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RESEARCH TOPICS

VASCULAR AGING: FACTS AND FACTORS

Hosted by
Elisabet Vila, Francesc Jiménez-Altayó and Ana Paula Dantas

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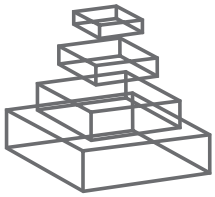

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VASCULAR AGING: FACTS AND FACTORS

Hosted By:
Elisabet Vila, Autonomous University of Barcelona, Spain
Francesc Jiménez-Altayó, Autonomous University of Barcelona, Spain
Ana Paula Dantas, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Spain

The increasing mean age of the population in developed countries has turned out to be an economic and social problem. Cardiovascular disease has long been considered to be age related in terms of their onset and progression. As such, we can say that the increase in life expectancy goes in parallel with increased incidence of cardiovascular disease. With age, a number of changes occur in the vasculature altering the homeostasis of the irrigated organs promoting target organ damage. While different adaptive mechanisms to protect vessels against mild stress have been described, the aging process induces a progressive failure of protective mechanisms, leading to vascular changes and higher susceptibility to cardiovascular diseases. Indeed, vascular aging is exacerbated by coexisting cardiovascular risk factors, such as hypertension, metabolic syndrome and diabetes.

Compelling evidence indicates that diminished endothelial relaxation and increase, decrease, or no change in contractile responses to several agonists is associated with aging. There is an increase of vasoconstrictor factors expression and a decrease of vasodilators. Morphologic changes include lumen diameter enlargement, wall thickening and alterations of matrix substances as increased collagen or decreased elastin deposition, ultimately leading to greater arterial stiffening (reduced compliance). Importantly, arterial stiffness is an independent predictor of cardiovascular morbidity and mortality. Cellular and molecular mechanisms have also been documented. Senescence at the cellular level involves alterations in Ca2+ signaling and down regulation of anti-aging proteins. Both endothelial and smooth muscle cells change their number, morphology, function and their regenerative ability. Aging is also associated with a gradual loss of antioxidant defense mechanisms, a proinflammatory shift in the cytokine expression profile and a production of reactive oxygen species such as superoxide (O2-) that promotes the breakdown of nitric oxide. Nitric oxide and O2- interact to form peroxynitrite known to nitrosylate proteins affecting their physiological function. However,

vascular wall proteins may also suffer from other potentially deleterious modifications as glycation (Maillard reaction) and glyco-oxidative reactions with increasing age, which could be linked to the age-associated changes in vascular function.

Various strategies have shown benefit in preventing, delaying or attenuating vascular aging. For instance, a healthy lifestyle including low fat diet and/or exercise have a favorable effect. Nevertheless, it yet remains to be fully demonstrated whether vascular aging can be pharmacologically prevented. This Research Topic is intended to bring together research efforts to understand the causes and consequences of vascular aging and propose new therapeutic strategies for the management of vascular senescence.

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EDITORIAL
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Vascular aging: facts and factors

Ana P. Dantas[1], Francesc Jiménez-Altayó[2]* and Elisabet Vila[2]

[1] Institut Clínic del Tòrax, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain
[2] Departament de Farmacologia, Terapèutica i Toxicologia, Facultat de Medicina, Institut de Neurociències, Universitat Autònoma de Barcelona, Bellaterra, Spain
*Correspondence: francesc.jimenez@uab.cat

Edited by:
Gerald A. Meininger, University of Missouri, USA

Reviewed by:
Gerald A. Meininger, University of Missouri, USA

"Man is as old as his arteries." This old aphorism has been widely confirmed by epidemiological and observational studies establishing that cardiovascular diseases can be age-related in terms of their onset and progression. Besides, with aging come a number of physiological and morphological changes that alters cardiovascular function and lead to subsequently increased risk of cardiovascular disease, even in health asymptomatic individuals. Even though different adaptive mechanisms to protect blood vessels against mild stress have been described, the aging process induces a progressive failure of protective mechanisms, leading to vascular changes. The outcomes of the aging-related modifications are the impairment of homeostasis of the irrigated organs and resultant target organ damage.

The increasing mean age of the population in industrialized countries has turned out to be an economic and public health problem, as the increase in life expectancy goes in parallel with high incidence of several pathological conditions, despite unprecedented advances in prevention, diagnostics, and treatment. Of all aging-related illness, cardiovascular diseases remain the leading cause of morbidity and mortality in the elderly, and thus it is imperative to understand the mechanism underlying cardiovascular senescence.

This Research Topic presents a forum of comprehensive reviews on distinct aspects of the pathophysiology of vascular aging to provide insights into the causes and consequences of this complex process and attempts to propose new therapeutic strategies for the management of vascular senescence. Such as the idea proposed by Ming et al. (2012) and discussed by Cau and Tostes (2012), which suggests that targeting mTORC1-S6K1 signaling could be a promising therapeutic modality to retard the aging process and treat cardiovascular disease in the elderly.

Vascular aging could be simply described as a consequence of natural physical stress and fatigue that could account for the major physical changes seen in elderly: dilation (after fracture of load-bearing material) and stiffening (by transfer of stress to the more rigid collagenous component of the arterial wall). Although aging-associated changes on vascular functioning are considered a set up for cardiovascular disease, modifications on cardiac, and central function could slow down or accelerate this set point. Therefore, it is crucial to understand how aging and other pathophysiological states affect the interaction between the heart and arterial network. The article by Wojciechowska et al. (2012) provides information on the changes with age in central and peripheral systolic blood pressure, based on data collected from randomly recruited European and Chinese subjects supporting a vicious circle between age-related stiffness, increasing systolic blood pressure, and cardiovascular complications. Chantler and Lakatta (2012) elegantly describe the concept of arterial ventricular coupling and provide valuable information on how aging, in the absence and presence of cardiovascular disease, affects the coupling both at rest and during exercise, and its pathophysiological consequences.

In addition, vascular aging has been largely associated with senescence of the vascular endothelium. El Assar et al. (2012) present a wide overview of the mechanisms that participate on endothelial dysfunction that accompanies vascular aging, analyzing the synergisms, and interactions between them, some of the cellular mechanisms related to endothelial senescence as well as the prevention or reversion of those mechanisms that produce endothelial dysfunction. Cau et al. (2012) based on evidence from experimental models review the contribution of NO synthase (NOS) isoform alterations on aging-associated vascular dysfunctions, addressing the potential prevention by some drugs that modulate the expression/activity of NOS. Finally, Blanco and Bernabéu (2012) summarize data supporting the link between the splicing factor SRSF1 and endothelial cell senescence and suggest the existence of a common genetic program involving alternative splicing of a cluster of genes, which contributes to a senescent environment in the vessels. Nonetheless, aging-associated damage to the endothelium may not simply be a consequence of the endothelial cell malfunctioning, but also as a result of impaired maintenance repair systems by endothelial progenitor cell (EPC). As discussed by Williamson et al. (2012), a deterioration of endogenous EPC function with age may culminate in a decreased capacity for neovascularization and/or reduced re-endothelialization of vascular lesions, facilitating the development, progression, and clinical sequelae of cardiovascular disease.

Growing evidence show that the progress of vascular aging in women follows a different chronology than in men. The gender-associated difference in the pathophysiology of cardiovascular disease has generated heated discussion, although a general consensus has validated a role of sex hormones in the modulation of vascular function and dysfunction. This research topic covers two fronts in this field: Novella et al. (2012) review clinical and experimental data to clarify how menopause and aging contribute jointly to vascular senescence and how estrogen modulates vascular response at different ages; Lopes et al. (2012) discuss the conflicting information on the role of testosterone to the regulation of vascular function in elder men and women.

Last but not least, as suggested by Gragasin et al. (2012) the elderly is more frequently represented in common medical procedures and surgeries. Thus, understanding the circulatory

changes that accompany the aging process is therefore becoming increasingly timely and relevant. These authors discuss aspects of vascular control in aging that are particularly relevant in the maintenance of intraoperative hemodynamic stability reviewing the effect of certain notable anesthetic agents with respect to the aging vasculature.

Given the growing clinical relevance of the subject, we are pleased that our Research Topic has brought together basic and clinical scientists to spotlight on one of the greatest enemies of elder population: i.e., the vascular senescence. We hope that this Research Topic also place a special call on the need of studies to establish treatments and procedures to reduce the detrimental effects of vascular aging. It has been a great pleasure to be involved in this Research Topic and we would like to thank all of the authors, reviewers, and Frontiers staff for helping to make this Research Topic possible.

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REFERENCES

Blanco, F. J., and Bernabéu, C. (2012). The splicing factor SRSF1 as a marker for endothelial senescence. *Front. Physiol.* 3:54. doi: 10.3389/fphys.2012.00054

Cau, S. B., Carneiro, F. S., and Tostes, R. C. (2012). Differential modulation of nitric oxide synthases in aging: therapeutic opportunities. *Front. Physiol.* 3:218. doi: 10.3389/fphys.2012.00218

Cau, S. B., and Tostes, R. C. (2012). mTOR inhibition: a promise for a young heart. *Front. Physiol.* 3:31. doi: 10.3389/fphys.2012.00031

Chantler, P. D., and Lakatta, E. G. (2012). Arterial-ventricular coupling with aging and disease. *Front. Physiol.* 3:90. doi: 10.3389/fphys.2012.00090

El Assar, M., Angulo, J., Vallejo, S., Peiró, C., Sánchez-Ferrer, C. F., and Rodríguez-Mañas, L. (2012). Mechanisms involved in the aging-induced vascular dysfunction. *Front. Physiol.* 3:132. doi: 10.3389/fphys.2012.00132

Gragasin, F. S., Bourque, S. L., and Davidge, S. T. (2012). Vascular aging and hemodynamic stability in the intraoperative period. *Front. Physiol.* 3:74. doi: 10.3389/fphys.2012.00074

Lopes, R. A., Neves, K. B., Carneiro, F. S., and Tostes, R. C. (2012). Testosterone and vascular function in aging. *Front. Physiol.* 3:89. doi: 10.3389/fphys.2012.00089

Ming, X. F., Montani, J. P., and Yang, Z. (2012). Perspectives of targeting mTORC1-S6K1 in cardiovascular aging. *Front. Physiol.* 3:5. doi: 10.3389/fphys.2012.00005

Novella, S., Dantas, A. P., Segarra, G., Medina, P., and Hermenegildo, C. (2012). Vascular aging in women: Is estrogen the fountain of youth? *Front. Physiol.* 3:165. doi: 10.3389/fphys.2012.00165

Williamson, K., Stringer, S. E., and Alexander, M. Y. (2012). Endothelial progenitor cells enter the aging arena. *Front. Physiol.* 3:30. doi: 10.3389/fphys.2012.00030

Wojciechowska, W., Li, Y., Stolarz-Skrzypek, K., Kawecka-Jaszcz, K., Staessen, J. A., Wang, J. G., on behalf of the European Project on Genes in Hypertension and the JingNing Study Investigators. (2012). Cross-sectional and longitudinal assessment of arterial stiffening with age in European and Chinese populations. *Front. Physiol.* 3:209. doi: 10.3389/fphys.2012.00209

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Perspectives of targeting mTORC1–S6K1 in cardiovascular aging

Xiu-Fen Ming*, Jean-Pierre Montani and Zhihong Yang*

Laboratory of Vascular Biology, Division of Physiology, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland

Edited by:
Ana Paula Dantas, Institut d'Investigacions Biomediques August Pi i Sunyer, Spain

Reviewed by:
Xu Peng, Texas A&M Health Science Center, USA
Mercè Roqué, University of Barcelona, Spain
Rita Tostes, University of Sao Paulo, Brazil

****Correspondence:***
Xiu-Fen Ming and Zhihong Yang, Laboratory of Vascular Biology, Department of Medicine, University of Fribourg, Rue du Musée 5, CH-1700 Fribourg, Switzerland.
e-mail: xiu-fen.ming@unifr.ch; zhihong.yang@unifr.ch

The global population aging is accelerating and age-associated diseases including cardiovascular diseases become more challenging. The underlying mechanisms of aging and age-associated cardiovascular dysfunction remain elusive. There are substantial evidences demonstrating a pivotal role of the mammalian target of rapamycin complex 1 (mTORC1) and its down-stream effector S6K1 signaling in mammalian lifespan regulation and age-related diseases such as type II diabetes mellitus and cancer. The role of mTORC1–S6K1 in age-related cardiovascular diseases is, however, largely unknown and the available experimental results are controversial. This review article primarily summarizes the most recent advances toward understanding the role of mTORC1–S6K1 in cardiovascular aging and discusses the future perspectives of targeting mTORC1–S6K1 signaling as a healthy lifespan extension modality in anti-aging and anti-cardiovascular aging.

Keywords: aging, endothelial senescence, eNOS, mTOR, S6K1, oxidative stress, rapamycin, resveratrol

INTRODUCTION

Global population aging is accelerating, which has been predicted to be a great challenge for our society in the twenty-first century (Christensen et al., 2009). This is due to the steady achievements of medicine and public healthcare over the past century, having resulted in an increased life expectancy, which is mainly attributed to improvement of infant and childhood survival and reduction of mortality in the elderly population (Christensen et al., 2008, 2009; Rau et al., 2008). It has been predicted that the global proportion above age 60 will increase from 10% of the total population in 2000 to 21.8% in 2050 and 32.2% in 2100 (Lutz et al., 2008). With the increased aging population, we are confronted with an increase in age-associated diseases including cardiovascular disease, cancer, diabetes, and neurodegenerative disease (Christensen et al., 2009). Although aging has been proven to be an prominent independent risk factor for cardiovascular disease (Najjar et al., 2005), the mechanisms of aging and age-associated cardiovascular dysfunction are still elusive. There are substantial evidences suggesting that oxidative stress plays a crucial role in cardiovascular aging (Ungvari et al., 2010). However, several prospective clinical trials with various combinations of antioxidants fail to show significant effects on the incidence of major adverse cardiovascular events (Vivekananthan et al., 2003; Sesso et al., 2008). The results suggest that more thorough research on the mechanisms of aging and age-associated cardiovascular diseases is required.

Research in the past years provides compelling evidences showing a potential role of the target of rapamycin (TOR) signaling pathway in lifespan regulation, which is remarkably conserved across various species. In model systems such as yeast, nematodes, fruit flies, and also recently in mice, inhibition of TOR signaling increases lifespan (Evans et al., 2011). A role of deregulated mammalian TOR (mTOR) signaling in mammalian aging and age-related diseases such as type II diabetes mellitus and cancer are demonstrated (Evans et al., 2011). The role of mTOR, especially its down-stream effector S6K1 in age-related cardiovascular diseases or cardiovascular aging is, however, largely unknown. The primary emphasis of this review article is to discuss emerging evidence and future perspectives for a role of mTOR and S6K1 signaling in mammalian aging and age-associated cardiovascular diseases.

BRIEF BIOCHEMISTRY OF mTOR SIGNALING

mTOR is a serine/threonine protein kinase which serves as intracellular sensor for energy, nutrients, and stress, regulating cellular and organism growth and metabolism. Therefore, dysfunctional mTOR signaling has been considered a central integral mechanism linking aging, metabolic disorders, and cancer (Zoncu et al., 2011). The detailed molecular signaling network and regulation of mTOR signaling have been reviewed comprehensively in several articles (Dann et al., 2007; Sengupta et al., 2010; Evans et al., 2011; Zoncu et al., 2011). A few important biochemical features of mTOR signaling network are summarized in **Figure 1**. mTOR, with other molecular components, forms two structurally and functionally distinct complexes namely mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). In these complexes, mTOR functions as the catalytic subunit and its enzymatic activity is regulated and distinguished by its unique accessory proteins Raptor and Rictor, respectively. Raptor and Rictor function as scaffold proteins for assembling mTORC1 and mTORC2 and also for binding substrates and regulators in the respective complex.

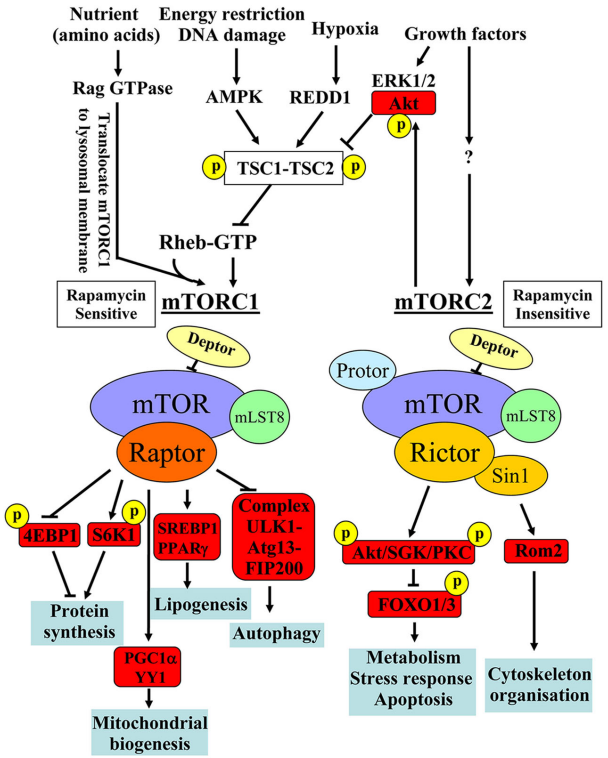

FIGURE 1 | Major distinct characteristics of the two known mTOR signaling complexes, mTORC1 and mTORC2, in composition, rapamycin sensitivity, upstream signals, substrates, and biological functions. Adapted from Foster and Finger (2010) and from Zoncu et al. (2011). 4E-BP1, eIF4E-binding protein 1; AMPK, AMP kinase; Atg, autophagy-related; FIP200, 200 kDa FAK family kinase-interacting protein; FOXO, forkhead box protein O; PPARγ, peroxisome proliferator-activated receptor-γ; REDD1, regulated in development and DNA damage response 1; Rom2, Rho1 GDP–GTP exchange protein-2; SGK, serum- and glucocorticoid-regulated kinase; SREBP, sterol regulatory element-binding protein; TSC, the tuberous sclerosis.

In addition to their different protein compositions, there are a few major important biochemical characteristics which distinguish the two complexes: (1) sensitivity to the immunosuppressant rapamycin: rapamycin inhibits mTORC1 but not mTORC2 activity, although prolonged treatment with rapamycin has been shown to be capable of inhibiting mTORC2 in certain cell types (Sarbassov et al., 2006); (2) the upstream signals they integrate: while mTORC2 seems to be regulated only by growth factors, mTORC1 is regulated by many stimuli including growth factors, energy status, stressors such as DNA damage, hypoxia, and nutrients. The small GTPase protein Rheb has been identified to be the key end point for mTORC1 activation by stimulating kinase activity of mTORC1. The TSC1–TSC2, a GTPase activating protein (GAP), negatively modulates Rheb by converting the active form Rheb-GTP into its inactive form Rheb-GDP through its GAP activity and thus reduces mTORC1 activity. Except nutrient amino acids, all of the above mentioned stimuli inputs regulate mTORC1 activity through modulation of TSC1–TSC2 activity. Growth factors inactivate TSC1–TSC2 via Akt/ERK1/2, leading to formation of Rheb-GTP and ultimately mTORC1 activation, whereas energy deficit, DNA damage, or hypoxia activate TSC1–TSC2 through activation of AMPK or REDD1 (regulated in development and DNA damage response 1), respectively, resulting in inactivation of Rheb and thus inhibition of mTORC1. Amino acids activate mTORC1 independently of TSC1–TSC2, but through action of Rag GTPases. In the presence of amino acids, Rag GTPases interact with mTORC1 and translocate the complex from cytoplasm to lysosomal membranes where it is activated by Rheb. For more detailed discussion about the regulatory mechanisms of mTORC1 activation by various inputs, please refer to the review article by Zoncu et al. (2011). In contrast to mTORC1, the regulatory mechanisms and functions of mTORC2 signaling are less well characterized; (3) the substrate they regulate and the biological processes they control: mTORC1 enhances protein synthesis through S6K1 and eIF4E-binding protein 1 (4E-BP1). Upon phosphorylation by mTORC1, 4E-BP1 dissociates from eIF4E, relieving its suppressing effect on mRNA translation, while S6K1, when phosphorylated by mTORC1, promotes mRNA translation. In addition, mTORC1 also induces lipogenesis in the liver through activating transcription factors SREBP1 and PPARγ, inhibits autophagy through phosphorylation of the ULK1–Atg13–FIP200 complex, and promotes mitochondrial biogenesis by activating PGC1α/YY1 (Zoncu et al., 2011). mTORC2 exerts its effects on metabolism, stress responses, apoptosis, and cytoskeleton organization through phosphorylation of many AGC kinases including Akt, serum-and glucocorticoid-induced protein kinase (SGK), protein kinase C-α (PKCα), and Rho1 GDP–GTP exchange protein-2 (Rom2; Sarbassov et al., 2005; Frost and Lang, 2011). Since mTORC2 activates Akt that in turn enhances mTORC1 activity through inactivation of TSC1–TSC2, mTORC2 is the upstream of mTORC1 upon stimulation by growth factors (see **Figure 1**).

EVIDENCE FOR A ROLE OF mTORC1–S6K1 IN REGULATION OF LONGEVITY

The role of mTORC1–S6K1 as a master determinant in longevity control stems from experiments in almost all model organisms including yeast, worms, flies, and mice. Genetic inactivation of TOR or core components of TOR signaling including S6K1 or pharmacological inhibition of the signaling pathway with rapamycin showed lifespan extension in yeast and invertebrates and also recently in mice (Chen et al., 2009; Harrison et al., 2009; Selman et al., 2009). These independent studies clearly verified the important role of mTORC1–S6K1 in mammalian aging. No data is available, yet, whether mTORC2 is also involved in regulation of longevity. The lifespan extension experiments with rapamycin may not fully exclude a possible role of mTORC2 in regulation of longevity, since long-term rapamycin treatment is able to inhibit mTORC2 in certain cell types (Sarbassov et al., 2006).

Furthermore, the effect of mTOR signaling inhibition on longevity can be mimicked by caloric restriction, i.e., reduction in calorie intake without malnutrition, across different species (Pan et al., 2007; Evans et al., 2011; Shinmura et al., 2011). A recent report demonstrating that caloric restriction also slows aging in rhesus monkeys (Colman et al., 2009) may suggest that caloric restriction could have anti-aging effects in humans (Kaeberlein and Kennedy, 2009). Indeed, it has been reported that the

Okinawan populations who consume 20% less calories compared to the average caloric consumption of the Japanese population live longer and display lower incidences of cancer and cardiovascular diseases in the elderly. The healthier aging status disappears in those who switched to Western diet (Kagawa, 1978; Willcox et al., 2007). A small caloric restriction study in adult humans over 6 years demonstrates less cardiovascular risk factors including lower body mass index, body fat deposition, blood pressure, fasting plasma levels of glucose and insulin, lower inflammation markers, i.e., tumor necrosis factor-α and C-reactive protein, and beneficial lipid profiles (Fontana et al., 2004) and shows better left ventricular diastolic function than healthy age-and sex-matched controls (Meyer et al., 2006). Also, caloric restriction improves cardiac function in patients with type 2 diabetes (Hammer et al., 2008). Whether the beneficial effects of caloric restriction in humans are due to healthier diet and could be translated to lifespan extension in healthy elderly subjects and improve survival in patients requires further investigation. The underlying mechanisms of caloric restriction on longevity may involve reduction of mTORC–S6K1 signaling. In yeast and flies, when TOR is inactivated, caloric restriction does not show further effects on lifespan extension (Kapahi and Zid, 2004; Powers et al., 2006). Whether this holds true also in humans needs to be confirmed.

Another piece of supporting evidence for mTORC1–S6K1 in regulation of longevity derives from experiments showing that down-regulation of insulin and insulin-like growth factor (IGF-1) signaling in mouse models such as haploinsufficiency of IGF-1 receptor and global deletion of insulin receptor substrate-1 (IRS-1) are associated with reduced mTORC1–S6K1 signaling and increased longevity in mice (Holzenberger et al., 2003; Selman et al., 2008) as in yeast, nematodes, and fruit flies (Stanfel et al., 2009). Moreover, caloric restriction prolongs lifespan in animal models, which is associated with reduced mTORC1–S6K1 mediated by IGF-1 signaling pathway (Estep et al., 2009; Fontana et al., 2010). These results further strengthen the role of mTORC1–S6K1 in lifespan regulation in mammals. The exact mechanisms of reduced signaling of mTORC1–S6K1 and IGF-1 under caloric restriction, however, remain elusive. It seems that AMPK and Sirt1, whose activities are augmented by caloric restriction, are involved in negative regulation of mTORC1–S6K1 signaling and in turn regulate aging process (Cohen et al., 2004; Canto et al., 2009; Shackelford and Shaw, 2009; **Figure 1**), although direct evidence for it in mammals is still lacking (Herranz and Serrano, 2010). It is also noteworthy that loss of *S6K1* in mice reciprocally results in activation of AMPK which has been proposed to mediate the lifespan extension (Selman et al., 2009). Intriguingly, persistent activation of AMPK seems to play an important role in vascular endothelial cell senescence (Zu et al., 2010). A working model of reciprocal regulatory effects of Sirt1 and AMPK on cellular senescence and aging is discussed by Wang et al. (2011). The molecular mechanisms of the reciprocal interplay between mTORC1–S6K1 and AMPK and Sirt1 are not clear. A mutual inhibition of mTORC1–S6K1 and AMPK has been reported (Lee et al., 2010).

Although the role of inhibition of mTORC1–S6K1 in lifespan extension is consistently demonstrated in animal models, very little information is available about the role of mTORC1–S6K1 in cardiovascular aging or age-associated cardiovascular diseases.

mTORC1–S6K1 SIGNALING IN CARDIOVASCULAR AGING

CARDIAC AGING

Age-associated cardiovascular diseases are accompanied by structural and functional changes in heart and blood vessels. These aging-associated changes in cardiovascular system are referred to as cardiovascular aging phenotypes. Cardiac aging is manifested by maladaptation to stress, cardiac dysfunction, and heart failure. Cardiac aging process involves cardiomyocytes and other cell types in the heart, such as interstitial fibroblasts and vascular cells. The morphological and functional changes of these cells with aging lead to cardiac hypertrophy and dilation, cardiac fibrosis due to chronic deposition, and remodeling of extracellular matrix produced mainly by fibroblasts. Moreover, a decreased regenerative capacity of cardiac stem cells, possibly due to impaired cell division and accelerated cell senescence and an increased cardiac myocyte death due to necrosis and apoptosis with aging, may also contribute to cardiac dysfunction in elderly. These pathological and clinical aspects of cardiac remodeling in aging have been recently reviewed in great details (Shih et al., 2011).

There are compelling evidences demonstrating that mTORC1–S6K1 signaling is involved in cardiac hypertrophy under various pathological conditions including diabetes and hypertension (Sadoshima and Izumo, 1995; Boluyt et al., 1997; Tu et al., 2002; McMullen et al., 2004; Soesanto et al., 2009; Kurdi and Booz, 2011; Sung et al., 2011). The underlying mechanisms have been proposed to be attributed to both an increase in protein synthesis upon stress stimulation and a decrease in protein degradation due to inhibition of autophagy by mTORC1 signaling (Hands et al., 2009). However, evidence for this hypothesis needs to be demonstrated. Inhibition of mTORC1–S6K1 pathway by rapamycin or activation of AMPK by 5-aminoimidazole-4-carboxamide riboside (AICAR) to negatively regulate mTORC1 signaling has been shown to attenuate pressure overload-induced cardiac hypertrophy *in vivo* (Li et al., 2007). Conversely, deficiency in AMPK enhances mTORC1–S6K1 signaling and exacerbates myocardial hypertrophy in response to pressure overload (Zhang et al., 2008).

Besides pressure overload, many hormonal factors, e.g., angiotensin-II, insulin, endothelin-1, catecholamine, etc., that are elevated in plasma and/or tissues under various cardiovascular pathologies and in aging, have been shown to stimulate mTORC1–S6K1 signaling in the cardiovascular system (Moschella et al., 2007; Muniyappa et al., 2007; Kim et al., 2012). Activation of mTORC1–S6K1 by the hormones, e.g., angiotensin-II and also by overnutrition (Glazer et al., 2009) participates in cardiac hypertrophy and vascular remodeling, and causes insulin resistance, an important cardiovascular risk (Reaven, 2011), through phosphorylation of IRS-1 at serine residues (Kim et al., 2012). Importantly, under the condition of cardiovascular insulin resistance, there is a selective inhibition of the metabolic pathway, i.e., Akt–eNOS in response to insulin, while the growth pathway, i.e., $p44/p42^{ERKs}$ remains active (Muniyappa et al., 2007). This selective insulin resistance may play an important role in decreased vascular relaxation due to impaired eNOS activation and enhanced cardiovascular remodeling in metabolic disorders such as type II diabetes (Muniyappa et al., 2007). mTORC1–S6K1 pathway is therefore

considered as an important molecular link between metabolic stress and cardiovascular abnormalities.

Despite the evidences for a role of mTORC1–S6K1 signaling in agonist or pressure-induced cardiac hypertrophy, only little information is available about the signaling of mTORC1–S6K1 in physiological process of aging heart. An early study using microarray analyses reported that gene expression pattern associated with mTOR is suppressed in aging heart of Fischer 344 rats (Linford et al., 2007), while a recent study shows no difference in mTORC1–S6K1 activity (measured by phosphorylated mTOR and S6K1 levels) in the heart between 8 and 30 month old rats of the same strain (Shinmura et al., 2011). There are two points that should be considered for interpretation of the inconsistent results. First, microarray has its limitation in elucidating signaling pathways, because signaling molecules are mainly enzymes whose activities could not be investigated with this experimental approach; second, aging-associated kinetics of mTORC1–S6K1 signaling may exist, meaning an enhanced signaling with time followed by a decreased signaling in old age. Indeed, a decreased mTOR signaling has been shown to play a role in sarcopenia in advanced age (Sakuma and Yamaguchi, 2010). Whether this also occurs in the heart requires investigation. Nevertheless, caloric restriction in the old rats showed improved diastolic function associated with reduced cell senescence and mTORC1–S6K1 signaling in the heart compared to the old animals fed *ad libitum* (Shinmura et al., 2011), suggesting that mTORC1–S6K1 is involved in caloric restriction-induced improvement of heart function in aging. It is also conceivable that the anti-cellular senescence effect and reduced mTORC1–S6K1 signaling contribute to the beneficial effects of caloric restriction on human heart function (Meyer et al., 2006; Hammer et al., 2008).

VASCULAR AGING

Endothelial dysfunction and inflammatory activation

In the vasculature, increased arterial wall thickening and generalized vascular stiffness occur with aging. This vascular aging phenotype is attributed to vascular calcification, increased collagen content and elastin breakdown, and elevated levels of advanced glycation end products. Vascular inflammation, oxidative stress, endothelial dysfunction, endothelial progenitor cell dysfunction, vascular cell apoptosis all intertwine with each other to affect vascular aging process, which accelerates coronary heart disease, heart failure, stroke, and dementia (Ungvari et al., 2010).

In the past decades, much attention has been devoted to endothelial dysfunction in aging. Evidence demonstrates that besides the enhanced production of endothelium-derived vasoconstrictor prostanoids, which is due to augmented expression and/or activity of cyclo-oxygenases in endothelial cells during aging (Vanhoutte et al., 2009), decreased endothelial nitric oxide (NO) bioavailability is a major characteristic of vascular aging, which is independent of other cardiovascular risk factors (Lakatta, 2001). Endothelial NO causes vascular relaxation, inhibits platelet aggregation, and leukocyte adhesion (Yang and Ming, 2006). Clinical studies provide evidence that endothelial dysfunction is not only highly associated with cardiovascular disease, it also predicts future cardiac events (Schachinger et al., 2000; Halcox et al., 2002; Bugiardini et al., 2004; Huang et al., 2007). eNOS dysfunction seems causally involved in cardiovascular aging, since eNOS−/− male mice have a significantly shorter lifespan than their wild type controls and exhibit accelerated cardiac dysfunction with age (Wei, 2004). Endothelial cells with dysfunctional eNOS in aging are also more vulnerable to apoptotic stimuli (Hoffmann et al., 2001; Csiszar et al., 2004). There is increasing evidence suggesting that the number and regenerative capacity of circulating endothelial progenitor cells which play a role in reendothelialization and repair after vascular injury are decreased in patients with cardiovascular diseases and risk factors including aging (Vasa et al., 2001; Hill et al., 2003; Rauscher et al., 2003; Werner et al., 2005). The underlying mechanisms of eNOS dysfunction in aging are multifactorial and have not been fully understood, yet. Decreased eNOS gene expression (Csiszar et al., 2002; Tanabe et al., 2003) or increased eNOS gene expression with impaired enzymatic activity due to oxidative stress are important mechanisms (Stockklauser-Farber et al., 2000; van der Loo et al., 2000; Ming et al., 2004; Desrois et al., 2010). The up-regulation of eNOS gene in aging and also in other vascular disease may represent a compensatory mechanism counteracting oxidative stress (Drummond et al., 2000; Stockklauser-Farber et al., 2000; Hink et al., 2001).

Oxidative stress has been proposed as the culminant mechanism impairing endothelial function via quenching of NO, i.e., inactivation of NO by increased production of O_2^-, which leads to formation of peroxynitrite, a strong oxidant that can further damage endothelial cells in aging (van der Loo et al., 2000; Brandes et al., 2005). Among other sources, eNOS itself produces significant amount of O_2^- in endothelial cells, when "eNOS uncoupling" occurs – that is, eNOS generates O_2^- instead of NO (Landmesser et al., 2003; Forstermann and Munzel, 2006; Rajapakse et al., 2011). It seems that normal function of eNOS requires homodimerization of the enzyme which is stabilized by the cofactor BH_4. The eNOS reductase domain generates electron flow from NADPH through FAD and FMN flavins, which are then transferred to the oxidase domain of other monomers in which L-arginine is metabolized to NO at the heme group in the active site (Forstermann and Munzel, 2006). In the absence of BH_4 due to oxidative inactivation, eNOS dimer/monomer ratio is decreased and the catalytic activity becomes uncoupled – that is – uncoupling of NADPH oxidation and NO synthesis, with oxygen instead of L-arginine as terminal electron acceptor, resulting in O_2^- generation (Forstermann and Munzel, 2006). In addition, limited specific pool of intracellular L-arginine bioavailability due to enhanced arginase activity (Csiszar et al., 2002; Berkowitz et al., 2003; Tanabe et al., 2003) or production of endogenous eNOS inhibitor asymmetric dimethylarginine (ADMA; Sydow and Munzel, 2003; Antoniades et al., 2009) have been also reported to contribute to endothelial dysfunction in aging. The finding that eNOS enzymatic dysfunction is the major mechanism for endothelial dysfunction in aging indicates that one should therapeutically focus on improving eNOS enzymatic function instead of increasing eNOS gene expression in blood vessels. Indeed, over-expression of eNOS under disease conditions for example in atherosclerosis prone $ApoE^{-/-}$ mice has been shown to accelerate atherosclerosis (Ozaki et al., 2002). Endothelial specific eNOS transgenic mice have enhanced O_2^- generation which can be inhibited by the eNOS inhibitor L-NAME or by endothelial-targeted GTP cyclohydrolase 1 over-expression to

increase BH_4 production (Bendall et al., 2005). The results further implicate that too much eNOS under pathological conditions where the enzyme is uncoupled, is detrimental. A recent study reveals that oxidative stress causes cysteine S-glutathionylation of the reductase domain of eNOS, resulting eNOS uncoupling (Chen et al., 2010). The eNOS S-glutathionylation level is increased in spontaneously hypertensive rats as compared to normotensive animals accompanied with impaired endothelium-dependent relaxations that can be reversed after the S-glutathionylation of eNOS is removed by thiol-specific reducing agents (Chen et al., 2010). Whether S-glutathionylation of eNOS also plays a role in endothelial dysfunction in aging requires further investigation.

Another important feature of endothelial aging is enhanced expression of inflammatory adhesion molecules such as ICAM-1 and VCAM-1 (Gorgoulis et al., 2003; Zhou et al., 2006; Ungvari et al., 2010), which leads to enhanced monocyte–endothelial interaction and accelerated atherogenesis. There are considerable evidences suggesting that oxidative stress is a major mechanism for promoting eNOS dysfunction as well as vascular inflammation which causes further endothelial dysfunction and aging in a positive-feedback manner (Herrera et al., 2010). However, evidence for a causal role of oxidative stress in vascular aging is still lacking, although there is no doubt that oxidative stress contributes to aging-associated vascular dysfunctions. Some authors suggest that mTOR signaling instead of oxidative stress might be the more powerful driving force for organismal aging (Blagosklonny, 2008).

Causal role of mTORC1–S6K1 signaling in endothelial aging

Although evidence for a role of mTOR–S6K1 signaling in regulation of organism lifespan has been well demonstrated (Evans et al., 2011; Zoncu et al., 2011), it is, however, not known, whether mTORC1–S6K1 signaling pathway participates in vascular aging. In contrast to the aging heart, an increased basal activity of mTORC1–S6K1 has been demonstrated in aortas of old Fischer 344xBrown Norway F1 hybrid rats (Rice et al., 2005), which implicates a potential role of mTORC1–S6K1 signaling in vascular aging. However, a study reported that inhibition of mTORC1–S6K1 by rapamycin and everolimus induces endothelial cellular senescence in culture (Ota et al., 2009), which does not support the role of mTORC1–S6K1 in endothelial aging. One has to take into account that the former study shows correlation between increased mTORC1–S6K1 signaling and vascular aging, while the latter used pharmacological inhibitors in young endothelial cells. The drugs may exert some non-specific effects which could interfere with the interpretation. Moreover, the function of mTORC1–S6K1 in young and old cells or organisms might be different. A regulated function of mTORC1–S6K1 signaling is necessary for organism development, while a persistent non-regulated mTORC1–S6K1 signaling is detrimental.

By pharmacological and genetic approaches, we demonstrate a hyperactive S6K1 activity in two aging model systems, i.e., in cultured senescent endothelial cells and in aortas of naturally aging rats as compared to young cells and young animals (Rajapakse et al., 2011). A persistent activation of mTORC1–S6K1 signaling is also shown to be associated with hematopoietic stem cells in old mice, which is responsible for decreased regenerative capacity of the stem cells in aging (Chen et al., 2009). Inhibition of mTORC1–S6K1 pathway either with rapamycin or with S6K1 silencing improves NO production and inhibits O_2^- production in senescent cells and old rat aortas. The enhanced O_2^- production in senescent cells and old rat aortas can be eliminated by the eNOS inhibitor L-NAME, demonstrating eNOS uncoupling in aging (Rajapakse et al., 2011). Conversely, over-expression of a S6K1 active mutant in young endothelial cells causes eNOS uncoupling and endothelial senescence, which provides the first evidence for a causative role of S6K1 in eNOS uncoupling and endothelial aging. How S6K1 drives endothelial senescence or aging and causes eNOS uncoupling, and whether S6K1 causes endothelial aging through eNOS uncoupling remain to be investigated.

Endothelial aging is also associated with increased expression of the coagulation factor tissue factor (TF) as well as adhesion molecules ICAM-1 and VCAM-1 (Csiszar et al., 2008), which may promote thrombosis and vascular inflammation in elderly subjects. Numerous studies including our own report the inhibitory effect of mTORC1 on TF expression based on the observation that rapamycin or silencing mTOR enhances TF expression in endothelial cells (Camici et al., 2010; Ming et al., 2010). However, silencing S6K1 reduces TF protein level in endothelial cells stimulated with thrombin or TNFα without affecting TF mRNA expression. Conversely, over-expression of a constitutively active S6K1 mutant enhances TF protein level even in the mTOR-silenced cells (Ming et al., 2010). The results reveal an unexpected opposing effect of mTOR and S6K1 on endothelial TF expression and are best explained by the mechanisms that the markedly enhanced TF mRNA expression under the condition of mTORC1 inhibition is translated by S6K1-independent pathways such as RhoA, NF-κB, and p38mapk (Ming et al., 2010), since blockade of RhoA, NF-κB, and p38mapk either pharmacologically or genetically is able to reduce the up-regulation of TF protein level (**Figure 2**). The uncoupling effect of mTOR and S6K1 has also been reported in skeletal muscle cells (Cunningham et al., 2007a). These findings may have potential clinical implications. TF is highly expressed in cells within atherosclerotic plaques (Hatakeyama et al., 1997). It has been reported that vascular injury after coronary intervention increases circulating TF activity in patients (Tutar et al., 2003), which favors thrombus formation after coronary intervention. With rapamycin (sirolimus)-eluting stents, TF expression in the vasculature and release from vascular cells including endothelial cells, smooth muscle cells, and macrophages might be exaggerated because of the stimulating effect of TF expression by the drug. This effect of rapamycin, may increase thrombotic risk, despite its property of preventing vascular restenosis in patients with coronary artery disease (Luscher et al., 2007), although inhibition of endothelial regeneration and in turn endothelialization of stent surface by drug-eluting stents may play a more prominent role in stent thrombosis (Inoue et al., 2011). It would be interesting to test whether inhibition of S6K1 alone, rather than mTORC1 as achieved by rapamycin, proves sufficient to prevent vascular restenosis and be superior in reducing thrombotic propensity by developing specific S6K1 inhibitors. The same consideration may be applied for anti-aging therapy. Moreover, silencing S6K1 in endothelial cells is able to prevent up-regulation of E-selectin induced by TNFα (Ming et al., 2009), implicating a possible role of

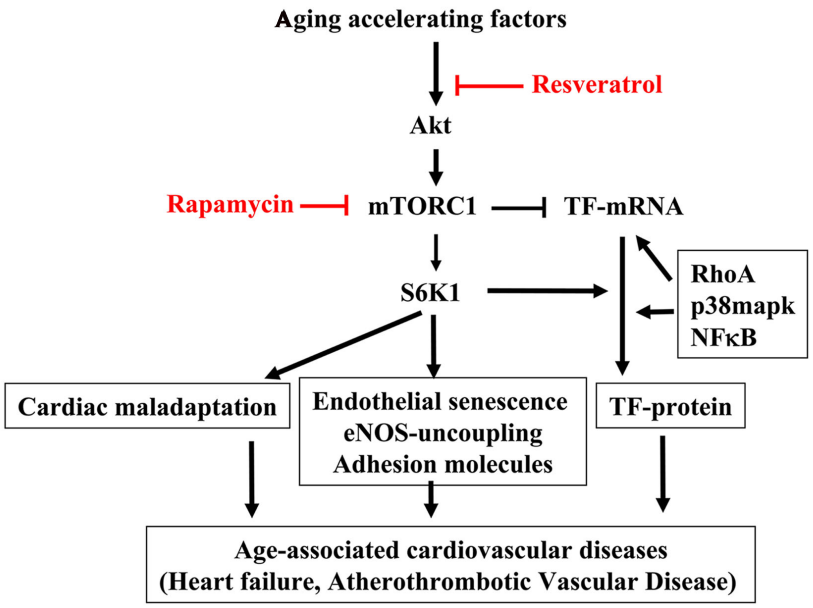

FIGURE 2 | mTORC1–S6K1 signaling in cardiovascular aging. Aging accelerating factors cause persistent activation of the Akt–mTORC1–S6K1 signaling. The hyperactive S6K1 has pleiotropic effects in cardiovascular system. In heart, it causes cardiomyocytes hypertrophy leading to cardiac maladaptation. In endothelium, it induces eNOS uncoupling, endothelial senescence, and adhesion molecule expression as well as TF protein expression. All of these effects contribute to age-associated cardiovascular diseases such as heart failure, atherosclerotic vascular disease. Thus inhibiting S6K1 with resveratrol or mTORC1 inhibitor rapamycin may have beneficial effect in treatment of age-associated cardiovascular diseases in clinic. It is important to recognize that there are some uncoupled biological function(s) of mTORC1 and S6K1 as reported for their opposing effect in regulation of TF expression. While mTOR suppresses endothelial TF mRNA expression, S6K1 is required for TF protein translation, which works in concert with other signaling pathways such as RhoA, p38mapk, and NF-κB. Given this finding, drugs more specifically inhibiting S6K1 rather than mTORC1 should be developed and the effects of targeting mTORC1 or S6K1 on aging-related cardiovascular diseases and cardiovascular aging and beyond should be investigated.

S6K1 in regulation of endothelial–leukocyte interaction. Whether S6K1 is involved in TF as well as adhesion molecule expression in aged endothelial cells and blood vessels warrants further investigation. The effects of mTORC1–S6K1 on cardiovascular functions are illustrated in **Figure 2**.

Resveratrol inhibits mTORC1–S6K1 and prevents endothelial aging

It is well demonstrated that resveratrol, a naturally occurring polyphenol, slows aging process and exerts protective effects on aging-associated pathologies including vascular diseases, type II diabetes in animal models (Baur et al., 2006; Lagouge et al., 2006; Barger et al., 2008; Pearson et al., 2008; Miller et al., 2011). Numerous studies including ours demonstrate that resveratrol inhibits ICAM-1 and VCAM-1 expression in endothelial cells in response to high glucose and TNFα (Csiszar et al., 2006; Park et al., 2009; Rajapakse et al., 2009). Interestingly, resveratrol is capable of recoupling eNOS, leading to inhibition of superoxide generation and increase in endothelial NO production in senescent endothelial cells and in aged rat aortas partly through inhibition of mTORC1–S6K1 signaling (Rajapakse et al., 2011), since NO production stimulated by acetylcholine is only partly restored by resveratrol and rapamycin in old rats. Additional defects in eNOS dysfunction as discussed must be present.

It also remains to be investigated whether resveratrol's inhibitory effect on mTORC1–S6K1–eNOS-uncoupling is mediated through activation of the NAD^+-dependent deacetylase Sirt1 (Lagouge et al., 2006; Zang et al., 2006; Baur, 2010), whose activation has been shown to exhibit protection against age-associated diseases including diabetes and atherosclerosis (Lagouge et al., 2006; Zang et al., 2006; Pearson et al., 2008), although current studies do not show life extending effect in mammals (Lagouge et al., 2006; Barger et al., 2008; Pearson et al., 2008; Miller et al., 2011). Recent studies provide evidence showing that resveratrol also exerts pleiotrophic effects independently of Sirt1 (Zhang, 2006; Pirola and Frojdo, 2008; Kitada et al., 2011). It has also been shown that resveratrol may indirectly activate Sirt1 through AMPK, which has been demonstrated to inhibit mTORC1–S6K1 pathway in different cell types (Ruderman et al., 2010). The underlying mechanisms by which resveratrol inhibits mTORC1–S6K1 in aging endothelial cells are not clear, yet. Since the hyperactive Akt, an upstream signaling of mTORC1–S6K1 pathway, is observed in senescent endothelial cells and is inhibited by resveratrol (Rajapakse et al., 2011), it seems that resveratrol negatively regulates mTORC1–S6K1 pathway through inhibition of mTORC2. The persistent activation of Akt–mTORC1–S6K1 signaling in endothelial aging observed in our study is consistent with the finding that a hyperactive Akt plays a role in endothelial cell senescence (Miyauchi et al., 2004). Further studies need to establish a role of mTORC2 in endothelial senescence and in regulation of longevity.

RAPAMYCIN AND RESVERATROL AS ANTI-AGING AND ANTI-CARDIOVASCULAR AGING DRUGS IN HUMANS?

Although caloric restriction seems the most promising approach to slow aging and onset of age-related diseases in humans with almost no recognizable adverse effects (Fontana et al., 2010), it is difficult to implement as a routine and long-term preventive or treatment modality in humans. The primary target of anti-aging strategy should focus on treatment of age-related diseases and not longevity, i.e., improvement of "healthy" lifespan. By treatment of age-related diseases, healthy lifespan extension, and improvement of life quality in elderly are expected.

In animal models, rapamycin is able to extend lifespan and prevents many age-related diseases such as cancer (Hudes et al., 2007), obesity (Um et al., 2004), cardiovascular diseases (Sadoshima and Izumo, 1995; Elloso et al., 2003; Waksman et al., 2003; McMullen et al., 2004; Pakala et al., 2005; Adelman, 2010). Moreover, a recent study reports that in cultured fibroblasts isolated from patients suffering Hutchinson–Gilford Progeria Syndrome (HGPS), a lethal genetic disease characterized by premature aging and death in adolescence or the teen years, rapamycin postpones cell senescence by activating autophagy, a process by which cells clear junk protein and trashed organelles and is inhibited by hyperactive mTORC1–S6K1 (Cao et al., 2011). These findings suggest an additional mechanism for the beneficial effects of rapamycin on aging.

Interestingly, many of the beneficial effects of rapamycin have been shared with resveratrol (Jang et al., 1997; Baur et al., 2006; Zang et al., 2006; Chan et al., 2008; Smoliga et al., 2011), although the lifespan extending effect could be demonstrated with rapamycin but not with resveratrol in mice (Pearson et al., 2008; Harrison et al., 2009; Miller et al., 2011). Both rapamycin and resveratrol inhibit hyperactive mTORC1–S6K1 signaling, improve endothelial function in senescent cells and aging rat aortas (Rajapakse et al., 2011), and also improve bone marrow-derived progenitor cell function and senescence (Chen et al., 2009; Huang et al., 2010). All the results suggest that rapamycin and resveratrol may be used as anti-aging therapeutics in humans. Rapamycin and analogs are indeed used in patients with organ transplantation and cancer and show clinical benefits (Kauffman et al., 2005; Law, 2005; Stallone et al., 2005; Zmonarski et al., 2005; Campistol et al., 2006). However, there is concern about some undesirable effects of rapamycin which may limit its systemic use as anti-aging or anti-cardiovascular aging drug. In contrast to the initial major concern about immunosuppressive effects, rapamycin has been shown to improve immune function and regenerative capacity of bone marrow stem cells in old mice (Chen et al., 2009). However, early studies in animal models treated with rapamycin reported multiple adverse effects including deregulated glucose homeostasis, hyperlipidemia (Cunningham et al., 2007b; Chang et al., 2009). In humans, rapamycin increases blood triglyceride and cholesterol resulting from lipolysis (Morrisett et al., 2002; Ribes et al., 2005), which may have negative impact on cardiovascular functions and metabolic homeostasis, although rapamycin has been shown to reduce atherosclerosis in mouse models (Basso et al., 2003; Elloso et al., 2003; Waksman et al., 2003; Pakala et al., 2005). The different effects of rapamycin in mice may be related to when the rapamycin therapy is initiated (early or late in life) or how long the therapy persists, or whether chronic persistent or pulsed therapy is instrumented. Finally, it may depend on whether young or old, healthy or diseased animals are treated with the drug. It is important to point out that rapamycin may be beneficial under the condition when mTORC1–S6K1 signaling is inappropriately consistently elevated, for example in aging, but detrimental if its activity under physiological conditions is abolished. Furthermore, taking into account that an opposing and uncoupling effect of mTORC1 and S6K1 has been demonstrated such as in regulation of endothelial TF expression (Ming et al., 2010), and that both rapamycin and resveratrol inhibit mTORC1 at the level and upstream of mTORC1, respectively (**Figure 2**), it would be worth to test whether targeting S6K1 directly would be a better approach than targeting mTORC1 as achieved by rapamycin or resveratrol. For this purpose, specific inhibitor of S6K1 should be developed. At the molecular level, since off-target effects of the drugs could not be excluded, more specific experimental approaches such as mutants (active or dominant negative), RNA interference, or targeted gene disruption of mTORC1–S6K1 signaling should be applied to evaluate their roles in cardiovascular systems.

So far, almost no undesirable effects of resveratrol have been reported, which might be due to the fact that this drug is newly investigated as compared to rapamycin. The first recently published human study with resveratrol confirmed metabolic beneficial effects which were previously observed in animals (Timmers et al., 2011). The study showed that treatment of healthy, obese men with 150 mg/day resveratrol for 30 days mimicked the effects of calorie restriction, significantly improved metabolic profiles on circulating glucose, triglycerides, liver lipid content, and decreased inflammation markers and systolic blood pressure (Timmers et al., 2011). The effects of resveratrol on cardiovascular diseases in patients remain to be demonstrated. Further, dosage dependent side effects of resveratrol should be investigated, especially for the newly developed derivatives which show much stronger effects than resveratrol on Sirt1 activity (Milne et al., 2007). The potency of a drug is usually associated with toxicity due to non-specific off-target effects.

CONCLUSION AND PERSPECTIVES

Emerging evidence demonstrates that targeting mTORC1–S6K1 signaling could be a promising therapeutic modality to slow aging process and treat cardiovascular disease in aging. Future work should further elucidate the mechanisms of persistent hyperactive mTORC–S6K1 and the mechanisms of cardiovascular aging driven by mTORC1–S6K1. Since mTORC1–S6K1 signaling is also essential for normal development and skeletal muscle mass growth, undesirable side effects of targeting mTOCR1–S6K1 may be avoided by short treatment instead long-term treatment. In addition, drugs more specifically inhibiting S6K1 rather than mTORC1 should be developed and the effects of targeting mTORC1 or S6K1 on aging-related cardiovascular diseases and cardiovascular aging and beyond should be investigated.

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REFERENCES

Adelman, S. J. (2010). Sirolimus and its analogs and its effects on vascular diseases. *Curr. Pharm. Des.* 16, 4002–4011.

Antoniades, C., Shirodaria, C., Leeson, P., Antonopoulos, A., Warrick, N., Van-Assche, T., Cunnington, C., Tousoulis, D., Pillai, R., Ratnatunga, C., Stefanadis, C., and Channon, K. M. (2009). Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur. Heart J.* 30, 1142–1150.

Barger, J. L., Kayo, T., Vann, J. M., Arias, E. B., Wang, J., Hacker, T. A., Wang, Y., Raederstorff, D., Morrow, J. D., Leeuwenburgh, C., Allison, D. B., Saupe, K. W., Cartee, G. D., Weindruch, R., and Prolla, T. A. (2008). A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS ONE* 3, e2264. doi:10.1371/journal.pone.0002264

Basso, M. D., Nambi, P., and Adelman, S. J. (2003). Effect of sirolimus on the cholesterol content of aortic arch in ApoE knockout mice. *Transplant. Proc.* 35, 3136–3138.

Baur, J. A. (2010). Resveratrol, sirtuins, and the promise of a DR mimetic. *Mech. Ageing Dev.* 131, 261–269.

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, C. D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de, C. R., and Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342.

Bendall, J. K., Alp, N. J., Warrick, N., Cai, S., Adlam, D., Rockett, K., Yokoyama, M., Kawashima, S., and Channon, K. M. (2005). Stoichiometric relationships between endothelial tetrahydrobiopterin, endothelial NO synthase (eNOS) activity, and eNOS coupling in vivo: insights from transgenic mice with endothelial-targeted GTP cyclohydrolase 1 and eNOS overexpression. *Circ. Res.* 97, 864–871.

Berkowitz, D. E., White, R., Li, D., Minhas, K. M., Cernetich, A., Kim, S., Burke, S., Shoukas, A. A., Nyhan, D., Champion, H. C., and Hare, J. M. (2003). Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* 108, 2000–2006.

Blagosklonny, M. V. (2008). Aging: ROS or TOR. *Cell Cycle* 7, 3344–3354.

Boluyt, M. O., Zheng, J. S., Younes, A., Long, X., O'Neill, L., Silverman, H., Lakatta, E. G., and Crow, M. T. (1997). Rapamycin inhibits alpha 1-adrenergic receptor-stimulated cardiac myocyte hypertrophy but not activation of hypertrophy-associated genes. Evidence for involvement of p70 S6 kinase. *Circ. Res.* 81, 176–186.

Brandes, R. P., Fleming, I., and Busse, R. (2005). Endothelial aging. *Cardiovasc. Res.* 66, 286–294.

Bugiardini, R., Manfrini, O., Pizzi, C., Fontana, F., and Morgagni, G. (2004). Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation* 109, 2518–2523.

Camici, G. G., Steffel, J., Amanovic, I., Breitenstein, A., Baldinger, J., Keller, S., Luscher, T. F., and Tanner, F. C. (2010). Rapamycin promotes arterial thrombosis in vivo: implications for everolimus and zotarolimus eluting stents. *Eur. Heart J.* 31, 236–242.

Campistol, J. M., Eris, J., Oberbauer, R., Friend, P., Hutchison, B., Morales, J. M., Claesson, K., Stallone, G., Russ, G., Rostaing, L., Kreis, H., Burke, J. T., Brault, Y., Scarola, J. A., and Neylan, J. F. (2006). Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J. Am. Soc. Nephrol.* 17, 581–589.

Canto, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., Elliott, P. J., Puigserver, P., and Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 458, 1056–1060.

Cao, K., Graziotto, J. J., Blair, C. D., Mazzulli, J. R., Erdos, M. R., Krainc, D., and Collins, F. S. (2011). Rapamycin reverses cellular phenotypes and enhances mutant protein clearance in hutchinson-gilford progeria syndrome cells. *Sci. Transl. Med.* 3, 89ra58.

Chan, A. Y., Dolinsky, V. W., Soltys, C. L., Viollet, B., Baksh, S., Light, P. E., and Dyck, J. R. (2008). Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt. *J. Biol. Chem.* 283, 24194–24201.

Chang, G. R., Wu, Y. Y., Chiu, Y. S., Chen, W. Y., Liao, J. W., Hsu, H. M., Chao, T. H., Hung, S. W., and Mao, F. C. (2009). Long-term administration of rapamycin reduces adiposity, but impairs glucose tolerance in high-fat diet-fed KK/HlJ mice. *Basic Clin. Pharmacol. Toxicol.* 105, 188–198.

Chen, C., Liu, Y., Liu, Y., and Zheng, P. (2009). mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci. Signal.* 2, ra75.

Chen, C. A., Wang, T. Y., Varadharaj, S., Reyes, L. A., Hemann, C., Talukder, M. A., Chen, Y. R., Druhan, L. J., and Zweier, J. L. (2010). S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature* 468, 1115–1118.

Christensen, K., Doblhammer, G., Rau, R., and Vaupel, J. W. (2009). Ageing populations: the challenges ahead. *Lancet* 374, 1196–1208.

Christensen, K., McGue, M., Petersen, I., Jeune, B., and Vaupel, J. W. (2008). Exceptional longevity does not result in excessive levels of disability. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13274–13279.

Cohen, H. Y., Miller, C., Bitterman, K. J., Wall, N. R., Hekking, B., Kessler, B., Howitz, K. T., Gorospe, M., de, C. R., and Sinclair, D. A. (2004). Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390–392.

Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kemnitz, J. W., and Weindruch, R. (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325, 201–204.

Csiszar, A., Smith, K., Labinskyy, N., Orosz, Z., Rivera, A., and Ungvari, Z. (2006). Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. *Am. J. Physiol. Heart Circ. Physiol.* 291, H1694–H1699.

Csiszar, A., Ungvari, Z., Edwards, J. G., Kaminski, P., Wolin, M. S., Koller, A., and Kaley, G. (2002). Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ. Res.* 90, 1159–1166.

Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., and Kaley, G. (2004). Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol. Genomics* 17, 21–30.

Csiszar, A., Wang, M., Lakatta, E. G., and Ungvari, Z. (2008). Inflammation and endothelial dysfunction during aging: role of NF-kappaB. *J. Appl. Physiol.* 105, 1333–1341.

Cunningham, J. T., Rodgers, J. T., Arlow, D. H., Vazquez, F., Mootha, V. K., and Puigserver, P. (2007a). mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 450, 736–740.

Cunningham, J. T., Rodgers, J. T., Arlow, D. H., Vazquez, F., Mootha, V. K., and Puigserver, P. (2007b). mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 450, 736–740.

Dann, S. G., Selvaraj, A., and Thomas, G. (2007). mTOR Complex1-S6K1 signaling: at the crossroads of obesity, diabetes and cancer. *Trends Mol. Med.* 13, 252–259.

Desrois, M., Clarke, K., Lan, C., Dalmasso, C., Cole, M., Portha, B., Cozzone, P. J., and Bernard, M. (2010). Upregulation of eNOS and unchanged energy metabolism in increased susceptibility of the aging type 2 diabetic GK rat heart to ischemic injury. *Am. J. Physiol. Heart Circ. Physiol.* 299, H1679–H1686.

Drummond, G. R., Cai, H., Davis, M. E., Ramasamy, S., and Harrison, D. G. (2000). Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. *Circ. Res.* 86, 347–354.

Elloso, M. M., Azrolan, N., Sehgal, S. N., Hsu, P. L., Phiel, K. L., Kopec, C. A., Basso, M. D., and Adelman, S. J. (2003). Protective effect of the immunosuppressant sirolimus against aortic atherosclerosis in apo E-deficient mice. *Am. J. Transplant.* 3, 562–569.

Estep, P. W. III, Warner, J. B., and Bulyk, M. L. (2009). Short-term calorie restriction in male mice feminizes gene expression and alters key regulators of conserved aging regulatory pathways. *PLoS ONE* 4, e5242. doi:10.1371/journal.pone.0005242

Evans, D. S., Kapahi, P., Hsueh, W. C., and Kockel, L. (2011). TOR signaling never gets old: aging, longevity and TORC1 activity. *Ageing Res. Rev.* 10, 225–237.

Fontana, L., Meyer, T. E., Klein, S., and Holloszy, J. O. (2004). Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6659–6663.

Fontana, L., Partridge, L., and Longo, V. D. (2010). Extending healthy life span – from yeast to humans. *Science* 328, 321–326.

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

Foster, K. G., and Finger, D. C. (2010). Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. *J. Biol. Chem.* 285, 14071–14077.

Frost, R. A., and Lang, C. H. (2011). mTor signaling in skeletal muscle during sepsis and inflammation: where does it all go wrong? *Physiology (Bethesda)* 26, 83–96.

Glazer, H. P., Osipov, R. M., Clements, R. T., Sellke, F. W., and Bianchi, C. (2009). Hypercholesterolemia is associated with hyperactive cardiac mTORC1 and mTORC2 signaling. *Cell Cycle* 8, 1738–1746.

Gorgoulis, V. G., Zacharatos, P., Kotsinas, A., Kletsas, D., Mariatos, G., Zoumpourlis, V., Ryan, K. M., Kittas, C., and Papavassiliou, A. G. (2003). p53 activates ICAM-1 (CD54) expression in an NF-kappaB-independent manner. *EMBO J.* 22, 1567–1578.

Halcox, J. P., Schenke, W. H., Zalos, G., Mincemoyer, R., Prasad, A., Waclawiw, M. A., Nour, K. R., and Quyyumi, A. A. (2002). Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 106, 653–658.

Hammer, S., Snel, M., Lamb, H. J., Jazet, I. M., van der Meer, R. W., Pijl, H., Meinders, E. A., Romijn, J. A., de, R. A., and Smit, J. W. (2008). Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J. Am. Coll. Cardiol.* 52, 1006–1012.

Hands, S. L., Proud, C. G., and Wyttenbach, A. (2009). mTOR's role in ageing: protein synthesis or autophagy? *Aging (Albany NY)* 1, 586–597.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., Carter, C. S., Pahor, M., Javors, M. A., Fernandez, E., and Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395.

Hatakeyama, K., Asada, Y., Marutsuka, K., Sato, Y., Kamikubo, Y., and Sumiyoshi, A. (1997). Localization and activity of tissue factor in human aortic atherosclerotic lesions. *Atherosclerosis* 133, 213–219.

Herranz, D., and Serrano, M. (2010). SIRT1: recent lessons from mouse models. *Nat. Rev. Cancer* 10, 819–823.

Herrera, M. D., Mingorance, C., Rodriguez-Rodriguez, R., and Alvarez de, S. M. (2010). Endothelial dysfunction and aging: an update. *Ageing Res. Rev.* 9, 142–152.

Hill, J. M., Zalos, G., Halcox, J. P., Schenke, W. H., Waclawiw, M. A., Quyyumi, A. A., and Finkel, T. (2003). Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348, 593–600.

Hink, U., Li, H., Mollnau, H., Oelze, M., Matheis, E., Hartmann, M., Skatchkov, M., Thaiss, F., Stahl, R. A., Warnholtz, A., Meinertz, T., Griendling, K., Harrison, D. G., Forstermann, U., and Munzel, T. (2001). Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ. Res.* 88, E14–E22.

Hoffmann, J., Haendeler, J., Aicher, A., Rossig, L., Vasa, M., Zeiher, A. M., and Dimmeler, S. (2001). Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. *Circ. Res.* 89, 709–715.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Even, P. C., Cervera, P., and Le, B. Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421, 182–187.

Huang, P. H., Chen, J. W., Lu, T. M., Yu-An, D. P., and Lin, S. J. (2007). Combined use of endothelial function assessed by brachial ultrasound and high-sensitive C-reactive protein in predicting cardiovascular events. *Clin. Cardiol.* 30, 135–140.

Huang, P. H., Chen, Y. H., Tsai, H. Y., Chen, J. S., Wu, T. C., Lin, F. Y., Sata, M., Chen, J. W., and Lin, S. J. (2010). Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler. Thromb. Vasc. Biol.* 30, 869–877.

Hudes, G., Carducci, M., Tomczak, P., Dutcher, J., Figlin, R., Kapoor, A., Staroslawska, E., Sosman, J., McDermott, D., Bodrogi, I., Kovacevic, Z., Lesovoy, V., Schmidt-Wolf, I. G., Barbarash, O., Gokmen, E., O'Toole, T., Lustgarten, S., Moore, L., and Motzer, R. J. (2007). Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N. Engl. J. Med.* 356, 2271–2281.

Inoue, T., Croce, K., Morooka, T., Sakuma, M., Node, K., and Simon, D. I. (2011). Vascular inflammation and repair: implications for re-endothelialization, restenosis, and stent thrombosis. *JACC Cardiovasc. Interv.* 4, 1057–1066.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., and Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275, 218–220.

Kaeberlein, M., and Kennedy, B. K. (2009). Ageing: a midlife longevity drug? *Nature* 460, 331–332.

Kagawa, Y. (1978). Impact of Westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. *Prev. Med.* 7, 205–217.

Kapahi, P., and Zid, B. (2004). TOR pathway: linking nutrient sensing to life span. *Sci. Aging Knowledge Environ.* 2004, E34.

Kauffman, H. M., Cherikh, W. S., Cheng, Y., Hanto, D. W., and Kahan, B. D. (2005). Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation* 80, 883–889.

Kim, J. A., Jang, H. J., Martinez-Lemus, L. A., and Sowers, J. R. (2012). Activation of mTOR/p70S6 kinase by ANG II inhibits insulin stimulated endothelial nitric oxide synthase and vasodilation. *Am. J. Physiol. Endocrinol. Metab.* 302, E201–E208.

Kitada, M., Kume, S., Imaizumi, N., and Koya, D. (2011). Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway. *Diabetes* 60, 634–643.

Kurdi, M., and Booz, G. W. (2011). Three 4-letter words of hypertension-related cardiac hypertrophy: TRPC, mTOR, and HDAC. *J. Mol. Cell. Cardiol.* 50, 964–971.

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., and Auwerx, J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127, 1109–1122.

Lakatta, E. G. (2001). Heart aging: a fly in the ointment? *Circ. Res.* 88, 984–986.

Landmesser, U., Dikalov, S., Price, S. R., McCann, L., Fukai, T., Holland, S. M., Mitch, W. E., and Harrison, D. G. (2003). Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest.* 111, 1201–1209.

Law, B. K. (2005). Rapamycin: an anti-cancer immunosuppressant? *Crit. Rev. Oncol. Hematol.* 56, 47–60.

Lee, J. H., Budanov, A. V., Park, E. J., Birse, R., Kim, T. E., Perkins, G. A., Ocorr, K., Ellisman, M. H., Bodmer, R., Bier, E., and Karin, M. (2010). Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. *Science* 327, 1223–1228.

Li, H. L., Yin, R., Chen, D., Liu, D., Wang, D., Yang, Q., and Dong, Y. G. (2007). Long-term activation of adenosine monophosphate-activated protein kinase attenuates pressure-overload-induced cardiac hypertrophy. *J. Cell. Biochem.* 100, 1086–1099.

Linford, N. J., Beyer, R. P., Gollahon, K., Krajcik, R. A., Malloy, V. L., Demas, V., Burmer, G. C., and Rabinovitch, P. S. (2007). Transcriptional response to aging and caloric restriction in heart and adipose tissue. *Aging Cell* 6, 673–688.

Luscher, T. F., Steffel, J., Eberli, F. R., Joner, M., Nakazawa, G., Tanner, F. C., and Virmani, R. (2007). Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. *Circulation* 115, 1051–1058.

Lutz, W., Sanderson, W., and Scherbov, S. (2008). The coming acceleration of global population ageing. *Nature* 451, 716–719.

McMullen, J. R., Sherwood, M. C., Tarnavski, O., Zhang, L., Dorfman, A. L., Shioi, T., and Izumo, S. (2004). Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* 109, 3050–3055.

Meyer, T. E., Kovacs, S. J., Ehsani, A. A., Klein, S., Holloszy, J. O., and Fontana, L. (2006). Long-term caloric restriction ameliorates the decline in diastolic function in humans. *J. Am. Coll. Cardiol.* 47, 398–402.

Miller, R. A., Harrison, D. E., Astle, C. M., Baur, J. A., Boyd, A. R., de, C. R., Fernandez, E., Flurkey, K., Javors, M. A., Nelson, J. F., Orihuela, C. J., Pletcher, S., Sharp, Z. D., Sinclair, D., Starnes, J. W., Wilkinson, J. E., Nadon, N. L., and Strong, R. (2011). Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 191–201.

Milne, J. C., Lambert, P. D., Schenk, S., Carney, D. P., Smith, J. J., Gagne, D. J., Jin, L., Boss, O., Perni, R. B., Vu, C. B., Bemis, J. E., Xie, R., Disch, J. S., Ng, P. Y., Nunes, J. J., Lynch, A. V., Yang, H., Galonek, H., Israelian, K., Choy, W., Iffland, A., Lavu, S., Medvedik, O., Sinclair, D. A., Olefsky, J. M., Jirousek, M. R., Elliott, P. J., and Westphal, C. H. (2007). Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 450, 712–716.

Ming, X. F., Barandier, C., Viswambharan, H., Kwak, B. R., Mach, F., Mazzolai, L., Hayoz, D., Ruffieux, J., Rusconi, S., Montani, J. P., and Yang, Z. (2004). Thrombin stimulates human endothelial arginase enzymatic activity via RhoA/ROCK pathway: implications for atherosclerotic endothelial dysfunction. *Circulation* 110, 3708–3714.

Ming, X. F., Rajapakse, A. G., Carvas, J. M., Ruffieux, J., and Yang, Z. (2009). Inhibition of S6K1 accounts partially for the anti-inflammatory effects of the arginase inhibitor L-norvaline. *BMC Cardiovasc. Disord.* 9, 12. doi:10.1186/1471-2261-9-12

Ming, X. F., Rajapakse, A. G., Carvas, J. M., Ruffieux, J., and Yang, Z. (2010). Opposing and uncoupling effects of mTOR and S6K1 in the regulation of endothelial tissue factor expression. *FEBS Lett.* 584, 135–140.

Miyauchi, H., Minamino, T., Tateno, K., Kunieda, T., Toko, H., and Komuro, I. (2004). Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway. *EMBO J.* 23, 212–220.

Morrisett, J. D., Abdel-Fattah, G., Hoogeveen, R., Mitchell, E., Ballantyne, C. M., Pownall, H. J., Opekun, A. R., Jaffe, J. S., Oppermann, S., and Kahan, B. D. (2002). Effects of sirolimus on plasma lipids, lipoprotein levels, and fatty acid metabolism in renal transplant patients. *J. Lipid Res.* 43, 1170–1180.

Moschella, P. C., Rao, V. U., McDermott, P. J., and Kuppuswamy, D. (2007). Regulation of mTOR and S6K1 activation by the nPKC isoforms, PKCepsilon and PKCdelta, in adult cardiac muscle cells. *J. Mol. Cell. Cardiol.* 43, 754–766.

Muniyappa, R., Montagnani, M., Koh, K. K., and Quon, M. J. (2007). Cardiovascular actions of insulin. *Endocr. Rev.* 28, 463–491.

Najjar, S. S., Scuteri, A., and Lakatta, E. G. (2005). Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 46, 454–462.

Ota, H., Eto, M., Ako, J., Ogawa, S., Iijima, K., Akishita, M., and Ouchi, Y. (2009). Sirolimus and everolimus induce endothelial cellular senescence via sirtuin 1 down-regulation: therapeutic implication of cilostazol after drug-eluting stent implantation. *J. Am. Coll. Cardiol.* 53, 2298–2305.

Ozaki, M., Kawashima, S., Yamashita, T., Hirase, T., Namiki, M., Inoue, N., Hirata, K., Yasui, H., Sakurai, H., Yoshida, Y., Masada, M., and Yokoyama, M. (2002). Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J. Clin. Invest.* 110, 331–340.

Pakala, R., Stabile, E., Jang, G. J., Clavijo, L., and Waksman, R. (2005). Rapamycin attenuates atherosclerotic plaque progression in apolipoprotein E knockout mice: inhibitory effect on monocyte chemotaxis. *J. Cardiovasc. Pharmacol.* 46, 481–486.

Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J., and Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 6, 111–119.

Park, J. S., Kim, K. M., Kim, M. H., Chang, H. J., Baek, M. K., Kim, S. M., and Jung, Y. D. (2009). Resveratrol inhibits tumor cell adhesion to endothelial cells by blocking ICAM-1 expression. *Anticancer Res.* 29, 355–362.

