

Accepted Manuscript

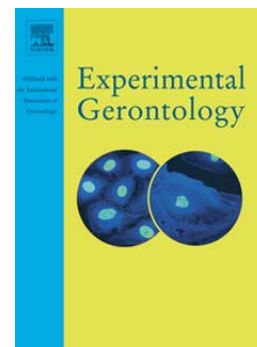
The Effects of Resveratrol on Aging Vessels

Miguel Diaz, Hans Degens, L Vanhees, Clare Austin, May Azzawi

PII: S0531-5565(16)30365-5
DOI: doi:[10.1016/j.exger.2016.09.016](https://doi.org/10.1016/j.exger.2016.09.016)
Reference: EXG 9915

To appear in: *Experimental Gerontology*

Received date: 21 December 2015
Revised date: 14 September 2016
Accepted date: 19 September 2016



Please cite this article as: Diaz, Miguel, Degens, Hans, Vanhees, L., Austin, Clare, Azzawi, May, The Effects of Resveratrol on Aging Vessels, *Experimental Gerontology* (2016), doi:[10.1016/j.exger.2016.09.016](https://doi.org/10.1016/j.exger.2016.09.016)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The Effects of Resveratrol on Aging Vessels

Miguel Diaz^{1,2}; Hans Degens^{1,3}; Vanhees L²; Clare Austin ⁴; May Azzawi^{1,*}.

¹ School of Healthcare Science, Faculty of Science and Engineering.
Manchester Metropolitan University. Manchester, UK.

² Department of Rehabilitation Sciences, University of Leuven, Belgium.

³ Sports Science and Innovation Institute, Lithuanian Sports University, Kaunas,
Lithuania

⁴ Faculty of Health and Social Care, Edge Hill University, Lancashire, UK

* Corresponding author

May Azzawi, PhD.

School of Healthcare Science
Faculty of Science & Engineering
Manchester Metropolitan University
Manchester M1 5GD
United Kingdom
Tel. +44 161 247 3332
Email m.azzawi@mmu.ac.uk

Abstract

Aging is a major risk factor for the development of cardiovascular disease. Despite a significant reduction in the mortality and morbidity rates over the last decade, the socio-economic burden of cardiovascular disease is still substantial. Consequently, there is a considerable need for alternative strategies, such as nutraceutical supplementation, that delay the functional vascular decline present in the elderly. Compromised autophagy and oxidative stress (OS) are considered major causes of the age-related endothelial dysfunction. OS reduces the bioavailability of nitric oxide (NO), which has been associated with hypertension, arteriosclerosis and a reduced vasodilatory response. High levels of free radicals and the low bioavailability of NO lead to a positive feedback loop of further OS, organelle damage, poor repair and endothelial dysfunction. Here we draw attention to the relationship between OS and autophagy in the aged vasculature. We have reviewed the published literature and provided arguments that support that treatment with resveratrol stimulates autophagy and thereby has the potential to restore oxidative balance in the endothelium, which indicates that treatment with resveratrol might have therapeutic potential to restore endothelial function in the elderly.

Keywords: Antioxidant; Autophagy; Endothelium; Oxidative Stress.

1. Introduction

Advances in medicine have allowed humans to become older. Life expectancy in developed countries is now on average 70 years and it is estimated that by 2030 20% of the world's population will be older than 65 years (North and Sinclair, 2012). However, aging is associated with a steady and rapid physiological decline. Aging, for instance, is a major risk factor for the development of cardiovascular disease. In 2014, cardiovascular disease was the second most common cause of death in the UK with approximately 155,000 deaths (Cardiovascular Disease Statistics 2015). Despite a significant reduction in the mortality and morbidity rates over the last decade, the socio-economic burden of cardiovascular disease is still substantial (Bhatnagar et al., 2015). Consequently, there is a considerable need for alternative strategies such as nutraceutical supplementation that delay the functional decline present in old age.

The aged vasculature is characterized by increased arterial thickness, stiffness and endothelial dysfunction. Such structural and functional changes result in hypertension, arteriosclerosis, stroke, poor tissue perfusion, among other pathological conditions (North and Sinclair, 2012). At the molecular level, aging is characterized by a reduced bioavailability of nitric oxide (NO), increased production of collagen by vascular smooth muscle cells, and changes in the expression of proteins that regulate calcium handling (Strait and Lakatta, 2012). NO plays a pivotal role in endothelium-dependent dilation. Further, NO is critical for the prevention of thrombosis and inhibition of platelet aggregation (Tousoulis et al., 2006; Vora et al., 1997). Hence, a reduced bioavailability of NO is associated with endothelial dysfunction. One of the most important factors affecting NO synthesis in the aged vasculature is oxidative stress (OS). Within the endothelial cell, cumulative oxidative damage leads to organelle dysfunction, and compromised repair or renewal (Irani, 2000).

In this regard, resveratrol (RV) has received considerable attention during the last decade. RV is a naturally occurring polyphenol found mostly in the skin of red grapes, peanuts, and blackberries (Li et al., 2012). Flow-mediated dilation (FMD) refers to the changes in vessel diameter caused by increases in shear stress. FMD is considered a surrogate of endothelial function and in humans is measured by ultrasound in superficial arteries such as the brachial and femoral arteries (Currie et al., 2014). A growing body of evidence suggests that RV improves FMD (Wong et al., 2013), reduces blood pressure (BP), the circulating levels of inflammatory molecules (Timmers et al., 2011), and myocardial damage during ischemia-reperfusion (Bradamante et al., 2004). The mechanisms by which RV confers vascular protection involve a higher biosynthesis of NO, increases in the activity of proteins that regulate oxidative metabolism, increases in the activity several antioxidant enzymes, as well as the stimulation of cellular self-repair processes (collectively known as autophagy) within the endothelial cell. Consequently, RV has become an attractive candidate in nutraceutical strategies that aim to improve vascular function in the elderly

Here we review how cumulative oxidative damage and impaired autophagy in old age might contribute to endothelial dysfunction. We discuss the potential of RV to protect against oxidative assault, promote autophagy and restore endothelial function in old vessels.

2. The vascular endothelium

The endothelium is a monolayer of cells that lines blood vessels and is the only cellular layer that separates the blood from surrounding tissue in capillaries. It acts as a permeable barrier to solutes and macromolecules, and is critical not only in the regulation of vessel diameter, but also in coagulation, angiogenesis and inflammation. Endothelial cells modulate vascular tone by releasing vasoactive molecules, such as NO (Furchgott and Vanhoutte, 1989). NO activates cGMP-dependent protein kinase, which induces vasodilation and prevents platelet

aggregation (McHugh and Cheek, 1998). Furthermore, NO enhances oxidative metabolism and oxygen consumption (Clementi et al., 1999; Parihar et al., 2008), and protects against ischemia-reperfusion injury and oxidative damage (Crouser, 2004; Gourine et al., 2002; Rakhit et al., 2001).

Under normal conditions, the vascular endothelium regulates arterial tone and blood flow to match the needs of the tissue. Vascular dysfunction is evident when either the release of, or the response to vasodilators is blunted. For instance, overproduction of vasoconstrictors with a reduced bioavailability of relaxing factors, such as NO, will lead to an increased vascular tone (Lerman and Burnett, 1992). During aging, the higher secretion of endothelial cell-derived pro-inflammatory and thrombogenic factors eventually results in irregular vasoreactivity and partial loss of endothelial function (Verma and Anderson, 2002), which underlies the increased prevalence of cardiovascular problems in old age.

