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## THE PRESSURE OF AGING

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### Abstract

Significant hemodynamic changes ensue with aging, leading to an ever-growing epidemic of hypertension. Alterations in central arterial properties play a major role in these hemodynamic changes. These alterations are characterized by an initial decline in aortic distensibility and a rise of diastolic blood pressure, followed by a sharp increase in pulse wave velocity (PWV), and a rise in pulse pressure (PP) beyond the sixth decade. However, the trajectories of PWV and PP diverge with advancing age, more profoundly in men than women, likely reflecting the more pronounced aortic dilatation in men. There is an increased prevalence of salt-sensitive hypertension with advancing age, that is, in part, mediated by marinobufagenin, an endogenous sodium pump ligand, which is also linked to central arterial stiffness. Within the arterial wall, biomechanical and humoral changes are accompanied by significant biomolecular alterations producing a proinflammatory state, in which activation of angiotensin II signaling plays a pivotal role. This proinflammatory state is in origin a reparatory response to a damaged arterial wall under a pulsatile injury. However the same reparatory process results in fibrosis, which in turn worsens arterial stiffness and produce more pulsatile hemodynamics; this relationship between the pulsatile damage and proinflammatory state is best described as a feed-forward loop. Effective efforts to counter the surging epidemic of hypertension with the aging of our population should be aimed at revealing early, pre-clinical hemodynamic and arterial wall alterations, and develop interventions that halt these processes before they reach the stage the medical community defined as “disease”.

### Keywords

Hemodynamics; aging; arterial wall remodeling; age-dependent salt-sensitive hypertension; pulse wave velocity; arterial fibrosis; angiotensin II; marinobufagenin; an endogenous steroidal Na pump inhibitor

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## Introduction

Major hemodynamic alterations ensue with aging and are primarily attributable to central arterial stiffening;<sup>1</sup> these alterations become manifest in the ever expanding epidemic of hypertension affecting 1 of every 3 Americans in general, and a staggering rate of 7 of every 10 of those aged 65 and older.<sup>2</sup> The burden of this epidemic is projected to increase with the aging of our population as the percentage of people aged 65 and older increasing from 15% in 2014 to 22% in 2030.<sup>3</sup> This shift in demographics makes predominantly systolic hypertension, a challenging form hypertension that becomes more prevalent with advancing age and that dominates the hypertension field.<sup>4</sup> All of these factors make hypertension a growing health burden with a predicted hypertension-related health care costs reaching \$389 billion in 2030.<sup>5</sup> Because aging is the major risk factor for predominantly systolic hypertension the pivotal question to effectively address this hypertension epidemic is: What underlies arterial aging?

Evolutionary biologists proclaim that most of us are wired to be very healthy until around the end of child-bearing age, because the main reason for our reality, they would say, is to perpetually insure the next generation of our species; after that, from an evolutionary perspective, there is no essential reason for us to be alive. However, we do remain alive longer well beyond our evolutionary life expectancy prescription because our environment has been enhanced by improved hygiene, better nutrition, better health care, etc. But, in outliving our paleolithic gene set, disorder among molecules within our body progressively increases and functional declines accumulate with advancing age and beyond 40 years we become vulnerable to what are referred to as “degenerative chronic diseases of aging”. Arterial aging and the associated alteration in hemodynamics are no exception.

The hypertension field has been struggling to better understand the complex relationship between arterial wall mechanical changes, i.e. arterial wall stiffness, and hemodynamics, i.e. arterial pressure alterations, and has been fixated on defining: “which factor is the culprit?” The failure to reach an unequivocal answer is not surprising and is probably a reflection of the naivety of the question is. In health “homeostasis”, a functional crosstalk between central and peripheral segments of the circulation is required for optimal operation. Once this homeostasis is broken, for any reason, a vicious cycle of minute alterations in central arterial mechanical and hemodynamics ensues and propagates, leading to the dramatic changes in arterial properties observed with aging. Thus, in this paradigm, it is close to impossible to detect the “initial” minute alteration and point to it as the “culprit”.

Given this extreme complexity, any efforts directed to treating or preventing the increase in blood pressure would be infertile without major efforts are committed to further explore the underpinning aging of the arterial wall. These efforts should be aimed at revealing early alterations, starting in young adulthood, before reaching the clinical threshold, and to develop stage/process-specific interventions rather than the “one-size fits all” approach that dominate the field of hypertension. In the meantime, targeting elements of this vicious cycle, “the master perpetuators” of arterial aging appears to be the most promising strategy to reduce the health burden of hypertension.

## Age-Associated Dysfunction of Central Arteries and Hemodynamic Alterations

Epidemiological studies have pursued description of changes in arterial stiffness with aging and to answer the question of whether central arterial stiffness is a cause or an effect of elevated systolic and pulse blood pressure. One of the difficulties in addressing this issue relates to a degree of ambiguity and restrictions of the terms “blood pressure” and “arterial stiffness”. This question might be better articulated if we expand these terms and rename “arterial stiffness” as “arterial mechanical alterations”, and “elevated blood pressure” as “hemodynamic alterations”; then, it becomes apparent that arterial mechanical properties and hemodynamics are inseparable and the question on what starts first, mechanical or hemodynamic alterations appears to be somewhat naïve.

### In the beginning... Early 3<sup>rd</sup> decade arterial mechanical alterations and the rise in diastolic blood pressure

The initial evidence of age-associated arterial mechanical alterations is observed by the third decade of age with sharp declines in aortic strain, the difference between aortic systolic and diastolic diameter relative to the diastolic diameter, and in aortic distensibility, i.e. aortic strain divided by pulse pressure; analysis of cardiac magnetic resonance (CMR) imaging of 111 healthy participants have shown that nearly 80% of the total decline of aortic strain occurs before the 5th decade of age after which the decline in strain is less dramatic<sup>6</sup> (Figure 1A), however the small decline in aortic strain beyond the age of 50 is associated with an exponential increase in PWV with aging (Figure 1B).

During the same stages of life, as central arterial strain is becoming reduced, there is an **increase** in diastolic blood pressure,<sup>7,8</sup> which could be associated with progression of increased endothelial dysfunction with aging, leading to increased peripheral vascular resistance. While such changes are not very well studied in normal human subjects, those with essential hypertension demonstrate eutrophic, inward remodeling of small arteries,<sup>9,10</sup> It is not clear whether the increase in diastolic blood pressure alters the optimal conformation of the arterial wall, making it prone to an increase in hemodynamic stress, and to additional mechanical alterations beyond those resulting from simply stretching elastin fibers at higher pressures and shifting the load to the stiffer collagen fibers.