Pearson, K. J., Baur, J. A., Lewis, K. N., Peshkin, L., Price, N. L., Labinskyy, N., Swindell, W. R., Kamara, D., Minor, R. K., Perez, E., Jamieson, H. A., Zhang, Y., Dunn, S. R., Sharma, K., Pleshko, N., Woollett, L. A., Csiszar, A., Ikeno, Y., Le, C. D., Elliott, P. J., Becker, K. G., Navas, P., Ingram, D. K., Wolf, N. S., Ungvari, Z., Sinclair, D. A., and de, C. R. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* 8, 157–168.

Pirola, L., and Frojdo, S. (2008). Resveratrol: one molecule, many targets. *IUBMB Life* 60, 323–332.

Powers, R. W. III, Kaeberlein, M., Caldwell, S. D., Kennedy, B. K., and Fields, S. (2006). Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 20, 174–184.

Rajapakse, A. G., Ming, X. F., Carvas, J. M., and Yang, Z. (2009). The hexosamine biosynthesis inhibitor azaserine prevents endothelial inflammation and dysfunction under hyperglycemic condition through antioxidant effects. *Am. J. Physiol. Heart Circ. Physiol.* 296, H815–H822.

Rajapakse, A. G., Yepuri, G., Carvas, J. M., Stein, S., Matter, C. M., Scerri, I., Ruffieux, J., Montani, J. P., Ming, X. F., and 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

Rau, R., Soroko, E., Jasilionis, D., and Vaupel, J. W. (2008). Continued reductions in mortality at advanced ages. *Popul. Dev. Rev.* 34, 747–768.

Rauscher, F. M., Goldschmidt-Clermont, P. J., Davis, B. H., Wang, T., Gregg, D., Ramaswami, P., Pippen, A. M., Annex, B. H., Dong, C., and Taylor, D. A. (2003). Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 108, 457–463.

Reaven, G. M. (2011). Insulin resistance: the link between obesity and cardiovascular disease. *Med. Clin. North Am.* 95, 875–892.

Ribes, D., Kamar, N., Esposito, L., and Rostaing, L. (2005). Combined use of tacrolimus and sirolimus in de novo renal transplant patients: current data. *Transplant. Proc.* 37, 2813–2816.

Rice, K. M., Kinnard, R. S., Harris, R., Wright, G. L., and Blough, E. R. (2005). Effects of aging on pressure-induced MAPK activation in the rat aorta. *Pflugers Arch.* 450, 192–199.

Ruderman, N. B., Xu, X. J., Nelson, L., Cacicedo, J. M., Saha, A. K., Lan, F., and Ido, Y. (2010). AMPK and SIRT1: a long-standing partnership? *Am. J. Physiol. Endocrinol. Metab.* 298, E751–E760.

Sadoshima, J., and Izumo, S. (1995). Rapamycin selectively inhibits angiotensin II-induced increase in protein synthesis in cardiac myocytes in vitro. Potential role of 70-kD S6 kinase in angiotensin II-induced cardiac hypertrophy. *Circ. Res.* 77, 1040–1052.

Sakuma, K., and Yamaguchi, A. (2010). Molecular mechanisms in aging and current strategies to counteract sarcopenia. *Curr. Aging Sci.* 3, 90–101.

Sarbassov, D. D., Ali, S. M., Sengupta, S., Sheen, J. H., Hsu, P. P., Bagley, A. F., Markhard, A. L., and Sabatini, D. M. (2006). Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell* 22, 159–168.

Sarbassov, D. D., Guertin, D. A., Ali, S. M., and Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307, 1098–1101.

Schachinger, V., Britten, M. B., and Zeiher, A. M. (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 101, 1899–1906.

Selman, C., Lingard, S., Choudhury, A. I., Batterham, R. L., Claret, M., Clements, M., Ramadani, F., Okkenhaug, K., Schuster, E., Blanc, E., Piper, M. D., Al-Qassab, H., Speakman, J. R., Carmignac, D., Robinson, I. C., Thornton, J. M., Gems, D., Partridge, L., and Withers, D. J. (2008). Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J.* 22, 807–818.

Selman, C., Tullet, J. M., Wieser, D., Irvine, E., Lingard, S. J., Choudhury, A. I., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., Woods, A., Robinson, I. C., Schuster, E., Batterham, R. L., Kozma, S. C, Thomas, G., Carling, D., Okkenhaug, K., Thornton, J. M., Partridge, L., Gems, D., and Withers, D. J. (2009). Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* 326, 140–144.

Sengupta, S., Peterson, T. R., and Sabatini, D. M. (2010). Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol. Cell* 40, 310–322.

Sesso, H. D., Buring, J. E., Christen, W. G., Kurth, T., Belanger, C., MacFadyen, J., Bubes, V., Manson, J. E., Glynn, R. J., and Gaziano, J. M. (2008). Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 300, 2123–2133.

Shackelford, D. B., and Shaw, R. J. (2009). The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat. Rev. Cancer* 9, 563–575.

Shih, H., Lee, B., Lee, R. J., and Boyle, A. J. (2011). The aging heart and post-infarction left ventricular remodeling. *J. Am. Coll. Cardiol.* 57, 9–17.

Shinmura, K., Tamaki, K., Sano, M., Murata, M., Yamakawa, H., Ishida, H., and Fukuda, K. (2011). Impact of long-term caloric restriction on cardiac senescence: caloric restriction ameliorates cardiac diastolic dysfunction associated with aging. *J. Mol. Cell. Cardiol.* 50, 117–127.

Smoliga, J. M., Baur, J. A., and Hausenblas, H. A. (2011). Resveratrol and health – a comprehensive review of human clinical trials. *Mol. Nutr. Food Res.* 0.55, 1129–1141.

Soesanto, W., Lin, H. Y., Hu, E., Lefler, S., Litwin, S. E., Sena, S., Abel,

E. D., Symons, J. D., and Jalili, T. (2009). Mammalian target of rapamycin is a critical regulator of cardiac hypertrophy in spontaneously hypertensive rats. *Hypertension* 54, 1321–1327.

Stallone, G., Schena, A., Infante, B., Di, P. S., Loverre, A., Maggio, G., Ranieri, E., Gesualdo, L., Schena, F. P., and Grandaliano, G. (2005). Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N. Engl. J. Med.* 352, 1317–1323.

Stanfel, M. N., Shamieh, L. S., Kaeberlein, M., and Kennedy, B. K. (2009). The TOR pathway comes of age. *Biochim. Biophys. Acta* 1790, 1067–1074.

Stockklauser-Farber, K., Ballhausen, T., Laufer, A., and Rosen, P. (2000). Influence of diabetes on cardiac nitric oxide synthase expression and activity. *Biochim. Biophys. Acta* 1535, 10–20.

Sung, M. M., Koonen, D. P., Soltys, C. L., Jacobs, R. L., Febbraio, M., and Dyck, J. R. (2011). Increased CD36 expression in middle-aged mice contributes to obesity-related cardiac hypertrophy in the absence of cardiac dysfunction. *J. Mol. Med.* 89, 459–469.

Sydow, K., and Munzel, T. (2003). ADMA and oxidative stress. *Atheroscler. Suppl.* 4, 41–51.

Tanabe, T., Maeda, S., Miyauchi, T., Iemitsu, M., Takanashi, M., Irukayama-Tomobe, Y., Yokota, T., Ohmori, H., and Matsuda, M. (2003). Exercise training improves ageing-induced decrease in eNOS expression of the aorta. *Acta Physiol. Scand.* 178, 3–10.

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., Auwer, J., and 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.

Tu, V. C., Bahl, J. J., and Chen, Q. M. (2002). Signals of oxidant-induced cardiomyocyte hypertrophy: key activation of p70 S6 kinase-1 and phosphoinositide 3-kinase. *J. Pharmacol. Exp. Ther.* 300, 1101–1110.

Tutar, E., Ozcan, M., Kilickap, M., Gulec, S., Aras, O., Pamir, G., Oral, D., Dandelet, L., and Key, N. S. (2003). Elevated whole-blood tissue factor procoagulant activity as a marker of restenosis after percutaneous transluminal coronary angioplasty and stent implantation. *Circulation* 108, 1581–1584.

Um, S. H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P. R., Kozma, S. C, Auwerx, J., and Thomas, G. (2004). Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431, 200–205.

Ungvari, Z., Kaley, G., de, C. R, Sonntag, W. E., and Csiszar, A. (2010). Mechanisms of vascular aging: new perspectives. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1028–1041.

van der Loo, B., Labugger, R., Skepper, J. N., Bachschmid, M., Kilo, J., Powell, J. M., Palacios-Callender, M., Erusalimsky, J. D., Quaschning, T., Malinski, T., Gygi, D., Ullrich, V., and Luscher, T. F. (2000). Enhanced peroxynitrite formation is associated with vascular aging. *J. Exp. Med.* 192, 1731–1744.

Vanhoutte, P. M., Shimokawa, H., Tang, E. H., and Feletou, M. (2009). Endothelial dysfunction and vascular disease. *Acta Physiol. (Oxf.)* 196, 193–222.

Vasa, M., Fichtlscherer, S., Aicher, A., Adler, K., Urbich, C., Martin, H., Zeiher, A. M., and Dimmeler, S. (2001). Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res.* 89, E1–E7.

Vivekananthan, D. P., Penn, M. S., Sapp, S. K., Hsu, A., and Topol, E. J. (2003). Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 361, 2017–2023.

Waksman, R., Pakala, R., Burnett, M. S., Gulick, C. P., Leborgne, L., Fournadjiev, J., Wolfram, R., and Hellinga, D. (2003). Oral rapamycin inhibits growth of atherosclerotic plaque in apoE knock-out mice. *Cardiovasc. Radiat. Med.* 4, 34–38.

Wang, Y., Liang, Y., and Vanhoutte, P. M. (2011). SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model. *FEBS Lett.* 585, 986–994.

Wei, L. (2004). Lysophospholipid signaling in cardiac myocyte hypertrophy. *J. Mol. Cell. Cardiol.* 36, 465–468.

Werner, N., Kosiol, S., Schiegl, T., Ahlers, P., Walenta, K., Link, A., Bohm, M., and Nickenig, G. (2005). Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* 353, 999–1007.

Willcox, B. J., Willcox, D. C., Todoriki, H., Fujiyoshi, A., Yano, K., He, Q., Curb, J. D., and Suzuki, M. (2007). Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span. *Ann. N. Y. Acad. Sci.* 1114, 434–455.

Yang, Z., and Ming, X. F. (2006). Recent advances in understanding endothelial dysfunction in atherosclerosis. *Clin. Med. Res.* 4, 53–65.

Zang, M., Xu, S., Maitland-Toolan, K. A., Zuccollo, A., Hou, X., Jiang, B., Wierzbicki, M., Verbeuren, T. J., and Cohen, R. A. (2006). Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes* 55, 2180–2191.

Zhang, J. (2006). Resveratrol inhibits insulin responses in a SirT1-independent pathway. *Biochem. J.* 397, 519–527.

Zhang, P., Hu, X., Xu, X., Fassett, J., Zhu, G., Viollet, B., Xu, W., Wiczer, B., Bernlohr, D. A., Bache, R. J., and Chen, Y. (2008). AMP activated protein kinase-alpha2 deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction in mice. *Hypertension* 52, 918–924.

Zhou, X., Perez, F., Han, K., and Jurivich, D. A. (2006). Clonal senescence alters endothelial ICAM-1 function. *Mech. Ageing Dev.* 127, 779–785.

Zmonarski, S. C., Boratynska, M., Rabczynski, J., Kazimierczak, K., and Klinger, M. (2005). Regression of Kaposi's sarcoma in renal graft recipients after conversion to sirolimus treatment. *Transplant. Proc.* 37, 964–966.

Zoncu, R., Efeyan, A., and Sabatini, D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell Biol.* 12, 21–35.

Zu, Y., Liu, L., Lee, M. Y., Xu, C., Liang, Y., Man, R. Y., Vanhoutte, P. M., and Wang, Y. (2010). SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. *Circ. Res.* 106, 1384–1393.

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mTOR inhibition: a promise for a young heart

Stefany B. A. Cau and Rita C. Tostes*

Department of Pharmacology, Medical School of Ribeirao Preto, Ribeirao Preto, São Paulo, Brazil
**Correspondence: rtostes@usp.br*

A Commentary on

Perspectives of targeting mTORC1–S6K1 in cardiovascular aging
by Ming, X.-F., Montani, J.-P., and Yang, Z. (2012). Front. Physiol. 3:5. doi: 10.3389/fphys.2012.00005

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that senses nutritional and cellular energy status and regulates cell growth, proliferation, and survival. Rapamycin (sirolimus), a naturally occurring antifungal macrolide isolated from the bacterium *Streptomyces hygroscopicus* in a soil sample from Easter Island of the Pacific Ocean (or Rapa Nui in the native language; Vezina et al., 1975), inhibits mTOR interaction with other molecular components (Brown et al., 1994). Rapamycin was shown to extend life span in mice, even when administered late in life (Harrison et al., 2009), suggesting that inhibition of the mTOR pathway may prolong human life span.

In this review, Ming et al. (2012) address the role of mTOR complex 1 and its downstream effector S6K1 (mTORC1–S6K1) signaling pathway in aging and age-associated diseases. The authors discuss the important new concept that augmented mTORC1–S6K1 signaling is not only critical in aging-related processes, but also provides a link between aging and cardiovascular disturbances, such as vascular and cardiac remodeling seen, e.g., in diabetes, arterial hypertension, atherosclerosis, and heart failure.

It has been hypothesized that some dietary regimes, like caloric restriction and methionine restriction, extends lifespan by decreasing mTOR activity (Kaeberlein et al., 2005). Accordingly, a comparison between the beneficial effects of pharmacological intervention with rapamycin and life style modification (caloric restriction) is also provided by the authors. One should keep in mind that the effects produced by caloric restriction and inhibition mTOR signaling are not straightforward correlated. Unlike caloric restriction, rapamycin treatment does not reduce animal size (Harrison et al., 2009) and caloric restriction fails to extend life span when initiated late in life (Masoro, 2005).

Mammalian target of rapamycin–S6K1 signaling and its specific inhibition emerge as a promising "treatment for aging," mainly through the prevention or reversion of cardiovascular aging. However, only intense research will clarify whether potential adverse side effects of mTOR inhibitors, such as suppression of the immune system (Weir et al., 2010), impairment of glucose tolerance (Houde et al., 2010), can be overcome by the beneficial effects in the treatment of age-related diseases (especially if they are to be used as a prophylactic treatment). Future research targeting mTOR downstream proteins, which would exhibit more specific actions, will also clarify the relevance of mTOR inhibition for the prophylactic treatment of aging or age-related diseases.

REFERENCES

Brown, E. J., Albers, M. W., Shin, T. B., Ichikawa, K., Keith, C. T., Lane, W. S., and Schreiber, S. L. (1994). A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 369, 756–758.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., Carter, C. S., Pahor, M., Javors, M. A., Fernandez, E., and Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395.

Houde, V. P., Brule, S., Festuccia, W. T., Blanchard, P. G., Bellmann, K., Deshaies, Y., and Marette, A. (2010). Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. *Diabetes* 59, 1338–1348.

Kaeberlein, M., Powers, R. W. III, Steffen, K. K., Westman, E. A., Hu, D., Dang, N., Kerr, E. O., Kirkland, K. T., Fields, S., and Kennedy, B. K. (2005). Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* 310, 1193–1196.

Masoro, E. J. (2005). Overview of caloric restriction and ageing. *Mech. Ageing Dev.* 126, 913–922.

Ming, X.-F., Montani, J.-P., and Yang, Z. (2012). Perspectives of targeting mTORC1-S6K1 in cardiovascular aging. *Front. Physiol.* 3:5. doi: 10.3389/fphys.2012.00005

Vezina, C., Kudelski, A., and Sehgal, S. N. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot.* 28, 721–726.

Weir, M. R., Diekmann, F., Flechner, S. M., Lebranchu, Y., Mandelbrot, D. A., Oberbauer, R., and Kahan, B. D. (2010). mTOR inhibition: the learning curve in kidney transplantation. *Transpl. Int.* 23, 447–460.

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Cross-sectional and longitudinal assessment of arterial stiffening with age in European and Chinese populations

Wiktoria Wojciechowska[1], Yan Li[2], Katarzyna Stolarz-Skrzypek[1], Kalina Kawecka-Jaszcz[1], Jan A. Staessen[3,4]*, Ji-Guang Wang[2] and on behalf of the European Project on Genes in Hypertension and the JingNing Study Investigators

[1] *The First Department of Cardiology and Hypertension, Jagiellonian University Medical College, Kraków, Poland*
[2] *Center for Epidemiologic Studies and Clinical Trials and Center for Vascular Evaluations, Shanghai Key Laboratory of Vascular Biology, Shanghai Institute of Hypertension, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China*
[3] *Division of Hypertension and Cardiovascular Rehabilitation, Department of Cardiovascular Diseases, Studies Coordinating Centre, University of Leuven, Leuven, Belgium*
[4] *Department of Epidemiology, Maastricht University, Maastricht, Netherlands*

Edited by:
Steffen-Sebastian Bolz, University of Toronto, Canada

Reviewed by:
Alun Hughes, Imperial College London, UK
Giovanni E. Mann, King's College London, UK

****Correspondence:***
Jan A. Staessen, Laboratory of Hypertension, Studies Coordinating Centre, Campus Sint Rafaël, Kapucijnenvoer 35, Block D, Box 7001, BE-3000 Leuven, Belgium.
e-mail: jan.staessen@med.kuleuven.be; jan.staessen@uzleuven.be; ja.staessen@epid.unimaas.nl

As arteries become stiffer with aging, reflected waves move faster and augment late systolic pressure. Few studies have described the age-related changes in both peripheral and central systolic blood pressures in populations. We investigated the age dependency of peripheral (pSBP) and central (cSBP) systolic pressure and pressure amplification (i.e., difference between peripheral and central SBP) in randomly selected participants from European and Chinese populations. Data were collected in 1420 Europeans (mean age, 41.7 years) and 2044 (mean age, 45.1 years) Chinese. In cross-sectional analyses of the population samples cSBP consistently increased more with age than pSBP with the age-related increases being greater in women than men. Repeat assessment of pSBP and cSBP in 398 Europeans and 699 Chinese at a median interval approximately 4 years of follow-up confirmed that also within subjects cSBP rose steeper with aging than pSBP. In conclusion, with aging, pSBP approximates to cSBP. This might explain why in older subjects pSBP becomes the main predictor of cardiovascular complications.

Keywords: aging, central blood pressure, peripheral blood pressure, cardiovascular disease, risk factors, epidemiology

INTRODUCTION

Systolic blood pressure substantially rises with aging across the full human life span. However, because arteries stiffen with higher age and because of differences in wave travel distance and wave reflections, the age-related increase in systolic blood pressure does not occur uniformly across the whole arterial tree (O'Rourke and Kelly, 1993). Indeed, during systole, the heart generates a forward running pressure wave, which is reflected at various sites in the peripheral arterial system (O'Rourke and Kelly, 1993). As the arteries become stiffer with advancing age, the reflected waves may cause an augmentation of late systolic pressure. In addition geometric changes of the aorta with aging may also contribute to age-related increase in systolic blood pressure (Redheuil et al., 2011). Additionally aortic reservoir function and other elastic changes markedly with aging, which accounts for the age-related changes in the aortic pressure waveform (Davies et al., 2010). Thus augmentation of systolic blood pressure in the central arteries increases with advancing age. Because of this phenomenon, there might be differences in the age dependency of peripheral systolic blood pressure, as measured at the brachial artery, and central systolic blood pressure, as estimated non-invasively by pulse wave analysis.

Systolic augmentation is now a generally accepted physiological concept. However, the initial evidence supporting the principle of the age dependency of the amplification of the central blood pressure came from animal experiments (O'Rourke, 1994) and invasive studies in selected subjects (Murgo et al., 1980; O'Rourke, 1994). Among the published population studies on the age dependency of arterial stiffness (Avolio et al., 1983, 1985; Wilkinson et al., 2001; Mitchell et al., 2004; McEniery et al., 2005; Li et al., 2008), most focused on pulse wave velocity (Avolio et al., 1983, 1985; Mitchell et al., 2004; McEniery et al., 2005), the ratio of pulse pressure in peripheral vs. central arteries (Wilkinson et al., 2001), or the augmentation index (McEniery et al., 2005; Li et al., 2008) in selected healthy subjects (Wilkinson et al., 2001; Mitchell et al., 2004; McEniery et al., 2005; Li et al., 2008) and patients at increased cardiovascular risk (McEniery et al., 2008). The predictive value of central pressure (both measured invasively and non-invasively) was proved in several studies (Roman et al., 2007; Jankowski et al., 2008). We previously reported reference values for the central and peripheral pulse pressures and augmentation indexes by age in healthy European (Wojciechowska et al., 2006) and Chinese (Li et al., 2008) reference populations. In the present review, we focused on the changes with age in central and peripheral systolic blood pressures, based on data collected from randomly recruited European (Wojciechowska et al., 2012) and Chinese (Li et al., 2012) subjects. These age-related changes were assessed cross-sectionally and in a subsample also longitudinally. Only participants without

antihypertensive drug treatment were included in all analyses to avoid confounding by blood pressure lowering medications.

CROSS-SECTIONAL STUDIES

Peripheral and central blood pressures were assessed among 1420 participants [731 women (51.5%) and 278 (19.6%) hypertensive patients] in Europe and 2044 [1066 women (52.2%) and 556 (27.2%) hypertensive patients] in China (Wojciechowska et al., 2012; Li et al., 2012).

In cross-sectional analyses of both populations, the peripheral and central systolic blood pressures increased with age (p for trend ≤ 0.01; **Figure 1** for Europe; Wojciechowska et al., 2012). In single regression analysis, the cross-sectionally assessed age-related increase in central systolic blood pressure was larger than that in peripheral systolic pressure both in women and in men. Additionally, in a subanalysis of the Chinese sample, systolic blood increased more with age in the central than peripheral arteries in women below age 50 (1.21 vs. 1.01 mm Hg per year; $p < 0.001$) and in men below age 60 (0.73 vs. 0.48 mm Hg per year; $p < 0.001$), whereas in older women (0.64 vs. 0.58 mmHg per year; $p = 0.27$) and older men (0.45 vs. 0.44 mm Hg per year; $p = 0.79$), the slopes of central and peripheral systolic blood pressures on age were similar (Li et al., 2012).

Our cross-sectional observations are in agreement with known physiologic concepts and also in line with several previously published cross-sectional population studies (Wilkinson et al., 2001; McEniery et al., 2005; Mitchell et al., 2010), including the Anglo-Cardiff Collaborative Trial (ACCT; McEniery et al., 2005). McEniery et al. (2005) studied 4001 healthy, normotensive individuals, aged 18–90 years. In both women and men, central systolic pressure increased more with age than did peripheral systolic blood pressure ($p < 0.001$). As in our current cross-sectional analyses, the increase in central systolic pressure was more prominent in women than men ($p = 0.01$). These consistent results were obtained based on White European populations with a western life style, high prevalence of obesity, and relatively high cholesterol levels. We cannot simply extrapolate them to other ethnicities or populations with different lifestyles. However, our findings were similar in lean Asian people, who generally have lower serum cholesterol levels.

Stiffening of the large arteries underlies the age-related increase in systolic blood pressure (Staessen et al., 1990). The loss of arterial elasticity over a person's life time is partly due to cyclic stress on the arterial wall with each heart beat (O'Rourke and Hashimoto, 2007). Over time, this causes fracture of elastin fibers, so that stress is transferred to the more rigid collagenous components of the arterial wall. At a young age, the aorta and proximal arteries dilate by approximately 10% with each heart beat, whereas the more distal muscular arteries dilate by only 2–3% with each heart beat (Boutouyrie et al., 1992). Atherosclerosis and inflammation thicken the arterial wall and contribute to arterial stiffening over and beyond the mechanical stress. To differentiate natural degeneration of the arterial wall from aging from disease, Avolio et al. (1983) highlighted the interest of studies of arterial properties in population studies with low cholesterol and low prevalence of atherosclerosis, such as Chinese. Avolio et al. (1985) contrasted the Chinese living in areas with low and high prevalence of hypertension, Guangzhou (4.9%) and Beijing (15.6%), respectively. In Guangzhou subjects, pulse wave velocity was consistently lower in the aorta, arm, and leg, and increased to a lesser degree with age, compared with Beijing subjects (Avolio et al., 1985). The contemporary cholesterol levels were 4.34 mmol l^{-1} in Guangzhou subjects and 4.49 mmol l^{-1} in Beijing subjects. In our Chinese population, cholesterol levels were of similar magnitude (4.73 mmol l^{-1}), but the prevalence of hypertension was much higher (27.2%). Our current observations strengthen Avolio's hypothesis (Avolio et al., 1983, 1985) that slowly progressing degeneration of the arterial wall through cyclic stress is the main cause of the age-related increase in systolic blood pressure.

The slopes of peripheral and central systolic blood pressures on age were consistently steeper ($p < 0.001$) in women than in men in both studied populations (Li et al., 2012; Wojciechowska et al., 2012). In the young and middle-aged subjects, the age-related increase in systolic blood pressure ran a steeper course in women than men. At all ages, women have a higher heart rate than men (Smulyan et al., 2001). The smaller height of women may be a cardiovascular risk factor, because of the early return of reflected waves to the central aorta in systole. The shorter stature of women also implies reduced length of the arterial tree, a factor believed to be responsible for the faster heart rate, a shorter diastolic period, a shorter diastolic time constant, and at the same peripheral resistance, lower arterial compliance (Smulyan et al., 2001). In older women, menopause might contribute to the continuing rise in systolic blood pressure, although it is difficult to differentiate the effects of aging from those of estrogen deprivation (Casiglia et al., 2008).

LONGITUDINAL ASSESSMENT OF AGE-DEPENDENT CHANGES IN ARTERIAL TREE

In European study 208 women (24.4%) and 190 men (27.6%), underwent a repeat arterial examination at a median interval of 4.79 years (5th–95th percentile interval, 3.96–5.98 years (Wojciechowska et al., 2012). In the Chinese study 369 women (34.6%) and 330 men (33.7%) underwent a repeat assessment of peripheral and central systolic blood pressure at a median interval of 3.60 years (5th–95th percentile interval, 3.56–3.96 years; Li et al., 2012). In the longitudinal analyses, all changes from baseline to follow-up were significant ($p \leq 0.039$). In the Caucasian populations, the annual increases in the peripheral and central systolic blood pressures averaged 0.91 and 1.06 mmHg in women, and 1.24 and 1.47 mmHg in men. The p-values for the sex differences were 0.12 and 0.08, respectively (Wojciechowska et al., 2012). In Chinese population peripheral systolic pressure increased more ($p \leq 0.025$) than the central systolic pressure both in women (2.35 vs. 2.12 mm Hg) and in men (1.37 vs. 1.16 mm Hg). On a relative scale, the percentage increases in peripheral and central systolic blood pressure from baseline to follow-up were similar in women (2.14 vs. 2.16% per year; $p = 0.76$), as well as in men (1.33 vs. 1.34% per year; $p = 0.96$;). In sensitivity analyses stratified by quartiles of the age distribution, the increase in peripheral systolic blood pressure was larger than that in central systolic blood pressure ($p \leq 0.02$) above median age in women and above the 75th percentile of age in men, whereas in all other sex–age subgroups, the increases of peripheral and central systolic pressures were similar ($p \geq 0.08$; **Figure 2**; Li et al., 2012).

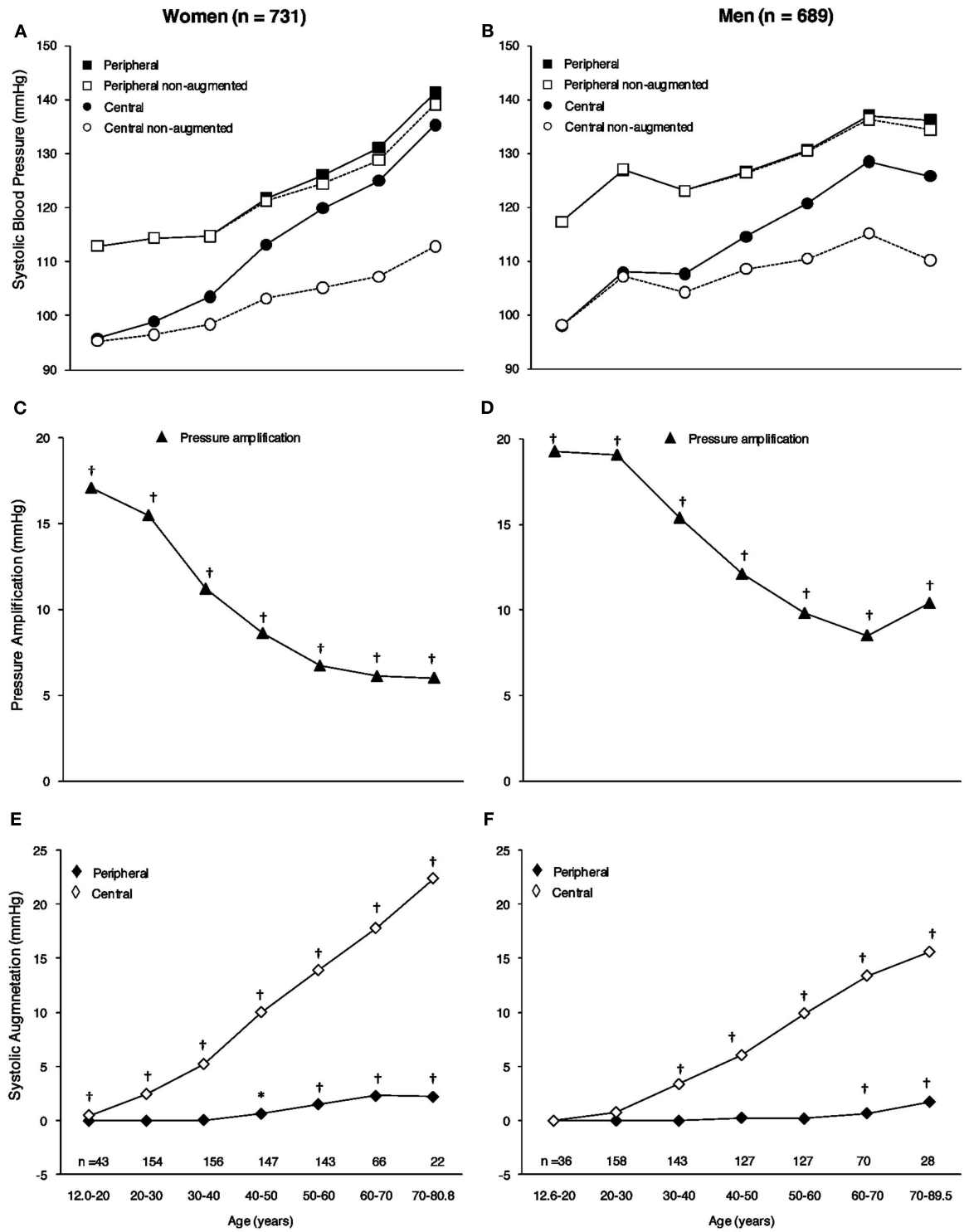

FIGURE 1 | European population. Association with age of peripheral and central systolic blood pressures **(A,B)**, peripheral, and central non-augmented systolic blood pressures **(A,B)**, pressure amplification **(C,D)**, and peripheral and central systolic augmentation **(E,F)** in women **(A,C,E)** and men **(B,D,F)**. Peripheral systolic blood pressure was the average of three blood pressure readings at the brachial artery. Central systolic blood pressure was the maximum pressure of the central waveform. Systolic augmentation was obtained by subtracting the first systolic peak from systolic blood pressure. Pressure amplification is peripheral minus central systolic blood pressure. Plotted values are means for each age group. Numbers indicate the subjects contributing to the group means. All *p*-values for trend with age were statistically significant ($p < 0.0001$). Significance of the difference with zero: $^{*}p < 0.05$ and $^{\dagger}p < 0.001$. Reproduced with permission from Blood Pressure Journal.

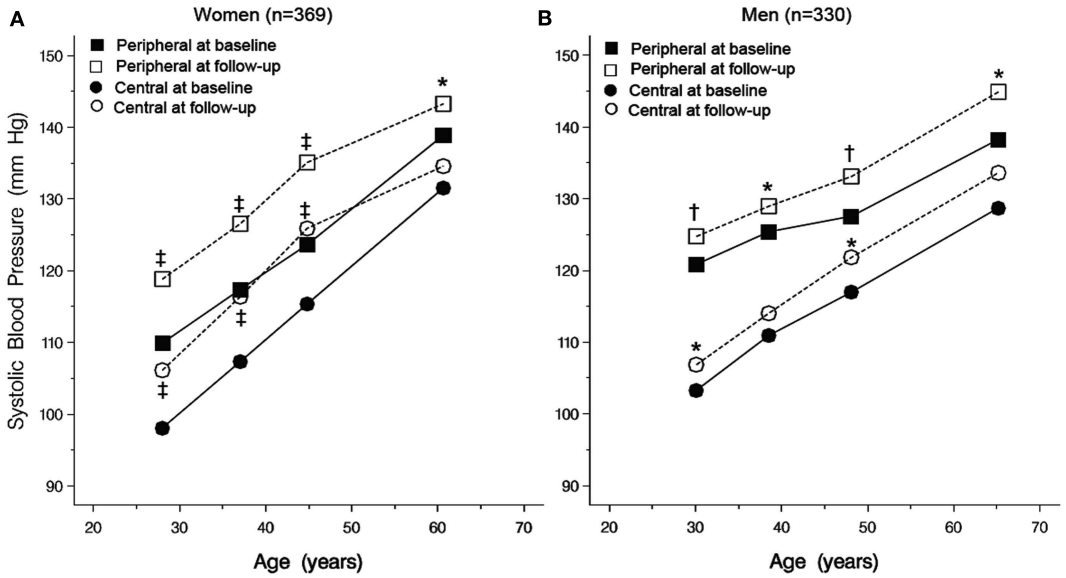

FIGURE 2 | Chinese population. Peripheral and central SBPs at baseline and follow-up by quartiles of the age distribution in 369 women **(A)** and 330 men **(B)**. All *p*-values for trend with age were statistically significant ($p < 0.0001$). Significance of the difference between baseline and follow-up: $^{*}p < 0.05$, $^{\ddagger}p < 0.01$, and $^{\dagger}p < 0.001$. Reproduced with permission from Hypertension Research Journal.

In an early Framingham report, Kannel and Gordan (1978) noticed that the age-related increase in systolic blood pressure was steeper on cross-sectional than longitudinal assessment in women, whereas the opposite was the case in men. The reasons for the difference in BP trends obtained cross-sectionally and longitudinally in the same Framingham cohort were not clear. One possible explanation is that subjects at the higher end of the distribution of systolic blood pressure are more likely to experience cardiovascular complications or die, and therefore to disappear from follow-up. The unmeasured attrition in our populations available for cross-sectional analysis might have contributed to the larger estimates of the age-related increase in systolic blood pressure in the longitudinal analyses.

CONCLUSION

The effects of aging on arterial function have often been underestimated, because of the sole reliance on the brachial cuff systolic pressure. With aging, peripheral systolic blood pressure approximates to the central systolic blood pressure. These findings support the point of view that age-related stiffness of arteries represents a vicious circle, in which increasing systolic blood pressure is, at the same time, the cause and the consequence of a self-sustaining process that leads to major cardiovascular complications. Breaking the vicious circle is the key to slowing the age-related rise in systolic blood pressure and preventing the associated cardiovascular complications.

REFERENCES

Avolio, A. P., Chen, S. G., Wang, R. P., Zhang, C. L., Li, M. F., and O'Rourke, M. F. (1983). Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 68, 50–58.

Avolio, A. P., Deng, F. A., Li, W. Q., Luo, Y. F., Huang, Z. D., Xing, L. F., and O'Rourke, M. F. (1985). Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 71, 202–210.

Boutouyrie, P. H., Laurent, S., Benetos, A., Girerd, X. J., Hoeks, A. P. G., and Safar, M. E. (1992). Opposing effects of ageing on distal and proximal large arteries in hypertensives. *J. Hypertens.* 10, S87–S91.

Casiglia, E., Tikhonoff, V., Caffi, S., Bascelli, A., Schiavon, L., Guidotti, F., Saugo, M., Giacomazzo, M., Martini, B., Mazza, A., D'Este, D., and Pessina, A. C. (2008). Menopause does not affect blood pressure and risk profile, and menopausal women do not become similar to men. *J. Hypertens.* 26, 1983–1992.

Davies, J. E., Baksi, J., Francis, D. P., Hadjiloizou, N., Whinnett, Z. I., Manisty, C. H., Aguado-Sierra, J., Foale, R. A., Malik, I. S., Tyberg, J. V., Parker, K. H., Mayet, J., and Hughes, A. D. (2010). The arterial reservoir pressure increases with aging and is the major determinant of the aortic augmentation index. *Am. J. Physiol. Heart Circ. Physiol.* 298, H580–H586.

Jankowski, P., Kawecka-Jaszcz, K., Czarnecka, D., Brzozowska-Kiszka, M., Styczkiewicz, K., Loster, M., Kloch-Badelek, M., Wiliński, J., Curylo, A. M., Dudek, D., and Aortic Blood Pressure and Survival Study Group. (2008). Pulsatile but not steady component of blood pressure predicts cardiovascular events in coronary patients. *Hypertension* 51, 848–855.

Kannel, W. B., and Gordan, T. (1978). Evaluation of cardiovascular risk in the elderly: the Framingham study. *Bull. N. Y. Acad. Med.* 54, 573–591.

Li, Y., Staessen, J. A., Li, L. H., Huang, Q. F., Lu, L., and Wang, J. G. (2008). Reference values for the arterial pulse wave in Chinese. *Am. J. Hypertens.* 21, 668–673.

Li, Y., Staessen, J. A., Sheng, C. S., Huang, Q. F., O'Rourke, M., and Wang, J. G. (2012). Age dependency of peripheral and central systolic blood pressures: cross-sectional and longitudinal observations in a Chinese population. *Hypertens. Res.* 35, 115–122.

McEniery, C. M., Yasmin, McDonnell, B., Munnery, M., Wallace, S. M., Rowe, C. V., Cockcroft, J. R., Wilkinson, I. B., and Anglo-Cardiff Collaborative Trial Investigators. (2008). Central pressure: variability and impact of cardiovascular risk factors: the Anglo-Cardiff Collaborative Trial II. *Hypertension* 51, 1476–1482.

McEniery, C. M., Yasmin Hall, I. R., Qasem, A., Wilkinson, I. B., and Cockcroft, J. R., and ACCT Investigators. (2005). Normal vascular

aging: differential effects on wave reflection and aortic pulse wave velocity. The Anglo-Cardiff Collaborative Trial (ACCT). *J. Am. Coll. Cardiol.* 46, 1753–1760.

Mitchell, G. F., Parise, H., Benjamin, E. J., Larson, M. G., Keyes, M. J., Vita, J. A., Vasan, R. S., and Levy, D. (2004). Changes in arterial stiffness and wave reflection with advancing age in healthy men and women. The Framingham Heart Study. *Hypertension* 43, 1239–1245.

Mitchell, G. F., Wang, N., Palmisano, J. N., Larson, M. G., Hamburg, N. M., Vita, J. A., Levy, D., Benjamin, E. J., and Vasan, R. S. (2010). Hemodynamic correlates of blood pressure across the adult age spectrum: noninvasive evaluation in the Framingham Heart Study. *Circulation* 122, 1379–1386.

Murgo, J. P., Westerhof, N., Giolma, J. P., and Altobelli, S. A. (1980). Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation* 62, 105–116.

O'Rourke, M. (1994). Arterial haemodynamics and ventricular-vascular interaction in hypertension. *Blood Press.* 3, 33–37.

O'Rourke, M. F., and Hashimoto, J. (2007). Mechanical factors in arterial aging. A clinical perspective. *J. Am. Coll. Cardiol.* 50, 1–13.

O'Rourke, M. F., and Kelly, P. J. (1993). Wave reflections in the systemic circulation and its implications in ventricular function. *J. Hypertens.* 11, 327–337.

Redheuil, A., Yu, W. C., Mousseaux, E., Harouni, A. A., Kachenoura, N., Wu, C. O., Bluemke, D., and Lima, J. A. (2011). Age-related changes in aortic arch geometry: relationship with proximal aortic function and left ventricular mass and remodeling. *J. Am. Coll. Cardiol.* 58, 1262–1270.

Roman, M. J., Devereux, R. B., Kizer, J. R., Lee, E. T., Galloway, J. M., Ali, T., Umans, J. G., and Howard, B. V. (2007). Central pressure more strongly relates to vascular disease and outcome than does brachial pressure. The Strong Heart study. *Hypertension* 50, 197–203.

Smulyan, H., Asmar, R. G., Rudnicki, A., London, G. M., and Safar, M. E. (2001). Comparative effects of aging in men and women on the properties of the arterial tree. *J. Am. Coll. Cardiol.* 37, 1374–1380.

Staessen, J., Amery, A., and Fagard, R. (1990). Editorial review. Isolated systolic hypertension in the elderly. *J. Hypertens.* 8, 393–405.

Wilkinson, I. B., Franklin, S. S., Hall, I. R., Tyrrell, S., and Cockcroft, J. R. (2001). Pressure amplification explains why pulse pressure is unrelated to risk in young subjects. *Hypertension* 38, 1461–1466.

Wojciechowska, W., Staessen, J. A., Nawrot, T., Cwynar, M., Seidlerová, J., Stolarz, K., Gasowski, J., Tichá, M., Richart, T., Thijs, L., Grodzicki, T., Kawecka-Jaszcz, K., Filipovský, J., and European Project on Genes in Hypertension (EPOGH) Investigators. (2006). Reference values in White Europeans for the arterial pulse wave recorded by means of the ShygmoCor device. *Hypertens. Res.* 29, 475–483.

Wojciechowska, W., Stolarz-Skrzypek, K., Tikhonoff, V., Richart, T., Seidlerová, J., Cwynar, M., Thijs, L., Li, Y., Kuznetsova, T., Filipovský, J., Casiglia, E., Grodzicki, T., Kawecka-Jaszcz, K., O'Rourke, M., Staessen, J. A., and European Project on Genes in Hypertension (Epogh) Investigators. (2012). Age dependency of central and peripheral systolic blood pressures: cross-sectional and longitudinal observations in European populations. *Blood Press.* 21, 58–68.

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Arterial–ventricular coupling with aging and disease

***Paul D. Chantler*[1]* and *Edward G. Lakatta*[2]**

[1] Division of Exercise Physiology, School of Medicine, West Virginia University, Morgantown, WV, USA
[2] Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Edited by:
Ana Paula Dantas, Institut d'Investigacions Biomediques August Pi i Sunyer, Spain

Reviewed by:
Roy Sutliff, Emory University, USA
Laurent Loufrani, CNRS, France

***Correspondence:**
Paul D. Chantler, Division of Exercise Physiology, School of Medicine, Robert C. Byrd Health Sciences Center, West Virginia University, P.O. Box 9227, Morgantown, WV 26506, USA.
e-mail: pchantler@hsc.wvu.edu

Age is the dominant risk factor for cardiovascular diseases. Understanding the coupling between the left ventricle (LV) and arterial system, termed arterial–ventricular coupling (E_A/E_{LV}), provides important mechanistic insights into the complex cardiovascular system and its changes with aging in the absence and presence of disease. E_A/E_{LV} can be indexed by the ratio of effective arterial elastance (E_A; a measure of the net arterial load exerted on the LV) to left ventricular end-systolic elastance (E_{LV}; a load-independent measure of left ventricular chamber performance). Age-associated alterations in arterial structure and function, including diameter, wall thickness, wall stiffness, and endothelial dysfunction, contribute to a gradual increase in resting E_A with age. Remarkably there is a corresponding increase in resting E_{LV} with age, due to alterations to LV remodeling (loss in myocyte number, increased collagen) and function. These age-adaptations at rest likely occur, at least, in response to the age-associated increase in E_A and ensure that E_A/E_{LV} is closely maintained within a narrow range, allowing for optimal energetic efficiency at the expense of mechanical efficacy. This optimal coupling at rest is also maintained when aging is accompanied by the presence of hypertension, and obesity, despite further increases in E_A and E_{LV} in these conditions. In contrast, in heart failure patients with either reduced or preserved ejection fraction, E_A/E_{LV} at rest is impaired. During dynamic exercise, E_A/E_{LV} decreases, due to an acute mismatch between the arterial and ventricular systems as E_{LV} increases disproportionate compared to E_A (≈200 vs. 40%), to ensure that sufficient cardiac performance is achieved to meet the increased energetic requirements of the body. However, with advancing age the reduction in E_A/E_{LV} during acute maximal exercise is blunted, due to a blunted increase E_{LV}. This impaired E_A/E_{LV} is further amplified in the presence of disease, and may explain, in part, the reduced cardiovascular functional capacity with age and disease. Thus, although increased stiffness of the arteries itself has important physiological and clinical relevance, such changes also have major implications on the heart, and vice versa, and the manner in the way they interact has important ramifications on cardiovascular function both at rest and during exercise. Examination of the alterations in arterial–ventricular coupling with aging and disease can yield mechanistic insights into the pathophysiology of these conditions and increase the effectiveness of current therapeutic interventions.

Keywords: left ventricular function, arterial system, exercise, aging, disease

INTRODUCTION

The population in the Western world is aging; by 2030, there will be 71 million individuals in the United States over 65 years of age, representing ≈20% of the U.S. population. Aging significantly increases cardiovascular morbidity even in the absence of other risk factors (e.g., hypertension, obesity, diabetes, hypercholesterolemia). Thus, the risk of death from heart disease is ≈60-fold greater in individuals in the eighth decade compared to individuals in the 4th decade of life. Not only does clinically overt cardiovascular disease increase dramatically with aging, but so do subclinical or occult diseases, such as silent coronary atherosclerosis. Therefore, the aging of the U.S. population is one of the major public health challenges that we face in the twenty-first century.

The cardiovascular system is modulated to provide sufficient pressure and flow to the tissues at rest and during exercise. Understanding the performance (pressure and flow output) of the left ventricle (LV) requires not only examining the properties of the LV itself (power and stroke capacity of the heart), but also investigating the modulating effects of the arterial system on left ventricular performance. These modulating effects of the vasculature include the capacitance and inertial properties of the aorta, along with the resistance capacity of the microcirculation. The interaction of the LV with the arterial system, termed arterial–ventricular coupling (E_A/E_{LV}), is a central determinant of cardiovascular performance and cardiac energetics. E_A/E_{LV} can be indexed by the ratio of effective arterial elastance (E_A) to left ventricular end-systolic elastance (E_{LV}) and is best displayed in the pressure–volume plane (**Figure 1**). This review will describe the concept of arterial ventricular coupling and how aging, in the absence and presence of cardiovascular disease, affects the coupling both at rest and during exercise, and

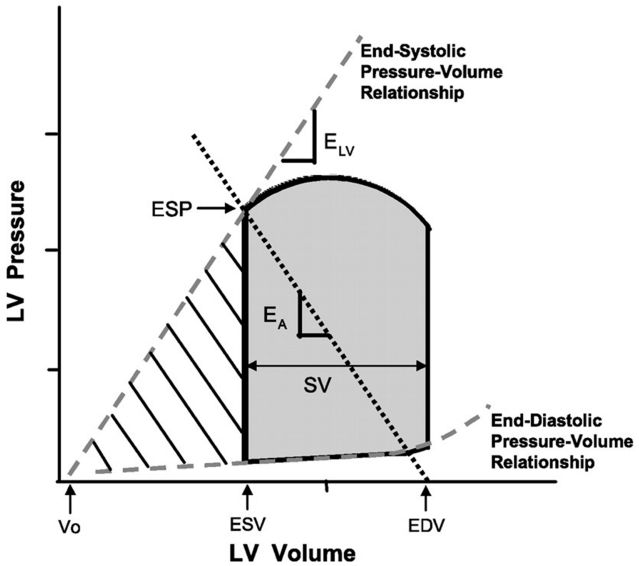

FIGURE 1 | Ventricular pressure–volume diagram from which effective arterial elastance (E_A) and left ventricular (LV) end-systolic elastance (E_{LV}) are derived. E_A represents the negative slope of the line joining the end-diastolic volume (EDV) and the end-systolic pressure (ESP) points. E_{LV} represents the slope of the ESP–volume relationship passing through the volume intercept ($V0$). The shaded area represents the cardiac stroke work, and the hatched area represents the potential energy. LV ESP is the LV pressure at the end of systole. EDV is the LV volume at the end of diastole. End-systolic volume (ESV) is the LV volume at the end of systole. Stroke volume (SV) is the volume of blood ejected by the LV with each beat and is obtained from subtracting ESV from EDV. From Chantler et al. (2008a) with permission.

its physiological consequences. Further, we will discuss potential therapeutic interventions to restore the coupling between the heart and arteries.

ARTERIAL–VENTRICULAR COUPLING

The gold standard to assess the arterial afterload that opposes left ventricular ejection independent of left ventricular function is through aortic input impedance derived from the Fourier analysis of aortic pressure and flow (Murgo et al., 1980). However, aortic input impedance is described in the frequency domain, whereas measures of left ventricular contraction are best described in the time domain; consequently making direct comparisons between arterial and left ventricular function difficult. The pioneering work from Sunagawa et al. (1983) conceived a measure of arterial load (E_A) that could be directly compared to a measure of left ventricular contraction (E_{LV}) in the same units (elastance; change in pressure for a given change in volume). Rather than specific arterial properties, E_A simplifies the arterial load into an integrative index that incorporates the principal elements of arterial load, including peripheral vascular resistance (which is determined, in large part, by the small arteries), total arterial compliance (which is determined, in large part, by the central elastic arteries), characteristic impedance, and systolic and diastolic time intervals (Sunagawa et al., 1983). Indeed E_A is directly related to heart rate and peripheral resistance, and is inversely related to compliance (Chemla et al., 2003). E_A can, therefore, be considered a measure of the net arterial load that is imposed on the LV. Further, E_A measured invasively as end-systolic pressure/stroke volume closely approximates the arterial load obtained from aortic input impedance and arterial compliance data based on a three-element Windkessel model (Kelly et al., 1992b). Invasively E_A is determined from pressure–volume loops as the negative slope of the line joining the end-diastolic volume and end-systolic pressure points (**Figure 1**).

The contractile function of the LV can also be expressed from the slope of the end-systolic pressure–volume relationship (E_{LV}; **Figure 1**), which can be obtained from a series of pressure–volume loops recorded while the preload of the heart is altered. E_{LV} reflects a relatively (within normal physiological limits) load-independent measure of left ventricular contraction (chamber stiffness at end systole). An increase in contractility is depicted by an increase in the slope and a shift in the end-systolic pressure–volume relationship to the left, which allows the ventricle to generate more pressure for a given left ventricular volume. However, in addition to the inotropic state, E_{LV} is also influenced by the geometric (structural remodeling) and biochemical properties (i.e., stiffness or compliance of myocytes, composition of muscle, fibrosis, collagen, etc., in the LV wall) that underlie end-systolic stiffness (Borlaug and Kass, 2008). Whereby a "stiffer" LV due to remodeling leads to a higher E_{LV}. Thus, caution should be exercised in interpreting the significance of an elevated E_{LV}, particularly when other measures of left ventricular systolic function are normal. E_{LV} should, therefore, be considered an integrated measure of left ventricular chamber performance that can be related to an integrated measure of arterial load (i.e., E_A). Importantly, E_A shares common units with E_{LV}, and their ratio E_A/E_{LV} is a measure of the interaction between the heart and the arterial system and provides information about how blood pressure and flow change due to different loading conditions. See Chantler et al. (2008a) for a more detailed review of the methods to measure E_A and E_{LV}.

At rest, in healthy individuals the properties of the heart and arteries are closely matched so that near maximal cardiac work, power, and chamber efficiency are achieved (Little and Cheng, 1991; De Tombe et al., 1993). Values obtained in isolated canine hearts show the efficiency and stroke work of the heart to be optimal over an E_A/E_{LV} ratio ranging 0.3–1.3 (**Figure 2**). Whereas, in healthy humans the optimal range of E_A/E_{LV} to cardiac efficiency and stroke work are generally ranging from 0.7 to 1.0 (Asanoi et al., 1992; Najjar et al., 2004, Redfield et al., 2005). During exercise, an acute mismatch between the arterial and ventricular systems occurs due to a disproportionate increase in E_{LV} vs. E_A (Najjar et al., 2004). As a result E_A/E_{LV} decreases to ensure that sufficient cardiac performance is achieved to meet the increased energetic requirements of the body. In the next section, we will review the alterations in E_A/E_{LV} at rest and during exercise due to aging and illustrate how the age–disease interaction accentuates the changes in E_A/E_{LV}. We will also discuss therapeutic interventions aimed to restore the relationship between

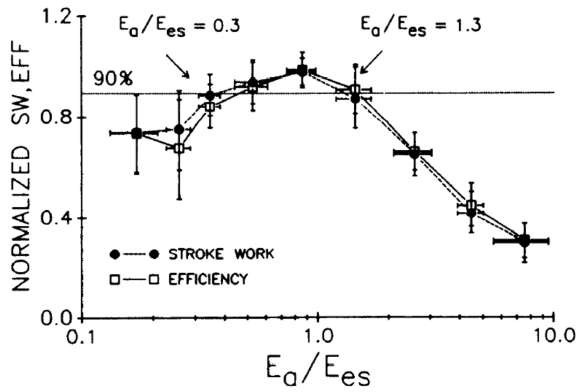

FIGURE 2 | Average normalized SW and SW/MVO2 (efficiency; EFF) as a function of arterial–ventricular coupling ratio. Both parameters reach optimal values between E_A/E_{es} ($E_{es} = E_{LV}$) ratios spanning 0.3–1.3. From De Tombe et al. (1993) with permission.

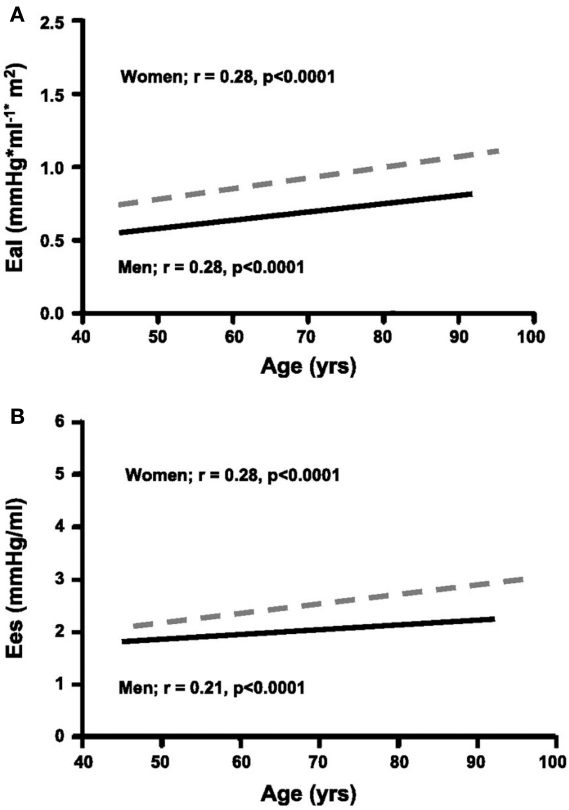

FIGURE 3 | The association between age and E_A indexed to body surface area (E_AI) (A) and E_{LV} ($E_{es} = E_{LV}$) (B) in men (solid line) and women (dashed line) at rest. Pearson correlation coefficients and probability values for each association are shown. With advancing age, both E_AI and E_{es} increase. However, the increase in E_{es} with age is significantly greater in women than men. Furthermore, E_AI and E_{es} are higher in women vs. men at all ages. Modified from Redfield et al. (2005) with permission.

E_A and E_{LV}, including the physiological meaning behind the adaptations.

HEALTHY AGING AND ARTERIAL VENTRICULAR COUPLING

AT REST

Numerous studies have documented a gradual increase in E_A with advancing age (Cohen-Solal et al., 1996; Chen et al., 1998, Redfield et al., 2005). Two small clinical cohort studies calculated E_A from invasive methods of flow and pressure and reported a significant rise in E_A (44–73%, $p < 001$) from ≈20 to 70 years of age (Cohen-Solal et al., 1996; Chen et al., 1998). Although the strength of these data lies in the invasive assessment of E_A, the small sample size (Cohen-Solal et al., 1996; Chen et al., 1998), and the inclusion of patients with coronary artery disease, and individuals on chronic medications (Chen et al., 1998), provides limited insight into the influence of healthy aging on E_A. Non-invasive studies of E_A have also reported an increase in E_A with age. In a larger epidemiological study consisting of 623 individuals from Olmsted County, Minnesota study, Redfield et al. (2005) observed an age-associated increase in E_AI (E_A normalized for body surface area) in both men ($r = 0.18$, $p < 0.01$) and women ($r = 0.13$, $p = 0.02$). Inclusion of individuals with existing cardiovascular disease further increased the age-associated change in E_AI in both men ($r = 0.28$, $p < 0.001$) and women ($r = 0.28$, $p < 0.001$; **Figure 3A**).

The specific mechanisms for the increased E_A with age reflect the age-associated changes in the arterial properties of individuals. E_A is a lumped parameter incorporating mean resistance and pulsatile properties of the arterial load, and is therefore influenced by changes in arterial compliance, wave reflection, and characteristic impedance (Kelly et al., 1992a). During the past two decades, we have characterized the effects of aging on multiple aspects of arterial structure and function in a single study population in the Baltimore Longitudinal Study on Aging (BLSA) who are rigorously screened to exclude both clinical and occult cardiovascular disease. Although at rest, peripheral resistance is the dominant factor affecting E_A (Chemla et al., 2003) the age-associated actions of peripheral resistance are heterogeneous to the extent that only an age-associated increase in resting peripheral resistance is noted in healthy women but not men (Fleg et al., 1995). Indeed, the increase in E_A with age noted by Redfield et al. (2005) is not attributed to an increase in peripheral resistance as no relationship between age and peripheral resistance existed. E_A is also altered by arterial stiffening and blood pressure pulsatility increases with age (Franklin et al., 1997) thus raising the end-systolic pressure required to eject blood thereby increasing E_A. Findings from the BLSA indicate that healthy aging is associated with an increase in aortic root diameter, an increase in carotid wall intimal media thickness, and an overall stiffening of the large elastic arteries (Najjar et al., 2005). Redfield et al. (2005) proposed the increase in E_A with age is attributable to an increase in arterial stiffness, manifested by an increase in pulse pressure (a surrogate measure of arterial stiffening). Thus, the age-associated increase in E_A is largely attributed to an increase arterial stiffness.

The heart seems to respond to an increase in E_A with a corresponding increase in E_{LV} with age (Cohen-Solal et al., 1996; Chen et al., 1998, Redfield et al., 2005). Cohen-Solal et al. (1996) noted a 28% higher E_{LV} in men ≈60 vs. 30 years of age. In the Olmsted County, Minnesota study, Redfield et al. (2005) noted a 10% ($r = 0.16$, $p < 0.001$) and 15% ($r = 23$, $p < 0.0001$) increase in E_{LV} in men and women without cardiovascular disease, respectively (**Figure 3B**). These results would suggest that E_{LV} increases to compensate for the increase in E_A ensuring that their ratio, E_A/E_{LV}, is matched for maximal efficiency at rest. Indeed, significant relationships exist between E_A and E_{LV} ranging from correlations of 0.50 to 0.73 (Chen et al., 1998; Borlaug et al., 2009) suggesting that a change in E_A accounts for a ≈25% change in E_{LV} (**Figure 4**). This small percentage is not surprising given that both components have multiple different determinants. Nevertheless, older individuals with increased arterial stiffness (E_A) are more likely to have an increased left ventricular stiffness (E_{LV}), i.e., a stiffer heart coupled to a stiffer vascular system. A consequence of the age-associated increase in E_{LV}, along with an increase in E_A, essentially permits the E_A/E_{LV} ratio to remain relatively matched (coupled; Cohen-Solal et al., 1996; Chen et al., 1998, Redfield et al., 2005). However, Redfield et al. (2005) did note a slight sex difference in E_A/E_{LV} with age whereby healthy women demonstrated a slight decline in E_A/E_{LV} with age, reflecting a disproportionate increase in E_{LV} compared with E_A. This suggests a greater impact of aging on ventricular vs. arterial properties in women compared to men, as indicated in **Figure 4**.

Given that E_{LV} is as a load-independent index of left ventricular contractility (Sagawa, 1978), one might suggest that an increase in E_{LV} with age (and more so in women) reflects enhanced contractility. This is unlikely given that other measures of left ventricular function do not increase with age (Lakatta, 1993). Thus, what does the increase in E_{LV} likely reflect? E_{LV} is also influenced by the structural/geometric and biochemical properties (i.e., stiffness or compliance of myocytes, composition of muscle, fibrosis, collagen, etc., in the heart wall) that underlie left ventricular end-systolic stiffness (Borlaug and Kass, 2008; Chantler et al., 2008a). Advancing age is also associated with alterations in left ventricular structure. Most notably, there is a reduction in myocyte number, and there is an increase in left ventricular wall thickness and collagen deposition in the heart (Olivetti et al., 1995; Lakatta, 2003). Thus, the increase in E_{LV} with age could represent differences in left ventricular geometry and/or structure between young and older individuals. For example, concentric remodeling leads to a higher E_{LV} (Borlaug et al., 2009). However, the increase in E_{LV} noted in Redfield et al. (2005) are unlikely to be due to differences in LV chamber size, as similar results are obtained when E_{LV} is normalized to end-diastolic volume (a crude estimate of chamber size). An increase in the amount (focal increases) and a change in the physical properties of collagen (purportedly due to non-enzymatic cross-linking) also occur within the myocardium with aging (Lakatta and Levy, 2003). Thus, the increase in E_{LV} with age is likely attributed to a combination of left ventricular remodeling but more so due to a loss of myocyte number and an increase in myocardial collagen content, thereby increasing myocardial stiffness.

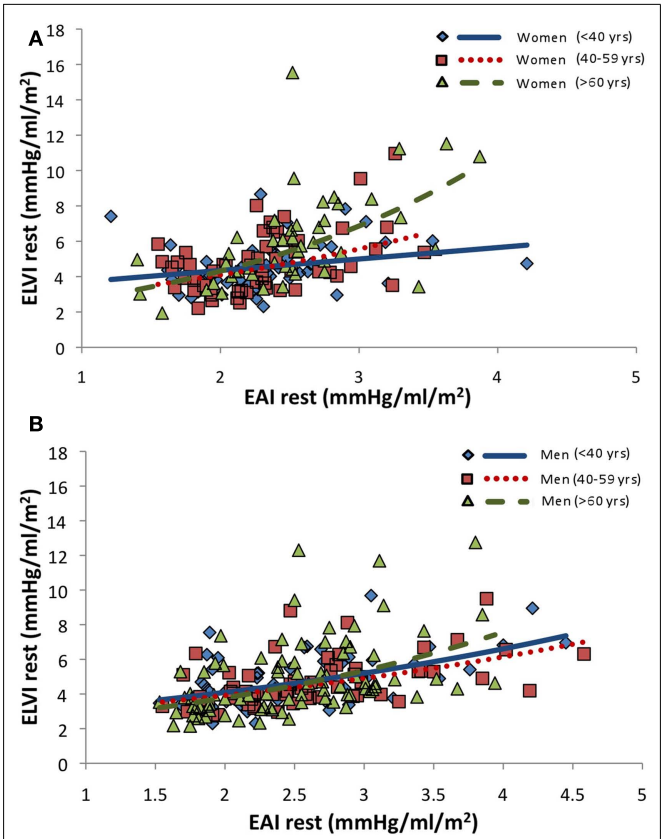

FIGURE 4 | Relationship between arterial (E_AI) and left ventricular (E_{LV}I) elastance indexed to body surface area in healthy men (A) and women (B) from the Baltimore longitudinal Study of Aging. Note the linear relationship between E_AI and E_{LV}I irrespective of age and gender (Chantler and Lakatta, unpublished data).

DURING EXERCISE

During exercise E_A has been shown to increase (Najjar et al., 2004; Otsuki et al., 2006), decline (Asanoi et al., 1992), or remain unchanged (Cohen-Solal et al., 1998). The response of E_A during exercise is dependent on the changes in its components and has important consequences on the Frank–Starling mechanism (Chantler et al., 2011). E_A is linearly related to heart rate and peripheral resistance, and inversely related to compliance (Segers et al., 2002; Chemla et al., 2003, Otsuki et al., 2006; Chantler et al., 2011). Both resistance and compliance usually decrease during exercise (reflecting less resistance to blood flow in the microcirculation, but increased stiffness of the conduit arteries) and the relative contribution of the pulsatile component (compliance) to E_A increases, so that by 80% of peak exercise the resistive and the pulsatile components provide nearly equal contributions to E_A (Otsuki et al., 2006). With advancing age, the ability to increase heart rate, and lower resistance during exercise is blunted. In addition the reduction in compliance (due to a greater increase in pulse

pressure) is also limited during exercise (Lakatta, 1993; Fleg et al., 1995). Although the blunted maximum cardiovascular responses with age are, in part, due to older individuals achieving a lower maximal workload, similar cardiovascular deficits are also evident at submaximal workloads (Lakatta, 1993; Fleg et al., 1995). The only study to directly examine the age-associated change in E_A/E_{LV} in the absence of cardiovascular disease reported that E_A, in general, increases during exercise and that E_A does not differ between young and older subjects (Najjar et al., 2004). Perhaps the blunted changes in resistance, compliance, and heart rate are compensated for by the greater increase in blood pressure during exercise in older vs. younger healthy individuals (Ogawa et al., 1992). Indeed, some of the components of the changes in E_A seem to be related to each other (Chantler et al., 2012). That is, greater preservation of compliance during exercise is associated with a greater reduction in resistance (and a smaller increase in mean and pulse pressure). This suggests that the tandem changes in resistance and compliance appear to be linked, and raises the possibility of a crosstalk between central and peripheral arteries. Further, the change in E_A during exercise is also linked to a specific pattern of change in ventricular volumes and function. The change in E_A is inversely related to the recruitment of end-diastolic volume, and the enhancement of stroke volume and cardiac output with exercise. Indeed individuals expressing a large increase in E_A during exercise demonstrate a blunted utilization of the Frank–Starling mechanism, irrespective of age, sex, and body size (Chantler et al., 2012). The cardiovascular system responds to exercise by increasing cardiac output predominately through an increase in heart rate, and in the upright position stroke volume is increased. The perfusion of the tissues depends both on the ability of the LV to produce flow and sustain perfusion pressure. Thus, the speed and force of left ventricular contraction increases during exercise and this is reflected by an increase in E_{LV} during exercise (Little and Cheng, 1993). Unlike E_A, aging impairs the increase in E_{LV} during exercise. Najjar et al. (2004) reports that the blunted increase in E_{LV} with aging begins to appear at 50% of maximal workload and that at maximal exercise older men and women (>60 years) have ≈40–55% smaller E_{LV} compared to men and women <40 years of age. A consequence of the blunted increase in E_{LV} during exercise in older healthy individuals is a corresponding blunted reduction in E_A/E_{LV} (Najjar et al., 2004). Furthermore, the altered coupling noted at peak exercise with age is also associated with a blunted increase in ejection fraction. Indeed, E_A/E_{LV} is inversely related to ejection fraction [$E_A/E_{LV} \approx (1/\text{ejection fraction}) - 1$; Cohen-Solal et al., 1994]. The advantage of E_A/E_{LV} over ejection fraction is that examining the components of E_A/E_{LV} permits evaluation of whether alterations in E_A/E_{LV} are due to alterations in arterial properties, left ventricular properties, or both. Consequently, advancing age is associated with a smaller E_A/E_{LV} (and ejection fraction) reserve ($E_AI/E_{LV}I$ peak $- E_A/E_{LV}$ rest) and E_{LV} reserve (E_{LV} peak $- E_{LV}$ rest; Najjar et al., 2004). Unlike at rest when an increase in E_{LV} could represent enhanced left ventricular contraction, decreased left ventricular mass (or concentric remodeling), or increased myocardial stiffness (due to alterations in biochemical properties), an increase in E_{LV} during exercise likely reflects an increase in inotropy. This would suggest a decrease in left ventricular contraction during exercise with increasing age.

AGING–CV DISEASE INTERACTIONS AFFECT ARTERIAL VENTRICULAR COUPLING

Unfortunately in today's society, for the most part, aging is highly linked to the occurrence of cardiovascular diseases. Further, cardiovascular disease risk factors (obesity, hypercholesterolemia, diabetes, and hypertension) often co-vary in number or severity with increasing age. Although measuring the age-associated changes in cardiovascular structure and function in a "healthy" aging population is important to provide insights into the normal aging process, the generalizability of these findings are sometimes limited. The age and disease interaction has important consequences on arterial ventricular coupling both at rest and during exercise as outlined below.

HYPERTENSION

The prevalence of hypertension markedly increases with advancing age, such that the relative risk of acquiring hypertension is ≈90% of individuals over 40 years of age (Lloyd-Jones et al., 2009; **Figure 5**). Age-associated changes in arterial and left ventricular structure and function are accelerated in the presence of hypertension. Hypertensive patients exhibit greater carotid wall thickness (Arnett et al., 1996), central arterial wall stiffness (Amar et al., 2001), and reflected waves (Nichols et al., 1992) than normotensive subjects, even after adjusting for age. Furthermore, hypertension is associated with left ventricular remodeling and fibrosis (Mayet and Hughes, 2003). As such, E_A and E_{LV} are reported to be increased, between 15–60 and 16–95%, respectively, in hypertensive patients compared with normotensive controls (Cohen-Solal et al., 1994; Saba et al., 1999, Lam et al., 2007). As with aging, however, the coupling ratio (E_A/E_{LV}) remains matched (coupled) between normotensives and hypertensives (Cohen-Solal et al., 1994; Saba et al., 1999). We found similar results (matched E_A/E_{LV} due to tandem increase in E_A and E_{LV}) when comparing men with predominantly systolic hypertension to normotensive men. In contrast, women with predominantly systolic hypertension, have a 23% lower resting E_A/E_{LV} than normotensive women, a finding that persisted even after adjusting for age (Chantler et al., 2008b). The lower E_A/E_{LV} in women with systolic hypertension is due to a disproportionate increase in E_{LV} compared with E_A (45 vs. 16%), suggesting an adaptation by these women to limit the impact of systolic hypertension on the vasculature or, alternatively, a more pronounced impact of systolic hypertension on ventricular vs. arterial elastance.

There are a limited number of studies that have examined the coupling response during dynamic exercise in hypertensive individuals. Borlaug et al. (2010) showed that hypertensive individuals express similar changes in E_A, E_{LV} and E_A/E_{LV} at submaximal and maximal exercise compared to normotensive individuals matched by age and sex. In contrast, Chantler et al. (2008b) reported that the effects of predominantly systolic hypertension on the changes in E_A/E_{LV} are similar between normotensive men and systolic hypertensive men and women at 50% of maximal workload and at peak upright bicycle exercise. In men, this is because E_A and

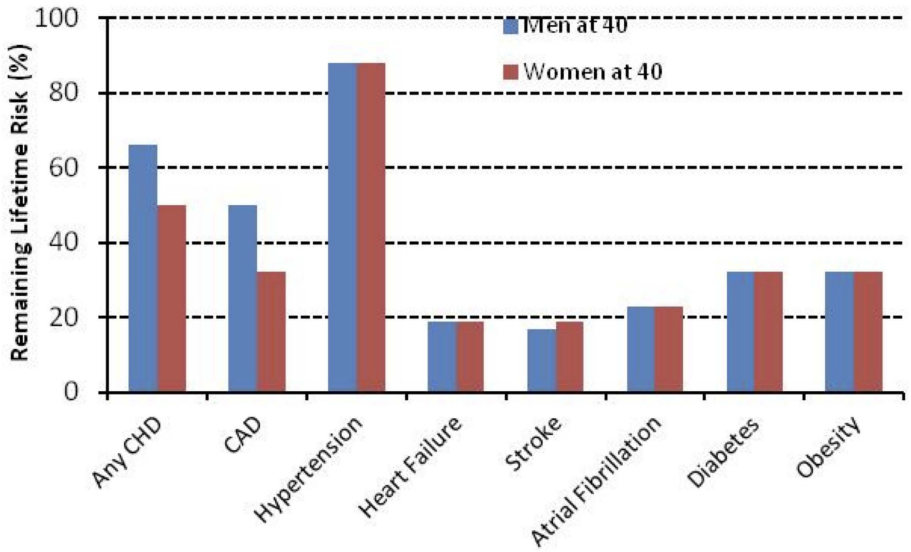

FIGURE 5 | Remaining lifetime risk for cardiovascular disease and other diseases among men and women free of disease at 40 years of age from the Framingham Study. Modified from Lloyd-Jones et al. (2009).

E_{LV} are proportionally higher at peak exercise in systolic hypertensive compared with normotensive individuals. In women, this is because E_A and E_{LV} are similar at peak exercise between systolic hypertensive and normotensive women. Thus, E_A/E_{LV} reserve is similar between systolic hypertensive and normotensive men, but 61% lower in systolic hypertensive vs. normotensive women because of the uncoupling in E_A/E_{LV} at rest in these systolic hypertensive women.