There is evidence that OS and mitochondrial dysfunction play a significant role in the age-related onset of endothelial dysfunction (Brandes et al., 2005). OS will over time result in an accumulation of molecules modified by reactive oxygen and nitrogen species, which leads to compromised organelle and ultimately cell and tissue function. One of the mechanisms whereby the cell protects itself from the accumulation of oxidatively modified protein and dysfunctional organelles is autophagy. During autophagy, pathogens, dysfunctional organelles and harmful cytoplasmic constituents are sequestered into vesicles, autophagosomes, and fused with the lysosome for degradation and recycling (Kroemer et al., 2010).

3. Oxidative stress, autophagy and the aged vasculature

The capacity of the vessel to dilate in response to increased flow or vasodilating agents diminishes with age (Brandes et al., 2005). Part of the problem is related to a disturbed regulation of release of vasodilatory and vasoconstricting factors from the endothelial cells. Many factors contribute to age-related endothelial dysfunction. Among those are decreased circulatory levels of growth factors and vasodilators,

increased circulatory levels of vasoconstrictors and inflammation (Csiszar et al., 2004; Daiber et al., 2016). Today, it is widely accepted that OS plays a pivotal role in age-related endothelial dysfunction (Brandes et al., 2005). Scavenging of free radicals is necessary for the appropriate functioning of the endothelium. With age, increased production of the anion superoxide ($O_2^{\bullet-}$) has been suggested to lead to a reduced bioavailability of NO, as $O_2^{\bullet-}$ will scavenge NO to produce peroxynitrite ($ONOO^-$) (Blackwell et al., 2004; Ferrer et al., 2003; Hamilton et al., 2001; Rodríguez-Mañas et al., 2009; van der Loo et al., 2000). The main sources of endothelium-derived $O_2^{\bullet-}$ appear to be the mitochondrion, NADPH oxidase and endothelial nitric oxide synthase (eNOS) itself (Hamilton et al., 2001). Tetrahydrobiopterin (BH_4) is an essential cofactor for the NO synthases. In high concentrations, $ONOO^-$ oxidizes BH_4 . In the absence of BH_4 , the NO synthases become uncoupled and produce $O_2^{\bullet-}$, which leads to further production of $ONOO^-$ and OS (Golbidi and Laher, 2013).

As mentioned above, aging is a major risk factor for the development of cardiovascular disease. The molecular and morphological profiles of the aged endothelial cell correspond to a state of disturbed homeostasis and poor repair (LaRocca et al., 2013). Considering that autophagy is reduced during aging and the close association between autophagy and OS, there is reason to believe that the inability to maintain autophagy is instrumental, if not critical, for the OS-induced endothelial dysfunction in old age (Lee et al., 2012). Autophagy is involved in the expression of eNOS under steady laminar shear stress (Guo et al., 2014). Endothelial cells with knockdown of autophagy related protein (Atg) 3 display significantly lower levels of eNOS phosphorylation and are not able to produce NO in response to shear stress. This is accompanied by higher levels of reactive oxygen species (ROS) and inflammation as indicated by the increase in monocyte chemoattractant protein-1 and interleukin 8 (IL-8) (Bharath et al., 2014). The reduced bioavailability of NO and subsequent compromise of endothelial function has been associated with

pathological conditions such as hypertension and atherosclerosis (Eren et al., 2013; Münzel et al., 2008).

Significant build-up of oxidative by-products results in endothelial cell death. However, such accumulation is largely prevented by autophagy. 4-hydroxynonenal (4-HNE) and acrolein, products of lipid peroxidation, have been shown to strongly induce autophagy in endothelial cells from Sprague–Dawley rat aortic explants. Further, failure to remove aldehyde-modified proteins by inhibiting autophagy accelerates aging and cell death (Hill et al., 2008; LaRocca et al., 2012). The importance of autophagy is indicated in these studies by a robust increase of microtubule-associated protein 1 light chain 3 II (LC3II) and significant vacuolization, formation of pinocytic bodies, crescent-shaped phagophores and multilamellar vesicles, all morphological characteristics of autophagy. (Hill et al., 2008; LaRocca et al., 2012). Thus, it is possible that the inability to maintain autophagy is responsible for the OS-induced endothelial dysfunction in old age.

Recently, LaRocca et al (2012), demonstrated in a compelling study that the decline in endothelial function in elderly subjects depends on NO availability. Further, that endothelial dysfunction is associated with higher circulating levels of oxidized low-density lipoprotein, IL-6 and C-reactive protein, and a reduced expression of beclin-1, a critical protein in the initiation of autophagy, in endothelial cells isolated from the brachial artery of these subjects. Similar to their findings in humans, their experiments on the aorta and carotid arteries from aged C57BL/6 mice revealed significantly lower endothelium-dependent dilation in response to acetylcholine when compared to young controls. As expected, this was consistent with a reduced expression of eNOS, beclin 1, LC3II, WD repeat domain phosphoinositide-interacting protein 1 (WIPI-1; a key mediator of autophagy), and markedly greater levels of O₂•. Interestingly, when the mice were supplemented, or the cultured cells treated with trehalose, a natural disaccharide that promotes autophagy, endothelial-dependent dilation and all markers of autophagy and OS were restored to the levels

of controls. In a similar study, they proceeded to test the effects of spermidine, a natural polyamine that strongly induces autophagy (LaRocca et al., 2013). As hypothesized, treatment with spermidine enhanced autophagy, reversed elastic stiffening of the aorta, restored endothelial-dependent dilation and reduced OS in old mice (LaRocca et al., 2013). Collectively, their findings provide strong evidence on the role that OS and impaired organelle turnover play in endothelial dysfunction during aging.

In addition to the increased production of free radicals, substantial evidence suggests that the reduction in antioxidant enzymes is also responsible for higher levels of OS in old age. In the aorta and femoral artery of rats, aging has been associated with a decrease in the activity of superoxide dismutase (SOD) and increased nitration of manganese superoxide dismutase (MnSOD) (Barton et al., 1997; Durrant et al., 2009; van der Loo et al., 2000; Zanetti et al., 2010). Evidence that the decline in antioxidant enzymes and a higher production of free radicals are associated with aging comes from MnSOD knockout models in which the animals suffered from age-dependent OS, endothelial dysfunction and cardiomyopathy (Ohashi et al., 2006; Roos et al., 2013; Strassburger et al., 2005; Wenzel et al., 2008). Further, treatment with the SOD mimetic Tempol restored endothelium-dependent dilation, and prevented the formation of atheroma plaques (Cannizzo et al., 2014; Lesniewski et al., 2009; Tatchum-Talom and Martin, 2004). Taken together, these data corroborate the effect of free radicals, particularly $O_2^{\bullet-}$ on the age-related endothelial dysfunction in humans and other animals.

