Antioxidant effects of resveratrol in cardiovascular, cerebral and metabolic diseases

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Article info
Article history:
Available online 18 July 2013

Keywords:
Oxidative stress
Nitric oxide
Antioxidant
Cardiovascular
Brain
Diabetes

Abstract
Resveratrol—a natural polyphenolic compound—was first discovered in the 1940s. Although initially used for cancer therapy, it has shown beneficial effects against most cardiovascular and cerebrovascular diseases. A large part of these effects are related to its antioxidant properties. Here we review: (a) the sources, the metabolism, and the bioavailability of resveratrol; (b) the ability of resveratrol to modulate redox signalling and to interact with multiple molecular targets of diverse intracellular pathways; (c) its protective effects against oxidative damage in cardio-cerebro-vascular districts and metabolic disorders such as diabetes; and (d) the evidence for its efficacy and toxicity in humans. The overall aim of this review is to discuss the frontiers in the field of resveratrol’s mechanisms, bioactivity, biology, and health-related use.

1. Introduction
1.1. Structure and history of resveratrol
Resveratrol—3,4',5-trihydroxy-trans-stilbene (MW: 228.2)—is a natural non-flavonoid polyphenol compound containing a stilbene structure similar to that of estrogen diethylstilbestrol (Fig. 1a). It is a fat-soluble compound existing in cis, trans-, and piceid isomeric forms (Fig. 1b). It was first isolated in 1940 from the roots of white hellebore (Veratrum grandiflorum O. Loes) and later, in 1963, from the roots of Polygonum cuspidatum, a plant used in traditional Chinese and Japanese medicine (Nonomura et al., 1963). Resveratrol has been in use since ancient times as an Indian herbal preparation termed ‘Darakchasava’, which is derived from fermented grapes. Remarkably, the effects described for Darakchasava more than 4500 years ago (Singh et al., 2013) are the same described for resveratrol today. Darakchasava is produced by several pharmaceutical companies and contains about 1.3–6.0 mg/L resveratrol (Paul et al., 1999). Despite its ancient discovery, the first real interest in resveratrol came in 1992 when it was postulated to explain some of the cardio-protective effects of red wine (Siemann and Creasy, 1992). It was suggested to be the solution to the “French Paradox”, a term used to describe the observation that the French population had a very low incidence of cardiovascular disease despite a high consumption of wine and saturated fat (Liu et al., 2007). In 1997, Jang and colleagues reported that resveratrol acts as a chemo-preventive agent, due to its ability to inhibit carcinogenesis at multiple stages (Jang et al., 1997). More recently, anti-inflammatory and antioxidant properties have been reported also (Baur and Sinclair, 2006; Vang et al., 2011), so today it has become a highly important natural active ingredient with potential therapeutic effects and market prospects.

1.2. Sources of resveratrol
Resveratrol is produced by various plants as a defense against stress, injury, excessive sunlight, ultraviolet radiation, infection, and invading fungi (Singh et al., 2013). For example, the roots of the plant P. cuspidatum, much cultivated in Asia, provides a rich source of resveratrol from which commercially available trans-resveratrol (98% pure) is isolated by high-speed counter-current chromatography (Yang et al., 2001). Resveratrol is also considered a nutraceutical present in grapes, peanuts, pine trees, cassia and other plants, and many food products (Ramprasath et al., 2010;...
Soleas et al., 1997). In wine, the concentration of resveratrol varies: red wines contain between 0.2 and 5.8 mg/L, depending upon the grape variety, whereas white wines contain ~0.68 mg/L (Romero-Pérez et al., 1999; Sato et al., 1997). This variation derives from the fact that red wine is extracted with the grape skin intact, whereas white wine is fermented after removal of the skin. Red wine contains more trans-resveratrol than white wine, whereas white has a higher concentration of cis-resveratrol (Feijóo et al., 2008). Concentrations of resveratrol in some natural foods are given in Table 1.

1.3. Metabolism of resveratrol

In rats and humans, resveratrol is a molecule involved in the enterohepatic cycle of metabolism. In particular, after resveratrol is taken up rapidly by enterocytes, it is metabolized to glucuronide- (3-O-glucuronide and 4′-O-glucuronide) and sulfate-conjugates (3-O-sulfate), which are secreted back into the intestine where they may be deconjugated and reabsorbed or excreted in the feces (Walle et al., 2004; Marier et al., 2002). The enterohepatic cycle thus reduces the concentration of the free compound reaching target tissues. So, the low concentration of resveratrol found in blood is likely explained by this enterohepatic cycle and its rapid metabolism in the liver. Apart from dihydroresveratrol, the major metabolites formed are the glucuronide- and sulfate-conjugates, including disulfates and mixed sulfate-glucuronides (Wang et al., 2005). Concentrations of these metabolites are reported to be higher than resveratrol post-absorption and to have longer half-lives (Andres-Lacueva et al., 2009; Polycarpou et al., 2013). In fact, the majority of orally dosed resveratrol is found in urine as sulfate- or glucuronic acid-conjugates (Singh et al., 2013). In particular, the proportion of glucuronide- and sulfate-metabolites are reported to change depending on the tissue and species considered (Juan et al., 2010; Azorín-Ortuño et al., 2011): glucuronide conjugates are reported to be the main metabolites in rodents, whereas primarily sulfates are found in humans (Walle, 2011); moreover, the quantity of glucuronides is higher than sulfates in rat testes and liver, but not in lung (Juan et al., 2010). As a result, many authors are beginning to investigate the effects of resveratrol metabolites on in vitro and in vivo models: for example, resveratrol 3-O-D-sulfate, as well as resveratrol 3-O-D-glucuronide and resveratrol 4-O-D-glucuronide, was found to inhibit cyclooxygenase (COX1 and COX2) (Calamini et al., 2010), whereas 3-O-D-sulfate and resveratrol 4-O-D-glucuronide were reported to reduce triacylglycerol content in 3T3-L1 adipocytes (Lasa et al., 2012). Recently, Polycarpou et al. demonstrated that resveratrol glucuronides are able to arrest the growth of different human colon cancer cells (Polycarpou et al., 2013). Thus, many of the effects reported for resveratrol may be due to the action of resveratrol’s metabolites.