OBESITY

About one-third of U.S. adults (33.8%) are obese and the prevalence of overweight/obesity increases with advancing age. Obesity-related conditions include heart disease, stroke, type 2 diabetes, and certain types of cancer, some of the leading causes of death (Flegal et al., 2010). The physiological changes that occur with obesity are similar to those in hypertension, and often hypertension and obesity co-exist in a given individual complicating interpretations due to obesity alone (Stamler et al., 1978, 1991). Indeed, Zebekakis et al. (2005) identified that obese individuals (14% on antihypertensive medications) have increased carotid, brachial, and femoral diameter with age, which remained significant after adjusting for differences in BP, medications, and smoking status. Further, increasing body mass index is associated with a reduced arterial distensibility (Zebekakis et al., 2005) and increased arterial stiffness (Toto-Moukouo et al., 1986; Zebekakis et al., 2005). In individuals with uncomplicated obesity (without the presence of cardiovascular disease), an increase in body mass index (45 ± 5 vs. 21 ± 2) is associated with an increase in left ventricular mass (irrespective of normalized to height or body surface area), and end-diastolic volume (unadjusted or indexed to height; Rider et al., 2011). Although such alterations in arterial and left ventricular structure/function increase resting E_A and E_{LV} in obese vs. non-obese controls, their coupling (E_A/E_{LV}), however, remains matched, irrespective of body size. Rider et al. (2011) noted a similar resting ejection fraction, which is inversely related to E_A/E_{LV}, between individuals of various body mass index categories. However, whether the increase in E_A and E_{LV} noted in obese individuals represents the pathologic effects of excess adipose tissue on cardiovascular function, or the normal physiological relationship between body size and cardiovascular function is important to decipher. In order to have meaningful clinical and scientific comparisons, differences in body size must be accounted for (Chantler et al., 2005; Chantler and Lakatta, 2009). Indeed, in the unadjusted form, resting E_A and E_{LV} are slightly reduced (4 and 3%, respectively) in obese vs. healthy non-obese controls (Chirinos et al., 2009). In contrast, when E_A and E_{LV} are scaled either ratiometrically or allometrically to body surface area, E_A and E_{LV} are between 16–20 and 18–19% higher ($p < 0.001$) in obese vs. controls. Irrespective of the scaling technique employed there was no relationship between obesity and E_A/E_{LV} (Chirinos et al., 2009). Unfortunately, the changes in E_A and E_{LV}, and subsequently E_A/E_{LV} have not been reported during dynamic exercise in obese vs. non-obese individuals.

HEART FAILURE

Heart failure (HF) is a syndrome that is characterized by an inability of the heart to pump a sufficient amount of blood to meet the demands of the metabolizing tissues, or can do so only at the expense of elevated filling pressures (Adams et al., 2006). The inability to meet the tissues' demands is attributed to the ability, or lack thereof, for the LV to fill (diastolic properties) or to eject (systolic properties) blood. Although there is considerable confusion as to the pathological mechanisms that describes individuals with systolic (HF with reduced ejection fraction), or diastolic (HF with preserved ejection fraction) the coupling between the heart and arteries does seem to be impacted differently by the type of HF.

HF WITH A REDUCED EF

Heart failure patients with systolic dysfunction are characterized by a diminished resting ejection fraction and left ventricular contractility (Asanoi et al., 1989). Patients with HF and a reduced ejection faction have a downward and rightward shift of the end-systolic pressure–volume relationship, reflecting a reduced E_{LV} (range 0.6–2.6 mmHg·ml^{-1}·m^{-2}; Asanoi et al., 1989). Patients with HF and a reduced ejection faction have an augmented E_A (range 1.7–3.7 mmHg·ml^{-1}·m^{-2}) due to a decrease in stroke volume and increases in heart rate and peripheral resistance (Asanoi et al., 1989). The increase in E_A and decrease in E_{LV} result in a three to fourfold increase in E_A/E_{LV} (range 1.3–4.3; Asanoi et al., 1989; Sasayama and Asanoi, 1991). This suboptimal coupling reflects diminished cardiovascular performance and efficiency of the failing heart.

During exercise, the traditional reduction in E_A/E_{LV} due to a substantial increase in E_{LV} vs. any change in E_A are virtually absent in HF patients with a reduced ejection fraction (Cohen-Solal et al., 1998). Thus the limited capacity of systolic HF patients to augment their cardiovascular function during times of stress (such as exercise) involve marked deficits in both left ventricular and arterial elastance reserves.

HF WITH A PRESERVED EF

Heart failure patients with a preserved ejection fraction ($\geq$50%) represent $\approx$40% of patients with HF (Owan et al., 2006). HF with a preserved ejection fraction is more prevalent with advancing age, in women, and in individuals with systolic hypertension (Klapholz et al., 2004). Recent interest has refocused attention on examining the coupling between the heart (systolic and diastolic properties) and arterial system in order to gain further insights into the pathophysiological mechanisms that underlies HF with a preserved ejection fraction. Some of the pathophysiological adaptations that occur in HF with a preserved ejection fraction can be attributed to normal adaptations evident with aging. Indeed the relative risk (controlled for gender, race, medical history, and admission characteristics) of developing HF with a preserved ejection fraction is $\approx$10–27% between 65 and 90 years of age (Masoudi et al., 2003). However, aging alone does not account for all the physiological changes noted in HF with a preserved ejection fraction. For example, compared to age-matched normotensive controls, HF patients with a preserved ejection fraction typically have an E_A that is $\approx$40% higher and an E_{LV} that is approximately twofold greater, and thus a lower E_A/E_{LV} is noted in HF patients with a preserved ejection fraction vs. healthy controls (Kawaguchi et al., 2003). However, when comparing HF patients with a preserved ejection fraction with age, and hypertensive matched controls without HF E_A/E_{LV} is similar because of a tandem rise in both E_A ($\approx$40%) and E_{LV} ($\approx$50%) in HF patients with a preserved ejection fraction (Kawaguchi et al., 2003). In larger epidemiological studies in whom E_A/E_{LV} is examined non-invasively, HF patients with a preserved ejection fraction have matched increases in E_A and E_{LV} (and thus similar E_A/E_{LV}) compared to non-hypertensive controls without HF (Lam et al., 2007; Borlaug et al., 2009). Further, no differences in E_A and E_{LV}, or E_A/E_{LV} are found between individuals with hypertension without HF and HF patients with a preserved ejection fraction (Lam et al., 2007; Borlaug et al., 2009). Also the increase in E_{LV} in HF patients with a preserved ejection fraction compared to non-hypertensive controls is not due to differences in left ventricular remodeling but more likely due to an increased passive myocardial stiffening (Borlaug et al., 2009). However, the vast majority of HF patients with a preserved ejection fraction in these epidemiological studies are hypertensive (>95%), which may contribute to the matched increases in E_A and E_{LV}. Indeed, some patients with HF patients with a preserved ejection fraction who are normotensive have values of E_A and E_{LV} that are similar to those of healthy normotensive controls (Maurer et al., 2005). These findings highlight the difficulty in understanding the pathophysiological mechanisms that are evident in a disease that is comprised of a very heterogeneous group of individuals.

Recent studies have highlighted the importance of altered arterial–ventricular interactions during exercise. HF patients with a preserved ejection fraction who have increased E_A and E_{LV} at rest exhibited a marked hypertensive response and elevated diastolic pressures to sustained handgrip exercise (Kawaguchi et al., 2003). Further, during upright cycle ergometry exercise at maximal effort, HF patients with a preserved ejection fraction have a threefold smaller increase in E_{LV} and a reduced ability to lower their peripheral resistance and increase their heart rate during exercise compared with hypertensive controls with left ventricular hypertrophy (Borlaug et al., 2006). Some of the peak cardiovascular deficits noted between HF patients with a preserved ejection fraction and hypertensive controls could be related to the fact that the HF patients with a preserved ejection fraction are unable to attain the same level of exercise workload compared to controls. In other words, are the peak cardiovascular deficits a mechanism or consequence of exercise limitation? However, similar deficits are also noted between HF patients with a preserved ejection fraction and hypertensive controls at matched submaximal workloads (Borlaug et al., 2010). Thus, HF patients with a preserved ejection fraction are characterized by a diminished increase in E_{LV} and a smaller reduction in E_A/E_{LV} compared to controls without hypertension, and to those with hypertension but without HF, both at submaximal exercise workloads (20 W) and at maximum exercise (Borlaug et al., 2010). In addition, the depressed reserve responses correlated with reduced exercise capacity and greater subjective symptoms at low-level workloads. Since female sex, systolic hypertension, and older age are risk factors for HF with a preserved ejection fraction (Klapholz et al., 2004), and as the pathophysiology of HF with a preserved ejection fraction involves a limited cardiovascular reserve (Kitzman et al., 1991), the diminished E_A/E_{LV} reserve observed in older systolic hypertensive women without HF (Chantler et al., 2008b) suggests that they may be exhibiting signs of subclinical (Stage B) HF. This raises the possibility that they may be on a trajectory to progressive exercise intolerance and perhaps functional limitations.

In summary, the pathophysiological mechanisms that contribute to HF with a preserved ejection fraction are due to the accumulation of multiple cardiovascular impairments that are expressed during exercise, reflecting impaired inotropic, chronotropic, lusitropic, and vasodilatory responses that impair E_A/E_{LV} (Kawaguchi et al., 2003; Borlaug et al., 2010).

CONSEQUENCES OF ALTERATIONS IN E_A/E_{LV} WITH AGING, HYPERTENSION, OR HEART FAILURE

In a young, healthy individual the coupling between the arteries and heart are well matched to: (1) maintain an optimal transfer of blood from heart to periphery without excessive changes in blood pressure and; (2) provide optimal cardiovascular flow reserve without compromising arterial pressures (Kass, 2005). However, the increased stiffness noted in both the arteries and heart with age, which is further exacerbated in the presence of disease (hypertension, HF, etc.) reflects a *coupling disease* (Kass, 2005). That is the stiff arteries and LV interact to limit the cardiovascular response to stress (reduced performance) and generates clinical symptoms (Kass, 2005).

As illustrated in **Figure 6**, a stiffer heart-arterial system increases load-sensitivity even if the coupling ratio is normal. That is, an increased resting E_A and E_{LV} means that systolic pressures are much more sensitive to changes in left ventricular volume. This is clearly observed in young vs. old individuals, whereby a decreased preload in younger individual results in a modest drop in systolic pressure but in older individuals there is a much greater change in systolic pressure (Chen et al., 1998). This exaggerated systolic pressure response is also evident in hypertensives and HF patients with a preserved ejection fraction compared to controls (Kawaguchi et al., 2003). Consequently, the stroke work (myocardial demand) required to perform this task is increased and can potently influence systolic and diastolic function, including coronary flow (i.e., greater dependence upon systolic pressure for coronary flow; Kawaguchi et al., 2003). Thus, older individuals are working at a higher setpoint regarding changes in pressure for a given change in loading conditions and this disadvantage is further exaggerated in hypertensives and HF patients with a preserved ejection fraction.

In addition to enhanced load-sensitivity, the global (systolic and diastolic) reserve capacity becomes blunted with arterial and left ventricular stiffening. Increased E_{LV} at rest translates into less effective changes in E_{LV} during exercise (or stress) thereby limiting cardiovascular performance (Borlaug et al., 2010). For example, individuals who start at a higher E_{LV} have a limited capacity to further increase stroke volume, and the limited stroke volume response is further exacerbated when a stiff heart is connected to a stiff artery (Chen et al., 1998; Kawaguchi et al., 2003). It is therefore not surprising that a stiffer LV and arterial system are linked to a reduced aerobic capacity (Borlaug et al., 2010). Further, in an isolated canine heart model, Kass et al. (1989) reported, larger reductions in E_{LV} after a myocardial infarction in hearts with a higher resting E_{LV}. This greater mechanical vulnerability to an ischemic insult may help to explain why older individuals may experience worse outcomes following a myocardial infarction (Maggioni et al., 1993).

Another a major consequence of arterial stiffening is an increased pulse pressure, which also increases cyclic changes of arterial flow. As such, the microcirculation receives larger pulsatile pressures which can damage the vascular beds and in turn, cause damage to the end organs (such as the kidney and brain). Indeed increased arterial stiffness is independently associated with dementia (Hanon et al., 2005). Increased arterial stiffness can also lead to endothelial dysfunction and an abnormal vasodilation response to stress.

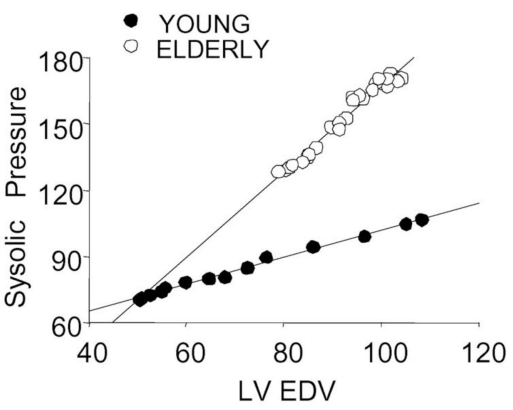

FIGURE 6 | Relationship between changes in systolic pressure to alterations in ventricular diastolic volume (induced by balloon obstruction of inferior vena caval inflow) in a young and in an elderly patient. There is much greater sensitivity of systolic pressure to volume changes in the elderly patient, indicated by the steeper slope. Modified from Chen et al. (1998) with permission.

THERAPEUTIC INTERVENTIONS

Interventions related to improving arterial ventricular coupling span from pharmacological to lifestyle (exercise and diet) approaches. We will briefly highlight some important interventions in this area. The abnormalities in combined arterial and ventricular stiffening leading to a mismatch in their interaction, as highlighted above, has important physiological consequences. Numerous approaches have been taken to reverse the age and disease associated changes. By reducing the increase in arterial and left ventricular systolic stiffness with age or disease we may improve arterial–ventricular interactions and thus cardiovascular performance by being more efficient blood pressure regulation for a given volume of blood. For example, acute administration of sodium nitroprusside, a balanced vasodilator, acutely reduces E_A (10%) and increases E_{LV} (47%) at rest in older (70 ± 8 years) individuals (Chantler et al., 2011). Further, at peak exercise, sodium nitroprusside leads to an increase in E_{LV} (68%) without a change in E_A and consequently the normal reduction in E_A/E_{LV} during exercise is enhanced (36%). Importantly, sodium nitroprusside acutely attenuates the age-associated deficits in E_A/E_{LV} and E_{LV} during exercise (Chantler et al., 2011). Similarly, acute intravenous verapamil (calcium-channel blocker) in healthy older (70 ± 10 years) volunteers reduces resting arterial–ventricular stiffening and E_A during exercise (though a decline in heart rate, pulsatile, and resistive arterial load), and improves (13%) aerobic exercise performance (Chen et al., 1999). These results in older persons highlight that, at least acutely, the abnormalities in arterial–ventricular interactions can be partly restored. Of note, verapamil also improved arterial ventricular interactions and exercise capacity in HF patients with a preserved ejection fraction and hypertrophic cardiomyopathy (Brown et al., 1985; Setaro et al., 1990). However, whether the acute effects of sodium nitroprusside or verapamil identified can be maintained with chronic drug administration is unknown.

Hypertensive patients on optimal brachial and central blood pressure antihypertensive therapies shifts arterial–ventricular coupling from blood flow maximization to left ventricular mechanical efficiency optimization (Osranek et al., 2008). Further, the effects of antihypertensive monotherapy on E_A/E_{LV} examined in 10,670 patients over a 6-month period indicated that angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (AIIRA), and dihydropyridine calcium antagonists decrease E_A/E_{LV}, whereas diuretics, α-blockers, both β-blocker groups (with and without intrinsic sympathomimetic activity), and non-dihydropyridines significantly increase E_A/E_{LV} compared to baseline measurements (**Figure 7**; Iakovou et al., 2004). Thus various antihypertensive drugs have a differential effect on E_A/E_{LV} with ACEI, AIIRA, and dihydropyridine calcium antagonists have the most favorable effect on this index, likely through their actions on causing vasodilatation and by actually inhibiting the vasoconstrictive results of neurohormonal activation (Iakovou et al., 2004). However, the effects of these drugs on the components of E_A/E_{LV} are not reported.

Other therapies shown to improve E_A/E_{LV} are exercise training. In healthy older men, 24–32 weeks of aerobic endurance exercise training does not alter baseline ejection fraction (inverse of E_A/E_{LV}) or left ventricular contractility (systolic blood pressure/end-systolic volume), but increases peak ejection fraction, suggesting that E_A/E_{LV} would have further decreased during exercise, due to a greater peak left ventricular contractility (Schulman et al., 1996). In the same study, eight master athletes stopped their endurance training for 12 weeks, which tended to decrease peak ejection fraction and LV contractility (Schulman et al., 1996). In patients with coronary artery disease, 12 months of aerobic endurance exercise training did not alter resting E_A/E_{LV}, or E_{LV}, but produced a slight 13% reduction in E_A at rest (Rinder et al., 1999). Further, exercise training led to a 37% increase in E_{LV} and a 23% decrease in E_A/E_{LV} during handgrip exercise performed at 30% of maximal voluntary contraction. However, the change in E_A during handgrip exercise remained unaltered after the exercise training. The results of this study suggest that long-term endurance exercise training induces significant cardiovascular adaptations both in the basal state and during an afterload stress in patients with coronary artery disease.

One year of progressive and vigorous endurance training in sedentary healthy older (70 ± 3 years) individuals resulted in slight reductions in E_A at rest (14%) and peak exercise (20%). This coincided with an improvement in compliance at rest (Fujimoto et al., 2010). However, the exercise training failed to reverse cardiac stiffening. One possible reason for the lack of changes in left ventricular stiffness with exercise training is the development of cross-linked advanced glycation end products in the left ventricular wall along with a loss in the number and the volume of cardiac myocytes that occur with older age and which are pathologically irreversible once formed (Aronson, 2003). Thus any improvement in left ventricular function could have been constrained by cross-linked collagen. A phase II drug, alagebrium, is a cross-link breaker and is known to improve left ventricular and arterial stiffness in animals (Kass, 2003), and a clinical trial is currently underway to examine the effects of exercise and alagebrium combined on cardiovascular stiffening in the elderly (NIH clinicaltrials.gov identifier NCT01014572). However, alagebrium administered to patients with HF and systolic dysfunction does not improve exercise tolerance or cardiac function (Hartog et al., 2011). Whether HF patients with a preserved ejection fraction, in whom the largest effect of alagebrium could be expected, would benefit from an advanced glycation end products breaker therapy remains unknown.

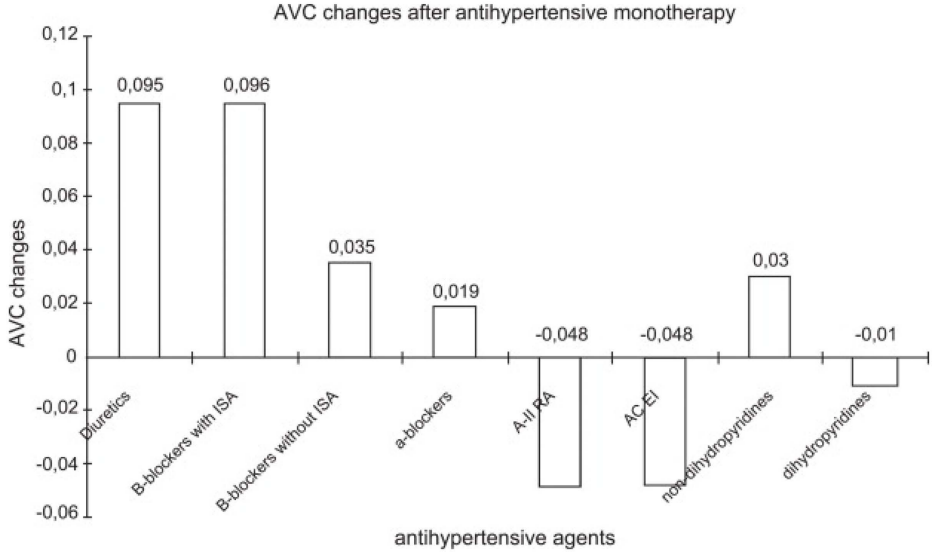

FIGURE 7 | Arterial–ventricular coupling (AVC) percentage changes after antihypertensive monotherapy in 10,670 patients over 6 months of treatment. Abbreviations: ACE inhibitors, angiotensin converting enzyme inhibitors; AIIRA, angiotensin II receptor antagonists; ISA, intrinsic sympathomimetic activity. From Iakovou et al. (2004) with permission.

CONCLUSION

Although increased stiffness of the arteries itself has important physiological and clinical relevance, such changes also have major implications on the heart, and vice versa, and the manner in the way they interact has important ramifications on cardiovascular function both at rest and during exercise. Examination of the alterations in arterial–ventricular coupling with aging and disease can yield mechanistic insights into the pathophysiology of these conditions and increase the effectiveness of current therapeutic interventions. Future studies should identify agents to chronically reverse increases in E_A and E_{LV} that occur with age and disease. Furthermore, longitudinal studies are needed to evaluate whether alterations in E_A/E_{LV}, E_A, and E_{LV} provide any prognostic information for adverse outcomes, such as HF.

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REFERENCES

Adams, K. F., Lindenfeld, J., and Arnold, J. M. O. (2006). Executive summary: HFSA 2006 comprehensive heart failure practice guideline. *J. Card. Fail.* 12, 10–38.

Amar, J., Ruidavets, J. B., Chamontin, B., Drouet, L., and Ferrieres, J. (2001). Arterial stiffness and cardiovascular risk factors in a population-based study. *J. Hypertens.* 19, 381–387.

Arnett, D. K., Tyroler, H. A., Burke, G., Hutchinson, R., Howard, G., and Heiss, G. (1996). Hypertension and subclinical carotid artery atherosclerosis in blacks and whites. The Atherosclerosis Risk in Communities Study. ARIC Investigators. *Arch. Intern. Med.* 156, 1983–1989.

Aronson, D. (2003). Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J. Hypertens.* 21, 3–12.

Asanoi, H., Kameyama, T., Ishizaka, S., Miyagi, K., and Sasayama, S. (1992). Ventriculoarterial coupling during exercise in normal human subjects. *Int. J. Cardiol.* 36, 177–186.

Asanoi, H., Sasayama, S., and Kameyama, T. (1989). Ventriculoarterial coupling in normal and failing heart in humans. *Circ. Res.* 65, 483–493.

Borlaug, B. A., and Kass, D. A. (2008). Ventricular-vascular interaction in heart failure. *Heart Fail. Clin.* 4, 23–36.

Borlaug, B. A., Lam, C. S., Roger, V. L., Rodeheffer, R. J., and Redfield, M. M. (2009). Contractility and ventricular systolic stiffening in hypertensive heart disease insights into the pathogenesis of heart failure with preserved ejection fraction. *J. Am. Coll. Cardiol.* 54, 410–418.

Borlaug, B. A., Melenovsky, V., Russell, S. D., Kessler, K., Pacak, K., Becker, L. C., and Kass, D. A. (2006). Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation* 114, 2138–2147.

Borlaug, B. A., Olson, T. P., Lam, C. S. P., Flood, K. S., Lerman, A., Johnson, B. D., and Redfield, M. M. (2010). Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. *J. Am. Coll. Cardiol.* 56, 845–854.

Brown, F., Campbell, W., Mitchell, P. J., and Randall, K. (1985). Dopamine autoreceptors and the effects of drugs on locomotion and dopamine synthesis. *Br. J. Pharmacol.* 84, 853–860.

Chantler, P. D., Clements, R. E., Sharp, L., George, K. P., Tan, L. B., and Goldspink, D. F. (2005). The influence of body size on measurements of overall cardiac function. *Am. J. Physiol. Heart Circ. Physiol.* 289, H2059–H2065.

Chantler, P. D., and Lakatta, E. G. (2009). Role of body size on cardiovascular function can we see the meat through the fat? *Hypertension* 54, 459–461.

Chantler, P. D., Lakatta, E. G., and Najjar, S. S. (2008a). Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise. *J. Appl. Physiol.* 105, 1342–1351.

Chantler, P. D., Melenovsky, V., Schulman, S. P., Gerstenblith, G., Becker, L. C., Ferrucci, L., Fleg, J. L., Lakatta, E. G., and Najjar, S. S. (2008b). The sex-specific impact of systolic hypertension and systolic blood pressure on arterial-ventricular coupling at rest and during exercise. *Am. J. Physiol. Heart Circ. Physiol.* 295, H145–H153.

Chantler, P. D., Nussbacher, A., Gerstenblith, G., Schulman, S. P., Becker, L. C., Ferrucci, L., Fleg, J. L., Lakatta, E. G., and Najjar, S. S. (2011). Abnormalities in arterial-ventricular coupling in older healthy persons are attenuated by sodium nitroprusside. *Am. J. Physiol. Heart Circ. Physiol.* 300, H1914–H1922.

Chantler, P. D., Melenovsky, V., Schulman, S. P., Gerstenblith, G., Becker, L. C., Ferrucci, L., Fleg, J. L., Lakatta, E. G., and Najjar, S. S. (2012). Use of the Frank-Starling mechanism during exercise is linked to exercise-induced changes in arterial load. *Am. J. Physiol. Heart Circ. Physiol.* 302, H349–H358.

Chemla, D., Antony, I., Lecarpentier, Y., and Nitenberg, A. (2003). Contribution of systemic vascular resistance and total arterial compliance to effective arterial elastance in humans. *Am. J. Physiol. Heart Circ. Physiol.* 285, H614–H620.

Chen, C. H., Nakayama, M., Nevo, E., Fetics, B. J., Maughan, W. L., and Kass, D. A. (1998). Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly. *J. Am. Coll. Cardiol.* 32, 1221–1227.

Chen, C.-H., Nakayama, M., Talbot, M., Nevo, E., Fetics, B., Gerstenblith, G., Becker, L. C., and Kass, D. A. (1999). Verapamil acutely reduces ventricular-vascular stiffening and improves aerobic exercise performance in elderly individuals. *J. Am. Coll. Cardiol.* 33, 1602–1609.

Chirinos, J. A., Rietzschel, E. R., De Buyzere, M. L., De Bacquer, D., Gillebert, T. C., Gupta, A. K., Segers, P., and Asklepios Investigators. (2009). Arterial load and ventricular-arterial coupling. *Hypertension* 54, 558–566.

Cohen-Solal, A., Caviezel, B., Himbert, D., and Gourgon, R. (1994). Left ventricular-arterial coupling in systemic hypertension: analysis by means of arterial effective and left ventricular elastances. *J. Hypertens.* 12, 591–600.

Cohen-Solal, A., Caviezel, B., Laperche, T., and Gourgon, R. (1996). Effects of aging on left ventricular-arterial coupling in man: assessment by means of arterial effective and left ventricular elastances. *J. Hum. Hypertens.* 10, 111–116.

Cohen-Solal, A., Faraggi, M., Czitrom, D., Le Guludec, D., Delahaye, N., and Gourgon, R. (1998). Left ventricular-arterial system coupling at peak exercise in dilated nonischemic cardiomyopathy. *Chest* 113, 870–877.

De Tombe, P. P., Jones, S., Burkhoff, D., Hunter, W. C., and Kass, D. A. (1993). Ventricular stroke work and efficiency both remain nearly optimal despite altered vascular loading. *Am. J. Physiol.* 264, H1817–H1824.

Fleg, J. L., O'Connor, F., Gerstenblith, G., Becker, L. C., Clulow, J., Schulman, S. P., and Lakatta, E. G. (1995). Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. *J. Appl. Physiol.* 78, 890–900.

Flegal, K. M., Carroll, M. D., Ogden, C. L., and Curtin, L. R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 303, 235–241.

Franklin, S. S., Gustin, W. IV, Wong, N. D., Larson, M. G., Weber, M. A., Kannel, W. B., and Levy, D. (1997). Hemodynamic patterns of age-related changes in blood pressure: the Framingham Heart Study. *Circulation* 96, 308–315.

Fujimoto, N., Prasad, A., Hastings, J. L., Arbab-Zadeh, A., Bhella, P. S., Shibata, S., Palmer, D., and Levine, B. D. (2010). Cardiovascular effects of 1 year of progressive and vigorous exercise training in previously sedentary individuals older than 65 years of age. *Circulation* 122, 1797–1805.

Hanon, O., Haulon, S., Lenoir, H., Seux, M.-L., Rigaud, A.-S., Safar, M., Girerd, X., and Forette, F. (2005). Relationship between arterial stiffness and cognitive function in elderly subjects with complaints of memory loss. *Stroke* 36, 2193–2197.

Hartog, J. W. L., Willemsen, S., van Veldhuisen, D. J., Posma, J. L., van Wijk, L. M., Hummel, Y. M., Hillege, H. L., and Voors, A. A. (2011). Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure. *Eur. J. Heart Fail.* 13, 899–908.

Iakovou, I., Karpanou, E. A., Vyssoulis, G. P., Toutouzas, P. K., and Cokkinos, D. V. (2004). Assessment of arterial ventricular coupling changes in

patients under therapy with various antihypertensive agents by a non-invasive echocardiographic method. *Int. J. Cardiol.* 96, 355–360.

Kass, D. A. (2003). Getting better without AGE: new insights into the diabetic heart. *Circ. Res.* 92, 704–706.

Kass, D. A. (2005). Ventricular arterial stiffening: integrating the pathophysiology. *Hypertension* 46, 185–193.

Kass, D. A., Marino, P., Maughan, W. L., and Sagawa, K. (1989). Determinants of end-systolic pressure-volume relations during acute regional ischemia in situ. *Circulation* 80, 1783–1794.

Kawaguchi, M., Hay, I., Fetics, B., and Kass, D. A. (2003). Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. *Circulation* 107, 714–720.

Kelly, R., Tunin, R., and Kass, D. (1992a). Effect of reduced aortic compliance on cardiac efficiency and contractile function of in situ canine left ventricle. *Circ. Res.* 71, 490–502.

Kelly, R. P., Ting, C. T., Yang, T. M., Liu, C. P., Maughan, W. L., Chang, M. S., and Kass, D. A. (1992b). Effective arterial elastance as index of arterial vascular load in humans. *Circulation* 86, 513–521.

Kitzman, D. W., Higginbotham, M. B., Cobb, F. R., Sheikh, K. H., and Sullivan, M. J. (1991). Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. *J. Am. Coll. Cardiol.* 17, 1065–1072.

Klapholz, M., Maurer, M., Lowe, A. M., Messineo, F., Meisner, J. S., Mitchell, J., Kalman, J., Phillips, R. A., and Steingart, R. (2004). Hospitalization for heart failure in the presence of a normal left ventricular ejection fraction: results of the New York heart failure registry. *J. Am. Coll. Cardiol.* 43, 1432–1438.

Lakatta, E. G. (1993). Cardiovascular regulatory mechanisms in advanced age. *Physiol. Rev.* 73, 413–467.

Lakatta, E. G. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part III: cellular and molecular clues to heart and arterial aging. *Circulation* 107, 490–497.

Lakatta, E. G., and Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part II: the aging heart in health: links to heart disease. *Circulation* 107, 346–354.

Lam, C. S., Roger, V. L., Rodeheffer, R. J., Bursi, F., Borlaug, B. A., Ommen, S. R., Kass, D. A., and Redfield, M. M. (2007). Cardiac structure and ventricular-vascular function in persons with heart failure and preserved ejection fraction from Olmsted County, Minnesota. *Circulation* 115, 1982–1990.

Little, W. C., and Cheng, C. P. (1991). Left ventricular-arterial coupling in conscious dogs. *Am. J. Physiol.* 261, H70–H76.

Little, W. C., and Cheng, C. P. (1993). Effect of exercise on left ventricular-arterial coupling assessed in the pressure-volume plane. *Am. J. Physiol.* 264, H1629–H1633.

Lloyd-Jones, D., Adams, R. J., Brown, T. M., Carnethon, M., Dai, S., De Simone, G., Ferguson, T. B., Ford, E., Furie, K., Gillespie, C., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, P. M., Howard, V., Kissela, B., Kittner, S., Lackland, D., Lisabeth, L., Marelli, A., McDermott, M. M., Meigs, J., Mozaffarian, D., Mussolino, M., Nichol, G., Roger, V., Rosamond, W., Sacco, R., Sorlie, P., Stafford, R., Thom, T., Wasserthiel-Smoller, S., Wong, N. D., Wylie-Rosett, J., and American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2009). Heart disease and stroke statistics – 2010 update. A report from the American Heart Association. *Circulation* 121, e1–e170.

Maggioni, A. P., Maseri, A., Fresco, C., Franzosi, M. G., Mauri, F., Santoro, E., and Tognoni, G. (1993). Age-related increase in mortality among patients with first myocardial infarctions treated with thrombolysis. The Investigators of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI-2). *N. Engl. J. Med.* 329, 1442–1448.

Masoudi, F. A., Havranek, E. P., Smith, G., Fish, R. H., Steiner, J. F., Ordin, D. L., and Krumholz, H. M. (2003). Gender, age, and heart failure with preserved left ventricular systolic function. *J. Am. Coll. Cardiol.* 41, 217–223.

Maurer, M. S., King, D. L., El-Khoury Rumbarger, L., Packer, M., and Burkhoff, D. (2005). Left heart failure with a normal ejection fraction: identification of different pathophysiologic mechanisms. *J. Card. Fail.* 11, 177–187.

Mayet, J., and Hughes, A. (2003). Cardiac and vascular pathophysiology in hypertension. *Heart* 89, 1104–1109.

Murgo, J. P., Westerhof, N., Giolma, J. P., and Altobelli, S. A. (1980). Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation* 62, 105–116.

Najjar, S. S., Schulman, S. P., Gerstenblith, G., Fleg, J. L., Kass, D. A., O'Connor, F., Becker, L. C., and Lakatta, E. G. (2004). Age and gender affect ventricular-vascular coupling during aerobic exercise. *J. Am. Coll. Cardiol.* 44, 611–617.

Najjar, S. S., Scuteri, A., and Lakatta, E. G. (2005). Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 46, 454–462.

Nichols, W. W., Nicolini, F. A., and Pepine, C. J. (1992). Determinants of isolated systolic hypertension in the elderly. *J. Hypertens.* 10, 573.

Ogawa, T., Spina, R. J., Martin, W. H. III, Kohrt, W. M., Schechtman, K. B., Holloszy, J. O., and Ehsani, A. A. (1992). Effects of aging, sex, and physical training on cardiovascular responses to exercise. *Circulation* 86, 494–503.

Olivetti, G., Giordano, G., Corradi, D., Melissari, M., Lagrasta, C., Gambert, S. R., and Anversa, P. (1995). Gender differences and aging: effects on the human heart. *J. Am. Coll. Cardiol.* 26, 1068–1079.

Osranek, M., Eisenach, J. H., Khandheria, B. K., Chandrasekaran, K., Seward, J. B., and Belohlavek, M. (2008). Arterioventricular coupling and ventricular efficiency after antihypertensive therapy. *Hypertension* 51, 275–281.

Otsuki, T., Maeda, S., Iemitsu, M., Saito, Y., Tanimura, Y., Ajisaka, R., and Miyauchi, T. (2006). Contribution of systemic arterial compliance and systemic vascular resistance to effective arterial elastance changes during exercise in humans. *Acta Physiol. (Oxf.)* 188, 15–20.

Owan, T. E., Hodge, D. O., Herges, R. M., Jacobsen, S. J., Roger, V. L., and Redfield, M. M. (2006). Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N. Engl. J. Med.* 355, 251–259.

Redfield, M. M., Jacobsen, S. J., Borlaug, B. A., Rodeheffer, R. J., and Kass, D. A. (2005). Age- and gender-related ventricular-vascular stiffening: a community-based study. *Circulation* 112, 2254–2262.

Rider, O. J., Petersen, S. E., Francis, J. M., Ali, M. K., Hudsmith, L. E., Robinson, M. R., Clarke, K., and Neubauer, S. (2011). Ventricular hypertrophy and cavity dilatation in relation to body mass index in women with uncomplicated obesity. *Heart* 97, 203–208.

Rinder, M. R., Miller, T. R., and Ehsani, A. A. (1999). Effects of endurance exercise training on left ventricular systolic performance and ventriculoarterial coupling in patients with coronary artery disease. *Am. Heart J.* 138, 169–174.

Saba, P. S., Ganau, A., Devereux, R. B., Pini, R., Pickering, T. G., and Roman, M. J. (1999). Impact of arterial elastance as a measure of vascular load on left ventricular geometry in hypertension. *J. Hypertens.* 17, 1007–1015.

Sagawa, K. (1978). The ventricular pressure-volume diagram revisited. *Circ. Res.* 43, 677–687.

Sasayama, S., and Asanoi, H. (1991). Coupling between the heart and arterial system in heart failure. *Am. J. Med.* 90, 14S–18S.

Schulman, S. P., Fleg, J. L., Goldberg, A. P., Busby-Whitehead, J., Hagberg, J. M., O'Connor, F. C., Gerstenblith, G., Becker, L. C., Katzel, L. I., Lakatta, L. E., and Lakatta, E. G. (1996). Continuum of cardiovascular performance across a broad range of fitness levels in healthy older men. *Circulation* 94, 359–367.

Segers, P., Stergiopulos, N., and Westerhof, N. (2002). Relation of effective arterial elastance to arterial system properties. *Am. J. Physiol. Heart Circ. Physiol.* 282, H1041–H1046.

Setaro, J. F., Zaret, B. L., Schulman, D. S., Black, H. R., and Soufer, R. (1990). Usefulness of verapamil for congestive heart failure associated with abnormal left ventricular diastolic filling and normal left ventricular systolic performance. *Am. J. Cardiol.* 66, 981–986.

Stamler, R., Ford, C. E., and Stamler, J. (1991). Why do lean hypertensives have higher mortality rates than other hypertensives? Findings of the Hypertension Detection and Follow-up Program. *Hypertension* 17, 553–564.

Stamler, R., Stamler, J., Riedlinger, W. F., Algera, G., and Roberts, R. H. (1978). Weight and blood pressure. Findings in hypertension screening of 1 million Americans. *JAMA* 240, 1607–1610.

Sunagawa, K., Maughan, W. L., Burkhoff, D., and Sagawa, K. (1983).

Left ventricular interaction with arterial load studied in isolated canine ventricle. *Am. J. Physiol.* 245, H773–H780.

Toto-Moukouo, J. J., Achimastos, A., Asmar, R. G., Hugues, C. J., and Safar, M. E. (1986). Pulse wave velocity in patients with obesity and hypertension. *Am. Heart J.* 112, 136–140.

Zebekakis, P. E., Nawrot, T., Thijs, L., Balkestein, E. J., van der Heijden-Spek, J., Van Bortel, L. M., Struijker-Boudier, H. A., Safar, M. E., and Staessen, J. A. (2005). Obesity is associated with increased arterial stiffness from adolescence until old age. *J. Hypertens.* 23, 1839–1846.

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Mechanisms involved in the aging-induced vascular dysfunction

Mariam El Assar[1], Javier Angulo[1,2], Susana Vallejo[3], Concepción Peiró[3], Carlos F. Sánchez-Ferrer[3] and Leocadio Rodríguez-Mañas[1,4]*

[1] *Fundación para la Investigación Biomédica, Hospital Universitario de Getafe, Madrid, Spain*
[2] *Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain*
[3] *Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain*
[4] *Servicio de Geriatría, Hospital Universitario de Getafe, Madrid, Spain*

Edited by:
Elisabet Vila, Autonomous University of Barcelona, Spain

Reviewed by:
Anthony Donato, University of Utah, USA
Junichi Sadoshima, University of Medicine and Dentistry of New, USA
Maria Alvarez De Sotomayor, Universidad de Sevilla, Spain
Anna Csiszar, University of Oklahoma, USA

***Correspondence:**
Leocadio Rodríguez-Mañas, Jefe de Servicio de Geriatría del Hospital Universitario de Getafe, Ctra de Toledo, Km 12,500, 28905, GETAFE, Madrid, España.
e-mail: lrodriguez.hugf@salud.madrid.org

Vascular aging is a key process determining health status of aged population. Aging is an independent cardiovascular risk factor associated to an impairment of endothelial function, which is a very early and important event leading to cardiovascular disease. Vascular aging, formerly being considered an immutable and inexorable risk factor, is now viewed as a target process for intervention in order to achieve a healthier old age. A further knowledge of the mechanisms underlying the age-related vascular dysfunction is required to design an adequate therapeutic strategy to prevent or restore this impairment of vascular functionality. Among the proposed mechanisms that contribute to age-dependent endothelial dysfunction, this review is focused on the following aspects occurring into the vascular wall: (1) the reduction of nitric oxide (NO) bioavailability, caused by diminished NO synthesis and/or by augmented NO scavenging due to oxidative stress, leading to peroxynitrite formation ($ONOO^-$); (2) the possible sources involved in the enhancement of oxidative stress; (3) the increased activity of vasoconstrictor factors; and (4) the development of a low-grade pro-inflammatory environment. Synergisms and interactions between all these pathways are also analyzed. Finally, a brief summary of some cellular mechanisms related to endothelial cell senescence (including telomere and telomerase, stress-induced senescence, as well as sirtuins) are implemented, as they are likely involved in the age-dependent endothelial dysfunction, as well as in the lower vascular repairing capacity observed in the elderly. Prevention or reversion of those mechanisms leading to endothelial dysfunction through life style modifications or pharmacological interventions could markedly improve cardiovascular health in older people.

Keywords: endothelial dysfunction, aging, cardiovascular disease, nitric oxide, oxidative stress, inflammation, senescence

The concept that a man (or a woman) is as old as his arteries are, coined by Georges Canabis and reformulated by Sir William Osler more than 100 years ago, can be considered nowadays quite a valid approximation. Vascular aging represents the process that more importantly impacts on the health status of elderly people. The aging process is the main risk factor for the development of cardiovascular diseases (CVD), explaining 50% of clinical CVD present in the elderly. In fact, aging is associated with complex structural and functional changes in the vasculature independently of other risk factors, such as hypertension, diabetes, or hypercholesterolemia (Barodka et al., 2011). The old vision of vascular aging considers it as an inevitable process generated through a series of inexorable mechanisms. These thoughts have evolved to the current position that assumes that the knowledge of the molecular mechanisms involved in the age-related vascular dysfunction will contribute to understand the extent and nature of these alterations. Therefore, strategies to attenuate the effect of aging in the vasculature could be potentially developed, preserving the quality of life, and alleviating CVD in the elderly population (Najjar et al., 2005). Furthermore, preservation of vascular function in aging should not only reduce deaths and disabilities secondary to cardiovascular events but it also should influence other aspects of the aging process that leads to loss of function and/or disability. In fact, loss of cardiovascular health is associated to increased risk of defective motor capacity and cognitive frailty (Panza et al., 2006; Kim et al., 2011; Watson et al., 2011). Thus, it seems to be now quite clear that robustness in advanced age cannot be achieved without preservation of vascular function. Dominant aspects of vascular aging include increased arterial stiffness, dilation of central elastic arteries, and endothelial dysfunction (Kotsis et al., 2011), although there is increasing evidence suggesting that all these processes are closely related (van Bussel et al., 2011). The present work will briefly review the most important mechanisms that have been related to the development of the age-dependent endothelial dysfunction.

VASCULAR DYSFUNCTION ASSOCIATED TO THE AGING PROCESS

Vascular aging is characterized by functional and structural changes of the endothelium and smooth muscle cells that form the vascular wall, as well as by alterations of the communication routes

between these two cell layers. Functional disturbances, together with other factors associated with aging, will contribute to the development of structural alterations, which in turn contribute to vascular stiffness and to an additional impairment of the endothelial function. Moreover, since endothelial dysfunction is associated with the major causes of morbidity and mortality, the maintenance of a correct function of this vascular layer is thought to be an essential determinant of healthy aging (Virdis et al., 2010; Toda, 2012). The evidence showing the presence of endothelial dysfunction in the aged vasculature is very important since the late 1980s in animal models and since the middle 1990s in humans. In particular, the reduction in the endothelium-dependent vasodilatations has been consistently described both *in vitro* and *in vivo* in different vascular beds from old animals and elderly humans (Matz and Andriantsitohaina, 2003; Brandes et al., 2005; Rodriguez-Mañas et al., 2009; Toda, 2012). These evidences demonstrated that aging is an independent factor associated with endothelial dysfunction even in the absence of other cardiovascular risk factors (Rodriguez-Mañas et al., 2009). The impairment of endothelial function is a progressive phenomenon starting in the middle age and, at present, it is considered as one of the main mechanisms by which aging increases the risk of CVD and the development of atherosclerosis in humans. Therefore, those approaches aimed to preserve or improve the endothelial function would be fundamental for the prevention of vascular diseases in the elderly. The reported scientific evidences indicate that the pathogenesis of the age-dependent endothelial dysfunction is clearly multifactorial, with several pathophysiological mechanisms contributing to the functional deterioration of vascular endothelial cells (**Figure 1**). These pathways are briefly summarized as follows.

ALTERATION OF THE NITRIC OXIDE (NO) PATHWAY

NO is the main vasodilator produced by the endothelium and exerts a protective role on the vessel wall. The reduction of NO availability deeply disturbs the vascular homeostasis, being involved in the development of hypertension, atherosclerosis, or diabetic vasculopathy (Sagach et al., 2006). NO is synthesized from L-arginine by the enzyme NO synthase (NOS). There are three known NOS isoforms: the constitutive endothelial (eNOS) and neuronal (nNOS) isoforms, producing regulated NO involved in regulatory or signaling pathways, and the inducible (iNOS) isoform, leading to massive NO synthesis and related with inflammatory responses. Aging is also associated with a reduction in the NO bioavailability, which is the result of the dynamic balance between its synthesis and degradation. Reduced NO production may be due to: (1) a deficiency in NOS substrates and cofactors; (2) the presence of endogenous eNOS inhibitors; and (3) a lower expression and/or activity of eNOS. On the other hand, enhanced NO degradation may be mostly due to excessive amounts of reactive oxygen species (ROS), such as superoxide anions ($O_2^{-\bullet}$) that quench NO hampering its functional activities.

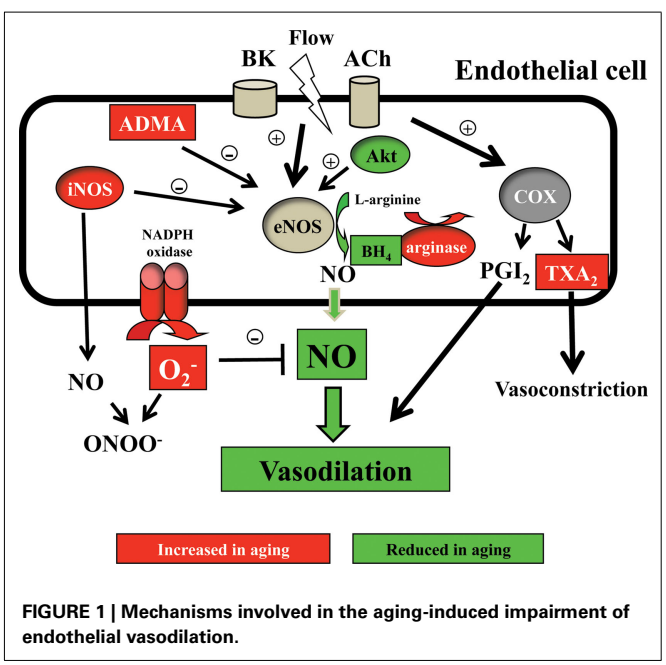

FIGURE 1 | Mechanisms involved in the aging-induced impairment of endothelial vasodilation.

DECREASE IN L-ARGININE AVAILABILITY

The reduction of available concentration of L-arginine to be used as eNOS substrate in aging was based on one study suggesting an improvement in endothelial function in older subjects after oral administration of L-arginine (Bode-Boger et al., 2003). However, this has not been further confirmed, as no significant improvement of the impaired flow-mediated dilation in the old subjects group has been observed after the intra-braquial infusion of L-arginine, despite a 23-fold increase of its plasmatic concentrations (Gates et al., 2007). From a biochemical point of view, a reduction in the availability of the substrate is difficult to sustain because the plasmatic concentration of L-arginine is more than one order of magnitude higher than the substrate concentration required for the optimal function of the enzyme. Although a lower availability could be explained by a reduction in the transport of L-arginine to endothelial cell, there is evidence showing that age-related deterioration of endothelial function is not associated to a change in the transport of L-arginine (Ahlers et al., 2004). Other possible explanation for the lower availability of L-arginine with aging could be related to an increased expression and/or activity of arginase, the enzyme that degrades L-arginine, leading to a decrease in substrate availability for eNOS and the consequent reduction of NO synthesis (Santhanam et al., 2008). In rats, an increase in the arginase-1 activity related to age-related endothelial dysfunction has also been proved by the use of arginase inhibitors, which restore NO-mediated vasodilation (Santhanam et al., 2007). Recovering of vasodilation after arginase inhibition in elderly subjects also supports a role for this enzyme in the endothelial dysfunction associated to human vascular aging (Holowatz et al., 2006).

THE ENDOGENOUS INHIBITOR OF NOS (ADMA)

The synthesis of NO is blocked by the inhibition of the NOS active site with guanidine-substituted analogs of L-arginine such as asymmetric dimethylarginine (ADMA). ADMA is a naturally occurring amino acid found in plasma and various tissues (Yamagishi and Matsui, 2011). An enhanced production of ADMA has been proposed to be a cardiovascular risk factor linked to different pathologies involving vascular disease and hypertension (Cooke, 2005). A possible role for this compound has been proposed in

the physiological process of aging, as a positive correlation has been reported in healthy subjects between the plasmatic levels of ADMA and age (Schulze et al., 2005). Moreover, it has been described an ADMA accelerating effect of endothelial cells senescence (Bode-Boger et al., 2005). However, other authors could not find any relationship between the endothelial expression of ADMA and the development of the age-related impairment of endothelium-dependent vasodilations (Gates et al., 2007).

REDUCTION OF TETRAHYDROBIOPTERIN (BH_4)

Tetrahydrobiopterin (BH_4) is a cofactor essential for NOS activity. Substantial evidence linked BH_4 deficiency as a condition leading eNOS to produce less NO with the consequent deterioration of endothelial function (Vasquez-Vivar et al., 2003). In humans, the participation of BH_4 deficiency in age-related endothelial dysfunction has been demonstrated, since the infusion of BH_4 restores the impaired endothelium-dependent vasodilation (Higashi et al., 2006).

ENDOTHELIAL NOS

There is no doubt about the existence of a deficit in the vascular NO bioavailability associated with aging, but a high number of studies evaluating the expression of eNOS in different vascular beds of aged animals have not displayed definitive results. While some authors have not observed changes of eNOS expression in mesenteric arteries (Sun et al., 2004), others have reported significant increases in the aorta (Cernadas et al., 1998) or the mesenteric arteries from old rats (Briones et al., 2005). The analysis of the eNOS mRNA levels has shown either an increase (Barton et al., 1997) or a decrease (Tang and Vanhoutte, 2008) in the aorta of aged rats. In the mesenteric microvessels from healthy young and old humans, no age-dependent changes have been detected concerning eNOS mRNA levels (Rodriguez-Mañas et al., 2009). Moreover, human endothelial cells from brachial artery and peripheral veins do not differ significantly with age in the eNOS expression (Donato et al., 2009).

In addition to transcriptional and translational regulations, eNOS presents a significant regulation at post-translational level involving PI3 kinase/Akt-dependent phosphorylation at Ser 1177, resulting in increased activity of eNOS. Although reduced Akt-dependent phosphorylation of eNOS in micro- and macrovasculature of aged animals has been demonstrated (Soucy et al., 2006; LeBlanc et al., 2008), the involvement of reduced eNOS phosphorylation in human vascular aging needs to be confirmed.

ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR

Further to the release of NO and prostacyclin, the endothelium controls vascular tone by causing hyperpolarization of underlying smooth muscle cells. This process is attributed to the endothelium-derived hyperpolarizing factor (EDHF). However, this generic term can refer to different mechanisms (with vascular territory- and species-dependent variability) including arachidonate metabolites derived from COX, lipoxygenase, or cytochrome P450 oxygenase activities, H_2O_2, CO, H_2S, and several peptides that can be released by endothelial cells. Furthermore, vasodilation can be generated by Ca^{2+}-induced hyperpolarization of endothelial cell causing the opening of Ca^{2+}-activated K^+-channels (K_{Ca}) that produce hyperpolarization and relaxation of smooth muscle through myo-endothelial gap junction-mediated electrical coupling or by K^+-efflux from the endothelial K_{Ca} and subsequent activation of inwardly rectifier K^+-channels or Na^+/K^+-ATPase in smooth muscle (Dora et al., 2008).

Contribution of EDHF to endothelial vasodilation of human arteries has been demonstrated in different vascular regions such as coronary arteries obtained from failing hearts at transplant surgery (Nakashima et al., 1993), omental and subcutaneous microvessels (Pascoal and Umans, 1996; MacKenzie et al., 2008), and penile resistance arteries (Angulo et al., 2003). Impaired EDHF-mediated vasodilation has been observed in mesenteric (Goto et al., 2000) and renal arteries (Long et al., 2005) from aged rats. In contrast, it has been reported that EDHF partially compensates for the loss of NO- and prostacyclin-mediated cutaneous vasodilation in old mice (Gaubert et al., 2007). These conflicting results could reflect either species differences or regional heterogeneity of vascular regulation with aging. In human gastroepiploic arteries, EDHF-induced relaxation inversely correlated with the age of the patients from whom the arteries were obtained (Urakami-Harasawa et al., 1997).

Age-induced decline in EDHF-mediated vasodilation seems to be related to an up-regulated renin-angiotensin system (RAS) since chronic angiotensin converting enzyme (ACE) inhibition, as well as angiotensin-II type 1 (AT1) receptor blockade, recovered EDHF-mediated responses in mesenteric arteries from old rats (Goto et al., 2004). In this sense, the impairment of EDHF-induced vasodilation in mesenteric arteries from middle aged rats (46 weeks old) is associated with increased AT1 receptor expression and reduced angiotensin-II type 2 (AT2) receptor and ACE expression, which were prevented by antioxidant treatment with red wine polyphenols (Idris Khodja et al., 2012). As occurs in angiotensin II (Ang II)-induced hypertension (Hilgers and Webb, 2007), age-related impairment of the EDHF component of endothelial vasodilation is likely due to reduced expression of small and intermediate conductance K_{Ca} (SK_{Ca} and IK_{Ca}; Idris Khodja et al., 2012). A decline on the responses mediated by large conductance K_{Ca} (BK_{Ca}) has been reported in coronary arteries from aged rats which can be recovered by exercise training (Albarwani et al., 2010).

CONTRACTILE FACTORS

COX PATHWAY ALTERATION

Cyclooxygenase (COX) plays an important role in the regulation of vascular tone under normal conditions by the synthesis of different vasoactive factors, which are particularly relevant since both vasodilators (prostacyclin, PGI_2) or vasoconstrictors (thromboxane A2, TXA_2) could be produced, those factors being in tight balance. During aging, a shift in the balance in favor of increased contractile factors occurs and, therefore, endothelium-dependent contractions increase. In humans, the lack of prostaglandin (PGI_2)-mediated vasodilatation has been reported *in vivo* (Schrage et al., 2007) and in mesenteric microvessels *in vitro* (Rodriguez-Mañas et al., 2009), while the existence of COX-derived vasoconstrictor factors associated with aging has been described *in vivo* by using plethysmographic studies (Taddei et al., 1997; Vanhoutte et al., 2005) and *in vitro* studying

isolated mesenteric arteries (Rodriguez-Mañas et al., 2009). The implication of the COX pathway in the endothelial dysfunction associated with aging is reinforced by the improvement of the impaired endothelium-dependent relaxations produced by TP-receptor antagonists (Rodriguez-Mañas et al., 2009). In humans, the nature of the endothelium-dependent vasoconstrictor factor is not entirely determined but thromboxane A_2 and prostaglandin H_2 are clear candidates. In aged hamsters, endothelial-dependent contractions are mediated by $PGF_{2\alpha}$ produced by COX-2 (Wong et al., 2009). A role for $O_2^{-\bullet}$ is also possible, as these ROSs have been described as COX activity-derived endothelium-dependent vasoconstrictors (Vanhoutte et al., 2005).

No consensus has so far been established regarding the COX isoform responsible for the age-related vasoconstrictions (Matz and Andriantsitohaina, 2003). The pre-incubation with the selective COX-1 inhibitor valeryl salicylate reduced the contractile response observed in the femoral artery form old rats (Shi et al., 2008), while the COX-2 inhibitor NS-398 improved endothelial dysfunction in the aged rat aorta and mesenteric arteries (de Sotomayor et al., 2005). However, other authors did not find an improvement of endothelial dysfunction by this same compound in mesenteric arteries from aged rats (Matz et al., 2000).

Controversial results have been also reported concerning the COX protein expression in the vasculature of old animals (Heymes et al., 2000; Stewart et al., 2000; Briones et al., 2005; Shi et al., 2008). No differences related to age have been detected in the expression of mRNA for COX-1 and COX-2 isoforms in human mesenteric microvessels (Rodriguez-Mañas et al., 2009). However, protein expression may be not the only factor accounting for COX-mediated effects, as the existence of post-translational changes in the activity of these enzymes cannot be ruled out. Thus, there is an important regulation of COX activity by NO and $ONOO^-$ (Upmacis et al., 2006). Furthermore, a physiological binding interaction between COX-2 and iNOS has recently been reported, bringing NO or $ONOO^-$ to activate COX-2 in a synergistic molecular interactions between these two inflammatory pathways (Kim et al., 2005).

THE RENIN-ANGIOTENSIN SYSTEM

The RAS is critical for cardiovascular control, impacting normal physiology and disease pathogenesis. Its major actions are mediated by Ang II, acting through its AT1 and AT2 receptors (Stegbauer and Coffman, 2011). At present, it is clear that both the increased generation of cellular ROS and activation of redox-sensitive signaling cascades are critical events involved in Ang II actions (Touyz, 2003). After binding to its AT1 receptors, Ang II triggers intracellular superoxide production by activating NAD(P)H (Kimura et al., 2005) and uncoupling endothelial NOS (eNOS; Mollnau et al., 2002). Under normal physiological conditions, Ang II-mediated signaling pathways are closely regulated. However, increased renin-angiotensin system activity is implicated in several vascular disorders and there is evidence for increased vascular expression of Ang II and ACE with aging (Rajagopalan et al., 2002; Wang et al., 2003). Furthermore a role for the angiotensin system in the vascular aging-related endothelial dysfunction has been observed. Indeed Ang II is a potent inducer of endothelial dysfunction and vascular oxidative stress (Idris Khodja et al., 2012). Moreover, treatment of rats with either an ACE inhibitor or an AT1 receptor antagonist improved endothelial dysfunction (mediated by the NO and the EDHF component) in aged blood vessels, in part, by decreasing oxidative stress (Goto et al., 2000; Kansui et al., 2002; Mukai et al., 2002). A recent study done by Benigni and associates showed that mice lacking AT1A receptors had prolonged life span compared to genetically matched wild-type controls; this enhanced longevity was associated with improved cardiovascular morphology, reduced ROS production, attenuated mitochondrial loss, and enhanced levels of nicotinamide phosphoribosyltransferase (Nampt) and sirtuin-3 (Sirt3; Benigni et al., 2009). However, losartan, an AT1 receptor antagonist, had no effect on brachial flow-mediated dilation in older adults, despite reducing blood pressure and circulating inflammatory markers (Rajagopalan et al., 2002) while other AT1 receptor antagonist, valsartan, improved vascular compliance in healthy normotensive elderly individuals without affecting flow-mediated dilation (Rajagopalan et al., 2006).

ENDOTHELIN-1

Endothelin-1 (ET-1) is the most potent vasoconstrictor protein synthesized and released by endothelial cells (Yanagisawa et al., 1988). ET-1 exerts vascular actions through activation of two distinct ET-1 receptor subtypes: ET_A, localized exclusively in vascular smooth muscle, and ET_B which are expressed in smooth muscle as well as endothelium (Masaki et al., 1991). Both ET_A and ET_B receptors are coupled to phospholipase C activation leading to increased cytosolic calcium and myosin kinase phosphorylation that results in long-lasting contraction of smooth muscle (Lüscher and Barton, 2000) and vasoconstriction (Seo et al., 1994; Haynes et al., 1995). Activation of ET_B receptors by ET-1 would increase intracellular calcium in endothelial cell promoting eNOS activation and vasodilation (Tsukahara et al., 1994).

Increased contractile responsiveness to ET-1 has been demonstrated in arteries from aged rats (Donato et al., 2005; Korzick et al., 2005) while ET_A blockade has been shown to reverse the impairment of endothelium-dependent relaxation in carotid arteries from old mice (Donato et al., 2009). Aging is also associated with elevated plasma concentrations of ET-1 in humans (Maeda et al., 2003; Donato et al., 2009). In humans, ET_A/ET_B antagonists produced larger leg blood flow increases in healthy sedentary old subjects than in young subjects, suggesting elevated ET-1-mediated vasoconstrictor tone in peripheral arteries from aged humans (Thijssen et al., 2007). Similarly, forearm vasoconstriction induced by ET-1 was blunted in older sedentary subjects while ET_A blockade only increased resting forearm blood flow in old sedentary subjects but not in young (Van Guilder et al., 2007). Furthermore, endothelium-dependent forearm vasodilation in older subjects negatively correlates with the expression of ET-1 in endothelial cells (Donato et al., 2009). Interestingly, exercise training decreases plasma ET-1 in old women (Maeda et al., 2003) and reduces ET-1-mediated increase in vascular tone in elderly subjects (Thijssen et al., 2007; Van Guilder et al., 2007).

OXIDATIVE STRESS

It is now clear that ROS are physiologically produced into the vascular wall in a controlled regulated manner. Under normal conditions, the endogenous antioxidant defense mechanisms, both

enzymatic, such as the manganese and copper/zinc superoxide dismutase (SOD), glutathione peroxidase (GP), catalase, and non-enzymatic, vitamin C, vitamin E, uric acid, inactivate ROS and repair the possible developed tissular damage. Indeed, together with NO, physiological ROS function as cell signaling initiators by their ability to introduce reversible post-translational protein modifications (Valko et al., 2007). Nevertheless, under pathological conditions, increased ROS levels lead to accumulation of damaged/misfolded proteins, increased mutagenesis rate, inflammation, and endothelial dysfunction (Malinin et al., 2011). This enhanced oxidative stress can consequence of either an increased ROS production and/or a reduction in the antioxidant defenses.

ROS AND VASCULAR DAMAGE

The implication of oxidative stress in the genesis of vascular damage has been described in several pathologies, including diabetes or hypertension, and also in aging process (Ungvari et al., 2008). A large body of evidence indicates that oxidative stress and ROS production are increased during the aging process (Ungvari et al., 2010b). The results obtained from studies in which antioxidant defense levels were determined were contradictory. While some authors observed an age-related decrease in the SOD and GP activities and a decline of plasma antioxidant capacity (Goraca, 2004), others found an increase in the plasma activity of SOD and GP (Mecocci et al., 2000). The first consequence of the increase in $O_2^{-\bullet}$ levels is a decrease of the NO availability, leading to a decrease in the endothelium-dependent relaxations (Pacher et al., 2007). In human mesenteric microvessels, this age–related endothelial dysfunction is improved by scavenging $O_2^{-\bullet}$with SOD or TEMPOL, which correlates with an increase in $O_2^{-\bullet}$ levels in these same vessels (Rodriguez-Mañas et al., 2009).

NO INACTIVATION

Accelerated degradation of NO by oxidative stress and especially by $O_2^{-\bullet}$ is one of the most widely accepted mechanisms involved in the alteration of the NO pathway. The diffusion-controlled reaction between NO and $O_2^{-\bullet}$ leads to the formation of the peroxynitrite anion ($ONOO^-$; Yang et al., 2009a). Thus, the NO produced in vascular cells from eNOS or iNOS reacts rapidly with $O_2^{-\bullet}$ to form $ONOO^-$, which is termed as a reactive nitrogen specie (RNS) because of its high reactivity with proteins, DNA, and lipids. Unlike $O_2^{-\bullet}$, $ONOO^-$ can easily penetrate into the cell causing oxidative modifications of macromolecules, especially lipids, DNA, and proteins via direct oxidative reactions through the nitrosylation of tyrosine and cysteine residues or via indirect radical-mediated mechanisms. These reactions trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury, committing cells to necrosis or apoptosis. Indeed, nitrotyrosine levels reflect the impact of $ONOO^-$ on proteins and constitute a cellular marker of oxidative stress. There are convincing data showing a substantially enhanced cardiovascular $ONOO^-$ formation during aging process (Francia et al., 2004), whereas increased nitrosative stress has been demonstrated in arteries from aged animals (van der Loo et al., 2000). Higher levels of nitrotyrosine have been found in endothelial cells obtained from brachial artery from aged subjects, while nitrotyrosine levels were inversely related to flow-mediated dilation (Donato et al., 2007). In human mesenteric microvessels, an age-dependent formation of nitrotyrosine has been reported, while the endothelial dysfunction associated to elderly is partially restored by scavenging $ONOO^-$ with uric acid (Rodriguez-Mañas et al., 2009). All these evidences suggest that the relevant changes related to age that are observed in the vascular wall are driven, at least partially, by these highly reactive molecules.

SOURCES OF OXIDATIVE STRESS

There are several main systems proposed to be the sources for the ROS increase production in the human vasculature, namely NADPH oxidase, xanthine oxidase, uncoupled NO synthase, and the mitochondrial respiratory chain (Cai and Harrison, 2000; Loscalzo, 2000; Guzik et al., 2002; Brandes et al., 2005; Lassegue and Griendling, 2010). Increased expression of the Nox-4 subunit of NADPH oxidase has been demonstrated in mesenteric microvessels from aged humans without other known cardiovascular risk factors; furthermore, the NADPH oxidase-derived ROS produced in these arteries impact vascular function, since the NADPH oxidase inhibitor apocynin improves the age-related endothelial dysfunction (Rodriguez-Mañas et al., 2009). In the aorta from aged rats, other authors noted that oxidative stress associated with aging might be related to the increased activity of the enzyme xanthine oxidase (Newaz et al., 2006). However, the role of this enzyme as a source of oxidative stress in vascular aging has not been confirmed in humans; thus, its expression in vascular endothelial cells from older subjects is not altered while *in vivo* inhibition of this enzyme with allopurinol does not improve aged-dependent endothelial dysfunction (Eskurza et al., 2006).

NOS uncoupling is a possible source for $O_2^{-\bullet}$ in vascular aging (Cai and Harrison, 2000; Brandes et al., 2005), which has been described after a decline in BH_4 bioavailability that is consequence of its oxidation to BH_2 by $O_2^{-\bullet}$ or peroxynitrite ($ONOO^-$; Milstien and Katusic, 1999; Laursen et al., 2001; Schiffrin, 2008). As BH_4 efficiently inhibits superoxide generation from the heme group at the oxygenase domain of eNOS, it can act as O_2^-a redox switch in the catalytic mechanism of the enzyme, which may have important consequences in the physiology of the endothelium (Vasquez-Vivar et al., 2003). High $O_2^{-\bullet}$ not only reduces NO bioavailability, but the ROS and RNS also oxidize proteins and lead to eNOS uncoupling (Munzel et al., 2005). Moreover, $ONOO^-$ further uncouples eNOS by oxidizing the zinc-thiolate complex (Zou et al., 2002). Mammalian target of rapamycin (mTOR)/SK61 signaling activation has been demonstrated to cause eNOS uncoupling in HUVEC and endothelial dysfunction in aged rats (Rajapakse et al., 2011). Interestingly, although most reports point to eNOS as the isoform that can be uncoupled, producing O_2^- (Brandes et al., 2005), the inducible iNOS isoform can also serve as a source of in the absence of sufficient substrate or BH_4 (Loscalzo, 2000). In addition, treatment of isolated mesenteric arteries from healthy aged subjects with BH_4 results in significant recovery of dysfunctional endothelium-dependent relaxations, suggesting that the enhancement of the intracellular levels of BH_4 would prevent NOS uncoupling and $O_2^{-\bullet}$ production, in this case likely involving the

inflammatory inducible iNOS isoform (Rodriguez-Mañas et al., 2009).

Another source for oxidative stress in the aging process is mitochondria. Under physiological conditions, the mitochondria continually produce large amounts of $O_2^{-\bullet}$ and H_2O_2, so that the mitochondrial DNA (mtDNA) is particularly exposed to oxidative damage. In aging there is a reduction in the number of mitochondria and an increase in the generation of dysfunctional proteins, which leads to a depletion in the energy supply and even to an increase in the $O_2^{-\bullet}$ production (Pang et al., 2008). Whatever the mechanism involved in the mitochondrial dysfunction, as it is the case for insulin-resistance (Abbatecola et al., 2011), the decline in mitochondrial dysfunction is able to enhance ROS production and further damage the mtDNA (Cui et al., 2012). An additional important link between mitochondrial oxidative stress and cardiovascular aging is the induction of programmed cell death. The available evidence suggests that age-associated increase in oxidative stress causes an increased rate of endothelial apoptosis (Ungvari et al., 2010b). Furthermore, recent studies show that mitochondria-derived ROS, in addition to causing oxidative damage, play an important role in activating numerous redox-sensitive transcription factors, including NF-κB and AP-1 (Ungvari et al., 2010b).

ANTIOXIDANT THERAPY IN ELDERLY HUMANS

Although some conflicting results showing ineffectiveness of vitamin E ingestion to improve endothelial function in older adults exist (Simons et al., 1999), more recent clinical evidences point to beneficial effects of antioxidant therapy on endothelial dysfunction related to aging. A recent clinical trial has shown the acute reversal of endothelial dysfunction in the elderly after oral administration of an antioxidant cocktail (vitamin C + vitamin E + α-lipoic acid; Wray et al., 2012), confirming previous results showing the recovery of exercise induced vasodilation of brachial artery in elderly men after administration of the same antioxidant cocktail (Donato et al., 2010). Administration of ascorbic acid has been reported to reverse vasoconstriction and improve resting leg blood in healthy older men (Jablonski et al., 2007) as well as to augment reflex cutaneous vasodilation in aged subjects (Holowatz et al., 2006). These clinical evidences demonstrate that oxidative stress may be an important target for recovering endothelial function in aging.

INFLAMMATION

In addition to oxidative stress, the development of the so-called low-grade chronic inflammatory state is another phenomenon linked to the aging process. This has been referred as "inflamm-aging" that could be caused by a continuous antigenic load and stress (Franceschi et al., 2000). Molecular inflammation is described as an underlying mechanism of aging and age-related diseases, which may constitute the link between normal aging and age-related pathological processes. Normal aging ("usual aging") is associated with higher levels of cytokines, especially TNF-α, IL-1β, and members of the super family of IL-6, as well as elevations of C-reactive protein (CRP) and fibrinogen (Ferrucci et al., 2005) that contribute to a pro-inflammatory microenvironment and facilitate the development of vascular dysfunction. Interactions between inflammation and oxidative stress occurs, as different redox-sensitive transcriptional factors such as AP-1 and NF-κB are activated by ROS, increasing the gene expression of cytokines (TNF-α, IL-1, and IL-6), adhesion molecules (ICAM, VCAM), and pro-inflammatory enzymes (iNOS, COX-2; Yu and Chung, 2006). Supporting the clinical relevance of these findings in humans, epidemiological data suggest an association between elevated inflammatory cytokines and mortality in the elderly people (Harris et al., 1999; Volpato et al., 2001). Inflammatory cells could also play a role in vascular dysfunction associated to aging. In this sense, in healthy subjects older than 55 years, neutrophil, eosinophil and monocyte count (within the normal ranges) as well as myeloperoxidase activity inversely correlated with forearm blood flow responses, which were improved after BH_4 administration (Walker et al., 2010). This suggests that moderate increment of inflammatory cells through myeloperoxidase activity would reduce BH_4 availability compromising NO-mediated vasodilation.

In experimental models, there is clear evidence concerning the relationship between age and vascular inflammation (Chung et al., 2002; Csiszar et al., 2003; d'Alessio, 2004; Ungvari et al., 2004). An age-related up-regulation in TNF-α has been described in coronary arteries, increasing endothelial cells apoptosis and leading to endothelial dysfunction (Csiszar et al., 2004). Many of the characteristics of vascular aging, including endothelial dysfunction, oxidative stress, increased apoptosis, and pro-inflammatory gene expression profile, can be mimicked by recombinant TNF-α (Csiszar et al., 2007). Moreover, chronic TNF-α inhibition improves flow-mediated arterial dilation in resistance arteries of aged animals, while reduced ICAM-1 and iNOS expression (Arenas et al., 2006).

The induction of the inflammatory iNOS isoform can be also related to the endothelial dysfunction associated with aging. Previous reports in rats suggest an age-dependent enhanced vascular expression of iNOS (Cernadas et al., 1998; Csiszar et al., 2002). Studies in human peritoneal mesothelial cells indicated an age-dependent enhancement of iNOS expression (Nevado et al., 2006), while in mesenteric microvessels from aged subjects there is an increased expression of mRNA for iNOS (Rodriguez-Mañas et al., 2009). Furthermore, the selective inhibition of this isoform partially restored the age-dependent endothelial dysfunction (Rodriguez-Mañas et al., 2009). The iNOS isoform generates high amounts of NO; therefore, its rapid reaction with $O_2^{-\bullet}$ generates high levels of $ONOO^-$. As the inducible iNOS isoform has much higher catalytic activity than eNOS and consumes more substrate and cofactors, this isoenzyme is also an important source of $O_2^{-\bullet}$ when uncoupled (Loscalzo, 2000).

During the last years, there is increasing evidence in humans confirming the relation between age and low-grade inflammation. The expression and activity of the inflammatory transcription nuclear factor-κB (NF-κB) is augmented in human peritoneal mesothelial cells accordingly to age (Nevado et al., 2006). Moreover, a pro-inflammatory profile has been reported in the grossly normal, aged, human aortic wall (Wang et al., 2007). Aging is also associated with greater nuclear NF-κB, lower IκBα, and increased expression of pro-inflammatory cytokines in vascular endothelial cells from healthy humans, which correlates with age-related endothelial dysfunction (Donato et al.,

2007, 2008, 2009). Furthermore, an *in situ* age-dependent NF-κB activation has been demonstrated in the vascular wall of human mesenteric microvessels from aged subjects by Southwestern histochemistry techniques, which clearly correlates with the development of endothelial dysfunction (Rodriguez-Mañas et al., 2009).