### Beyond the 6<sup>th</sup> Decade of Age: Central Arterial Mechanics and the Pulsatile Hemodynamic

Dramatic hemodynamic alterations ensue beyond the 6<sup>th</sup> decade of life, as increases in SBP and PP become the hallmarks of arterial aging.<sup>8</sup> PWV and blood pressure parameters have been shown to be strongly associated in cross-sectional and prospective studies.<sup>11,12</sup> The Framingham Heart Study, using data from Cycle 7 to predict SBP and PWV in Cycle 8, has shown that higher PWV in Cycle 7 is associated with higher SBP in Cycle 8;<sup>13</sup> however, the opposite was not true: SBP at Cycle 7 was not associated with higher PWV in Cycle 8. It is noteworthy, however, that one of the shortcomings of The Framingham Study design with a relatively short follow-up time of 7 years is that it does not address that the magnitude and the direction of association between SBP and PWV are not addressed and could change with aging and differ by gender; merely adjusting for these variables does not inform whether

these associations differed between the different categories of these variables. Hence, the findings reflect that the average associations over the age spectrum studied for both genders.

### **Longitudinal perspective on the conundrum of arterial wall stiffness, blood pressure, and aging: A vicious cycle between teammates that eventually diverge**

**Indications of a vicious cycle between arterial stiffness and systolic blood pressure**—The Baltimore Longitudinal Study of Aging (BLSA) is a cohort study of community-swelling populations with extended follow-up time and multiple repeated measures of PWV and blood pressure (1988–2013). Earlier analyses from the BLSA have shown that greater PWV was associated with larger increase in SBP with aging, and predicted the incidence of hypertension.<sup>14</sup> Recent analysis from the BLSA, however, using linear mixed effects models, shed light on the vicious cycle, showing that higher SBP, in a dose-dependent fashion, is also associated with a greater rate of increase in PWV; this association was more pronounced in men with accelerating rates of increase in PWV at higher SBP with advancing age.<sup>15</sup>

**Dissociation between PWV and SBP trajectories**—More recent insights on the longitudinal changes in PWV and SBP parameters came from the SardiNIA Study of concurrent trajectories of repeated measures of PWV and SBP; using linear mixed effects models allows the examination of whether the longitudinal changes of these parameters over time vary by starting age.<sup>7</sup> This analysis demonstrated a striking dissociation in the trajectories of these parameters with advancing age; a dissociation more pronounced in men than women.<sup>7</sup> Figures 2A and B illustrate the cross-sectional differences “beginning of the splines” and the longitudinal changes (slopes of the splines) with aging (Rates of changes are illustrated in the lower panels) of PWV and SBP in both men and women. In men (Figure 2) PWV increased with age at rates that increase linearly with advancing age; however, although cross-sectional SBP continues to increase, the longitudinal rates of change, while initially increasing begin to decline with time, thus the rates of change in SBP diverge from those of PWV by the fifth decade. A similar, but a less dramatic, divergence is observed in women (Figure 2); while, PWV showed the same pattern of longitudinal changes in men with linearly increasing rates of change with advancing age, SBP increased longitudinally at a steady, rather than increasing, rates throughout the age range studied. Preliminary analyses from the BLSA using the same approach of examining the concurrent trajectories showed a similar pattern of dissociations between PWV trajectories and those of SBP and PP, which were more pronounced in men than women.<sup>16</sup>

**Physiological explanations for the dissociation between PWV and the decline in SBP with aging in men**—Dissociations between the rates at which PWV and SBP change over time bring our attention again to terminology; “Arterial stiffness” usually implies an increase in arterial opposition to flow i.e. characteristics ( $Z_c$ ) impedance. However,  $Z_c$  is a function of both PWV and aortic diameter squared.<sup>17</sup> Hence, an explanation for the dissociation between SBP and PWV longitudinal trajectories in men is an increase in aortic diameter. Preliminary analysis from the BLSA show a greater increase in aortic root dilatation with increasing age in men than in women.<sup>16</sup> The net effect of increasing PWV and aortic diameter approximated by applying the water hammer equation

is, in fact, a less pronounced increase in calculated  $Z_c$  in men despite their more pronounced increase in PWV which was offset by the greater increase in diameter.<sup>16</sup> These results are in agreement with cross-sectional data from the Asklepios study showing that with advancing age, men have lower  $Z_c$  than women.<sup>18</sup> While aortic diameter and  $Z_c$  might explain how PWV and SBP/PP trajectories would diverge, the role of wave reflection in this dissociation is not well clear and it is worth further examination.

### Salt-sensitive hypertension and aging

The incidence of hypertension and salt-sensitivity increases with advancing age.<sup>19–23</sup> High NaCl intake in addition to its effect on blood pressure,<sup>24</sup> increases arterial stiffness by altering vascular structure, vascular smooth muscle cell (VSMC) and endothelial cell function, and producing arterial wall fibrosis.<sup>25, 26, 27</sup> Both clinical and experimental evidence indicate that NaCl induces hypertrophy of the arterial wall in the absence of changes in arterial pressure<sup>26</sup> and induces hypertrophy of cultured VSMC.<sup>27</sup> Excessive NaCl intake reduces the bioavailability of nitric oxide by interfering with the induction of nitric oxide synthase,<sup>28</sup> and by elevating levels of peroxynitrite due to an increase in NADPH oxidase activity, marker of oxidative stress, and production of reactive oxygen species.<sup>29</sup> These NaCl effects lead to hypertension by reducing arterial compliance and increasing peripheral vascular resistance (PVR),<sup>30</sup> and to oxidative damage to the arterial wall.<sup>31</sup> An age-associated decline in nitric oxide mediated dilation becomes particularly apparent during the 6<sup>th</sup> decade, a time when pulse pressure, a barometer of large artery stiffness, begins to appreciably elevate.<sup>32</sup>

### The endogenous steroids, sodium pump ligands

Evidence is mounting that a NaCl-induced signaling cascade involving tissue renin angiotensin aldosterone signaling (RAAS), initiates the production of an endogenous ouabain-like substance in the brain, which then acts as a neurohormone to activate brain  $AT_1R$  (Figure 3).<sup>33,34</sup> Sympathetic signaling to the adrenal cortex leads to production of marinobufagenin (MBG), another recently discovered Na pump ligand that is an endogenous inhibitor of the alpha-1 isoform of the Na/K-ATPase (NKA),<sup>35</sup> which is the exclusive NKA isoform in renal tubules, and a main isoform in VSMC (Figure 3). Inhibition of NKA in renal tubular cells leads to decreased reabsorption of Na and promotes natriuresis. However, Na-pump ligands are not selective for renal NKA, but also inhibit NKA in the vasculature, leading to arterial constriction, and an increase in PVR and arterial blood pressure (Figure 3).<sup>35,36,37,38,39</sup> Interestingly, compared to normotensive control, older patients with resistant hypertension demonstrate greater blood pressure and PWV, higher plasma MBG (an NKA inhibitor), and decreased erythrocyte NKA activity.<sup>40</sup> In this regard, it is of note that the increase in arterial pressure induced by a chronic high NaCl intake (i.e. salt-sensitive hypertension) in rodents is substantially reduced by an anti-MBG antibody.<sup>35,41</sup>