1.4. Bioavailability of resveratrol and plasma levels

Many studies have shown that resveratrol, like other polyphenols, has very low bioavailability (Goldberg et al., 2003). The bioavailability and pharmacokinetics of resveratrol have been studied in humans and in animal models. In humans, resveratrol is rapidly taken up after oral consumption of a low dose, with the plasma resveratrol concentration peaking about 30 min after consumption (Goldberg et al., 2003); in rats, the plasma half-life of resveratrol was reported to be 12–15 min after oral administration (Gescher and Steward, 2003). A study performed by Walle et al. using 14C-trans-resveratrol (25 mg orally) in humans showed that 70% of the resveratrol dose was absorbed by the body (Walle et al., 2004); a similar finding (~50%) was reported for rats (Marier et al., 2002). The glucuronide- and sulfate-conjugated metabolites of resveratrol peaked in plasma at 30–60 min post-administration, with a plasma half-life of 9.2 h. (Walle et al., 2004). In contrast, only small amounts of unmodified resveratrol (<5 ng/mL) were detected in plasma in a similar timeframe (Singh et al., 2013). In another study conducted on mice, rats, and humans, it was shown that within 24 h after administration of 0.03 mg/kg body weight (BW) resveratrol, nearly 50% of the resveratrol was excreted in the urine. However, because <25% of the resveratrol was found in the urine with a dose of 1 mg/kg BW, these results suggest that resveratrol undergoes rapid gastrointestinal absorption in all the three species studied (Meng et al., 2004).

The amount of resveratrol ingested from dietary sources, such as red wine and juices, rarely exceeds 5 mg/L and often results in plasma levels that are either not detectable or several orders of magnitude below the micromolar concentrations that are employed in experimentation in vitro, i.e., ~32 nM to 100 μM (Smolina et al., 2011). For example, administration of about 25 mg resveratrol resulted in plasma concentrations of the free form that ranged from 1 to 5 ng/mL (Almeida et al., 2009), and administration of higher doses (up to 5 g) increased the plasma resveratrol concentration to about 500 ng/mL (Boocock et al., 2007). The low doses of resveratrol observed in the plasma after ingestion are very low, as the concentrations used in vitro are not reached. However, due to its lipophilic character, tissue levels of resveratrol may be higher than those found in plasma (Timmers et al., 2012). Nonetheless, some of the biological effects of resveratrol are observed at very low concentrations (Waite et al., 2005; Pearce et al., 2008), bringing forward the idea that resveratrol exerts its major effects on intestinal tissue, affecting the rest of the body through secondary effects that are independent of the plasma levels reached by the compound (Baur et al., 2006). In rodent models, the doses employed normally range from as low as 0.1 mg/kg BW to up to 1000 mg/kg BW, with even higher or lower doses occasionally being used (Baur et al., 2006). Interestingly, studies show that the bioavailability of resveratrol can be

![Fig. 1. (a) Structure of resveratrol. (b) Structures of trans and cis isomers of resveratrol and piceid.](image)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Resveratrol content in certain natural foods (Prasad, 2012).</th>
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<tbody>
<tr>
<td>Food stuff</td>
<td>Concentration range</td>
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<tr>
<td>Grapes</td>
<td>0.16–3.54 μg/g</td>
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<tr>
<td>Dry grape skin</td>
<td>~24.06 μg/g</td>
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<tr>
<td>Red grape juice</td>
<td>~0.5 mg/L</td>
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<tr>
<td>White grape juice</td>
<td>~0.05 mg/L</td>
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<tr>
<td>Red wine</td>
<td>0.1–14.3 mg/L</td>
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<tr>
<td>White wine</td>
<td>0.1–2.1 mg/L</td>
</tr>
<tr>
<td>Peanuts</td>
<td>0.02–1.92 μg/g</td>
</tr>
<tr>
<td>Pistachios</td>
<td>0.09–1.67 μg/g</td>
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enhanced by using more potent resveratrol analogs (i.e. SRT501) (Howells et al., 2011), by enhancing delivery methods, such as liposomal encapsulation (Narayanan et al., 2009), or by combining it with piperine, a natural product from black pepper (Piper spp.) (Johnson et al., 2011).

1.5. Aims of the review

Studies performed in vivo and in vitro have shown that resveratrol exerts pleiotropic effects and can prevent or slow the progression of several pathological conditions, including cardiovascular and metabolic diseases, ischemic brain injuries, and cancer (Jang et al., 1997; Inoue et al., 2003), as well as extend lifespan in different organisms and enhance stress resistance (Yang et al., 2013). The aim of the present review is to highlight the antioxidant effects of resveratrol, focusing our attention on cardiovascular, cerebral, and metabolic disorders, such as diabetes, and reporting also the results of the main clinical trials.