SENESCENCE AND ENDOTHELIAL DYSFUNCTION

REPLICATIVE SENESCENCE

In the early 1960s, Hayflick and Moorhead defined cellular senescence as a condition where the cells lose its ability to proliferate, although it can be metabolically active. The underlying mechanism for this phenomenon is the telomere length shortening that occurs during each cell division until a critical length is exceeded. Without telomeres, genetic material would be lost every time a cell divides. DNA polymerase requires a RNA primer with a 3′ hydroxyl donor group to initiate DNA replication, during which the "end-replication problem" arises. Telomeres and telomerase ameliorate this problem by providing a repetitive template for enzymatic repair of the ends of chromosomes, thereby limiting the loss of genetic information during mitosis. When the telomeres are too short, cell signaling is triggered for the arrest of cell proliferation, senescence, and apoptosis. In addition to the lack of cell replication, senescent cells acquire distinct phenotypic characteristics associated to aging and age-related diseases (Ungvari et al., 2010a). Some of these phenotypic changes are related to the regenerative and angiogenic capacity of the vascular endothelium and involved in the development of atherosclerosis during aging (Erusalimsky, 2009). Indeed, more elastase and fibronectin are observed in senescent vascular smooth muscle (Minamino and Komuro, 2007).

Although senescence has been comprehensively characterized in cell culture, there is increasing evidence of the senescent phenotype *in vivo*, and its relevant pathophysiological implications, particularly in the cardiovascular system (Erusalimsky and Kurz, 2005). Thus, it has been described that telomere length is shortened in the endothelial cells from cadaveric samples of thoracic and iliac arteries, as well as from abdominal aorta, accordingly to the age of the patients (Aviv et al., 2001). On the other hand, telomere length is an independent predictor of heart disease related events, as its shortening is more pronounced in arteries derived from elderly patients with coronary disease and associated to endothelial dysfunction (Ogami et al., 2004; De Meyer et al., 2011). Telomere dysfunction and vascular senescence are related to enhanced ROS, decreased NO, and increased pro-inflammatory molecules, such as adhesion molecules (Minamino et al., 2004; Minamino and Komuro, 2007). In fact, ROS generation accelerates telomere shortening and senescence in HUVEC (Kurz et al., 2004).

In a search for biomarkers of CVD, the leukocyte telomere length has been related to several physiological or pathological conditions. Thus, cross sectional studies have shown that subjects with increased arterial stiffness have shorter leukocyte telomeres, indicating this may be an indicator of biological vascular aging in men (Benetos et al., 2001). It has been also reported that the leukocyte telomere length is shorter in a population of hypertensive patients than in the normotensive peers and the patients with shorter telomeres were more likely to develop atherosclerosis 5 years after (Yang et al., 2009b). Moreover, the rate of leukocyte telomere shortening predicts mortality from CVD in elderly men (Epel et al., 2009). Some criticism still exists, however, concerning the relations between telomere length and CVDs, as not always adequate correlations are found, neither an accepted pathophysiological link (Calado and Young, 2009).

STRESS-INDUCED SENESCENCE

Stress-induced senescence of endothelial cells can be produced as a result of various insults, including those causing intracellular oxidative stress and inflammation (Calado and Young, 2009; Erusalimsky and Skene, 2009). ROS are potential candidates responsible for senescence through varied actions but also by compromising NO availability since NO inhibits senescence in endothelial cells (Hayashi et al., 2006). In fact, ROS-induced senescence of HUVEC is antagonized by interventions resulting in increased eNOS activity (Ota et al., 2007). It has been suggested that inflammation may be a telomere-independent mechanism producing vascular cell senescence in human atherosclerotic plaques (Minamino et al., 2004). Exposure to AGE-modified proteins induces senescence of HUVEC without affecting telomeres length or telomerase activity (Patschan and Goligorsky, 2008). This type of stress-induced senescence would be secondary to ROS generation, lysosomal permeability and dysregulation of autophagy (Patschan et al., 2008). Oxidative stress seems to be involved in the genesis of both replicative and high glucose-induced senescence leading to dimethylaminohydrolase inactivation and ADMA accumulation which inhibits NO production (Scalera et al., 2008; Yuan et al., 2010). In addition, activation of Akt/mTOR pathway has been reported to contribute to vascular senescence associated to obesity (Wang et al., 2009).

SENESCENCE OF EPCs

Special relevance deserves the senescence occurring in the endothelial progenitor cells (EPCs), which are considered to contribute to endothelial repair (Griese et al., 2003; Takamiya et al., 2006; Kirton and Xu, 2010; Briasoulis et al., 2011), a phenomenon that is less efficient in aging (Williamson et al., 2012). However, although it is widely accepted the capacity of EPCs to promote neovascularization, the role of EPCs in regeneration of endothelial cells remains controversial (Pasquier and Dias, 2010; Sanchez-Guijo et al., 2010; Resch et al., 2011). Thus, advanced age is known to impair neovascularization, a process known to depend on the function of highly proliferative EPCs. There are evidences clearly demonstrating that aging impairs the function of *ex vivo* expanded EPCs (Keymel et al., 2008). The loss of EPCs associated to aging may be mediated by an imbalance between factors promoting growth, migration, and survival against those enhancing oxidative stress and promoting senescence (Chang et al., 2007). In this sense, hypoxia inducible factor-1α (HIF-1α) induces the expression of stromal cell-derived factor-1 (SDF-1) that enhances the recruitment of EPCs in injured or ischemic tissues in mice (Karshovska et al., 2007). There are evidences demonstrating that the levels or activity

of HIF-1α decreases with aging and thereby leads to reduced levels of SDF-1 (Hoenig et al., 2008). Telomere length declines with age in EPCs from healthy men (Minamino et al., 2004; Kushner et al., 2009). Moreover, EPCs senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension (Imanishi et al., 2005b). There is also a premature senescence in EPCs from diabetic patients (Rosso et al., 2006).

TELOMERASE

To avoid the attrition of telomeres, germinal cells and some somatic cells produces telomerase, an enzyme that catalyze DNA synthesis to maintain telomere length. Human endothelial cells and vascular smooth muscle express telomerase activity, which is activated by mitogen stimuli via PKC, this activity being reduced with age (Minamino and Komuro, 2007). Introduction of telomerase into EPCs extends life span and improves vasculogenesis of these cells (Minamino et al., 2004). It has been also described that NO activates telomerase in endothelial cells, delaying senescence (Vasa et al., 2000; Farsetti et al., 2009). Then, strategies aimed to increase endothelial NO bioavailability could be considered as possible therapies to prevent endothelial cell senescence associated with aging (Hayashi et al., 2008). By the contrary, angiotensin II-mediated oxidative stress (Imanishi et al., 2005a; Morris, 2005) and pro-atherogenic factors, like TNFα (Breitschopf et al., 2001), are major inducers for endothelial senescence, by inducing telomerase inactivation through an Akt-dependent mechanism. Antioxidants may prevent this telomerase inactivation by inhibiting the nuclear export of the catalytic subunit of the telomerase (Haendeler et al., 2004). The inactivation of telomerase induced by angiotensin II-mediated oxidative stress is related to the presence of nitrotyrosine, suggesting the involvement of peroxynitrite (Imanishi et al., 2005a; Morris, 2005). Interestingly, the endothelial cells senescence evoked by glycated collagen and the premature vascular senescence observed in type 2 diabetic rats can be prevented with peroxynitrite scavengers (Chen et al., 2002, 2007).

SIRTUINS

During the past decade, research on aging, based initially on simple laboratory organisms, has identified important genes and pathways that contribute to longevity. Among these is a family of nicotinamide adenine dinucleotide (NAD)-dependent proteins termed sirtuins, which can extend the life span in model organisms and are important in mediating the beneficial effects of low-calorie diets (Guarente, 2011). There are seven identified sirtuins in mammals (Sirt1 to Sirt7), which main function might be to promote survival and stress resistance, resulting in longevity (Sedding and Haendeler, 2007; Guarente, 2011). At present, endothelial dysfunction in arteries from aged mice and humans is associated with a reduction of vascular expression of Sirt1 (Donato et al., 2011) and there is increasing evidence that the beneficial effects produced by caloric restriction on endothelial function in aged animals are related, at least in part, by up-regulation of Sirt1 (Rippe et al., 2010; Weiss and Fontana, 2011). In fact, increased Sirt1 expression in cultured endothelial cells was driven by serum from aged rats under restricted calorie intake (Csiszar et al., 2009). In human endothelial cells, over-expression of Sirt1 prevented oxidative stress-induced senescence, while its inhibition leads to premature senescence-like phenotype. Interestingly, immunosuppressant drugs, like sirolimus and everolimus, induce endothelial cellular senescence via Sirt1 down-regulation (Ota et al., 2009). By the contrary, moderate over-expression of Sirt1 in mice hearts confers stress resistance to the heart *in vivo*, retarding aging phenotype (Alcendor et al., 2007). It has been suggested that NO upregulate Sirt1 in human endothelial cells, therefore inhibiting oxidative stress-induced premature cell senescence (Ota et al., 2008; Potente and Dimmeler, 2008). By the other hand, Sirt1 inhibition in HUVEC results in reduced eNOS activity and senescence-like phenotype while Sirt1 induction increases eNOS activity and prevents ROS-induced senescence (Ota et al., 2007). Sirt1 plays an important role in endothelial homeostasis by regulating eNOS activity; therefore, the Sirt1/eNOS axis may be quite relevant as a potential target against vascular senescence, endothelial dysfunction, and atherosclerosis (Ota et al., 2010). Moreover, Sirt1 enhancement inhibits the expression of AT1 receptors in vascular smooth muscle cells (Miyazaki et al., 2008), reducing cell hypertrophy and neointima formation following vascular injury (Li et al., 2011a,b). All the available data suggest that sirtuins can be a unique class of proteins exerting important effects on age-related CVDs and a promising target for drug development (Guarente, 2011).

CONCLUSION

The better understanding of the molecular and cellular mechanisms involved in vascular aging, as well as their potential interactions, provides a growing list of factors that can be targets for specific interventions aimed at preventing and delaying the vascular aging. The NO pathway and endothelial dysfunction, oxidative stress, inflammation, telomerase, and sirtuins are among the principal mechanisms likely involved in the vascular aging process, both in healthy and pathological conditions. A better comprehension of the complex interactions between them is an important objective for future research. Changes in lifestyle, dietary changes, loss of weight (if overweight), and particularly aerobic exercise may prevent or delay the onset of endothelial dysfunction. The pharmacological approaches could also be important tools for intervention throughout the aging process, either preventing endothelial dysfunction, or treating advanced stages of endothelial dysfunction, or even acting on structural alterations already evident in the vascular wall. Although we need more data in human beings about the role of telomerase and sirtuins in healthy aging and in disease, both may be new promising targets, in addition to mechanisms already investigated, including the activation of the NO pathway or the COX inhibition, or the interference with pro-oxidant and pro-inflammatory pathways.

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REFERENCES

Abbatecola, A. M., Paolisso, G., Fattoretti, P., Evans, W. J., Fiore, V., Dicioccio, L., and Lattanzio, F. (2011). Discovering pathways of sarcopenia in older adults: a role for insulin resistance on mitochondria dysfunction. *J. Nutr. Health Aging* 15, 890–895.

Ahlers, B. A., Parnell, M. M., Chin-Dusting, J. P., and Kaye, D. M. (2004). An age-related decline in endothelial function is not associated with alterations in L-arginine transport in humans. *J. Hypertens.* 22, 321–327.

Albarwani, S., Al-Siyabi, S., Baomar, H., and Hassan, M. O. (2010). Exercise training attenuates ageing-induced BKCa channel downregulation in rat coronary arteries. *Exp. Physiol.* 95, 746–755.

Alcendor, R. R., Gao, S., Zhai, P., Zablocki, D., Holle, E., Yu, X., Tian, B., Wagner, T., Vatner, S. F., and Sadoshima, J. (2007). Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ. Res.* 100, 1512–1521.

Angulo, J., Cuevas, P., Fernandez, A., Gabancho, S., Videla, S., and Saenz De Tejada, I. (2003). Calcium dobesilate potentiates endothelium-derived hyperpolarizing factor-mediated relaxation of human penile resistance arteries. *Br. J. Pharmacol.* 139, 854–862.

Arenas, I. A., Xu, Y., and Davidge, S. T. (2006). Age-associated impairment in vasorelaxation to fluid shear stress in the female vasculature is improved by TNF-alpha antagonism. *Am. J. Physiol. Heart Circ. Physiol.* 290, H1259–H1263.

Aviv, H., Khan, M. Y., Skurnick, J., Okuda, K., Kimura, M., Gardner, J., Priolo, L., and Aviv, A. (2001). Age dependent aneuploidy and telomere length of the human vascular endothelium. *Atherosclerosis* 159, 281–287.

Barodka, V. M., Joshi, B. L., Berkowitz, D. E., Hogue, C. W. Jr., and Nyhan, D. (2011). Review article: implications of vascular aging. *Anesth. Analg.* 112, 1048–1060.

Barton, M., Cosentino, F., Brandes, R. P., Moreau, P., Shaw, S., and Luscher, T. F. (1997). Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. *Hypertension* 30, 817–824.

Benetos, A., Okuda, K., Lajemi, M., Kimura, M., Thomas, F., Skurnick, J., Labat, C., Bean, K., and Aviv, A. (2001). Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 37, 381–385.

Benigni, A., Corna, D., Zoja, C., Sonzogni, A., Latini, R., Salio, M., Conti, S., Rottoli, D., Longaretti, L., Cassis, P., Morigi, M., Coffman, T. M., and Remuzzi, G. (2009). Disruption of the Ang II type 1 receptor promotes longevity in mice. *J. Clin. Invest.* 119, 524–530.

Bode-Boger, S. M., Muke, J., Surdacki, A., Brabant, G., Boger, R. H., and Frolich, J. C. (2003). Oral L-arginine improves endothelial function in healthy individuals older than 70 years. *Vasc. Med.* 8, 77–81.

Bode-Boger, S. M., Scalera, F., and Martens-Lobenhoffer, J. (2005). Asymmetric dimethylarginine (ADMA) accelerates cell senescence. *Vasc. Med.* 10(Suppl. 1), S65–S71.

Brandes, R. P., Fleming, I., and Busse, R. (2005). Endothelial aging. *Cardiovasc. Res.* 66, 286–294.

Breitschopf, K., Zeiher, A. M., and Dimmeler, S. (2001). Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. *FEBS Lett.* 493, 21–25.

Briasoulis, A., Tousoulis, D., Antoniades, C., Stefanadis, C., and Papageorgiou, N. (2011). The role of endothelial progenitor cells in vascular repair after arterial injury and atherosclerotic plaque development. *Cardiovasc. Ther.* 29, 125–139.

Briones, A. M., Montoya, N., Giraldo, J., and Vila, E. (2005). Ageing affects nitric oxide synthase, cyclooxygenase and oxidative stress enzymes expression differently in mesenteric resistance arteries. *Auton. Autacoid Pharmacol.* 25, 155–162.

Cai, H., and Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ. Res.* 87, 840–844.

Calado, R. T., and Young, N. S. (2009). Telomere diseases. *N. Engl. J. Med.* 361, 2353–2365.

Cernadas, M. R., Sanchez De Miguel, L., Garcia-Duran, M., Gonzalez-Fernandez, F., Millas, I., Monton, M., Rodrigo, J., Rico, L., Fernandez, P., De Frutos, T., Rodriguez-Feo, J. A., Guerra, J., Caramelo, C., Casado, S., and Lopez, F. (1998). Expression of constitutive and inducible nitric oxide synthases in the vascular wall of young and aging rats. *Circ. Res.* 83, 279–286.

Chang, E. I., Loh, S. A., Ceradini, D. J., Lin, S. E., Bastidas, N., Aarabi, S., Chan, D. A., Freedman, M. L., Giaccia, A. J., and Gurtner, G. C. (2007). Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation* 116, 2818–2829.

Chen, J., Brodsky, S. V., Goligorsky, D. M., Hampel, D. J., Li, H., Gross, S. S., and Goligorsky, M. S. (2002). Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. *Circ. Res.* 90, 1290–1298.

Chen, J., Park, H. C., Patschan, S., Brodsky, S. V., Gealikman, O., Kuo, M. C., Li, H., Addabbo, F., Zhang, F., Nasjletti, A., Gross, S. S., and Goligorsky, M. S. (2007). Premature vascular senescence in metabolic syndrome: could it be prevented and reversed by a selenorganic antioxidant and peroxynitrite scavenger ebselen? *Drug Discov. Today Ther. Strateg.* 4, 93–99.

Chung, H. Y., Kim, H. J., Kim, K. W., Choi, J. S., and Yu, B. P. (2002). Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. *Microsc. Res. Tech.* 59, 264–272.

Cooke, J. P. (2005). ADMA: its role in vascular disease. *Vasc. Med.* 10(Suppl. 1), S11–S17.

Csiszar, A., Labinskyy, N., Jimenez, R., Pinto, J. T., Ballabh, P., Losonczy, G., Pearson, K. J., De Cabo, R., and Ungvari, Z. (2009). Anti-oxidative and anti-inflammatory vasoprotective effects of caloric restriction in aging: role of circulating factors and SIRT1. *Mech. Ageing Dev.* 130, 518–527.

Csiszar, A., Labinskyy, N., Smith, K., Rivera, A., Orosz, Z., and Ungvari, Z. (2007). Vasculoprotective effects of anti-tumor necrosis factor-alpha treatment in aging. *Am. J. Pathol.* 170, 388–398.

Csiszar, A., Ungvari, Z., Edwards, J. G., Kaminski, P., Wolin, M. S., Koller, A., and Kaley, G. (2002). Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ. Res.* 90, 1159–1166.

Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., and Kaley, G. (2003). Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. *FASEB J.* 17, 1183–1185.

Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., and Kaley, G. (2004). Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol. Genomics* 17, 21–30.

Cui, H., Kong, Y., and Zhang, H. (2012). Oxidative stress, mitochondrial dysfunction, and aging. *J. Signal Transduct.* 2012, 646354.

d'Alessio, P. (2004). Aging and the endothelium. *Exp. Gerontol.* 39, 165–171.

De Meyer, T., Rietzschel, E. R., De Buyzere, M. L., Van Criekinge, W., and Bekaert, S. (2011). Telomere length and cardiovascular aging: the means to the ends? *Ageing Res. Rev.* 10, 297–303.

de Sotomayor, M. A., Perez-Guerrero, C., Herrrera, M. D., Jimenez, L., Marin, R., Marhuenda, E., and Andriantsitohaina, R. (2005). Improvement of age-related endothelial dysfunction by simvastatin: effect on NO and COX pathways. *Br. J. Pharmacol.* 146, 1130–1138.

Donato, A. J., Black, A. D., Jablonski, K. L., Gano, L. B., and Seals, D. R. (2008). Aging is associated with greater nuclear NF kappa B, reduced I kappa B alpha, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. *Aging Cell* 7, 805–812.

Donato, A. J., Eskurza, I., Silver, A. E., Levy, A. S., Pierce, G. L., Gates, P. E., and Seals, D. R. (2007). Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. *Circ. Res.* 100, 1659–1666.

Donato, A. J., Gano, L. B., Eskurza, I., Silver, A. E., Gates, P. E., Jablonski, K., and Seals, D. R. (2009). Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *Am. J. Physiol. Heart Circ. Physiol.* 297, H425–H432.

Donato, A. J., Lesniewski, L. A., and Delp, M. D. (2005). The effects of aging and exercise training on endothelin-1 vasoconstrictor responses in rat skeletal muscle arterioles. *Cardiovasc. Res.* 66, 393–401.

Donato, A. J., Magerko, K. A., Lawson, B. R., Durrant, J. R., Lesniewski, L. A., and Seals, D. R. (2011). SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans. *J. Physiol. (Lond.)* 589, 4545–4554.

Donato, A. J., Uberoi, A., Bailey, D. M., Wray, D. W., and Richardson, R. S. (2010). Exercise-induced brachial artery vasodilation: effects of antioxidants and exercise training in elderly men. *Am. J. Physiol. Heart Circ. Physiol.* 298, H671–H678.

Dora, K. A., Gallagher, N. T., Mcneish, A., and Garland, C. J. (2008). Modulation of endothelial cell KCa3.1

channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. *Circ. Res.* 102, 1247–1255.

Epel, E. S., Merkin, S. S., Cawthon, R., Blackburn, E. H., Adler, N. E., Pletcher, M. J., and Seeman, T. E. (2009). The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging (Albany NY)* 1, 81–88.

Erusalimsky, J. D. (2009). Vascular endothelial senescence: from mechanisms to pathophysiology. *J. Appl. Physiol.* 106, 326–332.

Erusalimsky, J. D., and Kurz, D. J. (2005). Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. *Exp. Gerontol.* 40, 634–642.

Erusalimsky, J. D., and Skene, C. (2009). Mechanisms of endothelial senescence. *Exp. Physiol.* 94, 299–304.

Eskurza, I., Kahn, Z. D., and Seals, D. R. (2006). Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. *J. Physiol. (Lond.)* 571, 661–668.

Farsetti, A., Grasselli, A., Bacchetti, S., Gaetano, C., and Capogrossi, M. C. (2009). The telomerase tale in vascular aging: regulation by estrogens and nitric oxide signaling. *J. Appl. Physiol.* 106, 333–337.

Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D. D., Guralnik, J. M., and Longo, D. L. (2005). The origins of age-related proinflammatory state. *Blood* 105, 2294–2299.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., and De Benedictis, G. (2000). Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908, 244–254.

Francia, P., Delli Gatti, C., Bachschmid, M., Martin-Padura, I., Savoia, C., Migliaccio, E., Pelicci, P. G., Schiavoni, M., Luscher, T. F., Volpe, M., and Cosentino, F. (2004). Deletion of p66shc gene protects against age-related endothelial dysfunction. *Circulation* 110, 2889–2895.

Gates, P. E., Boucher, M. L., Silver, A. E., Monahan, K. D., and Seals, D. R. (2007). Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. *J. Appl. Physiol.* 102, 63–71.

Gaubert, M. L., Sigaudo-Roussel, D., Tartas, M., Berrut, G., Saumet, J. L., and Fromy, B. (2007). Endothelium-derived hyperpolarizing factor as an in vivo back-up mechanism in the cutaneous microcirculation in old mice. *J. Physiol.* 585, 617–626.

Goraca, A. (2004). Assessment of total antioxidant capacity in human plasma. *Folia Med. (Plovdiv)* 46, 16–21.

Goto, K., Fujii, K., Kansui, Y., and Iida, M. (2004). Changes in endothelium-derived hyperpolarizing factor in hypertension and ageing: response to chronic treatment with renin-angiotensin system inhibitors. *Clin. Exp. Pharmacol. Physiol.* 31, 650–655.

Goto, K., Fujii, K., Onaka, U., Abe, I., and Fujishima, M. (2000). Angiotensin-converting enzyme inhibitor prevents age-related endothelial dysfunction. *Hypertension* 36, 581–587.

Griese, D. P., Ehsan, A., Melo, L. G., Kong, D., Zhang, L., Mann, M. J., Pratt, R. E., Mulligan, R. C., and Dzau, V. J. (2003). Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. *Circulation* 108, 2710–2715.

Guarente, L. (2011). Franklin H. Epstein lecture: sirtuins, aging, and medicine. *N. Engl. J. Med.* 364, 2235–2244.

Guzik, T. J., Mussa, S., Gastaldi, D., Sadowski, J., Ratnatunga, C., Pillai, R., and Channon, K. M. (2002). Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 105, 1656–1662.

Haendeler, J., Hoffmann, J., Diehl, J. F., Vasa, M., Spyridopoulos, I., Zeiher, A. M., and Dimmeler, S. (2004). Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ. Res.* 94, 768–775.

Harris, T. B., Ferrucci, L., Tracy, R. P., Corti, M. C., Wacholder, S., Ettinger, W. H. Jr., Heimovitz, H., Cohen, H. J., and Wallace, R. (1999). Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am. J. Med.* 106, 506–512.

Hayashi, T., Matsui-Hirai, H., Miyazaki-Akita, A., Fukatsu, A., Funami, J., Ding, Q. F., Kamalanathan, S., Hattori, Y., Ignarro, L. J., and Iguchi, A. (2006). Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 103, 17018–17023.

Hayashi, T., Yano, K., Matsui-Hirai, H., Yokoo, H., Hattori, Y., and Iguchi, A. (2008). Nitric oxide and endothelial cellular senescence. *Pharmacol. Ther.* 120, 333–339.

Haynes, W. G., Strachan, F. E., and Webb, D. J. (1995). Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. *Circulation* 92, 357–363.

Heymes, C., Habib, A., Yang, D., Mathieu, E., Marotte, F., Samuel, J., and Boulanger, C. M. (2000). Cyclooxygenase-1 and -2 contribution to endothelial dysfunction in ageing. *Br. J. Pharmacol.* 131, 804–810.

Higashi, Y., Sasaki, S., Nakagawa, K., Kimura, M., Noma, K., Hara, K., Jitsuiki, D., Goto, C., Oshima, T., Chayama, K., and Yoshizumi, M. (2006). Tetrahydrobiopterin improves aging-related impairment of endothelium-dependent vasodilation through increase in nitric oxide production. *Atherosclerosis* 186, 390–395.

Hilgers, R. H., and Webb, R. C. (2007). Reduced expression of SKCa and IKCa channel proteins in rat small mesenteric arteries during angiotensin II-induced hypertension. *Am. J. Physiol. Heart. Circ. Physiol.* 292, H2275–H2284.

Hoenig, M. R., Bianchi, C., Rosenzweig, A., and Sellke, F. W. (2008). Decreased vascular repair and neovascularization with ageing: mechanisms and clinical relevance with an emphasis on hypoxia-inducible factor-1. *Curr. Mol. Med.* 8, 754–767.

Holowatz, L. A., Thompson, C. S., and Kenney, W. L. (2006). L-Arginine supplementation or arginase inhibition augments reflex cutaneous vasodilatation in aged human skin. *J. Physiol. (Lond.)* 574, 573–581.

Idris Khodja, N., Chataigneau, T., Auger, C., and Schini-Kerth, V. B. (2012). Grape-derived polyphenols improve aging-related endothelial dysfunction in rat mesenteric artery: role of oxidative stress and the angiotensin system. *PLoS ONE* 7, e32039. doi:10.1371/journal.pone.0032039

Imanishi, T., Hano, T., and Nishio, I. (2005a). Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J. Hypertens.* 23, 97–104.

Imanishi, T., Moriwaki, C., Hano, T., and Nishio, I. (2005b). Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J. Hypertens.* 23, 1831–1837.

Jablonski, K. L., Seals, D. R., Eskurza, I., Monahan, K. D., and Donato, A. J. (2007). High-dose ascorbic acid infusion abolishes chronic vasoconstriction and restores resting leg blood flow in healthy older men. *J. Appl. Physiol.* 103, 1715–1721.

Kansui, Y., Fujii, K., Goto, K., Abe, I., and Iida, M. (2002). Angiotensin II receptor antagonist improves age-related endothelial dysfunction. *J. Hypertens.* 20, 439–446.

Karshovska, E., Zernecke, A., Sevilmis, G., Millet, A., Hristov, M., Cohen, C. D., Schmid, H., Krotz, F., Sohn, H. Y., Klauss, V., Weber, C., and Schober, A. (2007). Expression of HIF-1alpha in injured arteries controls SDF-1alpha mediated neointima formation in apolipoprotein E deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 27, 2540–2547.

Keymel, S., Kalka, C., Rassaf, T., Yeghiazarians, Y., Kelm, M., and Heiss, C. (2008). Impaired endothelial progenitor cell function predicts age-dependent carotid intimal thickening. *Basic Res. Cardiol.* 103, 582–586.

Kim, D. H., Newman, A. B., Hajjar, I., Strotmeyer, E. S., Klein, R., Newton, E., Sarnak, M. J., Burke, G. L., and Lipsitz, L. A. (2011). Retinal microvascular signs and functional loss in older persons: the cardiovascular health study. *Stroke* 42, 1589–1595.

Kim, S. F., Huri, D. A., and Snyder, S. H. (2005). Inducible nitric oxide synthase binds, S-nitrosylates, and activates cyclooxygenase-2. *Science* 310, 1966–1970.

Kimura, S., Zhang, G. X., Nishiyama, A., Shokoji, T., Yao, L., Fan, Y. Y., Rahman, M., Suzuki, T., Maeta, H., and Abe, Y. (2005). Role of NAD(P)H oxidase- and mitochondria-derived reactive oxygen species in cardioprotection of ischemic reperfusion injury by angiotensin II. *Hypertension* 45, 860–866.

Kirton, J. P., and Xu, Q. (2010). Endothelial precursors in vascular repair. *Microvasc. Res.* 79, 193–199.

Kotsis, V., Stabouli, S., Karafillis, I., and Nilsson, P. (2011). Early vascular aging and the role of central blood pressure. *J. Hypertens.* 29, 1847–1853.

Korzick, D. H., Muller-Delp, J. M., Dougherty, P., Heaps, C. L., Bowles, D. K., and Krick, K. K. (2005). Exaggerated coronary vasoreactivity to endothelin-1 in aged rats: role of protein kinase C. *Cardiovasc Res.* 66, 384–392.

Kurz, D. J., Decary, S., Hong, Y., Trivier, E., Akhmedov, A., and Erusalimsky, J. D. (2004). Chronic oxidative stress

compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J. Cell Sci.* 117, 2417–2426.

Kushner, E. J., Van Guilder, G. P., Maceneaney, O. J., Cech, J. N., Stauffer, B. L., and Desouza, C. A. (2009). Aging and endothelial progenitor cell telomere length in healthy men. *Clin. Chem. Lab. Med.* 47, 47–50.

Lassegue, B., and Griendling, K. K. (2010). NADPH oxidases: functions and pathologies in the vasculature. *Arterioscler. Thromb. Vasc. Biol.* 30, 653–661.

Laursen, J. B., Somers, M., Kurz, S., Mccann, L., Warnholtz, A., Freeman, B. A., Tarpey, M., Fukai, T., and Harrison, D. G. (2001). Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 103, 1282–1288.

LeBlanc, A. J., Shipley, R. D., Kang, L. S., and Muller-Delp, J. M. (2008). Age impairs Flk-1 signaling and NO-mediated vasodilation in coronary arterioles. *Am. J. Physiol. Heart Circ. Physiol.* 295, H2280–H2288.

Li, L., Gao, P., Zhang, H., Chen, H., Zheng, W., Lv, X., Xu, T., Wei, Y., Liu, D., and Liang, C. (2011a). SIRT1 inhibits angiotensin II-induced vascular smooth muscle cell hypertrophy. *Acta Biochim. Biophys. Sin. (Shanghai)* 43, 103–109.

Li, L., Zhang, H. N., Chen, H. Z., Gao, P., Zhu, L. H., Li, H. L., Lv, X., Zhang, Q. J., Zhang, R., Wang, Z., She, Z. G., Wei, Y. S., Du, G. H., Liu, D. P., and Liang, C. C. (2011b). SIRT1 acts as a modulator of neointima formation following vascular injury in mice. *Circ. Res.* 108, 1180–1189.

Long, D. A., Newaz, M. A., Prabhakar, S. S., Price, K. L., Truong, L. D., Feng, L., Mu, W., Oyekan, A. O., and Johnson, R. J. (2005). Loss of nitric oxide and endothelial-derived hyperpolarizing factor-mediated responses in aging. *Kidney Int.* 68, 2154–2163.

Loscalzo, J. (2000). What we know and don't know about L-arginine and NO. *Circulation* 101, 2126–2129.

Lüscher, T. F., and Barton, M. (2000). Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. *Circulation* 102, 2434–2440.

Mackenzie, A., Cooper, E. J., and Dowell, F. J. (2008). Differential effects of glucose on agonist-induced relaxations in human mesenteric and subcutaneous arteries. *Br. J. Pharmacol.* 153, 480–487.

Maeda, S., Tanabe, T., Miyauchi, T., Otsuki, T., Sugawara, J., Iemitsu, M., Kuno, S., Ajisaka, R., Yamaguchi, I., and Matsuda, M. (2003). Aerobic exercise training reduces plasma endothelin-1 concentration in older women. *J. Appl. Physiol.* 95, 336–341.

Malinin, N. L., West, X. Z., and Byzova, T. V. (2011). Oxidation as "the stress of life." *Aging (Albany NY)* 3, 906–910.

Masaki, T., Kimura, S., Yanagisawa, M., and Goto, K. (1991). Molecular and cellular mechanism of endothelin regulation. Implications for vascular function. *Circulation* 84, 1457–1468.

Matz, R. L., and Andriantsitohaina, R. (2003). Age-related endothelial dysfunction: potential implications for pharmacotherapy. *Drugs Aging* 20, 527–550.

Matz, R. L., De Sotomayor, M. A., Schott, C., Stoclet, J. C., and Andriantsitohaina, R. (2000). Vascular bed heterogeneity in age-related endothelial dysfunction with respect to NO and eicosanoids. *Br. J. Pharmacol.* 131, 303–311.

Mecocci, P., Polidori, M. C., Troiano, L., Cherubini, A., Cecchetti, R., Pini, G., Straatman, M., Monti, D., Stahl, W., Sies, H., Franceschi, C., and Senin, U. (2000). Plasma antioxidants and longevity: a study on healthy centenarians. *Free Radic. Biol. Med.* 28, 1243–1248.

Milstien, S., and Katusic, Z. (1999). Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem. Biophys. Res. Commun.* 263, 681–684.

Minamino, T., and Komuro, I. (2007). Vascular cell senescence: contribution to atherosclerosis. *Circ. Res.* 100, 15–26.

Minamino, T., Miyauchi, H., Yoshida, T., Tateno, K., and Komuro, I. (2004). The role of vascular cell senescence in atherosclerosis: antisenescence as a novel therapeutic strategy for vascular aging. *Curr. Vasc. Pharmacol.* 2, 141–148.

Miyazaki, R., Ichiki, T., Hashimoto, T., Inanaga, K., Imayama, I., Sadoshima, J., and Sunagawa, K. (2008). SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 28, 1263–1269.

Mollnau, H., Wendt, M., Szocs, K., Lassegue, B., Schulz, E., Oelze, M., Li, H., Bodenschatz, M., August, M., Kleschyov, A. L., Tsilimingas, N., Walter, U., Forstermann, U., Meinertz, T., Griendling, K., and Munzel, T. (2002). Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ. Res.* 90, E58–E65.

Morris, B. J. (2005). The scorching breath of angiotensin. *J. Hypertens.* 23, 33–35.

Mukai, Y., Shimokawa, H., Higashi, M., Morikawa, K., Matoba, T., Hiroki, J., Kunihiro, I., Talukder, H. M., and Takeshita, A. (2002). Inhibition of renin-angiotensin system ameliorates endothelial dysfunction associated with aging in rats. *Arterioscler. Thromb. Vasc. Biol.* 22, 1445–1450.

Munzel, T., Daiber, A., Ullrich, V., and Mulsch, A. (2005). Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler. Thromb. Vasc. Biol.* 25, 1551–1557.

Najjar, S. S., Scuteri, A., and Lakatta, E. G. (2005). Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 46, 454–462.

Nakashima, M., Mombouli, J. V., Taylor, A. A., and Vanhoutte, P. M. (1993). Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J. Clin. Invest.* 92, 2867–2871.

Nevado, J., Vallejo, S., El-Assar, M., Peiro, C., Sanchez-Ferrer, C. F., and Rodriguez-Manas, L. (2006). Changes in the human peritoneal mesothelial cells during aging. *Kidney Int.* 69, 313–322.

Newaz, M. A., Yousefipour, Z., and Oyekan, A. (2006). Oxidative stress-associated vascular aging is xanthine oxidase-dependent but not NAD(P)H oxidase-dependent. *J. Cardiovasc. Pharmacol.* 48, 88–94.

Ogami, M., Ikura, Y., Ohsawa, M., Matsuo, T., Kayo, S., Yoshimi, N., Hai, E., Shirai, N., Ehara, S., Komatsu, R., Naruko, T., and Ueda, M. (2004). Telomere shortening in human coronary artery diseases. *Arterioscler. Thromb. Vasc. Biol.* 24, 546–550.

Ota, H., Akishita, M., Eto, M., Iijima, K., Kaneki, M., and Ouchi, Y. (2007). Sirt1 modulates premature senescence-like phenotype in human endothelial cells. *J. Mol. Cell Cardiol.* 43, 571–579.

Ota, H., Eto, M., Ako, J., Ogawa, S., Iijima, K., Akishita, M., and Ouchi, Y. (2009). Sirolimus and everolimus induce endothelial cellular senescence via sirtuin 1 down-regulation: therapeutic implication of cilostazol after drug-eluting stent implantation. *J. Am. Coll. Cardiol.* 53, 2298–2305.

Ota, H., Eto, M., Kano, M. R., Ogawa, S., Iijima, K., Akishita, M., and Ouchi, Y. (2008). Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 28, 1634–1639.

Ota, H., Eto, M., Ogawa, S., Iijima, K., Akishita, M., and Ouchi, Y. (2010). SIRT1/eNOS axis as a potential target against vascular senescence, dysfunction and atherosclerosis. *J. Atheroscler. Thromb.* 17, 431–435.

Pacher, P., Beckman, J. S., and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 87, 315–424.

Pang, C. Y., Ma, Y. S., and Wei, Y. U. (2008). MtDNA mutations, functional decline and turnover of mitochondria in aging. *Front. Biosci.* 13, 3661–3675.

Panza, F., D'Introno, A., Colacicco, A. M., Capurso, C., Parigi, A. D., Capurso, S. A., Caselli, R. J., Pilotto, A., Scafato, E., Capurso, A., and Solfrizzi, V. (2006). Cognitive frailty: predementia syndrome and vascular risk factors. *Neurobiol. Aging* 27, 933–940.

Pascoal, I. F., and Umans, J. G. (1996). Effect of pregnancy on mechanisms of relaxation in human omental microvessels. *Hypertension* 28, 183–187.

Pasquier, D., and Dias, S. (2010). Endothelial progenitor cells: hope beyond controversy. *Curr. Cancer Drug Targets* 10, 914–921.

Patschan, S., Chen, J., Polotskaia, A., Mendelev, N., Cheng, J., Patschan, D., and Goligorsky, M. S. (2008). Lipid mediators of autophagy in stress-induced premature senescence of endothelial cells. *Am. J. Physiol. Heart. Circ. Physiol.* 294, H1119–H1129.

Patschan, S., and Goligorsky, M. S. (2008). Autophagy: the missing link between non-enzymatically glycated proteins inducing apoptosis and premature senescence of endothelial cells? *Autophagy* 4, 521–523.

Potente, M., and Dimmeler, S. (2008). NO targets SIRT1: a novel signaling network in endothelial senescence. *Arterioscler. Thromb. Vasc. Biol.* 28, 1577–1579.

Rajagopalan, S., Brook, R., Mehta, R. H., Supiano, M., and Pitt, B. (2002). Effect of losartan in aging-related endothelial impairment. *Am. J. Cardiol.* 89, 562–566.

Rajagopalan, S., Kariisa, M., Dellegrottaglie, S., Bard, R. L., Kehrer, C., Matlow, S., Daley, W., Pitt, B., and Brook, R. (2006). Angiotensin

receptor blockade improves vascular compliance in healthy normotensive elderly individuals: results from a randomized double-blind placebo-controlled trial. *J. Clin. Hypertens. (Greenwich)* 8, 783–790.

Rajapakse, A. G., Yepuri, G., Carvas, J. M., Stein, S., Matter, C. M., Scerri, I., Ruffieux, J., Montani, J. P., Ming, X. F., and Yang, Z. (2011). Hyperactive S6K1 mediates oxidative stress and endothelial dysfunction in aging: inhibition by resveratrol. *PLoS One* 6, e19237.

Resch, T., Pircher, A., Kähler, C. M., Pratschke, J., and Hilbe, W. (2011). Endothelial progenitor cells: current issues on characterization and challenging clinical applications. *Stem Cell Rev.* doi: 10.1007/s12015-011-9332-9. [Epub ahead of print].

Rippe, C., Lesniewski, L., Connell, M., LaRocca, T., Donato, A. J., and Seals, D. (2010). Short-term calorie restriction reverses vascular endothelial dysfunction in old mice by increasing nitric oxide and reducing oxidative stress. *Aging Cell* 9, 304–312.

Rodriguez-Mañas, L., El-Assar, M., Vallejo, S., Lopez-Doriga, P., Solis, J., Petidier, R., Montes, M., Nevado, J., Castro, M., Gomez-Guerrero, C., Peiro, C., and Sanchez-Ferrer, C. F. (2009). Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell* 8, 226–238.

Rosso, A., Balsamo, A., Gambino, R., Dentelli, P., Falcioni, R., Cassader, M., Pegoraro, L., Pagano, G., and Brizzi, M. F. (2006). p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *J. Biol. Chem.* 281, 4339–4347.

Sagach, V., Bondarenko, A., Bazilyuk, O., and Kotsuruba, A. (2006). Endothelial dysfunction: possible mechanisms and ways of correction. *Exp. Clin. Cardiol.* 11, 107–110.

Sanchez-Guijo, F. M., Oterino, E., Barbado, M. V., Carrancio, S., Lopez-Holgado, N., Muntion, S., Hernandez-Campo, P., Sanchez-Abarca, L. I., Perez-Simon, J. A., San Miguel, J. F., Briñon, J. G., and del Cañizo, M. C. (2010). Both CD133(+) cells and monocytes provide significant improvement for hindlimb ischemia, although they do not transdifferentiate into endothelial cells. *Cell Transplant.* 19, 103–112.

Santhanam, L., Christianson, D. W., Nyhan, D., and Berkowitz, D. E. (2008). Arginase and vascular aging. *J. Appl. Physiol.* 105, 1632–1642.

Santhanam, L., Lim, H. K., Miriel, V., Brown, T., Patel, M., Balanson, S., Ryoo, S., Anderson, M., Irani, K., Khanday, F., Di Costanzo, L., Nyhan, D., Hare, J. M., Christianson, D. W., Rivers, R., Shoukas, A., and Berkowitz, D. E. (2007). Inducible NO synthase dependent S-nitrosylation and activation of arginase1 contribute to age-related endothelial dysfunction. *Circ. Res.* 101, 692–702.

Scalera, F., Martens-Lobenhoffer, J., Bukowska, A., Lendeckel, U., Tager, M., and Bode-Boger, S. M. (2008). Effect of telmisartan on nitric oxide–asymmetrical dimethylarginine system: role of angiotensin II type 1 receptor gamma and peroxisome proliferator activated receptor gamma signaling during endothelial aging. *Hypertension* 51, 696–703.

Schiffrin, E. L. (2008). Oxidative stress, nitric oxide synthase, and superoxide dismutase: a matter of imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension* 51, 31–32.

Schrage, W. G., Eisenach, J. H., and Joyner, M. J. (2007). Ageing reduces nitric-oxide- and prostaglandin-mediated vasodilatation in exercising humans. *J. Physiol. (Lond.)* 579, 227–236.

Schulze, F., Maas, R., Freese, R., Schwedhelm, E., Silberhorn, E., and Boger, R. H. (2005). Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. *Eur. J. Clin. Invest.* 35, 622–626.

Sedding, D., and Haendeler, J. (2007). Do we age on Sirt1 expression? *Circ. Res.* 100, 1396–1398.

Seo, B., Oemar, B. S., Siebenmann, R., Von Segesser, L., and Luscher, T. F. (1994). Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation* 89, 1203–1208.

Shi, Y., Man, R. Y., and Vanhoutte, P. M. (2008). Two isoforms of cyclooxygenase contribute to augmented endothelium-dependent contractions in femoral arteries of 1-year-old rats. *Acta Pharmacol. Sin.* 29, 185–192.

Simons, L. A., Von Konigsmark, M., Simons, J., Stocker, R., and Celermajer, D. S. (1999). Vitamin E ingestion does not improve arterial endothelial dysfunction in older adults. *Atherosclerosis* 143, 193–199.

Soucy, K. G., Ryoo, S., Benjo, A., Lim, H. K., Gupta, G., Sohi, J. S., Elser, J., Aon, M. A., Nyhan, D., Shoukas, A. A., and Berkowitz, D. E. (2006). Impaired shear stress-induced nitric oxide production through decreased NOS phosphorylation contributes to age-related vascular stiffness. *J. Appl. Physiol.* 101, 1751–1759.

Stegbauer, J., and Coffman, T. M. (2011). New insights into angiotensin receptor actions: from blood pressure to aging. *Curr. Opin. Nephrol. Hypertens.* 20, 84–88.

Stewart, K. G., Zhang, Y., and Davidge, S. T. (2000). Aging increases PGHS-2-dependent vasoconstriction in rat mesenteric arteries. *Hypertension* 35, 1242–1247.

Sun, D., Huang, A., Yan, E. H., Wu, Z., Yan, C., Kaminski, P. M., Oury, T. D., Wolin, M. S., and Kaley, G. (2004). Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am. J. Physiol. Heart Circ. Physiol.* 286, H2249–H2256.

Taddei, S., Virdis, A., Mattei, P., Ghiadoni, L., Fasolo, C. B., Sudano, I., and Salvetti, A. (1997). Hypertension causes premature aging of endothelial function in humans. *Hypertension* 29, 736–743.

Takamiya, M., Okigaki, M., Jin, D., Takai, S., Nozawa, Y., Adachi, Y., Urao, N., Tateishi, K., Nomura, T., Zen, K., Ashihara, E., Miyazaki, M., Tatsumi, T., Takahashi, T., and Matsubara, H. (2006). Granulocyte colony-stimulating factor-mobilized circulating c-Kit+/Flk-1+ progenitor cells regenerate endothelium and inhibit neointimal hyperplasia after vascular injury. *Arterioscler. Thromb. Vasc. Biol.* 26, 751–757.

Tang, E. H., and Vanhoutte, P. M. (2008). Gene expression changes of prostanoid synthases in endothelial cells and prostanoid receptors in vascular smooth muscle cells caused by aging and hypertension. *Physiol. Genomics* 32, 409–418.

Thijssen, D. H., Rongen, G. A., Van Dijk, A., Smits, P., and Hopman, M. T. (2007). Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects. *J. Appl. Physiol.* 103, 852–857.

Toda, N. (2012). Age-related changes in endothelial function and blood flow regulation. *Pharmacol. Ther.* 133, 159–176.

Touyz, R. M. (2003). Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev. Cardiovasc. Ther.* 1, 91–106.

Tsukahara, H., Ende, H., Magazine, H. I., Bahou, W. F., and Goligorsky, M. S. (1994). Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells. Receptor coupling to nitric oxide synthase. *J. Biol. Chem.* 269, 21778–21785.

Ungvari, Z., Buffenstein, R., Austad, S. N., Podlutsky, A., Kaley, G., and Csiszar, A. (2008). Oxidative stress in vascular senescence: lessons from successfully aging species. *Front. Biosci.* 13, 5056–5070.

Ungvari, Z., Csiszar, A., and Kaley, G. (2004). Vascular inflammation in aging. *Herz* 29, 733–740.

Ungvari, Z., Kaley, G., De Cabo, R., Sonntag, W. E., and Csiszar, A. (2010a). Mechanisms of vascular aging: new perspectives. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1028–1041.

Ungvari, Z., Sonntag, W. E., and Csiszar, A. (2010b). Mitochondria and aging in the vascular system. *J. Mol. Med.* 88, 1021–1027.

Upmacis, R. K., Deeb, R. S., and Hajjar, D. P. (2006). Oxidative alterations of cyclooxygenase during atherogenesis. *Prostaglandins Other Lipid Mediat.* 80, 1–14.

Urakami-Harasawa, L., Shimokawa, H., Nakashima, M., Egashira, K., and Takeshita, A. (1997). Importance of endothelium-derived hyperpolarizing factor in human arteries. *J. Clin. Invest.* 100, 2793–2799.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39, 44–84.

van Bussel, B. C., Schouten, F., Henry, R. M., Schalkwijk, C. G., De Boer, M. R., Ferreira, I., Smulders, Y. M., Twisk, J. W., and Stehouwer, C. D. (2011). Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. *Hypertension* 58, 588–595.

van der Loo, B., Labugger, R., Skepper, J. N., Bachschmid, M., Kilo, J., Powell, J. M., Palacios-Callender, M., Erusalimsky, J. D., Quaschning, T., Malinski, T., Gygi, D., Ullrich, V., and Luscher, T. F. (2000). Enhanced peroxynitrite formation is associated with vascular aging. *J. Exp. Med.* 192, 1731–1744.

Van Guilder, G. P., Westby, C. M., Greiner, J. J., Stauffer, B. L., and Desouza, C. A. (2007). Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise. *Hypertension* 50, 403–409.

Vanhoutte, P. M., Feletou, M., and Taddei, S. (2005). Endothelium-dependent contractions in hypertension. *Br. J. Pharmacol.* 144, 449–458.

Vasa, M., Breitschopf, K., Zeiher, A. M., and Dimmeler, S. (2000). Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ. Res.* 87, 540–542.

Vasquez-Vivar, J., Kalyanaraman, B., and Martasek, P. (2003). The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. *Free Radic. Res.* 37, 121–127.

Virdis, A., Ghiadoni, L., Giannarelli, C., and Taddei, S. (2010). Endothelial dysfunction and vascular disease in later life. *Maturitas* 67, 20–24.

Volpato, S., Guralnik, J. M., Ferrucci, L., Balfour, J., Chaves, P., Fried, L. P., and Harris, T. B. (2001). Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study. *Circulation* 103, 947–953.

Walker, A. E., Seibert, S. M., Donato, A. J., Pierce, G. L., and Seals, D. R. (2010). Vascular endothelial function is related to white blood cell count and myeloperoxidase among healthy middle-aged and older adults. *Hypertension* 55, 363–369.

Wang, C. Y., Kim, H. H., Hiroi, Y., Sawada, N., Salomone, S., Benjamin, L. E., Walsh, K., Moskowitz, M. A., and Liao, J. K. (2009). Obesity increases vascular senescence and susceptibility to ischemic injury through chronic activation of Akt and mTOR. *Sci. Signal* 2, ra11.

Wang, M., Takagi, G., Asai, K., Resuello, R. G., Natividad, F. F., Vatner, D. E., Vatner, S. F., and Lakatta, E. G. (2003). Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. *Hypertension* 41, 1308–1316.

Wang, M., Zhang, J., Jiang, L. Q., Spinetti, G., Pintus, G., Monticone, R., Kolodgie, F. D., Virmani, R., and Lakatta, E. G. (2007). Proinflammatory profile within the grossly normal aged human aortic wall. *Hypertension* 50, 219–227.

Watson, N. L., Sutton-Tyrrell, K., Youk, A. O., Boudreau, R. M., Mackey, R. H., Simonsick, E. M., Rosano, C., Hardy, S. E., Windham, B. G., Harris, T. B., Najjar, S. S., Lakatta, E. G., Atkinson, H. H., Johnson, K. C., Bauer, D. C., and Newman, A. B. (2011). Arterial stiffness and gait speed in older adults with and without peripheral arterial disease. *Am. J. Hypertens.* 24, 90–95.

Weiss, E. P., and Fontana, L. (2011). Caloric restriction: powerful protection for the aging heart and vasculature. *Am. J. Physiol. Heart Circ. Physiol.* 301, H1205–H1219.

Williamson, K., Stringer, S. E., and Alexander, M. Y. (2012). Endothelial progenitor cells enter the aging arena. *Front. Physiol.* 3:30. doi:10.3389/fphys.2012.00030

Wong, S. L., Leung, F. P., Lau, C. W., Au, C. L., Yung, L. M., Yao, X., Chen, Z. Y., Vanhoutte, P. M., Gollasch, M., and Huang, Y. (2009). Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with increased impact during aging. *Circ. Res.* 104, 228–235.

Wray, D. W., Nishiyama, S. K., Harris, R. A., Zhao, J., Mcdaniel, J., Fjeldstad, A. S., Witman, M. A., Ives, S. J., Barrett-O'keefe, Z., and Richardson, R. S. (2012). Acute reversal of endothelial dysfunction in the elderly after antioxidant consumption. *Hypertension* 59, 818–824.

Yamagishi, S., and Matsui, T. (2011). Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy-friend or foe? *Pharmacol. Res.* 64, 187–194.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332, 411–415.

Yang, Y. M., Huang, A., Kaley, G., and Sun, D. (2009a). eNOS uncoupling and endothelial dysfunction in aged vessels. *Am. J. Physiol. Heart Circ. Physiol.* 297, H1829–H1836.

Yang, Z., Huang, X., Jiang, H., Zhang, Y., Liu, H., Qin, C., Eisner, G. M., Jose, P. A., Rudolph, L., and Ju, Z. (2009b). Short telomeres and prognosis of hypertension in a Chinese population. *Hypertension* 53, 639–645.

Yu, B. P., and Chung, H. Y. (2006). Adaptive mechanisms to oxidative stress during aging. *Mech. Ageing Dev.* 127, 436–443.

Yuan, Q., Peng, J., Liu, S. Y., Wang, C. J., Xiang, D. X., Xiong, X. M., Hu, C. P., and Li, Y. J. (2010). Inhibitory effect of resveratrol derivative BTM-0512 on high glucose-induced cell senescence involves dimethylaminohydrolase/asymmetric dimethylarginine pathway. *Clin. Exp. Pharmacol. Physiol.* 37, 630–635.

Zou, M. H., Shi, C., and Cohen, R. A. (2002). Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J. Clin. Invest.* 109, 817–826.

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Differential modulation of nitric oxide synthases in aging: therapeutic opportunities

Stefany B. A. Cau, Fernando S. Carneiro and Rita C. Tostes*

Department of Pharmacology, Medical School of Ribeirao Preto, Ribeirao Preto, Brazil

Edited by:
Elisabet Vila, Autonomous University of Barcelona, Spain

Reviewed by:
Elisabet Vila, Autonomous University of Barcelona, Spain
Maria Dolores Herrera, Universidad de Sevilla, Spain

Correspondence:
Rita C. Tostes, Department of Pharmacology, Medical School of Ribeirao Preto, University of Sao Paulo, Avenida Bandeirantes 3900, Ribeirao Preto, Sao Paulo 14049-900, Brazil.
e-mail: rtostes@usp.br

Vascular aging is the term that describes the structural and functional disturbances of the vasculature with advancing aging. The molecular mechanisms of aging-associated endothelial dysfunction are complex, but reduced nitric oxide (NO) bioavailability and altered vascular expression and activity of NO synthase (NOS) enzymes have been implicated as major players. Impaired vascular relaxation in aging has been attributed to reduced endothelial NOS (eNOS)-derived NO, while increased inducible NOS (iNOS) expression seems to account for nitrosative stress and disrupted vascular homeostasis. Although eNOS is considered the main source of NO in the vascular endothelium, neuronal NOS (nNOS) also contributes to endothelial cells-derived NO, a mechanism that is reduced in aging. Pharmacological modulation of NO generation and expression/activity of NOS isoforms may represent a therapeutic alternative to prevent the progression of cardiovascular diseases. Accordingly, this review will focus on drugs that modulate NO bioavailability, such as nitrite anions and NO-releasing non-steroidal anti-inflammatory drugs, hormones (dehydroepiandrosterone and estrogen), statins, resveratrol, and folic acid, since they may be useful to treat/to prevent aging-associated vascular dysfunction. The impact of these therapies on life quality in elderly and longevity will be discussed.

Keywords: aging, endothelial dysfunction, nitric oxide, nitric oxide synthases, uncoupled eNOS, statin, resveratrol, folic acid

INTRODUCTION

Many disorders emerge with advancing aging, and cardiovascular diseases (CVD) are a major cause of morbidity and mortality in the elderly[1] (Lakatta and Levy, 2003). The term vascular aging encompasses all the structural and functional alterations in the blood vessels with progressive aging (Herrera et al., 2010). Both smooth muscle cells and intima layers are affected. These vascular changes lead to endothelial dysfunction, arterial stiffness in consequence of intense remodeling and calcification, impaired angiogenesis, greater susceptibility to vascular injury and atherosclerotic lesions (Lakatta and Levy, 2003; Herrera et al., 2010).

The mechanisms underlying vascular aging are complex and involve multiple pathways and factors, and a comprehensive description of all mechanisms is beyond the scope of this review. Excellent and very recent reviews on the topic are available (Herrera et al., 2010; Seals et al., 2011; Toda, 2011). In this complex scenario, vascular function depends on the balanced production/bioavailability of nitric oxide (NO), which is maintained by the normal activity of endothelial nitric oxide synthase (eNOS). On the other hand, excessive amount of NO produced by inducible nitric oxide synthase (iNOS) up-regulation contributes to vascular dysfunction. Evidence obtained from experimental models indicates that decreased NO bioavailability as well as increased reactive nitrogen species (RNS) production contributes to aging-associated vascular dysfunction. These effects have been attributed to abnormal expression and activity of vascular NO synthase (NOS) isoforms. Accordingly, in this review, a very brief overview on NO signaling in cardiovascular aging is provided. The role of each NOS isoform on aging-associated vascular dysfunction is then discussed. The use of drugs that affect NO generation or modulate NOS expression/activity, including nitrite anions, NO-releasing non-steroidal anti-inflammatory drugs, statins, hormones (estrogen and dehydroepiandrosterone), resveratrol, and folic acid to prevent vascular dysfunction in elderly is also addressed.

BRIEF BIOCHEMISTRY OF NO SIGNALING

NO is a highly reactive signaling molecule, produced from the oxidation of the essential amino acid L-arginine by the NOS family (**Figure 1**). The eNOS and neuronal nitric oxide synthase (nNOS) isoforms are constitutively expressed in many tissues, including vessels and neurons (Melikian et al., 2009; Villanueva and Giulivi, 2010). Conversely, iNOS is expressed by vascular cells in response

[1]According to the Department of Health Statistics and Health Information Systems, World Health Organization (WHO), (http://www.who.int/healthinfo/survey/ageingdefnolder/en/index.html), "Most developed world countries have accepted the chronological age of 65 years as a definition of "elderly" or older person," although this concept does not adapt well to every country or population. Chronological or "official" definitions of aging can differ widely from traditional or community definitions of when a person is older. In many parts of the developing world, chronological time has little or no importance in the meaning of old age, which is seen to begin at the point when active contribution is no longer possible. Since in this review the aging process is being looked from a biological perspective, which has its own dynamic, largely beyond human control, the chronological aging definition may seem more appropriate.

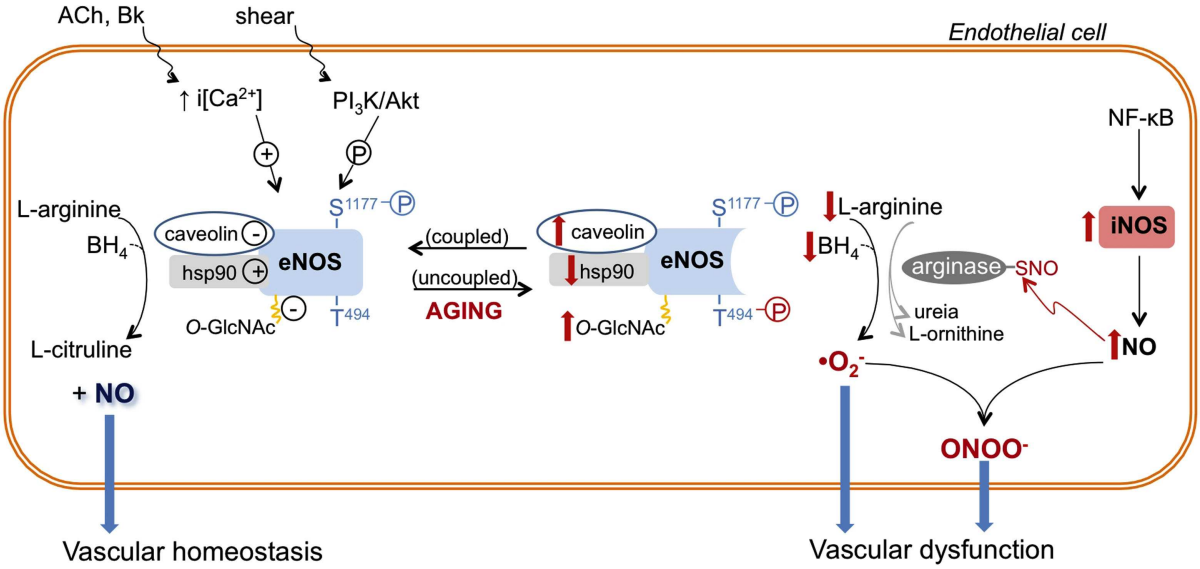

FIGURE 1 | Role of nitric oxide synthase (NOS) enzymes in endothelial function and aging-associated endothelial dysfunction. Endothelial (e)-NOS isoform converts l-arginine to nitric oxide (NO). eNOS activity is determined by intracellular calcium concentration [i(Ca^{2+})] and/or its phosphorylation (P) state at different sites (e.g., eNOS is positively and negatively modulated by phosphorylation of serine 1177 (S^{1177}) and threonine 494 (T^{494}), respectively). In addition, eNOS activity is inhibited by its interaction with caveolin-1 and by *O*-GlcNAc modification, while eNOS association with heat shock protein 90 (hsp90) favors its activation. In endothelial aging, NO synthesis is compromised because eNOS activity is decreased due to increased expression and interaction with caveolin, reduced expression and association with hsp90, reduced phosphorylation of S^{1177} and increased phosphorylation of T^{494}. In addition, reduced availability of l-arginine and tetrahydrobiopterin (BH_4) induces eNOS uncoupling or changes the enzyme to a state that favors superoxide anion ($\bullet O_2^-$) generation. In aged endothelial cells, up-regulated iNOS, which may be associated with NF-κB-induced vascular inflammation, produces high levels of NO. NO reacts with cysteine residues of proteins (arginase in this example), forming *S*-nitrosothiol (-SNO)–arginase, which increases arginase activity and l-arginine consumption. NO directly reacts with $\bullet O_2^-$ produced by uncoupled eNOS, and other vascular sources, generating the harmful reactive nitrogen specie peroxynitrite ($ONOO^-$), which contributes to vascular dysfunction. The aging–associated NOS alterations are depicted in red. ACh, acetylcholine; Bk, bradykinin; PI_3K, phosphoinositide-3-kinase.

to chemokines and invading microorganisms (Hecker et al., 1999). eNOS and nNOS are calcium (Ca^{2+})-dependent, while iNOS is Ca^{2+}-independent. Activation of specific receptors on endothelial cells (e.g., by bradykinin) increases the intracellular levels of Ca^{2+}, which in turn, promotes eNOS activation and NO generation in the vascular system (**Figure 1**). NO production is also increased as a consequence of mechanical distension (shear stress) caused by blood flow on the vascular wall. NO generated by nNOS and iNOS is classically described as a neurotransmitter and a reactive molecule involved in bacterial killing, respectively. However, recent advances highlight the role of nNOS and iNOS in cardiovascular (dys)function (Melikian et al., 2009; Tsutsui et al., 2009) as well as in aging, as discussed later.

Nitrite and nitrate anions are known as end products of NO oxidation and reflect endogenous NOS activity (Dejam et al., 2004; Moncada and Higgs, 2006). However, nitrite can be reduced to NO by either hypoxia, tissue acidosis conditions, or by a number of enzymes. This makes nitrite an important complementary reservoir of NO in physiological conditions (Dejam et al., 2004).

NOS-produced NO reacts rapidly with amino acids residues, ions, and superoxide anion ($\bullet O_2^-$), a reactive oxygen species (ROS; Moncada and Higgs, 2006). The latter reaction is particularly important since, as a $\bullet O_2^-$ scavenger, NO becomes an antioxidant molecule at the same time that its bioavailability is reduced in the presence of high $\bullet O_2^-$ levels. Under conditions of L-arginine or tetrahydrobiopterin (BH_4) deficiency, NOS becomes a radical generating enzyme, a phenomenon referred as NOS uncoupling.

EVIDENCE FOR A ROLE OF NO IN THE REGULATION OF LONGEVITY

NO seems to be a key molecule to longevity and cardiovascular health. This concept was partially derived from studies addressing the cardiovascular function in mice that do not express at least one, a combination of two, or all NOS isozymes genes ($nNOS^{-/-}$; $iNOS^{-/-}$; $eNOS^{-/-}$; $n/iNOSs^{-/-}$; $n/eNOSs^{-/-}$; $i/eNOSs^{-/-}$; $n/i/eNOSs^{-/-}$; Tsutsui et al., 2009). Accordingly, the survival rate of 10 month-old triple NOS knockout mice is reduced by ∼80%, mainly as a consequence of spontaneous myocardial infarction, coronary arteriosclerosis and mast cell infiltration in the coronary artery adventitia (Tsutsui et al., 2009). The role of each NOS is well established in some CVD. For instance, studies in mice models of atherogenesis demonstrated that eNOS and nNOS play protective roles, whereas iNOS is pro-atherosclerotic (Tsutsui et al., 2009). On the other hand, in particular conditions such as myocardial ischemia, iNOS up-regulation represents a compensatory molecular mechanism that protects myocardial cells from damage (Bolli, 2001). However, few studies have addressed whether chronic ablation of a specific NOS isoform affects lifespan. A role for eNOS in lifespan and spatial memory was suggested by a report showing that the survival rate of eNOS knockout mice was

reduced by 50%. Those mice also exhibit decreased exploratory behavior at 18–22 weeks of age when compared to age-matched controls (Dere et al., 2002). In addition, it has been reported that caloric restriction extends lifespan. However, this effect was strongly attenuated in eNOS knockout mice (Nisoli et al., 2005). This implicates eNOS-derived NO as one of the mechanisms by which caloric restriction extends lifespan.

In humans, an iNOS polymorphism is significantly more frequent in elderly hypertensive patients, indicating that abnormal iNOS-mediated NO formation may affect longevity in patients with moderate to severe hypertension (Glenn et al., 1999). In addition, cardiac overexpression of iNOS in mice leads to increased mortality, which is associated with gross ventricular dilation and hypertrophy, and sudden cardiac death due to bradyarrhythmia (Mungrue et al., 2002). Taken together, these data suggest that altered patterns of NO generation/turnover contribute to greater susceptibility to cardiovascular disorders in elderly, and that its regulation may decrease the risk of aging-associated diseases.

NO GENERATION IN CARDIOVASCULAR AGING

eNOS-derived NO is a fundamental mediator of vascular homeostasis (Moncada and Higgs, 2006). NO mediates vasodilation, inhibits leukocytes adhesion, and has antithrombotic and anti-apoptotic effects. Several studies in human and animal models have shown that endothelium-dependent vasodilatation, a marker of endothelial function, progressively declines with age (Gerhard et al., 1996; Taddei et al., 2001; Muller-Delp et al., 2002; Blackwell et al., 2004; Sun et al., 2004; Delp et al., 2008; Rodriguez-Manas et al., 2009). Age-associated infirmities such as arterial hypertension, atherosclerosis, heart failure, and neurodegenerative diseases have endothelial dysfunction as a common vascular component. Reduced NO bioavailability in aging is a consistent feature in experimental and clinical studies. Vascular aging is accompanied by reduced eNOS expression/activity or augmented breakdown of NO by ROS. In addition, senescent endothelial cells phenotype shifts toward an inflammatory state, with up-regulation of adhesion molecules, cytokines, and chemokines (Ungvari et al., 2004). This phenotype favors platelet aggregation and inflammatory cell adhesion, which may progress to thrombotic and atherosclerotic events. The expression of pro-inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and iNOS is induced by aging-associated endothelial dysfunction (Ungvari et al., 2004). COX-2-derived lipid mediators can contribute to enhanced reactivity to vasoconstrictors (Briones et al., 2005; Novella et al., 2011). In addition, iNOS-derived high levels of NO can induce post-translational modification of proteins or react with ROS to produce RNS, which increases vasoconstrictor tonus in aged arteries.

The major molecular mechanisms of age-related endothelial dysfunction involve at least three NO-associated events: NO consumption by $\bullet O_2^-$ overproduction, reduced vascular antioxidant ability, and altered NOS enzymes expression/activity. Vascular oxidative stress also interferes with the actions of endothelial cell-derived NO on vascular smooth muscle cells. Treatment of old animals with antioxidants or the exposure of arteries isolated from old mice to antioxidants restores endothelium-dependent dilation (Blackwell et al., 2004; Fleenor et al., 2011). The main vascular sources of ROS have been identified in aging: detaching mitochondria (Ungvari et al., 2010) and nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (NOx; Hamilton et al., 2001; Fleenor et al., 2011). As will be discussed, NOS can also act as a $\bullet O_2^-$ generator. Antioxidant enzymes such as superoxide dismutase, glutathione, and catalase are also down-regulated or exhibit decreased activity with aging (Sun et al., 2004; Lund et al., 2009; Denniss et al., 2011; Fleenor et al., 2011). The final consequence is an unbalanced ROS production and NO depletion in detriment of the antioxidant capacity.

Identifying the changes that each NOS isoform undergoes in vascular aging not only may help us to understand the natural adaptations of the NO pathways in the cardiovascular system with progressive aging, but may also provide novel targets for the treatment of aging-associated CVD.

EFFECTS OF AGING ON VASCULAR NOS ISOFORMS

EFFECTS OF AGING ON eNOS

The role of aging on eNOS expression is controversial. Vascular eNOS expression with advancing aging has been shown to be either unchanged (Sun et al., 2004; Rodriguez-Manas et al., 2009; Yang et al., 2009), decreased (Challah et al., 1997; Yoon et al., 2010), or increased (Cernadas et al., 1998; Matz et al., 2000; van der Loo et al., 2000; Goettsch et al., 2001). Although data on eNOS expression are contradictory, it is well accepted that the activity of eNOS is reduced in aging (Cernadas et al., 1998; Chou et al., 1998; Smith et al., 2006a,b; Yoon et al., 2010). Particularly, eNOS activity is decreased by deficient availability of substrates or cofactors, subcellular location, protein–protein interactions, and post-translational modifications such as acylation, nitrosylation, O-GlcNAcylation, and phosphorylation (Dudzinski and Michel, 2007; Villanueva and Giulivi, 2010).

Plasma membrane-associated eNOS represents an immediately available agonist-sensitive pool of the enzyme (Dudzinski and Michel, 2007; Villanueva and Giulivi, 2010). In old rats, the amount of eNOS localized in the endothelial cell plasma membrane is reduced (Smith et al., 2006b). In addition, senescent endothelial cells *in vitro* display reduced NO synthesis accompanied by increased eNOS linkage with caveolin-1 (Yoon et al., 2010), a protein whose interaction with eNOS keeps the enzyme inactivated in a region of the plasma membrane named caveolae (Dudzinski and Michel, 2007). eNOS activity is extended by protein–protein association with heat shock protein 90 (hsp90), a chaperone involved in protein trafficking and folding (Dudzinski and Michel, 2007). Hsp90 expression and binding to eNOS is reduced in old endothelial cells (Smith et al., 2006b; Yoon et al., 2010). Together, these may partially explain the decreased eNOS activity and the reduced NO-dependent vascular relaxation in elderly, as summarized in **Figure 1**.

In the presence of low intracellular Ca^{2+} levels, eNOS activity is maintained by phosphoinositide-3-kinase (PI_3K)/Akt-mediated phosphorylation of serine 1176 (S^{1176}) or 1177 (S^{1177}), in rat and human, respectively (Dudzinski and Michel, 2007). In contrast, phosphorylation of eNOS at threonine 494 (T^{494}) negatively regulates its activity (Dudzinski and Michel, 2007). Reduced eNOS phosphorylation at S^{1176}/S^{1177} and enhanced phosphorylation at T^{494} have been reported in endothelial cells from aged rats (Smith et al., 2006a) and senescent human endothelial cells *in vitro* (Yoon

et al., 2010). Together, these data further support a role for reduced eNOS-produced NO in aging-associated vascular dysfunction.

Glycosylation with *O*-linked-β-*N*-acetylglucosamine (*O*-GlcNAc) is a post-translational modification of nuclear and cytoplasmic proteins. *O*-GlcNAc modification of eNOS seems to reduce eNOS phosphorylation at Ser^{1177} and, consequently, eNOS activity (Lima et al., 2009). The vascular content of *O*-GlcNAc-modified proteins is augmented in the vasculature of old animals (Fulop et al., 2008). Although the amount of vascular *O*-GlcNAc-modified eNOS in old animals has not yet been determined, it is possible that senescence-associated increased vascular *O*-GlcNAc levels can also contribute to eNOS dysfunction in aging.

In addition to its reduced NO-releasing ability, eNOS seems to play a deleterious role in aging. Accordingly, endothelium removal reverses the increased vascular $\bullet O_2^-$ production associated with aging (van der Loo et al., 2000). Furthermore, aging-associated vascular oxidative stress is partially reversed by pharmacological NOS inhibition (Kim et al., 2009; Yang et al., 2009), which suggests that eNOS contributes to $\bullet O_2^-$ generation. As already mentioned, uncoupled eNOS is a source of $\bullet O_2^-$ in the vasculature. In fact, the ratio of monomeric eNOS is increased in mesenteric arteries from aged rats, indicating that eNOS uncoupling is associated with increased $\bullet O_2^-$ production (Yang et al., 2009). In aging, eNOS uncoupling has been associated with reduced BH_4 availability (Delp et al., 2008; Yang et al., 2009) rather than deficiency of L-arginine (Gates et al., 2007). Despite that, inhibition of arginase, an enzyme that degrades L-arginine, restores eNOS coupling in rat aortas (Kim et al., 2009). Moreover, addition of sepiapterin, a BH_4 precursor, ameliorates vascular relaxation in conduit and resistance arteries from aged animals (Blackwell et al., 2004; Delp et al., 2008) and reverses eNOS uncoupling (Yang et al., 2009).

