

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



## Female Vascular Senescence

Susana Novella<sup>1</sup>, Ana Paula Dantas<sup>2</sup>, Gloria Segarra<sup>1</sup>,  
Carlos Hermenegildo<sup>1</sup> and Pascual Medina<sup>1</sup>

<sup>1</sup>*Departamento de Fisiología, Universitat de València,  
Instituto de Investigación Sanitaria INCLIVA, Hospital Clínico Universitario, Valencia,*

<sup>2</sup>*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)  
Institut Clinic de Tòrax, Hospital Clinic Barcelona  
Spain*

### 1. Introduction

Long before the existence of cardiovascular imaging, Sir William Osler axiom that “*man is as old as his arteries*”. Followed by several physicians for decades, this aphorism has been widely confirmed by studies demonstrating that risk factors for cardiovascular disease increase as we age (Cooper et al., 1994; Lakatta & Levy, 2003). Nevertheless, a flaw in this statement is the generalization that men and women age similarly. Much data from clinical and basic research have established that vascular aging in women does not follow the same chronology as in men (Shaw et al., 2006; Pereira et al., 2010; Takenouchi et al., 2009). If known risk factors that influence cardiovascular aging are excluded (e.g. smoking, cholesterol, hypertension), men display a pattern of progressive vascular aging, while timing for vascular aging in women presents a clear hallmark, i.e. menopause (Taddei et al., 1996; Bucciarelli & Mannucci, 2009). Until menopause women are considered “hemodynamically younger” than men, based on epidemiological studies showing that the incidence of cardiovascular diseases in premenopausal women is markedly low compared to age-matched men (Messerli et al., 1987; Bairey Merz et al., 2006; Shaw et al., 2006). After menopause, however, these numbers rise to values that are close, or even higher, to those found in men (Lerner & Kannel, 1986; Eaker et al., 1993; Eaker et al., 1994). And so it one could say that “*man is as old as his arteries, although the arteries of a woman are as young as her hormones*”.

Cardiovascular disease is the primary cause of death among women after menopause (55%), compared to men (43%) even above all cancers combined (Rosamond et al., 2008). With increasing recognition of the importance of cardiovascular disease in women, the interest and emphasis on research concerning women and cardiovascular disease have grown substantially (Bairey Merz et al., 2006; Shaw et al., 2006). Despite this, there is still a concerning gap in the knowledge, understanding, and general awareness of mechanisms for cardiovascular aging in women. In this review, we will discuss clinical and experimental data that document the effects of aging, estrogens and hormonal replacement therapy on vascular function of females.

## 2. Effects of aging on vascular function

Vascular aging is a natural phenomenon that could be simply described as a consequence of physical stress. Arteries are elastic tissues, and as such are predisposed to fatigue and fracture with time, as a consequence of extension-relaxation cycles during heartbeats (Avolio et al., 1983; Avolio et al., 1985; O'Rourke & Hashimoto, 2007). In fact, fracture of elastic lamellae is observed with aging in aorta, and can account for the major physical changes seen in elder: dilation (after fracture of load-bearing material) and stiffening (by transfer of stress to the more rigid collagenous component of the arterial wall) (Lakatta, 2003).

There is growing evidence that vascular aging begins early in life, with evidence for alteration in vascular matrix proteins as early as the third decade in health individuals (Wallace, 2005; Tracy, 2006; Redheuil et al., 2010). This theory is mathematically supported by engineering studies establishing that fatigue and fracture of 10% of natural rubber occurs at  $8 \times 10^8$  extension-relaxation cycles, which is equivalent to 30 years at a heart rate of 70 beats/min (O'Rourke & Hashimoto, 2007). Biologically, a combination of imaging and histology studies have described age-associated increase in arterial thickening and a progressive reduction in aortic strain and distensibility, and have linked those changes to increased risk for cardiovascular disease (Lakatta & Levy, 2003; Lakatta, 2003; O'Rourke & Nichols, 2005; Redheuil et al., 2010). Although age-associated remodeling of arterial wall has been mostly described in patients with established risk for cardiovascular disease, few recent studies have shown similar age-related changes in healthy asymptomatic individuals (Redheuil et al., 2010). Similar age-related effects on arterial remodeling have been described in rodents and non-human primate without risk factors for cardiovascular disease, strengthening the hypothesis that aging *per se* can cause a series of alterations on mechanical properties that affect vascular function and lead to subsequent increased risk of cardiovascular disease.

Besides mechanical modifications, aging is also associated with several biochemical changes that are also implicated on the development and progression of cardiovascular disease. Dysfunction of both endothelial and smooth muscle molecular signaling appear to occur during aging process and favors vasospasm, thrombosis, inflammation and abnormal cell migration and proliferation (Lakatta, 2003; Briones et al., 2005; Barton, 2010; Herrera et al., 2010). The presence of endothelial dysfunction in the elder has been largely associated with malfunctioning of vascular tissue resulting, in turn, into cardiovascular disease (including atherosclerosis, hypertension or coronary artery disease) (Lakatta, 2003; Herrera et al., 2010), as well as renal dysfunction (Schmidt et al., 2001; Erdely et al., 2003), Alzheimer (Price et al., 2004) and erectile dysfunction (Burnett, 2006).

The mechanisms for age-associated endothelial dysfunction are multiple, though they are mostly associated to a decrease on nitric oxide (NO) bioavailability (Hayashi et al., 2008; Santhanam et al., 2008; Erusalimsky, 2009; Kim et al., 2009). NO is the major vascular messenger molecule involved in many physiological processes, including vasodilation and inhibition of thrombosis, cell migration and proliferation (Dudzinski & Michel, 2007; Lamas et al., 2007; Michel & Vanhoutte, 2010). Reduced endothelium-dependent and NO-mediated vasodilation has been described during aging in both human and animal models (Kim et al., 2009; Viridis et al., 2010).

A lower NO production in elderly may be based in either decreased NO synthesis or increased NO degradation. Several mechanisms to explain a reduction on NO production have been pointed out and include: 1) a decrease on the expression of endothelial NO synthase (eNOS) (Briones et al., 2005; Yoon et al., 2010); 2) a deficiency on NO precursor (L-arginine) (Santhanam et al., 2008) and eNOS cofactor (tetrahydrobiopterin - BH<sub>4</sub>) (Yoshida et al., 2000; Eskurza et al., 2005); or 3) an increase of endogenous eNOS inhibitors (asymmetric dimethylarginine - 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 superoxide anion (O<sub>2</sub><sup>-</sup>) is a potent contributor to lowering NO bioavailability and increasing endothelial dysfunction (Jacobson et al., 2007; Rodriguez-Manas et al., 2009).

Despite the decline in NO bioavailability could sufficiently explain most of the changes in the functioning of vascular cells, other molecules that are crucial to control vascular function have also been described to be modified by aging. In the regulation of vasomotion, cyclooxygenase (COX)-derived factors are of particular importance as they control both vascular relaxation and contraction. Under normal condition, COX-derived relaxing (PGI<sub>2</sub>) and contracting (TXA<sub>2</sub> and PGH<sub>2</sub>) are in perfect balance, and few studies have reported a prevalence in the production of relaxing COX factors in the vasculature of young and healthy individuals. During aging, however, a swap in this balance favoring to the release of contracting factors occurs, leading to an increase of vascular contraction. Moreover, activation of inflammatory pathways in the vascular wall plays a central role in the process of vascular aging. Several studies have created an important link between arterial aging and a pro-inflammatory endothelial phenotype, even in the absence of traditional risk factors for atherosclerosis. An age-associated shift to a pro-inflammatory gene expression profile, known as endothelial activation, induces up-regulation of cellular adhesion molecules and cytokines which increases endothelial-leukocyte interactions and permeability, mechanisms considered crucial on the initial steps for the development of atherosclerosis (Herrera et al., 2010; Seals et al., 2011).

Even though endothelial function is undoubtedly impaired in the elderly, how aging affects molecular biochemistry of vascular cells is largely unknown. Going back to the observation that vascular aging is a consequence of mechanical fatigue, one might speculate that the mechanical forces on the vascular wall could contribute to the damage on endothelial cell functioning. In fact, it is well known that blood vessels are under constant mechanical loading from flowing blood which cause internal stresses, known as endothelial shear stress (caused by flow) and circumferential stretch (caused by pressure). These mechanical forces not only cause morphological changes of endothelium and blood vessel wall, but also trigger a myriad of intracellular events in endothelial cells and activate biochemical and biological events (Lu & Kassab, 2011). The triggering of endothelial signaling by mechanic forces seems to be mostly determined by the cytoskeleton, which represents a highly dynamic network that constantly assembles and disassembles, playing an active role in responding to mechanical stimuli (Wong et al., 1983). The cytoskeleton rearranges upon changes on stress and stretch and activates signaling molecules, such as NO production, that are capable to regulate vascular tone in order to keep homeostasis (Su et al., 2005; Su et al., 2007). An increase in arterial wall stiffening by aging could alter the impact of a mechanical stimulus, and therefore induce a significant reduction or dysfunction in the signaling

pathways activated by shear stress (Kliche et al., 2011). In this regard, the chronically stiffed cells will lead to a decrease of NO, which will eventually lead to endothelial dysfunction.

