

## Arterial Stiffness and Hypertension

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During the past decade, increased aortic stiffness has emerged as an important risk factor for target organ damage and cardiovascular disease events. Aortic stiffness can be assessed as pulse wave velocity (PWV), which is a measure of aortic wall stiffness, and pulse pressure (PP), which is affected by wall stiffness and the interaction between flow and diameter. Because these stiffness measures have different sensitivities to geometry and other factors, they are only moderately correlated and play a complementary role in risk prediction. Arterial stiffness has long been viewed as a complication of hypertension that integrates long-term adverse effects of elevated blood pressure and other risk factors. However, PWV is only modestly correlated with risk factors other than age and blood pressure, which likely explains the ability of PWV to add to standard risk prediction models and reclassify risk in a clinically relevant manner. Recent studies have demonstrated that stiffness can antedate and can contribute to the pathogenesis of hypertension, raising the possibility that early assessment of arterial stiffness may provide insight into complications, including hypertension, that develop years later. The role that stiffness plays in the pathogenesis of hypertension and cardiovascular disease has sparked considerable interest in defining basic mechanisms that stiffen the aortic wall, increase PP, and contribute to target organ damage with a hope that elucidation of these mechanisms will allow for the development of more effective treatments.

### Mechanisms of Arterial Stiffening

#### Developmental and Early Life Contributions

In a young, healthy patient, elastic lamellae in the media bear most of the aortic wall stress at ambient pressure. These organized sheets of elastic fibers are produced early in life beginning in the fetus and continuing through early childhood.<sup>1</sup> After the lamellae are formed, the gene program required to produce elastic fibers is permanently silenced.<sup>2</sup> The aorta subsequently must adapt to changing conditions by remodeling the initial complement of elastic fibers produced during the critical elastogenic period in early life.<sup>1</sup> This unique aspect of aortic development may be relevant to the observation that uteroplacental insufficiency or impaired lactation in female rats was associated with intrauterine growth restriction and subsequent abnormalities in arterial stiffness and vascular function in their adult male offspring.<sup>3</sup> Increased arterial stiffness in adult

rats that were growth restricted was prevented by crossing the fetuses over to a normally lactating mother. Femoral artery stiffness was also increased in normal rats that were crossed over at birth to a mother with uteroplacental insufficiency and impaired lactation. Thus, perinatal exposures at a critical stage in the development may have long-lived effects on large arteries, possibly through alterations in elastin deposition that cannot be remediated later after the elastic fiber gene program has been silenced. An evaluation of aortic elastin content in this model of uteroplacental and lactational insufficiency may be informative. These early environmental factors may predispose to subsequent hypertension when challenged by a subsequent insult, such as obesity, that increases hemodynamic load on the aorta. Similar relationships between early life factors and adult vascular stiffness have been proposed although the relationships are modest.<sup>4-6</sup>

#### Vascular Growth Factors

Vascular growth factors contribute to the development of the arterial system and to the maintenance of normal vascular function throughout the lifespan. Lower levels of insulin-like growth factor 1 and higher levels of vascular endothelial growth factor (VEGF) were associated with higher mean arterial pressure (MAP) and higher carotid-femoral PWV (CFPWV) in the relatively young Framingham Third Generation cohort.<sup>7</sup> In models for CFPWV that further adjusted for MAP, relationships with insulin-like growth factor 1 persisted, whereas VEGF was no longer significant, suggesting that higher insulin-like growth factor 1 has favorable relationships with both small and large artery function. Inverse relationships between VEGF and vascular measures may indicate a counter-regulatory role for VEGF, given that VEGF inhibition promotes the development of hypertension.