The age-associated increase in the secretion of Na pump ligands, including MBG, linked to reduced renal NaCl excretion, could be an explanation for the moderate increases in PVR in older persons with predominantly systolic hypertension. The effects of MBG to increase PVR in salt-sensitive hypertension may be substantially enhanced via their interaction with

other vasoactive substances that are implicated in the pathogenesis of NaCl- dependent effects to increase arterial pressure. A NaCl induced up regulation of the tissue activity of RAAS, via PKC dependent phosphorylation of the Na/K-pump, may sensitize this pump to both vasoconstrictive and NaCl-dependent growth promoting MBG effects.<sup>42</sup> Additionally, NaCl-dependent angiotensin II (Ang II) signaling induced deficit in the bioavailability of endothelium-derived vasorelaxants, e.g., nitric oxide and C-type natriuretic peptide, which oppose the vasopressor action of MBG, but enhance its adaptive natriuretic action, may further reinforce the deleterious effects of the Na pump ligand.

Thus, Na pump ligands, including MBG, link high dietary salt intake to the increase in arterial stiffness and hypertension.<sup>20,21,34,36,43</sup> High salt intake is associated with an increase in MBG and is accompanied by marked salt-sensitivity of blood pressure.<sup>44,45</sup> Dietary sodium restriction is an effective lifestyle approach for reducing both blood pressure and arterial stiffness in middle-age and older individuals.<sup>46,47</sup> In older humans after 5 weeks of a low salt diet vs. 5 weeks of a normal salt diet a reduction in urinary MBG excretion was positively related to reductions in urinary Na excretion and arterial stiffness (Figure 4).<sup>48</sup> Furthermore, the expression of NADPH oxidase was correlated with MBG levels, indicating that low-salt-dependent reduction in MBG may contribute to the reductions in large elastic artery stiffness and SBP through decreased oxidative stress.<sup>48</sup> Thus, MBG is a molecular link of increased salt-sensitivity and arterial stiffness in aging.

### **Interaction of MBG and ANP in age-dependent salt-sensitivity**

In addition to MBG, high salt intake stimulates atrial natriuretic peptide (ANP) (Figure 5).<sup>49</sup> Inhibition of renal NKA by MBG is enhanced via ANP-dependent phosphorylation of NKA, whereas, in the aorta, ANP exerts the opposite effect.<sup>50</sup> Vasorelaxant ANP and vasoconstrictor MBG potentiate each other's natriuretic effects, but ANP may offset the deleterious vasoconstrictor effect of MBG.<sup>50</sup> An imbalance of ANP-MBG signaling increases with advancing age and is a factor that underlies the phenomenon of salt-sensitivity of blood pressure with aging.<sup>50</sup>

ANP sensitizes renal NKA to MBG inhibitory activity and reduces MBG-induced inhibition of vascular NKA via cyclic guanosine monophosphate/protein kinase G-dependent mechanism (cGMP/PKG). Because downregulation of cGMP/PKG signaling is associated with aging, ANP does not potentiate renal effects of MBG and does not oppose vasoconstrictive effects of MBG in older rats (Figure 6).<sup>49,50</sup>

The NaCl- dependence of blood pressure in older persons with predominantly systolic hypertension can be attributed to multiple mechanisms that underlie arterial compliance and vascular resistance. Excessive dietary NaCl may also alter vascular structure and function via Ang II or Na pump ligands driven mechanisms (Figure 7) in the setting of age-associated reductions in renal blood flow and in the ability to excrete Na. NaCl activates tissue Ang II and MBG, affects endothelial and vascular cell functions and affects arterial structural remodeling, which results in arterial stiffening (Figure 7). NaCl also activates ANP, via a cGMP/PKG-dependent mechanism that attenuates pro-fibrotic effect of MBG on vascular NKA (see below).<sup>50</sup> Remarkably, both phenomena, MBG-dependent activation of pro-fibrotic signaling and down-regulation of cGMP/PKG-dependent signaling, which



accelerates the pro-fibrotic and pro-hypertensive MBG effects, are the hallmarks of arterial aging (Figure 6).<sup>50</sup> Pro-fibrotic MBG activity, involving MBG/NKA association, is considered to be an important therapeutic target for immunoneutralization and modulation of MBG/NKA interactions in salt-sensitivity and in aging.

Excessive dietary NaCl, in fact, may be an etiologic factor in the increased central arterial stiffening that accompanies advancing age, and the attendant age-associated increase in pulse pressure, as this does not occur in populations that do not consume excess NaCl.<sup>24</sup> The combined effects of NaCl to increase arterial stiffness and resistance progresses to the point of raising systolic pressure, in nearly half of the individuals in our society, to the current epidemiologically defined hypertensive threshold (140 mm Hg). It is imperative, therefore, that we not lose sight of the reality that the NaCl baseline diet consumed in the US population at large, is 60% higher than the DASH recommendations<sup>47,51</sup> and appears to accelerate aging, and to increase the likelihood of salt-sensitive hypertension.

### **An age-associated increase in renin angiotensin aldosterone signaling (RAAS) in VSMC promotes central arterial wall remodeling**

Age-associated changes in vascular structure/function, per se, set the stage for the pathogenesis of vascular diseases such as hypertension in older persons.<sup>52–54</sup> The fact that the age-associated changes in arterial wall remodeling, including proinflammation, proliferation, migration/invasion of VSMCs, elastin fragmentation and calcification of Ang II signaling that are observed in rats also occur across a wide range of other species such as rabbits, nonhuman primates, and humans (Table 1; Figure 7), provides insights into the pathogenesis of hypertension with aging and age-associated hypertension.<sup>52–54</sup> The Table 1 and Figure 7 illustrate the proinflammatory profile of arterial wall remodeling. Components of the Ang II signaling cascade are “master perpetrators” of age-associated stress signaling that is linked to arterial wall remodeling.<sup>52–54</sup>