EFFECT OF AGING ON iNOS

iNOS expression is consistently reported in aged vessels, mainly in the intima layer (Cernadas et al., 1998; Chou et al., 1998; Goettsch et al., 2001; Csiszar et al., 2002; Rodriguez-Manas et al., 2009; Tian et al., 2010). Pharmacological selective inhibition of iNOS prevented age-related decrease of cardiac function (Yang et al., 2004) and reversed impaired endothelium-dependent vasorelaxation in old rats (Tian et al., 2010) and in elder subjects (Rodriguez-Manas et al., 2009). These exciting data reveal a new pathological mechanism involving iNOS in the abnormal constrictor vascular tone associated with aging. This is also reinforced by a recent report demonstrating iNOS up-regulation in microvessels of hypertensive subjects, and an impressive restoration of endothelium-dependent vasodilation by a selective iNOS inhibitor in hypertensive patients (Smith et al., 2011).

The mechanism of iNOS-induced vascular dysfunction in aging is not completely elucidated. Aging-associated iNOS up-regulation is accompanied by RNS peroxynitrite ($ONOO^-$) production (van der Loo et al., 2000), due to the reaction of NO with $\bullet O_2^-$, which is facilitated in the pro-oxidant environment of aged arteries. $ONOO^-$ is a potent and harmful molecule to cellular lipids, genetic material, and proteins (Peluffo and Radi, 2007). Intense immunostaining for nitrotyrosine, a marker of protein nitration by $ONOO^-$ (Peluffo and Radi, 2007), is found in aged vessels (Csiszar et al., 2002; Rodriguez-Manas et al., 2009; Tian et al., 2010). Furthermore, a pharmacological scavenger of $ONOO^-$ reversed the reduced endothelium-dependent dilation of arteries from old rats (van der Loo et al., 2000), implicating $ONOO^-$ in aging-associated endothelial dysfunction (**Figure 1**).

The amount of NO produced by iNOS is substantially greater than that produced by the constitutive isoforms, and NO itself may act as a cytotoxic agent and induce tissue damage (Hecker et al., 1999; Moncada and Higgs, 2006). The reaction between NO with cysteine residues of target proteins forming *S*-nitrosothiol (SNO) is denominated S-nitrosylation, and it modifies protein function (Moncada and Higgs, 2006). Arginase is a target protein for S-nitrosylation, and this post-translational modification increases the enzyme activity. Increased S-nitrosylation of arginase has been implicated in the decreased NO production and reduced endothelium-dependent relaxation in aortas from old rats (Santhanam et al., 2007). Interestingly, pharmacological inhibition of iNOS abrogated arginase S-nitrosylation and restored endothelium-dependent vasodilation (Santhanam et al., 2007), indicating that increased arginase activity depends on iNOS (**Figure 1**).

EFFECTS OF AGING ON nNOS

Although the regulatory roles of eNOS and iNOS in vascular aging have been studied, just a small amount of information on the contribution of nNOS to aging-associated vascular dysfunction is available. In addition, there is limited knowledge on how nNOS modulates vascular tone (Melikian et al., 2009).

Electrical stimulation of endothelium-denuded small arteries causes α-adrenergic vasoconstriction, which is attenuated by nNOS. However, this negative modulatory effect by nNOS is significantly impaired in old rats (Ferrer and Balfagon, 2001). Furthermore, old hypertensive rats exhibit just a minor increase of vasoconstriction after pre-incubation with a selective nNOS inhibitor (Ferrer et al., 2003). These findings suggest that nNOS-derived NO in perivascular nitrergic innervation modulates microvascular tone by reducing α-adrenergic vasoconstriction and that its absence may account for the abnormal vasoconstrictor tone in aged arterioles (Ferrer and Balfagon, 2001; Ferrer et al., 2003). Of importance, more studies on nNOS function in the vasculature of aged animals as well as in aging-associated vascular dysfunction are necessary and expected.

DRUGS THAT AFFECT NO GENERATION/TURNOVER: A TREATMENT FOR AGING-ASSOCIATED VASCULAR DYSFUNCTION IN HUMANS?

Due to its protective role on vascular homeostasis, adequate NO synthesis and signaling represents an important goal in the treatment of vascular dysfunction. Therefore it is important to understand the cellular and molecular mechanisms that control the function and expression of NOS isoforms as well as the implications this may have for aging-associated vascular dysfunction. Unfortunately, as specific NOS isoforms pharmacological inhibitors are still limited for experimental interventions or local administration in humans, the effects of selective NOS activity inhibition and/or stimulation remains unknown.

RESTORING NO LEVELS

Due to their ability to restore reduced NO bioavailability, NO-donors represent a therapeutic strategy to treat aging-associated CVD. Although acute administration of the NO-donor sodium nitroprusside improved cardiovascular performance at rest and during exercise in healthy older volunteers (Chantler et al., 2011), inorganic and organic nitrates (e.g., nitroglycerin) are a class of compounds with limited long-term therapeutic use due to the induction of tolerance. Experimentally, NO-releasing non-steroidal anti-inflammatory drugs (NO-NSAID), which are pro-drugs that contain a NO-donor moiety chemically attached to a parent NSAID, have shown promising anti-inflammatory activity and NO-releasing properties (Fiorucci et al., 2001). NO-releasing-aspirin, but not aspirin, reduced vascular smooth muscle cell proliferation in response to vascular injury in old rats (Napoli et al., 2002), an effect that may be clinically relevant in the treatment of aging-associated vascular remodeling.

An emerging alternative to directly increase NO levels is represented by nitrite anions. Different schedules of inorganic nitrite supplementation as well as nitrite-rich diets have been shown to induce vascular relaxation and to reduce blood pressure (Cosby et al., 2003; Larsen et al., 2006; Webb et al., 2008; Kapil et al., 2010). A traditional Japanese diet, for a 10-day-period, increased plasma levels of nitrite and lowered blood pressure in healthy volunteers (Sobko et al., 2010). These results suggest that the higher longevity in the Japanese population compared to the occidental population may be associated with high levels of nitrites on their diet. There is a general agreement that inorganic and dietary nitrites can be therapeutically used to treat CVD and possibly, cardiovascular aging. However, uncertainties about the safety of nitrites limit clinical interventions. To our knowledge, there is just one report showing an association between high nitrite consumption and increased regional brain perfusion in elderly subjects (Presley et al., 2011). Since cerebral hypoperfusion precedes and contributes to the onset of clinical dementia, this finding has clinical relevance.

STATINS

Inhibitors of cholesterol synthesis, the statins, represent a therapeutic alternative to treat vascular aging. In addition to improvement in lipid profile, these drugs increase NO bioavailability by many mechanisms that include: increased eNOS expression due to increased eNOS mRNA stability and post-transcriptional modifications (Laufs and Liao, 1998; Laufs et al., 1998), eNOS recoupling (Wenzel et al., 2008; Sabri et al., 2011), and reduced NO breakdown by ROS (**Figure 2**) (Wagner et al., 2000; Wenzel et al., 2008). Additionally, atorvastatin up-regulates nNOS expression in endothelial cells *in vitro*, and about a quarter of the total NO production in aortic rings were attributed to atorvastatin-induced nNOS expression (Nakata et al., 2007). These pleiotropic effects of statins help to justify the huge number of clinical trials addressing the benefits of statin treatment in CVD (revised by Baigent et al., 2005).

Experimentally, statins reverse impaired endothelium-dependent vasodilation in old rats and increase vascular levels of eNOS (de Sotomayor et al., 2005). Conversely, impaired endothelium-dependent vasodilatation was not modified by atorvastatin treatment in elderly subjects without associated morbidities (Weverling-Rijnsburger et al., 2004). Although this finding seems to be disappointing, observational studies reported that statin treatment lowered the ratio of fatal outcomes in elderly with CVD (Foody et al., 2006; Gransbo et al., 2010). In the double-blind, randomized, placebo-controlled multicenter study PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), pravastatin was found to reduce death caused by coronary heart disease and the risk for non-fatal myocardial infarction in the treated elderly group (Shepherd et al., 2002), reinforcing the suggestion that statins may prevent cardiovascular aging.

Transgenic mouse with endothelial-targeted overexpression of guanosine triphosphate cyclohydrolase-1 (GTPCH-1), the rate-limiting enzyme for *de novo* BH_4 synthesis, does not exhibit diabetes-associated vascular abnormalities (Alp et al., 2003). Whereas streptozotocin (STZ) increases vascular oxidative stress and impairs NO-mediated endothelium-dependent vasodilatation in control mice, STZ-treated transgenic GTPCH-1 mice exhibit less superoxide production and normal NO-mediated vasodilatation (Alp et al., 2003). Similarly, treatment of hypertensive rats with simvastatin restored GTPCH-1 activity and the BH_4/eNOS/NO pathway reversing the hypertension-associated endothelial dysfunction (Zhang et al., 2012). *In vitro*, statins rapidly increase BH_4 bioavailability and up-regulate GTPCH-1, leading to eNOS recoupling and NO generation in endothelial cells (Hattori et al., 2003; Aoki et al., 2012; Zhang et al., 2012). Of importance, patients with coronary artery disease treated with atorvastatin showed very early improvement of flow-mediated dilation in the brachial artery and restored BH_4 levels in mammary arteries compared with the placebo group (Antoniades et al., 2011). Moreover, the incubation of human mammary arteries with atorvastatin augmented GTPCH-1 expression (Antoniades et al., 2011). Collectively, experimental findings indicate that statins produce beneficial vascular effects via actions in the GTPCH/BH_4/NO pathway and may be used for the treatment of vascular aging.

HORMONE THERAPIES: DEHYDROEPIANDROSTERONE (DHEA) AND ESTROGEN

Therapy with hormones, mainly dehydroepiandrosterone (DHEA) and estrogen, has been considered a "treatment for aging" (**Figure 2**) (Lamberts et al., 1997). DHEA replacement therapy improved endothelium-dependent dilation in normotensive hypercholesterolemic men and post-menopause women (Kawano et al., 2003; Williams et al., 2004). One potential explanation for the above observations is based on experimental data on DHEA-treated old rats. These animals showed increased aortic eNOS expression and restored NO production, although the repercussion on vascular function was not investigated (Wu et al., 2007). Disappointingly, a set of clinical observations reported null effects with DHEA supplementation in men with CVD (Traish et al., 2011).

Despite of the controversy about the benefits of estrogen replacement therapy in post-menopausal women, cardiovascular benefits of estrogen have been attributed to its modulatory effects on NO generation by NOS isoforms (Xing et al., 2009). Estrogen deficiency, induced by ovariectomy, further increased aging-associated vasoconstriction and impaired NO signaling (Stice et al., 2009; Novella et al., 2010). Estrogen replacement also improved flow-induced vasodilation in coronary arterioles

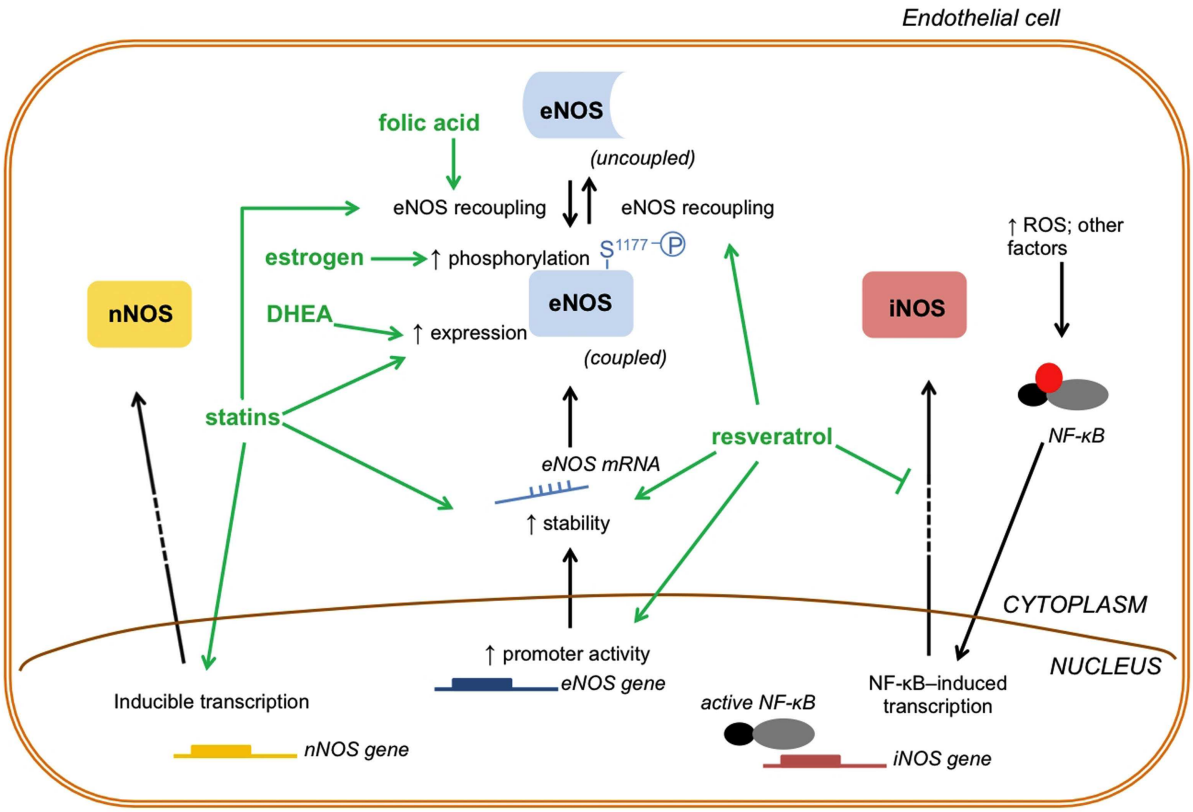

FIGURE 2 | Drugs that affect NO generation/turnover by modulating NOS enzymes. The drugs that modulate NOS enzymes and, consequently NO production, act by changing three important aspects of NOS enzymes function: activity, expression and transcription: (1) activity; estrogen induces eNOS phosphorylation at (S^{1177}) to increase enzyme activity; statins and folic acid may cause eNOS recoupling to increase eNOS activity and NO production; (2) expression; statins and DHEA increase eNOS expression and, consequently, NO generation; (3) transcription; statins and resveratrol act at the transcriptional level of eNOS increasing its mRNA stability. In addition, resveratrol activates the promoter gene of eNOS increasing its transcription. Whereas eNOS enzyme function can be altered in three different ways, nNOS and iNOS function can be modulated at the transcriptional level by some of these drugs. Statins may directly induce nNOS transcription and increase nNOS protein levels. On the other hand, resveratrol indirectly decreases iNOS transcription by inhibiting NF-κB. DHEA, dehydroepiandrosterone; ROS, reactive oxygen species; NF-κB, nuclear factor kappa B; mRNA, messenger RNA.

of aged and ovariectomized rats and restored eNOS phosphorylation (Ser^{1176}), suggesting a positive regulation of estrogen on eNOS activity in old females (LeBlanc et al., 2009). In addition, vascular dysfunction in aged female rats is associated with both decreased levels of estrogen and an altered pattern of estrogen receptor-mediated NO-induced vascular relaxation (Novensa et al., 2011). This provides a new insight for the refractoriness of age-related vascular dysfunction to estrogen replacement therapy.

Beneficial and harmful effects of estrogen on the vasculature are also associated to iNOS protein expression. Ovariectomized rats exhibited greater vascular iNOS expression than intact animals and estrogen replacement reduced iNOS expression in ovariectomized rats (Tamura et al., 2000). Therefore, it is possible that vascular dysfunction that accompanies estrogen withdrawal is related to increased iNOS levels. However, estrogen replacement reversed the vascular remodeling in injured arteries from ovariectomized mice that do not express iNOS, suggesting that estrogen-associated vascular protection is not only related to suppression of iNOS expression/activity (Tolbert et al., 2001). Conversely, estrogen has been shown to attenuate vasoconstriction by an estrogen receptor beta-mediated increase in iNOS expression (Zhu et al., 2002). The physiological significance of iNOS activation by estrogen on vascular tonus remains uncertain, since the absence of either alpha or beta estrogen receptors, which increases vascular iNOS, does not affect vascular sensitivity to vasoconstrictors (Liang et al., 2003). The protective effect associated with estrogen-induced iNOS expression may be related to a reduction in thrombotic events, since the gender-associated protection against experimentally induced thrombosis is lost in female mice that do not express iNOS (Upmacis et al., 2011). These data suggest that basal levels of estrogen can actively produce NO via iNOS and inhibit thrombus formation in females. Although yet untested, it is unlikely that estrogen replacement therapy elicits this effect, since a meta-analysis of observational studies reported a statistically increase in the risk for thromboembolic events among estrogen users (Nelson et al., 2002).

With regard to nNOS, it was demonstrated that endothelium-dependent relaxation of resistance arteries by estrogen is nNOS-

dependent in female, whereas it is eNOS-dependent in males (Lekontseva et al., 2011). Although the results of estrogen-induced relaxation via nNOS are extremely encouraging, the physiological significance of these findings has yet to be determined. Additionally, the effects of aging and estrogen replacement therapy in this phenomenon are poorly understood and more studies are needed. Finally, it must be considered that hormone replacement therapy in women is usually prescribed as a combination of estrogen and progestin (a synthetic analog of progesterone). The estrogen/progestin association may cause differential vascular effects than those produced by estrogen alone (Qiao et al., 2008).

RESVERATROL

Accumulating evidence support that resveratrol, a natural occurring polyphenol derived from plants such as grapes, exerts anti-aging effects. There are several genes and proteins known to influence longevity, such as the mammalian target of rapamycin (mTOR) – see a recent review and also a comment on topic (Cau and Tostes, 2012; Ming et al., 2012) – and sirtuin families pathways, which have been involved in the anti-aging properties of resveratrol (Pallàs et al., 2010). Whereas resveratrol extends lifespan in yeast and nematode, the polyphenol has been shown to increase survival rate in mice on a high-calorie diet. Resveratrol increased insulin sensitivity and improved motor function in these animals (Baur et al., 2006). In addition, resveratrol has been shown to inhibit gene expression profiles associated with cardiac and skeletal muscle aging, and prevent age-related cardiac dysfunction (Barger et al., 2008). Of special interest, resveratrol prevents vascular cell senescence by mechanisms that involve increased NO production by eNOS via multifaceted signaling pathways (Schmitt et al., 2010). Resveratrol not only restores eNOS activity, but it induces eNOS transcription via direct activation of the eNOS gene promoter and by increasing the stability of eNOS mRNA (**Figure 2**) (Wallerath et al., 2002).

Due to these various effects, resveratrol treatment has been experimentally tested and considered for a wide variety of CVD, including hypertension, atherosclerosis, diabetes and metabolic syndrome (Li et al., 2012). In general, there is a growing body of evidence to support the benefits of red wine consumption (a rich dietary source of resveratrol) to reduce CVD risk in obesity (Timmers et al., 2011; Wong et al., 2011), diabetes (Kar et al., 2009; Brasnyo et al., 2011), and coronary artery disease (Whelan et al., 2004; Lekakis et al., 2005). A pilot study in older adults treated with resveratrol reported a trend toward improved endothelial function (Crandall et al., 2012); however, larger studies in elderly are still awaited.

Although resveratrol-induced dilation of isolated small mesenteric arteries is similar in young and aged rats, treatment with resveratrol reduces aging-associated vascular $\bullet O_2^-$ production and inflammation (Labinskyy et al., 2006). Resveratrol also reversed eNOS uncoupling, reduced NO synthesis, and increased $\bullet O_2^-$ production in senescent endothelial cells *in vitro* as well as in aortas of aged rats (Rajapakse et al., 2011). Similarly, vascular and cardiac eNOS recoupling was reported in resveratrol-treated spontaneously hypertensive rats (Bhatt et al., 2011) and in a mouse model of atherogenesis (Xia et al., 2010), respectively. In addition, endothelium-dependent vasodilation was restored in resveratrol-treated old rats (Rajapakse et al., 2011).

The anti-inflammatory properties of resveratrol are related to its regulatory effects on the activity of transcription factors, particularly NF-kappaB (NF-κB; Labinskyy et al., 2006). NF-κB-induced expression of pro-inflammatory genes is involved in aging-associated vascular dysfunction (Donato et al., 2009). Therefore, inhibition of NF-κB by resveratrol may reverse the increased iNOS expression in aged arterioles, and consequently improve vascular function (Labinskyy et al., 2006). However, this effect has not been experimentally demonstrated.

AGENTS TO REVERSE eNOS UNCOUPLING

Reversing eNOS uncoupling represents a therapeutic option to treat CVD (Forstermann and Li, 2011; Zhang et al., 2011). While most interventions are experimentally restricted, eNOS cofactor BH_4 potentially leads to eNOS recoupling in the human vasculature (Forstermann and Li, 2011; Zhang et al., 2011). Infusion of BH_4 has been shown to recover eNOS bioactivity in hypercholesterolemic patients, hypertensive subjects, and smokers (revised by Forstermann and Li, 2011; Zhang et al., 2011). Importantly, improved endothelium-dependent vasodilation associated to BH_4 infusion was significantly more pronounced in elderly than in young subjects (Higashi et al., 2006). A recent study showed that an oral dose of BH_4 increased carotid artery compliance and brachial artery flow-mediated dilation in post-menopausal women (Moreau et al., 2012). The authors also addressed whether BH_4 and estrogen co-administration would bring additional improvement in vascular function, but no further effects were observed (Moreau et al., 2012). It is important to highlight that these studies were performed with acute doses of BH_4 and, although promising, BH_4 supplementation potentially leads to excessive and tissue unspecific NO production and toxicity (Moens and Kass, 2006).

Interactions of 5-methyltetrahydrofolate (5-MTHF), the active metabolite of folic acid, with eNOS protein have been described (Moens et al., 2008). Likewise, 5-MTHF restores the bioavailability of BH_4 by increasing the binding affinity of BH_4 to eNOS, by chemically stabilizing BH_4, and by enhancing the regeneration of BH_4 from its inactive form BH_2 (**Figure 2**) (Moens et al., 2008). 5-MTHF reversed eNOS uncoupling and restored NO-mediated vasodilation and reduced vascular superoxide, both *ex vivo* and *in vivo* (Antoniades et al., 2006). Interestingly, this study was performed in early elderly subjects (67.2 ± 0.97 years old) with coronary artery disease (Antoniades et al., 2006). Similarly, the treatment of hypertensive mice with folic acid prevented aneurism formation and promotes eNOS recoupling (Gao et al., 2012).

At least two limitations on folic acid enrichment must be pointed out. First, high levels of folic acid can mask the diagnosis of pernicious anemia (deficiency of vitamin B_{12}) and allow the neuropathy to progress undiagnosed. This could be particularly limiting for the elderly, where substantial ratio of plasmatic levels of non-metabolized folic acid was found after supplementation (Obeid et al., 2011). However, this can be easily corrected by co-supplementation with vitamin B_{12} which further ameliorates coronary flow parameters (Kurt et al., 2010). Second, the significance of folic acid supplementation to prevent major

cardiovascular events was recently questioned in a systematic revision of clinical studies (Zhou et al., 2011), and whether this mechanism on eNOS recoupling occurs in old people treated with folic acid and represents a rational therapeutic target remains unclear.

CONCLUSION AND PERSPECTIVES

The mechanisms by which NOS enzymes promote vascular dysfunction in aging are specific for each enzyme isoform. eNOS expression and activity are decreased in aging, resulting in reduced NO synthesis. Advancing aging is also accompanied by eNOS uncoupling, which contributes to vascular oxidative stress. iNOS expression is augmented in elderly, leading to reduced endothelium-dependent vasodilation and increased damage via formation of the reactive specie peroxynitrite. Possibly, an impairment of perivascular nNOS-released NO increases vasoconstrictor tone. These data suggest a differential regulation of the NOS family isoforms in the process of vascular aging, which is associated with reduced NO and increased $\bullet O_2^-$ production, and to the vascular dysfunction that lead to aging-related CVD.

Due to additional effects on the NOS pathway, e.g., restoration of eNOS activity and decreased RNS formation, drugs clinically used to treat hypercholesterolemia and post-menopause symptoms, such as statin and estrogen, respectively, have also been considered for the treatment of cardiovascular aging. Dietary alternatives to treat vascular aging include nitrite-rich meals and folic acid supplementation, although many questions on the safety and effectiveness of long-term regimes were not elucidated so far. Resveratrol is accepted as a key pharmacological agent for prevention and treatment of aging, which interferes at multiple molecular levels in aging-related vascular dysfunction. Although it is clear that all the pharmacological agents reviewed herein produce important and beneficial cardiovascular effects, it is necessary to clinically determine whether these treatments extend lifespan.

REFERENCES

Alp, N. J., Mussa, S., Khoo, J., Cai, S., Guzik, T., Jefferson, A., Goh, N., Rockett, K. A., and Channon, K. M. (2003). Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. *J. Clin. Invest.* 112, 725–735.

Antoniades, C., Bakogiannis, C., Leeson, P., Guzik, T. J., Zhang, M. H., Tousoulis, D., Antonopoulos, A. S., Demosthenous, M., Marinou, K., Hale, A., Paschalis, A., Psarros, C., Triantafyllou, C., Bendall, J., Casadei, B., Stefanadis, C., and Channon, K. M. (2011). Rapid, direct effects of statin treatment on arterial redox state and nitric oxide bioavailability in human atherosclerosis via tetrahydrobiopterin-mediated endothelial nitric oxide synthase coupling. *Circulation* 124, 335–345.

Antoniades, C., Shirodaria, C., Warrick, N., Cai, S., de Bono, J., Lee, J., Leeson, P., Neubauer, S., Ratnatunga, C., Pillai, R., Refsum, H., and Channon, K. M. (2006). 5-Methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation* 114, 1193–1201.

Aoki, C., Nakano, A., Tanaka, S., Yanagi, K., Ohta, S., Jojima, T., Kasai, K., Takekawa, H., Hirata, K., and Hattori, Y. (2012). Fluvastatin upregulates endothelial nitric oxide synthase activity via enhancement of its phosphorylation and expression and via an increase in tetrahydrobiopterin in vascular endothelial cells. *Int. J. Cardiol.* 156, 55–61.

Baigent, C., Keech, A., Kearney, P. M., Blackwell, L., Buck, G., Pollicino, C., Kirby, A., Sourjina, T., Peto, R., Collins, R., and Simes, R. (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 366, 1267–1278.

Barger, J. L., Kayo, T., Vann, J. M., Arias, E. B., Wang, J., Hacker, T. A., Wang, Y., Raederstorff, D., Morrow, J. D., Leeuwenburgh, C., Allison, D. B., Saupe, K. W., Cartee, G. D., Weindruch, R., and Prolla, T. A. (2008). A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS ONE* 3, e2264. doi:10.1371/journal.pone.0002264

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., and Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342.

Bhatt, S. R., Lokhandwala, M. F., and Banday, A. A. (2011). Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. *Eur. J. Pharmacol.* 667, 258–264.

Blackwell, K. A., Sorenson, J. P., Richardson, D. M., Smith, L. A., Suda, O., Nath, K., and Katusic, Z. S. (2004). Mechanisms of aging-induced impairment of endothelium-dependent relaxation: role of tetrahydrobiopterin. *Am. J. Physiol. Heart Circ. Physiol.* 287, H2448–H2453.

Bolli, R. (2001). Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. *J. Mol. Cell. Cardiol.* 33, 1897–1918.

Brasnyo, P., Molnar, G. A., Mohas, M., Marko, L., Laczy, B., Cseh, J., Mikolas, E., Szijarto, I. A., Merei, A., Halmai, R., Meszaros, L. G., Sumegi, B., and Wittmann, I. (2011). Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. *Br. J. Nutr.* 106, 383–389.

Briones, A. M., Montoya, N., Giraldo, J., and Vila, E. (2005). Ageing affects nitric oxide synthase, cyclooxygenase and oxidative stress enzymes expression differently in mesenteric resistance arteries. *Auton. Autacoid Pharmacol.* 25, 155–162.

Cau, S. B., and Tostes, R. C. (2012). mTOR Inhibition: a promise for a young heart. *Front. Physiol.* 3:31. doi:10.3389/fphys.2012.00031

Cernadas, M. R., Sanchez de Miguel, L., Garcia-Duran, M., Gonzalez-Fernandez, F., Millas, I., Monton, M., Rodrigo, J., Rico, L., Fernandez, P., de Frutos, T., Rodriguez-Feo, J. A., Guerra, J., Caramelo, C., Casado, S., and Farre, L. (1998). Expression of constitutive and inducible nitric oxide synthases in the vascular wall of young and aging rats. *Circ. Res.* 83, 279–286.

Challah, M., Nadaud, S., Philippe, M., Battle, T., Soubrier, F., Corman, B., and Michel, J. B. (1997). Circulating and cellular markers of endothelial dysfunction with aging in rats. *Am. J. Physiol.* 273(Pt 2), H1941–H1948.

Chantler, P. D., Nussbacher, A., Gerstenblith, G., Schulman, S. P., Becker, L. C., Ferrucci, L., Fleg, J. L., Lakatta, E. G., and Najjar, S. S. (2011). Abnormalities in arterial-ventricular coupling in older healthy persons are attenuated by sodium nitroprusside. *Am. J. Physiol. Heart Circ. Physiol.* 300, H1914–H1922.

Chou, T. C., Yen, M. H., Li, C. Y., and Ding, Y. A. (1998). Alterations of nitric oxide synthase expression with aging and Hypertension in rats. *Hypertension* 31, 643–648.

Cosby, K., Partovi, K. S., Crawford, J. H., Patel, R. P., Reiter, C. D., Martyr, S., Yang, B. K., Waclawiw, M. A., Zalos, G., Xu, X., Huang, K. T., Shields, H., Kim-Shapiro, D. B., Schechter, A. N., Cannon, R. O. III., and Gladwin, M. T. (2003). Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat. Med.* 9, 1498–1505.

Crandall, J. P., Oram, V., Trandafirescu, G., Reid, M., Kishore, P., Hawkins, M., Cohen, H. W., and Barzilai, N. (2012). Pilot study of resveratrol in older adults with impaired glucose tolerance. *J. Gerontol. A Biol. Sci. Med. Sci.* 67A, 1–6.

Csiszar, A., Ungvari, Z., Edwards, J. G., Kaminski, P., Wolin, M. S., Koller, A., and Kaley, G. (2002). Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ. Res.* 90, 1159–1166.

de Sotomayor, M. A., Perez-Guerrero, C., Herrrera, M. D., Jimenez, L., Marin, R., Marhuenda, E., and Andriantsitohaina, R. (2005). Improvement of age-related endothelial dysfunction by simvastatin: effect on NO and COX pathways. *Br. J. Pharmacol.* 146, 1130–1138.

Dejam, A., Hunter, C. J., Schechter, A. N., and Gladwin, M. T. (2004). Emerging role of nitrite in human biology. *Blood Cells Mol. Dis.* 32, 423–429.

Delp, M. D., Behnke, B. J., Spier, S. A., Wu, G., and Muller-Delp, J. M. (2008). Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. *J. Physiol. (Lond.)* 586, 1161–1168.

Denniss, S. G., Levy, A. S., and Rush, J. W. (2011). Effects of glutathione-depleting drug buthionine sulfoximine and aging on activity of endothelium-derived relaxing and contracting factors in carotid artery of sprague-dawley rats. *J. Cardiovasc. Pharmacol.* 58, 272–283.

Dere, E., De Souza Silva, M. A., Topic, B., Fiorillo, C., Li, J. S., Sadile, A. G., Frisch, C., and Huston, J. P. (2002). Aged endothelial nitric oxide synthase knockout mice exhibit higher mortality concomitant with impaired open-field habituation and alterations in forebrain neurotransmitter levels. *Genes Brain Behav.* 1, 204–213.

Donato, A. J., Pierce, G. L., Lesniewski, L. A., and Seals, D. R. (2009). Role of NFkappaB in age-related vascular endothelial dysfunction in humans. *Aging (Albany NY)* 1, 678–680.

Dudzinski, D. M., and Michel, T. (2007). Life history of eNOS: partners and pathways. *Cardiovasc. Res.* 75, 247–260.

Ferrer, M., and Balfagon, G. (2001). Aging alters neuronal nitric oxide release from rat mesenteric arteries: role of presynaptic beta-adrenoceptors. *Clin. Sci.* 101, 321–328.

Ferrer, M., Sanchez, M., Minoves, N., Salaices, M., and Balfagon, 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.

Fiorucci, S., Antonelli, E., Burgaud, J. L., and Morelli, A. (2001). Nitric oxide-releasing NSAIDs: a review of their current status. *Drug Saf.* 24, 801–811.

Fleenor, B. S., Seals, D. R., Zigler, M. L., and Sindler, A. L. (2011). Superoxide-lowering therapy with TEMPOL reverses arterial dysfunction with aging in mice. *Aging Cell* 11, 269–276.

Foody, J. M., Rathore, S. S., Galusha, D., Masoudi, F. A., Havranek, E. P., Radford, M. J., and Krumholz, H. M. (2006). Hydroxymethylglutaryl-CoA reductase inhibitors in older persons with acute myocardial infarction: evidence for an age-statin interaction. *J. Am. Geriatr. Soc.* 54, 421–430.

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

Fulop, N., Feng, W., Xing, D., He, K., Not, L. G., Brocks, C. A., Marchase, R. B., Miller, A. P., and Chatham, J. C. (2008). Aging leads to increased levels of protein O-linked N-acetylglucosamine in heart, aorta, brain and skeletal muscle in Brown-Norway rats. *Biogerontology* 9, 139–151.

Gao, L., Siu, K. L., Chalupsky, K., Nguyen, A., Chen, P., Weintraub, N. L., Galis, Z., and Cai, H. (2012). Role of uncoupled endothelial nitric oxide synthase in abdominal aortic aneurysm formation: treatment with folic acid. *Hypertension* 59, 158–166.

Gates, P. E., Boucher, M. L., Silver, A. E., Monahan, K. D., and Seals, D. R. (2007). Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. *J. Appl. Physiol.* 102, 63–71.

Gerhard, M., Roddy, M. A., Creager, S. J., and Creager, M. A. (1996). Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension* 27, 849–853.

Glenn, C. L., Wang, W. Y., and Morris, B. J. (1999). Different frequencies of inducible nitric oxide synthase genotypes in older hypertensives. *Hypertension* 33, 927–932.

Goettsch, W., Lattmann, T., Amann, K., Szibor, M., Morawietz, H., Munter, K., Muller, S. P., Shaw, S., and Barton, M. (2001). Increased expression of endothelin-1 and inducible nitric oxide synthase isoform II in aging arteries in vivo: implications for atherosclerosis. *Biochem. Biophys. Res. Commun.* 280, 908–913.

Gransbo, K., Melander, O., Wallentin, L., Lindback, J., Stenestrand, U., Carlsson, J., and Nilsson, J. (2010). Cardiovascular and cancer mortality in very elderly post-myocardial infarction patients receiving statin treatment. *J. Am. Coll. Cardiol.* 55, 1362–1369.

Hamilton, C. A., Brosnan, M. J., McIntyre, M., Graham, D., and Dominiczak, A. F. (2001). Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction. *Hypertension* 37(Pt 2), 529–534.

Hattori, Y., Nakanishi, N., Akimoto, K., Yoshida, M., and Kasai, K. (2003). HMG-CoA reductase inhibitor increases GTP cyclohydrolase I mRNA and tetrahydrobiopterin in vascular endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 23, 176–182.

Hecker, M., Cattaruzza, M., and Wagner, A. H. (1999). Regulation of inducible nitric oxide synthase gene expression in vascular smooth muscle cells. *Gen. Pharmacol.* 32, 9–16.

Herrera, M. D., Mingorance, C., Rodriguez-Rodriguez, R., and Alvarez de Sotomayor, M. (2010). Endothelial dysfunction and aging: an update. *Ageing Res. Rev.* 9, 142–152.

Higashi, Y., Sasaki, S., Nakagawa, K., Kimura, M., Noma, K., Hara, K., Jitsuiki, D., Goto, C., Oshima, T., Chayama, K., and Yoshizumi, M. (2006). Tetrahydrobiopterin improves aging-related impairment of endothelium-dependent vasodilation through increase in nitric oxide production. *Atherosclerosis* 186, 390–395.

Kapil, V., Milsom, A. B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., Arghandawi, S., Pearl, V., Benjamin, N., Loukogeorgakis, S., MacAllister, R., Hobbs, A. J., Webb, A. J., and Ahluwalia, A. (2010). Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. *Hypertension* 56, 274–281.

Kar, P., Laight, D., Rooprai, H. K., Shaw, K. M., and Cummings, M. (2009). Effects of grape seed extract in Type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. *Diabet. Med.* 26, 526–531.

Kawano, H., Yasue, H., Kitagawa, A., Hirai, N., Yoshida, T., Soejima, H., Miyamoto, S., Nakano, M., and Ogawa, H. (2003). Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J. Clin. Endocrinol. Metab.* 88, 3190–3195.

Kim, J. H., Bugaj, L. J., Oh, Y. J., Bivalacqua, T. J., Ryoo, S., Soucy, K. G., Santhanam, L., Webb, A., Camara, A., Sikka, G., Nyhan, D., Shoukas, A. A., Ilies, M., Christianson, D. W., Champion, H. C., and Berkowitz, D. E. (2009). Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. *J. Appl. Physiol.* 107, 1249–1257.

Kurt, R., Yilmaz, Y., Ermis, F., Kalayoglu Besisik, S., Polat, N., Elitok, A., Oflaz, H., and Karan, M. A. (2010). Folic Acid and vitamin B12 supplementation improves coronary flow reserve in elderly subjects with vitamin B12 deficiency. *Arch. Med. Res.* 41, 369–372.

Labinskyy, N., Csiszar, A., Veress, G., Stef, G., Pacher, P., Oroszi, G., Wu, J., and Ungvari, Z. (2006). Vascular dysfunction in aging: potential effects of resveratrol, an anti-inflammatory phytoestrogen. *Curr. Med. Chem.* 13, 989–996.

Lakatta, E. G., and Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circulation* 107, 139–146.

Lamberts, S. W., van den Beld, A. W., and van der Lely, A. J. (1997). The endocrinology of aging. *Science* 278, 419–424.

Larsen, F. J., Ekblom, B., Sahlin, K., Lundberg, J. O., and Weitzberg, E. (2006). Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.* 355, 2792–2793.

Laufs, U., La Fata, V., Plutzky, J., and Liao, J. K. (1998). Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97, 1129–1135.

Laufs, U., and Liao, J. K. (1998). Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. *J. Biol. Chem.* 273, 24266–24271.

LeBlanc, A. J., Reyes, R., Kang, L. S., Dailey, R. A., Stallone, J. N., Moningka, N. C., and Muller-Delp, J. M. (2009). Estrogen replacement restores flow-induced vasodilation in coronary arterioles of aged and ovariectomized rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R1713–R1723.

Lekakis, J., Rallidis, L. S., Andreadou, I., Vamvakou, G., Kazantzoglou, G., Magiatis, P., Skaltsounis, A. L., and Kremastinos, D. T. (2005). Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. *Eur. J. Cardiovasc. Prev. Rehabil.* 12, 596–600.

Lekontseva, O., Chakrabarti, S., Jiang, Y., Cheung, C. C., and Davidge, S. T. (2011). Role of neuronal nitric-oxide synthase in estrogen-induced relaxation in rat resistance arteries. *J. Pharmacol. Exp. Ther.* 339, 367–375.

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

Liang, M., Ekblad, E., Lydrup, M. L., and Nilsson, B. O. (2003). Combined lack of estrogen receptors alpha and beta affects vascular iNOS protein expression. *Cell Tissue Res.* 313, 63–70.

Lima, V. V., Rigsby, C. S., Hardy, D. M., Webb, R. C., and Tostes, R. C. (2009). O-GlcNAcylation: a novel post-translational mechanism to alter vascular cellular signaling in health and disease: focus on hypertension. *J. Am. Soc. Hypertens.* 3, 374–387.

Lund, D. D., Chu, Y., Miller, J. D., and Heistad, D. D. (2009). Protective effect of extracellular superoxide dismutase on endothelial function during aging. *Am. J. Physiol. Heart Circ. Physiol.* 296, H1920–H1925.

Matz, R. L., de Sotomayor, M. A., Schott, C., Stoclet, J. C., and Andriantsitohaina, R. (2000). Vascular bed heterogeneity in age-related endothelial dysfunction with respect to NO and eicosanoids. *Br. J. Pharmacol.* 131, 303–311.

Melikian, N., Seddon, M. D., Casadei, B., Chowienczyk, P. J., and Shah, A. M. (2009). Neuronal nitric oxide synthase and human vascular regulation. *Trends Cardiovasc. Med.* 19, 256–262.

Ming, X. F., Montani, J. P., and Yang, Z. (2012). Perspectives of targeting mTORC1-S6K1 in cardiovascular aging. *Front. Physiol.* 3:5. doi:10.3389/fphys.2012.00005

Moens, A. L., and Kass, D. A. (2006). Tetrahydrobiopterin and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* 26, 2439–2444.

Moens, A. L., Vrints, C. J., Claeys, M. J., Timmermans, J. P., Champion, H. C., and Kass, D. A. (2008). Mechanisms and potential therapeutic targets for folic acid in cardiovascular disease. *Am. J. Physiol. Heart Circ. Physiol.* 294, H1971–H977.

Moncada, S., and Higgs, E. A. (2006). The discovery of nitric oxide and its role in vascular biology. *Br. J. Pharmacol.* 147(Suppl. 1), S193–S201.

Moreau, K. L., Meditz, A., Deane, K. D., and Kohrt, W. M. (2012). Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. *Am. J. Physiol. Heart Circ. Physiol.* 302, H1211–H1218.

Muller-Delp, J. M., Spier, S. A., Ramsey, M. W., and Delp, M. D. (2002). Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. *Am. J. Physiol. Heart Circ. Physiol.* 283, H1662–H1672.

Mungrue, I. N., Gros, R., You, X., Pirani, A., Azad, A., Csont, T., Schulz, R., Butany, J., Stewart, D. J., and Husain, M. (2002). Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. *J. Clin. Invest.* 109, 735–743.

Nakata, S., Tsutsui, M., Shimokawa, H., Yamashita, T., Tanimoto, A., Tasaki, H., Ozumi, K., Sabanai, K., Morishita, T., Suda, O., Hirano, H., Sasaguri, Y., Nakashima, Y., and Yanagihara, N. (2007). Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-kappaB pathway. *Arterioscler. Thromb. Vasc. Biol.* 27, 92–98.

Napoli, C., Aldini, G., Wallace, J. L., de Nigris, F., Maffei, R., Abete, P., Bonaduce, D., Condorelli, G., Rengo, F., Sica, V., D'Armiento, F. P., Mignogna, C., de Rosa, G., Condorelli, M., Lerman, L. O., and Ignarro, L. J. (2002). Efficacy and age-related effects of nitric oxide-releasing aspirin on experimental restenosis. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1689–1694.

Nelson, H. D., Humphrey, L. L., Nygren, P., Teutsch, S. M., and Allan, J. D. (2002). Postmenopausal hormone replacement therapy: scientific review. *JAMA* 288, 872–881.

Nisoli, E., Tonello, C., Cardile, A., Cozzi, V., Bracale, R., Tedesco, L., Falcone, S., Valerio, A., Cantoni, O., Clementi, E., Moncada, S., and Carruba, M. O. (2005). Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310, 314–317.

Novella, S., Dantas, A. P., Segarra, G., Novensa, L., Bueno, C., Heras, M., Hermenegildo, C., and Medina, P. (2010). Gathering of aging and estrogen withdrawal in vascular dysfunction of senescent accelerated mice. *Exp. Gerontol.* 45, 868–874.

Novella, S., Dantas, A. P., Segarra, G., Novensa, L., Heras, M., Hermenegildo, C., and Medina, P. (2011). Aging enhances contraction to thromboxane A(2) in aorta from female senescence-accelerated mice. *Age (Dordr.).* PMID: 22102320. [Epub ahead of print].

Novensa, L., Novella, S., Medina, P., Segarra, G., Castillo, N., Heras, M., Hermenegildo, C., and Dantas, A. P. (2011). Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERalpha/ERbeta balance in female mice. *PLoS ONE* 6, e25335. doi:10.1371/journal.pone.0025335

Obeid, R., Kirsch, S. H., Kasoha, M., Eckert, R., and Herrmann, W. (2011). Concentrations of unmetabolized folic acid and primary folate forms in plasma after folic acid treatment in older adults. *Metab. Clin. Exp.* 60, 673–680.

Pallàs, M., Junyent, F., Verdaguer, E., Beas-Zarate, C., and Camin, A. (2010). Aging control with resveratrol. *Drug Discov. Today Ther. Strateg.* 7, 51–56.

Peluffo, G., and Radi, R. (2007). Biochemistry of protein tyrosine nitration in cardiovascular pathology. *Cardiovasc. Res.* 75, 291–302.

Presley, T. D., Morgan, A. R., Bechtold, E., Clodfelter, W., Dove, R. W., Jennings, J. M., Kraft, R. A., King, S. B., Laurienti, P. J., Rejeski, W. J., Burdette, J. H., Kim-Shapiro, D. B., and Miller, G. D. (2011). Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide* 24, 34–42.

Qiao, X., McConnell, K. R., and Khalil, R. A. (2008). Sex steroids and vascular responses in hypertension and aging. *Gend. Med.* 5(Suppl. A), S46–S64.

Rajapakse, A. G., Yepuri, G., Carvas, J. M., Stein, S., Matter, C. M., Scerri, I., Ruffieux, J., Montani, J. P., Ming, X. F., and 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

Rodriguez-Manas, L., El-Assar, M., Vallejo, S., Lopez-Doriga, P., Solis, J., Petidier, R., Montes, M., Nevado, J., Castro, M., Gomez-Guerrero, C., Peiro, C., and Sanchez-Ferrer, C. F. (2009). Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell* 8, 226–238.

Sabri, M., Ai, J., Marsden, P. A., and MacDonald, R. L. (2011). Simvastatin re-couples dysfunctional endothelial nitric oxide synthase in experimental subarachnoid hemorrhage. *PLoS ONE* 6, e17062. doi:10.1371/journal.pone.0017062

Santhanam, L., Lim, H. K., Miriel, V., Brown, T., Patel, M., Balanson, S., Ryoo, S., Anderson, M., Irani, K., Khanday, F., Di Costanzo, L., Nyhan, D., Hare, J. M., Christianson, D. W., Rivers, R., Shoukas, A., and Berkowitz, D. E. (2007). Inducible NO synthase dependent S-nitrosylation and activation of arginase1 contribute to age-related endothelial dysfunction. *Circ. Res.* 101, 692–702.

Schmitt, C. A., Heiss, E. H., and Dirsch, V. M. (2010). Effect of resveratrol on endothelial cell function: molecular mechanisms. *Biofactors* 36, 342–349.

Seals, D. R., Jablonski, K. L., and Donato, A. J. (2011). Aging and vascular endothelial function in humans. *Clin. Sci.* 120, 357–375.

Shepherd, J., Blauw, G. J., Murphy, M. B., Bollen, E. L., Buckley, B. M., Cobbe, S. M., Ford, I., Gaw, A., Hyland, M., Jukema, J. W., Kamper, A. M., MacFarlane, P. W., Meinders, A. E., Norrie, J., Packard, C. J., Perry, I. J., Stott, D. J., Sweeney, B. J., Twomey, C., and Westendorp, R. G. (2002). Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 360, 1623–1630.

Smith, A. R., Visioli, F., Frei, B., and Hagen, T. M. (2006a). Age-related changes in endothelial nitric oxide synthase phosphorylation and nitric oxide dependent vasodilation: evidence for a novel mechanism involving sphingomyelinase and ceramide-activated phosphatase 2A. *Aging Cell* 5, 391–400.

Smith, A. R., Visioli, F., and Hagen, T. M. (2006b). Plasma membrane-associated endothelial nitric oxide synthase and activity in aging rat aortic vascular endothelia markedly decline with age. *Arch. Biochem. Biophys.* 454, 100–105.

Smith, C. J., Santhanam, L., Bruning, R. S., Stanhewicz, A., Berkowitz, D. E., and Holowatz, L. A. (2011). Upregulation of inducible nitric oxide synthase contributes to attenuated cutaneous vasodilation in essential hypertensive humans. *Hypertension* 58, 935–942.

Sobko, T., Marcus, C., Govoni, M., and Kamiya, S. (2010). Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* 22, 136–140.

Stice, J. P., Eiserich, J. P., and Knowlton, A. A. (2009). Role of aging versus the loss of estrogens in the reduction in vascular function in female rats. *Endocrinology* 150, 212–219.

Sun, D., Huang, A., Yan, E. H., Wu, Z., Yan, C., Kaminski, P. M., Oury, T. D., Wolin, M. S., and Kaley, G. (2004). Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am. J. Physiol. Heart Circ. Physiol.* 286, H2249–H2256.

Taddei, S., Virdis, A., Ghiadoni, L., Salvetti, G., Bernini, G., Magagna, A., and Salvetti, A. (2001). Age-related

reduction of NO availability and oxidative stress in humans. *Hypertension* 38, 274–279.

Tamura, K., Yamaguchi, K., and Kogo, H. (2000). 17Beta-estradiol inhibits ovariectomy-induced expression of inducible nitric oxide synthase in rat aorta in vivo. *Life Sci.* 66, 259–264.

Tian, J., Yan, Z., Wu, Y., Zhang, S. L., Wang, K., Ma, X. R., Guo, L., Wang, J., Zuo, L., Liu, J. Y., Quan, L., and Liu, H. R. (2010). Inhibition of iNOS protects endothelial-dependent vasodilation in aged rats. *Acta Pharmacol. Sin.* 31, 1324–1328.

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., Kunz, I., Schrauwen-Hinderling, V. B., Blaak, E. E., Auwerx, J., and 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.

Toda, N. (2011). Age-related changes in endothelial function and blood flow regulation. *Pharmacol. Ther.* 133, 159–176.

Tolbert, T., Thompson, J. A., Bouchard, P., and Oparil, S. (2001). Estrogen-induced vasoprotection is independent of inducible nitric oxide synthase expression: evidence from the mouse carotid artery ligation model. *Circulation* 104, 2740–2745.

Traish, A. M., Kang, H. P., Saad, F., and Guay, A. T. (2011). Dehydroepiandrosterone (DHEA) – a precursor steroid or an active hormone in human physiology. *J. Sex. Med.* 8, 2960–2983.

Tsutsui, M., Shimokawa, H., Otsuji, Y., Ueta, Y., Sasaguri, Y., and Yanagihara, N. (2009). Nitric oxide synthases and cardiovascular diseases: insights from genetically modified mice. *Circ. J.* 73, 986–993.

Ungvari, Z., Csiszar, A., and Kaley, G. (2004). Vascular inflammation in aging. *Herz* 29, 733–740.

Ungvari, Z., Sonntag, W. E., and Csiszar, A. (2010). Mitochondria and aging in the vascular system. *J. Mol. Med. (Berl.)* 88, 1021–1027.

Upmacis, R. K., Shen, H., Benguigui, L. E., Lamon, B. D., Deeb, R. S., Hajjar, K. A., and Hajjar, D. P. (2011). Inducible nitric oxide synthase provides protection against injury-induced thrombosis in female mice. *Am. J. Physiol. Heart Circ. Physiol.* 301, H617–H624.

van der Loo, B., Labugger, R., Skepper, J. N., Bachschmid, M., Kilo, J., Powell, J. M., Palacios-Callender, M., Erusalimsky, J. D., Quaschning, T., Malinski, T., Gygi, D., Ullrich, V., and Luscher, T. F. (2000). Enhanced peroxynitrite formation is associated with vascular aging. *J. Exp. Med.* 192, 1731–1744.

Villanueva, C., and Giulivi, C. (2010). Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease. *Free Radic. Biol. Med.* 49, 307–316.

Wagner, A. H., Kohler, T., Ruckschloss, U., Just, I., and Hecker, M. (2000). Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. *Arterioscler. Thromb. Vasc. Biol.* 20, 61–69.

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

Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J., Benjamin, N., MacAllister, R., Hobbs, A. J., and Ahluwalia, A. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 51, 784–790.

Wenzel, P., Daiber, A., Oelze, M., Brandt, M., Closs, E., Xu, J., Thum, T., Bauersachs, J., Ertl, G., Zou, M. H., Forstermann, U., and Munzel, T. (2008). Mechanisms underlying recoupling of eNOS by HMG-CoA reductase inhibition in a rat model of streptozotocin-induced diabetes mellitus. *Atherosclerosis* 198, 65–76.

Weverling-Rijnsburger, A. W., Blauw, G. J., and Meinders, A. E. (2004). Effect of atorvastatin on impaired vascular function in healthy old men. *J. Clin. Pharm. Ther.* 29, 157–164.

Whelan, A. P., Sutherland, W. H., McCormick, M. P., Yeoman, D. J., de Jong, S. A., and Williams, M. J. (2004). Effects of white and red wine on endothelial function in subjects with coronary artery disease. *Intern. Med. J.* 34, 224–228.

Williams, M. R., Dawood, T., Ling, S., Dai, A, Lew, R., Myles, K., Funder, J. W., Sudhir, K., and Komesaroff, P. A. (2004). Dehydroepiandrosterone increases endothelial cell proliferation in vitro and improves endothelial function in vivo by mechanisms independent of androgen and estrogen receptors. *J. Clin. Endocrinol. Metab.* 89, 4708–4715.

Wong, R. H., Howe, P. R., Buckley, J. D., Coates, A. M., Kunz, I., and Berry, N. M. (2011). Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. *Nutr. Metab. Cardiovasc. Dis.* 21, 851–856.

Wu, S., Ruan, Y., Yin, M., and Lai, W. (2007). Research on the age-related changes in the nitric oxide pathway in the arteries of rats and the intervention effect of dehydroepiandrosterone. *Gerontology* 53, 234–237.

Xia, N., Daiber, A., Habermeier, A., Closs, E. I., Thum, T., Spanier, G., Lu, Q., Oelze, M., Torzewski, M., Lackner, K. J., Munzel, T., Forstermann, U., and Li, H. (2010). Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. *J. Pharmacol. Exp. Ther.* 335, 149–154.

Xing, D., Nozell, S., Chen, Y. F., Hage, F., and Oparil, S. (2009). Estrogen and mechanisms of vascular protection. *Arterioscler. Thromb. Vasc. Biol.* 29, 289–295.

Yang, B., Larson, D. F., and Watson, R. R. (2004). Modulation of iNOS activity in age-related cardiac dysfunction. *Life Sci.* 75, 655–667.

Yang, Y. M., Huang, A., Kaley, G., and Sun, D. (2009). eNOS uncoupling and endothelial dysfunction in aged vessels. *Am. J. Physiol. Heart Circ. Physiol.* 297, H1829–H1836.

Yoon, H. J., Cho, S. W., Ahn, B. W., and Yang, S. Y. (2010). Alterations in the activity and expression of endothelial NO synthase in aged human endothelial cells. *Mech. Ageing Dev.* 131, 119–123.

Zhang, Y., Janssens, S. P., Wingler, K., Schmidt, H. H., and Moens, A. L. (2011). Modulating endothelial nitric oxide synthase: a new cardiovascular therapeutic strategy. *Am. J. Physiol. Heart Circ. Physiol.* 301, H634–H646.

Zhang, Z., Wang, M., Xue, S. J., Liu, D. H., and Tang, Y. B. (2012). Simvastatin ameliorates angiotensin II-induced endothelial dysfunction through restoration of Rho-BH4-eNOS-NO pathway. *Cardiovasc. Drugs Ther.* 26, 31–40.

Zhou, Y. H., Tang, J. Y., Wu, M. J., Lu, J., Wei, X., Qin, Y. Y., Wang, C., Xu, J. F., and He, J. (2011). Effect of folic acid supplementation on cardiovascular outcomes: a systematic review and meta-analysis. *PLoS ONE* 6, e25142. doi:10.1371/journal.pone.0025142

Zhu, Y., Bian, Z., Lu, P., Karas, R. H., Bao, L., Cox, D., Hodgin, J., Shaul, P. W., Thoren, P., Smithies, O., Gustafsson, J. A., and Mendelsohn, M. E. (2002). Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. *Science* 295, 505–508.

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The splicing factor SRSF1 as a marker for endothelial senescence

Francisco Javier Blanco* and Carmelo Bernabéu

Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas and Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

Edited by:
Francesc Jiménez-Altayó, Universitat Autònoma de Barcelona, Spain

Reviewed by:
Michael Ladomery, University of the West of England Bristol, UK
Xiu-Fen Ming, University of Fribourg, Switzerland
Gerardo Ferbeyre, Université de Montréal, Canada

****Correspondence:***
Francisco Javier Blanco, Department of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, C/Ramiro de Maeztu 9, Madrid 28040, Spain.
e-mail: fjblanco@cib.csic.es

Aging is the major risk factor *per se* for the development of cardiovascular diseases. The senescence of the endothelial cells (ECs) that line the lumen of blood vessels is the cellular basis for these age-dependent vascular pathologies, including atherosclerosis and hypertension. During their lifespan, ECs may reach a stage of senescence by two different pathways; a replicative one derived from their preprogrammed finite number of cell divisions; and one induced by stress stimuli. Also, certain physiological stimuli, such as transforming growth factor-β, are able to modulate cellular senescence. Currently, the cellular aging process is being widely studied to identify novel molecular markers whose changes correlate with senescence. This review focuses on the regulation of alternative splicing mediated by the serine–arginine splicing factor 1 (SRSF1, or ASF/SF2) during endothelial senescence, a process that is associated with a differential subcellular localization of SRSF1, which typically exhibits a scattered distribution throughout the cytoplasm. Based on its senescence-dependent involvement in alternative splicing, we postulate that SRSF1 is a key marker of EC senescence, regulating the expression of alternative isoforms of target genes such as endoglin (*ENG*), vascular endothelial growth factor A (*VEGFA*), tissue factor (*T3*), or lamin A (*LMNA*) that integrate in a common molecular senescence program.

Keywords: alternative splicing, endothelial senescence, SRSF1, endoglin, progerin, VEGF, tissue factor

INTRODUCTION

It is well established that vascular physiology declines with aging due to the impairment of endothelial functions. This endothelial dysfunction is fairly evidenced by the stiffening of blood vessels that fail to control the dilatory responses associated with hypertension (Versari et al., 2009), and by a pro-inflammatory state of the endothelial microenvironment that favors the development of atherosclerosis (Erusalimsky, 2009). Hence the biology of endothelial cells (ECs) is a key element for elucidating and understanding the cellular and molecular basis of vascular pathologies associated with age.

Endothelial cells form a thin monolayer that lines the blood vessels and is in direct contact with the blood flow. During embryonic development, processes including vasculogenesis and angiogenesis require repeated division of ECs, but in adults these cells are in a quiescent state and divide only occasionally (Conway and Carmeliet, 2004; Semenza, 2007; Carmeliet and Jain, 2011). However, at this latter stage ECs remain highly responsive to mechanical stimuli, such as shear stress and injuries, as well as to chemical signals, including hypoxia, cytokines, and growth factors, among others. Thus, during vascular remodeling or upon angiogenic stimulation, ECs actively proliferate, but progressively lose their capacity to divide, reaching the stage of replicative senescence. This process involves telomere shortening until the cells reach the so-called Hayflick's limit (Brandes et al., 2005), that renders cell division impossible, constituting a mechanism to prevent genomic instability and the development of cancer (Shay and Wright, 2007). Endothelial senescence can also be triggered by telomere-independent events, including chemotherapy, proto-oncogene-induced senescence, alterations in nuclear lamina, and stress stimuli that usually involve DNA damage. In this sense, oxidative stress and ultraviolet radiation are major stimuli for the induction of this type of senescence; both ultimately generate reactive oxygen species (ROS; Debacq-Chainiaux et al., 2005; Erusalimsky and Skene, 2009; Collins and Tzima, 2011) and activate retinoblastoma protein family pathways, the final effectors of the senescence program (Campisi and d'Adda di Fagagna, 2007; Erusalimsky, 2009).

There is a growing body of evidence that vascular aging and senescence of ECs are the basis of the endothelial dysfunction associated with vascular pathologies. For example, it is well known that aging is associated with decreased angiogenesis (Brandes et al., 2005; Erusalimsky, 2009), and that senescent ECs are unable to form neoangiogenic networks in matrigel assays (Chang et al., 2005). In this regard, certain physiological regulators of angiogenesis, such as transforming growth factor-β (TGF-β), are able to modulate cellular senescence of ECs (Santibanez et al., 2011; Blanco and Bernabeu, 2012; Doetschman et al., 2012; Krieglstein et al., 2012).

Recently, growing interest has focused on the role of alternative processing of certain precursor mRNA transcripts during EC senescence (Meshorer and Soreq, 2002; Harries et al., 2011). Alternative splicing represents an advantageous mechanism by which cells enrich their transcriptome in order to synthesize a specialized proteome for a particular stage or process. An early link between

the role of alternative splicing and aging was described in the late 1980s, when it was reported that alternative isoforms from the elastin gene (*ELN*) could contribute to aging and pathological situations in the cardiovascular and pulmonary systems (Indik et al., 1989). Since then, numerous papers have confirmed this idea, not only in senescent ECs, but also in other cell types (Meshorer and Soreq, 2002).

Currently, active investigations are focused on the molecular components that orchestrate the modulation of alternative splicing events in aging. Briefly, splicing is carried out by the spliceosome, a complex molecular machinery integrated by a core of small nuclear ribonucleoproteins (snRNPs) plus a great variety of essential and accessory splicing factors, or *trans*-elements. These splicing factors recognize short consensus sequences in a precursor mRNA, or *cis*-elements, thereby modulating its activity. Thus, it is assumed that the splicing changes observed during aging are part of a genetically programmed mechanism which is not necessarily associated with deleterious mutations, but with the splicing factors that play a critical role affecting the expression of target genes.

SPLICING FACTORS INVOLVED IN ENDOTHELIAL SENESCENCE

Analyses of the splicing factors SNEV and serine–arginine splicing factor 1 (SRSF1) have demonstrated their role in EC senescence. Thus, upon replicative senescence of human umbilical vein endothelial cells (HUVECs), a down-regulation of SNEV was observed (Voglauer et al., 2006). This event takes places concomitantly with down-regulation of the catalytic subunit of the telomerase, hTERT, the classic cellular senescent marker. Conversely, overexpression of SNEV provokes extension of the replicative lifespan in these cells. In addition to being involved in spliceosome assembly and stabilization, SNEV regulates proteasome function due to its U3-ligase activity, and is involved in DNA repair (Grillari et al., 2010). Furthermore, the haploinsufficiency of SNEV has been demonstrated using a heterozygous mouse model, which showed that the proliferative and repopulating capacity of hematopoietic stem cells from these mice was clearly compromised (Schraml et al., 2008).

The splicing factor SRSF1, or ASF/SF2, is the prototypical member of the highly conserved family of serine/arginine-rich proteins (SR proteins). Structurally, these SR proteins are composed of one or two RNA recognition motifs plus a C-terminal serine/arginine-rich domain involved in protein–protein interactions (**Figure 1**). During the past decade, the role of SR proteins has been extended to include a diverse set of cellular processes, including mRNA nuclear export, mRNA stability and quality control, translation, maintenance of genomic stability, and oncogenic transformation (Manley and Krainer, 2010). Within this family, SRSF1 participates in constitutive and alternative splicing, and in a plethora of biological processes, including viral infections and cancer (Sanford et al., 2005; Biamonti and Caceres, 2009). Recently, the role of SRSF1 in senescence, regulating the expression of the short isoform of endoglin, has been described (Blanco and Bernabeu, 2011). In ECs, SRSF1 promotes retention of the last intron of the endoglin gene (*ENG*) by binding to an intronic *cis*-element that overlaps with the branch point consensus sequence, interfering with intron removal (Blanco and Bernabeu, 2011). This retained intron shifts the open reading frame, incorporating an early stop codon that produces an alternate cytoplasmic domain shorter than the one present in the predominantly expressed L-endoglin (Bellon et al., 1993). This process has been associated with aging, contributing to the development of vascular pathologies including hypertension and atherosclerosis (Blanco et al., 2008). The involvement of SRSF1 in senescence has also been studied using mouse embryonic fibroblasts as a cellular model (Verduci et al., 2010). These authors found that two microRNAs, miR-28 and miR-505, are induced during senescence and negatively modulate SRSF1 expression, promoting cell cycle arrest and/or apoptosis.

SPLICED GENES REGULATED BY SRSF1 AND VASCULAR AGING

The role of SRSF1 in the endothelial context has been addressed by analyzing the expression of alternative isoforms of the vascular endothelial growth factor A (*VEGFA*) gene. VEGF is expressed as a disulfide-linked homodimer that specifically acts on ECs. Some of its biological effects include an increase in vascular permeability, induction of growth and migration of ECs (vasculogenesis and angiogenesis), and inhibition of apoptosis. Of note, *VEGFA* encodes two families of isoforms that are generated by alternate splice-site selection in its terminal exon #8. Thus, selection of the proximal splice-site (PSS) results in pro-angiogenic VEGF isoforms, whereas selection of the distal splice-site (DSS) leads to anti-angiogenic VEGFb isoforms (Neufeld et al., 1999). Interestingly, SRSF1 is a key regulator in the selection of the PSS, an event induced by IGF-1 treatment and abolished by TGF-β stimulus (Nowak et al., 2008; **Figure 2A**). Also, selection of the pro-angiogenic PSS by SRSF1 is prompted by changes in the cellular microenvironment such as those that occur in solid tumors, including a decrease in pH, among others (Elias and Dias, 2008). Because upon stimulation, SRPK1/2 kinases activate and translocate SRSF1 into the nucleus, it is not surprising to find that inhibition of SRPK1/2 reduced angiogenesis in a mouse model of retinal neovascularization, launching the potential involvement of alternative splicing regulation as a therapeutic target in angiogenic disorders (Nowak et al., 2010; Carter et al., 2011).

In addition to being involved in the selection of splice-sites, SRSF1 also participates in the skipping of particular exonic sequences, as in the case of the tissue factor (TF) gene (*T3*), the primary initiator of blood coagulation. TF is mainly expressed by ECs, usually as a transmembrane glycoprotein, but is also occasionally produced as a soluble form in response to pro-inflammatory cytokines (Szotowski et al., 2005). The activation and overexpression of SRSF1, as well as other SR proteins, in ECs favor skipping exon #5 of TF. Consequently, exon #4 is spliced directly to exon #6, producing a translational frameshift that leads to the generation of a soluble variant that contributes to thrombus growth, thus initiating and propagating the coagulation process (Bogdanov et al., 2003; **Figure 2B**).

Recently, the involvement of SRSF1 in alternative splicing of the lamin A gene (*LMNA*) has been described (Lopez-Mejia et al., 2011). *LMNA* encodes lamins A and C, which are major components of the nuclear lamina in the inner membrane of the nuclear envelope. These proteins are involved in essential

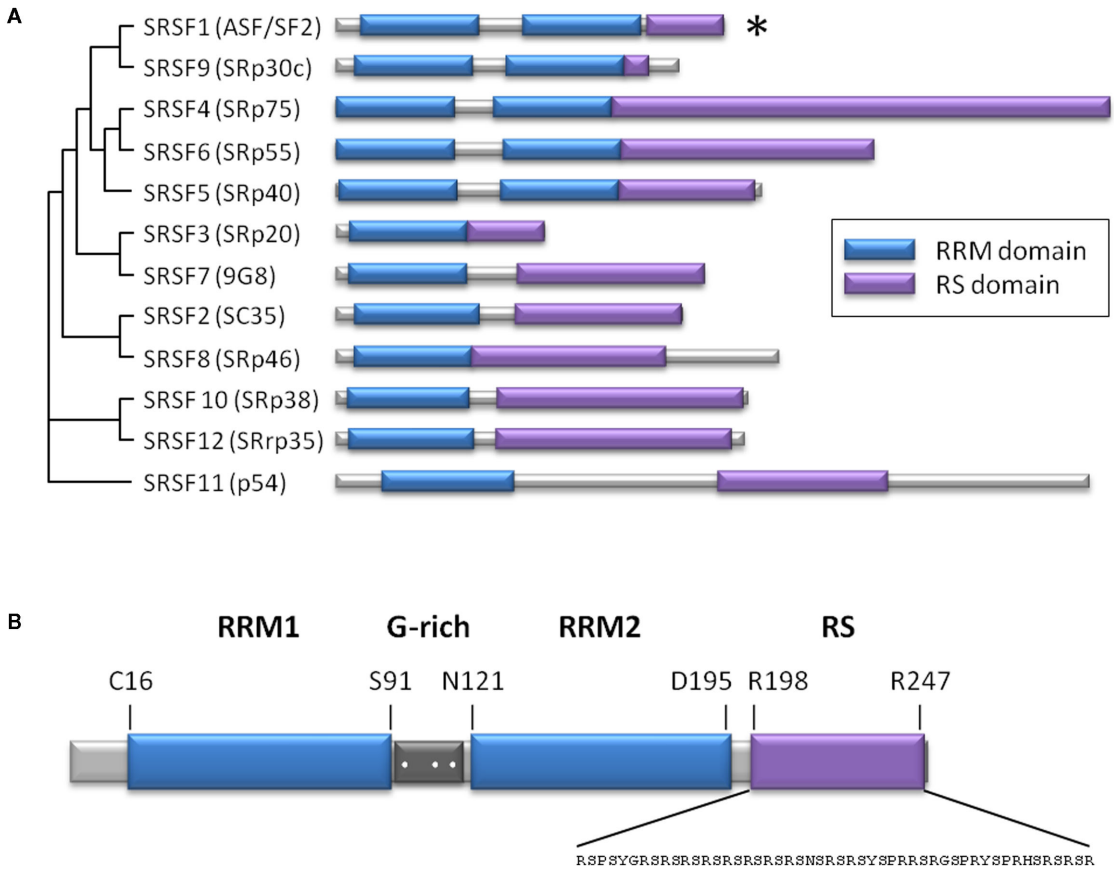

FIGURE 1 | The SR protein family. (A) Scheme representing the level of homology among human SR proteins (Manley and Krainer, 2010), based on the result of a multiple sequence alignment using ClustalW2. Alternate names are in parentheses. Both RS and RRMs are modular domains, which means that RRMs can be exchanged between SR proteins, and may still bind to RNA in the absence of the RS domain. The RS domain can bind to a heterologous RNA-binding domain and be still functional. The asterisk indicates the SRSF1 member. **(B)** Structural scheme of SRSF1 showing two RRM domains (blue) separated by a glycine-rich sequence (dark gray), where three arginine residues are susceptible to methylation (white dots). The sequence of the RS domain (violet) is indicated. RRM, RNA recognition motif; RS, arginine/serine-rich.

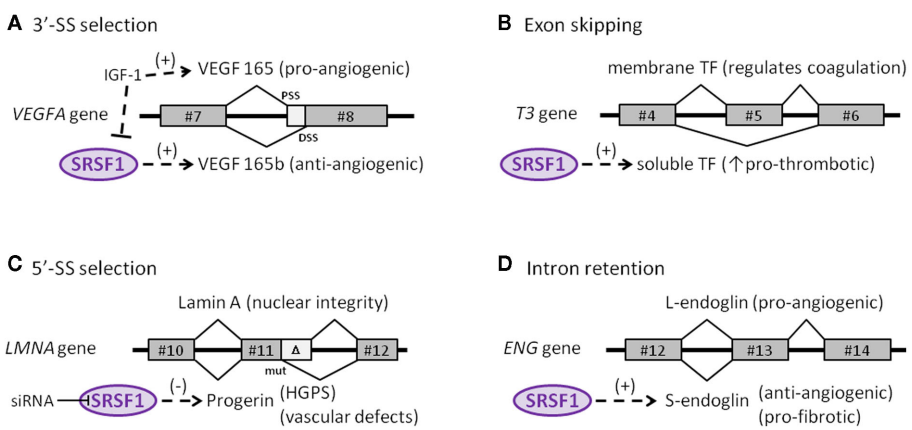

FIGURE 2 | Endothelial target genes of SRSF1. (A) Selection of the proximal (PSS) or distal (DSS) 3′-splice-site determines synthesis of either the pro- or anti-angiogenic isoform of the *VEGFA* gene, respectively. **(B)** The skipping of exon #5 in the tissue factor (*T3*) gene affects the reading frame and generates a soluble, pro-thrombotic isoform of tissue factor. **(C)** A reinforcing mutation near a cryptic 5′-splice-site in the *LMNA* gene truncates the final protein (progerin) that accumulates in the nucleus. **(D)** Retention of the final intron in the *ENG* gene alters the reading frame and generates the anti-angiogenic, pro-fibrotic isoform S-endoglin.

functions, including DNA replication, transcription, chromatin organization, and dis/assembly of the nucleus during cell division (Worman et al., 2009). Sporadically, a *de novo* germline mutation in exon #11 promotes the reinforcement of a weak 5′ splice-site that generates a truncated and pathological version of lamin A named progerin, which accumulates in cell nuclei in a tissue-specific manner (**Figure 2C**). Interestingly, SRSF1 is critically involved in the control of the lamin A/progerin splicing switch. Thus, specific knock-down of SRSF1 leads to a reduction in progerin expression, resulting in an important impact on the lifespan of the animal (Lopez-Mejia et al., 2011). Mutations in *LMNA*, that give rise to progerin, are responsible for Hutchinson–Gilford progeria syndrome, a rare disorder characterized by segmental premature aging and death (Gerhard-Herman et al., 2012). Progerin accumulates primarily in vascular cells, suggesting its direct involvement in vascular disease (McClintock et al., 2006), in agreement with the cardiovascular defects, including accelerated atherosclerosis, myocardial infarction, and stroke characteristic of Hutchinson–Gilford progeria syndrome (Trigueros-Motos et al., 2011; Gerhard-Herman et al., 2012).