4. The use of antioxidants

Considering the deleterious effects that cumulative oxidative damage causes in the vasculature, significant attention has been given during the last decade to the potential of antioxidants to restore oxidative balance (Conti et al., 2016). The role that free radicals play on such a broad spectrum of physiological functions has fueled the passionate debate on the potential beneficial effects of antioxidant

supplementation. As elegantly explained by Bast and Haenen (Bast and Haenen, 2013), the expectations for antioxidants have been so high that the results so far have been somewhat confusing and disappointing. As mentioned above, RV has received extensive attention in this regard. As an antioxidant, RV scavenges $O_2^{\bullet-}$ and the hydroxyl radical ($\bullet OH$), increases the activity of several antioxidant enzymes and protects lipid membranes and DNA from peroxidation and strand-breaks, respectively (Cao and Li, 2004; Leonard et al., 2003). In addition to its antioxidant effects, RV increases the activity of eNOS (Wallerath et al., 2002) and confers anti-inflammatory protection (Ungvari et al., 2009), which makes it an attractive candidate to reverse the age-related structural and functional changes in the blood vessel.

5. Considerations for the use of resveratrol

Thus far, most studies have focused on sirtuin 1 (SIRT1) as the key target of RV. Sirtuins are NAD^+ -dependent protein deacetylases that regulate oxidative metabolism. SIRT1 in particular is localized in the nucleus and has been shown to increase the activity of catalase and prevent apoptosis in human endothelial cells (Alcendor et al., 2007; Li et al., 2015). However, a growing body of evidence indicates that other important signaling pathways convey the antioxidant and anti-inflammatory properties of RV. Such pathways include the activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) via AMP-activated protein kinase (AMPK) (Baur et al., 2006; Lagouge et al., 2006; Olesen et al., 2014a; Ungvari et al., 2009), as well as the down-regulation nuclear factor κB (NF- κB), which signals pro-inflammatory responses in aging (Ungvari et al., 2009, 2007). In addition, the induction of the transcription factor erythroid 2-related factor 2 (Nrf2) is responsible for some of the antioxidant and anti-inflammatory effects of RV on the endothelial cells. Upon activation, Nrf2 induces the expression of genes coding for important enzymes involved in antioxidant processes such as NADPH:quinone oxidoreductase 1, heme oxygenase-1, and γ -

glutamylcysteine synthetase (Ungvari et al., 2010). On the other hand, downregulation of Nfr2 prevents the RV-induced protection from OS in human coronary arteries and impairs the dilation in response to acetylcholine in the arterioles in the gracilis muscle of aged mice (Ungvari et al., 2010).

Some of the initial enthusiasm for the nutraceutical potential of antioxidants has faded. This, mostly because we now understand that ROS serve a broad range of physiological functions. Under controlled balance free radicals regulate homeostasis and physiological angiogenesis (Bir et al., 2012). Furthermore, hydrogen peroxide (H₂O₂) stimulates cell proliferation (Simon and Stutzin, 2008), promotes the secretion of growth factors (Chua et al., 1998; Colavitti et al., 2002; González-Pacheco et al., 2006), regulates apoptosis (Chen et al., 2004) and acts as a vasodilating agent in human coronary arterioles (Zhang et al., 2012). Recent evidence suggests that the H₂O₂-induced dimerization of cGMP-dependent protein kinase (PKG)-I α leads to the opening of calcium-activated potassium channels and further dilation of the vessel (Zhang et al., 2012). Hence, the paradigm that 'the more antioxidants the better' is now changing to a complementary strategy aimed to normalize the production of free radicals (Bast and Haenen, 2013). Regarding the effects of RV on the vascular endothelium in old age, conflicting data between humans and other species have been published. For instance, RV has been shown to reverse oxidative damage by reducing the expression of NADPH oxidase while increasing the expression of SIRT1 in the aorta of aged rats (Tang et al., 2012). In the cardiovascular system, the reduced expression of SIRT1 is specifically limited to aged and/or atherosclerotic vessels (Kao et al., 2010). Also, it has been demonstrated that RV prevents free radical-induced senescence in human umbilical vein endothelial cells (HUVECs) but fails to protect from oxidative damage when the gene for SIRT1 is silenced (Kao et al., 2010).

Some of the benefits of RV are mediated by stimulation of autophagy. Treatment of HUVECs with RV up-regulated the expression of sequestosome 1 (SQSMT1), an autophagosome cargo protein that selectively targets proteins for autophagy, eNOS,

SIRT1, and several genes related to autophagy including *LC3B* and *AGT3* (Chen et al., 2013; Takizawa et al., 2013). In support of such protective effects, RV has also been shown to preserve telomere length and increase telomerase activity in aortic rings from aged rats (da Luz et al., 2012a). Of particular interest is the fact that endothelium-dependent dilation improved significantly after treatment with RV for six months (da Luz et al., 2012b). Recently, a causal relationship between the levels of the mammalian target of rapamycin (mTOR) and the ribosomal protein S6 kinase beta-1 (S6K1), and vascular aging was reported. Over activation of this pathway leads to aging-related disorders including cardiovascular disease (Stanfel et al., 2009). The activity of S6K1 is higher in aortic rings of old rats and in cultured senescent human endothelial cells (Rajapakse et al., 2011). Related to this, there is a higher production of free radicals and a concomitant reduced synthesis of NO. Treatment of the cells for one hour with RV restored excess production of mitochondrial O₂•⁻ and enhanced the synthesis of NO in response to acetylcholine (Rajapakse et al., 2011).

Recent studies in humans, by contrast, suggest that in aged, but healthy individuals RV blunts the positive cardiovascular effects of physical exercise (Gliemann et al., 2014, 2013; Olesen et al., 2014b). When submitted to eight weeks of high intensity training and a daily intake of either 250 mg of RV or placebo, the subjects that received RV showed no increase in the capillary-to-fiber ratio or the concentration of vascular endothelial growth factor in the vastus lateralis. Further, supplementation with RV inhibited the exercise-induced reduction in BP and blood lipids and attenuated the gains in maximal oxygen consumption observed in the group that exercised under the placebo. Likewise, no changes were reported in markers of metabolic and inflammation status such as 3-hydroxyacyl-CoA dehydrogenase, cytochrome c oxidase I, PCG-1 α , SIRT1, and TNF α . Finally, in HUVECs, RV inhibited cell migration and capillary tube formation by enhancing the nuclear translocation of the transcription factors forkhead box O (FOXO)1, FOXO3a and FOXO4 (Srivastava et al., 2010). Clearly, these results cast some doubt on the

current paradigm that RV mimics the metabolic and anti-inflammatory effects of exercise and caloric restriction that promote cardiovascular health. The studies in humans, particularly in the elderly, are still only a handful when compared to those in rodents. However, as will be discussed below they call for caution when attributing to RV, or any anti-oxidant for that matter, exceptional therapeutic properties. Table 1 summarizes the studies on the effects of RV on the blood vessel. Figure 1 shows some of the mechanisms by which RV confers vascular protection.

6. Perspective

Even though some might deem the therapeutic potential of RV to be overstated, we believe that the data published thus far reflect clinical promise. Healthy populations might not experience positive metabolic or cardiovascular effects after treatment with RV. Yet, there is no clinical justification to treat those subjects or expect such effects. Moreover, it is not clear how positive effects in an already healthy population should be interpreted (Smoliga et al., 2013). On the other hand, enough evidence has been gathered to suggest that compromised populations such as the elderly do benefit from treatment with RV (Smoliga et al., 2013). Even though a recent series of publications in aged humans (Gliemann et al., 2014, 2013; Olesen et al., 2014b) reveals no benefit from RV supplementation in a broad range of cardiovascular and metabolic parameters, further research needs to be conducted to definitely validate the claims both in favor and against RV.