#### **Proinflammation**

In a hybrid FXBN strain old rat (30 mo) aortae exhibit a dramatic increase in intimal-medial thickness compared as young adults (8 mo), due to increased VSMCs infiltration into the intima, collagen deposition, calcification, elastin fracture, known as arterial wall remodeling.<sup>55–57</sup> Arterial Ang II signaling components (Figure 7) are increased in age-associated aortic remodeling in FXBN rats, including an increased abundance of Ang II, the AT-1 receptor, angiotensin converting enzyme (ACE), and their downstream molecules aldosterone, endothelin-1 (ET-1), matrix metalloproteinase type II (MMP-2), calpain-1, transformation growth factor beta 1 (TGF- $\beta$ 1), monocyte chemoattractant protein-1 (MCP-1), milk fat globule EGF-8 (MFG-E8) in FXBN rat with aging (Figure 7).<sup>52,55–64</sup> In addition, aldosterone mineralocorticoid receptor (MR) activation is increased, promoting arterial wall remodeling.<sup>65</sup> Interestingly, aortic prorenin receptor (PPR) ACE, Ang II and AT1 receptor proteins are also upregulated in C57/BL6 mice with aging (Figure 7).<sup>66</sup>

Chronic (30 days) Ang II infusion into young FXBN rats increases the intimal-medial thickness, enhances calpain-1, MMP-2, MCP-1, TGF- $\beta$ 1, MFG-E8 expression or activities,

collagen deposition, and elastin network breakdown in the arterial wall, mimicking arterial wall remodeling that accompanies an advancing age.<sup>58,62,67</sup> In other terms in response to chronic administration of Ang II to young rats, the arterial VSM and matrix take on the appearance of their counterparts in old rats.

Chronic ACE inhibition or AT<sub>1</sub> receptor blockade, beginning at an early age, markedly inhibits the expression of proinflammatory molecules and delays the progression of age-associated aortic remodeling and senescence.<sup>68,69</sup> Interestingly, long-term AT<sub>1</sub> blockade improves endothelial function and decreases blood pressure, doubles lifespan of hypertensive rats rendering it similar to normotensives.<sup>70</sup> Disruption of the AT<sub>1</sub> receptor, retards arterial inflammation, promotes longevity and improves survival after MI in mice.<sup>71</sup> These findings provide strong support to the hypothesis that increased Ang II proinflammatory signaling is involved in arterial wall remodeling (Figure 7).

### Proliferation

VSMCs within the aged aortic wall have an enhanced proliferation capacity compared to young cells linked to proinflammation.<sup>60</sup> The proliferation rate in cultured VSMCs is increased in old vs. young adult rats.<sup>59</sup> An increase in CDK4 and PCNA, an increase in the acceleration of cell cycle S and G<sub>2</sub> phases, a decrease in the G<sub>1</sub> / G<sub>0</sub> phase, and an increase in PDGF and its receptors drive the elevated proliferative capacity in early passage old VSMC vs. young VSMC.<sup>60</sup>

Ang II signaling increases MFG-E8 expression in both arterial walls and VSMCs (Figure 7).<sup>67,72</sup> An increase in MFG-E8, a cell adhesion protein, is a signature of aging arterial walls.<sup>60,72-74</sup> Aortic MFG-E8 mRNA and protein levels and its integrin receptor,  $\alpha_v\beta_3/5$ , increase with aging.<sup>60,72</sup> MFG-E8 signaling via integrins activates proliferation of VSMC, PCNA and Ki67, markers of cell cycle activation.<sup>60</sup> In young VSMC in vitro, MFG-E8 treatment triggers p-ERK-1/2, augments levels of PCNA and CDK4, increases BrdU incorporation, and promotes proliferation via  $\alpha_v\beta_5$  integrins.<sup>60</sup> MFG-E8 silencing, or its receptor inhibition, or the blockade of p-ERK1 / 2 in these cells reduces PCNA and CDK4 levels, and decelerates the cell cycle S phase, conferring a reduction in proliferative capacity.<sup>60</sup> Collectively, MFG-E8 coordinates the expression of cell cycle molecules and facilitates VSMC proliferation via integrin / ERK1 / 2 signaling (Figure 7).

### Migration/Invasion

The migration/invasion of VSMCs, which is a key cellular event in age-associated diffuse intimal thickening, is driven by proinflammatory molecular signaling.<sup>52</sup> VSMCs from old vs. young adult rats also have a 50% increase in migration potential.<sup>59</sup> Ang II triggers the activation of MMP-2, calpain 1, MCP-1, and MFG-E8, which play a causal role in the migration/ invasion of VSMC due to its cleavage of the basement membrane and cytoskeletal remodeling (Figure 7).<sup>58,61,62,72</sup> In cultured young VSMCs, Ang II exposure increases VSMC migration to the level of isolated old cells.<sup>58,62</sup> The Ang II-mediated, age-associated increases in VSMC migration capacity is blocked by inhibition of MMP-2.<sup>58</sup> Calpain-1 is an intracellular Ca<sup>2+</sup>-activated cysteine protease and downstream molecule of Ang II signaling cascade. Its transcription, translation, and activation are significantly up-



regulated in rat aortae and VSMCs in culture with aging.<sup>55,58</sup> Calpain-1 and MMP-2 are colocalized within old VSMC.<sup>55</sup> Over-expression of calpain-1 in young VSMC results in cleavage of intact vimentin (as an index of calpain-1 activity) and an increased migratory capacity, mimicking old VSMC. These actions are blocked by the MMP inhibitor, GM6001 and its inhibitor calpastatin.<sup>58</sup> Thus, calpain-1 and MMP-2 activation are pivotal molecular events in the age-associated arterial Ang II signaling/migration cascade of VSMC migration.

In addition, MFG-E8 plays an important role as a relay element within the AngII/MCP-1 signaling cascade that modulates VSMC invasion with aging.<sup>72</sup> MCP-1 and its receptor, CCR2 (Figure 7), are upregulated in age-associated aortic remodeling.<sup>59,61</sup> Exposure of young VSMCs to Ang II markedly increases MFG-E8 and enhances their invasive capacity to old cell levels.<sup>72</sup> Treatment of VSMCs with MFG-E8 increases MCP-1 expression and VSMC invasion, and both are inhibited by the MCP-1 receptor blocker vCCI.<sup>72</sup> Exposure of young VSMC to MCP-1 also increases their migration, up to levels of old cells.<sup>59</sup> Silencing MFG-E8 substantially reduces MFG-E8 expression and VSMC invasion capacity.<sup>72</sup> Thus MFG-E8, a protein secreted by VSMC, significantly increases with aging and is a pivotal relay element within the Ang II/MCP-1/VSMC invasion signaling cascade (Figure 7).