#### Calcification

Calcification and stiffening of the aorta are associated although the predominant directionality of this likely bidirectional association remains uncertain. Calcification occurs at the site of structural damage in the aortic media, which is likely to be associated with altered stiffness, and in non-load-bearing plaque, where it is unlikely to have a major effect on overall mechanical properties of the aortic wall. Proteolytic cleavage of elastin releases elastin degradation



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products. Elevated circulating levels of matrix metalloproteinase (MMP)-2, cathepsin-S, and elastin degradation products have been found in patients with stage 3 to 4 chronic kidney disease and are associated with higher CFPWV and increased total mortality.<sup>8</sup> In addition, elastin degradation products activate innate immunity,<sup>9</sup> potentially contributing to inflammation in the aortic wall. In a twin study, asymptomatic aortic plaque was associated with calcification but not with aortic stiffness.<sup>10</sup> However, calcification burden was associated with stiffness with a large shared genetic component, suggesting that common genetic factors may contribute to stiffness and calcification in the aorta.

### Adaptive and Innate Immunity

Aortic stiffness and hypertension are associated with activation of innate and adaptive immunity, whereas mice with complete ablation of T and B cells are resistant to vascular inflammation and hypertension induced by chronic infusions of angiotensin II (A-II).<sup>11</sup> The leading genome-wide association for CFPWV lies in a gene enhancer for *BCL11B*, which has recently emerged as the master regulator of T-cell fate.<sup>12</sup> Enhanced function of CD4+/CD25+ T regulatory lymphocytes (Tregs), which modulate adaptive and innate immune responses, may limit aortic stiffening and the development of hypertension.<sup>13,14</sup> In mice, transfer of Tregs before the initiation of A-II or aldosterone infusion ameliorated the development of hypertension and prevented the infiltration of aortic adventitia and periaortic fat with monocytes and activated CD3+ T cells. In addition, Treg infusions attenuated oxidative stress in the aorta and reduced plasma levels of proinflammatory cytokines, including interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , and interleukin-6.

In addition to attacking elastin, MMPs can activate or can induce the expression of inflammatory mediators and vice versa. Knockout of the proinflammatory cytokine cardiotrophin-1 was associated with reduced MMP-2, MMP-9, and MMP-13 activities in the aortic media, higher isobaric carotid distensibility, reduced matrix type I collagen and fibronectin, and increased diameter and reduced media area.<sup>15</sup> Markers of apoptosis and senescence were reduced, and DNA repair pathways were upregulated in vascular smooth muscle in the knockout mice. Exogenous administration of cardiotrophin-1 to Wistar rats was associated with unchanged blood pressure but increased media thickness, collagen and fibronectin content, and stiffness of the aorta.<sup>16</sup> Similarly, overexpression of calpain-1, a calcium-activated, intracellular cysteine protease, increased the expression of membrane type 1 MMP and reduced the expression of tissue inhibitor of MMP-2, resulting in increased expression and activity of MMP-2.<sup>17</sup> These changes were accompanied by the activation of transforming growth factor- $\beta$  signaling and increased collagen deposition in the aorta. Calpain-1 is upregulated by age and A-II signaling and may mediate A-II-related aging of the vascular wall. MMP inhibition blunts age-associated arterial proinflammatory signaling via monocyte chemoattractant protein-1 and transforming growth factor- $\beta$ , resulting in preservation of intact elastin fibers and a reduction in collagen.<sup>18</sup> However, MMP inhibitors have intolerable side effects, including poor wound healing, impaired angiogenesis, and

skeletal myopathy. Increased selectivity of newer agents may reduce toxicity and may offer a new therapeutic approach to arterial stiffness and clinical sequelae.

Relationships between arterial stiffness and inflammatory markers were evaluated for a 6-year interval in participants in the Amsterdam Growth and Health Longitudinal Study, who were 36 and 42 years of age at the 2 visits.<sup>19</sup> PP fell significantly during the follow-up period, whereas CFPWV increased, which has been a consistent cross-sectional observation in this age range in various studies, including the Framingham Heart Study<sup>20</sup> and the Asklepios Study.<sup>21</sup> The aortic wall stiffens in young adults before midlife, whereas aortic remodeling to a larger diameter seems to offset the adverse effects of wall stiffening, leading to a reduction in characteristic impedance and forward wave amplitude and a reduction in PP that continues until midlife and then reverses rather dramatically thereafter, particularly in women.<sup>20</sup> In the Amsterdam cohort, markers of endothelial function were associated with femoral artery stiffness, whereas markers of inflammation were associated with carotid and femoral stiffness, suggesting that inflammation is associated with both elastic (carotid) and muscular (femoral) artery properties, whereas endothelial function is associated with muscular artery function. No relationship was found between C-reactive protein or interleukin-6 and CFPWV, which is at odds with previous observations<sup>22</sup> and may reflect a power limitation in a smaller study or the younger age of the cohort.