2. Antioxidant properties of resveratrol

A well-documented method for reducing oxidative stress is to reduce caloric intake by selecting appropriate foods (Nisoli et al., 2005). It is well known that nutrients, whether water soluble or lipid soluble, comprise an important aspect of the antioxidant defense system. Beyond their normal occurrence in cells and tissues of living organisms, free radicals and reactive species are present in the unhealthy foods that people consume every day, inducing undesirable reactions like oxidation of lipids, proteins, nucleic acids, and carbohydrates. An impaired ability to scavenge free radicals and reactive species, as a consequence of decreased levels of antioxidant cellular defense systems or excessive free-radical production, is common in cerebral and cardiovascular diseases in humans and animals (Alissa and Ferns, 2012).

Resveratrol plays a prominent role among the foods exerting an antioxidant activity. The main antioxidant activities of resveratrol are summarized in Table 2 (Rocha et al., 2009; Li et al., 2006; Juan et al., 2005; Kohnen et al., 2007). The data suggest that resveratrol exerts its action in different ways: it scavenges reactive oxygen species (ROS), increasing the activity of enzymes that metabolize ROS, such as superoxide dismutase (SOD), or decreases the activity of enzymes that play a role in ROS production.

2.1. Impact of resveratrol on cardiovascular diseases

2.1.1. Effects of resveratrol on lipid peroxidation

Oxidative stress is one of the risks of cardiovascular disease (CVD), such as atherosclerosis, and is characterized by the production of free radicals that lead to the oxidation of low density lipoprotein (LDL) (Kovanen and Pentikainen, 2003; Puca et al., 2013). It is well known that oxidized LDL accumulates at the site of atherosclerotic lesions (Ramprasath and Jones, 2010), contributing to the formation of macrophage foam cells that induce endothelial dysfunction (Mietus-Snyder et al., 2000), a common marker of CVD. Because it prevents lipid peroxidation, inhibits uptake of oxidized LDL, and inhibits lipoprotein activity (Maccarrone et al., 1999; Kovanen and Pentikainen, 2003), resveratrol is a good candidate for the fight against oxidative stress in atherosclerosis (Fremont et al., 1999; Leighton et al., 1999; Bhavnani et al., 2001; Olas and Wachowicz, 2002). Oxidation of LDL cholesterol is strongly associated with risk of CVD (Holvoet, 2004). In this regard, resveratrol was found in rat liver microsomes to inhibit iron-induced, as well as ultraviolet-irradiated, lipid peroxidation and to prevent LDL oxidation by copper (Fauconneau et al., 1997; Miura et al., 2000); moreover, Rocha et al. found a reduction in oxidized LDL in rats fed on a high fat diet when treated with resveratrol for 45 days at a dose of 1 mg/kg BW/day (Rocha et al., 2009).

Resveratrol also prevents the oxidation of polyunsaturated fatty acids found in LDL (Miller and Rice-Evans, 1995), inhibits the oxidized LDL uptake in the vascular wall in a dose-dependent manner (Fremont, 2000), and prevents damage caused to lipids by peroxidation (Frankel and Waterhouse, 1993; Leighton et al., 1999). Its effect was found to be stronger than the well-known antioxidant vitamin E (Frankel and Waterhouse, 1993). The protective effect of resveratrol against lipid peroxidation was also found in the heart of rats exposed to low doses of doxorubicin, an antitumor drug that causes oxidative stress (Dudka et al., 2012), and in the post-ischemic, re-perfused myocardium of rats (Ray et al., 1999).

2.1.2. Effects of resveratrol on antioxidant mechanisms protecting against oxidative cardiovascular pathophysiology

It has been recently demonstrated that resveratrol reduces endothelial dysfunction in vessel from dyslipidemic patients with hypertension; this antioxidant action of resveratrol was mediated by upregulation of manganese superoxide dismutase (Mn-SOD) via a mechanism dependent upon nuclear factor (erythroid-derived 2)-like 2 (NRF2) (Carrizzo et al., 2013). This finding in humans was in agreement with experimental models showing that resveratrol was able to increase Mn-SOD expression in the mouse myoblast line C2C12 via nuclear translocation and activation of sirtuin 1 (SIRT1), a NAD+-dependent class III histone deacetylase. In obese rats, Franco et al. found that the activity of both SOD and catalase (CAT) was increased in plasma by the administration of resveratrol, preventing oxidative stress and reducing the risk of hypertension (Franco et al., 2013). Similarly, hepatic expression of SIRT1 and
Mn-SOD genes was induced in wild-type rats by 0.02% resveratrol after 4 weeks of treatment (Nakata et al., 2012). The stimulation of Mn-SOD levels was also reported in cultured cardiomyocytes and in coronary artery endothelial cells (Movahed et al., 2012; Ungvari et al., 2009; Tanno et al., 2010). In human aortic smooth muscle cells, it increased the expression of heme oxygenase-1 (HO-1), which degrades pro-oxidant heme to biliverdin/bilirubin, iron, and carbon monoxide, consequently reducing ROS levels (Juan et al., 2005). In vascular smooth muscle cell, resveratrol reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity induced by angiotensin II, and enhanced SOD activity, promoting a significant decrease in ROS generation (Zhang et al., 2013). Moreover, resveratrol inhibits the expression of NADPH oxidase in cardiovascular tissues and reduces O$_2^-$ production from mitochondria (Li et al., 2013). Recently, it has been demonstrated that resveratrol-mediated upregulation of GCH-1 (GTP cyclohydrolase I) and BH4 (tetrahydrobiopterin) biosynthesis prevents endothelial nitric oxide synthase (eNOS) uncoupling and reduces ROS production in the vasculature (Carrizzo et al., 2013).