The role of SRSF1 in intron retention by the endoglin gene (*ENG*) that leads to formation of the senescence-associated S-endoglin isoform has been demonstrated (Blanco and Bernabeu, 2011). Endoglin is an auxiliary co-receptor for TGF-β expressed in ECs that plays a critical role in vascular remodeling and angiogenesis, as illustrated by the fact that mutations in *ENG* lead to hereditary hemorrhagic telangiectasia Type 1, a disease characterized by vascular malformations (Lopez-Novoa and Bernabeu, 2010; Shovlin, 2010). While endoglin is predominantly expressed as the long isoform (L-endoglin) which shows a pro-angiogenic role, expression during senescence of the alternatively spliced S-endoglin significantly affects the behavior of ECs, promoting anti-angiogenic and pro-fibrotic phenotypes, and compromising vasodilator responses (Perez-Gomez et al., 2005; Blanco et al., 2008; Velasco et al., 2008). In this context, S-endoglin tilts the TGF-β signaling pathway toward its pro-fibrotic side, via Smad3 signaling, inducing expression of plasminogen activator inhibitor (PAI)-1, a key regulator of synthesis and deposition of the extracellular matrix in tissue homeostasis (Ghosh and Vaughan, 2012). Moreover, the TGF-β/S-endoglin signaling pathway represses the expression of Id1 factor, which is essential for cellular proliferation (Tang et al., 2002; Blanco et al., 2008). Compatible with this finding, elevated levels of TGF-β have been reported in aging varicose veins; these likely favor the fibrotic process and subsequent venous insufficiency (Pascual et al., 2007). Because TGF-β-induced Smad3 is able to interact with transcription factor c-myc, thus repressing the promoter of the hTERT gene that encodes the catalytic subunit of telomerase, up-regulation of S-endoglin would be predicted to contribute to endothelial senescence by compromising telomere integrity and arresting the cell cycle (Li and Liu, 2007). It has been postulated that the S-endoglin mRNA, which bears the last intron, may escape from the activity of the major spliceosome in the nucleus. Under normal conditions, this intron would typically be removed by the minor spliceosome in the cytoplasm, giving rise to the predominant isoform L-endoglin. However, during senescence, SRSF1 translocates to the cytoplasm, binding to the consensus SRSF1 *cis*-element within the branch point of the S-endoglin mRNA, interfering with the activity of the minor spliceosome and leading to an increase in the level of S-endoglin (**Figure 2D**).

EFFECT OF SENESCENCE-ASSOCIATED STRESS SIGNALS ON THE ROLE OF SRSF1

Precursor mRNA splicing is one target of several environmental stress factors, including oxidative stress and ultraviolet radiation, that frequently cause DNA damage (Biamonti and Caceres, 2009). Depending on the intensity of the stress signal, cells may react by activating the check-point pathway mediated by p53 and entering into cell cycle arrest, which, in turn, prompts senescence (Borras et al., 2011). Many of these stress stimuli induce the cytoplasmic accumulation of several splicing factors, including SRSF1 (Biamonti and Caceres, 2009), hnRNP A1 (Guil et al., 2006), Slu7 (Shomron et al., 2005), and PTB (Xie et al., 2003). Furthermore, SRSF1 translocates to the cytoplasm during replicative or stress-induced senescence, suggesting that SRSF1 is a new senescence target (Blanco and Bernabeu, 2011).

Serine–arginine splicing factor 1 is a shuttling factor that localizes either in the nucleus or the cytoplasm, depending on the phosphorylation state of its RS domain (Cazalla et al., 2002; Sanford et al., 2005) and/or the methylation of three arginine residues between the RRM domains (Sinha et al., 2010; **Figure 1B**). The kinase SRPK1 is a major contributor to SRSF1 phosphorylation and translocation. Under normal conditions, SRPK1 is located in the cytoplasm where this kinase continuously phosphorylates SRSF1, favoring its transport to the nucleus. However, some stress factors may alter the subcellular distribution of SRPK1, triggering the nuclear import of the kinase (Giannakouros et al., 2011). Consequently, the cytoplasmic pool of SRSF1 is augmented by the absence of SRPK1 in the cytoplasm, as well as by the nuclear phosphatase activity that facilitates the cytoplasmic export of SRSF1. Thus, it can be postulated that the cytoplasmic localization of SRSF1 that occurs during endothelial senescence, whether replicative or induced by stress stimuli, may be due, at least in part, to nuclear translocation of SRPK1.

Importantly, cytoplasmic SRSF1 is able to modulate the alternative splicing of specific precursor mRNAs, as shown for *ENG* in a process that involves the minor spliceosome (m-Sp) machinery (Blanco and Bernabeu, 2011). This m-Sp, also known to be

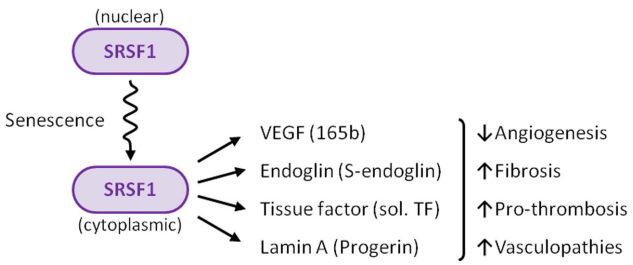

FIGURE 3 | Summary of the role of SRSF1 in ECs. Several stimuli, including senescence, induce translocation of SRSF1 to the cytoplasm. SRSF1 is a key factor that modulates the expression of critical regulators of vascular physiology, including VEGF, endoglin, tissue factor (TF), and lamin A.

U12-dependent, co-exists with the U2-dependent major spliceosome (m-Sp) in most organisms (Patel and Steitz, 2003). First described as responsible for the removal of a rare class of introns (U12-type), it is currently recognized that m-Sp is also involved in the processing of standard introns (Sheth et al., 2006) and may be localized in the cytoplasm (Konig et al., 2007). The m-Sp contains four unique small nuclear ribonucleic acids (snRNAs): U11, U12, U4atac, and U6atac, which are paralogs of the U1, U2, U4, and U6 snRNAs of the m-Sp, respectively, while U5 snRNA is shared between the two spliceosomes. While m-Sp is able to splice and remove the intron encoded in S-endoglin, the cytoplasmic localization of SRSF1 during endothelial senescence interferes with this splicing, promoting intron retention (Blanco and Bernabeu, 2011). This is in agreement with the findings that: (i) depletion of protein components essential for m-Sp activity leads to cellular growth arrest (Will et al., 2004); and (ii) during cell senescence, changes in gene expression levels affect the composition and activity of m-Sp (Meshorer and Soreq, 2002; Harries et al., 2011).

CONCLUSION

Numerous and increasing efforts are focused on aging research, to try to elucidate its underlying molecular mechanisms. Aging is one of the major risk factors for the development of cardiovascular diseases, and is associated with cellular senescence of the vascular endothelium. In this context, there is growing interest in the molecular components that orchestrate the modulation of alternative splicing events in senescent ECs. Due to its senescence-induced cytoplasmic localization, the splicing factor SRSF1 can be considered as a new marker for endothelial senescence. In addition, the cytoplasmic SRSF1 regulates alternative splicing of the endoglin pre-mRNA, leading to the senescence-associated S-endoglin isoform, which contributes to a senescent environment in the vessels. The data summarized in this review support the link between SRSF1 and senescence, and also suggest the existence of a common genetic program involving alternative splicing of a cluster of genes, including *ENG*, *VEGFA*, *T3*, and *LMNA*, whose resulting variants orchestrate vascular functions (**Figure 3**). Deciphering this senescent program will shed light on new therapeutic targets for cardiovascular diseases.

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REFERENCES

Bellon, T., Corbi, A., Lastres, P., Cales, C., Cebrian, M., Vera, S., Cheifetz, S., Massague, J., Letarte, M., and Bernabeu, C. (1993). Identification and expression of two forms of the human transforming growth factor-beta-binding protein endoglin with distinct cytoplasmic regions. *Eur. J. Immunol.* 23, 2340–2345.

Biamonti, G., and Caceres, J. F. (2009). Cellular stress and RNA splicing. *Trends Biochem. Sci.* 34, 146–153.

Blanco, F. J., and Bernabeu, C. (2011). Alternative splicing factor or splicing factor-2 plays a key role in intron retention of the endoglin gene during endothelial senescence. *Aging Cell* 10, 896–907.

Blanco, F. J., and Bernabeu, C. (2012). "Alternative splicing in endothelial senescence. Role of the TGF-β co-receptor endoglin," in *Senescence*, ed. T. Nagata (InTech). Available from: http://www.intechopen.com/articles/show/title/alternative-splicing-in-endothelial-senescence-role-of-the-tgf-beta-co-receptor-endoglin

Blanco, F. J., Grande, M. T., Langa, C., Oujo, B., Velasco, S., Rodriguez-Barbero, A., Perez-Gomez, E., Quintanilla, M., Lopez-Novoa, J. M., and Bernabeu, C. (2008). S-endoglin expression is induced in senescent endothelial cells and contributes to vascular pathology. *Circ. Res.* 103, 1383–1392.

Bogdanov, V. Y., Balasubramanian, V., Hathcock, J., Vele, O., Lieb, M., and Nemerson, Y. (2003). Alternatively spliced human tissue factor: a circulating, soluble, thrombogenic protein. *Nat. Med.* 9, 458–462.

Borras, C., Gomez-Cabrera, M. C., and Vina, J. (2011). The dual role of p53: DNA protection and antioxidant. *Free Radic. Res.* 45, 643–652.

Brandes, R. P., Fleming, I., and Busse, R. (2005). Endothelial aging. *Cardiovasc. Res.* 66, 286–294.

Campisi, J., and d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* 8, 729–740.

Carmeliet, P., and Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473, 298–307.

Carter, J. G., Cherry, J., Williams, K., Turner, S., Bates, D. O., and Churchill, A. J. (2011). Splicing factor polymorphisms, the control of VEGF isoforms and association with angiogenic eye disease. *Curr. Eye Res.* 36, 328–335.

Cazalla, D., Zhu, J., Manche, L., Huber, E., Krainer, A. R., and Caceres, J. F. (2002). Nuclear export and retention signals in the RS domain of SR proteins. *Mol. Cell. Biol.* 22, 6871–6882.

Chang, M. W., Grillari, J., Mayrhofer, C., Fortschegger, K., Allmaier, G., Marzban, G., Katinger, H., and Voglauer, R. (2005). Comparison of early passage, senescent and hTERT immortalized endothelial cells. *Exp. Cell Res.* 309, 121–136.

Collins, C., and Tzima, E. (2011). Hemodynamic forces in endothelial dysfunction and vascular aging. *Exp. Gerontol.* 46, 185–188.

Conway, E. M., and Carmeliet, P. (2004). The diversity of endothelial cells: a challenge for therapeutic angiogenesis. *Genome Biol.* 5, 207.

Debacq-Chainiaux, F., Borlon, C., Pascal, T., Royer, V., Eliaers, F., Ninane, N., Carrard, G., Friguet, B., De Longueville, F., Boffe, S., Remacle, J., and Toussaint, O. (2005). Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. *J. Cell Sci.* 118, 743–758.

Doetschman, T., Barnett, J. V., Runyan, R. B., Camenisch, T. D., Heimark, R. L., Granzier, H. L., Conway, S. J., and Azhar, M. (2012). Transforming growth factor beta signaling in adult cardiovascular diseases and repair. *Cell Tissue Res.* 347, 203–223.

Elias, A. P., and Dias, S. (2008). Microenvironment changes (in pH) affect VEGF alternative splicing. *Cancer Microenviron.* 1, 131–139.

Erusalimsky, J. D. (2009). Vascular endothelial senescence: from mechanisms to pathophysiology. *J. Appl. Physiol.* 106, 326–332.

Erusalimsky, J. D., and Skene, C. (2009). Mechanisms of endothelial senescence. *Exp. Physiol.* 94, 299–304.

Gerhard-Herman, M., Smoot, L. B., Wake, N., Kieran, M. W., Kleinman, M. E., Miller, D. T., Schwartzman, A., Giobbie-Hurder, A., Neuberg, D., and Gordon, L. B. (2012). Mechanisms of premature vascular aging in children with Hutchinson-Gilford progeria syndrome. *Hypertension* 59, 92–97.

Ghosh, A. K., and Vaughan, D. E. (2012). PAI-1 in tissue fibrosis. *J. Cell. Physiol.* 227, 493–507.

Giannakouros, T., Nikolakaki, E., Mylonis, I., and Georgatsou, E. (2011). Serine-arginine protein kinases: a small protein kinase family with a large cellular presence. *FEBS J.* 278, 570–586.

Grillari, J., Grillari-Voglauer, R., and Jansen-Durr, P. (2010). Post-translational modification of cellular proteins by ubiquitin and ubiquitin-like molecules: role in cellular senescence and aging. *Adv. Exp. Med. Biol.* 694, 172–196.

Guil, S., Long, J. C., and Caceres, J. F. (2006). hnRNP A1 relocalization to the stress granules reflects a role in the stress response. *Mol. Cell. Biol.* 26, 5744–5758.

Harries, L. W., Hernandez, D., Henley, W., Wood, A. R., Holly, A. C., Bradley-Smith, R. M., Yaghootkar, H., Dutta, A., Murray, A., Frayling, T. M., Guralnik, J. M., Bandinelli, S., Singleton, A., Ferrucci, L., and Melzer, D. (2011). Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. *Aging Cell* 10, 868–878.

Indik, Z., Yeh, H., Ornstein-Goldstein, N., Kucich, U., Abrams, W., Rosenbloom, J. C., and Rosenbloom, J. (1989). Structure of the elastin gene and alternative splicing of elastin mRNA: implications for human disease. *Am. J. Med. Genet.* 34, 81–90.

Konig, H., Matter, N., Bader, R., Thiele, W., and Muller, F. (2007). Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation. *Cell* 131, 718–729.

Krieglstein, K., Miyazono, K., Ten Dijke, P., and Unsicker, K. (2012). TGF-beta in aging and disease. *Cell Tissue Res.* 347, 5–9.

Li, H., and Liu, J. P. (2007). Mechanisms of action of TGF-beta in cancer: evidence for Smad3 as a repressor of the hTERT gene. *Ann. N. Y. Acad. Sci.* 1114, 56–68.

Lopez-Mejia, I. C., Vautrot, V., De Toledo, M., Behm-Ansmant, I., Bourgeois, C. F., Navarro, C. L., Osorio, F. G., Freije, J. M., Stevenin, J., De Sandre-Giovannoli, A., Lopez-Otin, C., Levy, N., Branlant, C., and Tazi, J. (2011). A conserved splicing mechanism of the LMNA gene controls premature aging. *Hum. Mol. Genet.* 20, 4540–4555.

Lopez-Novoa, J. M., and Bernabeu, C. (2010). The physiological role of endoglin in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* 299, H959–H974.

Manley, J. L., and Krainer, A. R. (2010). A rational nomenclature for serine/arginine-rich protein splicing factors (SR proteins). *Genes Dev.* 24, 1073–1074.

McClintock, D., Gordon, L. B., and Djabali, K. (2006). Hutchinson-Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-Lamin A G608G antibody. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2154–2159.

Meshorer, E., and Soreq, H. (2002). Pre-mRNA splicing modulations in senescence. *Aging Cell* 1, 10–16.

Neufeld, G., Cohen, T., Gengrinovitch, S., and Poltorak, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* 13, 9–22.

Nowak, D. G., Amin, E. M., Rennel, E. S., Hoareau-Aveilla, C., Gammons, M., Damodoran, G., Hagiwara, M., Harper, S. J., Woolard, J., Ladomery, M. R., and Bates, D. O. (2010). Regulation of vascular endothelial growth factor (VEGF) splicing from pro-angiogenic to anti-angiogenic isoforms: a novel therapeutic strategy for angiogenesis. *J. Biol. Chem.* 285, 5532–5540.

Nowak, D. G., Woolard, J., Amin, E. M., Konopatskaya, O., Saleem, M. A., Churchill, A. J., Ladomery, M. R., Harper, S. J., and Bates, D. O. (2008). Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. *J. Cell Sci.* 121, 3487–3495.

Pascual, G., Mendieta, C., Garcia-Honduvilla, N., Corrales, C., Bellon, J. M., and Bujan, J. (2007). TGF-beta1 upregulation in the aging varicose vein. *J. Vasc. Res.* 44, 192–201.

Patel, A. A., and Steitz, J. A. (2003). Splicing double: insights from the second spliceosome. *Nat. Rev. Mol. Cell Biol.* 4, 960–970.

Perez-Gomez, E., Eleno, N., Lopez-Novoa, J. M., Ramirez, J. R., Velasco, B., Letarte, M., Bernabeu, C., and Quintanilla, M. (2005). Characterization of murine S-endoglin isoform and its effects on tumor development. *Oncogene* 24, 4450–4461.

Sanford, J. R., Ellis, J. D., Cazalla, D., and Caceres, J. F. (2005). Reversible phosphorylation differentially affects nuclear and cytoplasmic functions of splicing factor 2/alternative splicing factor. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15042–15047.

Santibanez, J. F., Quintanilla, M., and Bernabeu, C. (2011). TGF-beta/TGF-beta receptor system and its role in physiological and pathological conditions. *Clin. Sci.* 121, 233–251.

Schraml, E., Voglauer, R., Fortschegger, K., Sibilia, M., Stelzer, I., Grillari, J., and Schauenstein, K. (2008). Haploinsufficiency of senescence evasion factor causes defects of hematopoietic stem cells functions. *Stem Cells Dev.* 17, 355–366.

Semenza, G. L. (2007). Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. *J. Cell. Biochem.* 102, 840–847.

Shay, J. W., and Wright, W. E. (2007). Hallmarks of telomeres in ageing research. *J. Pathol.* 211, 114–123.

Sheth, N., Roca, X., Hastings, M. L., Roeder, T., Krainer, A. R., and Sachidanandam, R. (2006). Comprehensive splice-site analysis using comparative genomics. *Nucleic Acids Res.* 34, 3955–3967.

Shomron, N., Alberstein, M., Reznik, M., and Ast, G. (2005). Stress alters the subcellular distribution of hSlu7 and thus modulates alternative splicing. *J. Cell Sci.* 118, 1151–1159.

Shovlin, C. L. (2010). Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. *Blood Rev.* 24, 203–219.

Sinha, R., Allemand, E., Zhang, Z., Karni, R., Myers, M. P., and Krainer, A. R. (2010). Arginine methylation controls the subcellular localization and functions of the oncoprotein splicing factor SF2/ASF. *Mol. Cell. Biol.* 30, 2762–2774.

Szotowski, B., Antoniak, S., Poller, W., Schultheiss, H. P., and Rauch, U. (2005). Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. *Circ. Res.* 96, 1233–1239.

Tang, J., Gordon, G. M., Nickoloff, B. J., and Foreman, K. E. (2002). The helix-loop-helix protein id-1 delays onset of replicative senescence in human endothelial cells. *Lab. Invest.* 82, 1073–1079.

Trigueros-Motos, L., Gonzalez, J. M., Rivera, J., and Andres, V. (2011). Hutchinson-Gilford progeria syndrome, cardiovascular disease and oxidative stress. *Front. Biosci. (Schol. Ed.)* 3, 1285–1297.

Velasco, S., Alvarez-Munoz, P., Peri-cacho, M., Dijke, P. T., Bernabeu, C., Lopez-Novoa, J. M., and Rodriguez-Barbero, A. (2008). L- and S-endoglin differentially modulate TGFbeta1 signaling mediated by ALK1 and ALK5 in L6E9 myoblasts. *J. Cell Sci.* 121, 913–919.

Verduci, L., Simili, M., Rizzo, M., Mercatanti, A., Evangelista, M., Mariani, L., Rainaldi, G., and Pitto, L. (2010). MicroRNA (miRNA)-mediated interaction between leukemia/lymphoma-related factor (LRF) and alternative splicing factor/splicing factor 2 (ASF/SF2) affects mouse embryonic fibroblast senescence and apoptosis. *J. Biol. Chem.* 285, 39551–39563.

Versari, D., Daghini, E., Virdis, A., Ghiadoni, L., and Taddei, S. (2009). Endothelium-dependent contractions and endothelial dysfunction in human hypertension. *Br. J. Pharmacol.* 157, 527–536.

Voglauer, R., Chang, M. W., Dampier, B., Wieser, M., Baumann, K., Sterovsky, T., Schreiber, M., Katinger, H., and Grillari, J. (2006). SNEV overexpression extends the life span of human endothelial cells. *Exp. Cell Res.* 312, 746–759.

Will, C. L., Schneider, C., Hossbach, M., Urlaub, H., Rauhut, R., Elbashir, S., Tuschl, T., and Luhrmann, R. (2004). The human 18S U11/U12 snRNP contains a set of novel proteins not found in the U2-dependent spliceosome. *RNA* 10, 929–941.

Worman, H. J., Fong, L. G., Muchir, A., and Young, S. G. (2009). Laminopathies and the long strange trip from basic cell biology to therapy. *J. Clin. Invest.* 119, 1825–1836.

Xie, J., Lee, J. A., Kress, T. L., Mowry, K. L., and Black, D. L. (2003). Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8776–8781.

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Endothelial progenitor cells enter the aging arena

K. Williamson, S. E. Stringer and M. Y. Alexander*

Cardiovascular Research Group, School of Biomedicine, University of Manchester, Manchester, UK

Edited by:
Ana Paula Dantas, Institut d'Investigacions Biomediques August Pi i Sunyer, Spain

Reviewed by:
Magda Heras, Hospital Clínic de Barcelona, Spain
Maria Helena C. Carvalho, Universidade de São Paulo, Brazil
Susana Novella, Research Foundation Hospital Clinico Valencia, Spain

***Correspondence:**
M. Y. Alexander, Cardiovascular Research Group, School of Biomedicine, University of Manchester, 46 Grafton Street, Manchester M13 9NT, UK.
e-mail: yvonne.alexander@manchester.ac.uk

Age is a significant risk factor for the development of vascular diseases, such as atherosclerosis. Although pharmacological treatments, including statins and anti-hypertensive drugs, have improved the prognosis for patients with cardiovascular disease, it remains a leading cause of mortality in those aged 65 years and over. Furthermore, given the increased life expectancy of the population in developed countries, there is a clear need for alternative treatment strategies. Consequently, the relationship between aging and progenitor cell-mediated repair is of great interest. Endothelial progenitor cells (EPCs) play an integral role in the cellular repair mechanisms for endothelial regeneration and maintenance. However, EPCs are subject to age-associated changes that diminish their number in circulation and function, thereby enhancing vascular disease risk. A great deal of research is aimed at developing strategies to harness the regenerative capacity of these cells. In this review, we discuss the current understanding of the cells termed "EPCs," examine the impact of age on EPC-mediated repair and identify therapeutic targets with potential for attenuating the age-related decline in vascular health via beneficial actions on EPCs.

Keywords: endothelial progenitor cells, age, vasculature, nitric oxide, estrogen, senescence, oxidative stress

INTRODUCTION

Despite significant advances in cardiovascular medicine over the past decade, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality throughout the developed world. Aging is a major risk factor for the onset and progression of CVD. The endothelium, which lines the lumen of all blood vessels, plays a pivotal role in maintaining cardiovascular homeostasis. This dynamic interface serves an enormous array of functions including the regulation of coagulation, arterial tone, permeability, vessel growth, and inflammation. As such, maintaining the functional integrity of the endothelial monolayer is of crucial importance for the prevention of CVDs, such as atherosclerosis. However, a characteristic of the aging process is the development of endothelial dysfunction, rendering the vasculature susceptible to the development of atherosclerosis and subsequent cardiovascular events. Although the mechanism of age-related impaired endothelial function remains unclear, an imbalance between the magnitude of vascular injury and the capacity for repair appears to play a role. Accumulating evidence suggests that bone-marrow derived circulating endothelial progenitor cells (EPCs) contribute to vascular repair and regeneration. In settings of tissue ischemia or endothelial damage, EPCs are mobilized from the bone marrow into the circulation and home to sites of vascular injury where they are able to contribute to new blood vessel formation and aid recovery. A deterioration of endogenous EPC function with age may culminate in a decreased capacity for neovascularization of ischemic tissues and/or reduced re-endothelialization of vascular lesions, facilitating the development, progression, and clinical sequelae of atherosclerotic disease.

THE DISCOVERY OF CIRCULATING EPCs

Asahara et al. (1997) published the first description of a candidate EPC; CD34+ circulating cells capable of differentiating into cells with endothelial characteristics and the ability to improve vascularization in a mouse model of hind limb ischemia. This discovery heralded a paradigm shift in vascular biology as it was recognized that vasculogenesis, a process by which new blood vessels form *de novo* through the differentiation of progenitor cells, and generally accepted as being restricted to embryonic development, may contribute to vascular repair and regeneration in adult life. The possibility of inducing new blood vessel formation and/or repairing damaged vessels by delivering/recruiting EPCs is an attractive idea that has stimulated much interest among the scientific community.

However, contradictory findings regarding the origin, identification, and contribution of EPCs to the neoangiogenic process has generated much debate within the field. The controversy is largely due to differences in the methodology used to isolate and culture EPCs. Consequently, the term EPC has encompassed a host of different cell types, which unsurprisingly, have demonstrated a mixed ability to contribute to blood vessel formation and repair.

In general, three methods have been described to isolate and expand EPCs from umbilical cord and peripheral blood mononuclear cells (MNCs; **Figure 1**; reviewed in Hirschi et al., 2008). The first method, originally described by Asahara et al. (1997) involves the plating of MNCs on fibronectin-coated tissue culture surfaces and re-plating the non-adherent cells after 48 h. Colonies form after 4–9 days and have been termed early EPCs. It is accepted that these are myeloid-derived cells that have a limited proliferative capacity and fail to form tube-like structures *in vitro*. The ability of these cells to augment new blood vessel formation appears to be through the production of angiogenic growth factors, chemokines, and cytokines without direct incorporation into vascular networks (Hur et al., 2004). The second approach involves plating MNCs on fibronectin-coated dishes for 4 days, but this time keeping the adherent cells which give rise to a heterogenous collection of cells termed circulating angiogenic cells

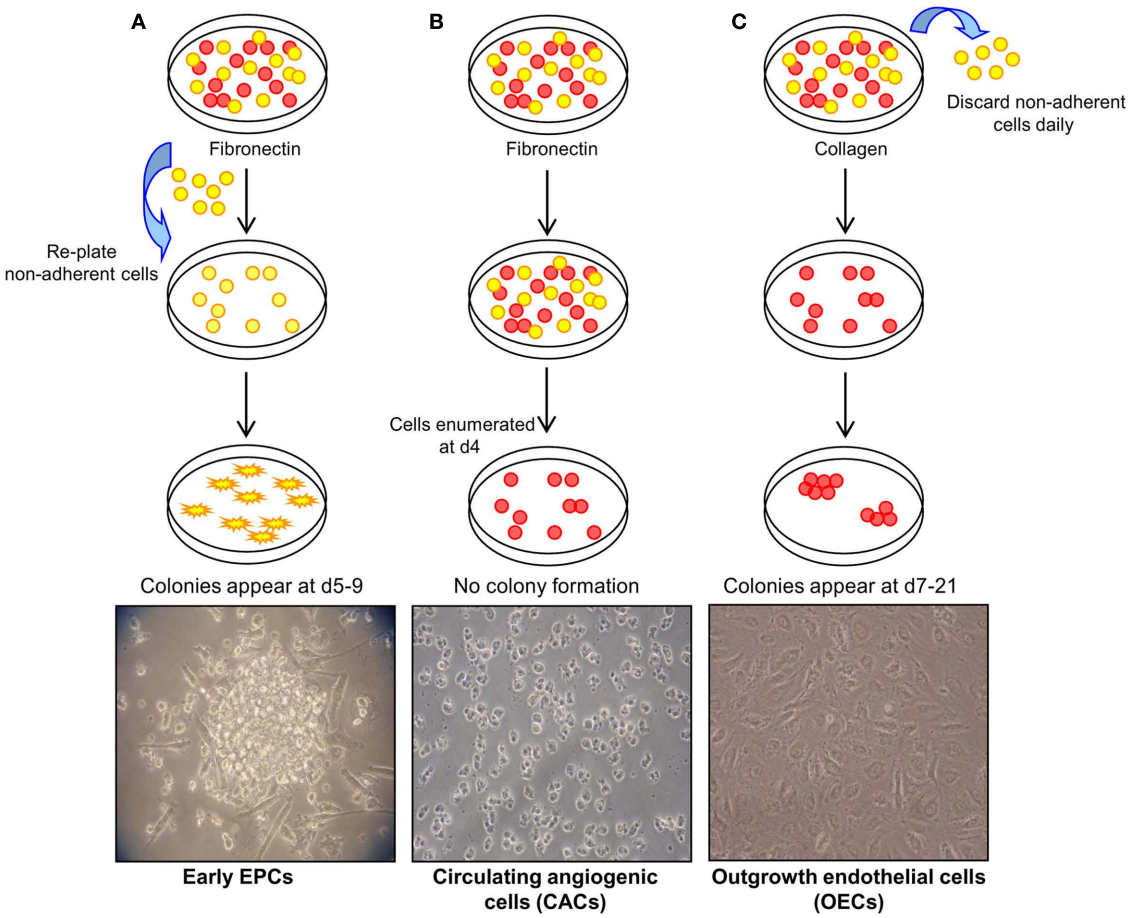

FIGURE 1 | Methods of endothelial progenitor cell (EPC) isolation. (A) Method 1: culture of early EPCs. Non-adherent MNCs are plated on fibronectin-coated tissue surfaces and form colonies after 4–9 days. These colonies have been termed early EPCs and are comprised of round cells surrounded by spindle-shaped cells. Image courtesy of Sahena Haque (×40 magnification). **(B)** Method 2: culture of circulating angiogenic cells (CACs). MNCs are plated on fibronectin-coated tissue surfaces for 4 days after which the non-adherent cells are removed. The adherent cells have been termed CACs and do not typically display colony formation. Image courtesy of John Reynolds (×20 magnification). **(C)** Method 3: culture of outgrowth endothelial cells (OECs). MNCs plated on collagen I-coated surfaces form colonies, termed OECs, with a cobblestone morphology after 7–21 days of culture (×20 magnification). Adapted from Hirschi et al. (2008).

(CACs). As with early EPCs, CACs express endothelial cell surface antigens (Vasa et al., 2001) but also retain monocytic characteristics and have a low proliferative potential (Rehman et al., 2003). The third method of EPC isolation involves a longer period of culture of MNCs on type I collagen coated surfaces. A population of cells originate from the adherent cells between 7 and 21 days, and have been referred to as outgrowth endothelial cells "OECs" (Lin et al., 2000; Gulati et al., 2003). OECs uniformly express endothelial markers, such as cluster of differentiation (CD) 31, vascular endothelial (VE)-cadherin, vascular endothelial growth factor receptor-2 (VEGFR-2), and uptake of acetylated low density lipoprotein (AcLDL), but of note, unlike early EPCs and CACs, this cell type is negative for hematopoietic or monocyte/macrophage cell surface antigens CD45, CD14, and CD115. OECs are capable of high rates of proliferation *in vitro* and vessel-forming activity when implanted in collagen gels *in vivo* (Ingram et al., 2004; Melero-Martin et al., 2007; Yoder et al., 2007; Au et al., 2008).

Considerable effort has been devoted to distinguishing these different cell types so that a definitive classification of an EPC phenotype can be established (Medina et al., 2010; Richardson and Yoder, 2011). It is important to note, that to gain quantitative data of putative circulating EPCs, flow cytometric analysis, in which EPCs are identified by cell surface phenotype, has been widely used. However, as the field has yet to reach agreement as to the definitive phenotype(s) of an EPC, much debate surrounds the choice of surface markers used in this approach. Indeed, many studies reporting to quantify circulating EPCs have in fact quantified hematopoietic cells with angiogenic capabilities (reviewed by Mund and Case, 2011).

EPCs AND REDUCED VASCULAR REPAIR WITH AGE

The mobilization of EPCs from their bone-marrow niche appears to constitute an integral part of the vascular homeostatic response, since acute ischemic events, such as myocardial infarction

(Shintani et al., 2001; Massa et al., 2005), acute coronary syndrome (George et al., 2004), and vascular injury secondary to burns or surgery (Gill et al., 2001) are associated with a significant and rapid increase in the levels of circulating EPCs. Consistent with the notion that EPCs serve an integral role in cardiovascular repair mechanisms, depletion of the circulating EPC pool has been shown to be a marker of cardiovascular damage and an independent predictor of cardiovascular events and death (Schmidt-Lucke et al., 2005; Werner et al., 2005). Whether the decrease in EPC number and impairment of function contributes to a loss of vasculoprotection, and the progression of CVD with age, remains to be fully elucidated. However, the link between age and EPC dysfunction is supported by a number of studies. Heiss et al. compared the number and function of early EPCs isolated from the peripheral blood of healthy young and old (average ages of 25 and 61 years respectively) individuals. Whilst there were no significant differences in the numbers of circulating EPCs (defined as $CD34^+/KDR^+$ or $CD133^{+/}KDR^+$) between the two groups, early EPCs from the old subjects were found to be significantly impaired in terms of proliferation, migration, and survival (Heiss et al., 2005). Rauscher et al. (2003) demonstrated that bone-marrow derived EPCs (defined as $CD31^+$ $CD45^-$) from young non-atherosclerotic ApoE−/− mice reduced atherosclerotic plaque size in ApoE−/− recipients despite persistent hypercholesterolemia, an effect not detected when using EPCs from old ApoE−/− mice, thus suggesting that the atheroprotective properties of EPCs are diminished with age. In addition, transplantation of bone-marrow derived EPCs from young, but not old donor mice, was seen to prevent a decline in the angiogenic platelet-derived growth factor (PDGF)-B signaling and cardiac angiogenesis in an aging murine model (Edelberg et al., 2002). It is likely that the age-associated impairment of EPC number and function is due to a variety of environmental changes that impair EPC generation, mobilization from the bone marrow, homing, and function, as well as intracellular alterations within the cells that induce a senescent phenotype (**Figure 2**).

INCREASED EPC SENESCENCE WITH AGE

With advancing age most, if not all, mammalian cell types are subject to internal alterations as well as environmental influences that ultimately cause the cell to enter a state of irreversible growth arrest, termed senescence. Internal changes include a reduction in telomere length, a region of repetitive DNA sequences that serve as the protective caps for chromosome ends. Telomere shortening beyond a critical length leads to genomic instability and ultimately cell cycle arrest. Decreased telomere length has been reported in the leukocytes of patients with CVD, such as, chronic heart failure (van der Harst et al., 2007), atherosclerosis (Samani et al., 2001), and ventricular dysfunction (Collerton et al., 2007). A reduction in telomere length with age has been shown in early EPCs of healthy, sedentary men (Kushner et al., 2009) This is, in part, due to an age-related decline in the expression of telomerase, a telomere elongating reverse transcriptase enzyme. A 57% reduction in

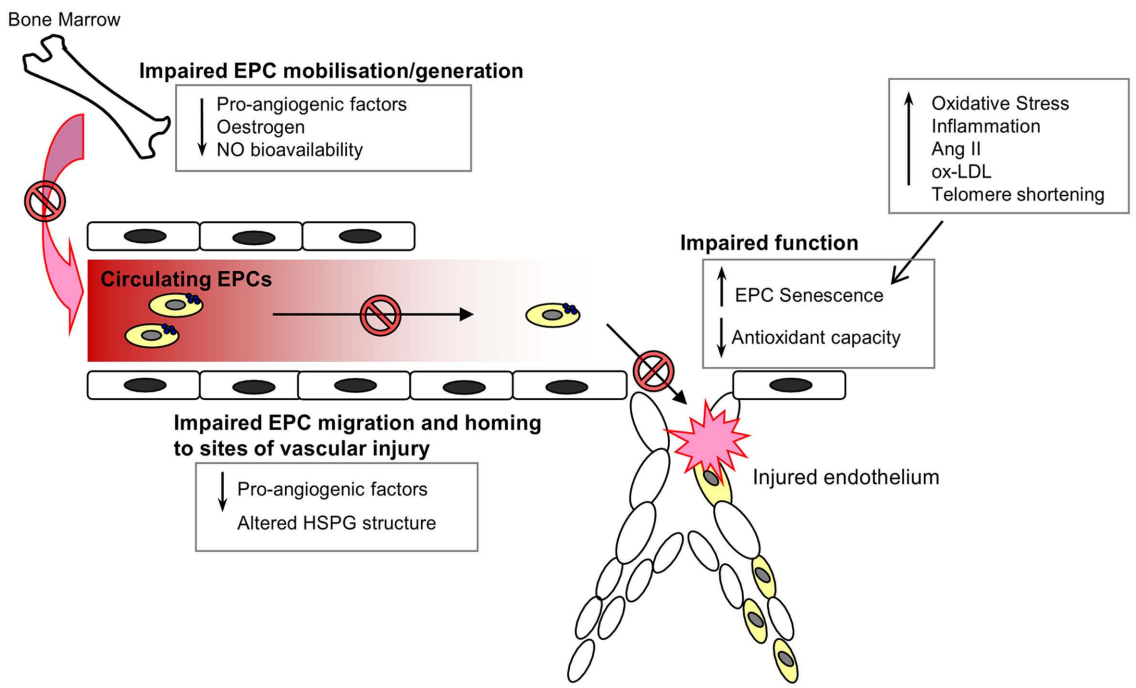

FIGURE 2 | Endothelial progenitor cells in the aging vasculature. A wide range of environmental and internal cellular changes occur with advancing age that impair EPC numbers in circulation and function at the site of injury. The generation, mobilization, and survival of circulating EPCs are dependent on the presence of various pro-angiogenic factors, estrogen, and nitric oxide, all of which are known to decline with increasing age. Furthermore, the angiogenic capacity of EPCs is also impaired with age owing to an increase in oxidative stress, inflammation and the induction of a senescent phenotype.

early EPC telomerase activity was found in older men as compared to younger counterparts (Kushner et al., 2011) and this is further modulated by oxidative stress. EPC senescence induced by telomere shortening may contribute to numerical and functional impairments of EPCs and thus diminished endothelial regeneration in the aging vasculature. Interestingly, overexpression of the human telomerase reverse transcriptase (hTERT) gene in CACs has been shown to conserve telomerase activity, delay cell senescence, and enhance EPC reparative functions in a murine ischemic hind limb model (Murasawa et al., 2002).

EPCs AND PRO-ANGIOGENIC FACTORS

An age-related decline in the expression of pro-angiogenic factors, including growth factors, cytokines, and hormones, is likely to contribute to impaired EPC generation, mobilization, migration, and survival. The mobilization of EPCs from the bone marrow and homing to sites of injury/neovascularization is dependent upon a number of factors, including stromal derived factor-1 (SDF-1), VEGF, granulocyte-colony stimulating factor (G-CSF), and estrogen. Decreased SDF-1 and VEGF expression in aged tissues has been shown to impair EPC ($Flk\text{-}1^+/CD11b^-$) trafficking to sites of ischemia, and is related to depressed hypoxia-inducible factor-1α (HIF-1α) signaling (Chang et al., 2007).

Premenopausal women have a lower risk of CVD than postmenopausal women or age-matched men (Grady et al., 1992; Grodstein et al., 1997). This is, in part, due to the dramatic decrease in levels of the female reproductive hormone estrogen at the onset of menopause. A number of studies have highlighted the role of estrogen in EPC generation, mobilization from the bone marrow, and incorporation at the sites of ischemia (Strehlow et al., 2003; Hamada et al., 2006; Iwakura et al., 2006; Ruifrok et al., 2009). Ovariectomy of young female mice was shown to significantly decrease the levels of EPCs ($Sca\text{-}1^+/Flk\text{-}1^+$) in the peripheral blood and residing bone marrow, but this effect was prevented by treatment with exogenous estrogen (Strehlow et al., 2003). In addition to augmenting EPC generation and mobilization, estrogen has been shown to inhibit the senescence of CACs *in vitro* by increasing telomerase activity, and also appears to promote EPC proliferation and their incorporation into vascular networks (Imanishi et al., 2005b).

The activity of a wide array of pro-angiogenic factors, including VEGF, basic fibroblast growth factor (bFGF), and SDF-1 is modulated by cell surface heparan sulfate (HS) proteoglycans (Fuster and Wang, 2010). Work from our laboratory suggests that age-associated changes of HS structure on the surface of OECs correlates with a significant reduction in migratory function (Williamson et al., 2011).

EPCs AND OXIDATIVE STRESS

The progressive accumulation of oxidative damage with age, due to increased production of reactive oxygen species (ROS) and decreased expression of antioxidant proteins, also promotes senescent cell changes. ROS lead to inactivation of nitric oxide (NO), cause damage to proteins, lipids, and DNA, and alter cellular redox state. Oxidative stress promotes the development of vascular pathologies (Higgins et al., 2011; Sugamura and Keaney, 2011). With increasing age, it is likely that oxidative cellular damage accumulates in EPCs, diminishing function and enhancing vascular disease risk. Early EPCs, CACs, and OECs have been shown to express high levels of antioxidant enzymes and as a result, are more resistant to oxidative stress, as compared with human umbilical vein endothelial cells (HUVECs) and adult microvascular endothelial cells (Dernbach et al., 2004; He et al., 2004, 2009; Cai et al., 2006). Such an enhanced antioxidant system may endow EPCs with improved resistance to oxidative stress and thus the ability to promote vascular regeneration in settings of ischemic injury. However, the antioxidant capacity of EPCs appears to be impaired with age, for example, He et al. (2009) demonstrated that early EPCs derived from old subjects had significantly reduced levels and activity of the antioxidant enzyme glutathione peroxidase-1 (GPx-1) and were more sensitive to oxidative stress-induced apoptosis as compared to EPCs of younger subjects. Together, these factors may reduce EPC survival capacity and their ability to promote endothelial repair in the aging host. Aging is also associated with an upregulation of proatherogenic stimuli including angiotensin II (Ang II) (Baylis et al., 1997; Wang et al., 2003), which has been shown to enhance the production of ROS in early EPCs and thereby accelerate cellular senescence (Imanishi et al., 2005a; Endtmann et al., 2011). Moreover, treatment of coronary artery disease patients with an Ang II type I receptor (AT1-R) antagonist was found to significantly increase the number of circulating EPCs ($CD34^+/KDR^+$) (Endtmann et al., 2011).

A DECREASE IN NITRIC OXIDE BIOAVAILABILITY IN EPCs WITH AGE

A decline in EPC levels and function may be related to the reduced capacity of the aging endothelium to generate nitric oxide (NO) and the increased production of ROS with age. The importance of endothelial nitric oxide synthase (eNOS) expression, and subsequent NO production, for EPC mobilization, was documented in eNOS null mice by Laufs and colleagues. Here, physical exercise, which increases NO bioavailability, was found to significantly increase EPC numbers ($Sca\text{-}1^+/Flk\text{-}1^+$) while this effect was attenuated in eNOS null mice and wild type mice treated with an NOS inhibitor (Laufs et al., 2004). The accumulation of oxidized low density lipoprotein (ox-LDL) with age may also contribute to a reduction in the number of circulating EPCs. ox-LDL has been shown to impair early EPC survival and function, due to its inhibitory effect on EPC eNOS expression and activity (Ma et al., 2006).

EPCs AND INFLAMMATION

Aging is known to be associated with the development of chronic low grade inflammation, which renders the vasculature susceptible to the development of CVD (Csiszar et al., 2008). Among the proinflammatory changes that occur with increasing age, dysregulation of the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) has been well established (Csiszar et al., 2003, 2004, 2007). This multifunctional proinflammatory cytokine is a key player in the pathogenesis of atherosclerosis. Indeed increased circulating levels of TNF-α is associated with a high prevalence of atherosclerosis in elderly patients (Bruunsgaard et al., 2000). Chronic TNF-α treatment has been found to induce premature senescence of highly proliferative OECs, which was related to an

increase in expression of the cell cycle inhibitor $p16^{INK4a}$ (Zhang et al., 2009). Thus, the shift to a proinflammatory state in the aged vasculature may contribute to EPC dysfunction and a reduced regenerative potential.

CONCLUDING REMARKS

Although age-related CVD may have multifactorial etiologies, a common element appears to be the deterioration of endogenous EPC function. Understanding EPC biology and identifying strategies to restore the factors that are depleted or impaired with age may provide a protective benefit to the cardiovascular system and limit vascular disease progression in the aging population. Indeed, pharmacological treatments including statins (Landmesser et al., 2004), estrogen (Iwakura et al., 2003, 2006), and peroxisome proliferator-activated receptor gamma agonists (Redondo et al., 2007; Werner et al., 2007) have been shown to augment EPC number and function, by increasing NO bioavailability in EPCs, among other mechanisms of action. Treatment with angiotensin receptor antagonists have been shown to reduce oxidative stress in EPCs and augment function by blocking the effect of AngII (Bahlmann et al., 2005). Other pharmacological strategies to reverse age-related EPC dysfunction include growth hormone treatment to increase insulin-like growth factor-1 (IGF-1) levels, the synthesis of which is attenuated with aging (Thum et al., 2007). Lifestyle modifications, such as regular aerobic exercise or cessation of smoking, are known to promote vascular health in the aging population and have direct effects on EPC number and function. Reports suggest that physical exercise increases the number and re-endothelialization capacity of EPCs in previously sedentary older men (Hoetzer et al., 2007; Xia et al., 2011; Yang et al., 2011), while smoking cessation has been found to induce rapid restoration of EPC levels (Kondo et al., 2004). Although long term follow up is necessary, these findings suggest that it is possible to increase EPC number and function in the aging host by restoring the pathways that govern EPC generation, mobilization, and homing. Such approaches have potential for attenuating the age-related decline in vascular health and improving the outcomes for older individuals with CVD. However, further work is needed to overcome the challenges associated with engraftment of these cells to the damaged tissue and the resulting cross-talk with the host endothelium and medial cells that will ultimately result in the repair of the damaged tissue.

REFERENCES

Asahara, T., Murohara, T., Sullivan, A., Silver, M., Van Der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., and Isner, J. M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275, 964–967.

Au, P., Daheron, L. M., Duda, D. G., Cohen, K. S., Tyrrell, J. A., Lanning, R. M., Fukumura, D., Scadden, D. T., and Jain, R. K. (2008). Differential in vivo potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. *Blood* 111, 1302–1305.

Bahlmann, F. H., De Groot, K., Mueller, O., Hertel, B., Haller, H., and Fliser, D. (2005). Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* 45, 526–529.

Baylis, C., Engels, K., Hymel, A., and Navar, L. G. (1997). Plasma renin activity and metabolic clearance rate of angiotensin II in the unstressed aging rat. *Mech. Ageing Dev.* 97, 163–172.

Bruunsgaard, H., Skinhoj, P., Pedersen, A. N., Schroll, M., and Pedersen, B. K. (2000). Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. *Clin. Exp. Immunol.* 121, 255–260.

Cai, H., Gehrig, P., Scott, T. M., Zimmermann, R., Schlapbach, R., and Zisch, A. H. (2006). MnSOD marks cord blood late outgrowth endothelial cells and accompanies robust resistance to oxidative stress. *Biochem. Biophys. Res. Commun.* 350, 364–369.

Chang, E. I., Loh, S. A., Ceradini, D. J., Chang, E. I., Lin, S. E., Bastidas, N., Aarabi, S., Chan, D. A., Freedman, M. L., Giaccia, A. J., and Gurtner, G. C. (2007). Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation* 116, 2818–2829.

Collerton, J., Martin-Ruiz, C., Kenny, A., Barrass, K., Von Zglinicki, T., Kirkwood, T., and Keavney, B. (2007). Telomere length is associated with left ventricular function in the oldest old: the Newcastle 85+ study. *Eur. Heart J.* 28, 172–176.

Csiszar, A., Labinskyy, N., Smith, K., Rivera, A., Orosz, Z., and Ungvari, Z. (2007). Vasculoprotective effects of anti-tumor necrosis factor-alpha treatment in aging. *Am. J. Pathol.* 170, 388–398.

Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., and Kaley, G. (2003). Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. *FASEB J.* 17, 1183–1185.

Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., and Kaley, G. (2004). Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol. Genomics* 17, 21–30.

Csiszar, A., Wang, M., Lakatta, E. G., and Ungvari, Z. (2008). Inflammation and endothelial dysfunction during aging: role of NF-kappaB. *J. Appl. Physiol.* 105, 1333–1341.

Dernbach, E., Urbich, C., Brandes, R. P., Hofmann, W. K., Zeiher, A. M., and Dimmeler, S. (2004). Antioxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. *Blood* 104, 3591–3597.

Edelberg, J. M., Tang, L., Hattori, K., Lyden, D., and Rafii, S. (2002). Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. *Circ. Res.* 90, E89–E93.

Endtmann, C., Ebrahimian, T., Czech, T., Arfa, O., Laufs, U., Fritz, M., Wassmann, K., Werner, N., Petoumenos, V., Nickenig, G., and Wassmann, S. (2011). Angiotensin II impairs endothelial progenitor cell number and function in vitro and in vivo: implications for vascular regeneration. *Hypertension* 58, 394–403.

Fuster, M. M., and Wang, L. (2010). Endothelial heparan sulfate in angiogenesis. *Prog. Mol. Biol. Transl. Sci.* 93, 179–212.

George, J., Goldstein, E., Abashidze, S., Deutsch, V., Shmilovich, H., Finkelstein, A., Herz, I., Miller, H., and Keren, G. (2004). Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. *Eur. Heart J.* 25, 1003–1008.

Gill, M., Dias, S., Hattori, K., Rivera, M. L., Hicklin, D., Witte, L., Girardi, L., Yurt, R., Himel, H., and Rafii, S. (2001). Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. *Circ. Res.* 88, 167–174.

Grady, D., Rubin, S. M., Petitti, D. B., Fox, C. S., Black, D., Ettinger, B., Ernster, V. L., and Cummings, S. R. (1992). Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann. Intern. Med.* 117, 1016–1037.

Grodstein, F., Stampfer, M. J., Colditz, G. A., Willett, W. C., Manson, J. E., Joffe, M., Rosner, B., Fuchs, C., Hankinson, S. E., Hunter, D. J., Hennekens, C. H., and Speizer, F. E. (1997). Postmenopausal hormone therapy and mortality. *N. Engl. J. Med.* 336, 1769–1775.

Gulati, R., Jevremovic, D., Peterson, T. E., Chatterjee, S., Shah, V., Vile, R. G., and Simari, R. D. (2003). Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ. Res.* 93, 1023–1025.

Hamada, H., Kim, M. K., Iwakura, A., Ii, M., Thorne, T., Qin, G., Asai, J., Tsutsumi, Y., Sekiguchi, H., Silver, M., Wecker, A., Bord, E., Zhu, Y., Kishore, R., and Losordo, D. W. (2006). Estrogen receptors alpha and beta mediate contribution of bone marrow-derived endothelial progenitor cells to functional recovery after myocardial infarction. *Circulation* 114, 2261–2270.

He, T., Joyner, M. J., and Katusic, Z. S. (2009). Aging decreases expression and activity of glutathione peroxidase-1 in human endothelial progenitor cells. *Microvasc. Res.* 78, 447–452.

He, T., Peterson, T. E., Holmuhamedov, E. L., Terzic, A., Caplice, N. M., Oberley, L. W., and Katusic, Z. S. (2004). Human endothelial progenitor cells tolerate oxidative stress due to intrinsically high expression of manganese superoxide dismutase. *Arterioscler. Thromb. Vasc. Biol.* 24, 2021–2027.

Heiss, C., Keymel, S., Niesler, U., Ziemann, J., Kelm, M., and Kalka, C. (2005). Impaired progenitor cell activity in age-related endothelial dysfunction. *J. Am. Coll. Cardiol.* 45, 1441–1448.

Higgins, P., Dawson, J., Lees, K. R., Mcarthur, K., Quinn, T. J., and Walters, M. R. (2011). Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. *Cardiovasc. Ther.* doi: 10.1111/j.1755-5922.2011.00277.x. [EPub ahead of print].

Hirschi, K. K., Ingram, D. A., and Yoder, M. C. (2008). Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler. Thromb. Vasc. Biol.* 28, 1584–1595.

Hoetzer, G. L., Van Guilder, G. P., Irmiger, H. M., Keith, R. S., Stauffer, B. L., and Desouza, C. A. (2007). Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *J. Appl. Physiol.* 102, 847–852.

Hur, J., Yoon, C. H., Kim, H. S., Choi, J. H., Kang, H. J., Hwang, K. K., Oh, B. H., Lee, M. M., and Park, Y. B. (2004). Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler. Thromb. Vasc. Biol.* 24, 288–293.

Imanishi, T., Hano, T., and Nishio, I. (2005a). Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J. Hypertens.* 23, 97–104.

Imanishi, T., Hano, T., and Nishio, I. (2005b). Estrogen reduces endothelial progenitor cell senescence through augmentation of telomerase activity. *J. Hypertens.* 23, 1699–1706.

Ingram, D. A., Mead, L. E., Tanaka, H., Meade, V., Fenoglio, A., Mortell, K., Pollok, K., Ferkowicz, M. J., Gilley, D., and Yoder, M. C. (2004). Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104, 2752–2760.

Iwakura, A., Luedemann, C., Shastry, S., Hanley, A., Kearney, M., Aikawa, R., Isner, J. M., Asahara, T., and Losordo, D. W. (2003). Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. *Circulation* 108, 3115–3121.

Iwakura, A., Shastry, S., Luedemann, C., Hamada, H., Kawamoto, A., Kishore, R., Zhu, Y., Qin, G., Silver, M., Thorne, T., Eaton, L., Masuda, H., Asahara, T., and Losordo, D. W. (2006). Estradiol enhances recovery after myocardial infarction by augmenting incorporation of bone marrow-derived endothelial progenitor cells into sites of ischemia-induced neovascularization via endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9. *Circulation* 113, 1605–1614.

Kondo, T., Hayashi, M., Takeshita, K., Numaguchi, Y., Kobayashi, K., Iino, S., Inden, Y., and Murohara, T. (2004). Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler. Thromb. Vasc. Biol.* 24, 1442–1447.

Kushner, E. J., Maceneaney, O. J., Weil, B. R., Greiner, J. J., Stauffer, B. L., and Desouza, C. A. (2011). Aging is associated with a proapoptotic endothelial progenitor cell phenotype. *J. Vasc. Res.* 48, 408–414.

Kushner, E. J., Van Guilder, G. P., Maceneaney, O. J., Cech, J. N., Stauffer, B. L., and Desouza, C. A. (2009). Aging and endothelial progenitor cell telomere length in healthy men. *Clin. Chem. Lab. Med.* 47, 47–50.

Landmesser, U., Engberding, N., Bahlmann, F. H., Schaefer, A., Wiencke, A., Heineke, A., Spiekermann, S., Hilfiker-Kleiner, D., Templin, C., Kotlarz, D., Mueller, M., Fuchs, M., Hornig, B., Haller, H., and Drexler, H. (2004). Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation* 110, 1933–1939.

Laufs, U., Werner, N., Link, A., Endres, M., Wassmann, S., Jurgens, K., Miche, E., Bohm, M., and Nickenig, G. (2004). Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. *Circulation* 109, 220–226.

Lin, Y., Weisdorf, D. J., Solovey, A., and Hebbel, R. P. (2000). Origins of circulating endothelial cells and endothelial outgrowth from blood. *J. Clin. Invest.* 105, 71–77.

Ma, F. X., Zhou, B., Chen, Z., Ren, Q., Lu, S. H., Sawamura, T., and Han, Z. C. (2006). Oxidized low density lipoprotein impairs endothelial progenitor cells by regulation of endothelial nitric oxide synthase. *J. Lipid Res.* 47, 1227–1237.

Massa, M., Rosti, V., Ferrario, M., Campanelli, R., Ramajoli, I., Rosso, R., De Ferrari, G. M., Ferlini, M., Goffredo, L., Bertoletti, A., Klersy, C., Pecci, A., Moratti, R., and Tavazzi, L. (2005). Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* 105, 199–206.

Medina, R. J., O'Neill, C. L., Sweeney, M., Guduric-Fuchs, J., Gardiner, T. A., Simpson, D. A., and Stitt, A. W. (2010). Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. *BMC Med. Genomics* 3, 18. doi:10.1186/1755-8794-3-18

Melero-Martin, J. M., Khan, Z. A., Picard, A., Wu, X., Paruchuri, S., and Bischoff, J. (2007). In vivo vasculogenic potential of human blood-derived endothelial progenitor cells. *Blood* 109, 4761–4768.

Mund, J. A., and Case, J. (2011). The ontogeny of endothelial progenitor cells through flow cytometry. *Curr. Opin. Hematol.* 18, 166–170.

Murasawa, S., Llevadot, J., Silver, M., Isner, J. M., Losordo, D. W., and Asahara, T. (2002). Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. *Circulation* 106, 1133–1139.

Rauscher, F. M., Goldschmidt-Clermont, P. J., Davis, B. H., Wang, T., Gregg, D., Ramaswami, P., Pippen, A. M., Annex, B. H., Dong, C., and Taylor, D. A. (2003). Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 108, 457–463.

Redondo, S., Hristov, M., Gumbel, D., Tejerina, T., and Weber, C. (2007). Biphasic effect of pioglitazone on isolated human endothelial progenitor cells: involvement of peroxisome proliferator-activated receptor-gamma and transforming growth factor-beta1. *Thromb. Haemost.* 97, 979–987.

Rehman, J., Li, J., Orschell, C. M., and March, K. L. (2003). Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 107, 1164–1169.

Richardson, M. R., and Yoder, M. C. (2011). Endothelial progenitor cells: quo vadis? *J. Mol. Cell. Cardiol.* 50, 266–272.

Ruifrok, W. P., De Boer, R. A., Iwakura, A., Silver, M., Kusano, K., Tio, R. A., and Losordo, D. W. (2009). Estradiol-induced, endothelial progenitor cell-mediated neovascularization in male mice with hind-limb ischemia. *Vasc. Med.* 14, 29–36.

Samani, N. J., Boultby, R., Butler, R., Thompson, J. R., and Goodall, A. H. (2001). Telomere shortening in atherosclerosis. *Lancet* 358, 472–473.

Schmidt-Lucke, C., Rossig, L., Fichtlscherer, S., Vasa, M., Britten, M., Kamper, U., Dimmeler, S., and Zeiher, A. M. (2005). Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 111, 2981–2987.

Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Honma, T., Katoh, A., Sasaki, K., Shimada, T., Oike, Y., and Imaizumi, T. (2001). Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 103, 2776–2779.

Strehlow, K., Werner, N., Berweiler, J., Link, A., Dirnagl, U., Priller, J., Laufs, K., Ghaeni, L., Milosevic, M., Bohm, M., and Nickenig, G. (2003). Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 107, 3059–3065.

Sugamura, K., and Keaney, J. F. Jr. (2011). Reactive oxygen species in cardiovascular disease. *Free Radic. Biol. Med.* 51, 978–992.

Thum, T., Hoeber, S., Froese, S., Klink, I., Stichtenoth, D. O., Galuppo, P., Jakob, M., Tsikas, D., Anker, S. D., Poole-Wilson, P. A., Borlak, J., Ertl, G., and Bauersachs, J. (2007). Age-dependent impairment of endothelial progenitor cells is corrected by growth-hormone-mediated increase of insulin-like growth-factor-1. *Circ. Res.* 100, 434–443.

van der Harst, P., Van Der Steege, G., De Boer, R. A., Voors, A. A., Hall, A. S., Mulder, M. J., Van Gilst, W. H., and Van Veldhuisen, D. J. (2007). Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 49, 1459–1464.

Vasa, M., Fichtlscherer, S., Aicher, A., Adler, K., Urbich, C., Martin, H., Zeiher, A. M., and Dimmeler, S. (2001). Number and migratory activity of

circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res.* 89, E1–E7.

Wang, M., Takagi, G., Asai, K., Resuello, R. G., Natividad, F. F., Vatner, D. E., Vatner, S. F., and Lakatta, E. G. (2003). Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. *Hypertension* 41, 1308–1316.

Werner, C., Kamani, C. H., Gensch, C., Bohm, M., and Laufs, U. (2007). The peroxisome proliferator-activated receptor-gamma agonist pioglitazone increases number and function of endothelial progenitor cells in patients with coronary artery disease and normal glucose tolerance. *Diabetes* 56, 2609–2615.

Werner, N., Kosiol, S., Schiegl, T., Ahlers, P., Walenta, K., Link, A., Bohm, M., and Nickenig, G. (2005). Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* 353, 999–1007.

Williamson, K., Stringer, S., Sipos, P., Crocker, I., and Alexander, M. (2011). Age-related modification of heparan sulphate proteoglycans on human endothelial progenitor cells. *Heart* 97, e7.

Xia, W. H., Li, J., Su, C., Yang, Z., Chen, L., Wu, F., Zhang, Y. Y., Yu, B. B., Qiu, Y. X., Wang, S. M., and Tao, J. (2011). Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. *Aging Cell.*

Yang, Z., Xia, W. H., Su, C., Wu, F., Zhang, Y. Y., Xu, S. Y., Liu, X., Zhang, X. Y., Ou, Z. J., Lai, G. H., Liao, X. X., Jin, Y. F., and Tao, J. (2011). Regular exercise-induced increased number and activity of circulating endothelial progenitor cells attenuates age-related decline in arterial elasticity in healthy men. *Int. J. Cardiol.*

Yoder, M. C., Mead, L. E., Prater, D., Krier, T. R., Mroueh, K. N., Li, F., Krasich, R., Temm, C. J., Prchal, J. T., and Ingram, D. A. (2007). Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 109, 1801–1809.

Zhang, Y., Herbert, B. S., Rajashekhar, G., Ingram, D. A., Yoder, M. C., Clauss, M., and Rehman, J. (2009). Premature senescence of highly proliferative endothelial progenitor cells is induced by tumor necrosis factor-alpha via the p38 mitogen-activated protein kinase pathway. *FASEB J.* 23, 1358–1365.

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Vascular aging in women: is estrogen the fountain of youth?

Susana Novella[1,2], Ana Paula Dantas[3], Gloria Segarra[1,2], Pascual Medina[1,2] and Carlos Hermenegildo[1,2]*

[1] *Departamento de Fisiología, Universitat de València, Valencia, Spain*
[2] *Instituto de Investigación Sanitaria INCLIVA, Hospital Clínico Universitario, Valencia, Spain*
[3] *Institut D'Investigacions Biomèdiques August Pi i Sunyer and Institut Clínic del Tòrax, Hospital Clinic, Barcelona, Spain*

Edited by:
Francesc Jiménez-Altayó, Universitat Autonoma de Barcelona, Spain

Reviewed by:
Rayna Gonzales, University of Arizona COM Phoenix, USA
Raouf Khalil, Brigham and Women's Hospital, USA
Eduardo Nava, University of Castilla-La Mancha, Spain

Correspondence:
Carlos Hermenegildo, Departamento de Fisiología, Facultad de Medicina y Odontología, Universitat de València, Av. Blasco Ibañez, 15, E 46010 Valencia, Spain.
e-mail: carlos.hermenegildo@uv.es

Aging is associated with structural and functional changes in the vasculature, including endothelial dysfunction, arterial stiffening and remodeling, impaired angiogenesis, and defective vascular repair, and with increased prevalence of atherosclerosis. Cardiovascular risk is similar for older men and women, but lower in women during their fertile years. This age- and sex-related difference points to estrogen as a protective factor because menopause is marked by the loss of endogenous estrogen production. Experimental and some clinical studies have attributed most of the protective effects of estrogen to its modulatory action on vascular endothelium. Estrogen promotes endothelial-derived NO production through increased expression and activity of endothelial nitric oxide synthase, and modulates prostacyclin and thromboxane A_2 release. The thromboxane A_2 pathway is key to regulating vascular tone in females. Despite all the experimental evidence, some clinical trials have reported no cardiovascular benefit from estrogen replacement therapy in older postmenopausal women. The "Timing Hypothesis," which states that estrogen-mediated vascular benefits occur only before the detrimental effects of aging are established in the vasculature, offers a possible explanation for these discrepancies. Nevertheless, a gap remains in current knowledge of cardiovascular aging mechanisms in women. This review comprises clinical and experimental data on the effects of aging, estrogens, and hormone replacement therapy on vascular function of females. We aim to clarify how menopause and aging contribute jointly to vascular aging and how estrogen modulates vascular response at different ages.

Keywords: endothelium, menopause, estradiol, nitric oxide, vascular protection

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in both men and women in developed countries. Nonetheless, sex-associated differences regarding the age of CVD onset and its progression are observed worldwide. Incidence of CVD in premenopausal women is markedly lower than age-matched men in epidemiological studies (Messerli et al., 1987; Bairey Merz et al., 2006; Shaw et al., 2006). After menopause, however, the incidence is comparable or even higher in women than in men (Lerner and Kannel, 1986; Eaker et al., 1993), making CVD the primary cause of death in postmenopausal women (55 versus 43% in men), exceeding all cancer deaths (Rosamond et al., 2008). The lower CVD risk among fertile women is often attributed to the protective role of estrogens at the vascular level. According to epidemiological observations and extensive basic research, estrogen and other sex steroids have direct cardiovascular benefits. Estrogen modulates a myriad of molecular pathways that improve vascular function, whether at the physiological level or when administered as hormone replacement therapy (HRT; Grodstein et al., 2000, 2001; Miller and Duckles, 2008).

Nevertheless, some clinical studies have questioned the protective value of HRT against vascular disease. Two randomized clinical trials, the Women's Health Initiative (WHI; Rossouw et al., 2002) and the Heart and Estrogen/Progestin Replacement Study (HERS I and II; Gambacciani et al., 2002), indicate that HRT may increase CVD risk and events in postmenopausal women. The reason for this paradox could be attributable to many patient characteristics, including age.

Although aging occurs progressively in both men and women, the onset of menopause marks a sudden increase in the appearance of aging-associated signs in women, and more specifically in the progress of vascular aging. Information about the role of age and menopause in the development of CVD in women is scarce. This review of clinical and experimental data on the effects of aging, estrogens, and HRT on vascular function of females aims to clarify how menopause and aging contribute jointly to vascular aging and how estrogen modulates vascular response at different ages.

HOW DO ESTROGENS AFFECT VASCULAR FUNCTION?

The numerous vascular effects of estrogens are triggered by complex genomic and non-genomic mechanisms. They include modulation of vascular function and inflammatory response as well as metabolic and hemodynamic effects. Estradiol, the most abundant and potent estrogen in humans, mainly binds and activates estrogen receptors (ERs). Vascular estrogen signaling involves at least three ERs identified in both vascular smooth muscle and

endothelium, reinforcing the idea that estrogen has a key role in controlling vascular function. The classical subtypes ERα (Soloff and Szego, 1969) and ERβ (Kuiper et al., 1996) vary not only in their tissue distributions, but also in their agonist/antagonist profile with respect to several compounds (Cano and Hermenegildo, 2000). These ER subtypes belong to the intracellular receptors classically defined as nuclear ligand-activated transcription factors. Activation of these receptors by the corresponding hormones affects gene expression by acting on estrogen-response elements in the target genes and modulating transcriptional events (Beato et al., 1995). Estrogen binding to ERα and ERβ regulates gene expression in a time- and tissue-dependent manner, generating controversy about the type of receptor involved in vascular protection (Murphy, 2011). In the cardiovascular system, both ERα and ERβ have been identified in the endothelium, smooth muscle cells, adventitia, and adrenergic nerve endings of arteries from various territories and several species, including humans (Karas et al., 1994; Kim-Schulze et al., 1996; Venkov et al., 1996; Register and Adams, 1998). Although it has been reported that cultured endothelial cells do not express ERα (Toth et al., 2009), other investigators have demonstrated the presence of both ERα and ERβ mRNA in endothelium (Wagner et al., 2001) and data from our group demonstrate the protein expression of both ERα and ERβ in HUVEC (Sobrino et al., 2009, 2010).

In addition to their classic nuclear location, ER can also target the plasma membrane, enabling estrogen activation of several signaling pathways, including those involved in calcium mobilization (Zhang et al., 1994; Nakajima et al., 1995; Prakash et al., 1999) and the phosphatidylinositol-3-kinase (PI3K) pathway (Hisamoto et al., 2001). A third type of ER, G-protein coupled, and mainly located in the plasma membrane, was initially named GPR30 (Takada et al., 1997), then renamed GPER by the International Union of Basic and Clinical Pharmacology, IUPHAR (Alexander et al., 2008). GPER is expressed in both endothelial and smooth muscle cells of human arteries and veins (Haas et al., 2007; Deschamps and Murphy, 2009). After estrogen binding, GPER activates rapid signaling cascades such as extracellular signal-related kinase and PI3K (Meyer et al., 2011). Several rapid and non-genomic estrogen effects formerly attributed to ERα have now been described as GPER-mediated (Prossnitz and Barton, 2011).

The vascular protection conferred by estrogen may be mediated indirectly by its influence on the metabolism of lipoproteins or by a direct action on the modulation of molecular pathways in the vessel wall, and more specifically on endothelial cells (Hermenegildo et al., 2002). Vascular endothelium not only regulates vascular tone through flow-mediated mechanisms, but also confers antithrombotic and antiinflammatory properties to the blood vessel. Nitric oxide (NO), the primary endothelial-derived mediator, is involved in many physiological processes, including vasodilation and inhibition of thrombosis, cell migration, and proliferation (Dudzinski and Michel, 2007; Lamas et al., 2007; Michel and Vanhoutte, 2010). Estrogen is known to increase NO bioavailability by mechanisms that either directly increase NO generation (**Figure 1**) or decrease superoxide anion (O_2^-) concentration, thereby attenuating O_2^--mediated NO inactivation. Mechanisms involved in estrogen-induced increases in NO availability include: (1) transcriptional stimulation of endothelial NO synthase (eNOS) gene expression (Huang et al., 1997; Sumi and Ignarro, 2003); (2) non-genomic activation of enzyme activity via a PI3K/phosphokinase B (PKB/AKT)-mediated signaling pathway (Hisamoto et al., 2001); (3) increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) in endothelial cells (Rubio-Gayosso et al., 2000); (4) decreased production of asymmetric dimethylarginine (ADMA), the eNOS endogenous inhibitor (Monsalve et al., 2007); and (5) attenuated O_2^- concentrations (Wassmann et al., 2001; Dantas et al., 2002; Ospina et al., 2002).

Estrogens such as 17β-estradiol, estrone, and estriol have been described to act as reactive oxygen species (ROS) scavengers by virtue of the hydrogen-donating capacity of their phenolic molecular structure (Halliwell and Grootveld, 1987; Dubey and Jackson, 2001). However, in these studies the direct effect of estrogens as scavengers can only be observed at concentrations above 1 μM (Arnal et al., 1996; Kim et al., 1996). Considering that plasma concentrations of estrogen in physiological conditions are within the nanomolar range, it is likely that direct scavenger action is not estrogen's main antioxidant mechanism. Estrogen modulates ROS concentration through a mechanism that involves interaction with its estrogenic nuclear receptors to decrease oxidative proteins and/or increase antioxidant enzymes expression. Many studies have associated changes in estrogen levels with altered levels of antioxidant enzymes including glutathione peroxidase, catalase, and superoxide dismutase (Capel et al., 1981; Robb and Stuart, 2011; Sivritas et al., 2011). Moreover, estrogen modulates NADH/NADPH oxidases and AT_1 receptor gene expression, both of which are major sources of O_2^- production (Wassmann et al., 2001; Dantas et al., 2002).

Estrogen also has a modulating effect on constrictive factors and positively upregulates the production of endothelium-derived relaxing factors such as PGI_2 (Sobrino et al., 2009, 2010) and the endothelium-derived hyperpolarizing factors (Golding and Kepler, 2001), both of which are important mediators of vascular relaxation in resistance-sized arteries. The beneficial effects of estrogen on the endothelium can be partially explained by an inhibitory effect on the production or action of the cyclooxygenase (COX)-derived vasoconstrictor agents prostaglandin H_2, PGH_2, and thromboxane A_2, TXA_2 (Davidge and Zhang, 1998; Dantas et al., 1999; Novella et al., 2010), and of endothelin-1 (ET-1; David et al., 2001).

Furthermore, estrogen can interfere with ion channels through non-genomic actions. It regulates contractile responses by a direct modulation of Ca^{2+} mobilization into the vascular smooth muscle cells. Direct interaction of estradiol with voltage-gated Maxi-K channel subunit beta, which confers higher Ca^{2+} sensitivity, may modulate vascular smooth muscle (Valverde et al., 1999). Estrogen does not inhibit Ca^{2+} release from the intracellular stores (Crews and Khalil, 1999; Murphy and Khalil, 1999). However, supraphysiological concentrations of estrogen impede Ca^{2+} influx from the extracellular space (Han et al., 1995; Crews and Khalil, 1999; Murphy and Khalil, 1999) by inhibiting Ca^{2+} entry through voltage-gated Ca^{2+} channels (Freay et al., 1997; Kitazawa et al., 1997; Crews and Khalil, 1999; Murphy and Khalil, 1999). Expression of the L-type Ca^{2+} channels in cardiac muscle is substantially increased in ER-deficient mice (Johnson et al., 1997), suggesting ER-mediated regulation of Ca^{2+} mobilization.