### Arterial Stiffening and Target Organ Damage Effects on Left Ventricular Structure and Function

Aortic stiffening loads the left ventricle by increasing pressure pulsatility, which increases early systolic load, and also by contributing to earlier return of the reflected wave, which increases late systolic load, particularly in younger adults. The resulting alteration in load and loading sequence may impair systolic and diastolic function and may contribute to ventricular remodeling. In the Asklepios cohort of middle-aged women and men, peak stress in early systole was associated with higher characteristic impedance of the aorta, which determines forward pressure wave amplitude.<sup>23</sup> Late systolic stress, which was much lower than peak or early systolic stress, was associated with reflected wave amplitude. Women had higher peak and end-systolic wall stress, which may render them more susceptible to heart failure in later life. Higher early systolic wall stress was associated with increased early peak filling assessed by mitral annulus tissue Doppler ( $e'$ ), whereas higher late systolic wall stress was associated with reduced early filling rate.<sup>24</sup> The latter association may relate to higher prevalence of heart failure with preserved ejection fraction in women, who have greater wave reflection than that in men even after considering differences in height.

### Effects on Baroreceptor Function and Blood Pressure Variability

Excessive blood pressure variability may represent 1 mechanism whereby increased arterial stiffness causes target organ damage. Baroreceptors sense strain in the walls of the aortic arch and carotid arteries and provide feedback for the regulation of blood pressure. The concept of baroreceptor encasement

by stiffened arterial walls in the vicinity of baroreceptors was once proposed as a potential mechanism for persistent hypertension. However, animal models demonstrated that ablation of baroreceptor afferents did not result in sustained hypertension but did produce excessive blood pressure variability and increased aortic stiffness. Schillaci et al<sup>25</sup> demonstrated that short-term systolic blood pressure variability was associated with higher CFPWV. They suggested that increased large artery stiffness could contribute to variability in blood pressure through a combination of an impaired baroreflex and increased volume sensitivity in a stiffened cardiovascular system.

Okada et al<sup>26</sup> examined the relationship between sympathetic baroreflex sensitivity and arterial stiffness in elderly men and women. Women had lower baroreflex sensitivity and greater stiffness of the carotid artery and aorta, where baroreceptors are located. Baroreflex sensitivity was associated with carotid and aortic stiffness in men and in women when central pressure was estimated by direct carotid tonometry but not when a transfer function was used to estimate central pressure. A post hoc analysis of data from the J-CORE (The Japan-Combined Treatment With Olmesartan and a Calcium Channel Blocker Versus Olmesartan and Diuretics Randomized Efficacy) study demonstrated that treatment with a combination of an A-II receptor blocker and a calcium channel blocker reduced blood pressure variability in proportion to a reduction in CFPWV, suggesting that a reduction in arterial stiffness may have contributed to the reduction in blood pressure variability.<sup>27</sup> In light of evidence that aortic stiffness is associated with impaired microvascular reactivity, enhanced stiffness may contribute to target organ damage by rendering an individual more susceptible to episodic fluctuations in blood pressure that result in transient ischemia in key organs, such as the brain, because of inability of the microcirculation to respond appropriately to the alteration in blood pressure.<sup>28,29</sup>