Continuous damage to the endothelium from the daily pounding of the cycling pressure can also activate maintenance repair systems. When maintenance system is efficient (as in young individuals), endothelial cells likely correct the defect and keep going. On the other hand, when an irreversible damage occur or when endothelial cells are senescent, those inefficient cells are eventually eliminated by a mechanism yet to be described, while a “sister” circulating progenitor endothelial cells assume some repair function and will divide to fill up the gap (Thorin & Thorin-Trescases, 2009). Recent findings on progenitor stem cell research suggest that continuous division of progenitor endothelial cells for maintenance is likely the main response of an injured endothelium (Hill et al., 2003; Van Craenenbroeck & Conraads, 2010). Continuous cell division during life causes shortening in telomeres, a region of repetitive DNA sequences at the end of a chromosome, which protects the chromosomes from deterioration (Allsopp et al., 1995). Increasing evidence have support a role for reduction on telomere length with changes on cellular function and cellular senescence that may contribute to increased risk of vascular damage. In the long term, therefore, the regenerated endothelium may become dysfunctional as senescent endothelial cells start to express a pro-inflammatory, pro-oxidative, and pro-atherogenic phenotype (Chang & Harley, 1995; Bekaert et al., 2007; De Meyer T. et al., 2011).

In addition to mechanical fatigue, the vascular endothelium also undergoes important oxidative damage. The free-radical theory of aging states that organisms age because cells accumulate oxidative stress damage over time (de Grey, 2006; Camici et al., 2011). In other words, one can say that the body literally “rusts” with time. Growing evidence from research studies have supported this theory and have described an intimate relationship of increased oxidative stress with vascular dysfunction and increased risk for cardiovascular disease (Touyz, 2003; Griendling & Alexander, 1997; Harrison, 1997). Numerous studies underscore the importance of dysregulated oxidant and antioxidant balance in advancing age (Moon et al., 2001) and in the development and progression of atherosclerosis (Wassmann et al., 2004). Aging-associated increase in reactive oxygen species (ROS) are common to many species and despite decades of investigation, the mechanisms for the aging-related increase in ROS and how they affect vascular function have yet to be defined.

The main ROS proposed to be implicated on vascular aging process is the  $O_2^{\cdot-}$ . Increased  $O_2^{\cdot-}$  in the vessel wall has been well associated with decrease of NO bioavailability due to its rapid interaction and inactivation by  $O_2^{\cdot-}$ . In this regard, an increase of oxidative stress, and more specifically  $O_2^{\cdot-}$ , during aging could cause vascular damage simply by reducing the protective effect of NO in the vessel wall (Squadrito & Pryor, 1998; Harrison, 1997). However, increased oxidative stress has been implicated in more complex modulatory mechanisms that may affect vascular function by aging. Numerous studies have demonstrated that increase of oxidative stress contributes to the activation of transcriptional factors (such as NF- $\kappa$ B) that are key regulators of endothelial activation. By this way, aging-associated increase of ROS could favor endothelial cells to express a pro-inflammatory phenotype and increase the risk for cardiovascular disease (Herrera et al., 2010).

But proper vascular function does not lean on endothelium only. Vascular smooth muscle cells comprised by medial layer of blood vessels represent a dynamic component of the



vasculature, and thus may also be affected by aging. In fact, vascular smooth muscle cells degenerate and decrease in number when subjects reach middle or advanced age. Smooth muscle cells are intercalated between the elastic lamina and the elastic fibers that also undergo a process of degeneration, thinning, sectioning, fracture and decrease in volume with aging. In parallel, there is a marked increase on collagen fibers, mucinous substrate, and calcification of the intercellular substrates begins (Toda et al., 1980).

Biochemical studies have shown that the content of elastin in human aorta decreases with age (Spina et al., 1983). Large amounts of elastin are produced during the fetal or neonatal period but not later (Godfrey et al., 1993). An age-related decrease in the cross-links in elastin contributes significantly to the reduction in arterial elasticity (Watanabe et al., 1996). As the turnover of elastin and collagen requires a very long period of time (lasting more than 10 years), these molecules are likely to undergo the addition of a sugar or a glycooxidative reaction. Thus, advanced glycation end-products accumulate in the arteries with age and partially contribute to age-related arterial stiffness (Konova et al., 2004; Semba et al., 2009). Type I, III, and V collagens are the major components of the collagen fibers of large conductance vessels such as aorta. During infancy or early childhood, collagen fibers are absent in the aorta and begin to accumulate with age; this process is known as fibrosis or sclerosis. Most studies have shown an age-related increase in the collagen content in the aorta (Spina et al., 1983) and increase in the number of collagen cross-links (Watanabe et al., 1996). Both an increase in the collagen content and the number of cross-links contributes significantly to the stiffening of the elastic arteries, namely atherosclerosis.

Senescent vascular smooth muscle cells have been shown to exhibit a pro-calcificatory/osteoblastic phenotype (Reid & Andersen, 1993; Burton, 2009; Nakano-Kurimoto et al., 2009), that could play a major role in the pathophysiology of age-related vascular calcification, a well-known major risk factor for the development of cardiovascular diseases (Adragao et al., 2004; Thompson & Partridge, 2004). Calcification in tunica media (medial calcification) increases throughout ageing, and accumulation of calcium in the elastin-rich layer of the media is  $\geq 30$ -times more in the thoracic aorta at 90 years of age than that at 20 years of age (Elliott & McGrath, 1994). The underlying mechanisms that lead to the development of vascular calcification currently remain elusive. Calcification in the media usually occurs in the absence of macrophages and lipids, and is associated with  $\alpha$ -smooth muscle actin-positive vascular smooth muscle cells, suggesting that vascular smooth muscle cells are the main key player in medial calcification (Luo et al., 1997). Alternatively, ROS may have some involvement in the osteoblastic transition of vascular smooth muscle cells (Byon et al., 2008).

Researchers have examined the role of the redox state in vascular smooth muscle cells in the pathogenesis of vascular disease (Clempus & Griendling, 2006; Lyle & Griendling, 2006). Vascular smooth muscle cells present in atherosclerotic lesions proliferate more rapidly and show increased expression of genes for growth factors and other molecules involved in extracellular matrix remodeling (Schwartz, 1997; Newby, 2006). Proliferation of vascular smooth muscle cells is part of the initiation and the progression of atherosclerosis (Ross, 1993) and may occur in response to injury or as a result of aberrant apoptosis (Clarke et al., 2006). Besides, vascular smooth muscle cells appear to undergo an age-associated phenotypic modulation toward a dedifferentiated and synthetic state. Smooth muscle cell migration from the medial to the intimal compartment is a plausible mechanism for the

increased number of vascular smooth muscle cells within the diffusely thickened intima of central arteries as animals age (Miller et al., 2007).

In general, growth factors and hormones are the most potent activators that stimulate vascular smooth muscle growth, migration, and extracellular matrix synthesis. For instance, angiotensin II (Ang II) signaling has been widely linked to an age-associated increase in the migratory capacity of vascular smooth muscle cells and to the proinflammatory features of arterial aging. Ang II increases within the aged arterial wall and activates matrix metalloproteinase type II (MMP2) (Wang et al., 2003; Jiang et al., 2008). Ang II appears to initiate growth-promoting signal transduction through ROS-sensitive tyrosine kinases (Frank & Eguchi, 2003; Touyz et al., 2003).

### 3. Gender differences on vascular aging

Although arteries from females are so exposed to mechanical and oxidative damage as arteries from males, they seem do not follow the same time course for vascular aging, or at least, they do not age in the same way. Experimental and clinical studies support the hypothesis that men are hemodynamically older than age-matched, premenopausal women (Messerli et al., 1987; Bairey Merz et al., 2006; Shaw et al., 2006). With aging, the progression of cardiovascular disease occurs at an earlier age and become more severe in males compared to age-matched premenopausal females (Taddei et al., 1996; Viridis et al., 2010).