### Elastin fragmentation

Rat aortic wall elastin fraction is significantly decreased (by 60%) with advancing age and the network of elastin is diminished.<sup>56</sup> The age-dependent, Ang II-mediated increase in arterial MMP-2 activity is involved in the cleavage of the elastin fibrillin-1 leading to the degradation of the elastin arterial network (Figure 8).<sup>55-57,62</sup> These age-associated changes within elastin lamina are associated with an imbalance of synthesis and degradation of tropoelastin. The level of tropoelastin production by old aortic VSMCs in vitro is markedly reduced and tropoelastin is also degraded more rapidly in tertiary culture with increased passage number than in primary culture.<sup>75,76</sup> Importantly, elastin fibers are cleaved by age-associated activation of the gelatinases MMP-2/9 and elastase.<sup>76,77</sup> Chronic administration of a broad-spectrum MMP inhibitor, PD166793, via a daily gavage, to 16-month-old rats for 8 months markedly blunted the expected age-associated increases in aortic MMP activity and a release of fibrillin-1 and preserved the elastic fiber network integrity.<sup>63</sup> Importantly, degraded fibrillin-1 initiates fibrosis, and ends of fractured fiber become an impetus to calcification (Figure 8).<sup>78</sup>

### Arterial calcification

Arterial calcification is also the calcium build-up consequence of a reparative or reactive process to chronic proinflammation. Arterial calcification, the deposition of calcium phosphate mineral, most often hydroxyapatite within or outside of arterial cells is a salient feature of age-associated arterial remodeling (Figure 8). Old cultured VSMCs, like osteoblasts, are able to produce large amounts of bone-like substrates, including collagen II, which become bio-mineralized and calcified.<sup>63</sup> Over-expression of calpain-1 reduces the calcification inhibitors, osteonectin, and osteopontin (OPN), and induces alkaline phosphatase activity in young VSMCs, mimicking that of old cells.<sup>63</sup> In addition, the activity of tissue transglutaminase (TG2), a protein crosslinking enzyme, increases in the old arterial wall, and is closely associated with reduction of NO bioavailability.<sup>79-82</sup> Activated

TG2 up-regulates calcification promoter genes, i.e. *Runx2* and down-regulates the expression of calcification inhibitor genes, i.e. *OPN* within VSMCs and increases arterial stiffness (Figure 8).<sup>80-82</sup>

### Arterial fibrosis

Arterial wall fibrosis is a hallmark of aging and vascular diseases.<sup>83,84</sup> Arterial fibrosis is the formation of excessive extracellular fibrous tissue in a reparative or reactive process in response to chronic proinflammation (Figures 7 and 8). VSMCs are stretched via longitudinal or circumferential strain causing an age-associated increase in Ang II and TGF- $\beta$ 1 signaling in the arterial wall over time.<sup>85,86</sup> Inflammation induces tissue repair,<sup>87</sup> which is important in the scenario of tissue damage or blood pressure increase, and TGF-beta is an important player in this reparative process.<sup>87,88</sup> Increased aortic calpain-1 associated MMP-2 activity mediates age-associated arterial Ang II profibrotic signaling effect (Figures 7 and 8).<sup>58</sup> Overexpression of calpain-1 induces MMP-2 transcription, following by an increase in protein levels and activity, in part, by increasing the ratio of MT1-MMPs to TIMP2.<sup>55</sup> The increased MMP-2 activity of the old rat aorta colocalizes with TGF- $\beta$ 1.<sup>57</sup> The latent TGF- $\beta$ 1 precursor linked to fibrillin 1, and its intermediate degradation form, latent associated protein (LAP), as well as active TGF- $\beta$ 1 within VSMC increase with aging via a stepwise of cleavage by MMP-2.<sup>57</sup> The expression of TGF- $\beta$ 1 receptor T $\beta$ RII also increases with aging. Downstream receptor signaling molecules (Figure 7) of p-SMAD2, 3 and 4 increases while the medial expression of the suppressor SMAD7 is decreased with aging.<sup>57</sup> The effect of calpain-1-induced MMP-2 activation (Figure 8) results in increased collagen I, II and III production in VSMCs.<sup>55</sup> Chronic inhibition of MMP markedly reduces arterial interstitial collagenase activity, TGF- $\beta$ 1 activation, the profibrogenic signaling molecule SMAD-2/3 phosphorylation, and collagen deposition<sup>63</sup>. Collectively, in vitro and in vivo results indicate that MMP inhibition retards age-associated arterial profibrotic signaling (Figures 7 and 8).

### The role of the endogenous NKA ligand, MBG, in pro-fibrotic signaling

It is known that aging is a predominant factor for most diseases.<sup>89</sup> Chronic diseases even in younger ages also resemble aging by alteration of expression of genes in pro-inflammatory and pro-fibrotic pathways, creating an aging profile at the genetic, molecular, cellular, physiological levels especially in cardiovascular system.<sup>83,90,91,92</sup> Fibrosis of arterial wall accompanies aging and vascular diseases.<sup>83,90,91,92</sup> Levels of the pro-fibrotic factor, MBG, are implicated in development of vascular fibrosis in preeclampsia, chronic renal failure, and salt-sensitive hypertension at any age.<sup>39,93-99</sup> Production of this steroid, MBG, is regulated by Ang II (Figure 7).<sup>34</sup> MBG links salt-sensitive arterial stiffness and hypertension. MBG initiates TGF $\beta$ - and Fli-1- dependent pro-fibrotic signaling via binding to NKA (Figure 8).<sup>100,101</sup> Activation of TGF $\beta$  pro-fibrotic signaling by MBG was demonstrated in the Dahl-S model of salt-sensitive hypertension and aging. *In vivo*, immunoneutralization of high MBG levels in old Dahl-S rats by an anti-MBG monoclonal antibody reverses arterial fibrosis, and down-regulates genes, implicated in TGF $\beta$  signaling.

Fli1-dependent signaling, another pro-fibrotic pathway, is also activated by MBG. MBG-NKA-Src complex activates of epidermal growth factor receptor (EGFR) signaling resulting

in a degradation of Fli1 (a negative nuclear regulator of the procollagen-1 gene) and induction of collagen-1 synthesis (Figure 8).<sup>101</sup> This mechanism could be relevant to the pathogenesis of several conditions, including preeclampsia, in which elevated plasma MBG levels are associated with development of fibrosis in umbilical arteries accompanied by the reduction of Fli-1.<sup>102</sup> Cardiovascular fibrosis activated by high endogenous MBG levels in rat model of chronic renal disease (CRD), was reversed by immunization of these rats with monoclonal anti-MBG antibody.<sup>103</sup> Of note, the *pro-fibrotic effect of MBG* and *anti-fibrotic effect of anti-MBG antibody* are pressure-independent.<sup>104</sup> High NaCl intake in normotensive rats increases MBG levels and induces aortic fibrosis in the absence of a hypertensive response.<sup>104</sup> In this study, immunoneutralization of MBG reduces aortic fibrosis and restores the aortic relaxation.<sup>104</sup>