### Hypertension and Arterial Stiffness: Chicken or Egg?

Arterial stiffness has long been viewed as a consequence of long-standing hypertension. However, recent studies have suggested that arterial stiffness may contribute to the pathogenesis of hypertension. Mice fed a high-fat, high-sucrose diet developed insulin resistance, chronic inflammation, oxidant stress, and microalbuminuria, consistent with changes seen in obese humans.<sup>30</sup> Aortic PWV increased within 1 to 2 months on high-fat, high-sucrose diet at a time when blood pressure was unchanged and was followed by an increase in blood pressure at 6 months, indicating that aortic wall stiffening preceded the increase in MAP and PP. Within 2 months, aortic NO availability was reduced and the activity of tissue transglutaminase-2, an NO-sensitive enzyme that contributes to protein crosslinking in the matrix, was increased. The human aorta is probably not appreciably contractile and thus factors, such as NO and vasoactive peptides, may not influence stiffness directly through effects on vascular smooth muscle tone. However, increased crosslinking by tissue transglutaminase-2 as a result of diminished NO bioavailability could play a role in the modulation of aortic stiffness. Inflammation in the aorta was increased as evidenced by increased expression of tumor necrosis factor- $\alpha$ , monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 $\alpha$ . When high-fat, high-sucrose mice were

reverted to normal diet, weight returned to normal within 2 months, as did PWV and blood pressure, indicating that mechanisms contributing to aortic stiffening and elevated blood pressure were reversible in this model.

In a summary of ongoing funding for hypertension research provided by the Vascular Biology and Hypertension Branch of the National Heart, Lung, and Blood Institute, Galis et al<sup>31</sup> underscored the importance of elucidating the role of arterial stiffness in the pathogenesis of hypertension. They discussed a recent Vascular Biology and Hypertension Branch-sponsored initiative Cellular and Molecular Mechanisms of Arterial Stiffening and Its Relationship to Development of HTN (RFA HL-10-027), which provided funding for the high-fat, high-sucrose mouse model.<sup>30</sup> They also referred to a recent publication from another Vascular Biology and Hypertension Branch-supported clinical study that reported the same temporal sequence in the Framingham offspring cohort, where aortic stiffness assessed by CFPWV was associated with blood pressure progression and incident hypertension 4 to 10 years later.<sup>32</sup> Thus, the mouse study and the clinical study both suggest that aortic stiffening can precede and can contribute to the development of hypertension. As noted in the Vascular Biology and Hypertension Branch review, this concordance of findings in animal and clinical studies solidifies the support for the hypothesis that aortic stiffening has a potential causal role in the pathogenesis of hypertension and emphasizes the need to investigate molecular pathways that contribute to aortic stiffening and subsequent hypertension.<sup>31</sup>

### Components of Pulsatile Load and Clinical Outcomes

CFPWV is a strong predictor of cardiovascular disease risk in patient- and community-based cohorts.<sup>33</sup> However, the role of wave reflection as a predictor of events remains controversial. Weber et al<sup>34</sup> used a Windkessel-based method to analyze noninvasive central pressure waveforms in a high-risk cohort referred for cardiac catheterization. Although their technique separates forward ( $P_f$ ) and reflected or backward ( $P_b$ ) waves, the separation uses a model-based rather than a measured flow. Reflected waves add to pressure and subtract from flow; however, the balance between pressure augmentation versus flow deceleration (in a measured flow) is determined, in part, by ventricular function, which confounds pressure-only measures of wave reflection. Weber et al<sup>34</sup> found that brachial PP and  $P_f$  were the strongest correlates of events in minimally adjusted models, whereas measures of relative wave reflection, such as augmentation index (AI) and reflection magnitude ( $RM=P_b/P_f$ ), were not consistently related to outcomes in minimally adjusted models.  $P_b$  was related to events in minimally adjusted models; however, it is important to note that  $P_b$  is heavily confounded by its dependence on  $P_f$  (rearranging RM gives  $P_b=P_f \times RM$ ). The observation that RM was not related to events in minimally adjusted models suggests that larger  $P_f$  rather than higher RM may have mediated the relationship between  $P_b$  and events. Indeed, measures of relative wave reflection (RM, AI, and reflection index) required adjustment for various invasively determined measures, such as left ventricular ejection fraction, to relate to events with a nominally significant  $P$  value. As the authors noted, their

study underscores a key weakness of measures of apparent wave reflection derived from pressure-only in that the values require adjustment for various confounders, including ventricular function, to reveal modest relationships with outcome.