Xanthine oxidase has been shown to be an important source of oxidative production in vascular endothelium (Sabán et al., 2013) and also a contributing factor to oxidative stress during strenuous exercise. On this issue, has been demonstrated that resveratrol inhibits hypoxanthine/xanthine oxidase in mice, reducing ROS generation (Ryan et al., 2010). Moreover, it has been reported that resveratrol treatment decreases ROS levels in high-capacity runner rats especially in endurance racing: in particular, it increased the aerobic performance and upper-limb strength of these rats. This beneficial effect is mediated by enhanced mitochondrial biogenesis, with activation of the AMPK–SIRT1–PGC-1α pathway (Hart et al., 2013). Also, resveratrol inhibited oxidative stress in vivo by scavenging ROS and attenuating peroxyl radicals, hydrogen peroxide (H$_2$O$_2$), and superoxide radical (−O$_2$) (Liu et al., 2003; Chen et al., 2004). In rat pheochromocytoma (PC12) cells, which are characterized by a high level of catecholamines, 1–100 mmol/L resveratrol inhibited production of ROS (Jang and Suh, 2001).

Resveratrol has also been shown to inhibit −O$_2$ and H$_2$O$_2$ produced in murine macrophages stimulated by lipopolysaccharides (LPS) or phorbol esters (Martinez and Moreno, 2000). In embryonic rat cardiac cells, it prevented mitochondrial damage induced by H$_2$O$_2$ (He et al., 2012). Moreover, resveratrol activates an important survival signal pathway consisting in A1 and A3 adenosine receptor-mediated activation of the PI3K–AKT pathway and the CAMP response element-binding protein (CREB), promoting upregulation of Bcl-2 and, hence, protecting cardiac tissue from cell death (Li et al., 2012).

In human platelets, resveratrol significantly lowers the levels of thiol proteins (Olas et al., 2004). It also hampers platelet aggregation and activation: phytoalexin seems to inhibit the interaction of platelets with collagen and thrombin in vitro in isolated platelets and in animal models. The mechanism remains unclear, but it seems that inhibition of prostaglandin H synthase 1 and cyclooxygenase-1 over COX2 represent possible mechanisms for the anti-platelet aggregation effect of resveratrol (Borriello et al., 2010).

In a wide variety of cells, such as myeloid, lymphoid, and epithelial cells, resveratrol has been shown to prevent the production of ROS induced by tumor necrosis factor (TNFα) (Manna et al., 2000). In aortic endothelial cells, resveratrol (100 nM) was found to prevent TNFα–induced oxidative stress through a reduction in NADPH oxidase activity and the production of H$_2$O$_2$ and −O$_2$ (Vecchione et al., 2009a,b). Recently, Wang et al. demonstrated that resveratrol decreases apoptosis induced by oxidative stress in vascular adventitial fibroblasts of rats treated with TNF-α, acting by activating SIRT1 (Wang et al., 2013).

Other mechanisms through which resveratrol has been suggested to exert CVD-preventing antioxidant effects are:
(a) competition with coenzyme Q, decreasing oxidative chain complex III, and increasing endogenous antioxidants and phase 2 enzymes in rat cardiomyocytes (Cao and Li, 2004);
(b) antioxidant effects against linoleic acid peroxidation in sodium dodecyl sulfate and cetyltrimethylammonium bromide micelles (Fang et al., 2002; Fang and Zhou, 2008);
(c) maintenance of glutathione levels in oxidatively stressed human peripheral blood mononuclear cells, and elevation of glutathione levels in human lymphocytes activated by H$_2$O$_2$ (Losa, 2003; Olas et al., 2004). A strong dose-dependent induction of antioxidant genes was demonstrated when rats were supplemented with 0.3, 1, and 3 g/kg BW/day resveratrol for 28 days (Hebbar et al., 2005);
(d) interaction with AMP-activated protein kinase (AMPK) in diabetic LDL-receptor-deficient mice (Zang et al., 2006), and PPARγ coactivator (PGC)-1α in mouse cardiac tissue (Lagouge et al., 2006);
(e) reduction in the rate of cytochrome C oxidation by hydroxyl radicals (Turrens et al., 1997). Jian et al. reported that resveratrol significantly reduced cytochrome C protein levels in the heart tissue of rats subjected to trauma-hemorrhage; the authors suggest that resveratrol may be important for mitochondrial membrane integrity, leading to a reduction of ROS generation (Jian et al., 2012).

2.1.3. Effects of resveratrol on nitric oxide metabolism
Nitric oxide (NO) plays a critical role in maintaining cardiovascular homeostasis (Dudzinski et al., 2006; Dudzinski and Michel, 2007; Puca et al., 2012). In the vasculature, NO is constitutively synthesized by eNOS and acts by relaxing vascular smooth muscle cells and upregulating blood flow, and so prevents thrombogenic and atherogenic processes. It has been demonstrated both in vitro and in vivo that resveratrol is involved in NO metabolism. For example, 30 μM resveratrol inhibited the contractile response to phenylephrine in isolated rat aorta (Chen and Pace-Asciak, 1996). Similarly, 70 μM resveratrol caused relaxation of isolated human saphenous vein and internal mammary artery rings (Rakici et al., 2005), and relaxed porcine arterial rings pre-contracted with KCl (Li et al., 2006). In those studies, the inhibitory effect of resveratrol was reversed by removal of the endothelium or by inhibition of eNOS. Orallo et al. reported that resveratrol (1–30 μM) relaxed the contractile response of rat aortic rings to phenylephrine and KCl in an NO-dependent manner (Orallo et al., 2002); however, it was suggested that resveratrol does not affect eNOS activity, but instead inhibits NADH/NADPH oxidase, with a decreased reduction in superoxide generation, leading to improved NO bioavailability. Resveratrol rapidly increased NO production in cultured endothelial EA.hy926 cells, although at a high concentration (10 μM) (Wallerath et al., 2002). In bovine aortic endothelial cells, 100 nM resveratrol for 15 min was found to increase NO production through phosphorylation of AKT, extracellular signal-regulated kinase (ERK)1/2, and eNOS (Wang et al., 2011). Klings et al. proposed that resveratrol increases NO production through membrane estrogen receptors (ERs) in bovine aortic cells, human umbilical vein cells, and human microvascular endothelial cells (Klinge et al., 2005, 2008) by rapid activation of Src and ERK1/2, leading to eNOS activation. However, as demonstrated by studies on isolated porcine coronary arteries (Li et al., 2006) and murine endothelial F-2 cells (Takahashi et al., 2009), ER antagonists do not inhibit resveratrol-stimulated NO production.

