British Journal of Pharmacology

British Journal of Pharmacology (2017) **174** 1633–1646 1633

Themed Section: Redox Biology and Oxidative Stress in Health and Disease

REVIEW ARTICLE

Antioxidant effects of resveratrol in the cardiovascular system

Correspondence Professor Dr Huige Li, Department of Pharmacology, Johannes Gutenberg University Medical Center, Obere Zahlbacher Str. 67, Mainz, 55131, Germany. E-mail: huigeli@uni-mainz.de

Received 9 September 2015; **Revised** 16 March 2016; **Accepted** 31 March 2016

Ning Xia[1], Andreas Daiber[2], Ulrich Förstermann[1] and Huige Li[1]

[1]Department of Pharmacology, Johannes Gutenberg University Medical Center, Mainz, Germany, and [2]2nd Medical Department, Cardiology and Angiology, Johannes Gutenberg University Medical Center, Mainz, Germany

The antioxidant effects of resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) contribute substantially to the health benefits of this compound. Resveratrol has been shown to be a scavenger of a number of free radicals. However, the direct scavenging activities of resveratrol are relatively poor. The antioxidant properties of resveratrol *in vivo* are more likely to be attributable to its effect as a gene regulator. Resveratrol inhibits NADPH oxidase-mediated production of ROS by down-regulating the expression and activity of the oxidase. This polyphenolic compound reduces mitochondrial superoxide generation by stimulating mitochondria biogenesis. Resveratrol prevents superoxide production from uncoupled endothelial nitric oxide synthase by up-regulating the tetrahydrobiopterin-synthesizing enzyme GTP cyclohydrolase I. In addition, resveratrol increases the expression of various antioxidant enzymes. Some of the gene-regulating effects of resveratrol are mediated by the histone/protein deacetylase sirtuin 1 or by the nuclear factor-E2-related factor-2. In this review article, we have also summarized the cardiovascular effects of resveratrol observed in clinical trials.

LINKED ARTICLES

This article is part of a themed section on Redox Biology and Oxidative Stress in Health and Disease. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.12/issuetoc

Abbreviations

ApoE, apolipoprotein E; BH_4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; GCH1, GTP cyclohydrolase I; GPx1, glutathione peroxidase 1; HO1, haem oxygenase 1; KO, knockout; NOX, NADPH oxidase catalytic subunit; NQO, NAD(P)H: quinoneoxidoreductase; Nrf2, nuclear factor-E2-related factor-2; SIRT1, histone/protein deacetylase sirtuin 1; SOD1, copper/zinc SOD; SOD2, mitochondrial manganese SOD; SOD3, extracellular SOD

Tables of Links

TARGETS	
Nuclear hormone receptors[a]	**Enzymes**[b]
Oestrogen receptors (ER)	Dihydrofolate reductase
ERα	Endothelial NOS (eNOS)
	Haem oxygenase 1 (HO1)
	PDE1-PDE5
	Sirtuin 1 (SIRT 1)
	Xanthine oxidase (XO)

LIGANDS	
Angiotensin II	Nitric oxide (NO)
Bilirubin	Paraquat
Biliverdin	Resveratrol
Cysteine	Tetrahydrobiopterin (BH_4)
Glutathione (GSH)	Uric acid
Hydrogen peroxide (H_2O_2)	Vitamin C
L-NAME	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b]Alexander *et al.*, 2015a,b).

DOI:10.1111/bph.13492

Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a polyphenol phytoalexin present in a variety of plant species and in red wine. Preclinical studies have demonstrated that resveratrol has protective effects in a number of disease models, including cardiovascular disease, diabetes, cancer and neurodegenerative diseases (Baur and Sinclair, 2006; Juhasz *et al.*, 2010b). Some beneficial effects have also been observed in clinical trials, although many discrepancies and conflicting information exist (Novelle *et al.*, 2015). The mechanisms of action of resveratrol are complex. Among these, antioxidant properties contribute substantially to the health benefits of resveratrol. In the present article we have reviewed the molecular mechanisms of resveratrol's antioxidant effects in the cardiovascular system.

Role of oxidative stress in cardiovascular disease

ROS, including free oxygen radicals, oxygen ions and peroxides, may have both physiological and pathological roles that are concentration-dependent. At moderate concentrations, ROS are important regulators of vascular homeostasis by acting as signalling molecules (Li *et al.*, 2014). In contrast, a high ROS concentration, due to excessive ROS production or malfunctioning antioxidant defence systems, causes oxidative stress. All cardiovascular risk factors lead to oxidative stress, which represents an important pathomechanism for cardiovascular disease. Therefore, pharmacological prevention of oxidative stress is of therapeutic interest (Li *et al.*, 2013).

ROS-producing systems

Among the ROS-producing enzyme systems in the vascular wall, NADPH oxidase, xanthine oxidase (XO), enzymes of the mitochondrial respiratory chain, and a dysfunctional endothelial NOS (eNOS) are of major importance (Li *et al.*, 2013; 2014).

NADPH oxidases

NADPH oxidases are multi-subunit enzyme complexes consisting of two membrane-bound subunits (NOX and p22phox) and several cytosolic regulatory subunits (Bedard and Krause, 2007; Drummond *et al.*, 2011; Wingler *et al.*, 2011). In the vascular wall, vascular smooth muscle cells express NOX4 and NOX1, whereas endothelial cells express predominantly NOX4 and NOX2 (Li *et al.*, 2013; Li *et al.*, 2014). NADPH oxidases are major sources of ROS in the vasculature, producing superoxide ($O_2^{\bullet-}$) as well as hydrogen peroxide (by NOX4). Importantly, NADPH oxidase can trigger ROS production from other sources including uncoupled eNOS, XO (Landmesser *et al.*, 2007) and mitochondria (Kroller-Schon *et al.*, 2014; Schulz *et al.*, 2014). An up-regulation of NADPH oxidase subunits has been observed in human atherosclerosis as well as in animal models of cardiovascular disease (Li *et al.*, 2014).

Xanthine oxidase

XO-catalysed chemical reactions lead to the production of $O_2^{\bullet-}$ and hydrogen peroxide. Endothelial cells express XO. In addition, XO can be released from the liver, and circulating XO can adhere to endothelial cells (White *et al.*, 1996). Atherosclerosis in human and experimental animals is associated with increased activity of both endothelial XO and plasma XO (Patetsios *et al.*, 2001; Guzik *et al.*, 2006), suggesting a contribution of XO-derived ROS to cardiovascular disease.

Mitochondria

Mitochondria produce substantial amounts of $O_2^{\bullet-}$ at electron transport chain complexes I and III. Complex I releases $O_2^{\bullet-}$ into the mitochondrial matrix and is considered the main producer of $O_2^{\bullet-}$. The matrix-localized mitochondrial manganese SOD (SOD2) converts $O_2^{\bullet-}$ to H_2O_2, which in turn is reduced to water by glutathione peroxidase (GPx) or catalase (Lubos *et al.*, 2011). The levels of mitochondrial ROS are of central importance to atherogenesis, heart function and other cardiovascular diseases. Loss of SOD2 causes perinatal lethality because of cardiac myopathy or congestive heart failure (Li *et al.*, 1995; Nojiri *et al.*, 2006). Moreover, mitochondrial ROS also promote the activity of other ROS sources (e.g. NADPH oxidases, eNOS uncoupling and XO) (Kroller-Schon *et al.*, 2014; Schulz *et al.*, 2014).

Dysfunctional, uncoupled endothelial NOS

Under physiological conditions, eNOS produces NO, which represents a key element in the vasoprotective function of the endothelium (Li and Forstermann, 2000; Li *et al.*, 2002). Under pathological conditions, however, eNOS may become dysfunctional (Forstermann and Munzel, 2006; Li and Forstermann, 2013; Li and Forstermann, 2014). Oxidative stress evidently contributes to endothelial dysfunction, primarily because of rapid oxidative inactivation of NO by an excess of $O_2^{\bullet-}$. In a second step, the persistant oxidative stress induces eNOS uncoupling (i.e. uncoupling of O_2 reduction from NO synthesis), thereby converting the eNOS enzyme to an $O_2^{\bullet-}$ producer.

