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Female Vascular Senescence

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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 $8x10^8$ 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; Virdis 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_4) (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_2^-) 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_2) and contracting (TXA_2 and PGH_2) 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^-. Increased O_2^- in the vessel wall has been well associated with decrease of NO bioavailability due to its rapid interaction and inactivation by O_2^-. In this regard, an increase of oxidative stress, and more specifically O_2^-, 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-κB) 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 ≥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 α-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; Virdis 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 Ca^{2+} concentration ($[Ca^{2+}]i$) 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 $[Ca^{2+}]i$ and the Ca^{2+}-mobilization mechanisms (i.e. Ca^{2+} release from the intracellular stores and 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_2 and TXA_2) (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^{2+} into the vascular smooth muscle cells. Although some studies have shown that estrogen does not inhibit Ca^{2+} release from the intracellular stores (Crews & Khalil, 1999; Murphy & Khalil, 1999), others have described that, supraphysiological concentrations of estrogen inhibit Ca^{2+} influx from the extracellular space (Han et al., 1995; Crews & Khalil, 1999; Murphy & Khalil, 1999) by inhibiting Ca^{2+} entry through voltage-gated Ca^{2+} channels (Freay et al., 1997; Kitazawa et al., 1997; Crews & Khalil, 1999; Murphy & Khalil, 1999). The expression of the L-type Ca^{2+} channels in cardiac muscle is substantially increased in estrogen receptor-deficient mice (Johnson et al., 1997), suggesting that estrogen may regulate Ca^{2+} mobilization by a receptor-mediated system.

Although a genomic action of physiological concentrations of estrogen on the expression of the Ca^{2+} channels may underlie the reduced cell contraction and $[Ca^{2+}]i$ 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 $[Ca^{2+}]i$ *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^{2+} entry into vascular smooth muscle (Kitazawa et al., 1997; Crews & Khalil, 1999; Murphy & Khalil, 1999). Whether estrogen inhibits Ca^{2+} entry by a direct or indirect action on plasmalemmal Ca^{2+} channels remains unclear. Some studies have shown that estrogen blocks Ca^{2+} channels in smooth muscle cells (Zhang et al., 1994; Nakajima et al., 1995) and others have shown that estrogen activates large conductance Ca^{2+}-activated K^+ channels, which could lead to hyperpolarization and decreased Ca^{2+} entry through voltage-gated channels (White et al., 1995; Wellman et al., 1996). Estrogen may also decrease $[Ca^{2+}]i$ by stimulating Ca^{2+} extrusion via the plasmalemmal Ca^{2+} pump (Prakash et al., 1999). However, this mechanism seems less likely because the rate of decay of $[Ca^{2+}]i$ transients in smooth muscle incubated in Ca^{2+}-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_1) 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 α. 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 17β-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 giving 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).

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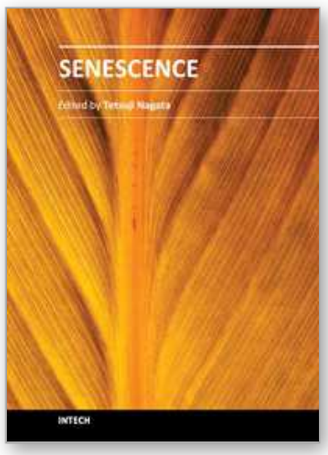

Senescence
Edited by Dr. Tetsuji Nagata

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

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