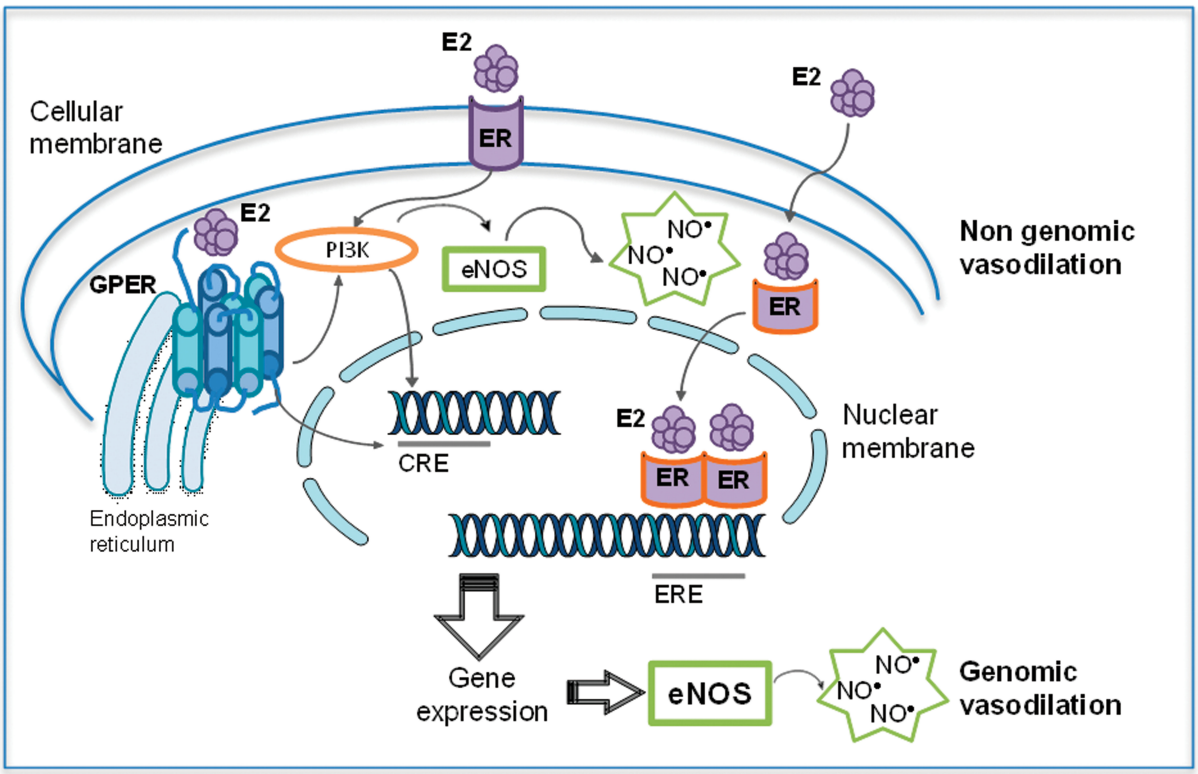

FIGURE 1 | Dual effects of estradiol (E2) on eNOS expression and activity. Estradiol effects on eNOS-mediated nitric oxide (NO) production include both genomic and non-genomic effects. Genomic effects include the classical intracellular estrogen receptors (ER), which after binding of E2 interact with estrogen-response element (ERE) in DNA, resulting in an increased eNOS expression. Moreover, E2 binding to GPER leads to activation of different transcriptional factors such as cAMP response element (CRE) which also induces eNOS expression. Among non-genomic effects, ER and GPER regulate the E2-induced eNOS activity (modified from Sobrino, 2011).

Estrogen also exerts direct modulation on the components of the renin-angiotensin system (RAS), a key regulator of blood pressure and smooth muscle cell growth. Production of angiotensin II (Ang II), the active hormone of the RAS, is reduced by estrogen inhibition of angiotensin-converting enzyme (ACE) expression. In animal models of menopause and in postmenopausal women, chronic estrogen replacement reduces ACE activity in the circulation and in tissues including the kidney and aorta (Brosnihan et al., 1999; Seely et al., 2004). Furthermore, estrogen attenuates expression of and tissue response to type 1 angiotensin receptor (AT_1) in the aorta, heart, and kidney (Silva-Antonialli et al., 2000; Wu et al., 2003).

THE PROCESS OF VASCULAR AGING: HOW ARE FEMALES AFFECTED?

Vascular aging is associated with endothelial dysfunction, arterial stiffening and remodeling, impaired angiogenesis, defective vascular repair, and an increasing prevalence of atherosclerosis (Lakatta and Levy, 2003; Erusalimsky, 2009). Aging-associated changes in structure and function of large elastic arteries are seen even in the absence of clinical CVD (Moreau et al., 2003). Although aging *per se* has detrimental effects in the vasculature, the lack of estrogen due to menopause may add an aggravating CVD risk factor in women, compared to arterial aging in men. In middle-aged females, aging-associated vascular dysfunction is potentiated by lack of estrogen due to menopause or ovariectomy and improves with estrogen replacement (Harman, 2004; Stice et al., 2009; Novella et al., 2010). Unfortunately, the onset of menopause coincides with a time when aging-associated damage may be noted, making it particularly difficult to distinguish between the contributions of aging and the lack of estrogen.

Vascular aging is a natural phenomenon that could be simply described as a consequence of physical stress, beginning early in life. Arteries are elastic tissues, susceptible to fatigue, and fracture over time as a consequence of extension-relaxation cycles during heartbeats (Avolio et al., 1983). In cross-sectional studies, postmenopausal females taking HRT have less arterial stiffness than their non-treated peers (Moreau et al., 2003; Sumino et al., 2005, 2006). Radial artery distensibility fluctuates in accordance with estrogen levels during menstrual cycles (Giannattasio et al., 1999). Basic research using animal models of estrogen withdrawal and aging suggests a modulatory role for estrogen in the molecular mechanisms to prevent arterial stiffening (Zhang et al., 2000). A recent study reported that HRT improves arterial compliance, an effect related in part to estrogen actions in the control of endothelial-dependent vasodilatory tone (Moreau et al., 2012). Collagen and elastin content of arterial walls is a key factor in arterial thickening and stiffening. It is mostly regulated by matrix

metalloproteinases (MMP), enzymes capable of degrading components of the extracellular matrix. During aging, MMP activity decreases markedly, collagen accumulates, and stiffening increases. Estrogen replacement in ovariectomized rats increases MMP activity and restores aged arteries to structural properties similar to those of younger animals studied (Zhang et al., 2000).

Aging is also associated with biochemical changes implicated in CVD development and progression. Dysfunction of both endothelial and smooth muscle molecular signaling appears during the aging process and favors vasospasm, thrombosis, inflammation, and abnormal cell migration and proliferation (Lakatta and Levy, 2003; Briones et al., 2005; Barton, 2010; Herrera et al., 2010). Endothelial dysfunction in the elderly has been associated with malfunctioning of vascular tissue, resulting in atherosclerosis, hypertension, and coronary artery disease (Lakatta and Levy, 2003; Herrera et al., 2010), renal dysfunction (Schmidt et al., 2001; Erdely et al., 2003), and Alzheimer disease (Price et al., 2004). In women, a slight age-related decrease in endothelium-dependent relaxation persists until middle age (around 50 years). After that, the declining response to the endothelium-dependent vasodilator hastens, even exceeding the rate experienced by men (Taddei et al., 1996).

The mechanisms for age-associated endothelial dysfunction are multiple, although most are associated with decreased NO bioavailability (Hayashi et al., 2008; Santhanam et al., 2008; Erusalimsky, 2009; Kim et al., 2009). Reduced endothelium-dependent and NO-mediated vasodilation has been described in both human and animal models of aging (Kim et al., 2009; Virdis et al., 2010). Lower NO production in the elderly may be based on decreased NO synthesis or increased NO degradation. Suggested mechanisms to explain reduced NO production include: (1) decreased expression of eNOS (Briones et al., 2005; Yoon et al., 2010); (2) a lack of NO precursor (L-arginine; Santhanam et al., 2008) and eNOS cofactor tetrahydrobiopterin (BH_4; Yoshida et al., 2000; Eskurza et al., 2005; Meyer et al., 2011); and (3) increased endogenous eNOS inhibitor ADMA (Xiong et al., 2001; Kielstein et al., 2003). On the other hand, strong evidences support the hypothesis that age-associated increase in oxidative stress and consequent production of O_2^- is a potent contributor to lower NO bioavailability and increased endothelial dysfunction (Jacobson et al., 2007; Rodriguez-Manas et al., 2009). There is little information to correlate the progression of aging with the production/degradation of NO in women. Although several studies have described decreased expression of eNOS in senile female rats and mice (Wynne et al., 2004; Novensa et al., 2011), aging-associated effects on eNOS in women can be easily confounded with the effects of lack of estrogen, since most of these studies grouped women into just two time-points: premenopausal and menopausal groups.

Even though the decline in NO bioavailability could sufficiently explain most of the changes in the functioning of vascular cells, other molecules crucial to control of vascular function are also modified by aging. In the regulation of vascular tone, COX-derived factors are particularly important as they can induce both vascular relaxation (PGI_2) and contraction (TXA_2 and PGH_2). Some studies have reported a prevalence in the production of relaxing COX factors in the vasculature of young and healthy individuals (Tang and Vanhoutte, 2008). With aging, COX-dependent vasoconstrictors production becomes evident, leading to increased vascular contraction (Taddei et al., 1997; Rodriguez-Manas et al., 2009). However, the COX isoform involved in the generation of contractile prostanoids remains unclear. In functional studies developed in femoral arteries of aged rats, oxygen free radicals participate in the augmented endothelium-dependent contractions mediated by COX-derived prostanoids. Both the constitutive and inducible isoforms of COX contribute to this endothelial dysfunction (Shi et al., 2008). Molecular studies performed in endothelial cells from aged rats showed an increase in mRNA levels of COX-1, COX-2, and other enzymes involved in the synthesis of prostanoids (Tang and Vanhoutte, 2008), demonstrating the importance of the arachidonic acid–COX cascade in the endothelial and vascular dysfunction associated with aging. Moreover, functional studies have demonstrated an interaction between NO and prostanoids pathways. In aorta from aged female mice, NO bioavailability increases when the COX pathway is inhibited; both gene and protein expression of COX-1 are increased (Novella et al., 2011). Furthermore, activation of inflammatory pathways in the vascular wall plays a central role in the process of vascular aging.

Even in the absence of traditional risk factors for atherosclerosis, an age-associated shift to a proinflammatory gene expression profile, known as endothelial activation, induces upregulation of cellular adhesion molecules and cytokines, which increases endothelial–leukocyte interactions and permeability, mechanisms considered crucial to initial steps in the development of atherosclerosis (Herrera et al., 2010; Seals et al., 2011). Accordingly, a sex-associated difference in inflammatory responses during aging has been proposed. Inflammatory atherosclerosis and associated acute coronary heart disease develop earlier in life in men than in women (Roger et al., 2011) and are associated with earlier death, although men and women present the same overall plaque burden (Frink, 2009). In animal models of atherosclerosis, male sex contributes to a faster and more severe progression of lipid deposition, remodeling, and aortic lesions (Pereira et al., 2010; Surra et al., 2010).

COULD ESTROGEN DECREASE VASCULAR AGING IN WOMEN?

With the wide-ranging data from experimental research, estrogens might appear to promise protection against the progression of vascular aging and CVD in women. Epidemiological observational studies also suggest that postmenopausal women on HRT are less likely to develop CVD than non-users at the same age (Grodstein et al., 2000, 2001) Nevertheless, these studies contrast with the large prospective clinical trials, HERS and WHI, which failed to show reduced cardiovascular events in postmenopausal women on HRT. In fact, WHI suggested that HRT was associated with increased risk to the cardiovascular system (Rossouw et al., 2002). Possible reasons for this discrepancy have been extensively discussed and include the average age of women entering most HRT clinical trials, 65 years and older, which results in a study population with some degree of aging-associated vascular damage. In addition, participants had been estrogen-deficient for an average of 10 years before starting HRT, a relatively late start that could modify the status of ERs and molecular signaling so as to attenuate the benefits of estrogen. For instance, during aging ERs can undergo posttranslational modifications such as methylation,

which decreases their expression and activity. We recently reported that aging contributes to increased DNA methylation in female mice aorta, which could be associated with the decrease in the modulatory effects of estrogen (Novensa et al., 2011). A few clinical studies also provide evidence for aging-associated dysregulation of ER methylation and suggest that focal epigenetic changes in ER could contribute to decreased estrogen activity and to the development of atherosclerosis in elderly women (Post et al., 1999; Kim et al., 2007).

Detailed examination of WHI data reveals that early initiation of estrogen replacement produces more favorable results than the later average initiation employed in the WHI studies overall (Grodstein et al., 2006; Rossouw et al., 2007; Prentice et al., 2009). These findings, together with observational studies, have led scientists to create the so-called "timing hypothesis" that estrogen-mediated benefits to prevent CVD only occur when treatment is initiated before the detrimental effects of aging are established on vascular walls (Harman, 2006). These effects include endothelial dysfunction and pathophysiological actions, such as increased vascular calcification and generalized stiffening of the arterial tree that increase the prevalence of hypertension and atherosclerosis (Lakatta and Levy, 2003; Erusalimsky, 2009; Kovacic et al., 2011).

Little information is available on whether and how vascular effects of estrogen are modified with aging in females. Aging has been associated with significant reductions in the direct estrogen-mediated mechanisms of vascular relaxation (Wynne et al., 2004; LeBlanc et al., 2009; Lekontseva et al., 2010) and inflammation (Pechenino et al., 2011). The lack of estrogen responses in these animal studies was not related to age-associated changes in the plasma levels of estrogen or activity of ER, but rather to possible age-related changes in estrogen-mediated signaling pathways in the vasculature. Modifications in the ratio between ERα and ERβ in older female mice are associated with the lack of protective effects of estrogen on NO production and with a reversal in its antioxidant effect to a pro-oxidant profile (Novensa et al., 2011). Moreover, clinical studies have revealed that CVD risk factors in postmenopausal women were lower among women aged 50–59 years at HRT initiation (Manson et al., 2007; Sherwood et al., 2007). These studies clearly establish the complexity of estrogen effects, which may be influenced by pathophysiological conditions including aging and subclinical CVD. Despite convincing arguments by the followers of the "timing hypothesis" the potential extrapolation of the protective effects of estrogen replacement, well described in young females, to older women remains controversial. The field still lacks detailed experimental and clinical research on the long-term effects of estrogen and how it modulates cardiovascular function during aging.

CONCLUSION AND FUTURE DIRECTION

Our society is aging progressively, and increased life expectancy enhances the risks for diseases associated with the natural fatigue of the body, including CVD. Despite this undeniable reality, there is evidence that vascular aging in women does not follow the same chronology as in men. The vascular protective effects exerted by estrogens have been proposed as the major reason for reduced signs of vascular aging and CVD risk in premenopausal women, compared to men. When natural estrogen withdrawal occurs and a woman enters her climacteric stage, effects of sudden vascular aging become evident, leading to vascular dysfunction and increased risk of a cardiovascular event. The lack of crucial information from clinical trials and the discrepancies in the available data on the regulation of the female cardiovascular system can lead to inappropriate diagnosis and treatment of CVD in older women. Women have been treated like men, despite the notable sex-associated differences in the elements of aging and disease processes. Much research effort is still needed to understand age- and sex-related differences in cardiovascular control, establish the impact of the menstrual cycle and HRT on vascular function, and propose new therapeutic strategies to improve CVD diagnosis and treatment and the overall management of vascular senescence in women.

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REFERENCES

Alexander, S. P., Mathie, A., and Peters, J. A. (2008). Guide to receptors and channels (GRAC), 3rd edition. *Br. J. Pharmacol.* 153(Suppl. 2), S1–S209.

Arnal, J. F., Clamens, S., Pechet, C., Negre-Salvayre, A., Allera, C., Girolami, J. P., Salvayre, R., and Bayard, F. (1996). Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. *Proc. Natl. Acad. Sci. U.S.A.* 93, 4108–4113.

Avolio, A. P., Chen, S. G., Wang, R. P., Zhang, C. L., Li, M. F., and O'Rourke, M. F. (1983). Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 68, 50–58.

Bairey Merz, C. N., Shaw, L. J., Reis, S. E., Bittner, V., Kelsey, S. F., Olson, M., Johnson, B. D., Pepine, C. J., Mankad, S., Sharaf, B. L., Rogers, W. J., Pohost, G. M., Lerman, A., Quyyumi, A. A., and Sopko, G. (2006). Insights from the NHLBI-sponsored women's ischemia syndrome evaluation (WISE) study: part II: gender differences in presentation, diagnosis, and outcome with regard to gender-based pathophysiology of atherosclerosis and macrovascular and microvascular coronary disease. *J. Am. Coll. Cardiol.* 47, S21–S29.

Barton, M. (2010). Obesity and aging: determinants of endothelial cell dysfunction and atherosclerosis. *Pflugers Arch.* 460, 825–837.

Beato, M., Herrlich, P., and Schutz, G. (1995). Steroid hormone receptors: many actors in search of a plot. *Cell* 83, 851–857.

Briones, A. M., Montoya, N., Giraldo, J., and Vila, E. (2005). Ageing affects nitric oxide synthase, cyclooxygenase and oxidative stress enzymes expression differently in mesenteric resistance arteries. *Auton. Autacoid Pharmacol.* 25, 155–162.

Brosnihan, K. B., Senanayake, P. S., Li, P., and Ferrario, C. M. (1999). Bi-directional actions of estrogen on the renin-angiotensin system. *Braz. J. Med. Biol. Res.* 32, 373–381.

Cano, A., and Hermenegildo, C. (2000). Modulation of the oestrogen receptor: a process with distinct susceptible steps. *Hum. Reprod. Update* 6, 207–211.

Capel, I. D., Jenner, M., Williams, D. C., Donaldson, D., and Nath, A. (1981). The effect of prolonged oral contraceptive steroid use on erythrocyte glutathione peroxidase activity. *J. Steroid Biochem.* 14, 729–732.

Crews, J. K., and Khalil, R. A. (1999). Antagonistic effects of 17b-estradiol, progesterone, and testosterone on Ca^{2+} entry mechanisms of coronary vasoconstriction. *Arterioscler. Thromb. Vasc. Biol.* 19, 1034–1040.

Dantas, A. P., Scivoletto, R., Fortes, Z. B., Nigro, D., and Carvalho, M. H. (1999). Influence of female sex hormones on endothelium-derived vasoconstrictor prostanoid generation in microvessels of spontaneously hypertensive rats. *Hypertension* 34, 914–919.

Dantas, A. P., Tostes, R. C., Fortes, Z. B., Costa, S. G., Nigro, D., and Carvalho, M. H. (2002). In vivo evidence for antioxidant potential of estrogen in microvessels of female spontaneously hypertensive rats. *Hypertension* 39, 405–411.

David, F. L., Carvalho, M. H., Cobra, A. L., Nigro, D., Fortes, Z. B., Reboucas, N. A., and Tostes, R. C. (2001). Ovarian hormones modulate endothelin-1 vascular reactivity and mRNA expression in DOCA-salt hypertensive rats. *Hypertension* 38, 692–696.

Davidge, S. T., and Zhang, Y. (1998). Estrogen replacement suppresses a prostaglandin H synthase-dependent vasoconstrictor in rat mesenteric arteries. *Circ. Res.* 83, 388–395.

Deschamps, A. M., and Murphy, E. (2009). Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am. J. Physiol. Heart Circ. Physiol.* 297, H1806–H1813.

Dubey, R. K., and Jackson, E. K. (2001). Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. *Am. J. Physiol. Renal Physiol.* 280, F365–F388.

Dudzinski, D. M., and Michel, T. (2007). Life history of eNOS: partners and pathways. *Cardiovasc. Res.* 75, 247–260.

Eaker, E. D., Chesebro, J. H., Sacks, F. M., Wenger, N. K., Whisnant, J. P., and Winston, M. (1993). Cardiovascular disease in women. *Circulation* 88, 1999–2009.

Erdely, A., Greenfeld, Z., Wagner, L., and Baylis, C. (2003). Sexual dimorphism in the aging kidney: effects on injury and nitric oxide system. *Kidney Int.* 63, 1021–1026.

Erusalimsky, J. D. (2009). Vascular endothelial senescence: from mechanisms to pathophysiology. *J. Appl. Physiol.* 106, 326–332.

Eskurza, I., Myerburgh, L. A., Kahn, Z. D., and Seals, D. R. (2005). Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *J. Physiol.* 568, 1057–1065.

Freay, A. D., Curtis, S. W., Korach, K. S., and Rubanyi, G. M. (1997). Mechanism of vascular smooth muscle relaxation by estrogen in depolarized rat and mouse aorta. Role of nuclear estrogen receptor and Ca^{2+} uptake. *Circ. Res.* 81, 242–248.

Frink, R. J. (2009). Gender gap, inflammation and acute coronary disease: are women resistant to atheroma growth? Observations at autopsy. *J. Invasive Cardiol.* 21, 270–277.

Gambacciani, M., Rosano, G. M., Monteleone, P., Fini, M., and Genazzani, A. R. (2002). Clinical relevance of the HERS trial. *Lancet* 360, 641.

Giannattasio, C., Failla, M., Grappiolo, A., Stella, M. L., Del, B. A., Colombo, M., and Mancia, G. (1999). Fluctuations of radial artery distensibility throughout the menstrual cycle. *Arterioscler. Thromb. Vasc. Biol.* 19, 1925–1929.

Golding, E. M., and Kepler, T. E. (2001). Role of estrogen in modulating EDHF-mediated dilations in the female rat middle cerebral artery. *Am. J. Physiol. Heart Circ. Physiol.* 280, H2417–H2423.

Grodstein, F., Manson, J. E., Colditz, G. A., Willett, W. C., Speizer, F. E., and Stampfer, M. J. (2000). A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann. Intern. Med.* 133, 933–941.

Grodstein, F., Manson, J. E., and Stampfer, M. J. (2001). Postmenopausal hormone use and secondary prevention of coronary events in the nurses' health study. A prospective, observational study. *Ann. Intern. Med.* 135, 1–8.

Grodstein, F., Manson, J. E., and Stampfer, M. J. (2006). Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. *J. Womens Health (Larchmt.)* 15, 35–44.

Haas, E., Meyer, M. R., Schurr, U., Bhattacharya, I., Minotti, R., Nguyen, H. H., Heigl, A., Lachat, M., Genoni, M., and Barton, M. (2007). Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension* 49, 1358–1363.

Halliwell, B., and Grootveld, M. (1987). The measurement of free radical reactions in humans. Some thoughts for future experimentation. *FEBS Lett.* 213, 9–14.

Han, S. Z., Karaki, H., Ouchi, Y., Akishita, M., and Orimo, H. (1995). 17b-Estradiol inhibits Ca^{2+} influx and Ca^{2+} release induced by thromboxane A2 in porcine coronary artery. *Circulation* 91, 2619–2626.

Harman, S. M. (2004). What do hormones have to do with aging? What does aging have to do with hormones? *Ann. N. Y. Acad. Sci.* 1019, 299–308.

Harman, S. M. (2006). Estrogen replacement in menopausal women: recent and current prospective studies, the WHI and the KEEPS. *Gend. Med.* 3, 254–269.

Hayashi, T., Yano, K., Matsui-Hirai, H., Yokoo, H., Hattori, Y., and Iguchi, A. (2008). Nitric oxide and endothelial cellular senescence. *Pharmacol. Ther.* 120, 333–339.

Hermenegildo, C., Garcia-Martinez, M. C., Tarin, J. J., and Cano, A. (2002). Inhibition of low-density lipoprotein oxidation by the pure antiestrogens ICI 182780 and EM-652 (SCH 57068). *Menopause* 9, 430–435.

Herrera, M. D., Mingorance, C., Rodriguez-Rodriguez, R., and Alvarez de Sotomayor, M. (2010). Endothelial dysfunction and aging: an update. *Ageing Res. Rev.* 9, 142–152.

Hisamoto, K., Ohmichi, M., Kurachi, H., Hayakawa, J., Kanda, Y., Nishio, Y., Adachi, K., Tasaka, K., Miyoshi, E., Fujiwara, N., Taniguchi, N., and Murata, Y. (2001). Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. *J. Biol. Chem.* 276, 3459–3467.

Huang, A., Sun, D., Kaley, G., and Koller, A. (1997). Estrogen maintains nitric oxide synthesis in arterioles of female hypertensive rats. *Hypertension* 29, 1351–1356.

Jacobson, A., Yan, C., Gao, Q., Rincon-Skinner, T., Rivera, A., Edwards, J., Huang, A., Kaley, G., and Sun, D. (2007). Aging enhances pressure-induced arterial superoxide formation. *Am. J. Physiol. Heart Circ. Physiol.* 293, H1344–H1350.

Johnson, B. D., Zheng, W., Korach, K. S., Scheuer, T., Catterall, W. A., and Rubanyi, G. M. (1997). Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. *J. Gen. Physiol.* 110, 135–140.

Karas, R. H., Patterson, B. L., and Mendelsohn, M. E. (1994). Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation* 89, 1943–1950.

Kielstein, J. T., Bode-Boger, S. M., Frolich, J. C., Ritz, E., Haller, H., and Fliser, D. (2003). Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 107, 1891–1895.

Kim, J., Kim, J. Y., Song, K. S., Lee, Y. H., Seo, J. S., Jelinek, J., Goldschmidt-Clermont, P. J., and Issa, J. P. (2007). Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and in-vitro vascular senescence. *Biochim. Biophys. Acta* 1772, 72–80.

Kim, J. H., Bugaj, L. J., Oh, Y. J., Bivalacqua, T. J., Ryoo, S., Soucy, K. G., Santhanam, L., Webb, A., Camara, A., Sikka, G., Nyhan, D., Shoukas, A. A., Ilies, M., Christianson, D. W., Champion, H. C., and Berkowitz, D. E. (2009). Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. *J. Appl. Physiol.* 107, 1249–1257.

Kim, Y. D., Chen, B., Beauregard, J., Kouretas, P., Thomas, G., Farhat, M. Y., Myers, A. K., and Lees, D. E. (1996). 17b-Estradiol prevents dysfunction of canine coronary endothelium and myocardium and reperfusion arrhythmias after brief ischemia/reperfusion. *Circulation* 94, 2901–2908.

Kim-Schulze, S., McGowan, K. A., Hubchak, S. C., Cid, M. C., Martin, M. B., Kleinman, H. K., Greene, G. L., and Schnaper, H. W. (1996). Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. *Circulation* 94, 1402–1407.

Kitazawa, T., Hamada, E., Kitazawa, K., and Gaznabi, A. K. (1997). Non-genomic mechanism of 17b-oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. *J. Physiol.* 499, 497–511.

Kovacic, J. C., Moreno, P., Nabel, E. G., Hachinski, V., and Fuster, V. (2011). Cellular senescence, vascular disease, and aging: part 2 of a 2-part review: clinical vascular disease in the elderly. *Circulation* 123, 1900–1910.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J. A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. U.S.A.* 93, 5925–5930.

Lakatta, E. G., and Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. *Circulation* 107, 139–146.

Lamas, S., Lowenstein, C. J., and Michel, T. (2007). Nitric oxide signaling

comes of age: 20 years and thriving. *Cardiovasc. Res.* 75, 207–209.

LeBlanc, A. J., Reyes, R., Kang, L. S., Dailey, R. A., Stallone, J. N., Moningka, N. C., and Muller-Delp, J. M. (2009). Estrogen replacement restores flow-induced vasodilation in coronary arterioles of aged and ovariectomized rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R1713–R1723.

Lekontseva, O. N., Rueda-Clausen, C. F., Morton, J. S., and Davidge, S. T. (2010). Ovariectomy in aged versus young rats augments matrix metalloproteinase-mediated vasoconstriction in mesenteric arteries. *Menopause* 17, 516–523.

Lerner, D. J., and Kannel, W. B. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. *Am. Heart J.* 111, 383–390.

Manson, J. E., Allison, M. A., Rossouw, J. E., Carr, J. J., Langer, R. D., Hsia, J., Kuller, L. H., Cochrane, B. B., Hunt, J. R., Ludlam, S. E., Pettinger, M. B., Gass, M., Margolis, K. L., Nathan, L., Ockene, J. K., Prentice, R. L., Robbins, J., and Stefanick, M. L. (2007). Estrogen therapy and coronary-artery calcification. *N. Engl. J. Med.* 356, 2591–2602.

Messerli, F. H., Garavaglia, G. E., Schmieder, R. E., Sundgaard-Riise, K., Nunez, B. D., and Amodeo, C. (1987). Disparate cardiovascular findings in men and women with essential hypertension. *Ann. Intern. Med.* 107, 158–161.

Meyer, M. R., Prossnitz, E. R., and Barton, M. (2011). The G protein-coupled estrogen receptor GPER/GPR30 as a regulator of cardiovascular function. *Vascul. Pharmacol.* 55, 17–25.

Michel, T., and Vanhoutte, P. M. (2010). Cellular signaling and NO production. *Pflugers Arch.* 459, 807–816.

Miller, V. M., and Duckles, S. P. (2008). Vascular actions of estrogens: functional implications. *Pharmacol. Rev.* 60, 210–241.

Monsalve, E., Oviedo, P. J., Garcia-Perez, M. A., Tarin, J. J., Cano, A., and Hermenegildo, C. (2007). Estradiol counteracts oxidized LDL-induced asymmetric dimethylarginine production by cultured human endothelial cells. *Cardiovasc. Res.* 73, 66–72.

Moreau, K. L., Donato, A. J., Seals, D. R., DeSouza, C. A., and Tanaka, H. (2003). Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc. Res.* 57, 861–868.

Moreau, K. L., Meditz, A., Deane, K., and Kohrt, W. M. (2012). Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. *Am. J. Physiol. Heart Circ. Physiol.* 302, H1211–H1218.

Murphy, E. (2011). Estrogen signaling and cardiovascular disease. *Circ. Res.* 109, 687–696.

Murphy, J. G., and Khalil, R. A. (1999). Decreased $[Ca^{2+}]$i during inhibition of coronary smooth muscle contraction by 17b-estradiol, progesterone, and testosterone. *J. Pharmacol. Exp. Ther.* 291, 44–52.

Nakajima, T., Kitazawa, T., Hamada, E., Hazama, H., Omata, M., and Kurachi, Y. (1995). 17b-Estradiol inhibits the voltage-dependent L-type Ca^{2+} currents in aortic smooth muscle cells. *Eur. J. Pharmacol.* 294, 625–635.

Novella, S., Dantas, A. P., Segarra, G., Novensa, L., Bueno, C., Heras, M., Hermenegildo, C., and Medina, P. (2010). Gathering of aging and estrogen withdrawal in vascular dysfunction of senescent accelerated mice. *Exp. Gerontol.* 45, 868–874.

Novella, S., Dantas, A. P., Segarra, G., Novensa, L., Heras, M., Hermenegildo, C., and Medina, P. (2011). Aging enhances contraction to thromboxane A(2) in aorta from female senescence-accelerated mice. *Age (Dordr.).* (in press).

Novensa, L., Novella, S., Medina, P., Segarra, G., Castillo, N., Heras, M., Hermenegildo, C., and Dantas, A. P. (2011). Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERalpha/ERbeta balance in female mice. *PLoS ONE* 6, e25335. doi:10.1371/journal.pone.0025335

Ospina, J. A., Krause, D. N., and Duckles, S. P. (2002). 17b-estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke* 33, 600–605.

Pechenino, A. S., Lin, L., Mbai, F. N., Lee, A. R., He, X. M., Stallone, J. N., and Knowlton, A. A. (2011). Impact of aging vs. estrogen loss on cardiac gene expression: estrogen replacement and inflammation. *Physiol. Genomics* 43, 1065–1073.

Pereira, T. M., Nogueira, B. V., Lima, L. C., Porto, M. L., Arruda, J. A., Vasquez, E. C., and Meyrelles, S. S. (2010). Cardiac and vascular changes in elderly atherosclerotic mice: the influence of gender. *Lipids Health Dis.* 9, 87.

Post, W. S., Goldschmidt-Clermont, P. J., Wilhide, C. C., Heldman, A. W., Sussman, M. S., Ouyang, P., Milliken, E. E., and Issa, J. P. (1999). Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovasc. Res.* 43, 985–991.

Prakash, Y. S., Togaibayeva, A. A., Kannan, M. S., Miller, V. M., Fitzpatrick, L. A., and Sieck, G. C. (1999). Estrogen increases Ca^{2+} efflux from female porcine coronary arterial smooth muscle. *Am. J. Physiol.* 276, H926–H934.

Prentice, R. L., Manson, J. E., Langer, R. D., Anderson, G. L., Pettinger, M., Jackson, R. D., Johnson, K. C., Kuller, L. H., Lane, D. S., Wactawski-Wende, J., Brzyski, R., Allison, M., Ockene, J., Sarto, G., and Rossouw, J. E. (2009). Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. *Am. J. Epidemiol.* 170, 12–23.

Price, J. M., Hellermann, A., Hellermann, G., and Sutton, E. T. (2004). Aging enhances vascular dysfunction induced by the Alzheimer's peptide beta-amyloid. *Neurol. Res.* 26, 305–311.

Prossnitz, E. R., and Barton, M. (2011). The G-protein-coupled estrogen receptor GPER in health and disease. *Nat. Rev. Endocrinol.* 7, 715–726.

Register, T. C., and Adams, M. R. (1998). Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. *J. Steroid Biochem. Mol. Biol.* 64, 187–191.

Robb, E. L., and Stuart, J. A. (2011). Resveratrol interacts with estrogen receptor-beta to inhibit cell replicative growth and enhance stress resistance by upregulating mitochondrial superoxide dismutase. *Free Radic. Biol. Med.* 50, 821–831.

Rodriguez-Manas, L., El-Assar, M., Vallejo, S., Lopez-Doriga, P., Solis, J., Petidier, R., Montes, M., Nevado, J., Castro, M., Gomez-Guerrero, C., Peiro, C., and Sanchez-Ferrer, C. F. (2009). Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell* 8, 226–238.

Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Adams, R. J., Berry, J. D., Brown, T. M., Carnethon, M. R., Dai, S., de, S. G., Ford, E. S., Fox, C. S., Fullerton, H. J., Gillespie, C., Greenlund, K. J., Hailpern, S. M., Heit, J. A., Ho, P. M., Howard, V. J., Kissela, B. M., Kittner, S. J., Lackland, D. T., Lichtman, J. H., Lisabeth, L. D., Makuc, D. M., Marcus, G. M., Marelli, A., Matchar, D. B., McDermott, M. M., Meigs, J. B., Moy, C. S., Mozaffarian, D., Mussolino, M. E., Nichol, G., Paynter, N. P., Rosamond, W. D., Sorlie, P. D., Stafford, R. S., Turan, T. N., Turner, M. B., Wong, N. D., and Wylie-Rosett, J. (2011). Heart disease and stroke statistics – 2011 update: a report from the American Heart Association. *Circulation* 123, e18–e209.

Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S. M., Ho, M., Howard, V., Kissela, B., Kittner, S., Lloyd-Jones, D., McDermott, M., Meigs, J., Moy, C., Nichol, G., O'Donnell, C., Roger, V., Sorlie, P., Steinberger, J., Thom, T., Wilson, M., and Hong, Y. (2008). Heart disease and stroke statistics – 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 117, e25–e146.

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., Jackson, R. D., Beresford, S. A., Howard, B. V., Johnson, K. C., Kotchen, J. M., and Ockene, J. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA* 288, 321–333.

Rossouw, J. E., Prentice, R. L., Manson, J. E., Wu, L., Barad, D., Barnabei, V. M., Ko, M., LaCroix, A. Z., Margolis, K. L., and Stefanick, M. L. (2007). Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* 297, 1465–1477.

Rubio-Gayosso, I., Sierra-Ramirez, A., Garcia-Vazquez, A., Martinez-Martinez, A., Munoz-Garcia, O., Morato, T., and Ceballos-Reyes, G. (2000). 17b-estradiol increases intracellular calcium concentration through a short-term and nongenomic mechanism in rat vascular endothelium in culture. *J. Cardiovasc. Pharmacol.* 36, 196–202.

Santhanam, L., Christianson, D. W., Nyhan, D., and Berkowitz, D. E. (2008). Arginase and vascular aging. *J. Appl. Physiol.* 105, 1632–1642.

Schmidt, R. J., Beierwaltes, W. H., and Baylis, C. (2001). Effects of aging and alterations in dietary sodium intake on total nitric oxide production. *Am. J. Kidney Dis.* 37, 900–908.

Seals, D. R., Jablonski, K. L., and Donato, A. J. (2011). Aging and vascular

endothelial function in humans. *Clin. Sci.* 120, 357–375.

Seely, E. W., Brosnihan, K. B., Jeunemaitre, X., Okamura, K., Williams, G. H., Hollenberg, N. K., and Herrington, D. M. (2004). Effects of conjugated oestrogen and droloxifene on the renin-angiotensin system, blood pressure and renal blood flow in postmenopausal women. *Clin. Endocrinol. (Oxf.)* 60, 315–321.

Shaw, L. J., Bairey Merz, C. N., Pepine, C. J., Reis, S. E., Bittner, V., Kelsey, S. F., Olson, M., Johnson, B. D., Mankad, S., Sharaf, B. L., Rogers, W. J., Wessel, T. R., Arant, C. B., Pohost, G. M., Lerman, A., Quyyumi, A. A., and Sopko, G. (2006). Insights from the NHLBI-sponsored women's ischemia syndrome evaluation (WISE) study: part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. *J. Am. Coll. Cardiol.* 47, S4–S20.

Sherwood, A., Bower, J. K., McFetridge-Durdle, J., Blumenthal, J. A., Newby, L. K., and Hinderliter, A. L. (2007). Age moderates the short-term effects of transdermal 17b-estradiol on endothelium-dependent vascular function in postmenopausal women. *Arterioscler. Thromb. Vasc. Biol.* 27, 1782–1787.

Shi, Y., Man, R. Y., and Vanhoutte, P. M. (2008). Two isoforms of cyclooxygenase contribute to augmented endothelium-dependent contractions in femoral arteries of 1-year-old rats. *Acta Pharmacol. Sin.* 29, 185–192.

Silva-Antonialli, M. M., Fortes, Z. B., Carvalho, M. H., Scivoletto, R., and Nigro, D. (2000). Sexual dimorphism in the response of thoracic aorta from SHRs to losartan. *Gen. Pharmacol.* 34, 329–335.

Sivritas, D., Becher, M. U., Ebrahimian, T., Arfa, O., Rapp, S., Bohner, A., Mueller, C. F., Umemura, T., Wassmann, S., Nickenig, G., and Wassmann, K. (2011). Antiproliferative effect of estrogen in vascular smooth muscle cells is mediated by Kruppel-like factor-4 and manganese superoxide dismutase. *Basic Res. Cardiol.* 106, 563–575.

Sobrino, A. (2011). *New Expression Pathways Regulated by Estradiol in Human Endothelial Cells.* Doctoral Thesis. University of Valencia.

Sobrino, A., Mata, M., Laguna-Fernandez, A., Novella, S., Oviedo, P. J., Garcia-Perez, M. A., Tarin, J. J., Cano, A., and Hermenegildo, C. (2009). Estradiol stimulates vasodilatory and metabolic pathways in cultured human endothelial cells. *PLoS ONE* 4, e8242. doi:10.1371/journal.pone.0008242

Sobrino, A., Oviedo, P. J., Novella, S., Laguna-Fernandez, A., Bueno, C., Garcia-Perez, M. A., Tarin, J. J., Cano, A., and Hermenegildo, C. (2010). Estradiol selectively stimulates endothelial prostacyclin production through estrogen receptor-{alpha}. *J. Mol. Endocrinol.* 44, 237–246.

Soloff, M. S., and Szego, C. M. (1969). Purification of estradiol receptor from rat uterus and blockade of its estrogen-binding function by specific antibody. *Biochem. Biophys. Res. Commun.* 34, 141–147.

Stice, J. P., Eiserich, J. P., and Knowlton, A. A. (2009). Role of aging versus the loss of estrogens in the reduction in vascular function in female rats. *Endocrinology* 150, 212–219.

Sumi, D., and Ignarro, L. J. (2003). Estrogen-related receptor alpha 1 up-regulates endothelial nitric oxide synthase expression. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14451–14456.

Sumino, H., Ichikawa, S., Kasama, S., Kumakura, H., Takayama, Y., Sakamaki, T., and Kurabayashi, M. (2005). Effect of transdermal hormone replacement therapy on carotid artery wall thickness and levels of vascular inflammatory markers in postmenopausal women. *Hypertens. Res.* 28, 579–584.

Sumino, H., Ichikawa, S., Kasama, S., Takahashi, T., Kumakura, H., Takayama, Y., Kanda, T., and Kurabayashi, M. (2006). Different effects of oral conjugated estrogen and transdermal estradiol on arterial stiffness and vascular inflammatory markers in postmenopausal women. *Atherosclerosis* 189, 436–442.

Surra, J. C., Guillen, N., rbones-Mainar, J. M., Barranquero, C., Navarro, M. A., Arnal, C., Orman, I., Segovia, J. C., and Osada, J. (2010). Sex as a profound modifier of atherosclerotic lesion development in apolipoprotein E-deficient mice with different genetic backgrounds. *J. Atheroscler. Thromb.* 17, 712–721.

Taddei, S., Virdis, A., Ghiadoni, L., Magagna, A., and Salvetti, A. (1997). Cyclooxygenase inhibition restores nitric oxide activity in essential hypertension. *Hypertension* 29, 274–279.

Taddei, S., Virdis, A., Ghiadoni, L., Mattei, P., Sudano, I., Bernini, G., Pinto, S., and Salvetti, A. (1996). Menopause is associated with endothelial dysfunction in women. *Hypertension* 28, 576–582.

Takada, Y., Kato, C., Kondo, S., Korenaga, R., and Ando, J. (1997). Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochem. Biophys. Res. Commun.* 240, 737–741.

Tang, E. H., and Vanhoutte, P. M. (2008). Gene expression changes of prostanoid synthases in endothelial cells and prostanoid receptors in vascular smooth muscle cells caused by aging and hypertension. *Physiol. Genomics* 32, 409–418.

Toth, B., Scholz, C., Saadat, G., Geller, A., Schulze, S., Mylonas, I., Friese, K., and Jeschke, U. (2009). Estrogen receptor modulators and estrogen receptor beta immunolabelling in human umbilical vein endothelial cells. *Acta Histochem.* 111, 508–519.

Valverde, M. A., Rojas, P., Amigo, J., Cosmelli, D., Orio, P., Bahamonde, M. I., Mann, G. E., Vergara, C., and Latorre, R. (1999). Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. *Science* 285, 1929–1931.

Venkov, C. D., Rankin, A. B., and Vaughan, D. E. (1996). Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. *Circulation* 94, 727–733.

Virdis, A., Ghiadoni, L., Giannarelli, C., and Taddei, S. (2010). Endothelial dysfunction and vascular disease in later life. *Maturitas* 67, 20–24.

Wagner, A. H., Schroeter, M. R., and Hecker, M. (2001). 17beta-estradiol inhibition of NADPH oxidase expression in human endothelial cells. *FASEB J.* 15, 2121–2130.

Wassmann, S., Baumer, A. T., Strehlow, K., van Eickels, M., Grohe, C., Ahlbory, K., Rosen, R., Bohm, M., and Nickenig, G. (2001). Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation* 103, 435–441.

Wu, Z., Maric, C., Roesch, D. M., Zheng, W., Verbalis, J. G., and Sandberg, K. (2003). Estrogen regulates adrenal angiotensin AT1 receptors by modulating AT1 receptor translation. *Endocrinology* 144, 3251–3261.

Wynne, F. L., Payne, J. A., Cain, A. E., Reckelhoff, J. F., and Khalil, R. A. (2004). Age-related reduction in estrogen receptor-mediated mechanisms of vascular relaxation in female spontaneously hypertensive rats. *Hypertension* 43, 405–412.

Xiong, Y., Yuan, L. W., Deng, H. W., Li, Y. J., and Chen, B. M. (2001). Elevated serum endogenous inhibitor of nitric oxide synthase and endothelial dysfunction in aged rats. *Clin. Exp. Pharmacol. Physiol.* 28, 842–847.

Yoon, H. J., Cho, S. W., Ahn, B. W., and Yang, S. Y. (2010). Alterations in the activity and expression of endothelial NO synthase in aged human endothelial cells. *Mech. Ageing Dev.* 131, 119–123.

Yoshida, Y. I., Eda, S., and Masada, M. (2000). Alterations of tetrahydrobiopterin biosynthesis and pteridine levels in mouse tissues during growth and aging. *Brain Dev.* 22(Suppl. 1), S45–S49.

Zhang, F., Ram, J. L., Standley, P. R., and Sowers, J. R. (1994). 17b-Estradiol attenuates voltage-dependent Ca^{2+} currents in A7r5 vascular smooth muscle cell line. *Am. J. Physiol.* 266, C975–C980.

Zhang, Y., Stewart, K. G., and Davidge, S. T. (2000). Estrogen replacement reduces age-associated remodeling in rat mesenteric arteries. *Hypertension* 36, 970–974.

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Testosterone and vascular function in aging

Rhéure A. M. Lopes*, Karla B. Neves, Fernando S. Carneiro and Rita C. Tostes*

Department of Pharmacology, Medical School of Ribeirao Preto, Ribeirao Preto, São Paulo, Brazil

Edited by:
Ana Paula Dantas, Institut d'Investigacions Biomediques August Pi i Sunyer, Spain

Reviewed by:
Raghvendra K. Dubey, University Hospital Zurich, Switzerland
Juan Duarte, University of Granada, Spain

****Correspondence:***
Rhéure A. M. Lopes and Rita C. Tostes, Department of Pharmacology, Medical School of Ribeirao Preto, University of Sao Paulo, Av Bandeirantes 3900, Ribeirao Preto, São Paulo 14049-900, Brazil.
e-mail: rheurealves@usp.br; rtostes@usp.br

Androgen receptors are widely distributed in several tissues, including vascular endothelial and smooth muscle cells. Through classic cytosolic androgen receptors or membrane receptors, testosterone induces genomic and non-genomic effects, respectively. Testosterone interferes with the vascular function by increasing the production of pro-inflammatory cytokines and arterial thickness. Experimental evidence indicates that sex steroid hormones, such as testosterone modulate the synthesis and bioavailability of NO and, consequently, endothelial function, which is key for a healthy vasculature. Of interest, aging itself is accompanied by endothelial and vascular smooth muscle dysfunction. Aging-associated decline of testosterone levels is accompanied by age-related diseases, such as metabolic and cardiovascular diseases, indicating that very low levels of androgens may contribute to cardiovascular dysfunction observed in these age-related disorders or, in other words, that testosterone may have beneficial effects in the cardiovascular system. However, testosterone seems to play a negative role in the severity of renal disease. In this mini-review, we briefly comment on the interplay between aging and testosterone levels, the vascular actions of testosterone and its implications for vascular aging. Renal effects of testosterone and the use of testosterone to prevent vascular dysfunction in elderly are also addressed.

Keywords: testosterone, aging, vascular function, cardiovascular disease

INTRODUCTION

Sex hormones, including testosterone, have important extragonadal effects and experimental and clinical data point to important effects of sex hormones on the cardiovascular system (Reckelhoff, 2005). There is intense debate on the role of testosterone on cardiovascular function and cardiovascular disease (CVD). Whereas high doses of testosterone have been associated with sudden cardiac death and liver disease (Bagatell and Bremner, 1996), low levels are associated with the progression of atherosclerosis, production of pro-inflammatory cytokines, increased arterial thickness, increased levels of glucose, total cholesterol, and low-density lipoprotein, all important in CVD (Hak et al., 2002; Miller et al., 2004; Francomano et al., 2010).

Although reduced testosterone levels in aging men are intimately associated with several aspects of vascular injury, the male sex[1,2] is extensively mentioned as a risk factor for CVD, with males having an earlier and higher prevalence of many cardiovascular and metabolic-related diseases (Yang and Reckelhoff, 2011).

In this mini-review, we will briefly comment on the interplay between aging and testosterone levels, the vascular actions of testosterone and its implications for vascular aging. Renal effects of testosterone and the use of testosterone to prevent vascular dysfunction in elderly are also addressed.

BRIEF BIOCHEMISTRY OF TESTOSTERONE SYNTHESIS AND SIGNALING

More than 95% of testosterone, the principal male steroid hormone from the androgens group, is produced by the testes and secreted by the Leydig cells, after a series of enzymatic reactions in the cholesterol molecule. Small amounts of testosterone are also secreted by the zona reticularis of the adrenal glands (Sa et al., 2009). In men, normal levels of testosterone range from 10 to 30 nM, while much lower levels are found in women (0.6–2.5 nM; Burger, 2002). Testosterone plays a major role in the development of male reproductive tissues and is found in mammals, reptiles, birds, and other vertebrates. In men, testosterone also promotes secondary sexual characteristics, such as increased muscle, bone mass, and the growth of body hair (Mooradian et al., 1987). Although adult human males produce about 10 times more testosterone than the female counterparts, females are very sensitive to the hormone. In women testosterone is synthesized by the theca cells of the ovaries, by

[1]According to the Department of Reproductive Health and Research, World Health Organization (WHO), http://www.who.int/gender/whatisgender/en/), "Sex" refers to the biological and physiological characteristics that define men and women; "Gender" refers to the socially constructed roles, behaviors, activities, and attributes that a given society considers appropriate for men and women."

[2]As recently reviewed by Miller et al. (2011): "Sex," a biological construct, refers to biological differences defined by sex chromosomes (XX, XY) and the presence of functional reproductive organs and sex steroids. "Gender," a cultural construct, refers to behaviors thought to be directed by specific stimuli or by psychosocial expectations that result or accrue on the basis of assigned or perceived sex. Whereas sex is considered a dichotomous variable; gender is a continuous variable as defined by a range of characteristics that might vary with age, species (animals), or ethnicity (humans), geographical location, education, and culture. Most studies using animals categorized by anatomical features and chromosomes can be described as studies of "sex" differences.

the placenta, as well as by the adrenal cortex (Bassil et al., 2009).

In men, whereas around 7% of testosterone is reduced to 5α-dihydrotestosterone (DHT) by the cytochrome P450 enzyme 5α-reductase (an enzyme highly expressed in male accessory sex organs and hair follicles), small amounts (around 0.5%) are converted into estradiol by aromatase (CYP19A1, an enzyme expressed in the brain, liver, adipose, and cardiovascular tissues). DHT is more biologically active than testosterone since it binds to the androgen receptor with a 15-fold higher affinity than testosterone (Thigpen et al., 1993; Randall, 1994; Meinhardt and Mullis, 2002; Askew et al., 2007).

Androgen receptors are widely distributed in several cells/tissues, including vascular smooth muscle cells and endothelial cells (Negro-Vilar, 1999). Through classic cytosolic androgen receptors or membrane receptors, testosterone induces genomic and non-genomic effects, respectively. The classical effects of testosterone depends on its binding to the androgen receptor, which acts as a transcription factor that upon association to the androgen response element, modulates transcription, and protein synthesis. The non-genomic effects are rapidly produced, do not require the association of androgen receptors to DNA and therefore are insensitive to the inhibition of RNA and protein synthesis, and involve the activation of various signaling pathways, including calcium-, protein kinase A-, protein kinase C-, and mitogen-activated protein kinase (MAPK)-activated pathways (Bhasin and Jasuja, 2009). It is important to mention that activation of androgen receptors may vary considering whether naturally produced or commercially available hormones are being used, as well as whether metabolic products of androgens are being generated (e.g., testosterone, but not DHT, can be aromatized to 17β-estradiol) or whether androgen effects were tested on tissues/cells derived from male or female animals (Elbaradie et al., 2011; Toufexis and Wilson, 2012).

EVIDENCE FOR A ROLE OF TESTOSTERONE IN REGULATION OF LONGEVITY

While the decrease in estrogen and progesterone occurs abruptly in women, testosterone levels decline in men starts in the early years of adulthood, with a 1–2% reduction per year, and persists throughout life (Harman et al., 2001; Araujo and Wittert, 2011). This reduction is often associated with CVD, metabolic syndrome, insulin resistance, and atherosclerosis. The increased longevity has also been accompanied by an increase in the number of men with hypogonadism (Bassil et al., 2009). Commonly, testosterone replacement improves cardiovascular and metabolic functions (Ruige et al., 2011; Hyde et al., 2012). In addition, a prospective observational study with men aged 70–96 years demonstrated that low testosterone in men, independent of pre-existing health conditions, and other risk factors, is associated with increased mortality risk (Fukai et al., 2011).

Decreased expression of cardiac β1-adrenergic receptors is involved in many models of decompensate hypertrophy and heart failure (Bisognano et al., 2000). A study conducted in male Sprague-Dawley rats showed that low levels of testosterone reduced β1-adrenoceptor mRNA, while testosterone replacement produced a fourfold increase in β1-adrenoceptor mRNA levels. Testosterone replacement also increased mRNA levels of androgen receptor, Na^+/Ca^{2+} exchanger, and L-type calcium channel as well as heart weight (Golden et al., 2002). Therefore it is possible that aging-associated testosterone deficiency may contribute to cardiovascular dysfunction in the elderly.

Although many studies indicate that low levels of testosterone represent a risk factor for CVD, men have a higher prevalence of CVD than women and this ratio narrows after menopause (Lerner and Kannel, 1986). In general, explanations for the sex differences in CVD point to a deleterious effect of testosterone or the absence of the cardioprotective effects of estrogen in men (Wu and von Eckardstein, 2003). As elegantly reviewed by Wu and von Eckardstein (2003), a global evaluation of the relationship between androgens and coronary heart disease (CAD) revealed that the data are contradictory, and that is not yet possible to say whether there is a direct association between levels of testosterone or other androgens and CAD. In addition, although testosterone levels in men are positively related to HDL and negatively to LDL, in women this ratio is negative, suggesting that males and females respond differently to androgens, and that some of the beneficial effects of testosterone in men, may be deleterious in women.

Aging-associated CVDs are accompanied by structural and functional changes in the heart and blood vessels. Cardiovascular aging is manifested by maladaptation to stress, cardiac and vascular dysfunction, and heart failure. Aging-associated morphological and functional changes of cardiac and vascular myocytes lead to cardiac and vascular hypertrophy, fibrosis, decreased regenerative capacity of cardiac stem cells/endothelial progenitor cells (EPCs), increased myocyte death due to necrosis and apoptosis (Ungvari et al., 2010; Shih et al., 2011). In the vasculature, increased arterial wall thickening and generalized vascular stiffness are attributed to vascular calcification, increased collagen content/elastin breakdown, and elevated levels of advanced glycation end products (AGEs). In addition, endothelial dysfunction, vascular inflammation, oxidative stress, vascular cell apoptosis also contribute to aging-associated vascular dysfunction, which accelerates CAD, heart failure, stroke, and dementia (Ungvari et al., 2010).

Aging is not only a prominent independent risk factor for CVD, but it is also associated with decreased cognitive function. Of importance, there is strong evidence for a protective role of testosterone in brain regions, including regions susceptible to Alzheimer's disease, such as the hippocampus (Pike et al., 2009). Neuronal damage and apoptosis caused by oxidative stress, a hallmark in aging-associated cardiovascular dysfunction, can also be inhibited by testosterone. Testosterone induces the activation of extracellular-signal-regulated kinases (ERKs), which phosphorylate the pro-apoptotic protein Bad, a member of the pro-apoptotic Bcl-2 family, making it inactive, and increasing neuronal activity. It is known that regions like the hippocampus and cortical regions have a large amount of androgen receptors (Simerly et al., 1990). Interestingly, whereas testosterone inhibits oxidative stress and apoptosis in the brain, our group has shown that testosterone increases reactive oxygen species (ROS) generation (Chignalia et al., 2012) and activates apoptotic pathways (unpublished data) in vascular smooth muscle cells.

Sexual health has also become more important with the increasing longevity in men and women. Erectile dysfunction markedly

increases with age and men with increased sexual activity have a higher level of bioavailable testosterone than men with erectile dysfunction. Although erectile dysfunction is not a life threatening condition, it directly affects the well-being, self-steam, interpersonal relationship and overall quality of life. It has been suggested that approximately 15–25% of men over the age of 50 years are expected to have serum testosterone levels that fall below that of the threshold considered normal in men between 20 and 40 years of age (Jockenhovel, 2004). Testosterone improves a great number of androgen deficiency symptoms in the aging male (Giuliano et al., 2004; Amar et al., 2005). Several studies have shown that normal testosterone levels are important for sexual function, and the correlation between hypogonadism and sexual dysfunction is well documented (Shabsigh et al., 2006; Blute et al., 2009; Buvat et al., 2010). For example, testosterone is important for normal erectile function because it regulates nitric oxide (NO), phosphodiesterase-5 (PDE5) levels, and it maintains penile muscle cells (Blute et al., 2009). However, it is important to point out that the main effects of testosterone are not to maintain an erection *per se*. Conversely, testosterone is much more important for the global aspects of sexual function including libido, orgasm, ejaculatory volume, preserving the general function of the cavernosum and the vasculature involved in the erectile process (Lazarou and Morgentaler, 2005). Accordingly, clinical trials have demonstrated that replacement of testosterone levels in hypogonadal men improves overall sexual function and restores vascular damage in the cavernosal tissue (Seftel et al., 2004; Bassil et al., 2009; Romanelli et al., 2010). In addition, testosterone is essential for health and well-being (Bassil et al., 2009) as well as the prevention of osteoporosis (Tuck and Francis, 2009).

TESTOSTERONE SIGNALING IN VASCULAR AGING

Experimental data demonstrate that testosterone induces relaxation of many vascular beds, including coronary, mesenteric, iliac, renal, and femoral arteries in several animal species such as rabbit, dog, rat, pig, both *in vivo* and *in vitro* conditions. In general, most studies indicate that the relaxation induced by testosterone involves endothelium-independent mechanisms, potassium channel-opening actions and calcium antagonistic effects (Yue et al., 1995; Chou et al., 1996; Crews and Khalil, 1999; Murphy and Khalil, 1999; English et al., 2000, 2002; Deenadayalu et al., 2001).

However, testosterone, as well as other sex steroid hormones (e.g., estrogen) also modulate NO release and, consequently, influence endothelial function (Miller and Mulvagh, 2007). Physiological concentrations of testosterone (and DHT) have been shown to increase endothelial synthesis of NO via activation of the extracellular-signal-regulated kinase (ERK) 1/2 and phosphatidylinositol 3-OH kinase (PI3K)/Akt cascades (Goglia et al., 2010). Similarly, in rat aortic strips, testosterone significantly increased NO production, via androgen receptor and calcium influx (Campelo et al., 2012). Using endothelial cell cultures these authors demonstrated that testosterone enhances NO production by directly acting in the endothelial cells via PKC- and MAPK-dependent pathways. Testosterone also significantly increased DNA synthesis indicating that androgens may also modulate vascular endothelial cell growth (Campelo et al., 2012). In addition, testosterone, at physiological concentrations and via androgen receptor activation, induces proliferation, migration, and colony formation activity of EPCs (Foresta et al., 2008), indicating that the release of EPCs by bone marrow may be an additional mechanism by which testosterone modulates endothelial function (Foresta et al., 2006).

A criticism to the studies reporting the relaxation effects of testosterone in blood vessels is that generally the effects are observed at supraphysiological doses/concentrations, and when physiological concentrations of testosterone were used, the studies were inconclusive, with some showing positive effects, and others neutral or even deleterious effects of testosterone (Wu and von Eckardstein, 2003). Furthermore, since most of the studies on the effects of androgens on NO production have been conducted in co-culture systems, it is necessary to assess the direct impact of testosterone on endothelial cell growth and function.

Sader et al. (2001) observed that although low doses of testosterone *per se* induce vasodilation (brachial artery flow-mediated dilatation, FMD) in healthy men, estradiol supplementation is associated with enhanced arterial vasodilation. The mechanisms by which testosterone and estrogen induce NO release are intertwined, since through the P450 aromatase, testosterone can be converted to estrogen. An excellent review by Miller and Mulvagh (2007) has addressed the mechanisms by which testosterone and estrogen modulate endothelial cells function and NO release. As summarized in **Figure 1**, the mechanism of action of testosterone in endothelial cell includes classic steroid receptor activation via modulation of gene transcription (genomic), and also membrane receptor activation coupled to fast intracellular signaling (non-genomic). Activation of both estrogen and androgen receptors modulates endothelial function by mechanisms involving activation of Akt, MAPK, tyrosine kinase, and $G_i\alpha$, culminating in NO synthase activation and release of NO. The aging-associated decrease in testosterone levels may thus compromise this important pathway in vascular tone control.

English and colleagues, in a study with arteries from young and elderly male Wistar rats demonstrated that despite normal contractile responses to prostaglandin F2α, vessels of elderly animals were less sensitive to the vasodilatory effects of testosterone compared to vessels from young animal. This was accompanied by hypertrophy of vascular myocytes, and greater thickening of the smooth muscle layer, in aged animals (English et al., 2000).

Yildiz et al. (2005) demonstrated that the relaxation induced by testosterone in human internal mammary artery is dependent on large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}). This channel is key in the regulation of vascular tone and arterial diameter and is target to various vasoconstrictor and vasodilator agents. As shown in **Figure 1**, aging decreases the expression of BK_{Ca} channels in coronary smooth muscle and increases endothelial responses to vasoconstrictor agents, which can contribute to the reduced vasodilator response to testosterone in elderly and also increase the risk of vasospasm, myocardial ischemia, and infarction in these individuals (Marijic et al., 2001).

Ripple et al. (1999) showed that, in androgen-sensitive human prostate cancer (LNCaP), androgens increase mitochondrial dehydrogenase activity, levels of cellular peroxides and hydroxyl radicals and the area fraction of mitochondria per cell. The oxidative stress in LNCaP was abolished in the presence of rotenone and antimycin

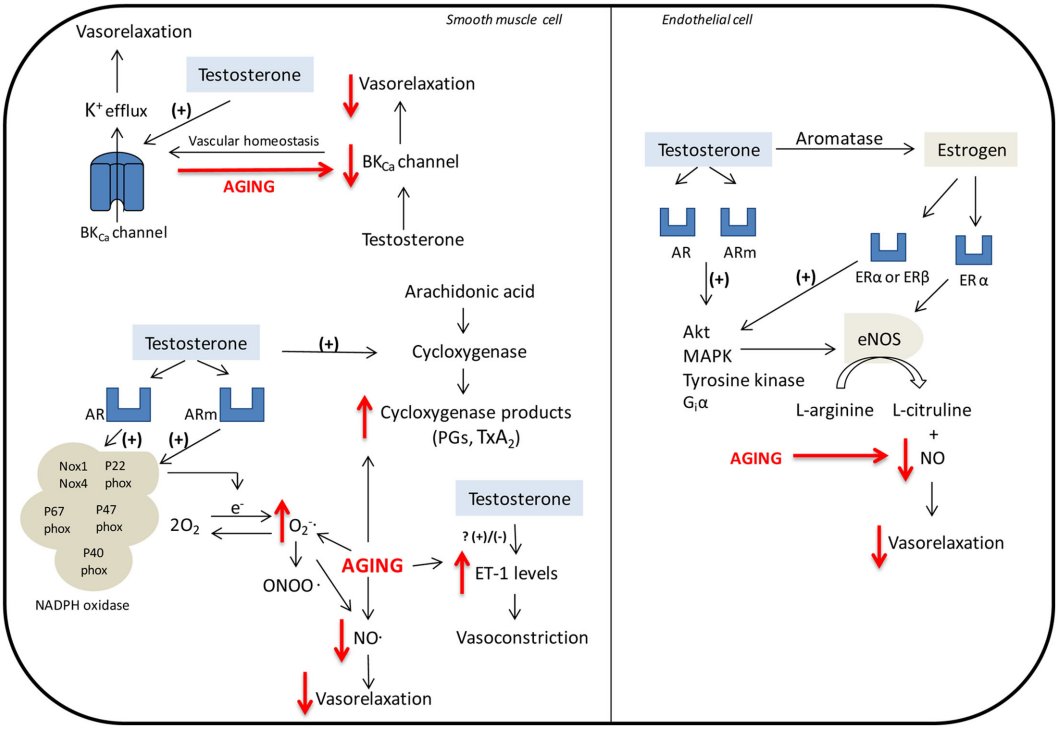

FIGURE 1 | Mechanisms involved in the regulation of vascular function by testosterone. Smooth muscle cell: The relaxation induced by testosterone is dependent on large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}). Aging decreases the expression of BK_{Ca}, which can determine the reduced vasodilator response to testosterone in elderly. Testosterone induces NADPH oxidase-dependent ROS generation. NO can react with $\bullet O_2^-$ forming peroxynitrite ($ONOO^-$). This decreases NO availability and consequently reduces vasodilator responses. Testosterone also stimulates COX-1/COX-2 and ET-1 pathways. Endothelial cell: Through the P450 aromatase, testosterone can be converted to estrogen. Activation of estrogen and androgen receptors modulates endothelial function by mechanisms involving release of NO via NO synthase. Abbreviations: AR, androgen receptor; ER estrogen receptor; Akt, serine/threonine kinase; BK_{Ca}, large conductance Ca^{2+}-activated K^+ channels; ERKs, extracellular-signal-regulated kinases; eNOS, endothelial NO synthase; $G_i\alpha$, guanine nucleotide regulator protein subunit that inhibits guanylate cyclase; MAPK, mitogen-activated protein kinase; $\bullet O_2^{-\bullet}$, superoxide anion; $ONOO^-$, peroxynitrite; PGs, prostaglandins; TxA_2, thromboxane A_2. Aging-associated decline in testosterone levels, as well as mechanisms that contribute to aging-associated endothelial dysfunction are depicted in red.

A (electron transport system inhibitors), and abrogated in the presence of alpha-tocopherol succinate. These data indicate that ROS generation induced by androgen in LNCaP involves mitochondrial pathways and is sensitive to antioxidants. On the other hand, Hwang et al. (2011) demonstrated that treatment of Leydig cells with low doses of testosterone has cytoprotective effects by decreasing ROS and lipid peroxides. However, cytotoxic effects, due to increased ROS generation, were found with high doses of testosterone. Although data from our laboratory demonstrate that testosterone induces ROS generation in VSMCs (Chignalia et al., 2012), which can culminate in decreased NO bioavailability and reduce vasodilator responses, it is not known whether this effect is altered in senescent VSMCs.

Testosterone also stimulates thromboxane synthase as well as COX-1 and COX-2, which are key enzymes in the synthesis of prostaglandins (Cheuk et al., 2000; Song et al., 2004). Accordingly, the aging-associated decrease in testosterone levels may interfere with vascular function via changes in the thromboxane/COX pathway (**Figure 1**).

de Souza Rossignoli et al. (2010), in a study to determine the effects of declining levels of testosterone, as occurs in aging, and testosterone replacement on vascular function, found that orchidectomy enhanced the maximum responses of the portal vein to phenylephrine. However, testosterone replacement had no effect on these responses. Yet in this study, orchidectomy-induced increased maximum response to phenylephrine was not altered by a NOS inhibitor alone or combined with indomethacin. Interestingly, the increased response was abolished in the presence of ETA and ETB receptor antagonists (BQ-123 and BQ-788, respectively), suggesting that increased contractile responses to phenylephrine induced by decreases in plasma testosterone, involves activation of ETA and ETB receptors. The relationship between testosterone and endothelin-1 (ET-1) is complex. Whereas substantial data support the idea that testosterone positively modulates ET-1 levels and ET-1-mediated vasoconstrictor responses, other reports indicate that ET-1 levels are higher in conditions associated with low levels of testosterone, as in hypogonadism (Tostes et al., 2008).

The regulation of resting membrane potential is mostly dependent on the voltage-dependent potassium channels (Kv), and functional changes of these channels may impair vascular function. Kv plays a key role in endothelial cells function and is decreased in stroke-prone spontaneously hypertensive rats (Sadanaga et al.,

2002). Zhou et al. (2008) investigated the effects of *testosterone* deprivation (castration – 6 months) as well as the effects of *testosterone* replacement therapy on the function of Kv channels in rat *thoracic aortic VSMCs*. In this study long-term deprivation of endogenous *testosterone* decreased the expression of *Kv1.5* protein, which culminated in reduced function of Kv channels. *Testosterone* replacement restored the impaired *function* of Kv, which suggests that testosterone deficiency in aging may impair vascular function via a decrease of endothelial and vascular smooth muscle Kv channels.

As already mentioned, aging is accompanied by endothelial dysfunction (Yildiz, 2007). There is extensive discussion on the mechanisms by which the aging process attenuates endothelium-dependent dilatation. Enhanced production of endothelium-derived vasoconstrictor prostanoids, decreased NO bioavailability, increased expression/activity of ET-1, and augmented ROS generation are all potential mechanisms involved in aging-associated endothelial injury and vascular dysfunction (Mombouli and Vanhoutte, 1999; Blackwell et al., 2004; Vanhoutte et al., 2009). The generation of superoxide anion ($\bullet O_2^-$) results in rapid formation of hydrogen peroxide (H_2O_2) via mitochondrial manganese superoxide dismutase (MnSOD). NO can react with $\bullet O_2^-$ forming peroxynitrite ($ONOO^-$) that inactivates MnSOD and may also uncouple NO synthase. Both reactions decrease the availability of NO and consequently reduce vasodilator responses (Yildiz, 2007). Decreased NO bioavailability is also associated with endothelial NO synthase (eNOS) dysfunction since uncoupled eNOS is a source of $\bullet O_2^-$ in the vasculature. Accordingly, removal of endothelial cells, as well as pharmacological inhibition of NOS, reverses the vascular increased $\bullet O_2^-$ production of aged vessels. In aging, eNOS uncoupling seems to be associated with a reduction of the cofactor tetrahydrobiopterin availability (Delp et al., 2008; Yang et al., 2009) rather than to a deficiency of L-arginine substrate concentration (Gates et al., 2007). In addition, eNOS knockout male mice have a significantly shorter lifespan than their wild type controls and exhibit accelerated aging-dependent cardiac dysfunction. Regarding ET-1 and aging, plasma ET-1 concentrations increase with age (Battistelli et al., 1996; Donato et al., 2009; Goel et al., 2010), ET-1-mediated vasoconstriction is augmented in older adults (Thijssen et al., 2007; Van Guilder et al., 2007) and synthesis of ET-1 is greater in cultured aortic endothelial cells obtained from older donors compared with young ones (Tokunaga et al., 1992). Finally, inhibition of ET-1 signaling with an ETA receptor antagonist improves endothelial dysfunction in arteries from old mice, while not affecting dilation in young controls (Donato et al., 2009). As discussed above, testosterone has been shown to modulate each of these mechanisms that contribute to aging-associated endothelial dysfunction. However, whether decreased testosterone levels are directly associated with the endothelial dysfunction and increased incidence of CVDs remains controversial.

Hypogonadism is a condition associated with endothelial dysfunction (Akishita et al., 2007; Foresta et al., 2008). A study where male patients were submitted to examination of vasomotor function of the brachial artery and intima-media thickness (IMT) of the carotid artery, showed that low levels of testosterone are associated with endothelial dysfunction, independent of age, body mass index, hypertension, hyperlipidemia, diabetes mellitus, or current smoking, suggesting a protective effect of testosterone on endothelial function (Akishita et al., 2007).

THE USE OF TESTOSTERONE TO PREVENT VASCULAR DYSFUNCTION IN ELDERLY

Testosterone replacement therapy has been shown to decrease total cholesterol, low-density lipoprotein, and triglycerides, to increase high density lipoprotein, and also to inhibit fatty streak formation, indicating that androgens may have protective effects against atherosclerosis (Nettleship et al., 2007, 2009; Saad et al., 2008).

Likewise, testosterone has been shown to improve hemodynamic parameters in patients and animal models of heart failure, especially via a reduction in peripheral vascular resistance and increased coronary blood flow through vasodilation and via direct effects in the cardiac tissue (e.g., by inhibition of cardiac cAMP-phosphodiesterases; Bordallo et al., 2011; Nguyen et al., 2011).

Through the measurement of pulse wave velocity, a technique that allows to analyze arterial stiffness (decreased compliance), vascular disease, and also to predict future cardiovascular events (Boutouyrie et al., 2002; Pizzi et al., 2006), Yaron and colleagues reported that pulse wave velocity was significantly higher in men with hypogonadism. Transdermal testosterone replacement therapy increased androgen bioavailability and decreased pulse wave velocity, indicating that testosterone replacement can diminish arterial stiffness associated with male hypogonadism (Yaron et al., 2009).

The decline in testosterone levels observed with aging occurs at a time when prostate diseases start to emerge. Excellent results have been found with new testosterone preparations via different routes of administration (Titus et al., 2005). 5α-reductase inhibition (with finasteride), which is used in the treatment of men with benign prostatic hyperplasia (McConnell et al., 1992), also improves the quality of life of these patients.

Decreased levels of dehydroepiandrosterone (DHEA), a precursor of testosterone, are also associated with aging-related diseases. Although its mechanisms of action remain poorly understood, DHEA replacement therapy exerts positive anti-aging, anti-obesity, anti-atherosclerosis, anti-diabetic, and anti-osteoporosis effects (Gomez-Santos et al., 2011).

The above-mentioned data indicate that testosterone actions on the vasculature are beneficial and that low levels of testosterone are detrimental to cardiovascular function. Testosterone replacement may be beneficial in the treatment of diseases characteristic of elderly, but the positive cardiovascular benefits resulting from it need to be clearly demonstrated in large clinical trials.