A number of mechanisms are implicated in eNOS uncoupling (Forstermann and Munzel, 2006; Li and Forstermann, 2009; Forstermann and Li, 2011). Among these, tetrahydrobiopterin (BH_4) deficiency is likely to represent a major cause of eNOS uncoupling. BH_4 is biosynthesized from GTP with GTP cyclohydrolase I (GCH1) acting as the rate-limiting enzyme (Schmidt and Alp, 2007). Under conditions associated with oxidative stress, peroxynitrite (and $O_2^{\bullet-}$ less effectively) oxidizes BH_4 to BH_2, leading to a deficiency of BH_4 (Laursen *et al.*, 2001). BH_2 can be reduced back to BH_4 by the enzyme dihydrofolate reductase. Thus, a deficit in BH_4 can be caused by enhanced BH_4 oxidation, by reduced BH_4 *de novo* synthesis (i.e. due to the down-regulation of GCH1) or by reduced BH_4 recycling from BH_2 (i.e. due to the down-regulation of dihydrofolate reductase) (Chalupsky and Cai, 2005).

Uncoupling of eNOS is a crucial mechanism contributing to atherogenesis. It not only reduces NO production but also potentiates the pre-existing oxidative stress. The overproduction of ROS (e.g. $O_2^{\bullet-}$ and subsequently peroxynitrite) by uncoupled eNOS in turn enhances the oxidation of BH_4, creating a vicious

circle (Forstermann and Munzel, 2006; Li and Forstermann, 2013; Li and Forstermann, 2014).

Antioxidant systems

The vascular wall contains a variety of enzymes, which can act as antioxidant defence systems and reduce the ROS burden.

SOD

SOD enzymes catalyse the dismutation of $O_2^{\bullet-}$ into hydrogen peroxide, thereby providing a key antioxidant effect. There are three mammalian isoforms of SOD. The copper/zinc SOD (SOD1) is a soluble enzyme located in the cytoplasm and in the mitochondrial intermembrane space. SOD2 is found in the mitochondrial matrix. In contrast, the extracellular SOD (SOD3) is expressed in extracellular matrix, on the cell surface and in extracellular fluids (Li *et al.*, 2014).

Catalase

Catalase is an important cellular antioxidant enzyme and catalyses the decomposition of hydrogen peroxide to oxygen and water. The overexpression of catalase reduces atherosclerosis in apolipoprotein E-knockout (ApoE-KO) mice (Yang *et al.*, 2004).

Glutathione peroxidases

GSH peroxidase proteins convert hydrogen peroxide to water and lipid peroxides to their respective alcohols. GSH peroxidase 1 (GPx1) is the most abundant selenoperoxidase and is a key antioxidant enzyme in many cell types (Lubos *et al.*, 2011).

NAD(P)H:quinone oxidoreductase 1

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a flavoprotein that catalyses two-electron reduction of a broad range of substrates, including quinones. Quinonoid compounds generate aggressive ROS via redox cycling mechanisms and arylating nucleophiles. NQO1 reduces quinones to hydroquinones without the formation of semiquinones and ROS that are deleterious to cells. Therefore, the removal of quinones from a biological system by NQO1 is considered an important detoxification reaction (Ross and Siegel, 2004).

Haem oxygenase 1

Haem oxygenase 1 (HO1) catalyses the degradation of the prooxidant haem into carbon monoxide, iron and biliverdin, which is subsequently converted to bilirubin. The antioxidant effects of HO1 also include the activation of transcriptional machinery that induces a range of antioxidant genes. The catabolism of haem provides protection to cells via numerous routes, including the induction of ferritin to store redox-active iron, the antioxidant actions of biliverdin and bilirubin and the anti-inflammatory and anti-apoptotic effects of carbon monoxide (Dunn *et al.*, 2014).

Nonenzymatic antioxidants

In addition to the antioxidant enzymes, nonenzymatic antioxidants are also important for the cell to control ROS levels. Nonenzyme low molecular weight antioxidant compounds include vitamins C and E, GSH, β-carotene and uric acid.

Resveratrol as a ROS scavenger

As a polyphenolic compound, resveratrol has been shown in *in vitro* systems to directly scavenge a variety of oxidants, including hydroxyl radical ($^{\bullet}OH$), $O_2^{\bullet-}$, H_2O_2 and peroxynitrite.

In a cell-free system using the Fenton reaction as a source of $^{\bullet}OH$, resveratrol (at concentrations ≥300 μM) has been shown to act as a scavenger and not an inhibitor of the Fenton reaction (Leonard *et al.*, 2003). The calculated resveratrol reaction rate of $^{\bullet}OH$ (9.45×10^8 $M^{-1}{\cdot}s^{-1}$), however, is significantly less than that of well-established antioxidants, including ascorbate (1.2×10^{10} $M^{-1}{\cdot}s^{-1}$), GSH (1.5×10^{10} $M^{-1}{\cdot}s^{-1}$) and cysteine (1.3×10^{10} $M^{-1}{\cdot}s^{-1}$). The hydroxyl radical-scavenging property of resveratrol is proposed to be due to its phenolic groups (Leonard *et al.*, 2003).

Resveratrol (at concentrations ≥100 μM) has been shown to scavenge $O_2^{\bullet-}$ directly in a nonenzymatic, cell-free system (the potassium $O_2^{\bullet-}$ system) (Jia *et al.*, 2008). Interestingly, the $O_2^{\bullet-}$ scavenging activity of resveratrol is higher when the xanthine/XO system is used to generate $O_2^{\bullet-}$ (Table 1) (Hung *et al.*, 2002; Jia *et al.*, 2008). The difference in EC_{50} values in these two systems can be explained by the fact that resveratrol not only has an $O_2^{\bullet-}$ scavenging activity but also suppresses $O_2^{\bullet-}$ generation by inhibiting XO activity (at concentrations ≥50 μM) (Jia *et al.*, 2008).

Table 1

Direct scavenging effects of resveratrol

		Scavenging effect			Reference
	IC_{50} (μM)	1 μM	10 μM	100 μM	
$O_2^{\bullet-}$ (XXO)	245	n.d.	2.8%	n.d.	Hung *et al.* (2002)
$O_2^{\bullet-}$ (XXO)	252	n.d.	4%	23%	Jia *et al.* (2008)
$O_2^{\bullet-}$ (KO_2)	458	n.d.	2%	18%	Jia *et al.* (2008)
H_2O_2	11	26%	48%	84%	Ungvari *et al.* (2007)
$ONOO^-$	63	10%	23%	57%	Holthoff *et al.* (2010)

Superoxide ($O_2^{\bullet-}$) is produced by either xanthine/xanthine oxidase (XXO) or by the potassium superoxide system (KO_2). $ONOO^-$, peroxynitrite; n.d., no data available.

In cell-free assays, resveratrol effectively attenuates H_2O_2 levels (Table 1). Resveratrol (1 or 24 h incubation) also dose-dependently decreases H_2O_2 concentration in cultured arteries treated with paraquat or UV light (Ungvari *et al.*, 2007).

When incubated with authentic peroxynitrite in a cell-free system, resveratrol directly scavenges peroxynitrite, blocking the nitration of bovine serum albumin 20-fold more potently than *N*-acetyl-L-cysteine (Holthoff *et al.*, 2010). Resveratrol inhibits peroxynitrite-induced LDL oxidation (Brito *et al.*, 2002) and inhibits peroxynitrite-induced cytotoxicity (Holthoff *et al.*, 2010).

In general, however, the direct antioxidant effects of resveratrol are rather poor (Table 1). The effects of resveratrol against oxidative injury *in vivo* are more likely to be attributable to its effects as a gene regulator rather than its direct ROS scavenging activity (Li *et al.*, 2012; Xia *et al.*, 2014b).

Resveratrol as a gene regulator of the redox system

Many of resveratrol's protective effects *in vivo* are mediated by gene regulation. In whole-genome microarray experiments using liver samples from mice fed a high-calorie diet, the expression patterns of 782 out of 41 534 individual genes are changed significantly by resveratrol treatment. Remarkably, resveratrol prevents the effects of high caloric intake in 144 out of 153 significantly altered pathways (Baur *et al.*, 2006). These results indicate that resveratrol is a powerful gene regulator (Xia *et al.*, 2014b).

Direct molecular targets of resveratrol

Resveratrol has been shown to induce various biological effects in preclinical studies. This is probably because resveratrol is a molecule with many targets (Bollmann *et al.*, 2014). Resveratrol is relatively hydrophobic because of its planar stilbene motif. Therefore, resveratrol has a relatively high affinity for hydrophobic pockets and binding sites in proteins. Moreover, the polar OH groups act as both hydrogen-bond donors and acceptors, which can form multiple interactions with amino acid side chains as well as backbone amide groups (Britton *et al.*, 2015). There have been around 20 proteins identified as having a specific affinity for resveratrol to date (Britton *et al.*, 2015).