We have discussed here how RV scavenges free radicals, increases antioxidant activity, and promotes autophagy and the synthesis of proteins central to oxidative metabolism in aged vessels. We have also highlighted the fact that free radicals serve as signaling molecules and as such, a controlled production is necessary for adequate functioning of the blood vessel. Interestingly, supplementation with thiol-based and other antioxidants such as tocopherol prevents ROS-induced autophagy (Underwood et al., 2010). With that in mind, some have questioned whether a higher production of free radicals during aging is in fact detrimental to cardiovascular health (Gliemann et al., 2013). Some evidence suggests that in mammalian cultured cells NO, a free radical, inhibits autophagy by nitrosylation of c-Jun N-terminal kinase 1. This ultimately leads to the disruption of the Beclin/1-hVps34 complex, which is necessary during autophagosome formation (Sarkar et al., 2011). At first, these findings might seem in direct contrast to some of the reports discussed above. However, it is worth mentioning at this point that the production of free radicals, degradation of proteins and organelles via autophagy and the synthesis of antioxidants enzymes can be either beneficial or detrimental depending

on the energy and redox status of the cell. Different oxidative modifications are particular to different types of free radicals in the same way that different antioxidants scavenge different ROS. For instance, during nutrient deprivation the cell promotes an oxidative environment by expelling glutathione (Desideri et al., 2012; Filomeni et al., 2015). Oxidized proteins become then a target of autophagy so amino acids can enter the tricarboxylic acid cycle to be used to synthesize ATP (Desideri et al., 2012; Filomeni et al., 2015). Hence, supplementation with antioxidants needs to be carefully designed. The elderly constitute that *rancid* population with high levels of inflammation and oxidative damage to which Bast and Haenen referred (Bast and Haenen, 2013). In the elderly, and in the appropriate dose, RV might *enhance* endothelial function when used as a secondary strategy to a balanced diet and regular exercise and thus, contribute to a healthier yet unavoidable, aging.

Conflict of Interest: none declared

Funding: This research was funded by the European Commission through MOVE-AGE, an Erasmus Mundus Joint Doctorate program (2011-2015).

ACCEPTED MANUSCRIPT

References

- Alcendor, R.R., Gao, S., Zhai, P., Zablocki, D., Holle, E., Yu, X., Tian, B., Wagner, T., Vatner, S.F., Sadoshima, J., 2007. Sirt1 Regulates Aging and Resistance to Oxidative Stress in the Heart. *Circ. Res.* 100, 1512–1521. doi:10.1161/01.RES.0000267723.65696.4a
- Barton, M., Cosentino, F., Brandes, R.P., Moreau, P., Shaw, S., Lüscher, T.F., 1997. Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. *Hypertension* 30, 817–824.
- Bast, A., Haenen, G.R.M.M., 2013. Ten misconceptions about antioxidants. *Trends Pharmacol. Sci.* 34, 430–436. doi:10.1016/j.tips.2013.05.010
- Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V.V., Allard, J.S., Lopez-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S., Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W., Spencer, R.G., Lakatta, E.G., Le Couteur, D., Shaw, R.J., Navas, P., Puigserver, P., Ingram, D.K., de Cabo, R., Sinclair, D.A., 2006. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342. doi:10.1038/nature05354
- Bharath, L.P., Mueller, R., Li, Y., Ruan, T., Kunz, D., Goodrich, R., Mills, T., Deeter, L., Sargsyan, A., Anandh Babu, P.V., Graham, T.E., Symons, J.D., 2014. Impairment of autophagy in endothelial cells prevents shear-stress-induced increases in nitric oxide bioavailability. *Can. J. Physiol. Pharmacol.* 92, 605–612. doi:10.1139/cjpp-2014-0017
- Bhatnagar, P., Wickramasinghe, K., Williams, J., Rayner, M., Townsend, N., 2015. The epidemiology of cardiovascular disease in the UK 2014. *Heart* heartjnl-2015-307516. doi:10.1136/heartjnl-2015-307516
- Bir, S.C., Kolluru, G.K., Fang, K., Kevil, C.G., 2012. Redox balance dynamically regulates vascular growth and remodeling. *Semin. Cell Dev. Biol.* 23, 745–757. doi:10.1016/j.semcdb.2012.05.003
- Blackwell, K.A., Sorenson, J.P., Richardson, D.M., Smith, L.A., Suda, O., Nath, K., Katusic, Z.S., 2004. Mechanisms of aging-induced impairment of endothelium-dependent relaxation: role of tetrahydrobiopterin. *Am. J. Physiol. Heart Circ. Physiol.* 287, H2448-2453. doi:10.1152/ajpheart.00248.2004
- Bradamante, S., Barengi, L., Villa, A., 2004. Cardiovascular protective effects of resveratrol. *Cardiovasc. Drug Rev.* 22, 169–188.
- Brandes, R.P., Fleming, I., Busse, R., 2005. Endothelial aging. *Cardiovasc. Res.* 66, 286–294. doi:10.1016/j.cardiores.2004.12.027
- Cannizzo, B., Quesada, I., Militello, R., Amaya, C., Miatello, R., Cruzado, M., Castro, C., 2014. Tempol attenuates atherosclerosis associated with metabolic syndrome via decreased vascular inflammation and NADPH-2 oxidase expression. *Free Radic. Res.* 48, 526–533. doi:10.3109/10715762.2014.889295
- Cao, Z., Li, Y., 2004. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: protection against oxidative and electrophilic injury. *Eur. J. Pharmacol.* 489, 39–48. doi:10.1016/j.ejphar.2004.02.031