Consistent with the foregoing observations, CFPWV and augmented pressure, but not AI, predicted events in a renal transplant cohort.<sup>35</sup> Similarly, Wang et al<sup>36</sup> showed that  $P_f$  and  $P_b$  from triangulation, but not AI or reflection index, were associated with outcomes in men and in women in a community-based cohort from Asia. These studies underscore the mixed nature of absolute measures of reflected wave amplitude, such as  $P_b$  or augmented pressure, and underscore the need to look at specific components of the reflected wave to determine whether effects of a larger  $P_b$  are attributable to greater wave reflection per se (RM) or simply a larger  $P_f$  encountering the same amount of relative wave reflection. The latter distinction has important pathophysiologic implications that may translate into important treatment implications.

### Effects on the Microcirculation

Large artery stiffness and excessive pressure pulsatility have important relationships with various measures of microvascular structure and function, particularly in high-flow organs, such as the brain and kidney.<sup>28,29,37</sup> In risk factor adjusted models, higher PP, primary pressure wave, and aortic PWV—but not AI, pressure amplification, or muscular artery stiffness—were associated with increased renal blood flow pulsatility.<sup>38</sup> Higher pulsatility was also associated with albuminuria, suggesting that factors that favor penetration of excessive flow pulsatility into the kidney microcirculation are associated with kidney damage. PP, but not MAP, was associated with increased wall/lumen ratio in the retinal vessels in a relatively young and healthy nondiabetic cohort with at most mild-to-moderate hypertension.<sup>39</sup> In fat biopsy specimens taken in an exclusively hypertensive cohort, the media/lumen ratio of small arteries was associated with higher CFPWV, central PP, MAP, and AI. The relationship between media/lumen ratio and CFPWV persisted after adjustment for MAP.<sup>40</sup> The foregoing studies suggest that stiffening of the aorta may trigger damage and remodeling of the microcirculation. Excessive pulsatility in obligate high-flow organs, such as the brain and kidney, may cause microvascular target organ damage, whereas resistance vessel remodeling in demand flow beds, such as fat and muscle, may drive up resting MAP and wave reflection, leading to a vicious cycle of progressive aortic stiffening and microvascular remodeling that culminates in clinical hypertension and target organ damage.

## Treatment

### Diet and Activity

Recent observational studies offer insight into lifestyle modifications that may favorably affect large artery stiffness. Higher historical dairy consumption was associated with lower CFPWV, PP, and systolic blood pressure.<sup>41</sup> Relationships between dairy intake and CFPWV showed a dose-response that persisted after adjusting for confounders, including MAP. The authors speculated that dairy-derived peptides may inhibit angiotensin-converting enzyme and noted additional

potentially beneficial components in milk, including potassium, magnesium, phosphorus, and calcium.

In a salt-sensitive African cohort, higher urinary sodium/potassium ratio (Na/K) was associated with higher PP because of separate relationships with higher primary (forward) pressure wave amplitude (P1) and AI.<sup>42</sup> Consistent with the foregoing, in a short-term intervention study, potassium supplementation was associated with a reduction in CFPWV.<sup>43</sup> Similarly, the low-sodium dietary approaches to stop hypertension diet reduced blood pressure, CFPWV, and oxidative stress in a small sample of patients with hypertension and heart failure with preserved ejection fraction.<sup>44</sup>

Modest weight loss associated with 12 weeks of caloric restriction alone (with no change in activity level) in an overweight-to-obese sample of middle-aged and older individuals was associated with a reduction in CFPWV that correlated with the degree of reduction in measures of adiposity after adjusting for change in MAP.<sup>45</sup> A brief course of statin therapy was associated with comparable reductions in CFPWV in the absence of a change in MAP.<sup>46</sup> These studies demonstrate that it may be possible to achieve modest reductions in aortic stiffness after a relatively brief intervention. However, these preliminary results in small samples underscore the need for adequately powered, potentially nested studies that can efficiently compare various interventions and clarify the roles of lifestyle and drugs in favorable modification of aortic stiffness.