For the antioxidant effects of resveratrol in the cardiovascular system, the NAD^+-dependent histone/protein deacetylase sirtuin 1 (SIRT1) and the nuclear factor-E2-related factor-2 (Nrf2) are particularly important (see below). Another important resveratrol target for its cardiovascular effects is the oestrogen receptor (ER). A subpopulation of ERα is associated with caveolae in the endothelial plasma membrane and coupled to the eNOS in endothelial cells via a G protein (Wyckoff *et al.*, 2001). Resveratrol has been shown to rapidly activate eNOS by stimulating the membrane ER (Klinge *et al.*, 2005; Klinge *et al.*, 2008). This represents one of the many mechanisms by which resveratrol enhances endothelial NO production (Xia *et al.*, 2014a). In addition to the direct vasoprotective effects of endothelial NO (Xia *et al.*, 2014a), ER-mediated NO production is likely to be involved in resveratrol-induced up-regulation of antioxidant proteins such as thioredoxin-1 and also HO1 (Thirunavukkarasu *et al.*, 2007; Yu *et al.*, 2010).

SIRT1 as a resveratrol target

Among the known resveratrol targets, SIRT1 has received much attention. Resveratrol has been identified as a SIRT1 activator in an *in vitro* assay (Howitz *et al.*, 2003). However, later studies indicate that resveratrol directly activates SIRT1 only on certain substrates (Hubbard *et al.*, 2013). Indirectly, resveratrol may activate SIRT1 either through a signalling cascade involving PDE inhibition and subsequent elevation of cellular NAD^+ (Park *et al.*, 2012) or by enhancing the binding of SIRT1 to lamin A, a protein activator of SIRT1 (Liu *et al.*, 2012). Resveratrol inhibits PDE1, PDE3 and PDE4 with IC_{50} values of 6, 10 and 14 μM, respectively, without affecting the activity of PDE2 or PDE5 (Park *et al.*, 2012). Finally, the SIRT1-dependent effects of resveratrol *in vivo* may be also partially attributable to an up-regulation of SIRT1 expression (Csiszar *et al.*, 2009; Xia *et al.*, 2013).

Like resveratrol, SIRT1 is also a molecule with many targets, which is the molecular basis by which SIRT1 regulates a broad range of biological processes. SIRT1 modulates gene expression by targeting molecules such as histones, non-histone substrates (e.g. transcription factors and co-regulators) and SIRT1-interacting proteins (Zhang and Kraus, 2010). For instance, SIRT1 deacetylates the RelA/p65 subunit of NF-κB, thereby suppressing inflammation (Yeung *et al.*, 2004). By targeting p53, sterol regulatory element-binding proteins, forkhead box O (FOXO) transcription factors and proliferator-activated receptor–coactivator (PGC)-1α, SIRT1 modifies the expression of a number of enzymes involved in cell cycle/apoptosis, stress defence, anti-ageing processes, lipid metabolism and metabolic adaptation (Liang *et al.*, 2009; Sinclair and Guarente, 2014). In addition, SIRT1 target molecules also include some cytosolic proteins that are not transcription factors or cofactors including eNOS (Mattagajasingh *et al.*, 2007).

SIRT1-dependent up-regulation of antioxidant enzymes

Resveratrol regulates the expression and activity of a number of redox enzymes, thereby inhibiting ROS production and facilitating ROS detoxification (Figure 1).

The up-regulation of SOD enzymes by resveratrol has been observed in cultured cells (Spanier *et al.*, 2009; Ungvari *et al.*, 2009; Xia *et al.*, 2010) as well as in laboratory animals *in vivo* (Xia *et al.*, 2010). In cultured human endothelial cells, resveratrol (10–100 μM) increases the mRNA and protein levels of all three SOD enzymes (Spanier *et al.*, 2009; Ungvari *et al.*, 2009; Xia *et al.*, 2010). The up-regulation of SOD1 and SOD2, but not that of SOD3, by resveratrol is likely to be mediated by SIRT1 (Ungvari *et al.*, 2009; Xia *et al.*, 2010). A recent study has shown that the SIRT1-induced up-regulation of SOD2 is partially mediated by FOXO1, a transcription factor regulated by SIRT1 (Hsu *et al.*, 2010).

Resveratrol enhances the expression of GPx1 and catalase in cultured human endothelial cells (Ungvari *et al.*, 2007; Xia *et al.*, 2010) as well as in cardiac tissue of ApoE-KO mice (Xia *et al.*, 2010). SIRT1 is likely to be involved in the up-regulation of GPx1 (Xia *et al.*, 2010) and catalase (Alcendor *et al.*, 2007) by resveratrol. The transcription factor FOXO3a, which is a

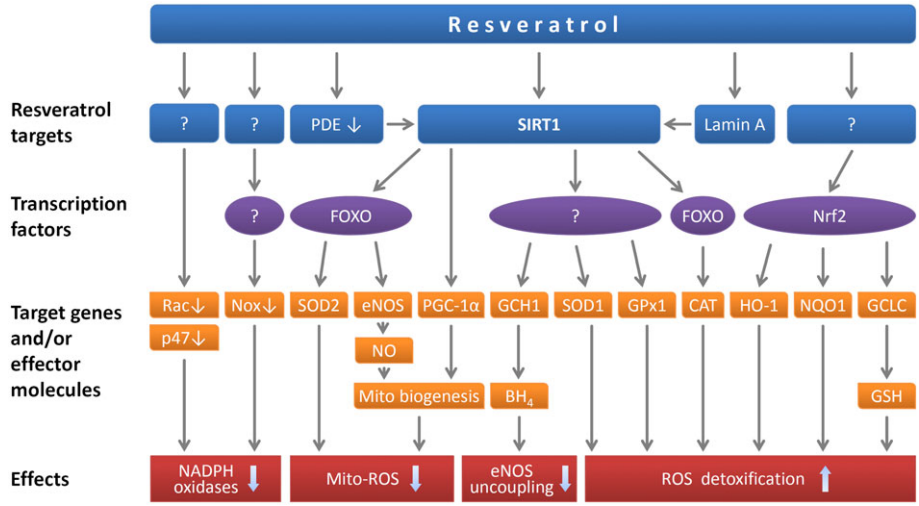

Figure 1

Antioxidant effects of resveratrol. Resveratrol inhibits NADPH oxidase-mediated ROS production by down-regulation of the catalytic subunits (NOX proteins) and by inhibiting membrane translocation of Rac1 and inhibiting phosphorylation of p47phox. Resveratrol directly activates SIRT1 on certain substrates. It can also activate SIRT1 indirectly by potentiating the activation effect of lamin A or via a pathway involving PDE inhibition that leads to elevation of cellular NAD^+. Among the established SIRT1 targets, FOXO transcription factors contribute to the antioxidative effects of resveratrol by up-regulating antioxidative enzymes (e.g. SOD2 and catalase, CAT) and eNOS. SIRT1 inhibits mitochondrial $O_2^{\bullet -}$ production by stimulating mitochondrial biogenesis, which is mediated by proliferator-activated receptor–coactivator-1α (PGC-1α) deacetylation and by NO-dependent mechanisms. The up-regulation of GCH1 leads to enhancement of BH_4 biosynthesis and prevention of eNOS uncoupling. In addition, resveratrol up-regulates a number of antioxidant enzymes by activating Nrf2.

target molecule of SIRT1, has been implicated in the SIRT1-mediated up-regulation of catalase (Hasegawa *et al.*, 2008; Liang *et al.*, 2009).

Nrf2-dependent up-regulation of antioxidant enzymes

Nrf2 is an indirect target of resveratrol (Ungvari *et al.*, 2010). Under quiescent conditions, Nrf2 is localized in the cytoplasm through binding to Kelch-like erythroid cap'n'collar homologue (ECH) associated protein 1 (Keap 1). This interaction facilitates the ubiquitination and subsequent degradation of Nrf2. Treatment of cells with resveratrol leads to Nrf2 release from Keap 1 and Nrf2 translocation to the nucleus. The binding of Nrf2 to antioxidant response elements triggers antioxidant response element-dependent transcription of phase II and antioxidant defence enzymes. In cultured endothelial cells, resveratrol up-regulates gene expression of antioxidant defence enzymes NQO1 and HO1 in an Nrf2-dependent manner (Ungvari *et al.*, 2010), although the molecular mechanism by which resveratrol activates Nrf2 is still unclear.