- Cardiovascular Disease Statistics 2015 [WWW Document], n.d. URL <https://www.bhf.org.uk/publications/statistics/cvd-stats-2015> (accessed 7.29.16).
- Chen, K., Thomas, S.R., Albano, A., Murphy, M.P., Keaney, J.F., 2004. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. *J. Biol. Chem.* 279, 35079–35086. doi:10.1074/jbc.M404859200
- Chen, M.-L., Yi, L., Jin, X., Liang, X.-Y., Zhou, Y., Zhang, T., Xie, Q., Zhou, X., Chang, H., Fu, Y.-J., Zhu, J.-D., Zhang, Q.-Y., Mi, M.-T., 2013. Resveratrol attenuates vascular endothelial inflammation by inducing autophagy through the cAMP signaling pathway. *Autophagy* 9, 2033–2045. doi:10.4161/auto.26336
- Chua, C.C., Hamdy, R.C., Chua, B.H., 1998. Upregulation of vascular endothelial growth factor by H₂O₂ in rat heart endothelial cells. *Free Radic. Biol. Med.* 25, 891–897.
- Clementi, E., Brown, G.C., Foxwell, N., Moncada, S., 1999. On the mechanism by which vascular endothelial cells regulate their oxygen consumption. *Proc. Natl. Acad. Sci.* 96, 1559–1562. doi:10.1073/pnas.96.4.1559
- Colavitti, R., Pani, G., Bedogni, B., Anzevino, R., Borrello, S., Waltenberger, J., Galeotti, T., 2002. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J. Biol. Chem.* 277, 3101–3108. doi:10.1074/jbc.M107711200
- Conti, V., Izzo, V., Corbi, G., Russomanno, G., Manzo, V., De Lise, F., Di Donato, A., Filippelli, A., 2016. Antioxidant Supplementation in the Treatment of Aging-Associated Diseases. *Front. Pharmacol.* 7, 24. doi:10.3389/fphar.2016.00024
- Crouser, E.D., 2004. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion, Mitochondrial Medicine - Developing the Scientific Basis to Medical Management of Mitochondrial Disease* 4, 729–741. doi:10.1016/j.mito.2004.07.023
- Csiszar, A., Ungvari, Z., Koller, A., Edwards, J.G., Kaley, G., 2004. Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol. Genomics* 17, 21–30. doi:10.1152/physiolgenomics.00136.2003
- Currie, K.D., McKelvie, R.S., Macdonald, M.J., 2014. Brachial artery endothelial responses during early recovery from an exercise bout in patients with coronary artery disease. *BioMed Res. Int.* 2014, 591918. doi:10.1155/2014/591918
- da Luz, P.L., Tanaka, L., Brum, P.C., Dourado, P.M.M., Favarato, D., Krieger, J.E., Laurindo, F.R.M., 2012a. Red wine and equivalent oral pharmacological doses of resveratrol delay vascular aging but do not extend life span in rats. *Atherosclerosis* 224, 136–142. doi:10.1016/j.atherosclerosis.2012.06.007
- da Luz, P.L., Tanaka, L., Brum, P.C., Dourado, P.M.M., Favarato, D., Krieger, J.E., Laurindo, F.R.M., 2012b. Red wine and equivalent oral pharmacological doses of resveratrol delay vascular aging but do not extend life span in rats. *Atherosclerosis* 224, 136–142. doi:10.1016/j.atherosclerosis.2012.06.007
- Daiber, A., Steven, S., Weber, A., Shuvaev, V.V., Muzykantov, V.R., Laher, I., Li, H., Lamas, S., Münzel, T., 2016. Targeting vascular (endothelial) dysfunction. *Br. J. Pharmacol.* n/a-n/a. doi:10.1111/bph.13517

- Desideri, E., Filomeni, G., Ciriolo, M.R., 2012. Glutathione participates in the modulation of starvation-induced autophagy in carcinoma cells. *Autophagy* 8, 1769–1781. doi:10.4161/auto.22037
- Durrant, J.R., Seals, D.R., Connell, M.L., Russell, M.J., Lawson, B.R., Folian, B.J., Donato, A.J., Lesniewski, L.A., 2009. Voluntary wheel running restores endothelial function in conduit arteries of old mice: direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. *J. Physiol.* 587, 3271–3285. doi:10.1113/jphysiol.2009.169771
- Eren, E., Yilmaz, N., Aydin, O., 2013. Functionally defective high-density lipoprotein and paraoxonase: a couple for endothelial dysfunction in atherosclerosis. *Cholesterol* 2013.
- Ferrer, M., Sánchez, M., Minoves, N., Salaices, M., Balfagón, G., 2003. Aging increases neuronal nitric oxide release and superoxide anion generation in mesenteric arteries from spontaneously hypertensive rats. *J. Vasc. Res.* 40, 509–519. doi:75183
- Filomeni, G., De Zio, D., Cecconi, F., 2015. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ.* 22, 377–388. doi:10.1038/cdd.2014.150
- Furchgott, R.F., Vanhoutte, P.M., 1989. Endothelium-derived relaxing and contracting factors. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 3, 2007–2018.
- Gliemann, L., Olesen, J., Biensø, R.S., Schmidt, J.F., Akerstrom, T., Nyberg, M., Lindqvist, A., Bangsbo, J., Hellsten, Y., 2014. Resveratrol modulates the angiogenic response to exercise training in skeletal muscles of aged men. *Am. J. Physiol. Heart Circ. Physiol.* 307, H1111-1119. doi:10.1152/ajpheart.00168.2014
- Gliemann, L., Schmidt, J.F., Olesen, J., Biensø, R.S., Peronard, S.L., Grandjean, S.U., Mortensen, S.P., Nyberg, M., Bangsbo, J., Pilegaard, H., Hellsten, Y., 2013. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J. Physiol.* 591, 5047–5059. doi:10.1113/jphysiol.2013.258061
- Golbidi, S., Laher, I., 2013. Exercise and the Aging Endothelium. *J. Diabetes Res.* 2013, e789607. doi:10.1155/2013/789607
- González-Pacheco, F.R., Deudero, J.J.P., Castellanos, M.C., Castilla, M.A., Alvarez-Arroyo, M.V., Yagüe, S., Caramelo, C., 2006. Mechanisms of endothelial response to oxidative aggression: protective role of autologous VEGF and induction of VEGFR2 by H2O2. *Am. J. Physiol. Heart Circ. Physiol.* 291, H1395-1401. doi:10.1152/ajpheart.01277.2005
- Gourine, A.V., Bulhak, A.A., Gonon, A.T., Pernow, J., Sjöquist, P.-O., 2002. Cardioprotective effect induced by brief exposure to nitric oxide before myocardial ischemia-reperfusion in vivo. *Nitric Oxide Biol. Chem. Off. J. Nitric Oxide Soc.* 7, 210–216.
- Guo, F., Li, X., Peng, J., Tang, Y., Yang, Q., Liu, L., Wang, Z., Jiang, Z., Xiao, M., Ni, C., Chen, R., Wei, D., Wang, G., 2014. Autophagy regulates vascular endothelial cell eNOS and ET-1 expression induced by laminar shear stress in an ex vivo

- perfused system. *Ann. Biomed. Eng.* 42, 1978–1988. doi:10.1007/s10439-014-1033-5
- Hamilton, C.A., Brosnan, M.J., McIntyre, M., Graham, D., Dominiczak, A.F., 2001. Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction. *Hypertension* 37, 529–534.
- Hill, B.G., Haberkzettel, P., Ahmed, Y., Srivastava, S., Bhatnagar, A., 2008. Unsaturated lipid peroxidation-derived aldehydes activate autophagy in vascular smooth-muscle cells. *Biochem. J.* 410, 525–534. doi:10.1042/BJ20071063
- Irani, K., 2000. Oxidant Signaling in Vascular Cell Growth, Death, and Survival A Review of the Roles of Reactive Oxygen Species in Smooth Muscle and Endothelial Cell Mitogenic and Apoptotic Signaling. *Circ. Res.* 87, 179–183. doi:10.1161/01.RES.87.3.179
- Kao, C.-L., Chen, L.-K., Chang, Y.-L., Yung, M.-C., Hsu, C.-C., Chen, Y.-C., Lo, W.-L., Chen, S.-J., Ku, H.-H., Hwang, S.-J., 2010. Resveratrol Protects Human Endothelium from H₂O₂-Induced Oxidative Stress and Senescence via Sirt1 Activation. *J. Atheroscler. Thromb.* 17, 970–979. doi:10.5551/jat.4333
- Kroemer, G., Mariño, G., Levine, B., 2010. Autophagy and the Integrated Stress Response. *Mol. Cell* 40, 280–293. doi:10.1016/j.molcel.2010.09.023
- Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., Auwerx, J., 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 127, 1109–1122. doi:10.1016/j.cell.2006.11.013
- LaRocca, T.J., Gioscia-Ryan, R.A., Hearon Jr., C.M., Seals, D.R., 2013. The autophagy enhancer spermidine reverses arterial aging. *Mech. Ageing Dev.* 134, 314–320. doi:10.1016/j.mad.2013.04.004
- LaRocca, T.J., Henson, G.D., Thorburn, A., Sindler, A.L., Pierce, G.L., Seals, D.R., 2012. Translational evidence that impaired autophagy contributes to arterial ageing. *J. Physiol.* 590, 3305–3316. doi:10.1113/jphysiol.2012.229690
- Lee, J., Giordano, S., Zhang, J., 2012. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem. J.* 441, 523–540. doi:10.1042/BJ20111451
- Leonard, S.S., Xia, C., Jiang, B.-H., Stinefelt, B., Klandorf, H., Harris, G.K., Shi, X., 2003. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. *Biochem. Biophys. Res. Commun.* 309, 1017–1026. doi:10.1016/j.bbrc.2003.08.105
- Lerman, A., Burnett, J.C.J., 1992. Intact and altered endothelium in regulation of vasomotion. *Circulation* 86, III12–19.
- Lesniewski, L.A., Connell, M.L., Durrant, J.R., Folan, B.J., Anderson, M.C., Donato, A.J., Seals, D.R., 2009. B6D2F1 Mice are a suitable model of oxidative stress-mediated impaired endothelium-dependent dilation with aging. *J. Gerontol. A. Biol. Sci. Med. Sci.* 64, 9–20. doi:10.1093/gerona/gln049
- Li, H., Xia, N., Förstermann, U., 2012. Cardiovascular effects and molecular targets of resveratrol. *Nitric Oxide* 26, 102–110. doi:10.1016/j.niox.2011.12.006