If, in one hand, androgens seem to confer cardioprotection, on the other hand androgens can increase blood pressure and induce renal dysfunction, which may adversely influence the cardiovascular system. For example, chronic infusion of DHT increased sodium and water reabsorption and subsequently blood pressure in Sprague-Dawley rats (Quan et al., 2004). Further support to the idea that testosterone may have deleterious effects on renal function derives from studies in animals with arterial hypertension. Orchidectomy in male SHR prevents aging-associated increase in renal vascular resistance and reduces renal superoxide production (Reckelhoff et al., 2005). In addition, physiological concentrations of DHT increase dihydroethidium fluorescence in cultured

mesangial cells from SHR (Reckelhoff et al., 2005), whereas tempol, a SOD mimetic, reduces ROS generation and blood pressure in SHR (Fortepiani and Reckelhoff, 2005). Considering that testosterone also induces ROS generation in VSMCs from SHR (Chignalia et al., 2012) and that superoxide, the major ROS found in kidneys, reduces NO bioavailability and increases renal vasoconstriction (Reckelhoff and Romero, 2003; Yildiz, 2007), it is possible that testosterone may directly contribute to renal damage and vascular dysfunction via ROS generation. Furthermore, in the renal wrap model of hypertension, castration of male rats decreases renal damage, and DHT treatment of castrated males, reverses it, suggesting that androgens may directly contribute to the development of renal failure (Ji et al., 2005).

However, studies also suggest a renoprotective role for testosterone. For example, reduced levels of testosterone correlates with the development of albuminuria, a marker of diabetic renal disease, and castration of streptozotocin-treated rats potentiates diabetes-associated albuminuria (Xu et al., 2008). In addition, clinical studies show reduced levels of testosterone in diabetic patients (Laaksonen et al., 2004; Ding et al., 2006; Kapoor et al., 2007), raising the possibility that the severity of renal disease in these patients is partially due to a decrease in testosterone levels. A study conducted in 36 outpatient centers in Belgium, France, Germany, Italy, the Netherlands, Spain, Sweden, and the UK between February 2006 and March 2007 evaluated the effects of testosterone replacement therapy on insulin resistance in hypogonadal men with type 2 diabetes. Testosterone replacement reduced insulin resistance and improved glycemic control in the overall population (Jones et al., 2011). Furthermore, promising results arose from a study in which testosterone replacement was used for 2 years in type 2 diabetic male patients, who had the first ischemic stroke. Several parameters were improved by testosterone replacement on those patients, such as body mass index, glycated hemoglobin, cholesterol, triglycerides, and low-density lipoproteins. Furthermore, only 7.1% of the patients receiving testosterone replacement had a secondary stroke compared with 16.6% in the control group (Morgunov et al., 2011). These data indicate that testosterone replacement may be important to reduce cardiovascular risk factors, especially in type 2 diabetes associated with the hypogonadism condition.

It is unknown whether renal dysfunction or renal protection by testosterone is associated with direct effects of testosterone on the renal cells, or indirect effects of the hormone (e.g., in the renal vasculature). Since human renal proximal tubule and cortical collecting duct cells express enzymes involved in androgen synthesis (Quinkler et al., 2003) as well as androgen receptors (Kimura et al., 1993), one may speculate that testosterone regulates renal function by direct mechanisms.

Although data on testosterone and androgens being bad/deleterious or good/beneficial to the cardiovascular and renal systems are still very controversial, in general, experimental evidence indicates that testosterone is essential for the equilibrium in the actions of many endothelium-derived vasoactive agents as well as for the appropriate function of structural components of these systems (**Table 1**). The aging-associated decrease in testosterone levels may disrupt these signals and therefore contribute to cardiovascular and renal dysfunction observed in elderly.

CONCLUSION AND PERSPECTIVES

Even though evidence indicates that vascular damage characteristic of aging may be improved or even reversed with testosterone replacement therapy, more studies are needed to confirm the putative beneficial cardiovascular effects of testosterone therapy. In addition, the long-term effects of testosterone replacement therapy need to be carefully addressed. For example, whereas short-term testosterone treatment has been shown to produce a wide range of benefits (it improves hemodynamic parameters, cognitive and sexual function, mood, muscle mass and strength), the beneficial effects may not be maintained (the gain of muscle mass, strength, and quality of life are not maintained at 6 months post-treatment

Table 1 | Cardiovascular and renal effects mediated by androgens.

Androgen	Cell/tissue	Effect	Reference
Testosterone	Heart	↑ β1-Adrenoceptor, ↑ androgen receptor, ↑ Na^+/Ca^{2+} exchanger, ↑ L-type calcium channel	Golden et al. (2002)
Testosterone	Cultured VSMCs	↑ ROS	Chignalia et al. (2012)
Testosterone/ dihydrotestosterone	Human endothelial cells, blood vessels	↑ ERK ½, ↑ PI3K, ↑ eNOS, ↑ NO	Goglia et al. (2010), Miller and Mulvagh (2007)
Testosterone	Brachial artery	↑ FMD	Sader et al. (2001)
Testosterone	Human internal mammary artery	↑ BK_{Ca} activation	Yildiz et al. (2005)
Testosterone	Rat epididymis, thoracic aortae, and mesenteric arteries	↑ COX-1 and COX-2	Cheuk et al. (2000), Song et al. (2004)
Testosterone	Rat thoracic aorta	↑ Kv channels	Zhou et al. (2008)
Testosterone	Endothelial cells	↑ EPCs	Foresta et al. (2008)
Dihydrotestosterone	Kidney	↑ Sodium and water reabsorption	Quan et al. (2004)
Dihydrotestosterone	Mesangial cells	↑ ROS	Reckelhoff et al. (2005)

FMD, brachial artery flow-mediated dilatation; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthases; EPC, endothelial progenitor cells; ERK 1/2, extracellular-signal-regulated kinase (ERK) 1/2; PI3K, phosphatidylinositol 3-OH kinase; ROS, reactive oxygen species; NO, nitric oxide; VSMC, vascular smooth muscle cell.

with testosterone in frail elderly men; O'Connell et al., 2011). Does the same happen with the cardiovascular effects (are the benefits sustained or not)? What is the optimal duration of anabolic hormonal intervention? Are any possible side effects (e.g., prostatic disease and erythrocytosis) overruled by the beneficial cardiovascular effects? Do physical exercise, diet, and other lifestyle options, in conjunction with pharmacological testosterone replacement, improve beneficial cardiovascular effects of hormone therapy? These questions clearly illustrate that the cardiovascular benefits of testosterone therapy for age-related declines are not yet as clear as they may seem.

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REFERENCES

Akishita, M., Hashimoto, M., Ohike, Y., Ogawa, S., Iijima, K., Eto, M., and Ouchi, Y. (2007). Low testosterone level is an independent determinant of endothelial dysfunction in men. *Hypertens. Res.* 30, 1029–1034.

Amar, E., Grivel, T., Hamidi, K., Lemaire, A., and Giuliano, F. (2005). Ageing men's sexual functions decline and the erectile dysfunction (ED) increase. *Prog. Urol.* 15, 6–9.

Araujo, A. B., and Wittert, G. A. (2011). Endocrinology of the aging male. *Best Pract. Res. Clin. Endocrinol. Metab.* 25, 303–319.

Askew, E. B., Gampe, R. T. Jr., Stanley, T. B., Faggart, J. L., and Wilson, E. M. (2007). Modulation of androgen receptor activation function 2 by testosterone and dihydrotestosterone. *J. Biol. Chem.* 282, 25801–25816.

Bagatell, C. J., and Bremner, W. J. (1996). Androgens in men – uses and abuses. *N. Engl. J. Med.* 334, 707–714.

Bassil, N., Alkaade, S., and Morley, J. E. (2009). The benefits and risks of testosterone replacement therapy: a review. *Ther. Clin. Risk. Manag.* 5, 427–448.

Battistelli, S., Gori, S., Borgogni, T., and Manasse, G. (1996). Variation in the plasma endothelin levels in relation to age. *Minerva Cardioangiol.* 44, 111–114.

Bhasin, S., and Jasuja, R. (2009). Selective androgen receptor modulators as function promoting therapies. *Curr. Opin. Clin. Nutr. Metab. Care* 12, 232–240.

Bisognano, J. D., Weinberger, H. D., Bohlmeyer, T. J., Pende, A., Raynolds, M. V., Sastravaha, A., Roden, R., Asano, K., Blaxall, B. C., Wu, S. C., Communal, C., Singh, K., Colucci, W., Bristow, M. R., and Port, D. J. (2000). Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. *J. Mol. Cell. Cardiol.* 32, 817–830.

Blackwell, K. A., Sorenson, J. P., Richardson, D. M., Smith, L. A., Suda, O., Nath, K., and Katusic, Z. S. (2004). Mechanisms of aging-induced impairment of endothelium-dependent relaxation: role of tetrahydrobiopterin. *Am. J. Physiol. Heart Circ. Physiol.* 287, H2448–H2453.

Blute, M., Hakimian, P., Kashanian, J., Shteynshluyger, A., Lee, M., and Shabsigh, R. (2009). Erectile dysfunction and testosterone deficiency. *Front. Horm. Res.* 37, 108–122.

Bordallo, J., Cantabrana, B., Suarez, L., and Sanchez, M. (2011). Testosterone inhibits cAMP-phosphodiesterases in heart extracts from rats and increases cAMP levels in isolated left atria. *Pharmacology* 87, 155–160.

Boutouyrie, P., Tropeano, A. I., Asmar, R., Gautier, I., Benetos, A., Lacolley, P., and Laurent, S. (2002). Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 39, 10–15.

Burger, H. G. (2002). Androgen production in women. *Fertil. Steril.* 77(Suppl. 4), S3–S5.

Buvat, J., Maggi, M., Gooren, L., Guay, A. T., Kaufman, J., Morgentaler, A., Schulman, C., Tan, H. M., Torres, L. O., Yassin, A., and Zitzmann, M. (2010). Endocrine aspects of male sexual dysfunctions. *J. Sex. Med.* 7(Pt 2), 1627–1656.

Campelo, A. E., Cutini, P. H., and Massheimer, V. L. (2012). Testosterone modulates platelet aggregation and endothelial cell growth through nitric oxide pathway. *J. Endocrinol.* 213, 77–87.

Cheuk, B. L., Leung, P. S., Lo, A. C., and Wong, P. Y. (2000). Androgen control of cyclooxygenase expression in the rat epididymis. *Biol. Reprod.* 63, 775–780.

Chignalia, A. Z., Schuldt, E., Camargo, L., Montezano, A. C. I., Callera, G. E., Laurindo, F. R., Lopes, L. R., Avellar, M. C. W., Carvalho, M. H. C., Fortes, Z. B., Touyz, R. M., and Tostes, R. C. (2012). Testosterone induces vascular smooth muscle cell migration by NADPH oxidase and c-SRC-dependent pathways. *Hypertension* (in press).

Chou, T. M., Sudhir, K., Hutchison, S. J., Ko, E., Amidon, T. M., Collins, P., and Chatterjee, K. (1996). Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. *Circulation* 94, 2614–2619.

Crews, J. K., and Khalil, R. A. (1999). Antagonistic effects of 17 beta-estradiol, progesterone, and testosterone on Ca^{2+} entry mechanisms of coronary vasoconstriction. *Arterioscler. Thromb. Vasc. Biol.* 19, 1034–1040.

de Souza Rossignoli, P., Pereira, O. C., and Chies, A. B. (2010). Orchidectomy enhances the effects of phenylephrine in rat isolated portal vein. *Clin. Exp. Pharmacol. Physiol.* 37, 368–374.

Deenadayalu, V. P., White, R. E., Stallone, J. N., Gao, X., and Garcia, A. J. (2001). Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am. J. Physiol. Heart Circ. Physiol.* 281, H1720–H1727.

Delp, M. D., Behnke, B. J., Spier, S. A., Wu, G., and Muller-Delp, J. M. (2008). Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. *J. Physiol. (Lond.)* 586, 1161–1168.

Ding, E. L., Song, Y., Malik, V. S., and Liu, S. (2006). Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 295, 1288–1299.

Donato, A. J., Gano, L. B., Eskurza, I., Silver, A. E., Gates, P. E., Jablonski, K., and Seals, D. R. (2009). Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *Am. J. Physiol. Heart Circ. Physiol.* 297, H425–H432.

Elbaradie, K., Wang, Y., Boyan, B. D., and Schwartz, Z. (2011). Rapid membrane responses to dihydrotestosterone are sex dependent in growth plate chondrocytes. *J. Steroid Biochem. Mol. Biol.* doi: 10.1016/j.jsbmb.2011.12.009. [Epub ahead of print].

English, K. M., Jones, R. D., Jones, T. H., Morice, A. H., and Channer, K. S. (2000). Aging reduces the responsiveness of coronary arteries from male Wistar rats to the vasodilatory action of testosterone. *Clin. Sci.* 99, 77–82.

English, K. M., Jones, R. D., Jones, T. H., Morice, A. H., and Channer, K. S. (2002). Testosterone acts as a coronary vasodilator by a calcium antagonistic action. *J. Endocrinol. Invest.* 25, 455–458.

Foresta, C., Caretta, N., Lana, A., De Toni, L., Biagioli, A., Ferlin, A., and Garolla, A. (2006). Reduced number of circulating endothelial progenitor cells in hypogonadal men. *J. Clin. Endocrinol. Metab.* 91, 4599–4602.

Foresta, C., Zuccarello, D., De Toni, L., Garolla, A., Caretta, N., and Ferlin, A. (2008). Androgens stimulate endothelial progenitor cells through an androgen receptor-mediated pathway. *Clin. Endocrinol. (Oxf.)* 68, 284–289.

Fortepiani, L. A., and Reckelhoff, J. F. (2005). Role of oxidative stress in the sex differences in blood pressure in spontaneously hypertensive rats. *J. Hypertens.* 23, 801–805.

Francomano, D., Bruzziches, R., Natali, M., Aversa, A., and Spera, G. (2010). Cardiovascular effect of testosterone replacement therapy in aging male. *Acta Biomed.* 81(Suppl. 1), 101–106.

Fukai, S., Akishita, M., Yamada, S., Ogawa, S., Yamaguchi, K., Kozaki, K., Toba, K., and Ouchi, Y. (2011). Plasma sex hormone levels and mortality in disabled older men and women. *Geriatr. Gerontol. Int.* 11, 196–203.

Gates, P. E., Boucher, M. L., Silver, A. E., Monahan, K. D., and Seals, D. R. (2007). Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. *J. Appl. Physiol.* 102, 63–71.

Giuliano, F., Tostain, J., and Rossi, D. (2004). Testosterone and male sexuality: basic research and clinical data. *Prog. Urol.* 14, 783–790.

Goel, A., Su, B., Flavahan, S., Lowenstein, C. J., Berkowitz, D. E., and Flavahan, N. A. (2010). Increased endothelial exocytosis and generation of endothelin-1 contributes to constriction of aged arteries. *Circ. Res.* 107, 242–251.

Goglia, L., Tosi, V., Sanchez, A. M., Flamini, M. I., Fu, X. D., Zullino, S., Genazzani, A. R., and Simoncini, T. (2010). Endothelial regulation of eNOS, PAI-1 and t-PA by testosterone and dihydrotestosterone in vitro and in vivo. *Mol. Hum. Reprod.* 16, 761–769.

Golden, K. L., Marsh, J. D., and Jiang, Y. (2002). Castration reduces mRNA levels for calcium regulatory proteins in rat heart. *Endocrine* 19, 339–344.

Gomez-Santos, C., Larque, E., Granero, E., Hernandez-Morante, J. J., and Garaulet, M. (2011). Dehydroepiandrosterone-sulphate replacement improves the human plasma fatty acid profile in plasma of obese women. *Steroids* 76, 1425–1432.

Hak, A. E., Witteman, J. C., de Jong, F. H., Geerlings, M. I., Hofman, A., and Pols, H. A. (2002). Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J. Clin. Endocrinol. Metab.* 87, 3632–3639.

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., and Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J. Clin. Endocrinol. Metab.* 86, 724–731.

Hwang, T. I., Liao, T. L., Lin, J. F., Lin, Y. C., Lee, S. Y., Lai, Y. C., and Kao, S. H. (2011). Low-dose testosterone treatment decreases oxidative damage in TM3 Leydig cells. *Asian J. Androl.* 13, 432–437.

Hyde, Z., Norman, P. E., Flicker, L., Hankey, G. J., Almeida, O. P., McCaul, K. A., Chubb, S. A., and Yeap, B. B. (2012). Low free testosterone predicts mortality from cardiovascular disease but not other causes: the health in men study. *J. Clin. Endocrinol. Metab.* 97, 179–189.

Ji, H., Menini, S., Mok, K., Zheng, W., Pesce, C., Kim, J., Mulroney, S., and Sandberg, K. (2005). Gonadal steroid regulation of renal injury in renal wrap hypertension. *Am. J. Physiol. Renal Physiol.* 288, F513–F520.

Jockenhovel, F. (2004). Testosterone therapy – what, when and to whom? *Aging Male* 7, 319–324.

Jones, T. H., Arver, S., Behre, H. M., Buvat, J., Meuleman, E., Moncada, I., Morales, A. M., Volterrani, M., Yellowlees, A., Howell, J. D., and Channer, K. S. (2011). Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care* 34, 828–837.

Kapoor, D., Aldred, H., Clark, S., Channer, K. S., and Jones, T. H. (2007). Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: correlations with bioavailable testosterone and visceral adiposity. *Diabetes Care* 30, 911–917.

Kimura, N., Mizokami, A., Oonuma, T., Sasano, H., and Nagura, H. (1993). Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. *J. Histochem. Cytochem.* 41, 671–678.

Laaksonen, D. E., Niskanen, L., Punnonen, K., Nyyssonen, K., Tuomainen, T. P., Valkonen, V. P., Salonen, R., and Salonen, J. T. (2004). Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care* 27, 1036–1041.

Lazarou, S., and Morgentaler, A. (2005). Hypogonadism in the man with erectile dysfunction: what to look for and when to treat. *Curr. Urol. Rep.* 6, 476–481.

Lerner, D. J., and Kannel, W. B. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. *Am. Heart J.* 111, 383–390.

Marijic, J., Li, Q., Song, M., Nishimaru, K., Stefani, E., and Toro, L. (2001). Decreased expression of voltage- and Ca(2+)-activated K(+) channels in coronary smooth muscle during aging. *Circ. Res.* 88, 210–216.

McConnell, J. D., Wilson, J. D., George, F. W., Geller, J., Pappas, F., and Stoner, E. (1992). Finasteride, an inhibitor of 5 alpha-reductase, suppresses prostatic dihydrotestosterone in men with benign prostatic hyperplasia. *J. Clin. Endocrinol. Metab.* 74, 505–508.

Meinhardt, U., and Mullis, P. E. (2002). The essential role of the aromatase/p450arom. *Semin. Reprod. Med.* 20, 277–284.

Miller, V. M., Kaplan, J. R., Schork, N. J., Ouyang, P., Berga, S. L., Wenger, N. K., Shaw, L. J., Webb, R. C., Mallampalli, M., Steiner, M., Taylor, D. A., Bairey Merz, C. N., and Reckelhoff, J. F. (2011). Strategies and methods to study sex differences in cardiovascular structure and function: a guide for basic scientists. *Biol. Sex Differ.* 2, 14.

Miller, V. M., and Mulvagh, S. L. (2007). Sex steroids and endothelial function: translating basic science to clinical practice. *Trends Pharmacol. Sci.* 28, 263–270.

Miller, V. M., Tindall, D. J., and Liu, P. Y. (2004). Of mice, men, and hormones. *Arterioscler. Thromb. Vasc. Biol.* 24, 995–997.

Mombouli, J. V., and Vanhoutte, P. M. (1999). Endothelial dysfunction: from physiology to therapy. *J. Mol. Cell. Cardiol.* 31, 61–74.

Mooradian, A. D., Morley, J. E., and Korenman, S. G. (1987). Biological actions of androgens. *Endocr. Rev.* 8, 1–28.

Morgunov, L., Denisova, I. A., Rozhkova, T. I., Stakhovskaia, L. V., and Skvortsova, V. I. (2011). Androgenic deficit and its treatment in stroke male patients with diabetes mellitus type II. *Zh. Nevrol. Psikhiatr. Im. S. S. Korsakova.* 111(8 Pt 2), 21–24.

Murphy, J. G., and Khalil, R. A. (1999). Decreased [Ca(2+)](i) during inhibition of coronary smooth muscle contraction by 17beta-estradiol, progesterone, and testosterone. *J. Pharmacol. Exp. Ther.* 291, 44–52.

Negro-Vilar, A. (1999). Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J. Clin. Endocrinol. Metab.* 84, 3459–3462.

Nettleship, J. E., Jones, R. D., Channer, K. S., and Jones, T. H. (2009). Testosterone and coronary artery disease. *Front. Horm. Res.* 37, 91–107.

Nettleship, J. E., Jones, T. H., Channer, K. S., and Jones, R. D. (2007). Physiological testosterone replacement therapy attenuates fatty streak formation and improves high-density lipoprotein cholesterol in the Tfm mouse: an effect that is independent of the classic androgen receptor. *Circulation* 116, 2427–2434.

Nguyen, C. T., Aaronson, A., Morrissey, R. P., Agarwal, M., Willix, R. D., and Schwarz, E. R. (2011). Myths and truths of growth hormone and testosterone therapy in heart failure. *Expert Rev. Cardiovasc. Ther.* 9, 711–720.

O'Connell, M. D., Roberts, S. A., Srinivas-Shankar, U., Tajar, A., Connolly, M. J., Adams, J. E., Oldham, J. A., and Wu, F. C. (2011). Do the effects of testosterone on muscle strength, physical function, body composition, and quality of life persist six months after treatment in intermediate-frail and frail elderly men? *J. Clin. Endocrinol. Metab.* 96, 454–458.

Pike, C. J., Carroll, J. C., Rosario, E. R., and Barron, A. M. (2009). Protective actions of sex steroid hormones in Alzheimer's disease. *Front. Neuroendocrinol.* 30, 239–258.

Pizzi, O., Brandão, A. A., Magalhães, M. E. C., Pozzan, R., and Brandão, A. P. (2006). Pulse wave velocity. *Braz. J. Hypertens.* 13, 4.

Quan, A., Chakravarty, S., Chen, J. K., Chen, J. C., Loleh, S., Saini, N., Harris, R. C., Capdevila, J., and Quigley, R. (2004). Androgens augment proximal tubule transport. *Am. J. Physiol. Renal Physiol.* 287, F452–F459.

Quinkler, M., Bumke-Vogt, C., Meyer, B., Bahr, V., Oelkers, W., and Diederich, S. (2003). The human kidney is a progesterone-metabolizing and androgen-producing organ. *J. Clin. Endocrinol. Metab.* 88, 2803–2809.

Randall, V. A. (1994). Role of 5 alpha-reductase in health and disease. *Baillieres Clin. Endocrinol. Metab.* 8, 405–431.

Reckelhoff, J. F. (2005). Sex steroids, cardiovascular disease, and hypertension: unanswered questions and some speculations. *Hypertension* 45, 170–174.

Reckelhoff, J. F., and Romero, J. C. (2003). Role of oxidative stress in angiotensin-induced hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R893–R912.

Reckelhoff, J. F., Yanes, L. L., Iliescu, R., Fortepiani, L. A., and Granger, J. P. (2005). Testosterone supplementation in aging men and women: possible impact on cardiovascular-renal disease. *Am. J. Physiol. Renal Physiol.* 289, F941–F948.

Ripple, M. O., Hagopian, K., Oberley, T. D., Schatten, H., and Weindruch, R. (1999). Androgen-induced oxidative stress in human LNCaP prostate cancer cells is associated with multiple mitochondrial modifications. *Antioxid. Redox Signal.* 1, 71–81.

Romanelli, F., Sansone, A., and Lenzi, A. (2010). Erectile dysfunction in aging male. *Acta Biomed.* 81(Suppl. 1), 89–94.

Ruige, J. B., Mahmoud, A. M., De Bacquer, D., and Kaufman, J. M. (2011). Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis. *Heart* 97, 870–875.

Sa, E. Q., Sa, F. C., Guedes, A. D., and Verreschi, I. T. (2009). Serum testosterone and cardiovascular disease in men. *Arq. Bras. Endocrinol. Metabol.* 53, 915–922.

Saad, F., Gooren, L. J., Haider, A., and Yassin, A. (2008). A dose-response study of testosterone on sexual dysfunction and features of the metabolic syndrome using testosterone gel and parenteral testosterone undecanoate. *J. Androl.* 29, 102–105.

Sadanaga, T., Ohya, Y., Ohtsubo, T., Goto, K., Fujii, K., and Abe, I. (2002). Decreased 4-aminopyridine sensitive K+ currents in endothelial cells from hypertensive rats. *Hypertens. Res.* 25, 589–596.

Sader, M. A., McCredie, R. J., Griffiths, K. A., Wishart, S. M., Handelsman, D. J., and Celermajer, D. S. (2001). Oestradiol improves arterial endothelial function in healthy men receiving testosterone. *Clin. Endocrinol. (Oxf.)* 54, 175–181.

Seftel, A. D., Mack, R. J., Secrest, A. R., and Smith, T. M. (2004). Restorative increases in serum testosterone levels are significantly correlated to improvements in sexual functioning. *J. Androl.* 25, 963–972.

Shabsigh, R., Rajfer, J., Aversa, A., Traish, A. M., Yassin, A., Kalinchenko, S. Y., and Buvat, J. (2006). The evolving role of testosterone in the treatment of erectile dysfunction. *Int. J. Clin. Pract.* 60, 1087–1092.

Shih, H., Lee, B., Lee, R. J., and Boyle, A. J. (2011). The aging heart and post-infarction left ventricular remodeling. *J. Am. Coll. Cardiol.* 57, 9–17.

Simerly, R. B., Chang, C., Muramatsu, M., and Swanson, L. W. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J. Comp. Neurol.* 294, 76–95.

Song, D., Arikawa, E., Galipeau, D., Battell, M., and McNeill, J. H. (2004). Androgens are necessary for the development of fructose-induced hypertension. *Hypertension* 43, 667–672.

Thigpen, A. E., Silver, R. I., Guileyardo, J. M., Casey, M. L., McConnell, J. D., and Russell, D. W. (1993). Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. *J. Clin. Invest.* 92, 903–910.

Thijssen, D. H., Rongen, G. A., van Dijk, A., Smits, P., and Hopman, M. T. (2007). Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects. *J. Appl. Physiol.* 103, 852–857.

Titus, M. A., Schell, M. J., Lih, F. B., Tomer, K. B., and Mohler, J. L. (2005). Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin. Cancer Res.* 11, 4653–4657.

Tokunaga, O., Fan, J., Watanabe, T., Kobayashi, M., Kumazaki, T., and Mitsui, Y. (1992). Endothelin. Immunohistologic localization in aorta and biosynthesis by cultured human aortic endothelial cells. *Lab. Invest.* 67, 210–217.

Tostes, R. C., Fortes, Z. B., Callera, G. E., Montezano, A. C., Touyz, R. M., Webb, R. C., and Carvalho, M. H. (2008). Endothelin, sex and hypertension. *Clin. Sci.* 114, 85–97.

Toufexis, D. J., and Wilson, M. E. (2012). Dihydrotestosterone differentially modulates the cortisol response of the hypothalamic-pituitary-adrenal axis in male and female rhesus macaques, and restores circadian secretion of cortisol in females. *Brain Res.* 1429, 43–51.

Tuck, S. P., and Francis, R. M. (2009). Testosterone, bone and osteoporosis. *Front. Horm. Res.* 37, 123–132.

Ungvari, Z., Kaley, G., de Cabo, R., Sonntag, W. E., and Csiszar, A. (2010). Mechanisms of vascular aging: new perspectives. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1028–1041.

Van Guilder, G. P., Westby, C. M., Greiner, J. J., Stauffer, B. L., and DeSouza, C. A. (2007). Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise. *Hypertension* 50, 403–409.

Vanhoutte, P. M., Shimokawa, H., Tang, E. H., and Feletou, M. (2009). Endothelial dysfunction and vascular disease. *Acta Physiol. (Oxf.)* 196, 193–222.

Wu, F. C., and von Eckardstein, A. (2003). Androgens and coronary artery disease. *Endocr. Rev.* 24, 183–217.

Xu, Q., Wells, C. C., Garman, J. H., Asico, L., Escano, C. S., and Maric, C. (2008). Imbalance in sex hormone levels exacerbates diabetic renal disease. *Hypertension* 51, 1218–1224.

Yang, X. P., and Reckelhoff, J. F. (2011). Estrogen, hormonal replacement therapy and cardiovascular disease. *Curr. Opin. Nephrol. Hypertens.* 20, 133–138.

Yang, Y. M., Huang, A., Kaley, G., and Sun, D. (2009). eNOS uncoupling and endothelial dysfunction in aged vessels. *Am. J. Physiol. Heart Circ. Physiol.* 297, H1829–H1836.

Yaron, M., Greenman, Y., Rosenfeld, J. B., Izkhakov, E., Limor, R., Osher, E., Shenkerman, G., Tordjman, K., and Stern, N. (2009). Effect of testosterone replacement therapy on arterial stiffness in older hypogonadal men. *Eur. J. Endocrinol.* 160, 839–846.

Yildiz, O. (2007). Vascular smooth muscle and endothelial functions in aging. *Ann. N. Y. Acad. Sci.* 1100, 353–360.

Yildiz, O., Seyrek, M., Gul, H., Un, I., Yildirim, V., Ozal, E., Uzun, M., and Bolu, E. (2005). Testosterone relaxes human internal mammary artery in vitro. *J. Cardiovasc. Pharmacol.* 45, 580–585.

Yue, P., Chatterjee, K., Beale, C., Poole-Wilson, P. A., and Collins, P. (1995). Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91, 1154–1160.

Zhou, P., Fu, L., Pan, Z., Ma, D., Zhang, Y., Qu, F., Guo, L., Cao, J., Gao, Q., and Han, Y. (2008). Testosterone deprivation by castration impairs expression of voltage-dependent potassium channels in rat aorta. *Eur. J. Pharmacol.* 593, 87–91.

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Vascular aging and hemodynamic stability in the intraoperative period

Ferrante S. Gragasin[1,2,3,4]*†, Stephane L. Bourque[3,4,5]† and Sandra T. Davidge[2,3,4,5]

[1] *Department of Anesthesiology and Pain Medicine, University of Alberta, Edmonton, AB, Canada*
[2] *Department of Physiology, University of Alberta, Edmonton, AB, Canada*
[3] *Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada*
[4] *Women and Children's Health Research Institute, University of Alberta, Edmonton, AB, Canada*
[5] *Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada*

Edited by:
Elisabet Vila, Autonomous University of Barcelona, Spain

Reviewed by:
Akira Nishiyama, Kagawa University Medical School, Japan
Shenyuan Zhang, Texas A&M University, USA

****Correspondence:***
Ferrante S. Gragasin, Department of Anesthesiology and Pain Medicine, University of Alberta, Edmonton, AB, Canada T6G 2G3.
e-mail: gragasin@ualberta.ca

†Ferrante S. Gragasin and Stephane L. Bourque have contributed equally to this work.

The proportion of elderly people in the population is steadily increasing, and the inevitable consequence is that this subpopulation is more frequently represented in common medical procedures and surgeries. Understanding the circulatory changes that accompany the aging process is therefore becoming increasingly timely and relevant. In this short review, we discuss aspects of vascular control in aging that are particularly relevant in the maintenance of intraoperative hemodynamic stability. We subsequently review the effects of certain notable anesthetic agents with respect to the aging vasculature.

Keywords: aging, anesthesia, vascular, hemodynamics, intraoperative

INTRODUCTION

The percentage of the population over the age of 65 is steadily increasing and is expected to continue in the foreseeable future (United-Nations, 2002), with people over the age of 80 now among the fastest growing subset of the population (Jarad, 2011). Currently, cardiovascular diseases (CVDs) are the most common cause of death among elderly patients in the Western world, accounting for more than 40% of all mortalities among people aged 65–74, and 60% of people 85 years and older (Ungvari et al., 2010). Moreover, almost 70% of the population over the age of 70 has some degree of hypertension, which reflects the fact that blood pressure increases with age (Pedelty and Gorelick, 2008; Wills et al., 2011).

Despite the increased risk of CVD associated with aging, evidence indicates that health among the elderly population is improving, as illustrated by falling rates of ischemic heart disease, heart failure, and cerebrovascular disease among people over the age of 80 (National Heart, Lung and Blood Institute, 2006; Perls, 2009). The inevitable consequence of increasing life expectancy is that the aging population constitutes a greater proportion of patients undergoing medical treatments and presenting for surgery.

Since the elderly are associated with a higher incidence of disease and concurrent use of medications, intraoperative management of these patients can be challenging. For example, diabetes and hypertension are highly prevalent in this population and are known to impact various aspects of circulatory function. Medications such as beta blockers and angiotensin converting enzyme (ACE) inhibitors are commonly used in this age group, and the resultant drug interactions may also compromise hemodynamic stability and lead to untoward events. Even in the absence of these complicating factors, elderly patients are intrinsically more susceptible to the circulatory effects of anesthetic agents and other cardiovascular stressors due to the progressive structural and functional changes in the circulation that invariably occur with aging.

Due to increasing life expectancies in an ever-growing elderly population, studies pertaining to the circulatory changes in these patients are becoming increasingly timely and relevant. The focus of the present review is to consolidate the available information on aging as it pertains to vascular function and the intraoperative maintenance of hemodynamic stability in the elderly patient. Thus, rather than focusing on the mechanisms of aging that leads to progressive deterioration of circulatory function over a long period of time, we discuss aspects of the aging vasculature that are more relevant in the acute regulation of blood pressure – an important aspect in the intraoperative setting. We subsequently discuss the vascular effects of certain notable anesthetic agents, and where evidence is available, discuss some of the mechanisms underlying their effects. For more detailed reviews on the progressive vascular changes that occur with aging, see the following references: Seals et al. (2006), Mitchell (2008), Lekontseva et al. (2010), as well as those included in this issue of *Frontiers in Physiology*.

HEMODYNAMIC INSTABILITY AND AGING

One of the greatest challenges in intraoperative care for elderly patients is the maintenance of stable hemodynamic parameters. In particular, prevention of hypotension resulting in reduced end-organ perfusion is a primary concern. Elderly patients are

more prone to developing hypotension in general (Johnson et al., 1965; Caird et al., 1973; Lipsitz et al., 1983; Shannon et al., 1986), but this may be exacerbated in surgery due to the fact that many anesthetic agents are, by nature, hypotensive-inducing. Hypoperfusion may be particularly harmful in elderly patients for several reasons. First, organs in elderly patients are accustomed to perfusion at higher pressures (Hoffman et al., 1981; Harrison et al., 1988; Tomanek, 1990); thus, in a patient with ischemic heart disease and long-standing hypertension, a decrease in blood pressure intraoperatively toward the "low-normal range" of healthy adults may actually be detrimental. Second, due to an age-associated decline in function, organs in the elderly appear more susceptible to loss of perfusion (Corcoran and Hillyard, 2011).

Given the prevalence of hypertension in the elderly population, it is perhaps counterintuitive that these patients are more susceptible to intraoperative hypotension (Reich et al., 2005). This hemodynamic "instability" stems from multiple factors acting in concert. First, the circulation in elderly patients is highly dependent on preload, such that alterations in vascular tone can have a large influence on cardiac output and end-organ perfusion (Corcoran and Hillyard, 2011). Second, evidence indicates that "physiologic reserve" declines with age (Lipsitz, 2004). Physiologic reserve broadly refers to a system's capacity to cope with homeostatic perturbations. In the context of acute blood pressure regulation, changes from the operating setpoint are buffered by two principal mechanisms: (1) baroreceptor reflex, which modulates autonomic nervous system activity; and (2) vascular responsiveness to shear stress, which is coupled to endothelial nitric oxide (NO) production (Park, 2002). Both baroreceptor capacity to buffer acute blood pressure changes and shear stress-induced production of NO are markedly attenuated in elderly patients (Mancia et al., 1991), resulting in a diminished capacity to cope with alterations in the blood pressure.

A third factor that contributes to the hemodynamic instability in the elderly is the downregulation of vasodilatory mechanisms (e.g., NO, prostaglandins) and concomitant upregulation of vascular constrictor mechanisms [endothelin (ET)-1, sympathetic nervous system (SNS) stimulation, renin–angiotensin system (RAS) activity]. The sudden removal of these vasoconstrictor signaling mechanisms by anesthetic agents (see below) may contribute to profound and prolonged hypotension. A case in point is the increased tonic SNS activity that occurs with aging (Seals and Esler, 2000). The consequences of this increased SNS activity are several-fold, but perhaps the most important implications are that (1) there is a greater autonomic support of arterial pressure in elderly patients compared to young patients (Jones et al., 2001), and (2) there is reduced responsiveness of alpha and beta receptors to further increases in catecholamine release (Mack, 2004), which may implicate receptor downregulation and post-receptor desensitization mechanisms (Xiao and Lakatta, 1992; Pilotti et al., 2004). Iatrogenic removal of this sympathetic "overstimulation" may therefore result in an exaggerated reduction in vascular tone, due to the increased reliance of peripheral vascular resistance on autonomic stimulation, thereby resulting in hypotension. Moreover, there is a compounding effect of reduced baroreceptor sensitivity which results in reduced sympathetic discharge (McConnell et al., 2009), coupled with reduced peripheral tissue responsiveness to released catecholamines (Ramos Macias et al., 1992; Sugiyama et al., 1996; Davy et al., 1998; Dinenno et al., 2001). In all, the elderly circulatory system has a significantly reduced capacity to cope with hemodynamic disturbances, particularly in the case when SNS activity is reduced.

VASCULAR CHANGES ASSOCIATED WITH AGING AND EFFECTS OF ANESTHESIA: SPECIFIC AGENTS

Anesthetics can influence hemodynamic stability directly by altering cardiac function, vascular reactivity, or affecting cardiovascular reflexes (Mallow et al., 1976; Westenskow et al., 1978; Bennett and Stanley, 1979; Waxman et al., 1980; Brismar et al., 1982; Fairfield et al., 1991). Because cardiac and vascular function are in many ways compromised with aging, the circulatory effects of anesthetics are exacerbated in the elderly (Tokics et al., 1985). Given the emphasis of the present review, we focus on the vascular effects of two primary classes of general anesthetics: inhalational and intravenous. It is also noteworthy that due to changes in pharmacokinetics, elderly patients tend to be more sensitive to the neurological effects of anesthetic agents. Although not discussed herein, it is possible that the circulatory effects of anesthetic agents may also be exacerbated in the elderly due to greater distribution to the brain, particularly if greater concentrations are achieved in the vasoregulatory centers; for information on these aspects of anesthetic agents in the aging population, see the following reviews (Kalker et al., 1991; Shafer, 2000; Kruijt Spanjer et al., 2011).

INHALATIONAL ANESTHETICS

While many of the vascular effects of anesthetic agents have been well-characterized in young patients and animals, relatively few studies have been done in the elderly or in animal models of aging. Inhalational anesthetic agents such as sevoflurane, isoflurane, and desflurane are widely acknowledged to cause greater hypotension in elderly patients than in young patients, albeit the scientific studies supporting this observation are generally lacking (Ebert, 2002). The principal cause of hypotension appears to implicate a loss vascular tone and reduction in total peripheral resistance (Lynch, 1986; Housmans and Murat, 1988; Pagel et al., 1991, 1993; Kikura and Ikeda, 1993), although the specific mechanisms are not clear. There is evidence that inhalational anesthetic agents decrease SNS activity, resulting in reduced vascular tone and diminished baroreceptor responsiveness in the wake of blood pressure fluctuations (Seagard et al., 1984; Tanaka et al., 1996). This is important because sudden decreases in SNS activity may cause excessive and prolonged vasodilation in the elderly, as described above. Yu et al. (2004) have also shown that inhalational anesthetics may decrease vascular tone through inhibition of the RAS, which is known to be upregulated in aging (Baruch, 2004; Diz, 2008). Given that angiotensin II is a strong vasoconstrictor and may lead to increased vascular tone, it is tempting to speculate that the sudden withdrawal of this system may produce a profound hypotension, analogous to the situation involving the SNS, as described above. However, evidence that directly implicates the RAS in this exaggerated hypotensive effect is presently lacking.

Inhalational anesthetics have also been shown to have direct vasodilatory effects on the vasculature, which may be implicated in the exaggerated effects in the elderly. In isolated vessels of several

species, inhalational anesthetics mitigate the contractile responses to potassium chloride or norepinephrine; this effect was observed whether the endothelium was intact or not (Akata, 2007), suggesting a direct effect on vascular smooth muscle. Indeed, studies have shown that many such anesthetic agents influence calcium mobilization and sensitization (Tsuneyoshi et al., 2003; Akata, 2007) as well as potassium channel function (Tanaka et al., 2007) in vascular smooth muscle cells, with corresponding changes in vessel contractility. Given the importance of calcium channels and potassium channels in vascular tone, direct actions on these targets could potentially influence vascular responses to anesthetics in aging patients, although little information is available on the age-related changes with ion channels in the vasculature. There is evidence for diminished expression of large-conductance calcium-activated potassium channels in aging in both rodents and humans (Marijic et al., 2001; Toro et al., 2002), although their contribution to the vascular effects of inhalational anesthetic agents in aging is not currently known.

Inhalational anesthetics also have vasodilatory-promoting effects on endothelial function. Isoflurane has been shown to decrease ET-1 production (Boillot et al., 1995), which may have a profound effect in the elderly population given the increased ET-1 contribution to vascular tone with age (Stauffer et al., 2008; Goel et al., 2010). Inhalational anesthetics could also cause excessive vasodilation in the elderly via an antioxidant effect. While these agents do not appear to have intrinsic antioxidant effects *per se*, certain agents such as isoflurane and sevoflurane have been shown to increase endogenous antioxidant enzyme activity (Yang et al., 2011; Crystal et al., 2012), which may have a role in decreasing vascular tone. Indeed, in aging patients, a progressive increase in reactive oxygen species production is a well-defined etiological mechanism of vascular dysfunction and increased vasoconstriction (Herrera et al., 2010; Ungvari et al., 2010).

Interestingly, in contrast to those aforementioned studies, inhalational anesthetics have also been shown to increase vascular responsiveness to norepinephrine and KCl in rat mesenteric arteries, an effect that is entirely dependent on an intact endothelium (Stone and Johns, 1989; Izumi et al., 2000, 2001; Yoshino et al., 2005). Similarly, several groups have shown that these agents also inhibit endothelial-dependent vasodilation (Muldoon et al., 1988; Toda et al., 1992; Uggeri et al., 1992; Yoshida and Okabe, 1992; Park et al., 1997). The mechanisms underlying this latter observation are not clear, although neither NO, endothelium-derived hyperpolarizing factor, prostaglandin H synthase, and lipoxygenase pathways, nor ET-1, angiotensin II, or serotonin receptors appear to be implicated; calcium, potassium, and sodium channels are obvious potential targets. Taken together, these studies demonstrate that anesthetic effects on the endothelium, are in part, vasoconstrictor in nature. Although puzzling, this apparent disparity may be reconciled by the fact that the net effect of an agent is the sum of all simultaneous effects on the vessel (Muldoon et al., 1988). Therefore, while the endothelial effects of anesthetics appears to be vasoconstrictor in nature, the net effect, taking into account the effects of inhaled anesthetics on smooth muscle function, SNS, RAS, and other local and humoral factors, is vasodilatory. Since elderly patients are known to have altered endothelial secretory profiles, it is tempting to speculate that the hypotensive effects of inhalational anesthetics stem, at least in part, from mitigated endothelial vasoconstrictor effects producing an overall enhanced vasodilation. Studies are needed to directly test this hypothesis.

INTRAVENOUS ANESTHETICS

Etomidate

In vessels from young rats, etomidate causes direct vasodilation *ex vivo* (Bazin et al., 1998), involving increased vasodilator prostaglandins (Asher et al., 1992) and decreased ET-1 production (Hayashi et al., 1999). However, *in vivo*, administration of etomidate has little effect on blood pressure in young patients; in fact, its hemodynamic stability during induction of general anesthesia make it a preferred agent in many clinical scenarios (Gooding and Corssen, 1977; Criado et al., 1980). The hemodynamic stability of etomidate appears to stem from a lack of inhibition of SNS function (Ebert et al., 1992; Robinson et al., 1997). Thus, despite causing direct vasodilation, hemodynamic perturbations are transient and easily corrected by baroreceptor stimulation of heart rate and contractility. However, when administered to elderly patients, etomidate causes a 20–30% decrease in blood pressure (Larsen et al., 1988). This hypotensive effect is not well understood, although recent studies have provided insights into its mechanism. The reason may be due in part to the interaction between etomidate and adrenoceptor signaling. Etomidate enhances norepinephrine-induced constriction in mesenteric resistance arteries in young rats, resulting in increased vascular tone; this effect appears to be lost in aged rats (Shirozu et al., 2009). The authors of this study suggested that hypotension with etomidate in aged patients is not due to direct actions on the vasculature but rather due to interference with norepinephrine-induced vasoconstriction. Interestingly, Ebert et al. (1992) demonstrated that etomidate does not reduce overall release of catecholamines from sympathetic nerve terminals, suggesting that the majority of the blood pressure-lowering effects in the elderly may stem from altering the adrenoceptor responsiveness to catecholamines. Thus, despite increased NE levels in aging patients, etomidate-induced decrease in adrenoceptor responsiveness results in a more profound vasodilatory effect.

Etomidate is also known to cause adrenocortical suppression (Allolio et al., 1985; Lamberts et al., 1987), resulting in diminished cortisol release which can be problematic in certain patients (e.g., septic patients). Cortisol increases blood pressure principally by increasing sensitivity of the vasculature to catecholamines. Thus, etomidate-induced reduction in cortisol production may also play a role in causing hypotension in the intraoperative period, again making elderly patients more susceptible to this effect due to the progressive loss of catecholamine responsiveness with aging. This is supported by a recent case report of an elderly patient given a single dose of etomidate causing refractory hypotension that was only reversible with cortisol administration (Lundy et al., 2007). It is noteworthy that this mechanism is likely in addition to that described by Shirozu et al. (2009) since those experiments were done in isolated vessels.

Propofol

It was recently reported that the use of propofol over other general anesthetics, as well as age of the patient, are independent predictors

of clinically significant hypotension in patients undergoing general anesthesia (Reich et al., 2005). The majority of hypotensive effects of propofol appear to occur via reduced systemic vascular resistance. Part of this effect may stem from the fact that propofol, in contrast to etomidate, diminishes SNS function (Robinson et al., 1997), which may result in vasodilation. Moreover, loss of SNS activity results in decreased compensatory heart rate and contractility in the wake of hypotension, may be an important contributor to its hypotensive effects in the elderly, as described above. Propofol has also been shown to cause direct relaxation in several vascular beds, [e.g., aorta in rat (Boillot et al., 1999), coronary artery in the pig (Park et al., 1995), and mesenteric arteries in humans and rats (Moreno et al., 1997; Yamazaki et al., 2002)], although studies by Yamashita et al. (1999) and Kamitani et al. (1995) have shown opposite effects in rabbits , which may suggest species differences. In young animals, propofol increases NO production in isolated endothelial cells concomitant with decreased ET-1 release from the endothelium (Cheng et al., 2009). Tanabe et al. (1998) also showed that propofol inhibits ET-1 signaling in vascular smooth muscle. Given the role of diminished NO and increased ET-1 as important mechanisms in vascular aging, it seems plausible that these mediators may be implicated in the exaggerated hypotensive effects in elderly patients.

In our studies, we demonstrated that propofol causes enhanced mesenteric artery relaxation in aged rats when compared to young, and this effect was attributed to increased NO bioavailability (Gragasin and Davidge, 2009). We also demonstrated that the combination of antioxidant enzymes superoxide dismutase and catalase enhance vasodilation to acetylcholine similar to propofol in aged rats (Gragasin and Davidge, 2009). On the basis of these studies, we suggested that propofol may exert part of its vasodilatory effects via intrinsic antioxidant effects (Ansley et al., 1998), since it is structurally similar to α-tocopherol (vitamin E). Thus, we speculate that the vasodilatory effect may be more pronounced because aging vessels have increased oxidative stress. These data may also suggest that the use of NO donors to treat acute hypertensive crises intraoperatively may be harmful in elderly patients anesthetized with propofol. Indeed, it is already known that elderly patients have an increased sensitivity to nitroglycerin causing hypotension (Cahalan et al., 1992), and the presence of propofol may cause an unwanted synergistic blood pressure-lowering effect.

More recently, we investigated the effects of propofol in aged animals treated with the ACE inhibitor captopril. ACE inhibitors are commonly prescribed medications for blood pressure control in elderly patients, and it is well documented that these treated patients exhibit greater hypotension under general anesthesia (Bertrand et al., 2001; Comfere et al., 2005; Kheterpal et al., 2008). It has been suggested that there may be an interaction between ACE inhibitors and propofol which causes severe hypotension (Malinowska-Zaprzalka et al., 2005). In an aging rodent model, chronic treatment with captopril increases endothelial-dependent vasodilation in the presence of propofol (unpublished data). Interestingly, NO was not responsible for the differential response observed between control and captopril-treated rats (unpublished data); thus there may be alterations in other vasodilating mechanisms such as endothelial-derived hyperpolarizing factor (Hutri-Kahonen et al., 1997; Goto et al., 2000). The caveat is that isolated vessels may not recapitulate *in vivo* vascular responses, and therefore further investigation using integrated, whole-animal approaches are needed to identify the mechanisms underlying this enhanced relaxation.

CONCLUSION

Aging is associated with a multitude of changes at the vascular level, including structural changes, enhanced oxidative stress, and altered secretory profiles of vasoconstrictor and vasodilators. Anesthetic agents may acutely alter this balance leading to excessive and unwanted vasodilation in the elderly, and elucidating the underlying mechanisms dictating vascular function and its changes with age are therefore crucial. Moreover, given that elderly patients often have existing co-morbidities, additional studies are needed to elucidate the mechanisms of anesthetic-induced changes in hemodynamics in the presence of overt disease, such as hypertension, diabetes, chronic kidney disease, or more surreptitious conditions associated with aging, such as nutritional deficiencies (e.g., iron deficiency), which may have additional effects on vascular function. From a clinical perspective, the Anesthesiologist is tasked with making important decisions about drug use in elderly patients, considering factors such as patient safety, neurological recovery, and drug availability. Gaining further knowledge of the mechanisms of action of anesthetic agents at the vascular level as well in intact animal models can potentially be translated to the bedside, possibly changing clinical practice by enhancing management of circulatory health in the elderly during the intraoperative period.

REFERENCES

Akata, T. (2007). General anesthetics and vascular smooth muscle: direct actions of general anesthetics on cellular mechanisms regulating vascular tone. *Anesthesiology* 106, 365–391.

Allolio, B., Dorr, H., Stuttmann, R., Knorr, D., Engelhardt, D., and Winkelmann, W. (1985). Effect of a single bolus of etomidate upon eight major corticosteroid hormones and plasma ACTH. *Clin. Endocrinol. (Oxf.)* 22, 281–286.

Ansley, D. M., Lee, J., Godin, D. V., Garnett, M. E., and Qayumi, A. K. (1998). Propofol enhances red cell antioxidant capacity in swine and humans. *Can. J. Anaesth.* 45, 233–239.

Asher, E. F., Alsip, N. L., Zhang, P. Y., and Harris, P. D. (1992). Prostaglandin-related microvascular dilation in pentobarbital- and etomidate-anesthetized rats. *Anesthesiology* 76, 271–278.

Baruch, L. (2004). Hypertension and the elderly: more than just blood pressure control. *J. Clin. Hypertens. (Greenwich)* 6, 249–255.

Bazin, J. E., Dureuil, B., Danialou, G., Vicaut, E., Aubier, M., Desmonts, J. M., and Boczkowski, J. (1998). Effects of etomidate, propofol and thiopental anaesthesia on arteriolar tone in the rat diaphragm. *Br. J. Anaesth.* 81, 430–435.

Bennett, G. M., and Stanley, T. H. (1979). Cardiovascular effects of fentanyl during enflurane anesthesia in man. *Anesth. Analg.* 58, 179–182.

Bertrand, M., Godet, G., Meersschaert, K., Brun, L., Salcedo, E., and Coriat, P. (2001). Should the angiotensin II antagonists be discontinued before surgery? *Anesth. Analg.* 92, 26–30.

Boillot, A., Laurant, P., Berthelot, A., and Barale, F. (1999). Effects of propofol on vascular reactivity in isolated aortae from normotensive and spontaneously hypertensive rats. *Br. J. Anaesth.* 83, 622–629.

Boillot, A., Vallet, B., Marty, J., Auclerc, A., and Barale, F. (1995). Effects of halothane, enflurane and isoflurane on contraction of rat aorta induced by endothelin-1. *Br. J. Anaesth.* 75, 761–767.

Brismar, B., Hedenstierna, G., Lundh, R., and Tokics, L. (1982). Oxygen uptake, plasma catecholamines and cardiac output during neuroleptnitrous oxide and halothane anaesthesias. *Acta Anaesthesiol. Scand.* 26, 541–549.

Cahalan, M. K., Hashimoto, Y., Aizawa, K., Verotta, D., Ionescu, P., Balea, M., Eger, E. I. II, Benet, L. Z., Ehrenfeld, W. K., Goldstone, J., Reilly, L. M., Stoney, R. J., and Sheiner, L. B. (1992). Elderly, conscious patients have an accentuated hypotensive response to nitroglycerin. *Anesthesiology* 77, 646–655.

Caird, F. I., Andrews, G. R., and Kennedy, R. D. (1973). Effect of posture on blood pressure in the elderly. *Br. Heart J.* 35, 527–530.

Cheng, T. H., Chen, J. J., Chen, C. H., and Wong, K. L. (2009). Effects of propofol on cyclic strain-induced endothelin-1 expression in human umbilical vein endothelial cells. *Anesthesiology* 110, 74–80.

Comfere, T., Sprung, J., Kumar, M. M., Draper, M., Wilson, D. P., Williams, B. A., Danielson, D. R., Liedl, L., and Warner, D. O. (2005). Angiotensin system inhibitors in a general surgical population. *Anesth. Analg.* 100, 636–644, table of contents.

Corcoran, T. B., and Hillyard, S. (2011). Cardiopulmonary aspects of anaesthesia for the elderly. *Best Pract. Res. Clin. Anaesthesiol.* 25, 329–354.

Criado, A., Maseda, J., Antón, S., Gilsanz, F. J., and Avello, F. (1980). Cerebral activity during anesthesia induction with etomidate. *Rev. Esp. Anestesiol. Reanim.* 27, 15–20.

Crystal, G. J., Malik, G., Yoon, S. H., and Kim, S. J. (2012). Isoflurane late preconditioning against myocardial stunning is associated with enhanced antioxidant defenses. *Acta Anaesthesiol. Scand.* 56, 39–47.

Davy, K. P., Seals, D. R., and Tanaka, H. (1998). Augmented cardiopulmonary and integrative sympathetic baroreflexes but attenuated peripheral vasoconstriction with age. *Hypertension* 32, 298–304.

Dinenno, F. A., Tanaka, H., Stauffer, B. L., and Seals, D. R. (2001). Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. *J. Physiol. (Lond.)* 536, 977–983.

Diz, D. I. (2008). Lewis K. Dahl memorial lecture: the renin-angiotensin system and aging. *Hypertension* 52, 37–43.

Ebert, T. J. (2002). "Physiology of the cardiovascular effects of general anesthesia in the elderly," in *Syllabus on Geriatric Anesthesiology*, American Society of Anesthesiologists. Available at: http://asatest.asahq.org/clinical/geriatrics/phy.htm

Ebert, T. J., Muzi, M., Berens, R., Goff, D., and Kampine, J. P. (1992). Sympathetic responses to induction of anesthesia in humans with propofol or etomidate. *Anesthesiology* 76, 725–733.

Fairfield, J. E., Dritsas, A., and Beale, R. J. (1991). Haemodynamic effects of propofol: induction with 2.5 mg kg^{-1}. *Br. J. Anaesth.* 67, 618–620.

Goel, A., Su, B., Flavahan, S., Lowenstein, C. J., Berkowitz, D. E., and Flavahan, N. A. (2010). Increased endothelial exocytosis and generation of endothelin-1 contributes to constriction of aged arteries. *Circ. Res.* 107, 242–251.

Gooding, J. M., and Corssen, G. (1977). Effect of etomidate on the cardiovascular system. *Anesth. Analg.* 56, 717–719.

Goto, K., Fujii, K., Onaka, U., Abe, I., and Fujishima, M. (2000). Angiotensin-converting enzyme inhibitor prevents age-related endothelial dysfunction. *Hypertension* 36, 581–587.

Gragasin, F. S., and Davidge, S. T. (2009). The effects of propofol on vascular function in mesenteric arteries of the aging rat. *Am. J. Physiol. Heart Circ. Physiol.* 297, H466–H474.

Harrison, D. G., Florentine, M. S., Brooks, L. A., Cooper, S. M., and Marcus, M. L. (1988). The effect of hypertension and left ventricular hypertrophy on the lower range of coronary autoregulation. *Circulation* 77, 1108–1115.

Hayashi, Y., Minamino, N., Isumi, Y., Kangawa, K., Kuro, M., and Matsuo, H. (1999). Effects of thiopental, ketamine, etomidate, propofol and midazolam on the production of adrenomedullin and endothelin-1 in vascular smooth muscle cells. *Res. Commun. Mol. Pathol. Pharmacol.* 103, 325–331.

Herrera, M. D., Mingorance, C., Rodríguez-Rodríguez, R., and Alvarez de Sotomayor, M. (2010). Endothelial dysfunction and aging: an update. *Ageing Res. Rev.* 9, 142–152.

Hoffman, W. E., Albrecht, R. F., and Miletich, D. J. (1981). The influence of aging and hypertension on cerebral autoregulation. *Brain Res.* 214, 196–199.

Housmans, P. R., and Murat, I. (1988). Comparative effects of halothane, enflurane, and isoflurane at equipotent anesthetic concentrations on isolated ventricular myocardium of the ferret. I. Contractility. *Anesthesiology* 69, 451–463.

Hutri-Kahonen, N., Kahonen, M., Tolvanen, J. P., Wu, X., Sallinen, K., and Pörsti, I. (1997). Ramipril therapy improves arterial dilation in experimental hypertension. *Cardiovasc. Res.* 33, 188–195.

Izumi, K., Akata, T., and Takahashi, S. (2000). The action of sevoflurane on vascular smooth muscle of isolated mesenteric resistance arteries (part 1): role of endothelium. *Anesthesiology* 92, 1426–1440.

Izumi, K., Akata, T., and Takahashi, S. (2001). Role of endothelium in the action of isoflurane on vascular smooth muscle of isolated mesenteric resistance arteries. *Anesthesiology* 95, 990–998.

Jarad, N. (2011). The size of current and future problems. *Chron. Respir. Dis.* 8, 141–142.

Johnson, R. H., Smith, A. C., Spalding, J. M., and Wollner, L. (1965). Effect of posture on blood-pressure in elderly patients. *Lancet* 1, 731–733.

Jones, P. P., Shapiro, L. F., Keisling, G. A., Jordan, J., Shannon, J. R., Quaife, R. A., and Seals, D. R. (2001). Altered autonomic support of arterial blood pressure with age in healthy men. *Circulation* 104, 2424–2429.

Kalker, U., Leppek, S., and Peters, M. (1991). Screening for cardiovascular risk factors in public screening programs: are target groups being reached? *Offentl. Gesundheitswes.* 53, 604–605.

Kamitani, K., Yamazaki, M., Yukitaka, M., Ito, Y., and Momose, Y. (1995). Effects of propofol on isolated rabbit mesenteric arteries and veins. *Br. J. Anaesth.* 75, 457–461.

Kheterpal, S., Khodaparast, O., Shanks, A., O'Reilly, M., and Tremper, K. K. (2008). Chronic angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy combined with diuretic therapy is associated with increased episodes of hypotension in noncardiac surgery. *J. Cardiothorac. Vasc. Anesth.* 22, 180–186.

Kikura, M., and Ikeda, K. (1993). Comparison of effects of sevoflurane/nitrous oxide and enflurane/nitrous oxide on myocardial contractility in humans. load-independent and noninvasive assessment with transesophageal echocardiography. *Anesthesiology* 79, 235–243.

Kruijt Spanjer, M. R., Bakker, N. A., and Absalom, A. R. (2011). Pharmacology in the elderly and newer anaesthesia drugs. *Best Pract. Res. Clin. Anaesthesiol.* 25, 355–365.

Lamberts, S. W., Bons, E. G., Bruining, H. A., and de Jong, F. H. (1987). Differential effects of the imidazole derivatives etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursors by human adrenocortical cells. *J. Pharmacol. Exp. Ther.* 240, 259–264.

Larsen, R., Rathgeber, J., Bagdahn, A., Lange, H., and Rieke, H. (1988). Effects of propofol on cardiovascular dynamics and coronary blood flow in geriatric patients. a comparison with etomidate. *Anaesthesia* 43, 25–31.

Lekontseva, O., Chakrabarti, S., and Davidge, S. T. (2010). Endothelin in the female vasculature: a role in aging? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298, R509-R516.

Lipsitz, L. A. (2004). Physiological complexity, aging, and the path to frailty. *Sci. Aging Knowledge Environ.* 2004, pe16.

Lipsitz, L. A., Nyquist, R. P. J., Wei, J. Y., and Rowe, J. W. (1983). Postprandial reduction in blood pressure in the elderly. *N. Engl. J. Med.* 309, 1255–1256.

Lundy, J. B., Slane, M. L., and Frizzi, J. D. (2007). Acute adrenal insufficiency after a single dose of etomidate. *J. Intensive Care Med.* 22, 111–117.

Lynch, C. III. (1986). Differential depression of myocardial contractility by halothane and isoflurane in vitro. *Anesthesiology* 64, 620–631.

Mack, G. W. (2004). Hypothalamic control of body temperature: insights from the past. *J. Appl. Physiol.* 97, 1593–1594.

Malinowska-Zaprzalka, M., Wojewodzka, M., Dryl, D., Grabowska, S. Z., and Chabielska, E. (2005). Hemodynamic effect of propofol in enalapril-treated hypertensive patients during induction of general anesthesia. *Pharmacol. Rep.* 57, 675–678.

Mallow, J. E., White, R. D., Cucchiara, R. F., and Tarhan, S. (1976). Hemodynamic effects of isoflurane and halothane in patients with coronary artery disease. *Anesth. Analg.* 55, 135–138.

Mancia, G., Cleroux, J., Daffonchio, A., Ferrari, A. U., Giannattasio, C., and Grassi, G. (1991). Reflex control of circulation in the elderly. *Cardiovasc. Drugs Ther.* 4, 1223–1228.

Marijic, J., Li, Q., Song, M., Nishimaru, K., Stefani, E., and Toro, L. (2001). Decreased expression of voltage- and Ca2+-activated K+- channels in coronary smooth muscle during aging. *Circ. Res.* 88, 210–216.

McConnell, K., Somers, V. K., Kimball, T., Daniels, S., VanDyke, R., Fenchel, M., Cohen, A., Willging, P., Shamsuzzaman, A., and Amin, R. (2009). Baroreflex gain in children with obstructive sleep apnea. *Am. J. Respir. Crit. Care Med.* 180, 42–48.

Mitchell, G. F. (2008). Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J. Appl. Physiol.* 105, 1652–1660.

Moreno, L., Martinez-Cuesta, M. A., Muedra, V., Beltrán, B., and Esplugues, J. (1997). Role of the endothelium in the relaxation induced by propofol and thiopental in isolated arteries from man. *J. Pharm. Pharmacol.* 49, 430–432.

Muldoon, S. M., Hart, J. L., Bowen, K. A., and Freas, W. (1988). Attenuation of endothelium-mediated vasodilation by halothane. *Anesthesiology* 68, 31–37.

National Heart, Lung and Blood Institute. (2006). *Incidence and Prevalence: 2006 Chartbook on Cardiovascular and Lung Diseases.* Available at: http://www.nhlbi.nih.gov/resources/docs/cht-book_ip.htm. 2011

Pagel, P. S., Kampine, J. P., Schmeling, W. T., and Warltier, D. C. (1991). Influence of volatile anesthetics on myocardial contractility in vivo: desflurane versus isoflurane. *Anesthesiology* 74, 900–907.

Pagel, P. S., Kampine, J. P., Schmeling, W. T., and Warltier, D. C. (1993). Evaluation of myocardial contractility in the chronically instrumented dog with intact autonomic nervous system function: effects of desflurane and isoflurane. *Acta Anaesthesiol. Scand.* 37, 203–210.

Park, K. W. (2002). Cardiovascular effects of inhalational anesthetics. *Int. Anesthesiol. Clin.* 40, 1–14.

Park, K. W., Dai, H. B., Lowenstein, E., Darvish, A., and Sellke, F. W. (1997). Isoflurane and halothane attenuate endothelium-dependent vasodilation in rat coronary microvessels. *Anesth. Analg.* 84, 278–284.

Park, K. W., Dai, H. B., Lowenstein, E., and Sellke, F. W. (1995). Propofol-associated dilation of rat distal coronary arteries is mediated by multiple substances, including endothelium-derived nitric oxide. *Anesth. Analg.* 81, 1191–1196.

Pedelty, L., and Gorelick, P. B. (2008). Management of hypertension and cerebrovascular disease in the elderly. *Am. J. Med.* 121, S23–S31.

Perls, T. (2009). Health and disease in people over 85. *BMJ* 339, b4715.

Pilotti, M., Chodorow, M., and Thornton, K. C. (2004). Asymmetric interference effects in fragment completion: the consequences of recognition failures and successes. *Am. J. Psychol.* 117, 565–578.

Ramos Macias, A., de Miguel Martinez, I., Martín Sánchez, A. M., Gómez González, J. L., and Martín Galán, A. (1992). The incorporation of acyclovir into the treatment of peripheral paralysis: a study of 45 cases. *Acta Otorrinolaringol. Esp.* 43, 117–120.

Reich, D. L., Hossain, S., Krol, M., Baez, B., Patel, P., Bernstein, A., and Bodian, C. A. (2005). Predictors of hypotension after induction of general anesthesia. *Anesth. Analg.* 101, 622–628, table of contents.

Robinson, B. J., Ebert, T. J., O'Brien, T. J., Colinco, M. D., and Muzi, M. (1997). Mechanisms whereby propofol mediates peripheral vasodilation in humans. sympathoinhibition or direct vascular relaxation? *Anesthesiology* 86, 64–72.

Seagard, J. L., Hopp, F. A., Bosnjak, Z. J., Osborn, J. L., and Kampine, J. P. (1984). Sympathetic efferent nerve activity in conscious and isoflurane-anesthetized dogs. *Anesthesiology* 61, 266–270.

Seals, D. R., and Esler, M. D. (2000). Human ageing and the sympathoadrenal system. *J. Physiol. (Lond.)* 528, 407–417.

Seals, D. R., Moreau, K. L., Gates, P. E., and Eskurza, I. (2006). Modulatory influences on ageing of the vasculature in healthy humans. *Exp. Gerontol.* 41, 501–507.

Shafer, S. L. (2000). The pharmacology of anesthetic drugs in elderly patients. *Anesthesiol. Clin. North America* 18, 1–29.

Shannon, R. P., Wei, J. Y., Rosa, R. M., Epstein, F. H., and Rowe, J. W. (1986). The effect of age and sodium depletion on cardiovascular response to orthostasis. *Hypertension* 8, 438–443.

Shirozu, K., Akata, T., Yoshino, J., Setoguchi, H., Morikawa, K., and Hoka, S. (2009). The mechanisms of the direct action of etomidate on vascular reactivity in rat mesenteric resistance arteries. *Anesth. Analg.* 108, 496–507.

Stauffer, B. L., Westby, C. M., and DeSouza, C. A. (2008). Endothelin-1, aging and hypertension. *Curr. Opin. Cardiol.* 23, 350–355.

Stone, D. J., and Johns, R. A. (1989). Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. *Anesthesiology* 71, 126–132.

Sugiyama, Y., Matsukawa, T., Shamsuzzaman, A. S., Okada, H., Watanabe, T., and Mano, T. (1996). Delayed and diminished pressor response to muscle sympathetic nerve activity in the elderly. *J. Appl. Physiol.* 80, 869–875.

Tanabe, K., Kozawa, O., Kaida, T., Matsuno, H., Niwa, M., Ohta, S., Dohi, S., and Uematsu, T. (1998). Inhibitory effects of propofol on intracellular signaling by endothelin-1 in aortic smooth muscle cells. *Anesthesiology* 88, 452–460.

Tanaka, K., Kawano, T., Nakamura, A., Nazari, H., Kawahito, S., Oshita, S., Takahashi, A., and Nakaya, Y. (2007). Isoflurane activates sarcolemmal adenosine triphosphate-sensitive potassium channels in vascular smooth muscle cells: a role for protein kinase A. *Anesthesiology* 106, 984–991.

Tanaka, S., Tsuchida, H., Nakabayashi, K., Seki, S., and Namiki, A. (1996). The effects of sevoflurane, isoflurane, halothane, and enflurane on hemodynamic responses during an inhaled induction of anesthesia via a mask in humans. *Anesth. Analg.* 82, 821–826.

Toda, H., Nakamura, K., Hatano, Y., Nishiwada, M., Kakuyama, M., and Mori, K. (1992). Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. *Anesth. Analg.* 75, 198–203.

Tokics, L., Brismar, B., and Hedenstierna, G. (1985). Halothane-relaxant anaesthesia in elderly patients. *Acta Anaesthesiol. Scand.* 29, 303–308.

Tomanek, R. J. (1990). Response of the coronary vasculature to myocardial hypertrophy. *J. Am. Coll. Cardiol.* 15, 528–533.

Toro, L., Marijic, J., Nishimaru, K., Tanaka, Y., Song, M., and Stefani, E. (2002). Aging, ion channel expression, and vascular function. *Vascul. Pharmacol.* 38, 73–80.

Tsuneyoshi, I., Zhang, D., and Boyle, W. A. III. (2003). Ca2+- and myosin phosphorylation-independent relaxation by halothane in K+-depolarized rat mesenteric arteries. *Anesthesiology* 99, 656–665.

Uggeri, M. J., Proctor, G. J., and Johns, R. A. (1992). Halothane, enflurane, and isoflurane attenuate both receptor- and non-receptor-mediated EDRF production in rat thoracic aorta. *Anesthesiology* 76, 1012–1017.

Ungvari, Z., Kaley, G., de Cabo, R., Sonntag, W. E., and Csiszar, A. (2010). Mechanisms of vascular aging: new perspectives. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1028–1041.

United-Nations. (2002). *World Population Aging: 1950–2050.* New York: Department of Economic and Social Affairs Population Division.

Waxman, K., Shoemaker, W. C., and Lippmann, M. (1980). Cardiovascular effects of anesthetic induction with ketamine. *Anesth. Analg.* 59, 355–358.

Westenskow, D. R., Jordan, W. S., Hodges, M. R., and Stanleym, T. H. (1978). Correlation of oxygen uptake and cardiovascular dynamics during N2O-fentanyl and N2O-thiopental anesthesia in the dog. *Anesth. Analg.* 57, 37–41.

Wills, A. K., Lawlor, D. A., Matthews, F. E., Sayer, A. A., Bakra, E., Ben-Shlomo, Y., Benzeval, M., Brunner, E., Cooper, R., Kivimaki, M., Kuh, D., Muniz-Terrera, G., and Hardy, R. (2011). Life course trajectories of systolic blood pressure using longitudinal data from eight UK cohorts. *PLoS Med.* 8, e1000440. doi:10.1371/journal.pmed.1000440

Xiao, R. P., and Lakatta, E. G. (1992). Deterioration of beta-adrenergic modulation of cardiovascular function with aging. *Ann. N. Y. Acad. Sci.* 673, 293–310.

Yamashita, A., Kajikuri, J., Ohashi, M., Kanmura, Y., and Itoh, T. (1999). Inhibitory effects of propofol on acetylcholine-induced, endothelium-dependent relaxation and prostacyclin synthesis in rabbit mesenteric resistance arteries. *Anesthesiology* 91, 1080–1089.

Yamazaki, M., Nagakawa, T., Deng, J., Wang, Q., Ye, R., Li, X., Hu, S., Dong, H., and Xiong, L. (2002). The effects of propofol on neural and endothelial control of in situ rat mesenteric vascular smooth muscle transmembrane potentials. *Anesth. Analg.* 94, 892–897, table of contents.

Yang, Q., Dong, H., Deng, J., Wang, Q., Ye, R., Li, X., Hu, S., Dong, H., and Xiong, L. (2011). Sevoflurane preconditioning induces neuroprotection through reactive oxygen species-mediated up-regulation of antioxidant enzymes in rats. *Anesth. Analg.* 112, 931–937.

Yoshida, K., and Okabe, E. (1992). Selective impairment of endothelium-dependent relaxation by sevoflurane: oxygen free radicals participation. *Anesthesiology* 76, 440–447.

Yoshino, J., Akata, T., Izumi, K., and Takahashi, S. (2005). Multiple actions of halothane on contractile response to noradrenaline in isolated mesenteric resistance arteries.

Naunyn Schmiedebergs Arch. Pharmacol. 371, 500–515.

Yu, J., Tokinaga, Y., Ogawa, K., Iwahashi, S., and Hatano, Y. (2004). Sevoflurane inhibits angiotensin II-induced, protein kinase c-mediated but not Ca2+-elicited contraction of rat aortic smooth muscle. *Anesthesiology* 100, 879–884.

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