A history of habitual activity during childhood and adolescence was related to carotid stiffness measured at 36 years of age.<sup>47</sup> Differences in carotid stiffness related more strongly to the duration of vigorous activity, possibly because of favorable effects on risk factor profile. Those with stiffer carotid arteries experienced a more substantial decrease in duration of vigorous activities in the transition from adolescence to adulthood. At the opposite end of the age spectrum, triaxial accelerometry was used to show that a high frequency of light physical activity during the day may be associated with lower CFPWV in older people, even after adjusting for the amounts of moderate or vigorous activity.<sup>48</sup> In older people with heart failure with preserved ejection fraction, greater carotid artery stiffness was correlated with lower peak oxygen consumption and 6-minute walk distance.<sup>49</sup> Data from the Whitehall II study demonstrated a relationship of CFPWV with physical functioning, including pulmonary function tests, in middle-aged and older adults.<sup>50</sup> Relationships persisted when adjusted for age and various potential confounders, including MAP and PP. The foregoing studies suggest that PWV and PP are potentially valuable markers of vascular age that may predict risk for various degrees of premature disability.

Despite the substantial age range examined across the foregoing cross-sectional studies, one cannot ascertain whether physical function was a cause or consequence of aortic stiffening. Findings in the younger cohort suggest that early activity may have modified aortic stiffness at midlife, whereas those in the older Whitehall II cohort suggests that premature vascular aging may limit physical functioning. However, the opposite conclusions are potentially valid for each cohort in these cross-sectional studies. Serial measures of aortic stiffness and structured intervention studies will be required to understand

the cause and effect in the likely bidirectional relationship between aortic stiffness and physical activity better.

### A-II Type 2 Receptor Activation

Potentially favorable effects of A-II type 2 receptor activation by compound 21 were evaluated in animal models of hypertension. Compound 21 alone did not prevent the development of hypertension but reduced collagen and fibronectin deposition and macrophage infiltration in the aorta<sup>51</sup> and attenuated aortic wall stiffening and fibrosis.<sup>52</sup> The A-II type 1 receptor antagonist olmesartan reduced blood pressure, aortic PWV, and remodeling to control levels but did not restore hydroxyproline content to control levels, whereas the addition of the A-II type 2 receptor agonist had no further effect on blood pressure but reduced hydroxyproline content to control levels. Direct anti-inflammatory, antiproliferative, or antifibrotic effects of A-II type 2 receptor agonists have been demonstrated and may have contributed to the observed MAP-independent effects of A-II type 2 receptor stimulation on aortic structure and function.

### Arterial Stiffness as a Surrogate End Point for Cardiovascular Disease

There is strong evidence that aortic stiffness is related to clinical events even in models that adjust for effects of well-known, modifiable risk factors. As noted above, aortic stiffness may antedate and may contribute to the development of hypertension, which is a leading cause of potentially preventable disability and premature death. However, additional work is needed before PWV can be considered a true surrogate end point for cardiovascular disease. Future studies will be needed to demonstrate that an intervention strategy that targets PWV can successfully reduce PWV and that the reduction in PWV is associated with a proportional reduction in events.<sup>53</sup>

### Summary

During the past 2 decades, arterial stiffness has emerged as an important, prevalent risk factor for premature disability and death. Aging of the world population in combination with high relative risk and prevalence of arterial stiffness with advancing age guarantees that the stiffness-related disease burden will increase substantially for the next 2 decades unless measures are identified and implemented that are effective at preventing or reversing excessive aortic stiffness. An optimal approach likely will require that we promote aortic health starting at prenatal and developmental stages and continuing throughout the life course. Specific pharmacotherapy designed to target established aortic stiffness is needed, but in light of the rather severe abnormalities in aortic structure and function that are seen in a substantial proportion of older people, effective prevention strategies likely will be essential for optimal benefit.

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Dr Mitchell is the owner of Cardiovascular Engineering, Inc, a company that develops and manufactures devices to measure vascular

stiffness and serves as a consultant to and receives honoraria from Novartis and Merck.

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