- Li, P., Zhang, L., Zhou, C., Lin, N., Liu, A., 2015. Sirt 1 activator inhibits the AGE-induced apoptosis and p53 acetylation in human vascular endothelial cells. *J. Toxicol. Sci.* 40, 615–624. doi:10.2131/jts.40.615
- McHugh, J., Cheek, D.J., 1998. Nitric oxide and regulation of vascular tone: pharmacological and physiological considerations. *Am. J. Crit. Care Off. Publ. Am. Assoc. Crit.-Care Nurses* 7, 131-140-142.
- Münzel, T., Sinning, C., Post, F., Warnholtz, A., Schulz, E., 2008. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Ann. Med.* 40, 180–196.
- North, B.J., Sinclair, D.A., 2012. The Intersection Between Aging and Cardiovascular Disease. *Circ. Res.* 110, 1097–1108. doi:10.1161/CIRCRESAHA.111.246876
- Ohashi, M., Runge, M.S., Faraci, F.M., Heistad, D.D., 2006. MnSOD deficiency increases endothelial dysfunction in ApoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 26, 2331–2336. doi:10.1161/01.ATV.0000238347.77590.c9
- Olesen, J., Gliemann, L., Biensø, R., Schmidt, J., Hellsten, Y., Pilegaard, H., 2014a. Exercise training, but not resveratrol, improves metabolic and inflammatory status in skeletal muscle of aged men. *J. Physiol.* 592, 1873–1886. doi:10.1113/jphysiol.2013.270256
- Olesen, J., Gliemann, L., Biensø, R., Schmidt, J., Hellsten, Y., Pilegaard, H., 2014b. Exercise training, but not resveratrol, improves metabolic and inflammatory status in skeletal muscle of aged men. *J. Physiol.* 592, 1873–1886. doi:10.1113/jphysiol.2013.270256
- Parihar, M.S., Nazarewicz, R.R., Kincaid, E., Bringold, U., Ghafourifar, P., 2008. Association of mitochondrial nitric oxide synthase activity with respiratory chain complex I. *Biochem. Biophys. Res. Commun.* 366, 23–28. doi:10.1016/j.bbrc.2007.11.056
- Rajapakse, A.G., Yepuri, G., Carvas, J.M., Stein, S., Matter, C.M., Scerri, I., Ruffieux, J., Montani, J.-P., Ming, X.-F., Yang, Z., 2011. Hyperactive S6K1 Mediates Oxidative Stress and Endothelial Dysfunction in Aging: Inhibition by Resveratrol. *PLoS ONE* 6, e19237. doi:10.1371/journal.pone.0019237
- Rakhit, R.D., Mojet, M.H., Marber, M.S., Duchon, M.R., 2001. Mitochondria as targets for nitric oxide-induced protection during simulated ischemia and reoxygenation in isolated neonatal cardiomyocytes. *Circulation* 103, 2617–2623.
- Rodríguez-Mañas, L., El-Assar, M., Vallejo, S., López-Dóriga, P., Solís, J., Petidier, R., Montes, M., Nevado, J., Castro, M., Gómez-Guerrero, C., Peiró, C., Sánchez-Ferrer, C.F., 2009. Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell* 8, 226–238. doi:10.1111/j.1474-9726.2009.00466.x
- Roos, C.M., Hagler, M., Zhang, B., Oehler, E.A., Arghami, A., Miller, J.D., 2013. Transcriptional and phenotypic changes in aorta and aortic valve with aging and MnSOD deficiency in mice. *Am. J. Physiol. Heart Circ. Physiol.* 305, H1428-1439. doi:10.1152/ajpheart.00735.2012
- Sarkar, S., Korolchuk, V.I., Renna, M., Imarisio, S., Fleming, A., Williams, A., Garcia-Arencibia, M., Rose, C., Luo, S., Underwood, B.R., Kroemer, G., O’Kane, C.J.,

- Rubinsztein, D.C., 2011. Complex Inhibitory Effects of Nitric Oxide on Autophagy. *Mol. Cell* 43, 19–32. doi:10.1016/j.molcel.2011.04.029
- Simon, F., Stutzin, A., 2008. Protein kinase C-mediated phosphorylation of p47 phox modulates platelet-derived growth factor-induced H₂O₂ generation and cell proliferation in human umbilical vein endothelial cells. *Endothel. J. Endothel. Cell Res.* 15, 175–188. doi:10.1080/10623320802174480
- Smoliga, J.M., Colombo, E.S., Campen, M.J., 2013. A healthier approach to clinical trials evaluating resveratrol for primary prevention of age-related diseases in healthy populations. *Aging* 5, 495–506.
- Srivastava, R.K., Unterman, T.G., Shankar, S., 2010. FOXO transcription factors and VEGF neutralizing antibody enhance antiangiogenic effects of resveratrol. *Mol. Cell. Biochem.* 337, 201–212. doi:10.1007/s11010-009-0300-5
- Stanfel, M.N., Shamieh, L.S., Kaeberlein, M., Kennedy, B.K., 2009. The TOR pathway comes of age. *Biochim. Biophys. Acta BBA - Gen. Subj., Includes Special Section: Biochemical and Molecular Mechanisms of Aging: from Model Systems to Human Longevity 1790*, 1067–1074. doi:10.1016/j.bbagen.2009.06.007
- Strait, J.B., Lakatta, E.G., 2012. Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Fail. Clin.* 8, 143–164. doi:10.1016/j.hfc.2011.08.011
- Strassburger, M., Bloch, W., Sulyok, S., Schüller, J., Keist, A.F., Schmidt, A., Wenk, J., Peters, T., Wlaschek, M., Lenart, J., Krieg, T., Hafner, M., Kumin, A., Werner, S., Müller, W., Scharffetter-Kochanek, K., 2005. Heterozygous deficiency of manganese superoxide dismutase results in severe lipid peroxidation and spontaneous apoptosis in murine myocardium in vivo. *Free Radic. Biol. Med.* 38, 1458–1470. doi:10.1016/j.freeradbiomed.2005.02.009
- Takizawa, Y., Kosuge, Y., Awaji, H., Tamura, E., Takai, A., Yanai, T., Yamamoto, R., Kokame, K., Miyata, T., Nakata, R., Inoue, H., 2013. Up-regulation of endothelial nitric oxide synthase (eNOS), silent mating type information regulation 2 homologue 1 (SIRT1) and autophagy-related genes by repeated treatments with resveratrol in human umbilical vein endothelial cells. *Br. J. Nutr.* 110, 2150–2155. doi:10.1017/S0007114513001670
- Tang, Y., Xu, J., Qu, W., Peng, X., Xin, P., Yang, X., Ying, C., Sun, X., Hao, L., 2012. Resveratrol reduces vascular cell senescence through attenuation of oxidative stress by SIRT1/NADPH oxidase-dependent mechanisms. *J. Nutr. Biochem.* 23, 1410–1416. doi:10.1016/j.jnutbio.2011.08.008
- Tatchum-Talom, R., Martin, D.S., 2004. Tempol improves vascular function in the mesenteric vascular bed of senescent rats. *Can. J. Physiol. Pharmacol.* 82, 200–207. doi:10.1139/y04-010
- Timmers, S., Konings, E., Bilet, L., Houtkooper, R.H., van de Weijer, T., Goossens, G.H., Hoeks, J., van der Krieken, S., Ryu, D., Kersten, S., Moonen-Kornips, E., Hesselink, M.K.C., Kunz, I., Schrauwen-Hinderling, V.B., Blaak, E.E., Auwerx, J., Schrauwen, P., 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. doi:10.1016/j.cmet.2011.10.002