Wallerath et al. reported that the treatment of cultured endothelial cells with resveratrol (10–100 μM) for 24–72 h upregulated eNOS mRNA and protein expression levels, resulting in increased...
production of NO (Wallerath et al., 2002; Conti et al., 2012). Similarly, other studies confirmed that high concentrations of resveratrol significantly enhanced eNOS gene expression and enzyme activity, and hence NO production, in in vitro assays (Räthel et al., 2007; Appeldoor et al., 2009). In contrast, Nicholson et al. (Nicholson et al., 2010) reported that exposure of HUVECs to nanomolar concentrations of resveratrol for 24 h increased the eNOS mRNA level, although eNOS protein and NO production were not affected. In the same cell line, Takahashi et al. demonstrated that 50 nM resveratrol did not alter the eNOS protein level or NO production after 24 h of treatment, whereas daily treatment for 5 days significantly increased both eNOS protein and NO production without producing any cytotoxic effects (Takahashi and Nakashima, 2011). Resveratrol also increased the synthesis of NO in ischemic re-perfused rat tissue (Hattori et al., 2002) and preserved eNOS phosphorylation in diabetic type 2 (db/db) mice (Zhang et al., 2009).

3. Impact of resveratrol on cerebrovascular diseases

Many studies have reported that the central nervous system is targeted by resveratrol. This compound is in fact able to pass the blood-brain barrier (Baur et al., 2006). Regarding its radical-scavenging activity, structural studies demonstrated that the hydroxyl group at the 4’ position of resveratrol is much easier to subject to oxidation than other hydroxyl groups in the antioxidant reaction (Caruso et al., 2004). Intraperitoneal administration of resveratrol exerted neuroprotective effects, upregulating several endogenous antioxidant enzymes, such as SOD and CAT, in the brain of healthy rats (Mokni et al., 2007). Regarding the various isoforms of SOD, SOD2 plays a more important role against oxidant-induced mitochondrial oxidative stress and cytotoxicity in neuronal cells (Vincent et al., 2007). Fukui et al. demonstrated in HT22 neural cells that the neuroprotective effect of resveratrol after glutamate-induced cytotoxicity is largely independent of its direct antioxidant activity; rather, this effect was mediated by induction of SOD2 expression via activation of the PI3K–AKT–GSK-3β–β-catenin signaling pathway (Fukui et al., 2010). In rats, prolonged administration of resveratrol improved colchicine-induced cognitive impairment, reduced malondialdehyde—an indicator of lipid per-oxidation and nitrite levels—and restored depleted glutathione (GSH), a ROS scavenger (Kumar et al., 2007).

It is interesting that resveratrol might be involved in the attenuation of neuroinflammatory responses because it is able to reduce the concentration of 8-iso-prostaglandin F2α, an indicator of free-radical generation in rat microglia (Candelario-Jalil et al., 2007). It has also been shown that resveratrol inhibits COX1, but does not affect the expression of COX2 (Davinelli et al., 2012). Since nuclear factor-κB (NF-κB) signaling activation also plays an important role in neurodegeneration, a link between Alzheimer’s Disease (AD) and the neuroprotective activity of resveratrol is its ability to reduce, in neurodegeneration, a link between Alzheimer’s Disease (AD) and some-mediated degradation. KEAP1 functions as a sensor of stress activating cascade that resveratrol can be considered a neuroprotective compound in the context of AD, it can be speculated that the ability to counteract Aβ toxicity is due to its antioxidant properties, but also due to SIRT1 activation.

The anti-amyloidogenic activity of resveratrol has been reported in several studies: for example, Riviére et al. showed that more than other stilbenes, resveratrol inhibits β-amyloid peptide polymerization in vitro, even though its anti-amyloidogenic mechanism remained unknown (Riviére et al., 2007). As illustrated by Marambaud and colleagues, resveratrol promotes clearance of intracellular Aβ by activating proteasomal degradation (Marambaud et al., 2005). Moreover, SIRT1 overexpression reduces Aβ pathology in APP-expressing neuronal cultures by delaying Aβ synthesis (Marambaud et al., 2005; Tang and Chua, 2008). Feng et al. demonstrated that resveratrol disrupts Aβ hydrogen binding, preventing fibril formation by destabilizing preformed fibrils without affecting oligomerization (Feng et al., 2009). Furthermore, studies have shown that the protective effects of resveratrol on β-amyloid-induced toxicity are related to activation of PKC or AMPK (Han et al., 2004; Karuppagounder et al., 2009).

