.; Cunningham, D.; Flynn, D.; Griffith, D.A.; Griffor, M.; Loulakis, P.; Pabst, B.; Qiu, X.; Stockman, B.; Thanabal, V.; Varghese, A.; Ward, J.; Withka, J.; Ahn, K. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *J. Biol. Chem.,* **2010**, *285*(11), 8340-8351.

[62] Stefani, M.; Markus, M.A.; Lin, R.C.; Pinese, M.; Dawes, I.W.; Morris, B.J. The effect of resveratrol on a cell model of human aging. *Ann. N.Y. Acad. Sci.,* **2007**, *1114*, 407-418.

[63] Brunet, A.; Sweeney, L.B.; Sturgill, J.F.; Chua, K.F.; Greer, P.L.; Lin, Y.; Tran, H.; Ross, S.E.; Mostoslavsky, R.; Cohen, H.Y.; Hu, L.S.; Cheng, H.L.; Jedrychowski, M.P.; Gygi, S.P.; Sinclair, D.A.; Alt, F.W.; Greenberg, M.E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*, **2004**, *303*(5666), 2011-2015.

[64] Kops, G.J.; Dansen, T.B.; Polderman, P.E. ; Saarloos, I.; Wirtz, K.W.; Coffer, P.J.; Huang, T.T.; Bos, J.L.; Medema, R.H.; Burgering, B.M. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature,* **2002**, *419*(6904), 316-321.

[65] Macmillan-Crow, L.A.; Cruthirds, D.L. Invited review: manganese superoxide dismutase in disease. *Free Radic. Res.,* **2001**, *34*(4), 325-336.

[66] Das, D.K. Thioredoxin regulation of ischemic preconditioning. *Antioxid. Redox Signal*., **2004**, *6*(2), 405-412.

[67] Turoczi, T.; Chang, V.W.; Engelman, R.M.; Maulik, N.; Ho, Y.S.; Das, D.K. Thioredoxin redox signaling in the ischemic heart: an insight with transgenic mice overexpressing Trx1. *J. Mol. Cell Cardiol*., **2003**, *35*(6), 695-704.

[68] Edwards, M.; Rassin, D.k.; Izumi, T.; Mitra, S.; Perez-Polo, J.R. APE/Ref-1 responses to oxidative stress in aged rats. *J. Neurosci. Res.,* **1998**, *54*(5), 635-638.

[69] Gustafsson, A.B.; Gottlieb, R.A. Recycle or die: the role of autophagy in cardioprotection. *J. Mol. Cell Cardiol.,* **2008**, *44*(4), 654-661.

[70] Brito, P.M.; Devillard, R.; Negre-Salvayre, A.; Almeida, L.M.; Dinis, T.C.; Salvayre, R.; Augé, N. Resveratrol inhibits the mTOR mitogenic signaling evoked by oxidized LDL in smooth muscle cells. *Atherosclerosis*, **2009**, *205*(1), 126-134.

[71] Vaz-da-Silva, M.; Loureiro, A.I.; Falcao, A.; Nunes, T.; Rocha, J.F.; Fernandes-Lopes, C.; Soares, E.; Wright, L.; Almeida, L.; Soares-da-Silva, P. Effect of food on the pharmacokinetic profile of transresveratrol. *Int. J. Clin. Pharmacol. Ther.,* **2008**, *46*(11), 564-570.

[72] Almeida, L.; Vaz-da-Silva, M.; Falcao, A.; Soares, E.; Costa, R.; Loureiro, A.I.; Fernandes-Lopes, C.; Rocha, J.F.; Nunes, T.; Wright, L.; Soares-da-Silva, P. Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers. *Mol. Nutr. Food Res.,* **2009**, *53*(Suppl 1), S7-S15.

[73] Crowell, J.A.; Korytko, P.J.; Morrissey, R.L.; Booth, T.D.; Levine, B.S. Resveratrol-associated renal toxicity. *Toxicol. Sci.,* **2004**, *82*(2), 614-619.

[74] Juan, M.E.; Vinardell, M.P.; Planas, J.M. The daily oral administration of high doses of trans-resveratrol to rats for 28 days is not harmful. *J. Nutr.,* **2002**, *132*(2), 257-260.

[75] Williams, L.D.; Burdock, G.A.; Edwards, J.A.; Beck, M.; Bausch, J. Safety studies conducted on high-purity transresveratrol in experimental animals. *Food Chem. Toxicol.,* **2009**, *47*(9), 2170-2182.

[76] Mukherjee, S.; Dudley, J.I.; Das, D.K. Dose-dependency of resveratrol in providing health benefits. *Dose-response,* **2010** (in press) doi: 10.2203/dose-response.09-015. Mukherjee.

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