Lower concentrations of resveratrol (0.1–1 μM) are able to produce this effect (Ungvari *et al.*, 2010), whereas higher concentrations are needed to activate SIRT1 (high μM) (Howitz *et al.*, 2003; Milne *et al.*, 2007). The *in vivo* relevance of resveratrol-induced Nrf2 activation has been demonstrated in mice fed a high-fat diet, in which the endothelial protective effects of resveratrol are largely diminished by genetic Nrf2 depletion (Ungvari *et al.*, 2010). Nrf2 may be also involved in GPx1 up-regulation (Gounder *et al.*, 2012; Howden, 2013), although direct evidence is still unavailable.

Effects of resveratrol on nonenzymatic antioxidants

Nrf2 activation by resveratrol also leads to an up-regulation of γ-glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis (Ungvari *et al.*, 2010). Consistently, resveratrol increases endothelial GSH content (Ungvari *et al.*, 2009).

Reduction of ROS production from vascular NADPH oxidases

The expression of NOX2 and NOX4 in the heart of hypercholesterolemic ApoE-KO mouse is reduced by resveratrol (100 $mg \cdot kg^{-1}$) (Xia *et al.*, 2010). This effect is likely to be independent of SIRT1; the down-regulation of NOX4 by resveratrol in endothelial cells (by 10–100 μM resveratrol) was not affected by SIRT1 inhibition or SIRT1 knockdown (Spanier *et al.*, 2009; Xia *et al.*, 2010). Trauma haemorrhage in rats leads to an up-regulation of vascular NOX1, NOX2, NOX4, p22phox and p47phox. All these expressional changes can be normalized by resveratrol (30 $mg \cdot kg^{-1}$ i.v.) treatment (Yu *et al.*, 2010). The effect of resveratrol in trauma haemorrhagic rats is abolished by an ER antagonist or by a haem oxygenase enzyme inhibitor. Thus, it is possible that an ER-dependent up-regulation of HO1 is involved in the regulation of NADPH oxidase by resveratrol (Yu *et al.*, 2010).

In addition to its effect on NOX expression, resveratrol also modulates the activity of the NADPH oxidase enzyme complex. The activity of NOX4 relies on its association with p22phox, whereas the activity of NOX1 and NOX2 in

vascular cells requires not only p22phox but also p47phox (or NOXO1), p67phox (or NOXA1) and Rac proteins (Brandes and Kreuzer, 2005). Resveratrol (5 μM) reduces angiotensin II- and oxLDL-induced NADPH oxidase activation in cultured endothelial cells by inhibiting the membrane translocation of Rac (Chow *et al.*, 2007). In platelets, protein kinase C-mediated phosphorylation and activation of p47phox are prevented by resveratrol (0.15–0.25 μM) (Shen *et al.*, 2007).

Reduction of ROS production from mitochondria

Resveratrol stimulates mitochondrial biogenesis and thereby decreases mitochondrial ROS generation because mitochondrial proliferation reduces the flow of electrons per unit of mitochondria (Csiszar *et al.*, 2009; Beauloye *et al.*, 2011). Mitochondrial biogenesis is impaired in the aorta of type 2 diabetic db/db mice, and this impairment can be normalized by resveratrol treatment (20 $mg \cdot kg^{-1}$) (Csiszar *et al.*, 2009). Resveratrol (10 μM) increases mitochondrial mass and mitochondrial DNA content and up-regulates the electron transport chain constituents and mitochondrial biogenesis factors in human cultured coronary arterial endothelial cells (Csiszar *et al.*, 2009). SIRT1-dependent NO production (Csiszar *et al.*, 2009; Xia *et al.*, 2013) and SIRT1-mediated PGC-1α deacetylation (Beauloye *et al.*, 2011) are implicated in resveratrol-stimulated mitochondrial biogenesis in endothelial cells.

Resveratrol decreases mitochondrial ROS levels not only by reducing ROS production but also by up-regulating antioxidant defence systems and thus accelerating ROS detoxification. The expression of SOD2 is enhanced by resveratrol in a SIRT1-dependent manner (Ungvari *et al.*, 2009). An up-regulation of SOD2 *per se* increases mitochondrial generation of H_2O_2, which can easily penetrate mitochondrial membranes and diffuse into the cytoplasm. Interestingly, resveratrol treatment results in lower cytoplasmic H_2O_2 levels (Ungvari *et al.*, 2009), which may result from increased H_2O_2 detoxification by GPx1 in mitochondria and/or by enhanced H_2O_2 inactivation by GPx1 and catalase in the cytoplasm. Both antioxidant enzymes are up-regulated by resveratrol (see above).

Reduction of ROS production from uncoupled eNOS

In the hypercholesterolaemic, atherosclerosis-prone ApoE-KO mice, uncoupled eNOS contributes significantly to the oxidative stress in cardiovascular tissues. ApoE-KO mice show increased ROS production in the aorta (Alp *et al.*, 2004; Wohlfart *et al.*, 2008) and the heart (Xia *et al.*, 2010). The NOS inhibitor L-NAME decreases $O_2^{\bullet -}$ production in both organs (Alp *et al.*, 2004; Wohlfart *et al.*, 2008; Xia *et al.*, 2010), indicating eNOS uncoupling in this pathological model. The major molecular mechanism of eNOS uncoupling in ApoE-KO mice has been found to be a deficiency of BH_4, very likely due to increased oxidative degradation of the molecule (Alp *et al.*, 2004).

Treatment of ApoE-KO mice with resveratrol (30 or 100 $mg \cdot kg^{-1}$) enhances the expression of the BH_4-synthesizing enzyme GCH1, increases the biosynthesis of BH_4 and reverses eNOS uncoupling (Xia *et al.*, 2010). Findings from cell culture studies demonstrate that the up-regulation of GCH1 by resveratrol is a SIRT1-dependent effect, because it can be reduced by the SIRT1 inhibitor sirtinol or by siRNA-mediated SIRT1 knockdown (Xia *et al.*, 2010). At the same time, resveratrol also prevents BH_4 oxidation by reducing ROS levels, through both SIRT1-dependent (up-regulation of SOD1, SOD2, GPx1 and catalase) and SIRT1-independent (up-regulation of SOD3 and down-regulation of NOX4) mechanisms (Xia *et al.*, 2010). Resveratrol-induced up-regulation of GCH1 and elevation of BH_4 levels have also been observed in superior thyroid arteries obtained from patients with hypertension and dyslipidaemia (Carrizzo *et al.*, 2013).

Resveratrol doses and pharmacokinetics

The optimal resveratrol dose is not known. Because of the low bioavailability of resveratrol (Baur and Sinclair, 2006; Cottart *et al.*, 2014), very high resveratrol doses (up to 3000 mg) have been used in some clinical trials (Table 2). A recent study indicates that such high doses may be unnecessary. Interestingly, the low dose (5 mg in humans or 0.07 $mg \cdot kg^{-1}$ in mice) has been shown to have even superior cancer chemopreventive efficacy than the high dose (1000 mg in humans or 14 $mg \cdot kg^{-1}$ in mice) (Cai *et al.*, 2015). Under certain conditions, resveratrol may display a hormetic action, protecting cells at lower doses while being detrimental at higher doses (Juhasz *et al.*, 2010a).

About 1 h after oral ingestion of a single resveratrol dose by healthy volunteers, the maximum peak plasma concentrations (C_{max}) of 0.6 and 137 μM (for intakes of 5 mg and 1 g respectively) are reached (Cai *et al.*, 2015). Circulating resveratrol is still detectable as late as 24 h after resveratrol administration (average resveratrol concentrations 0.08 and 14 μM in the 5 mg and 1 g dose groups respectively) (Cai *et al.*, 2015).

The following mechanisms may contribute to the phenomenon that low resveratrol doses are effective, despite the rapid and extensive metabolism of resveratrol into sulfate and glucuronide conjugates. (i) Some resveratrol metabolites are biologically active, although not as effective as the parent molecule (Miksits *et al.*, 2009; Lu *et al.*, 2013). (ii) Resveratrol and its metabolites can accumulate in tissues, resulting in enhanced concentrations compared to those in serum (Bresciani *et al.*, 2014; Cai *et al.*, 2015). (iii) Some metabolites can be converted back to resveratrol in tissues (Miksits *et al.*, 2009).