- Tousoulis, D., Charakida, M., Stefanadis, C., 2006. Endothelial function and inflammation in coronary artery disease. *Heart* 92, 441–444. doi:10.1136/hrt.2005.066936
- Underwood, B.R., Imarisio, S., Fleming, A., Rose, C., Krishna, G., Heard, P., Quick, M., Korolchuk, V.I., Renna, M., Sarkar, S., García-Arencibia, M., O’Kane, C.J., Murphy, M.P., Rubinsztein, D.C., 2010. Antioxidants can inhibit basal autophagy and enhance neurodegeneration in models of polyglutamine disease. *Hum. Mol. Genet.* 19, 3413–3429. doi:10.1093/hmg/ddq253
- Ungvari, Z., Bagi, Z., Feher, A., Recchia, F.A., Sonntag, W.E., Pearson, K., Cabo, R. de, Csiszar, A., 2010. Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. *Am. J. Physiol. - Heart Circ. Physiol.* 299, H18–H24. doi:10.1152/ajpheart.00260.2010
- Ungvari, Z., Labinsky, N., Mukhopadhyay, P., Pinto, J.T., Bagi, Z., Ballabh, P., Zhang, C., Pacher, P., Csiszar, A., 2009. Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. *Am. J. Physiol. - Heart Circ. Physiol.* 297, H1876–H1881. doi:10.1152/ajpheart.00375.2009
- Ungvari, Z., Orosz, Z., Labinsky, N., Rivera, A., Xiangmin, Z., Smith, K., Csiszar, A., 2007. Increased mitochondrial H₂O₂ production promotes endothelial NF- κ B activation in aged rat arteries. *Am. J. Physiol. - Heart Circ. Physiol.* 293, H37–H47. doi:10.1152/ajpheart.01346.2006
- van der Loo, B., Labugger, R., Skepper, J.N., Bachschmid, M., Kilo, J., Powell, J.M., Palacios-Callender, M., Erusalimsky, J.D., Quaschnig, T., Malinski, T., Gygi, D., Ullrich, V., Lüscher, T.F., 2000. Enhanced peroxynitrite formation is associated with vascular aging. *J. Exp. Med.* 192, 1731–1744.
- Verma, S., Anderson, T.J., 2002. Fundamentals of Endothelial Function for the Clinical Cardiologist. *Circulation* 105, 546–549. doi:10.1161/hc0502.104540
- Vora, D.K., Fang, Z.-T., Liva, S.M., Tyner, T.R., Parhami, F., Watson, A.D., Drake, T.A., Territo, M.C., Berliner, J.A., 1997. Induction of P-Selectin by Oxidized Lipoproteins Separate Effects on Synthesis and Surface Expression. *Circ. Res.* 80, 810–818. doi:10.1161/01.RES.80.6.810
- Wallerath, T., Deckert, G., Ternes, T., Anderson, H., Li, H., Witte, K., Förstermann, U., 2002. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation* 106, 1652–1658.
- Wenzel, P., Schuhmacher, S., Kienhofer, J., Muller, J., Hortmann, M., Oelze, M., Schulz, E., Treiber, N., Kawamoto, T., Scharffetter-Kochanek, K., Munzel, T., Burkle, A., Bachschmid, M.M., Daiber, A., 2008. Manganese superoxide dismutase and aldehyde dehydrogenase deficiency increase mitochondrial oxidative stress and aggravate age-dependent vascular dysfunction. *Cardiovasc. Res.* 80, 280–289. doi:10.1093/cvr/cvn182
- Wong, R.H.X., Berry, N.M., Coates, A.M., Buckley, J.D., Bryan, J., Kunz, I., Howe, P.R.C., 2013. Chronic resveratrol consumption improves brachial flow-mediated dilatation in healthy obese adults. *J. Hypertens.* 31, 1819–1827. doi:10.1097/HJH.0b013e328362b9d6
- Zanetti, M., Gortan Cappellari, G., Burekovic, I., Barazzoni, R., Stebel, M., Guarnieri, G., 2010. Caloric restriction improves endothelial dysfunction during vascular

aging: Effects on nitric oxide synthase isoforms and oxidative stress in rat aorta. *Exp. Gerontol.* 45, 848–855. doi:10.1016/j.exger.2010.07.002

Zhang, D.X., Borbouse, L., Gebremedhin, D., Mendoza, S.A., Zinkevich, N.S., Li, R., Gutterman, D.D., 2012. H₂O₂-induced dilation in human coronary arterioles: role of protein kinase G dimerization and large-conductance Ca²⁺-activated K⁺ channel activation. *Circ. Res.* 110, 471–480. doi:10.1161/CIRCRESAHA.111.258871

ACCEPTED MANUSCRIPT

Figure Legends

Figure 1: Schematic representation of the molecular, biochemical and physiological effects of RV on the blood vessel. RV activates PCG1- α , SIRT1 and Nfr2 through the cAMP-AMPK signaling pathway. Improvements in vasodilation are the consequence of enhanced activity of eNOS and antioxidant enzymes, substrate oxidation and organelle quality control together with a reduction in ROS and pro-inflammatory molecules. During aging, there is a natural decline in autophagy, which results in higher levels of oxidative stress, inflammation, and eventually, endothelial dysfunction.

Table 1. Effects of resveratrol on the blood vessel.