Anti-fibrotic effects of the MR antagonists, spironolactone, and its common metabolite, canrenone,<sup>105</sup> are also associated with Na pump ligands.<sup>106,107</sup> The MR antagonist, canrenone, blocks the pro-fibrotic activity of an endogenous MBG in animal models of CRD, and also suppresses cardiac fibrosis in rats chronically treated by MBG in the absence of changes in aldosterone levels.<sup>108</sup> In addition, canrenone significantly attenuates pressure-independent pro-fibrotic activity of MBG in cultures aortic VSMCs *in vitro*. Incubation of rat aortic rings with MBG reduces aortic Fli1, increases collagen-1, and attenuates relaxation of aorta. Canrenone restores aortic relaxation, restores Fli1 levels and reduces collagen abundance in aortic wall.<sup>40</sup>

Older patients with resistant hypertension exhibit an increase in PWV, higher plasma MBG, and inhibited erythrocyte NKA vs. normotensive age-matched control.<sup>40</sup> Administration of spironolactone to these patients with resistant hypertension for 6 months in addition to the conventional triple anti-hypertensive therapy restores of NKA activity, decreased SBP and DBP, and significantly reduced PWV. These data further demonstrate the intimate relationship of MBG and arterial stiffness. Ang II-driven MBG is a novel target for MR antagonists in MBG-induced arterial remodeling and arterial stiffness. In addition, the immunoneutralization of pro-fibrotic steroid MBG and also blocking its inhibiting effect on NKA by MR antagonists are additional approaches in treatment of salt-sensitive hypertension and conditions when heightened MBG level cause a devastating pro-fibrotic and pro-hypertensive effect, which is accelerated by aging.

## Summary

The aforementioned evidence indicates that accelerated arterial wall remodeling via Ang II signaling within the arteries, per se, ought to be considered a type of hypertensive effect, because the molecular disorder and the inflammatory milieu it creates within the arteries with advancing age are the roots of the pathophysiology of hypertension (Table 1). Thus, therapies to prevent or reduce signaling that drive arterial wall remodeling may ultimately reduce the epidemic of hypertension in the older population by reducing the undisputed major risk factor for hypertension i.e., arterial aging, per se. Targeting “the master perpetuators” of arterial aging, i.e. therapies to prevent or delay Ang II signaling related vascular changes that accompany aging, appears to be the most promising strategy to reduce

the health burden of hypertension and ultimately reduce the **prevalence** of hypertension and arterial fibrosis.

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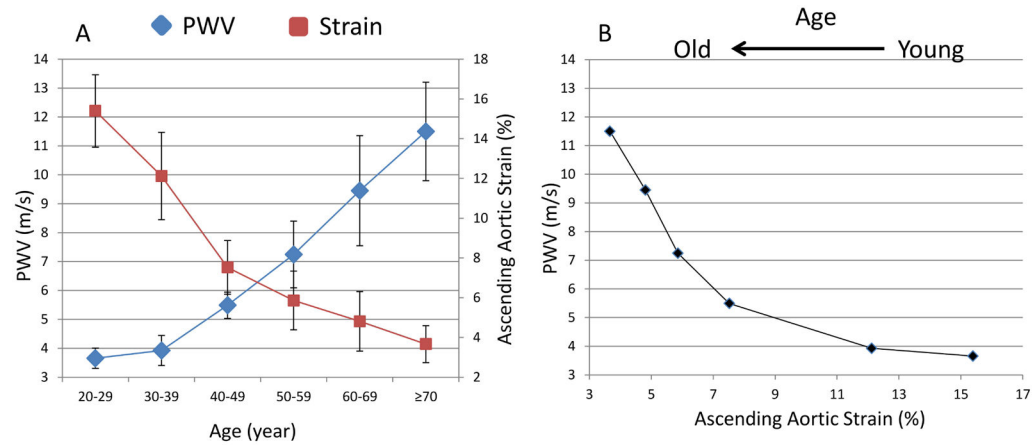
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### Key Points

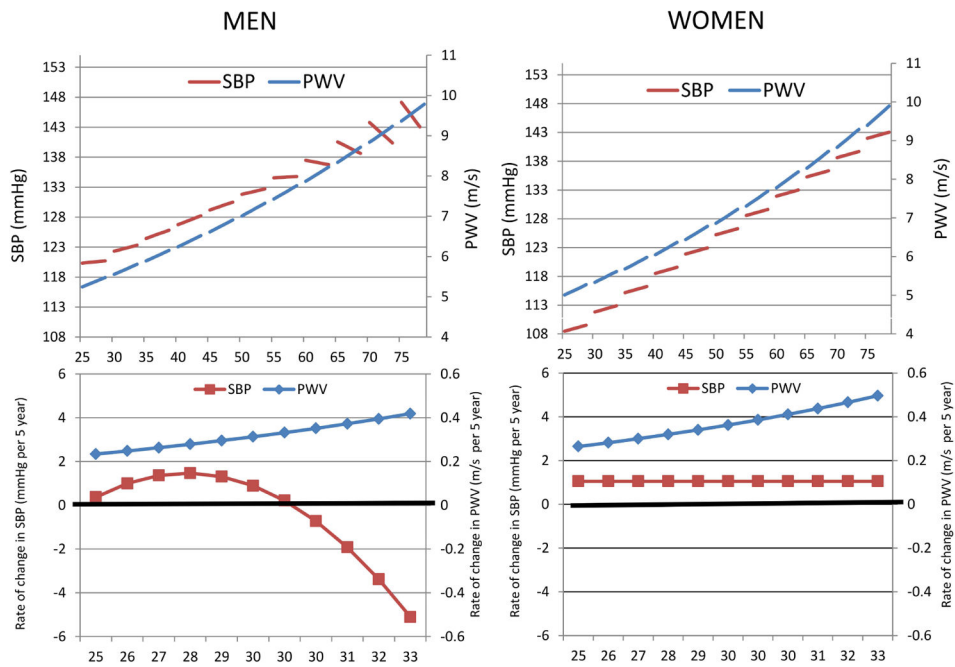
- While pre-clinical hemodynamic alterations observed early in life are not directly harmful, they form the basis for the deleterious hemodynamic effects observed with aging.
- The increase of salt-sensitivity with advancing age appears to be linked to aortic biomechanics.
- A proinflammatory state in the arterial wall, with a pivotal role for angiotensin II, is a key component of arterial aging
- An endogenous sodium pump ligand, marinobufagenin is a novel marker that links aging, salt-sensitivity, and arterial stiffness.
- Pulsatile damage to arterial wall and the proinflammatory state within the arterial wall interact in a vicious cycle, resulting in increasing arterial wall fibrosis.
- Interventions should be aimed at breaking the vicious cycle at its early stages.
- Therapies to prevent or delay Ang II signaling related vascular changes that accompany aging may ultimately reduce the prevalence of hypertension.