Cardiovascular effects of resveratrol in humans

Preclinical studies have demonstrated a variety of protective effects in animal models of cardiovascular disease, including hypertension (Mizutani *et al.*, 2000; Dolinsky *et al.*, 2009; Dolinsky *et al.*, 2013), hypercholesterolaemia (Penumathsa *et al.*, 2007; Juhasz *et al.*, 2011), atherosclerosis (Wang *et al.*, 2005; Do *et al.*, 2008), ischaemic heart disease (Andreadou *et al.*, 2015; Novelle *et al.*, 2015), diabetes (Su *et al.*, 2006; Um *et al.*, 2010) and metabolic syndrome (Novelle *et al.*, 2015;

Table 2

Cardiovascular effects of resveratrol in humans

	Resveratrol treatment		Resveratrol effects						References
Study subjects	**Daily dose**	**Duration**	**BP**	**Lipid**	**Glucose**	**Anthropometry**	**Inflammation**	**Other effects**	
Healthy subjects (n = 20)	400 mg	30 days			Glucose ↔ Insulin ↓ HOMA-IR ↓		TNF-α ↔ IL-6 ↔ IFN-γ ↓	Plasma from resveratrol-treated subjects down-regulates, *in vitro*, endothelial VCAM and ICAM expression	Agarwal *et al.* (2013)
Healthy firefighters (n = 30)	100 mg	90 days		TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔		IL-6 ↓ TNF-α ↓	ALT ↔; AST ↔; GGT ↔; thiol ↔; 8-isoprostane ↔; 8-OHdG ↔; erythrocyte GPx activity ↓	Macedo *et al.* (2015)
Non-obese, postmenopausal women (n = 15)	75 mg	84 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose↔ Insulin↔ HOMA-IR↔	BW ↔ BMI ↔	CRP ↔ IL-6 ↔ Leptin ↔ ADPN ↔		Yoshino *et al.* (2012)
Healthy aged physically inactive men (n = 14)	250 mg	56 days	MAP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔	BW ↔ BMI ↔	SIRT1 ↔	Resveratrol blunts the positive effects (MAP, TG and LDL) of exercise training in aged men	Gliemann *et al.* (2013)
Healthy aged physically inactive men (n = 9)	250 mg	56 days	MAP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔	BW ↔ BMI ↔	CRP ↓ TNF-α ↔ IL-6 ↔	Resveratrol impairs exercise training-induced effects in skeletal muscle	Olesen *et al.* (2014)
Healthy obese men (n = 11)	150 mg	30 days	SBP ↓ DBP ↔	TG ↓	Glucose ↓ Insulin ↓ HOMA-IR ↓	BW ↔	CRP ↔ IL-6 ↔ TNF-α ↓ Leptin ↓ ADPN ↔	Metabolic rate ↓; activation of AMPK and SIRT1	Timmers *et al.* (2011)
Healthy obese men (n = 28)	75 mg	42 days	SBP ↔ DBP ↔			BMI ↔		FMD ↑	Wong *et al.* (2013)
Healthy obese men (n = 12)	500 mg	28 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↔ HbA1c ↔	BMI ↔	hsCRP ↔ IL-6 ↔ TNF-α ↔ MCP-1 ↔	No effect on resting energy expenditure or lipid oxidation rates	Poulsen *et al.* (2013)

(Continues)

Table 2 (Continued)

Study subjects	Resveratrol treatment		Resveratrol effects						References
	Daily dose	Duration	BP	Lipid	Glucose	Anthropometry	Inflammation	Other effects	
Healthy obese men (n = 10)	150 mg	30 days						GLP-1 ↔ GIP ↔ Glucagon ↓	Knop *et al.* (2013)
Overweight older adults (n = 10–12)	300 or 1000 mg	90 days	SBP ↔ DBP ↔		Glucose ↓	BW ↔ BMI ↔ WC ↔			Anton *et al.* (2014)
Overweight/obese individuals with mild hypertriglyceridaemia (n = 8)	1–2 g	14 days		TG ↔ TC ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↔			Intestinal and hepatic lipoprotein particle production ↓	Dash *et al.* (2013)
Older adults with impaired glucose tolerance (n = 10)	1–2 g	28 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↓	BW ↔	hsCRP ↔ ADPN ↔		Crandall *et al.* (2012)
Patients with metabolic syndrome (n = 34)	100 mg	90 days	SBP ↔ DBP ↔	TG ↔ LDL ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↔ HbA1c ↔	BW ↔ BMI ↔ WC ↔	hsCRP ↔ IL-6 ↔	FMD↑	Fujitaka *et al.* (2011)
Patients with metabolic syndrome (n = 12)	1.5 g	90 days			Insulin ↓	BW ↓ BMI ↓ WC ↓			Mendez-del Villar *et al.* (2014)
Patients with type 2 diabetes mellitus (n = 28)	250 mg	90 days	SBP ↓ DBP ↓	TG ↔ TC ↓ LDL ↓ HDL ↔	Glucose ↓ HbA1c ↓	BW ↔ BMI ↔			Bhatt *et al.* (2012)
Patients with type 2 diabetes (n = 33)	1 g	45 days	SBP ↓ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↑	Glucose ↓ Insulin ↓ HOMA-IR ↓ HbA1c ↓	BW ↔ BMI ↔			Movahed *et al.* (2013)
Patients with type 2 diabetes (n = 5)	3 g	84 days		TG ↔ TC ↔ LDL ↑ HDL ↔	Glucose ↔ Insulin ↔ HbA1c ↔ HOMA-IR ↔	BW ↔ BMI ↔	ADPN ↔	SIRT1 ↑; p-AMPK/AMPK ↑ in skeletal muscle	Goh *et al.* (2014)

(Continues)

Table 2 (Continued)

Study subjects	Resveratrol treatment		Resveratrol effects						References
	Daily dose	Duration	BP	Lipid	Glucose	Anthropometry	Inflammation	Other effects	
Patients with type 2 diabetes and hypertension (n = 13)	8 mg	365 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↓ HbA1c ↓		hsCRP ↔ ADPN ↔ TNF-α ↔ PAI-1 ↔ IL-6 ↓	Expression changes of cytokines and microRNAs in PBMC	Tome-Carneiro *et al.* (2013b)
Patients undergoing primary CVD prevention (n = 25)	8 mg	365 days		TG ↔ TC ↔ LDL ↔ HDL ↔			hsCRP ↓ TNF-α ↓ ADPN ↔ IL-6 ↔ PAI-1 ↓		Tome-Carneiro *et al.* (2012)
Patients with stable CAD (n = 25)	8–16 mg	365 days					hsCRP ↓ TNF-α ↔ ADPN ↓ IL-6 ↔ PAI-1 ↓	Expression of pro-inflammatory gene in PBMCs ↓	Tome-Carneiro *et al.* (2013a)
Patients with stable angina pectoris (n = 29)	20 mg	60 days		TG ↓ TC ↓ LDL ↓ HDL ↑			hsCRP ↓	NT-proBNP ↓	Militaru *et al.* (2013)
Post-infarction patients with stable CAD (n = 20)	10 mg	90 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↓ HDL ↔	HbA1c ↔		CRP ↔ TNF-α ↔	Left ventricular diastolic function ↑; FMD ↑; platelet activity ↓	Magyar *et al.* (2012)
Overweight or obese men with NAFLD (n = 10)	3 g	56 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↔	BW ↔ BMI ↔	CRP ↔ TNF-α ↔ IL-6 ↓	ALT ↑; AST ↔ Liver steatosis ↔ F2-isoprostanes ↔ Total antioxidant capacity ↔	Chachay *et al.* (2014)
Overweight patients with NAFLD (n = 25)	500 mg	84 days	SBP ↔ DBP ↔	TG ↔ TC ↔ LDL ↔ HDL ↔	Glucose ↔ Insulin ↔ HOMA-IR ↔	BW ↓ BMI ↓ WC ↓	hsCRP ↓ TNF-α ↔ IL-6 ↓	ALT ↓; AST ↓; GGT ↓ Liver steatosis ↓	Faghihzadeh *et al.* (2015), Faghihzadeh *et al.* (2014)

(Continues)

Table 2 (Continued)

Study subjects	Resveratrol treatment		Resveratrol effects						References
	Daily dose	Duration	BP	Lipid	Glucose	Anthropometry	Inflammation	Other effects	
Patients with NAFLD (n = 30)	600 mg	90 days	SBP ↔ DBP ↔	TG ↔ TC ↓ LDL ↓ HDL ↔	Glucose ↓ Insulin ↔ HOMA-IR ↓	BW ↔ BMI ↔ WC ↔	hsCRP ↓ ADPN ↑ IL-6 ↓	ALT ↓; AST ↓; GGT ↔	Chen *et al.* (2015)

ADPN, adiponectin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BW, body weight; CAD, coronary artery disease; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; GGT, γ-glutamyl transferase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GPx, GSH peroxidase; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity CRP; ICAM, intercellular adhesion molecule; MAP, mean arterial pressure; MCP-1, monocyte chemoattractant protein-1; n, number of subjects treated with resveratrol; NAFLD, non-alcoholic fatty liver disease; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAI-1, plasminogen activator inhibitor type 1; PBMC, peripheral blood mononuclear cells; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; VCAM, vascular cell adhesion molecule; WC, waist circumference.