Arterial stiffening and distensibility are established markers for vascular aging and have been found to progressively increase with aging in both men and women. Studies in rodents indicate that there are gender differences in aging vessels, with stiffness increasing more in male than in females (Ruiz-Feria et al., 2009; Chan et al., 2011). Also in nonhuman primates, aortic stiffness has shown to be increased more in old male monkeys than in old females (Qiu et al., 2007). However, gender-associated relationship with those markers in humans remains unclear and currently limited studies have addressed to the evaluation of age-related vascular changes in man and women separately. Even though, many studies have performed their analysis on aging correlation with arterial stiffness and distensibility in men and women separately, their statistical models generally mask the gender differences in the influence of these variables (Breithaupt-Grogler & Belz, 1999; Segers et al., 2007; Redheuil et al., 2010; Miyoshi et al., 2011). In most clinical studies using small population group, the data do not provide sufficient power to detect significant gender-related differences in the rate of age-dependent change in vascular wall structure. The field still misses a large multi-centric populational study to identify whether aging-related effects are modulated by gender.

When it comes to the endothelium, sexual dimorphism on endothelial dysfunction and the progression of cardiovascular disease has also been well documented in various animal models (Ouchi et al., 1987; Ashton & Balment, 1991; Dantas et al., 2004a). With aging, males exhibit signals of impairment on endothelium-dependent relaxation at earlier age than do females (Kauser & Rubanyi, 1995; Huang et al., 1997). Thus far, the mechanisms better established to explain the gender- and aging-associated differences involve: 1) increased NO production by females (Huang et al., 1997); and 2) increased oxidative stress in male blood vessels (Dantas et al., 2004a). In this area of age-associated effects, a translation of animal

models to humans can be performed. Early clinical studies on gender- and aging-related effects on endothelium-dependent relaxation in forearm blood flow have identified a constant age-related decline in maximal vasodilation to acetylcholine per year (Taddei et al., 1996). In contrast, women were found to show a slight decrease per year in vasodilation to acetylcholine up to middle-age (around 50's). After that, the vascular decline in the responses to the endothelium-dependent vasodilator hasten, and even decline more quickly in comparison with men (Taddei et al., 1996).

Gender modulation of vascular tone is also observed in functional studies. Contractile responses are greater in the aorta of male than female rats (Stallone et al., 1991; Crews et al., 1999; Tostes et al., 2000). These differences may be related to the vasodilatory effects of estrogens (Crews et al., 1999; Kanashiro & Khalil, 2001) through a direct action on vascular smooth muscle (Jiang et al., 1992; Mugge et al., 1993; Gerhard & Ganz, 1995; Crews & Khalil, 1999). Expression of estrogen receptors in smooth muscle may vary depending on the gender and the gonadal status (Tamaya et al., 1993). The decreased vascular responses to constrictors may be related to 1) the higher relative abundance of estrogen receptors in females arteries (Collins et al., 1995), 2) estrogen-induced down-regulation of gene expression of vasoconstrictor receptors, such as Ang II (Nickenig et al., 2000), and 3) signaling mechanisms of vascular smooth muscle contraction downstream from receptor activation.

As intracellular free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) is important for the initiation of smooth muscle contraction (Horowitz et al., 1996), several studies have used isolated vascular preparations and smooth muscle cells from control and gonadectomized male and female animals to investigate the effect of estrogen on  $[\text{Ca}^{2+}]_i$  and the  $\text{Ca}^{2+}$ -mobilization mechanisms (i.e.  $\text{Ca}^{2+}$  release from the intracellular stores and  $\text{Ca}^{2+}$  entry from the extracellular space) (Zhang et al., 1994; Crews & Khalil, 1999; Crews et al., 1999; Murphy & Khalil, 1999; Murphy & Khalil, 2000; Novella et al., 2010).

Taken together those studies can suggest that, with aging, women are more protected against its deleterious consequences in the cardiovascular system than men. After menopause, however, this protection seems to be lost, since the incidence of cardiovascular disease increases considerably to levels similar (or higher) to those found in men. Because the onset of menopause is marked by the loss of endogenous estrogen production from the ovaries, estrogen is felt to confer the premenopausal protection.

#### **4. Vascular aging in females: Effects of estrogen on vascular function and aging**

In women, arterial aging includes an aggravating risk factor in comparison to men. The decrease in estrogen production by menopause is thought to contribute to increased cardiovascular risk. Although aging *per se* has detrimental effects in the vasculature of middle aged female, these effects seem to be potentiated by the lack of estrogen with menopause, and restored by estrogen replacement (Harman, 2004; Stice et al., 2009; Novella et al., 2010). For this reason it is particularly difficult to distinguish what would be the contribution of aging and the lack of estrogen in the control of vascular function in menopausal women.



Epidemiological observations and extensive basic laboratory research has shown that female sex hormones, and more specifically estrogen, has direct beneficial effects in the cardiovascular system (Staessen et al., 1989; Dantas et al., 1999; Tostes et al., 2003; Dantas et al., 2004b; Hinojosa-Laborde et al., 2004). Estrogen has been described to display a myriad of metabolic, hemodynamic, and vascular effects, which have been largely associated to cardiovascular protection in females. For instance, estrogen can promote cardiovascular protection by indirectly influence on the metabolism of lipoproteins or directly by acting on the modulation of molecular pathways in the vessel wall (Miller & Duckles, 2008). Receptors for estrogen have been identified biochemically and show a plentiful expression in both vascular smooth muscle and endothelium, reinforcing the idea that estrogen play a key role in the control of vascular function (Couse et al., 1997; Pau et al., 1998; Arnal et al., 2010).

When considering the major structural changes caused by aging, cross-sectional studies have shown that postmenopausal females taking hormone replacement therapy present lower arterial stiffness compared with their peers not taken estrogen (Moreau et al., 2003; Sumino et al., 2005; Sumino et al., 2006). Besides, radial artery distensibility fluctuates in accordance with estrogen levels during menstrual cycles (Giannattasio et al., 1999). Basic research using animal models for estrogen withdrawn and aging have proposed that estrogen play a modulatory role in the molecular mechanisms to prevent stiffening of arterial wall. As mentioned above, content of collagen and elastin into arterial wall is a key factor that contributes to arterial wall thickening and stiffening, and is mostly regulated by the activity of matrix metalloproteinases (MMP), a family of enzymes capable of degrading components of the extracellular matrix. During aging there is a marked decrease of MMP activity which results in increase of collagen accumulation and consequent stiffening. Data from studies in female rodents have found that estrogen replacement in ovariectomized animals increases MMP activity and restores structural properties of aged arteries similar to that of the young group (Zhang et al., 2000). Altogether these studies suggest that estrogen can exert a favorable modulatory effect on arterial stiffness with aging in females.

Endothelial dysfunction secondary to estrogen deprivation has been largely described and has been mostly associated with reductions in NO availability. Estrogen is known to increases NO bioavailability by mechanisms that involve either increase of NO generation directly or by decreasing  $O_2^-$  concentration, and thereby attenuating  $O_2^-$  mediated inactivation of NO. The 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 & Ignarro, 2003); 2) non-genomic activation of enzyme activity via a phosphatidylinositol-3-OH kinase (PI3-kinase)/phosphokinase B (PKB/AKT) mediated signaling pathway (Hisamoto et al., 2001); 3) increased  $[Ca^{2+}]_i$  in endothelial cells (Rubio-Gayosso et al., 2000); 4) decreased production of eNOS endogenous inhibitor, ADMA (Monsalve et al., 2007), and 5) attenuated  $O_2^-$  concentrations (Wassmann et al., 2001; Dantas et al., 2002; Ospina et al., 2002).

In addition to NO, actions of estrogen in the vasculature also influence the metabolism of other endothelium-derived factors (EDF). Estrogen has been described to positively up-regulate the production of endothelium-derived relaxing factors (EDRF), such as  $PGI_2$  (Sobrino et al., 2009; Sobrino et al., 2010) and the endothelium-derived hyperpolarizing factors (EDHF) (Golding & Kepler, 2001), both of which are important mediators of vascular relaxation in resistance-sized arteries. Concomitantly, a modulating role of estrogen on

constrictor factors (EDCF) is observed. Studies have shown that the beneficial effects of estrogen on the endothelium can be partially explained by an inhibitory effect on the production or action of the COX-derived vasoconstrictor agents (PGH<sub>2</sub> and TXA<sub>2</sub>) (Davidge & Zhang, 1998; Dantas et al., 1999; Novella et al., 2010) and endothelin-1 (ET-1) (David et al., 2001).

Estrogen has been shown to be a modulator of contractile responses by directly interfering with Ca<sup>2+</sup> into the vascular smooth muscle cells. Although some studies have shown that estrogen does not inhibit Ca<sup>2+</sup> release from the intracellular stores (Crews & Khalil, 1999; Murphy & Khalil, 1999), others have described that, supraphysiological concentrations of estrogen inhibit Ca<sup>2+</sup> influx from the extracellular space (Han et al., 1995; Crews & Khalil, 1999; Murphy & Khalil, 1999) by inhibiting Ca<sup>2+</sup> entry through voltage-gated Ca<sup>2+</sup> channels (Freay et al., 1997; Kitazawa et al., 1997; Crews & Khalil, 1999; Murphy & Khalil, 1999). The expression of the L-type Ca<sup>2+</sup> channels in cardiac muscle is substantially increased in estrogen receptor-deficient mice (Johnson et al., 1997), suggesting that estrogen may regulate Ca<sup>2+</sup> mobilization by a receptor-mediated system.