**Figure 1.**

Aortic strain (red) decreases sharply between the 3<sup>rd</sup> and 5<sup>th</sup> decade of life after which there is a sharp rise in aortic pulse wave velocity (blue) (A). Aortic strain and PWV plotted against each other showing an exponential increase in PWV with declining aortic strain with aging (B)

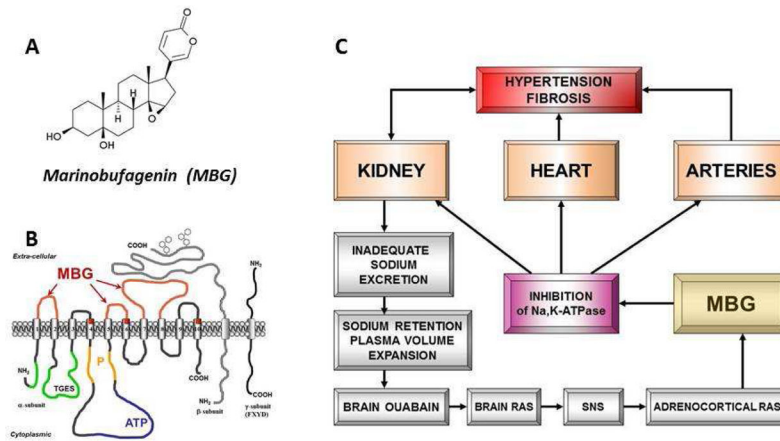
*Adapted from Redheuil A, Yu WC, Wu CO, et al. Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. Hypertension 2010;55(2): 319–26; with permission.*



**Figure 2.** Linear mixed-effects models predicted PWV and SBP values illustrating gender-specific cross-sectional differences “beginning of the splines” and the longitudinal changes (slopes of the splines) with aging (Rates of changes are illustrated in the lower panels) in men and women from the SardinIA project.

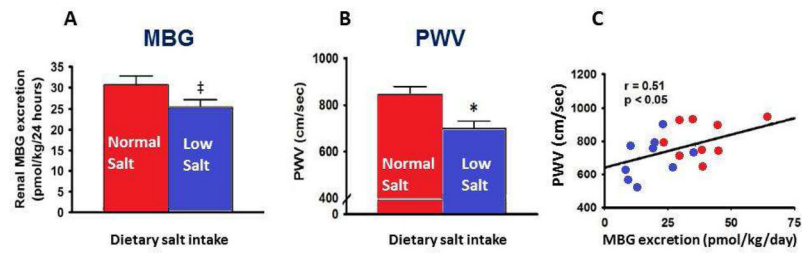
*Adapted from* Scuteri A, Morrell CH, Orru M, et al. Longitudinal perspective on the conundrum of central arterial stiffness, blood pressure, and aging. *Hypertension* 2014;64(6): 1219–27; with permission.





**Figure 3.** Structures of marinobufagenin (MBG) (A) and Na/K-ATPase with binding sites for MBG (B). Interaction between RAAS and MBG in the pathogenesis of salt-sensitive hypertension (C).

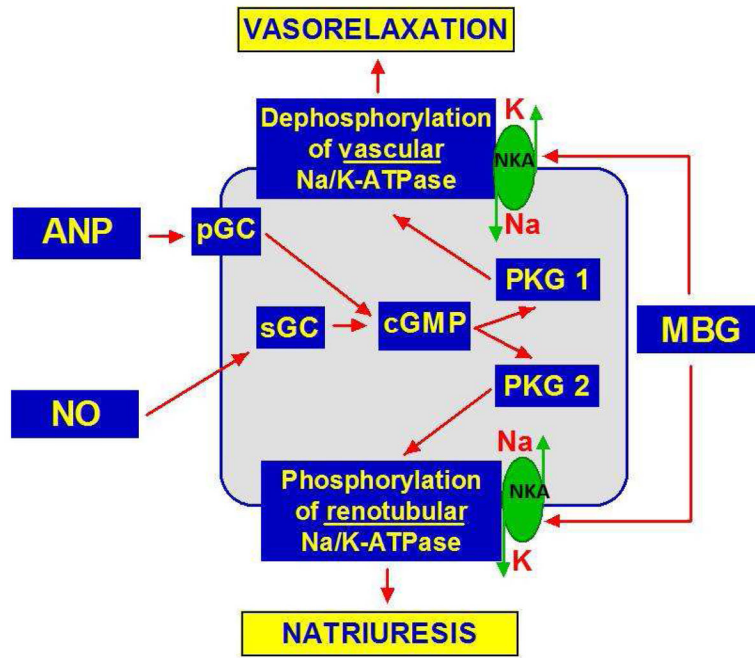
*Adapted from* Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotoxic steroids: physiology, pharmacology, and novel therapeutic targets. *Pharmacological reviews* 2009;61(1):9–38; with permission.



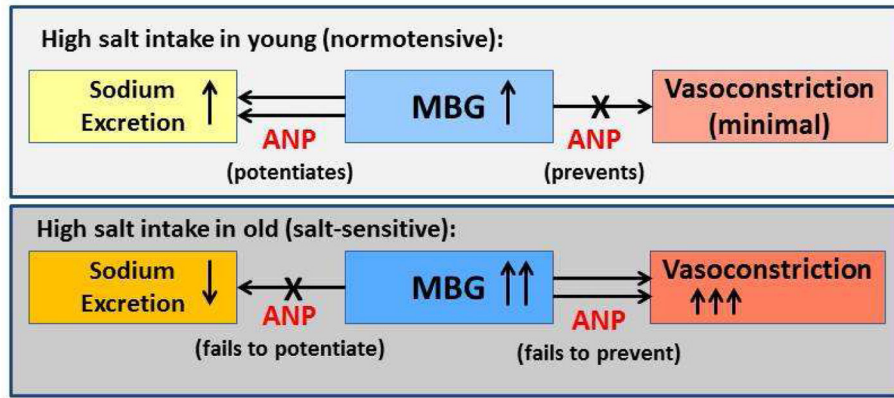
**Figure 4.**

Effect of low and normal dietary salt intake on urinary MBG excretion (A), aortic pulse-wave velocity (PWV) (B), and correlation between urinary MBG and PWV (C) in older patients

*Adapted from* Jablonski KL, Fedorova OV, Racine ML, et al. Dietary sodium restriction and association with urinary marinobufagenin, blood pressure, and aortic stiffness. *Clin J Am Soc Nephrol* 2013;8(11):1952–9; with permission.



**Figure 5.** Factors implicated in the modulation of cGMP-dependent phosphorylation / dephosphorylation of renal and vascular Na/K-ATPase (NKA).