Pechanova *et al.*, 2015). These cardiovascular effects of resveratrol in laboratory animals have been reviewed in our previous article (Li *et al.*, 2012) and in a recent publication (Zordoky *et al.*, 2015). Therefore, here we have summarized only the cardiovascular effects of resveratrol observed in clinical trials.

As shown in Table 2, the results of these studies are not always consistent, sometimes even contradictory. Moreover, the antioxidant effect of resveratrol does not always lead to a beneficial effect on cardiovascular health. For instance, exercise training induces a number of beneficial cardiovascular effects in healthy aged men, probably mediated partly through ROS-dependent mechanisms. A concomitant oral resveratrol supplementation, however, blunts part of these positive effects of exercise training (Gliemann *et al.*, 2013; Olesen *et al.*, 2014).

Overall, the major limitation of the clinical studies currently available is the small sample size (Table 2). Large clinical trials are clearly warranted to establish the clinical significance of resveratrol in humans.

Conclusion

The antioxidant effects of resveratrol are implicated in the health benefits of the compound. The direct ROS-scavenging effects of resveratrol are relatively poor. Resveratrol's antioxidant effects *in vivo* are more likely to be attributable to its regulation of redox genes leading to reduced ROS production from NADPH oxidases, uncoupled eNOS and the mitochondria. At the same time, an up-regulation of antioxidant enzymes by resveratrol accelerates the detoxification of ROS.

Acknowledgements

Original work from our own laboratory contributing to this review was supported by the Collaborative Research Center SFB 553 and by grants LI-1042/1-1 and LI-1042/3-1 from the DFG (Deutsche Forschungsgemeinschaft), Bonn, Germany. The present work was supported by the European Cooperation in Science and Research (COST Action BM1203/EU-ROS).

Author contributions

N.X. and H.L. wrote the initial draft of the manuscript. All authors critically reviewed and revised the manuscript and agreed to its publication.

Conflict of interest

The authors declare no conflicts of interest.

References

Agarwal B, Campen MJ, Channell MM, Wherry SJ, Varamini B, Davis JG *et al.* (2013). Resveratrol for primary prevention of atherosclerosis:

clinical trial evidence for improved gene expression in vascular endothelium. Int J Cardiol 166: 246–248.

Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X *et al.* (2007). Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res 100: 1512–1521.

Alexander SP, Cidlowski JA, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The concise guide to PHARMACOLOGY 2015/16: nuclear hormone receptors. Br J Pharmacol 172: 5956–5978.

Alexander SP, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015b). The concise guide to PHARMACOLOGY 2015/16: enzymes. Br J Pharmacol 172: 6024–6109.

Alp NJ, McAteer MA, Khoo J, Choudhury RP, Channon KM (2004). Increased endothelial tetrahydrobiopterin synthesis by targeted transgenic GTP-cyclohydrolase I overexpression reduces endothelial dysfunction and atherosclerosis in ApoE-knockout mice. Arterioscler Thromb Vasc Biol 24: 445–450.

Andreadou I, Iliodromitis EK, Rassaf T, Schulz R, Papapetropoulos A, Ferdinandy P (2015). The role of gasotransmitters NO, H2S and CO in myocardial ischaemia/reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. Br J Pharmacol 172: 1587–1606.

Anton SD, Embry C, Marsiske M, Lu X, Doss H, Leeuwenburgh C *et al.* (2014). Safety and metabolic outcomes of resveratrol supplementation in older adults: results of a twelve-week, placebo-controlled pilot study. Exp Gerontol 57: 181–187.

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A *et al.* (2006). Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444: 337–342.

Baur JA, Sinclair DA (2006). Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5: 493–506.

Beauloye C, Bertrand L, Horman S, Hue L (2011). AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure. Cardiovasc Res 90: 224–233.

Bedard K, Krause KH (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87: 245–313.

Bhatt JK, Thomas S, Nanjan MJ (2012). Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res 32: 537–541.

Bollmann F, Art J, Henke J, Schrick K, Besche V, Bros M *et al.* (2014). Resveratrol post-transcriptionally regulates pro-inflammatory gene expression via regulation of KSRP RNA binding activity. Nucleic Acids Res 42: 12555–12569.

Brandes RP, Kreuzer J (2005). Vascular NADPH oxidases: molecular mechanisms of activation. Cardiovasc Res 65: 16–27.

Bresciani L, Calani L, Bocchi L, Delucchi F, Savi M, Ray S *et al.* (2014). Bioaccumulation of resveratrol metabolites in myocardial tissue is dose-time dependent and related to cardiac hemodynamics in diabetic rats. Nutr Metab Cardiovasc Dis 24: 408–415.

Brito P, Almeida LM, Dinis TC (2002). The interaction of resveratrol with ferrylmyoglobin and peroxynitrite; protection against LDL oxidation. Free Radic Res 36: 621–631.

Britton RG, Kovoor C, Brown K (2015). Direct molecular targets of resveratrol: identifying key interactions to unlock complex mechanisms. Ann N Y Acad Sci 1348: 124–133.

Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A *et al.* (2015). Cancer chemoprevention: evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Sci Transl Med 7: 298ra117.

Carrizzo A, Puca A, Damato A, Marino M, Franco E, Pompeo F *et al.* (2013). Resveratrol improves vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism. Hypertension 62: 359–366.

Chachay VS, Macdonald GA, Martin JH, Whitehead JP, O'Moore-Sullivan TM, Lee P *et al.* (2014). Resveratrol does not benefit patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 12: 2092–2103 e2091-2096.

Chalupsky K, Cai H (2005). Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 102: 9056–9061.

Chen S, Zhao X, Ran L, Wan J, Wang X, Qin Y*et al.* (2015). Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: a randomized controlled trial. Dig Liver Dis 47: 226–232.

Chow SE, Hshu YC, Wang JS, Chen JK (2007). Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. J Appl Physiol 102: 1520–1527.

Cottart CH, Nivet-Antoine V, Beaudeux JL (2014). Review of recent data on the metabolism, biological effects, and toxicity of resveratrol in humans. Mol Nutr Food Res 58: 7–21.

Crandall JP, Oram V, Trandafirescu G, Reid M, Kishore P, Hawkins M *et al.* (2012). Pilot study of resveratrol in older adults with impaired glucose tolerance. J Gerontol A Biol Sci Med Sci 67: 1307–1312.

Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G *et al.* (2009). Resveratrol induces mitochondrial biogenesis in endothelial cells. Am J Physiol Heart Circ Physiol 297: H13–H20.

Dash S, Xiao C, Morgantini C, Szeto L, Lewis GF (2013). High-dose resveratrol treatment for 2 weeks inhibits intestinal and hepatic lipoprotein production in overweight/obese men. Arterioscler Thromb Vasc Biol 33: 2895–2901.

Do GM, Kwon EY, Kim HJ, Jeon SM, Ha TY, Park T*et al.* (2008). Long-term effects of resveratrol supplementation on suppression of atherogenic lesion formation and cholesterol synthesis in apo E-deficient mice. Biochem Biophys Res Commun 374: 55–59.

Dolinsky VW, Chakrabarti S, Pereira TJ, Oka T, Levasseur J, Beker D *et al.* (2013). Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rats and mice. Biochim Biophys Acta 1832: 1723–1733.

Dolinsky VW, Chan AY, Robillard Frayne I, Light PE, Des Rosiers C, Dyck JR (2009). Resveratrol prevents the prohypertrophic effects of oxidative stress on LKB1. Circulation 119: 1643–1652.