Although a genomic action of physiological concentrations of estrogen on the expression of the Ca<sup>2+</sup> channels may underlie the reduced cell contraction and [Ca<sup>2+</sup>]<sub>i</sub> observed in vascular smooth muscle cells of females, it is less likely to account for the acute inhibitory effects of 17β-estradiol on cell contraction and [Ca<sup>2+</sup>]<sub>i</sub> *in vitro*. The acute nature of the vasorelaxant effects of exogenous estrogen may represent additional non-genomic effects of estrogen on the mechanisms of Ca<sup>2+</sup> entry into vascular smooth muscle (Kitazawa et al., 1997; Crews & Khalil, 1999; Murphy & Khalil, 1999). Whether estrogen inhibits Ca<sup>2+</sup> entry by a direct or indirect action on plasmalemmal Ca<sup>2+</sup> channels remains unclear. Some studies have shown that estrogen blocks Ca<sup>2+</sup> channels in smooth muscle cells (Zhang et al., 1994; Nakajima et al., 1995) and others have shown that estrogen activates large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels, which could lead to hyperpolarization and decreased Ca<sup>2+</sup> entry through voltage-gated channels (White et al., 1995; Wellman et al., 1996). Estrogen may also decrease [Ca<sup>2+</sup>]<sub>i</sub> by stimulating Ca<sup>2+</sup> extrusion via the plasmalemmal Ca<sup>2+</sup> pump (Prakash et al., 1999). However, this mechanism seems less likely because the rate of decay of [Ca<sup>2+</sup>]<sub>i</sub> transients in smooth muscle incubated in Ca<sup>2+</sup>-free solution are not affected by estrogen (Crews & Khalil, 1999; Murphy & Khalil, 1999).

Other systems critically involved in the control of vascular function are also known to undergo estrogen modulation. For example, estrogen has been described to exert direct modulation on the components of renin-angiotensin system (RAS), which is a key regulator of blood pressure and smooth muscle cell growth. Estrogen reduces production of the active hormone of the RAS, Ang II in part, by inhibiting angiotensin-converting enzyme (ACE) expression. ACE activity in the circulation and in tissues, including the kidney and aorta, is reduced upon chronic estrogen replacement in animal models of menopause as well as in postmenopausal women (Brosnihan et al., 1999; Seely et al., 2004). Furthermore, estrogen attenuates the expression and tissue response to type 1 (AT<sub>1</sub>) angiotensin receptor in several cardiovascular tissues including the aorta, heart and kidney (Silva-Antonialli et al., 2000; Wu et al., 2003).

Because increased oxidative stress play a crucial role on aging-associated vascular damage, numerous studies have assessed the antioxidant potential of estrogens. Basic research in

human cultured endothelial cells revealed an antioxidant effect of estradiol (Hermenegildo et al., 2002a). In addition, clinical experimental studies have shown that different estrogens are capable of reducing oxidation of LDL- cholesterol and consequently the development of atherosclerosis (Keaney, Jr. et al., 1994; Shwaery et al., 1998; Hermenegildo et al., 2001; Hermenegildo et al., 2002b). In addition to its antioxidant role, estradiol exerts a direct effect by restoring the ADMA levels rise induced by oxidized LDL in human cultured endothelial cells acting through estrogen receptor  $\alpha$ . Estrogen also attenuates the deleterious effects induced by increased generation of ROS follow ischemia/reperfusion in distinct research models (Kim et al., 1996; Kim et al., 2006; Guo et al., 2010).

As a result of their phenolic molecular structure, several estrogens, such as  $17\beta$ -estradiol, estrone or estriol, have been described to act as ROS scavengers by virtue of the hydrogen-donating capacity of their phenolic groups (Halliwell & Grootveld, 1987; Dubey & Jackson, 2001). However, in these studies the direct effect of estrogens as scavenger can only be observed at concentrations above 1 micromolar (Arnal et al., 1996; Kim et al., 1996). Considering that plasma concentrations of estrogen in physiological conditions are in the nanomolar range is likely that the direct action as a scavenger is not the main anti-oxidant mechanism by estrogen. In fact, studies have established that estrogen modulates ROS concentration a mechanism that involves interaction with its nuclear receptor to decrease oxidative proteins and/or increase antioxidant enzymes expression. Many studies have shown that changes in estrogen levels are associated with altered levels of anti-oxidant enzymes including glutathione peroxidase, catalase and superoxide dismutase (Capel et al., 1981; Robb & Stuart, 2011; Sivritas et al., 2011). Moreover, recent studies have shown a modulatory effect of estrogen on  $O_2^-$ , via modulation of NADH/NADPH oxidases and  $AT_1$  receptor gene expression (major sources of  $O_2^-$  production) (Wassmann et al., 2001; Dantas et al., 2002).

Among all research on cellular aging process and its complication, there is a growing interest on mechanisms to delay or decrease telomere shortening by aging, and therefore, keeping cellular integrity and function (Allsopp et al., 1995). In this sense, few studies have explored the effects of estrogen on telomere shortening, and even fewer have addressed this issue in association with vascular aging. Mechanistic studies have found that estrogen treatment up-regulates transcription of hTERT, the catalytic subunit of human telomerase, in distinct cell lines, including endothelial cells (Farsetti et al., 2009). Intriguingly, activation of hTERT by NO signaling has also been reported (Vasa et al., 2000). Considering that estrogen augments NO production, one can suggest that estrogens doubly prevent vascular senescence: by directly interacting with its receptor and by increasing NO.

Although estrogen modulates several mechanisms that are closely associated with vascular aging, assuming that estrogen put a break on vascular aging in females would be rather speculative. There is no sufficient data available to correlate estrogen levels with a delay on progression of vascular aging and recent clinical trials have questioned the value of estrogen replacement therapy in protecting vascular function. The benefits of hormone replacement therapy on the life expectancy and vascular health of women have dramatically lost consensus since publication of the results of the Women's Health Initiative study (WHI) (Rossouw et al., 2002). The WHI trial did not find any cardiovascular benefit from estrogen in postmenopausal women and in fact, showed hormone replacement therapy was associated with increased risk to the cardiovascular system (Rossouw et al., 2002).

There is much controversy over the interpretation of WHI. Concerns raised include that the estrogens used in those trials are not naturally occurring and thus would not act identically to natural estrogens. Most importantly was the fact that the WHI, as well as the majority of clinical trial on hormone replacement therapy, studied a population of women that were estrogen deficient for, on average, 10 years before hormone replacement was initiated. Currently, it is not known if the vascular effects of estrogen are modified by aging in females. These observations, together with observational studies, have led scientists to create the so-called “timing hypothesis”. This theory states that estrogen-mediated benefits to prevent cardiovascular disease only occur when treatment is initiated before the detrimental effects of aging are established on vascular wall (Harman, 2006). In this regard, few recent basic studies have shown that aging is 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). The lack of estrogen responses in those animals was not related to age-associated changes in the plasma levels of estrogen or activity of estrogen receptors, but rather by possible age-related changes in estrogen-mediated signaling pathways in the vasculature.

Moreover, recent clinical studies have revealed that different risk factors for cardiovascular disease in postmenopausal women were lower among women 50 to 59 years old at enrolment for estrogen replacement therapy (Manson et al., 2007; Sherwood et al., 2007). Nevertheless, the field lacks detailed research on the long-term effects by estrogen and how it modulates cardiovascular function during aging. It remains unclear to what extent the protective effects of estrogen replacement well described in young females can be extrapolated to older ones. The aging issue still needs to be addressed in both experimental and clinical studies, and together, these studies demonstrate that estrogen has complex biologic effects and may influence the risk of cardiovascular events and other outcomes through multiple pathways. Therefore aging of a living organism should always be taken into account when the pharmacological and physiological responses by estrogens are determined.

## 5. Conclusion

We live in an aging society, with life expectancy far greater today than a century ago. The increasing incidence of older-age people in our society represents the culmination of centuries of medical, scientific, and social accomplishments. The challenge for modern medicine is how to increase the number of disease-free years in elderly people and improve quality of life in later years. However, a disproportionate number of people who reach old age suffer from cardiovascular diseases.

Clinical and basic studies have established that vascular aging in women does not follow the same chronology as in men. Men display a pattern of progressive vascular aging, while timing for vascular aging in women presents a clear hallmark, i.e. menopause. Several studies have shown that the incidence of cardiovascular diseases in premenopausal women is markedly low compared to age-matched men. After menopause, however, these figures increase to values that are close, or even higher, to those found in men. Cardiovascular disease is the primary cause of death among women after menopause. Despite this, there is still a concerning gap in the knowledge, understanding, and general awareness of mechanisms for cardiovascular aging in women.