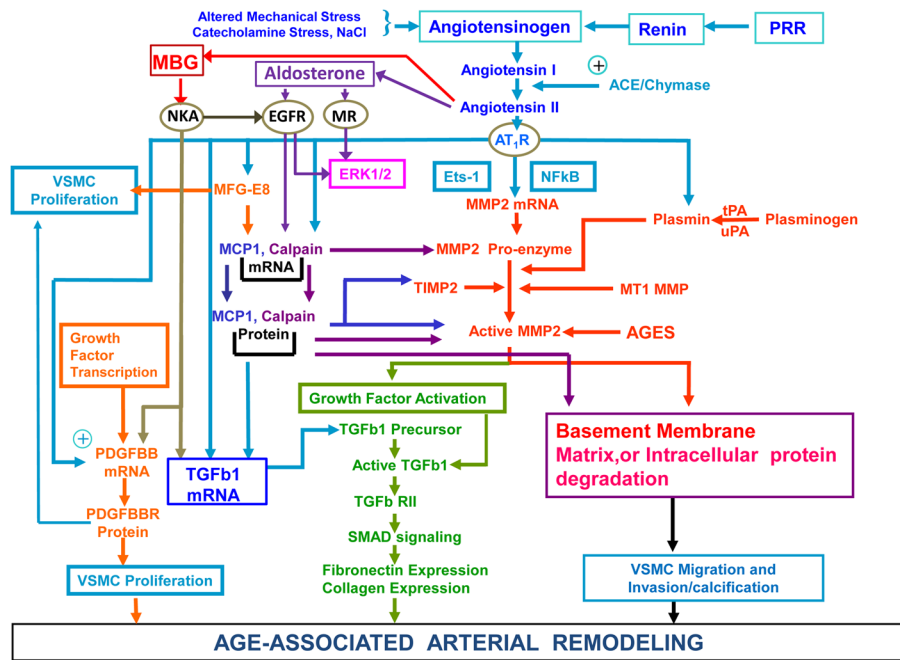
**Figure 6.** Scheme of age-associated shift of the modulation of renal and vascular effects of MBG by ANP.

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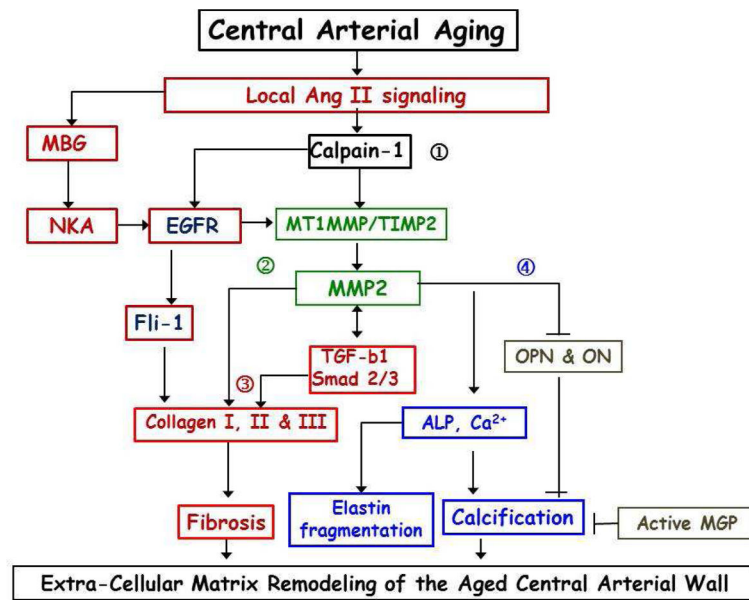
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**Figure 7.** Pathways that participate in age-associated remodeling of large arteries. Ang II initiates both inflammatory and repair processes *From* Lakatta EG. The reality of aging viewed from the arterial wall. *Artery research* 2013;7(2):73–80; with permission.



**Figure 8.** Age-Associated Matrix Remodeling of Central Arteries. MBG – marinobufagenin; NKA – Na/K-ATPase (MBG receptor); EGFR – epidermal growth factor receptor; MT1 – metallothionein 1; MMP – matrix metalloprotease; TIMP – metalloprotease inhibitor; TGF-β1 – transforming growth factor beta 1; OPN – osteoponin; ON – osteonectine; ALP – alkaline phosphatase; MGP – matrix Gla protein. Numerous other molecules, not shown on that diagram, have important role in fibrosis, calcification and elastin fragmentation.



**Table 1**

Age-associated proinflammatory arterial remodeling

	Aging					Hyper-tension	Ang II Signaling
	Humans >56 vs. <20 yrs	Monkeys 15–20 vs. <10 yrs	Rats 24–30 vs. 3–8 mo	Rabbits 2–6 yrs vs. <10 mo			
Local Ang II/AT1	↑	↑	↑	?	↑	↑	↑
ET-1	↑	?	↑	↑	↑	↑	↑
MMPs	↑	↑	↑	?	↑	↑	↑
Calpain-1	↑	↑	↑	?	↑	↑	↑
MCP-1/CCR2	↑	↑	↑	↑	↑	↑	↑
TGF-β1/TβIR	↑	↑	↑	↑	↑	↑	↑
NADPH Oxidase	↑	↑	↑	↑	↑	↑	↑
NO Bioavailability	↓	↓	↓	↓	↓	↓	↓
TNF-α1	↑	↑	↑	↑	↑	↑	↑
ICAM-1	↑	↑	↑	↑	↑	↑	↑
MFG-E8	↑	↑	↑	↑	?	↑	↑
PDGF/PDGF-R	↑	?	↑	?	↑	↑	↑
tPA/uPA	?	?	↑	?	↑	↑	↑
AGEs/RAGE	↑	↑	↑	↑	↑	↑	↑
IL-1/-6/-8	↑	?	↑	?	↑	↑	↑
MR	?	?	↑	?	?	?	↑
NF-κB	↑	↑	↑	↑	↑	↑	↑
Ets-1	?	?	↑	?	↑	↑	↑
Sirt1	↓	?	↓	?	↓	↓	↓
EC dysfunction	↑	↑	↑	↑	↑	↑	↑
Diffuse IMT	↑	↑	↑	↑	↑	↑	↑
Stiffness	↑	↑	↑	↑	↑	↑	↑
Matrix	↑	↑	↑	↑	↑	↑	↑
Calcification	↑	↑	↑	?	↑	↑	↑
FN/Collagen	↑	↑	↑	↑	↑	↑	↑
VSMC migration	↑	↑	↑	↑	↑	↑	↑

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Aging						
Humans	Monkeys	Rats	Rabbits	Hyper-tension	Ang II Signaling	
>56 yrs, <20 yrs	15-20 yrs, <10 yrs	24-30 vs. 3-8 mo	2-6 yrs vs. <10 mo			
↑	↑	↑	↑	↑	↑	↑
VSMC proliferation						

Adapted from Wang M, Jiang L, Monticone RE, Lakatta EG. Proinflammation: the key to arterial aging. Trends in endocrinology and metabolism 2014;25(2):72-9; with permission.