Drummond GR, Selemidis S, Griendling KK, Sobey CG (2011). Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. Nat Rev Drug Discov 10: 453–471.

Dunn LL, Midwinter RG, Ni J, Hamid HA, Parish CR, Stocker R (2014). New insights into intracellular locations and functions of heme oxygenase-1. Antioxid Redox Signal 20: 1723–1742.

Faghihzadeh F, Adibi P, Hekmatdoost A (2015). The effects of resveratrol supplementation on cardiovascular risk factors in patients with non-alcoholic fatty liver disease: a randomised, double-blind, placebo-controlled study. Br J Nutr 114: 796–803.

Faghihzadeh F, Adibi P, Rafiei R, Hekmatdoost A (2014). Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. Nutr Res 34: 837–843.

Forstermann U, Li H (2011). Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling. Br J Pharmacol 164: 213–223.

Forstermann U, Munzel T (2006). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113: 1708–1714.

Fujitaka K, Otani H, Jo F, Jo H, Nomura E, Iwasaki M *et al.* (2011). Modified resveratrol Longevinex improves endothelial function in adults with metabolic syndrome receiving standard treatment. Nutr Res 31: 842–847.

Gliemann L, Schmidt JF, Olesen J, Bienso RS, Peronard SL, Grandjean SU *et al.* (2013). Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. J Physiol 591: 5047–5059.

Goh KP, Lee HY, Lau DP, Supaat W, Chan YH, Koh AF (2014). Effects of resveratrol in patients with type 2 diabetes mellitus on skeletal muscle SIRT1 expression and energy expenditure. Int J Sport Nutr Exerc Metab 24: 2–13.

Gounder SS, Kannan S, Devadoss D, Miller CJ, Whitehead KJ, Odelberg SJ *et al.* (2012). Impaired transcriptional activity of Nrf2 in age-related myocardial oxidative stress is reversible by moderate exercise training. PLoS One 7: e45697.

Guzik TJ, Sadowski J, Guzik B, Jopek A, Kapelak B, Przybylowski P *et al.* (2006). Coronary artery superoxide production and nox isoform expression in human coronary artery disease. Arterioscler Thromb Vasc Biol 26: 333–339.

Hasegawa K, Wakino S, Yoshioka K, Tatematsu S, Hara Y, Minakuchi H *et al.* (2008). Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. Biochem Biophys Res Commun 372: 51–56.

Holthoff JH, Woodling KA, Doerge DR, Burns ST, Hinson JA, Mayeux PR (2010). Resveratrol, a dietary polyphenolic phytoalexin, is a functional scavenger of peroxynitrite. Biochem Pharmacol 80: 1260–1265.

Howden R (2013). Nrf2 and cardiovascular defense. Oxid Med Cell Longev 2013: 104308.

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG *et al.* (2003). Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. Nature 425: 191–196.

Hsu CP, Zhai P, Yamamoto T, Maejima Y, Matsushima S, Hariharan N *et al.* (2010). Silent information regulator 1 protects the heart from ischemia/reperfusion. Circulation 122: 2170–2182.

Hubbard BP, Gomes AP, Dai H, Li J, Case AW, Considine T*et al.* (2013). Evidence for a common mechanism of SIRT1 regulation by allosteric activators. Science 339: 1216–1219.

Hung LM, Su MJ, Chu WK, Chiao CW, Chan WF, Chen JK (2002). The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. Br J Pharmacol 135: 1627–1633.

Jia Z, Zhu H, Misra BR, Mahaney JE, Li Y, Misra HP (2008). EPR studies on the superoxide-scavenging capacity of the nutraceutical resveratrol. Mol Cell Biochem 313: 187–194.

Juhasz B, Das DK, Kertesz A, Juhasz A, Gesztelyi R, Varga B (2011). Reduction of blood cholesterol and ischemic injury in the hypercholesteromic rabbits with modified resveratrol, longevinex. [corrected]. Mol Cell Biochem 348: 199–203.

Juhasz B, Mukherjee S, Das DK (2010a). Hormetic response of resveratrol against cardioprotection. Exp Clin Cardiol 15: e134–e138.

Juhasz B, Varga B, Gesztelyi R, Kemeny-Beke A, Zsuga J, Tosaki A (2010b). Resveratrol: a multifunctional cytoprotective molecule. Curr Pharm Biotechnol 11: 810–818.

Klinge CM, Blankenship KA, Risinger KE, Bhatnagar S, Noisin EL, Sumanasekera WK *et al.* (2005). Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells. J Biol Chem 280: 7460–7468.

Klinge CM, Wickramasinghe NS, Ivanova MM, Dougherty SM (2008). Resveratrol stimulates nitric oxide production by increasing estrogen receptor alpha–Src–caveolin-1 interaction and phosphorylation in human umbilical vein endothelial cells. FASEB J 22: 2185–2197.

Knop FK, Konings E, Timmers S, Schrauwen P, Holst JJ, Blaak EE (2013). Thirty days of resveratrol supplementation does not affect postprandial incretin hormone responses, but suppresses postprandial glucagon in obese subjects. Diabet Med 30: 1214–1218.

Kroller-Schon S, Steven S, Kossmann S, Scholz A, Daub S, Oelze M *et al.* (2014). Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models. Antioxid Redox Signal 20: 247–266.

Landmesser U, Spiekermann S, Preuss C, Sorrentino S, Fischer D, Manes C *et al.* (2007). Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. Arterioscler Thromb Vasc Biol 27: 943–948.

Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA *et al.* (2001). Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. Circulation 103: 1282–1288.

Leonard SS, Xia C, Jiang BH, Stinefelt B, Klandorf H, Harris GK *et al.* (2003). Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem Biophys Res Commun 309: 1017–1026.

Li H, Forstermann U (2000). Nitric oxide in the pathogenesis of vascular disease. J Pathol 190: 244–254.

Li H, Forstermann U (2009). Prevention of atherosclerosis by interference with the vascular nitric oxide system. Curr Pharm Des 15: 3133–3145.

Li H, Forstermann U (2013). Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. Curr Opin Pharmacol 13: 161–167.

Li H, Forstermann U (2014). Pharmacological prevention of eNOS uncoupling. Curr Pharm Des 20: 3595–3606.

Li H, Horke S, Forstermann U (2013). Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 34: 313–319.

Li H, Horke S, Forstermann U (2014). Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 237: 208–219.

Li H, Wallerath T, Forstermann U (2002). Physiological mechanisms regulating the expression of endothelial-type NO synthase. Nitric Oxide 7: 132–147.

Li H, Xia N, Forstermann U (2012). Cardiovascular effects and molecular targets of resveratrol. Nitric Oxide 26: 102–110.

Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL *et al.* (1995). Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 11: 376–381.

Liang F, Kume S, Koya D (2009). SIRT1 and insulin resistance. Nat Rev Endocrinol 5: 367–373.

Liu B, Ghosh S, Yang X, Zheng H, Liu X, Wang Z *et al.* (2012). Resveratrol rescues SIRT1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based progeria. Cell Metab 16: 738–750.

Lu DL, Ding DJ, Yan WJ, Li RR, Dai F, Wang Q *et al.* (2013). Influence of glucuronidation and reduction modifications of resveratrol on its biological activities. Chembiochem 14: 1094–1104.

Lubos E, Loscalzo J, Handy DE (2011). Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 15: 1957–1997.

Macedo RC, Vieira A, Marin DP, Otton R (2015). Effects of chronic resveratrol supplementation in military firefighters undergo a physical fitness test–a placebo-controlled, double blind study. Chem Biol Interact 227: 89–95.

Magyar K, Halmosi R, Palfi A, Feher G, Czopf L, Fulop A *et al.* (2012). Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc 50: 179–187.

Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB *et al.* (2007). SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 104: 14855–14860.

Mendez-del Villar M, Gonzalez-Ortiz M, Martinez-Abundis E, Perez-Rubio KG, Lizarraga-Valdez R (2014). Effect of resveratrol administration on metabolic syndrome, insulin sensitivity, and insulin secretion. Metab Syndr Relat Disord 12: 497–501.

Miksits M, Wlcek K, Svoboda M, Kunert O, Haslinger E, Thalhammer T*et al.* (2009). Antitumor activity of resveratrol and its sulfated metabolites against human breast cancer cells. Planta Med 75: 1227–1230.