It has become apparent that to improve diagnosis and treatment of vascular aging, the gender differences in cardiovascular control must be addressed. The impact of the menstrual cycle and hormonal replacement therapy on vascular function of females should also be taken into consideration. Different strategies have shown benefit in preventing, delaying or attenuating vascular aging. Nevertheless, it yet remains to be fully demonstrated whether vascular aging can be pharmacologically prevented. Further research efforts are needed to understand the causes and consequences of female vascular aging and propose new therapeutic strategies for the management of vascular senescence in women.

## 6. Acknowledgment

This work was supported by the Spanish Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III - FEDER-ERDF (grants FIS PI10/00518, FIS PI080176 and Red HERACLES RD06/0009), Consellería de Sanidad, Generalitat Valenciana (grants AP 097/2011, AP 104/2011 and GE 027/2011), and Spanish Society of Cardiology (DN040480).

## 7. References

- Adragao, T.; Pires, A.; Lucas, C.; Birne, R.; Magalhaes, L.; Goncalves, M., & Negrao, A.P. (2004). A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. *Nephrol Dial Transplant*, 19, 1480-1488.
- Allsopp, R.C.; Chang, E.; Kashefi-Aazam, M.; Rogaev, E.I.; Piatyszek, M.A.; Shay, J.W., & Harley, C.B. (1995). Telomere shortening is associated with cell division in vitro and in vivo. *Exp Cell Res*, 220, 194-200.
- Arnal, J.F.; Clamens, S.; Pechet, C.; Negre-Salvayre, A.; Allera, C.; Girolami, J.P.; Salvayre, R., & 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.
- Arnal, J.F.; Fontaine, C.; Billon-Gales, A.; Favre, J.; Laurell, H.; Lenfant, F., & Gourdy, P. (2010). Estrogen receptors and endothelium. *Arterioscler Thromb Vasc Biol*, 30, 1506-1512.
- Ashton, N., & Balment, R.J. (1991). Sexual dimorphism in renal function and hormonal status of New Zealand genetically hypertensive rats. *Acta Endocrinol (Copenh)*, 124, 91-97.
- Avolio, A.P.; Chen, S.G.; Wang, R.P.; Zhang, C.L.; Li, M.F., & 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.Q.; Li, W.Q.; Luo, Y.F.; Huang, Z.D.; Xing, L.F., & 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.
- 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., & 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.
- Bekaert, S.; De Meyer T.; Rietzschel, E.R.; De Buyzere, M.L.; De Bacquer D.; Langlois, M.; Segers, P.; Cooman, L.; Van Damme P.; Cassiman, P.; Van Criekeing W.; Verdonck, P.; De Backer, G.G.; Gillebert, T.C., & Van Oostveldt P. (2007). Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. *Aging Cell*, 6, 639-647.
- Breithaupt-Grogler, K., & Belz, G.G. (1999). Epidemiology of the arterial stiffness. *Pathol Biol (Paris)*, 47, 604-613.
- Briones, A.M.; Montoya, N.; Giraldo, J., & 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., & Ferrario, C.M. (1999). Bi-directional actions of estrogen on the renin-angiotensin system. *Braz J Med Biol Res*, 32, 373-381.
- Bucciarelli, P., & Mannucci, P.M. (2009). The hemostatic system through aging and menopause. *Climacteric*, 12 Suppl 1, 47-51.
- Burnett, A.L. (2006). The role of nitric oxide in erectile dysfunction: implications for medical therapy. *J Clin Hypertens (Greenwich)*, 8, 53-62.
- Burton, D.G. (2009). Cellular senescence, ageing and disease. *Age (Dordr)*, 31, 1-9.
- Byon, C.H.; Javed, A.; Dai, Q.; Kappes, J.C.; Clemens, T.L.; Darley-USmar, V.M.; McDonald, J.M., & Chen, Y. (2008). Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J Biol Chem*, 283, 15319-15327.
- Camici, G.G.; Shi, Y.; Cosentino, F.; Francia, P., & Luscher, T.F. (2011). Anti-aging medicine: molecular basis for endothelial cell-targeted strategies - a mini-review. *Gerontology*, 57, 101-108.
- Capel, I.D.; Jenner, M.; Williams, D.C.; Donaldson, D., & Nath, A. (1981). The effect of prolonged oral contraceptive steroid use on erythrocyte glutathione peroxidase activity. *J Steroid Biochem*, 14, 729-732.
- Chan, V.; Fenning, A.; Levick, S.P.; Loch, D.; Chunduri, P.; Iyer, A.; Teo, Y.L.; Hoey, A.; Wilson, K.; Burstow, D., & Brown, L. (2011). Cardiovascular changes during maturation and ageing in male and female spontaneously hypertensive rats. *J Cardiovasc Pharmacol*, 57, 469-478.
- Chang, E., & Harley, C.B. (1995). Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci U S A*, 92, 11190-11194.
- Clarke, M.C.; Figg, N.; Maguire, J.J.; Davenport, A.P.; Goddard, M.; Littlewood, T.D., & Bennett, M.R. (2006). Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nat Med*, 12, 1075-1080.
- Clempus, R.E., & Griendling, K.K. (2006). Reactive oxygen species signaling in vascular smooth muscle cells. *Cardiovasc Res*, 71, 216-225.
- Collins, P.; Rosano, G.M.; Sarrel, P.M.; Ulrich, L.; Adamopoulos, S.; Beale, C.M.; McNeill, J.G., & Poole-Wilson, P.A. (1995). 17 $\beta$ -Estradiol attenuates acetylcholine-induced

- coronary arterial constriction in women but not men with coronary heart disease. *Circulation*, 92, 24-30.
- Cooper, L.T.; Cooke, J.P., & Dzau, V.J. (1994). The vasculopathy of aging. *J Gerontol*, 49, B191-B196.
- Couse, J.F.; Lindzey, J.; Grandien, K.; Gustafsson, J.A., & Korach, K.S. (1997). Tissue distribution and quantitative analysis of estrogen receptor-alpha (ER $\alpha$ ) and estrogen receptor-beta (ER $\beta$ ) messenger ribonucleic acid in the wild-type and ER $\alpha$ -knockout mouse. *Endocrinology*, 138, 4613-4621.
- Crews, J.K., & Khalil, R.A. (1999). Antagonistic effects of 17 $\beta$ -estradiol, progesterone, and testosterone on Ca<sup>2+</sup> entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol*, 19, 1034-1040.
- Crews, J.K.; Murphy, J.G., & Khalil, R.A. (1999). Gender differences in Ca<sup>2+</sup> entry mechanisms of vasoconstriction in Wistar-Kyoto and spontaneously hypertensive rats. *Hypertension*, 34, 931-936.
- Dantas, A.P.; Franco, M.C.; Silva-Antonialli, M.M.; Tostes, R.C.; Fortes, Z.B.; Nigro, D., & Carvalho, M.H. (2004a). Gender differences in superoxide generation in microvessels of hypertensive rats: role of NAD(P)H-oxidase. *Cardiovasc Res*, 61, 22-29.
- Dantas, A.P.; Franco, M.C.; Tostes, R.C.; Fortes, Z.B.; Costa, S.G.; Nigro, D., & Carvalho, M.H. (2004b). Relative contribution of estrogen withdrawal and gonadotropins increase secondary to ovariectomy on prostaglandin generation in mesenteric microvessels. *J Cardiovasc Pharmacol*, 43, 48-55.
- Dantas, A.P.; Scivoletto, R.; Fortes, Z.B.; Nigro, D., & 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., & 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., & 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., & Zhang, Y. (1998). Estrogen replacement suppresses a prostaglandin H synthase-dependent vasoconstrictor in rat mesenteric arteries. *Circ Res*, 83, 388-395.
- de Grey, A.D. (2006). Free radicals in aging: causal complexity and its biomedical implications. *Free Radic Res*, 40, 1244-1249.
- De Meyer T.; Rietzschel, E.R.; De Buyzere, M.L.; Van Criekinge W., & Bekaert, S. (2011). Telomere length and cardiovascular aging: the means to the ends? *Ageing Res Rev*, 10, 297-303.
- Dubey, R.K., & 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., & Michel, T. (2007). Life history of eNOS: partners and pathways. *Cardiovasc Res*, 75, 247-260.