3.2. Resveratrol and Nrf2

NRF2 is a key regulator of cellular antioxidant responses and appears to be a good candidate for neuroprotection in AD. In fact, NRF2 regulates the expression of genes encoding antioxidant and detoxifying proteins, such as glutathione S-transferase (GST), glutathione synthetase (GSS), HO-1, and NADP(H) quinone oxidoreductase (Scapagnini et al., 2011). Under basal conditions, NRF2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (KEAP1), which facilitates its polyubiquitylation and proteasome-mediated degradation. KEAP1 functions as a sensor of stress signals. Exposure to oxidants disrupts the KEAP1–NRF2 complex, stabilizing NRF2 and allowing it to accumulate in the nucleus. NRF2 activates the transcription of its target genes via antioxidant response elements (AREs) in their promoter regions, binding as a heterodimer with members of the Maf and Jun families (Davinelli et al., 2012). To date, only few studies have shown that the activation of NRF2 and of its antioxidant genes by resveratrol treatment is sufficient to protect against AD. However, Chen et al. reported that resveratrol is able to increase the expression of HO-1 and GSH, protecting PC12 cells from oxidative stress via activation of the NRF2–ARE signaling pathway (Chen et al., 2005), which does...
suggest a potential for the treatment of AD. Similarly, resveratrol was able to induce HO-1 in primary neuronal cultures, presumably through the activation of NRF2 (Zhuang et al., 2003). The neuroprotective actions of HO-1 are attributable to the formation of biliverdin and bilirubin during heme degradation, both of which can serve as ROS scavengers (Otterbein and Choi, 2000; Stocker et al., 2000). In conclusion, NRF2 is an attractive target for the discovery of natural neuroprotective agents, such as resveratrol.

4. Impact of resveratrol on diabetes

It has been proposed that oxidative stress caused especially by a sedentary lifestyle and an unhealthy diet is an important risk factor for the development of diabetes. Some studies have proposed resveratrol as a possible candidate for diabetes prevention.

4.1. Resveratrol and NAD(P)H oxidase

Activation of NAD(P)H oxidase contributes to vascular oxidative stress in experimental diabetes (Vecchione et al., 2006). In particular, TNFα-mediated activation of NAD(P)H oxidase is mainly responsible for the generation of the oxidative stress encountered in the coronary microcirculation in type 2 diabetes (Gao et al., 2007; Vecchione et al., 2007). The \(\cdot\text{O}_2\) derived from NAD(P)H oxidase can be dismutated to produce \(\text{H}_2\text{O}_2\) (Papa and Skulachev, 1997) or can be the cause of nitrative stress: in fact, the interaction of \(\cdot\text{O}_2\) with NO produces peroxynitrite, which leads to protein tyrosine nitration generating nitrotyrosine, an index of reactive nitrogen species; it also reduces NO bioavailability, causing endothelial dysfunction (Shah and Channon, 2004). Increased nitrotyrosine stress and peroxynitrite formation are associated with diabetes development, as demonstrated in various studies (Pacher et al., 2007; Frustaci et al., 2000; Pacher and Szabo, 2006). For example, nitrotyrosine content was found to be high in microvasculature endothelial cells of diabetic patients (Cerillo et al., 2002). In the aorta of diabetic mice, resveratrol was found to downregulate NAD(P)H oxidase expression, and thus contributed to a reduction in \(\cdot\text{O}_2\) production (Zhang et al., 2009). Aortic nitrotyrosine protein and \(\text{H}_2\text{O}_2\) levels also attenuate after treatment with resveratrol (Zhang et al., 2009). In type 2 diabetic mice, Kitada et al. demonstrated that resveratrol normalized Mn-SOD activity, through a reduction in tyrosine-nitrate modifications and decreased urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative stress, and \(\text{O}_2^\cdot\) levels (Kitada et al., 2011).

4.2. Resveratrol and nuclear factor-κB

Hyperglycemia—the hallmark of diabetes—can also induce oxidative stress via several pathways that converge on NF-κB, the activation of which in turn contributes to a further enhancement of pro-inflammatory cytokines, oxidative stress, and apoptosis (Kern, 2007; Singh et al., 2011). In this context, resveratrol was demonstrated to produce several anti-diabetic effects, such as reduction of circulatory pro-inflammatory cytokines, inhibition of apoptosis, and concomitant enhancement of antioxidant defenses (Lee et al., 2011; Palsamy and Subramanian, 2010; Sharma et al., 2009, 2011; Zhang et al., 2010). It has been documented that short-term treatment of diabetic subjects with resveratrol inhibited the activation of NF-κB at transcriptional or post-transcriptional levels (Lee et al., 2009; Zhang et al., 2010). Resveratrol may attenuate the inflammatory process through a reduction of oxidative damage and NF-κB activity (Kubota et al., 2009).

4.3. Resveratrol and oxidative markers

Oxidation of glucose is another mechanism occurring in diabetes (Maritim et al., 2003). Proteins such as hemoglobin and antioxidant enzymes can be glycate in the presence of a high concentration of oxidized glucose. This leads to a reduction in detoxification of ROS, resulting in lipid-, protein-, and DNA-peroxidation, and, finally, apoptosis (Rains and Jain, 2011). Glycated hemoglobin (HbA1c) is a good marker for diagnosis and prognosis of complications in diabetes, such as retinopathy, nephropathy, and neuropathy (Howlett and Ashwell, 2008). For example, it was shown that reduction of HbA1c by only 1 unit (8–7%) can reduce the risk of retinopathy by over 30% (Kowluu and Chan, 2007). Four months of resveratrol supplementation was found to reduce HbA1c levels in diabetic rats (Soufi et al., 2012).