Militaru C, Donoiu I, Craciun A, Scorei ID, Bulearca AM, Scorei RI (2013). Oral resveratrol and calcium fructoborate supplementation in subjects with stable angina pectoris: effects on lipid profiles, inflammation markers, and quality of life. Nutrition 29: 178–183.

Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ *et al.* (2007). Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 450: 712–716.

Mizutani K, Ikeda K, Kawai Y, Yamori Y (2000). Resveratrol attenuates ovariectomy-induced hypertension and bone loss in stroke-prone spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo) 46: 78–83.

Movahed A, Nabipour I, Lieben Louis X, Thandapilly SJ, Yu L, Kalantarhormozi M *et al.* (2013). Antihyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. Evid Based Complement Alternat Med 2013: 851267.

Nojiri H, Shimizu T, Funakoshi M, Yamaguchi O, Zhou H, Kawakami S *et al.* (2006). Oxidative stress causes heart failure with impaired mitochondrial respiration. J Biol Chem 281: 33789–33801.

Novelle MG, Wahl D, Dieguez C, Bernier M, de Cabo R (2015). Resveratrol supplementation: where are we now and where should we go? Ageing Res Rev 21: 1–15.

Olesen J, Gliemann L, Bienso R, Schmidt J, Hellsten Y, Pilegaard H (2014). Exercise training, but not resveratrol, improves metabolic and inflammatory status in skeletal muscle of aged men. J Physiol 592: 1873–1886.

Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H *et al.* (2012). Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. Cell 148: 421–433.

Patetsios P, Song M, Shutze WP, Pappas C, Rodino W, Ramirez JA *et al.* (2001). Identification of uric acid and xanthine oxidase in atherosclerotic plaque. Am J Cardiol 88: 188–191 A186.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.* (2014). The IUPHAR/BPS guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. Nucleic Acids Res 42: D1098–D1106.

Pechanova O, Varga ZV, Cebova M, Giricz Z, Pacher P, Ferdinandy P (2015). Cardiac NO signalling in the metabolic syndrome. Br J Pharmacol 172: 1415–1433.

Penumathsa SV, Thirunavukkarasu M, Koneru S, Juhasz B, Zhan L, Pant R *et al.* (2007). Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. J Mol Cell Cardiol 42: 508–516.

Poulsen MM, Vestergaard PF, Clasen BF, Radko Y, Christensen LP, Stodkilde-Jorgensen H *et al.* (2013). High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 62: 1186–1195.

Ross D, Siegel D (2004). NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase), functions and pharmacogenetics. Methods Enzymol 382: 115–144.

Schmidt TS, Alp NJ (2007). Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease. Clin Sci (Lond) 113: 47–63.

Schulz E, Wenzel P, Munzel T, Daiber A (2014). Mitochondrial redox signaling: Interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. Antioxid Redox Signal 20: 308–324.

Shen MY, Hsiao G, Liu CL, Fong TH, Lin KH, Chou DS *et al.* (2007). Inhibitory mechanisms of resveratrol in platelet activation: pivotal roles of p38 MAPK and NO/cyclic GMP. Br J Haematol 139: 475–485.

Sinclair DA, Guarente L (2014). Small-molecule allosteric activators of sirtuins. Annu Rev Pharmacol Toxicol 54: 363–380.

Spanier G, Xu H, Xia N, Tobias S, Deng S, Wojnowski L *et al.* (2009). Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). J Physiol Pharmacol 60 (Suppl. 4): 111–116.

Su HC, Hung LM, Chen JK (2006). Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 290: E1339–E1346.

Thirunavukkarasu M, Penumathsa SV, Koneru S, Juhasz B, Zhan L, Otani H *et al.* (2007). Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. Free Radic Biol Med 43: 720–729.

Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH *et al.* (2011). Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 14: 612–622.

Tome-Carneiro J, Gonzalvez M, Larrosa M, Yanez-Gascon MJ, Garcia-Almagro FJ, Ruiz-Ros JA *et al.* (2012). One-year consumption of a grape nutraceutical containing resveratrol improves the inflammatory and fibrinolytic status of patients in primary prevention of cardiovascular disease. Am J Cardiol 110: 356–363.

Tome-Carneiro J, Gonzalvez M, Larrosa M, Yanez-Gascon MJ, Garcia-Almagro FJ, Ruiz-Ros JA *et al.* (2013a). Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in

peripheral blood mononuclear cells: a triple-blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease. Cardiovasc Drugs Ther 27: 37–48.

Tome-Carneiro J, Larrosa M, Yanez-Gascon MJ, Davalos A, Gil-Zamorano J, Gonzalvez M *et al.* (2013b). One-year supplementation with a grape extract containing resveratrol modulates inflammatory-related microRNAs and cytokines expression in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease. Pharmacol Res 72: 69–82.

Um JH, Park SJ, Kang H, Yang S, Foretz M, McBurney MW*et al*. (2010). AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. Diabetes 59: 554–563.

Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K *et al.* (2010). Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am J Physiol Heart Circ Physiol 299: H18–H24.

Ungvari Z, Labinskyy N, Mukhopadhyay P, Pinto JT, Bagi Z, Ballabh P *et al.* (2009). Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. Am J Physiol Heart Circ Physiol 297: H1876–H1881.

Ungvari Z, Orosz Z, Rivera A, Labinskyy N, Xiangmin Z, Olson S *et al.* (2007). Resveratrol increases vascular oxidative stress resistance. Am J Physiol Heart Circ Physiol 292: H2417–H2424.

Wang Z, Zou J, Cao K, Hsieh TC, Huang Y, Wu JM (2005). Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. Int J Mol Med 16: 533–540.

White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA *et al.* (1996). Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. Proc Natl Acad Sci U S A 93: 8745–8749.

Wingler K, Hermans JJ, Schiffers P, Moens A, Paul M, Schmidt HH (2011). NOX1, 2, 4, 5: counting out oxidative stress. Br J Pharmacol 164: 866–883.

Wohlfart P, Xu H, Endlich A, Habermeier A, Closs EI, Hubschle T*et al.* (2008). Antiatherosclerotic effects of small-molecular-weight compounds enhancing endothelial nitric-oxide synthase (eNOS) expression and preventing eNOS uncoupling. J Pharmacol Exp Ther 325: 370–379.

Wong RH, Berry NM, Coates AM, Buckley JD, Bryan J, Kunz I *et al.* (2013). Chronic resveratrol consumption improves brachial flow-mediated dilatation in healthy obese adults. J Hypertens 31: 1819–1827.

Wyckoff MH, Chambliss KL, Mineo C, Yuhanna IS, Mendelsohn ME, Mumby SM *et al.* (2001). Plasma membrane estrogen receptors are coupled to endothelial nitric-oxide synthase through Galpha(i). J Biol Chem 276: 27071–27076.

Xia N, Daiber A, Habermeier A, Closs EI, Thum T, Spanier G *et al.* (2010). Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. J Pharmacol Exp Ther 335: 149–154.

Xia N, Forstermann U, Li H (2014a). Resveratrol and endothelial nitric oxide. Molecules 19: 16102–16121.

Xia N, Forstermann U, Li H (2014b). Resveratrol as a gene regulator in the vasculature. Curr Pharm Biotechnol 15: 401–408.

Xia N, Strand S, Schlufter F, Siuda D, Reifenberg G, Kleinert H *et al.* (2013). Role of SIRT1 and FOXO factors in eNOS transcriptional activation by resveratrol. Nitric Oxide 32: 29–35.

Yang H, Roberts LJ, Shi MJ, Zhou LC, Ballard BR, Richardson A *et al.* (2004). Retardation of atherosclerosis by overexpression of catalase or both Cu/Zn-superoxide dismutase and catalase in mice lacking apolipoprotein E. Circ Res 95: 1075–1081.

Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA *et al.* (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23: 2369–2380.

Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman KB *et al.* (2012). Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 16: 658–664.

Yu HP, Hwang TL, Hwang TL, Yen CH, Lau YT (2010). Resveratrol prevents endothelial dysfunction and aortic superoxide production after trauma hemorrhage through estrogen receptor-dependent hemeoxygenase-1 pathway. Crit Care Med 38: 1147–1154.

Zhang T, Kraus WL (2010). SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. Biochim Biophys Acta 1804: 1666–1675.

Zordoky BN, Robertson IM, Dyck JR (2015). Preclinical and clinical evidence for the role of resveratrol in the treatment of cardiovascular diseases. Biochim Biophys Acta 1852: 1155–1177.