- Eaker, E.; Chesebro, J.H.; Sacks, F.M.; Wenger, N.K.; Whisnant, J.P., & Winston, M. (1994). Special report: cardiovascular disease in women. Special writing group. *Heart Dis Stroke*, 3, 114-119.
- Eaker, E.D.; Chesebro, J.H.; Sacks, F.M.; Wenger, N.K.; Whisnant, J.P., & Winston, M. (1993). Cardiovascular disease in women. *Circulation*, 88, 1999-2009.
- Elliott, R.J., & McGrath, L.T. (1994). Calcification of the human thoracic aorta during aging. *Calcif Tissue Int*, 54, 268-273.
- Erdely, A.; Greenfeld, Z.; Wagner, L., & 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., & Seals, D.R. (2005). Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *J Physiol*, 568, 1057-1065.
- Farsetti, A.; Grasselli, A.; Bacchetti, S.; Gaetano, C., & Capogrossi, M.C. (2009). The telomerase tale in vascular aging: regulation by estrogens and nitric oxide signaling. *J Appl Physiol*, 106, 333-337.
- Frank, G.D., & Eguchi, S. (2003). Activation of tyrosine kinases by reactive oxygen species in vascular smooth muscle cells: significance and involvement of EGF receptor transactivation by angiotensin II. *Antioxid Redox Signal*, 5, 771-780.
- Freay, A.D.; Curtis, S.W.; Korach, K.S., & Rubanyi, G.M. (1997). Mechanism of vascular smooth muscle relaxation by estrogen in depolarized rat and mouse aorta. Role of nuclear estrogen receptor and  $\text{Ca}^{2+}$  uptake. *Circ Res*, 81, 242-248.
- Gerhard, M., & Ganz, P. (1995). How do we explain the clinical benefits of estrogen? From bedside to bench. *Circulation*, 92, 5-8.
- Giannattasio, C.; Failla, M.; Grappiolo, A.; Stella, M.L.; Del, B.A.; Colombo, M., & Mancia, G. (1999). Fluctuations of radial artery distensibility throughout the menstrual cycle. *Arterioscler Thromb Vasc Biol*, 19, 1925-1929.
- Godfrey, M.; Nejezchleb, P.A.; Schaefer, G.B.; Minion, D.J.; Wang, Y., & Baxter, B.T. (1993). Elastin and fibrillin mRNA and protein levels in the ontogeny of normal human aorta. *Connect Tissue Res*, 29, 61-69.
- Golding, E.M., & 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.
- Griendling, K.K., & Alexander, R.W. (1997). Oxidative stress and cardiovascular disease. *Circulation*, 96, 3264-3265.
- Guo, J.; Krause, D.N.; Horne, J.; Weiss, J.H.; Li, X., & Duckles, S.P. (2010). Estrogen-receptor-mediated protection of cerebral endothelial cell viability and mitochondrial function after ischemic insult in vitro. *J Cereb Blood Flow Metab*, 30, 545-554.
- Halliwell, B., & 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., & Orimo, H. (1995).  $17\beta$ -Estradiol inhibits  $\text{Ca}^{2+}$  influx and  $\text{Ca}^{2+}$  release induced by thromboxane  $\text{A}_2$  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.
- Harrison, D.G. (1997). Endothelial function and oxidant stress. *Clin Cardiol*, 20, II-7.
- Hayashi, T.; Yano, K.; Matsui-Hirai, H.; Yokoo, H.; Hattori, Y., & Iguchi, A. (2008). Nitric oxide and endothelial cellular senescence. *Pharmacol Ther*, 120, 333-339.
- Hermenegildo, C.; Garcia-Martinez, M.C.; Tarin, J.J., & Cano, A. (2002a). Estradiol reduces F<sub>2α</sub>-isoprostane production in cultured human endothelial cells. *Am J Physiol Heart Circ Physiol*, 283, H2644-H2649.
- Hermenegildo, C.; Garcia-Martinez, M.C.; Tarin, J.J.; Llacer, A., & Cano, A. (2001). The effect of oral hormone replacement therapy on lipoprotein profile, resistance of LDL to oxidation and LDL particle size. *Maturitas*, 38, 287-295.
- Hermenegildo, C.; Garcia-Martinez, M.C.; Valldecabres, C.; Tarin, J.J., & Cano, A. (2002b). Transdermal estradiol reduces plasma myeloperoxidase levels without affecting the LDL resistance to oxidation or the LDL particle size. *Menopause*, 9, 102-109.
- Herrera, M.D.; Mingorance, C.; Rodriguez-Rodriguez, R., & Alvarez de Sotomayor 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., & Finkel, T. (2003). Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med*, 348, 593-600.
- Hinojosa-Laborde, C.; Craig, T.; Zheng, W.; Ji, H.; Haywood, J.R., & Sandberg, K. (2004). Ovariectomy augments hypertension in aging female Dahl salt-sensitive rats. *Hypertension*, 44, 405-409.
- Hisamoto, K.; Ohmichi, M.; Kurachi, H.; Hayakawa, J.; Kanda, Y.; Nishio, Y.; Adachi, K.; Tasaka, K.; Miyoshi, E.; Fujiwara, N.; Taniguchi, N., & Murata, Y. (2001). Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. *J Biol Chem*, 276, 3459-3467.
- Horowitz, A.; Menice, C.B.; Laporte, R., & Morgan, K.G. (1996). Mechanisms of smooth muscle contraction. *Physiol Rev*, 76, 967-1003.
- Huang, A.; Sun, D.; Kaley, G., & 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., & Sun, D. (2007). Aging enhances pressure-induced arterial superoxide formation. *Am J Physiol Heart Circ Physiol*, 293, H1344-H1350.
- Jiang, C.; Sarrel, P.M.; Poole-Wilson, P.A., & Collins, P. (1992). Acute effect of 17β-estradiol on rabbit coronary artery contractile responses to endothelin-1. *Am J Physiol*, 263, H271-H275.
- Jiang, L.; Wang, M.; Zhang, J.; Monticone, R.E.; Telljohann, R.; Spinetti, G.; Pintus, G., & Lakatta, E.G. (2008). Increased aortic calpain-1 activity mediates age-associated angiotensin II signaling of vascular smooth muscle cells. *PLoS One*, 3, e2231.
- Johnson, B.D.; Zheng, W.; Korach, K.S.; Scheuer, T.; Catterall, W.A., & Rubanyi, G.M. (1997). Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. *J Gen Physiol*, 110, 135-140.



- Kanashiro, C.A., & Khalil, R.A. (2001). Gender-related distinctions in protein kinase C activity in rat vascular smooth muscle. *Am J Physiol Cell Physiol*, 280, C34-C45.
- Kauser, K., & Rubanyi, G.M. (1995). Gender difference in endothelial dysfunction in the aorta of spontaneously hypertensive rats. *Hypertension*, 25, 517-523.
- Keaney, J.F., Jr.; Shwaery, G.T.; Xu, A.; Nicolosi, R.J.; Loscalzo, J.; Foxall, T.L., & Vita, J.A. (1994). 17 $\beta$ -estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. *Circulation*, 89, 2251-2259.
- Kielstein, J.T.; Bode-Boger, S.M.; Frolich, J.C.; Ritz, E.; Haller, H., & Fliser, D. (2003). Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation*, 107, 1891-1895.
- Kim, J.; Kil, I.S.; Seok, Y.M.; Yang, E.S.; Kim, D.K.; Lim, D.G.; Park, J.W.; Bonventre, J.V., & Park, K.M. (2006). Orchiectomy attenuates post-ischemic oxidative stress and ischemia/reperfusion injury in mice. A role for manganese superoxide dismutase. *J Biol Chem*, 281, 20349-20356.
- 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., & 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., & Lees, D.E. (1996). 17 beta-Estradiol prevents dysfunction of canine coronary endothelium and myocardium and reperfusion arrhythmias after brief ischemia/reperfusion. *Circulation*, 94, 2901-2908.
- Kitazawa, T.; Hamada, E.; Kitazawa, K., & Gaznabi, A.K. (1997). Non-genomic mechanism of 17 $\beta$ -oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. *J Physiol*, 499 ( Pt 2), 497-511.
- Kliche, K.; Jeggle, P.; Pavenstadt, H., & Oberleithner, H. (2011). Role of cellular mechanics in the function and life span of vascular endothelium. *Pflugers Arch*, 462, 209-217.
- Konova, E.; Baydanoff, S.; Atanasova, M., & Velkova, A. (2004). Age-related changes in the glycation of human aortic elastin. *Exp Gerontol*, 39, 249-254.
- 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., & 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., & 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.; Moninga, N.C., & 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., & 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., & 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.
- Lu, D., & Kassab, G.S. (2011). Role of shear stress and stretch in vascular mechanobiology. *J R Soc Interface*, 8, 1379-1385.
- Luo, G.; Ducky, P.; McKee, M.D.; Pinero, G.J.; Loyer, E.; Behringer, R.R., & Karsenty, G. (1997). Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*, 386, 78-81.
- Lyle, A.N., & Griendling, K.K. (2006). Modulation of vascular smooth muscle signaling by reactive oxygen species. *Physiology (Bethesda)*, 21, 269-280.
- 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., & 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., & Amodeo, C. (1987). Disparate cardiovascular findings in men and women with essential hypertension. *Ann Intern Med*, 107, 158-161.
- Michel, T., & Vanhoutte, P.M. (2010). Cellular signaling and NO production. *Pflugers Arch*, 459, 807-816.
- Miller, S.J.; Watson, W.C.; Kerr, K.A.; Labarrere, C.A.; Chen, N.X.; Deeg, M.A., & Unthank, J.L. (2007). Development of progressive aortic vasculopathy in a rat model of aging. *Am J Physiol Heart Circ Physiol*, 293, H2634-H2643.
- Miller, V.M., & Duckles, S.P. (2008). Vascular actions of estrogens: functional implications. *Pharmacol Rev*, 60, 210-241.
- Miyoshi, H.; Mizuguchi, Y.; Oishi, Y.; Iuchi, A.; Nagase, N.; Ara, N., & Oki, T. (2011). Early detection of abnormal left atrial-left ventricular-arterial coupling in preclinical patients with cardiovascular risk factors: evaluation by two-dimensional speckle-tracking echocardiography. *Eur J Echocardiogr*, 12, 431-439.
- Monsalve, E.; Oviedo, P.J.; Garcia-Perez, M.A.; Tarin, J.J.; Cano, A., & Hermenegildo, C. (2007). Estradiol counteracts oxidized LDL-induced asymmetric dimethylarginine production by cultured human endothelial cells. *Cardiovasc Res*, 73, 66-72.
- Moon, S.K.; Thompson, L.J.; Madamanchi, N.; Ballinger, S.; Papaconstantinou, J.; Horaist, C.; Runge, M.S., & Patterson, C. (2001). Aging, oxidative responses, and proliferative capacity in cultured mouse aortic smooth muscle cells. *Am J Physiol Heart Circ Physiol*, 280, H2779-H2788.
- Moreau, K.L.; Donato, A.J.; Seals, D.R.; DeSouza, C.A., & 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.
- Mugge, A.; Riedel, M.; Barton, M.; Kuhn, M., & Lichtlen, P.R. (1993). Endothelium independent relaxation of human coronary arteries by 17 $\beta$ -oestradiol in vitro. *Cardiovasc Res*, 27, 1939-1942.