Another good marker of oxidative and antioxidant homeostasis is 8-isoprostane (8-isoprostaglandin F2α), a product of the oxidation of arachidonic acid present in phospholipids (Morrow et al., 1995). It was reported that plasma levels of 8-isoprostane increased with diabetes-induced lipid peroxidation and oxidative stress (Ndisang et al., 2010; Salim et al., 2010). Moreover, retinal 8-isoprostane increased during hypoxia-induced retinopathy (Kimura et al., 2007), and resveratrol reduced 8-isoprostane levels in blood and retinal tissue of normal and diabetic rats, demonstrating that resveratrol has a strong antioxidant effect and attenuates oxidative stress (Soufi et al., 2012).

In diabetes, the attenuation of oxidative stress reduces the level of activated caspases and, thus, reduces apoptosis. In fact, resveratrol was found to modulate embryonic oxidative stress and apoptosis in diabetic pregnancy: in particular, it reduced oxidative stress by restoring the level of reduced glutathione, total thiol, lipid peroxidation, and 4-hydroxy-2-non-enal (HNE) in diabetic dams (Singh et al., 2013).

5. Toxicity of resveratrol

Many studies have investigated the toxic effect of resveratrol. Most of the data available, both in human and in animal models, suggest that resveratrol does not have a significant toxic effect in the wide range of concentrations tested (Ramprashath and Jones, 2010). For example, no toxic effects were found in rats after oral administration of 20 mg/kg BW/day for 28 days, a dose higher than that produced by one glass of red wine per day (Jian et al., 2002). Moreover, no toxic effects were observed in rats given a supplementation of 300 mg resveratrol/day for 4 weeks. In humans, Boocock et al. found no toxicity after administration of a single dose of up to 5 g resveratrol (Boocock et al., 2007). In addition, clinical, biochemical, and hematological indices revealed no serious toxic effects in 44 healthy volunteers (10–12 per group) administered resveratrol for 29 days at a daily dose of 0.5, 1.0, 2.5, or 5.0 g (Brown et al., 2010). However, adverse effects found in 28 participants were considered possibly due to resveratrol: common symptoms were gastrointestinal in nature, particularly diarrhea, nausea, and abdominal pain, at a dose of 1 g. Typically, gastrointestinal symptoms occurred ~1 h after administration, and improved during the course of the day. However, all the events were graded as mild, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) (Ramprashath and Jones, 2010). Based on the these findings, the authors suggested that daily doses of resveratrol for subsequent clinical evaluation should not exceed 1 g (Brown et al., 2010). Chow et al. reinforced those findings and demonstrated that 1 g resveratrol taken once daily for 4 week was generally well tolerated in healthy participants (Chow et al., 2010): all the reported adverse events were CTC grade 1 or 2, with many being mild and transient. The frequency of the side
effects experienced was consistent with that observed in a trial described by Brown et al. (Brown et al., 2010) and in shorter-term studies involving fractionated daily doses (la Porte et al., 2010; Almeida et al., 2009; Nunes et al., 2009).

Finally, it is important to underline that resveratrol can exhibit pro-oxidant activities in the presence of transition metal ions, such as copper, leading to oxidative breakage of cellular DNA (de la Llastera and Villegas, 2007).

6. Clinical trials on the antioxidant effects of resveratrol

To date, only a small number of clinical trials on the antioxidant effects of resveratrol have been reported. The most significant clinical trials are summarized in Table 3. Ghanim et al. (2010) investigated the effects of resveratrol on different markers of inflammation and oxidative stress in a randomized placebo-controlled trial: the study was performed on 20 healthy adults receiving a 200 mg P. cuspidatum extract supplement containing 40 mg of resveratrol, for 6 weeks. Resveratrol did not alter fasting plasma concentrations of cholesterol (total, LDL, and HDL), triglycerides, or leptin compared with placebo. However, the treatment reduced ROS levels, TNF-α, and IL-6, and suppressed NF-κB in mononuclear cells. Additionally, C-reactive protein (CRP)—another important marker of inflammation—was significantly reduced.

Ghanim et al. also conducted a separate crossover placebo-controlled trial on 10 healthy humans fed with a high-fat, high-carbohydrate meal (Ghanim et al., 2011). The 100 mg resveratrol supplementation used significantly increased NRF2-binding activity following the meal, and significantly increased mRNA expression of important antioxidant enzymes, such as the NAD(P)H dehydrogenase [quinone] 1 (NQO-1) and glutathione S-transferase P1 (GST-P1). Resveratrol also attenuated the postprandial rise in cluster of differentiation 14 (CD14), IL-1β mRNA, and toll-like receptor 4 (TLR4) protein in mononuclear cells, while also

<table>
<thead>
<tr>
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<th>Duration</th>
<th>Molecular-level effects</th>
</tr>
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<tr>
<td>Ghanim et al. (2010)</td>
<td>20 healthy adults</td>
<td>40 mg</td>
<td>6 weeks</td>
<td>↑ ROS; ↑ P47(phox); ↑ NFκB; ↓ JNK-1, ↓ PTP-1B; ↓ SOCS-3</td>
</tr>
<tr>
<td>Ghanim et al. (2011)</td>
<td>4 healthy men and 6 women</td>
<td>100 mg + 75 mg grapeskin polyphenols</td>
<td>1 week</td>
<td>↑ ROS; ↑ TLR-4; ↑ CD14; ↑ IL-1β; ↑ SOCS-3</td>
</tr>
<tr>
<td>Brasnyo et al. (2011)</td>
<td>19 diabetic men</td>
<td>5 mg twice daily</td>
<td>4 weeks</td>
<td>↑ ROS; ↑ Nrf-2; ↑ NQO-1; ↑ GST-P1</td>
</tr>
<tr>
<td>Timmers et al. (2011)</td>
<td>11 healthy obese men</td>
<td>150 mg twice daily</td>
<td>30 days</td>
<td>↑ glucose; ↑ AMPK; ↑ SIRT1; ↑ PGC1α</td>
</tr>
<tr>
<td>Bo et al. (2013)</td>
<td>50 healthy adult smokers</td>
<td>500 mg</td>
<td>30 days</td>
<td>↑ ROS; ↑ CRP; ↑ TG</td>
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![Figure 2](image.png)