Experimental Model	Cells / Tissue	Dose and Duration of Treatment	Effects	Reference
<i>in vivo</i> studies				
Male Fisher 344 rats	Aorta	10 mg·kg ⁻¹ ·day ⁻¹ for 1 week	/ NF-κB, / monocyte adhesiveness ↓ inflammatory gene expression.	Ungvari et al., 2007
Wistar rats on HFS	Aorta	50 and 100 mg/kg bw for 14 weeks	↓ gains in BW ↓ SA-β-gal-positive cells ↓ HFS-induced increase in ROS ↓ HFS-induced down-regulation of SIRT1	Tang et al., 2012
Male Wistar rats	Aorta	0.0015 mg/kg & 4 mg/kg of chow for 6 months	↑ endothelium-dependent dilation ↑ telomere length and telomerase activity ↓ expression of p53 and p16	da Luz et al., 2012a
Male Wistar Kyoto rats	Aorta	10 μmol/L for 1 hour	↑ NO production ↑ endothelium-dependent dilation ↓ activation of Akt and S6K1 ↓ production of O ₂ •-	Rajapakse et al., 2011
Male ICR wild-type mice (Nrf2 ^{+/+})	Arterioles in the gracilis muscle	2.4 g resveratrol per kg diet for 16 weeks.	↓ gains in BW ↑ <i>NQO1</i> , <i>GCLC</i> , and <i>HMOX1</i> mRNA	Ungvari et al., 2010
<i>in vitro</i> studies				
Cell culture	HCAEC treated with high glucose	10 ⁻⁶ – 10 ⁻⁴ mol/l for 24 h	↓ high glucose-induced mtROS production	Ungvari et al., 2009
	HCAEC treated with high glucose	10 ⁻⁷ – 10 ⁻⁴ mol/L for 24 h	– vascular ROS production to control levels ↓ high glucose-induced mitochondrial O ₂ •- production	Ungvari et al., 2010
	BAEC	0.01, 0.1, 1.0, 10 μM for 24 h	↓ p47phox ↓ high glucose-induced ROS production	Tang et al., 2012
	HUVEC treated with H ₂ O ₂	50 μM for 48 h	↓ H ₂ O ₂ -induced down-regulation of SIRT1 ↓ SA-β-gal-positive cells ↓ ROS production / senescence process	Kao et al., 2010
	HUVEC	1 μM for 6 days	↑ eNOS and SIRT1 mRNA ↑ <i>GABARAP</i> , <i>LC3II</i> and <i>AGT3</i> , <i>MT1X</i> and <i>ANXA2</i> genes	Takizawa et al., 2013
	HUVEC treated with TNF-α	10 μM for 2 h	↓ SQSMT1, ICAM1, PTGS2 and MMP9 protein levels ↑ SIRT1, LC3B2 and cAMP concentration ↑ MAP1LC3B2-to-actin ratio	Chen et al., 2013

crose diet (HFS); Human coronary arterial endothelial cells (HCAEC); Human umbilical vein endothelial cells (HUVEC); Bovine aortic endothelial cells (BAEC).

Inc
rea
sed
(↑)
Re
du
ced
(↓
);
Ma
int
ain
ed
(–
):
Inh
ibit
ed
(/)
;
Bo
dy
we
igh
t
(B
W)
;
Hig
h-
fat
/su

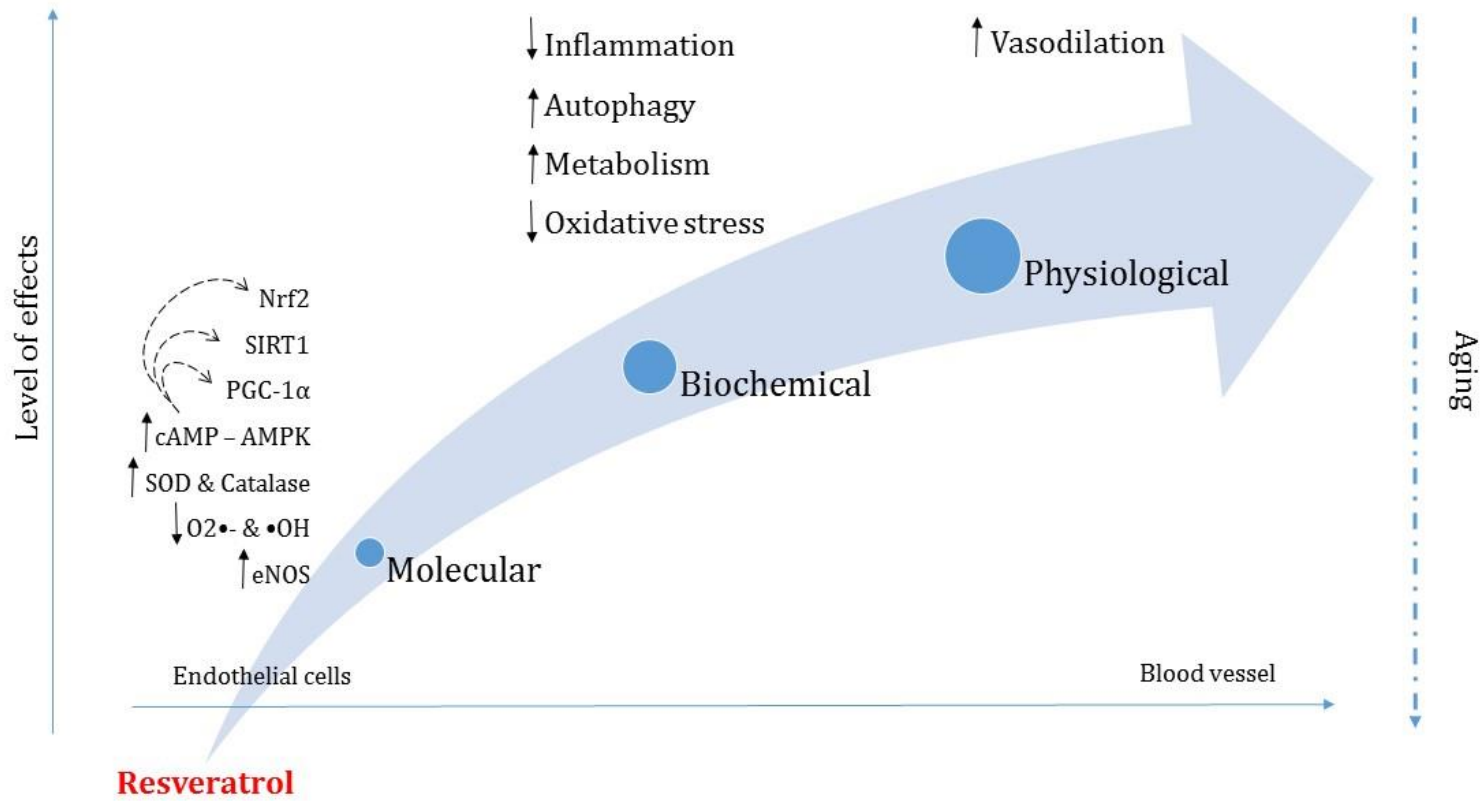


Figure 1

The Effects of Resveratrol on Aging Vessels

Highlights

- Aging is associated with a significant cardiovascular decline.
- Oxidative stress plays a critical role in such degenerative condition.
- Oxidative stress leads to compromised autophagy and poor organelle repair.
- Resveratrol promotes autophagy and protects from oxidative damage and inflammation.
- Resveratrol bears potential to restore vascular function in the elderly.

ACCEPTED MANUSCRIPT