- Murphy, J.G., & Khalil, R.A. (1999). Decreased  $[Ca^{2+}]_i$  during inhibition of coronary smooth muscle contraction by  $17\beta$ -estradiol, progesterone, and testosterone. *J Pharmacol Exp Ther*, 291, 44-52.
- Murphy, J.G., & Khalil, R.A. (2000). Gender-specific reduction in contractility and  $[Ca^{2+}]_i$  in vascular smooth muscle cells of female rat. *Am J Physiol Cell Physiol*, 278, C834-C844.
- Nakajima, T.; Kitazawa, T.; Hamada, E.; Hazama, H.; Omata, M., & Kurachi, Y. (1995).  $17\beta$ -Estradiol inhibits the voltage-dependent L-type  $Ca^{2+}$  currents in aortic smooth muscle cells. *Eur J Pharmacol*, 294, 625-635.
- Nakano-Kurimoto, R.; Ikeda, K.; Uraoka, M.; Nakagawa, Y.; Yutaka, K.; Koide, M.; Takahashi, T.; Matoba, S.; Yamada, H.; Okigaki, M., & Matsubara, H. (2009). Replicative senescence of vascular smooth muscle cells enhances the calcification through initiating the osteoblastic transition. *Am J Physiol Heart Circ Physiol*, 297, H1673-H1684.
- Newby, A.C. (2006). Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. *Cardiovasc Res*, 69, 614-624.
- Nickenig, G.; Strehlow, K.; Wassmann, S.; Baumer, A.T.; Albory, K.; Sauer, H., & Bohm, M. (2000). Differential effects of estrogen and progesterone on AT(1) receptor gene expression in vascular smooth muscle cells. *Circulation*, 102, 1828-1833.
- Novella, S.; Dantas, A.P.; Segarra, G.; Novensa, L.; Bueno, C.; Heras, M.; Hermenegildo, C., & Medina, P. (2010). Gathering of aging and estrogen withdrawal in vascular dysfunction of senescent accelerated mice. *Exp Gerontol*, 45, 868-874.
- O'Rourke, M.F., & Hashimoto, J. (2007). Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol*, 50, 1-13.
- O'Rourke, M.F., & Nichols, W.W. (2005). Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension*, 45, 652-658.
- Ospina, J.A.; Krause, D.N., & Duckles, S.P. (2002).  $17\beta$ -estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke*, 33, 600-605.
- Ouchi, Y.; Share, L.; Crofton, J.T.; Iitake, K., & Brooks, D.P. (1987). Sex difference in the development of deoxycorticosterone-salt hypertension in the rat. *Hypertension*, 9, 172-177.
- Pau, C.Y.; Pau, K.Y., & Spies, H.G. (1998). Putative estrogen receptor beta and alpha mRNA expression in male and female rhesus macaques. *Mol Cell Endocrinol*, 146, 59-68.
- Pereira, T.M.; Nogueira, B.V.; Lima, L.C.; Porto, M.L.; Arruda, J.A.; Vasquez, E.C., & Meyrelles, S.S. (2010). Cardiac and vascular changes in elderly atherosclerotic mice: the influence of gender. *Lipids Health Dis*, 9, 87.
- Prakash, Y.S.; Togaibayeva, A.A.; Kannan, M.S.; Miller, V.M.; Fitzpatrick, L.A., & Sieck, G.C. (1999). Estrogen increases  $Ca^{2+}$  efflux from female porcine coronary arterial smooth muscle. *Am J Physiol*, 276, H926-H934.
- Price, J.M.; Hellermann, A.; Hellermann, G., & Sutton, E.T. (2004). Aging enhances vascular dysfunction induced by the Alzheimer's peptide beta-amyloid. *Neurol Res*, 26, 305-311.

- Qiu, H.; Depre, C.; Ghosh, K.; Resuello, R.G.; Natividad, F.F.; Rossi, F.; Peppas, A.; Shen, Y.T.; Vatner, D.E., & Vatner, S.F. (2007). Mechanism of gender-specific differences in aortic stiffness with aging in nonhuman primates. *Circulation*, 116, 669-676.
- Redheuil, A.; Yu, W.C.; Wu, C.O.; Mousseaux, E.; de Cesare A.; Yan, R.; Kachenoura, N.; Bluemke, D., & Lima, J.A. (2010). Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. *Hypertension*, 55, 319-326.
- Reid, J.D., & Andersen, M.E. (1993). Medial calcification (whitlockite) in the aorta. *Atherosclerosis*, 101, 213-224.
- Robb, E.L., & 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., & Sanchez-Ferrer, C.F. (2009). Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. *Aging Cell*, 8, 226-238.
- 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., & 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, e25-146.
- Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, 362, 801-809.
- 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., & 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.
- Rubio-Gayosso, I.; Sierra-Ramirez, A.; Garcia-Vazquez, A.; Martinez-Martinez, A.; Munoz-Garcia, O.; Morato, T., & Ceballos-Reyes, G. (2000). 17 $\beta$ -estradiol increases intracellular calcium concentration through a short-term and nongenomic mechanism in rat vascular endothelium in culture. *J Cardiovasc Pharmacol*, 36, 196-202.
- Ruiz-Feria, C.A.; Yang, Y.; Thomason, D.B.; White, J.; Su, G., & Nishimura, H. (2009). Pulse wave velocity and age- and gender-dependent aortic wall hardening in fowl. *Comp Biochem Physiol A Mol Integr Physiol*, 154, 429-436.
- Santhanam, L.; Christianson, D.W.; Nyhan, D., & Berkowitz, D.E. (2008). Arginase and vascular aging. *J Appl Physiol*, 105, 1632-1642.
- Schmidt, R.J.; Beierwaltes, W.H., & Baylis, C. (2001). Effects of aging and alterations in dietary sodium intake on total nitric oxide production. *Am J Kidney Dis*, 37, 900-908.
- Schwartz, S.M. (1997). Smooth muscle migration in atherosclerosis and restenosis. *J Clin Invest*, 100, S87-S89.
- Seals, D.R.; Jablonski, K.L., & Donato, A.J. (2011). Aging and vascular endothelial function in humans. *Clin Sci (Lond)*, 120, 357-375.