Fig. 2. Representative scheme of the biological effects recruited by resveratrol and their involvement in cardiovascular, metabolic and cerebrovascular diseases.

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Table 3
Summary of clinical trials on antioxidant effects of resveratrol. ↑, downregualtion; ↑, upregulation.
decreasing plasma endotoxin. These data suggest strong antioxidant and anti-inflammatory effects of resveratrol in response to the high-fat, high-carbohydrate meal and a potential use in reducing the risk of atherosclerosis and diabetes.

Interestingly, in a randomized double-blind placebo-controlled crossover study, 5 mg trans-resveratrol supplementation given twice daily for 4 weeks improved insulin sensitivity and lowered blood glucose levels, delaying its peak (Brasnyo et al., 2011). Among the mechanisms suggested to exert these beneficial effects, the authors indicated decreased oxidative stress and increased AKT phosphorylation.

The metabolic effects of resveratrol have also been studied in obese men (Timmers et al., 2011): supplementation with 75 mg resveratrol for 30 days reduced sleeping- and resting-metabolic rate in the absence of body weight changes; moreover, resveratrol increased SIRT1 protein levels in muscle and reduced blood inflammation markers.

Finally, Bo et al. evaluated the effects of resveratrol on healthy smokers: they found that 500 mg resveratrol for 30 days significantly increased total antioxidant status values (Bo et al., 2013). The authors suggested that resveratrol may reduce the risk of cardiovascular diseases in smokers.

7. Conclusion and recommendations

In this review, we have focused our attention on the antioxidant effects of resveratrol and on its molecular mechanisms. The neutralization of free radicals prevents the activation of redox-sensitive molecules involved in the modulation of biological processes, such as cell cycle and mitochondrial biogenesis, and of a wide range of chronic diseases, including cardiovascular, neurological, and metabolic disorders (Fig. 2). It is necessary to underline that all antioxidant substances must be used at the proper dose, since high concentrations may induce undesirable effects, such as non-specific reactions with proteins, and decrease antioxidant properties. Although a beneficial "in vitro" antioxidant effect of resveratrol on vessels from patients showing vascular dysfunction is well defined, further clinical trials need to determine resveratrol’s mechanism of action, its safety, and its toxicity. In the light of existing data, it is clear that grapes—and wine—should be considered an integral component of fruit- and vegetable-enriched diets that are recommended by health authorities and widely accepted as beneficial for human health and disease prevention.

8. Conflict of Interest

The authors declare that there are no conflicts of interest.

List of abbreviations

- **O₂** Superoxide anion
- 8- OHG 
- 8- hydroxy-2′-deoxyguanosine
- AMPK 5′ adenosine monophosphate-activated protein kinase
- AREs Antioxidant response elements
- Aβ Beta amyloid
- BBB Blood-brain barrier
- Bcl-2 B-cell lymphoma 2
- BH4 Tetrahydrobiopterin
- BW Body weight
- CAT Catalase
- CD14 Cluster of differentiation 14
- COX Cycloxygenase
- CREB cAMP response element-binding protein
- CRP C-reactive protein
- CTCAE Common Terminology Criteria for Adverse Events
- CVD Cardiovascular diseases
- eNOS Endothelial nitric oxide synthase
- ER Estrogen receptors
- ERK Extracellular signal-regulated kinase
- GCH-1 GTP cyclohydrolase I
- GSH Reduced glutathione
- GSK-3β Glycogen kinase 3 beta
- GSS Glutathione synthetase
- GST Glutathione S-transferase
- GST-p1 Glutathione S-transferase p1
- H₂O₂ Hydrogen peroxide
- HbA1c Glycated hemoglobin
- HCR High capacity runner
- HDL High density lipoprotein
- HNE 4-hydroxy-2-nonenal
- HO-1 Heme oxygenase-1
- IL-6 Interleukin 6
- iNOS Inducible nitric oxide synthase
- Keap1 Kelch-like ECH-associating protein 1
- LDL Low density lipoprotein
- LPS Lipopolysaccharides
- Mn- Manganese superoxide dismutase
- NADPH Reduced nicotinamide adenine dinucleotide phosphate
- NF-kβ Nuclear factor-kβ
- NO Nitric oxide
- NQO-1 NAD(P)H dehydrogenase [quinone] 1
- NRF-2 Nuclear factor (erythroid-derived 2)-like 2
- PGC-1α Peroxisome-proliferator-activated receptor gamma
- PGE2 Prostaglandin E2
- PI3K Phosphatidylinositol 3-kinases
- PKC Protein kinase C
- PPAR Peroxisome proliferator-activated receptors
- ROS Reactive oxygen species
- SIRT-1 Sirtuin-1 NAD^+ -dependent class III histone deacetylases
- SOD Superoxide dismutase
- TAS Total antioxidant status
- TLR4 Toll-like receptor 4
- TNF-α Tumor necrosis factor alpha

References