- Seely, E.W.; Brosnihan, K.B.; Jeunemaitre, X.; Okamura, K.; Williams, G.H.; Hollenberg, N.K., & 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.
- Segers, P.; Rietzschel, E.R.; De Buyzere, M.L.; Vermeersch, S.J.; De Bacquer D.; Van Bortel, L.M.; De Backer G.; Gillebert, T.C., & Verdonck, P.R. (2007). Noninvasive (input) impedance, pulse wave velocity, and wave reflection in healthy middle-aged men and women. *Hypertension*, 49, 1248-1255.
- Semba, R.D.; Najjar, S.S.; Sun, K.; Lakatta, E.G., & Ferrucci, L. (2009). Serum carboxymethyllysine, an advanced glycation end product, is associated with increased aortic pulse wave velocity in adults. *Am J Hypertens*, 22, 74-79.
- 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., & 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., & Hinderliter, A.L. (2007). Age moderates the short-term effects of transdermal 17 $\beta$ -estradiol on endothelium-dependent vascular function in postmenopausal women. *Arterioscler Thromb Vasc Biol*, 27, 1782-1787.
- Shwaery, G.T.; Vita, J.A., & Keaney, J.F., Jr. (1998). Antioxidant protection of LDL by physiologic concentrations of estrogens is specific for 17 $\beta$ -estradiol. *Atherosclerosis*, 138, 255-262.
- Silva-Antonialli, M.M.; Fortes, Z.B.; Carvalho, M.H.; Scivoletto, R., & Nigro, D. (2000). Sexual dimorphism in the response of thoracic aorta from SHR 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., & 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.; Mata, M.; Laguna-Fernandez, A.; Novella, S.; Oviedo, P.J.; Garcia-Perez, M.A.; Tarin, J.J.; Cano, A., & Hermenegildo, C. (2009). Estradiol stimulates vasodilatory and metabolic pathways in cultured human endothelial cells. *PLoS One*, 4, e8242.
- Sobrino, A.; Oviedo, P.J.; Novella, S.; Laguna-Fernandez, A.; Bueno, C.; Garcia-Perez, M.A.; Tarin, J.J.; Cano, A., & Hermenegildo, C. (2010). Estradiol selectively stimulates endothelial prostacyclin production through estrogen receptor- $\alpha$ . *J Mol Endocrinol*, 44, 237-246.
- Spina, M.; Garbisa, S.; Hinnie, J.; Hunter, J.C., & Serafini-Fracassini, A. (1983). Age-related changes in composition and mechanical properties of the tunica media of the upper thoracic human aorta. *Arteriosclerosis*, 3, 64-76.
- Squadrito, G.L., & Pryor, W.A. (1998). Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med*, 25, 392-403.



- Staessen, J.; Bulpitt, C.J.; Fagard, R.; Lijnen, P., & Amery, A. (1989). The influence of menopause on blood pressure. *J Hum Hypertens*, 3, 427-433.
- Stallone, J.N.; Crofton, J.T., & Share, L. (1991). Sexual dimorphism in vasopressin-induced contraction of rat aorta. *Am J Physiol*, 260, H453-H458.
- Stice, J.P.; Eiserich, J.P., & 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.
- Su, Y.; Kondrikov, D., & Block, E.R. (2005). Cytoskeletal regulation of nitric oxide synthase. *Cell Biochem Biophys*, 43, 439-449.
- Su, Y.; Kondrikov, D., & Block, E.R. (2007). Beta-actin: a regulator of NOS-3. *Sci STKE*, 2007, e52.
- Sumi, D., & 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., & 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., & 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.
- Taddei, S.; Virdis, A.; Ghiadoni, L.; Mattei, P.; Sudano, I.; Bernini, G.; Pinto, S., & Salvetti, A. (1996). Menopause is associated with endothelial dysfunction in women. *Hypertension*, 28, 576-582.
- Takenouchi, Y.; Kobayashi, T.; Matsumoto, T., & Kamata, K. (2009). Gender differences in age-related endothelial function in the murine aorta. *Atherosclerosis*, 206, 397-404.
- Tamaya, T.; Wada, K.; Nakagawa, M.; Misao, R.; Itoh, T.; Imai, A., & Mori, H. (1993). Sexual dimorphism of binding sites of testosterone and dihydrotestosterone in rabbit model. *Comp Biochem Physiol Comp Physiol*, 105, 745-749.
- Thompson, G.R., & Partridge, J. (2004). Coronary calcification score: the coronary-risk impact factor. *Lancet*, 363, 557-559.
- Thorin, E., & Thorin-Trescases, N. (2009). Vascular endothelial ageing, heartbeat after heartbeat. *Cardiovasc Res*, 84, 24-32.
- Toda, T.; Tsuda, N.; Nishimori, I.; Leszczynski, D.E., & Kummerow, F.A. (1980). Morphometrical analysis of the aging process in human arteries and aorta. *Acta Anat (Basel)*, 106, 35-44.
- Tostes, R.C.; David, F.L.; Carvalho, M.H.; Nigro, D.; Scivoletto, R., & Fortes, Z.B. (2000). Gender differences in vascular reactivity to endothelin-1 in deoxycorticosterone-salt hypertensive rats. *J Cardiovasc Pharmacol*, 36, S99-101.
- Tostes, R.C.; Nigro, D.; Fortes, Z.B., & Carvalho, M.H. (2003). Effects of estrogen on the vascular system. *Braz J Med Biol Res*, 36, 1143-1158.
- Touyz, R.M. (2003). Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev Cardiovasc Ther*, 1, 91-106.
- Touyz, R.M.; Cruzado, M.; Tabet, F.; Yao, G.; Salomon, S., & Schiffrin, E.L. (2003). Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. *Can J Physiol Pharmacol*, 81, 159-167.



- Tracy, R.P. (2006). The five cardinal signs of inflammation: Calor, Dolor, Rubor, Tumor ... and Penuria (Apologies to Aulus Cornelius Celsus, De medicina, c. A.D. 25). *J Gerontol A Biol Sci Med Sci*, 61, 1051-1052.
- Van Craenenbroeck, E.M., & Conraads, V.M. (2010). Endothelial progenitor cells in vascular health: focus on lifestyle. *Microvasc Res*, 79, 184-192.
- Vasa, M.; Breitschopf, K.; Zeiher, A.M., & Dimmeler, S. (2000). Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ Res*, 87, 540-542.
- Virdis, A.; Ghiadoni, L.; Giannarelli, C., & Taddei, S. (2010). Endothelial dysfunction and vascular disease in later life. *Maturitas*, 67, 20-24.
- Wallace, D.C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*, 39, 359-407.
- Wang, M.; Takagi, G.; Asai, K.; Resuello, R.G.; Natividad, F.F.; Vatner, D.E.; Vatner, S.F., & Lakatta, E.G. (2003). Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. *Hypertension*, 41, 1308-1316.
- Wassmann, S.; Baumer, A.T.; Strehlow, K.; van Eickels M.; Grohe, C.; Ahlbory, K.; Rosen, R.; Bohm, M., & Nickenig, G. (2001). Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation*, 103, 435-441.
- Wassmann, S.; Wassmann, K., & Nickenig, G. (2004). Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. *Hypertension*, 44, 381-386.
- Watanabe, M.; Sawai, T.; Nagura, H., & Suyama, K. (1996). Age-related alteration of cross-linking amino acids of elastin in human aorta. *Tohoku J Exp Med*, 180, 115-130.
- Wellman, G.C.; Bonev, A.D.; Nelson, M.T., & Brayden, J.E. (1996). Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca<sup>2+</sup>-dependent K<sup>+</sup> channels. *Circ Res*, 79, 1024-1030.
- White, R.E.; Darkow, D.J., & Lang, J.L. (1995). Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. *Circ Res*, 77, 936-942.
- Wong, A.J.; Pollard, T.D., & Herman, I.M. (1983). Actin filament stress fibers in vascular endothelial cells in vivo. *Science*, 219, 867-869.
- Wu, Z.; Maric, C.; Roesch, D.M.; Zheng, W.; Verbalis, J.G., & Sandberg, K. (2003). Estrogen regulates adrenal angiotensin AT<sub>1</sub> receptors by modulating AT<sub>1</sub> receptor translation. *Endocrinology*, 144, 3251-3261.
- Wynne, F.L.; Payne, J.A.; Cain, A.E.; Reckelhoff, J.F., & 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., & 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., & 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., & 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., & Sowers, J.R. (1994).  $17\beta$ -Estradiol attenuates voltage-dependent  $\text{Ca}^{2+}$  currents in A7r5 vascular smooth muscle cell line. *Am J Physiol*, 266, C975-C980.
- Zhang, Y.; Stewart, K.G., & Davidge, S.T. (2000). Estrogen replacement reduces age-associated remodeling in rat mesenteric arteries. *Hypertension*, 36, 970-974.

IntechOpen

IntechOpen



## **Senescence**

Edited by Dr. Tetsuji Nagata

ISBN 978-953-51-0144-4

Hard cover, 850 pages

**Publisher** InTech

**Published online** 29, February, 2012

**Published in print edition** February, 2012

The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Susana Novella, Ana Paula Dantas, Gloria Segarra, Carlos Hermenegildo and Pascual Medina (2012). Female Vascular Senescence, *Senescence*, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: <http://www.intechopen.com/books/senescence/female-vascular-senescence>

**INTech**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen