Recent Advances in Hypertension

Mechanisms and Subclinical Consequences of Aortic Stiffness

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The number of published articles on PubMed with arterial stiffness or aortic stiffness in the title during the past 20 years exceeds 4000 articles published, with more than one-half published in the past 5 years (see Figure). The studies published on the topic of arterial stiffness span the full translational continuum from basic mechanistic studies in cell and animal models to small experimental studies in humans and large observational cross-sectional and prospective studies relating arterial stiffness to subclinical target organ damage or clinical cardiovascular disease (CVD) events. The reason for this rapidly growing area of investigation is likely attributed to the strong body of evidence indicating that arterial stiffness, specifically aortic stiffness as measured by the reference standard carotid-femoral pulse wave velocity (CFPWV), is a robust predictor of clinical CVD events and BP result in reductions in compliance and endothelial function of peripheral muscular arteries, but the influence of transient elevations in SNS activity on the large elastic arteries (eg, aorta and carotid arteries) shows conflicting results. For example, Mäki-Petäjä et al had a group of young healthy adults perform 4 minutes of static handgrip exercise, a model well established to increase SNS activity and BP. As expected, handgrip exercise resulted in small but significant elevations in BP and CFPWV; however, the rise in CFPWV was completely abrogated after adjustment of increase mean arterial BP. In contrast, acute experimental increases in SNS activity in the absence of increases in BP using the lower body negative pressure technique resulted in no changes in aortic or carotid elastic compliance in young healthy humans in 2 studies, therefore suggesting a rise in BP is likely necessary to alter elastic artery stiffness.

In 2015, the American Heart Association published a comprehensive Scientific Statement addressing the nomenclature, methodologies, utility, limitations, and gaps in knowledge related to arterial stiffness to inform the research community of the state of field and help guide future directions for investigation, including identifying targets for intervention. Because the putative mechanisms that contribute to the development of aortic stiffness remain elusive, the current review article will focus on the recent advances during the past 2 to 3 years describing some novel mechanisms that contribute to elevated aortic stiffness that may be therapeutic targets for intervention. Then, a few of the most exciting recent investigations about the subclinical negative consequences of aortic stiffness on the brain and kidney will be discussed.

Sympathetic and Parasympathetic Nervous System Function

Chronic sympathetic nervous system (SNS) hyperactivation is linked to cardiovascular end-organ damage, such as vascular hypertrophy, left ventricular hypertrophy, and endothelial dysfunction. However, the degree to which the SNS activation acutely or chronically modulates mechanical properties of large elastic arteries remains controversial. Experimental maneuvers that acutely increase SNS activity and BP result in reductions in compliance and endothelial function of peripheral muscular arteries, but the influence of transient elevations in SNS activity on the large elastic arteries (eg, aorta and carotid arteries) shows conflicting results. For example, Mäki-Petäjä et al had a group of young healthy adults perform 4 minutes of static handgrip exercise, a model well established to increase SNS activity and BP. As expected, handgrip exercise resulted in small but significant elevations in BP and CFPWV; however, the rise in CFPWV was completely abrogated after adjustment of increase mean arterial BP. In contrast, acute experimental increases in SNS activity in the absence of increases in BP using the lower body negative pressure technique resulted in no changes in aortic or carotid elastic compliance in young healthy humans in 2 studies, therefore suggesting a rise in BP is likely necessary to alter elastic artery stiffness.

In relation to chronic elevations in SNS activity, higher resting tonic SNS activity, as measured by muscle sympathetic nerve activity, was positively associated with elevated CFPWV in young/middle-aged healthy men even after accounting for mean arterial BP. Similarly, Tanaka et al demonstrated an inverse relationship between muscle sympathetic nerve activity and carotid artery compliance. After adjusting for muscle sympathetic nerve activity, the age-related differences in compliance between young and older adults were abolished, suggesting that chronically elevated SNS activity mediates the age-related reduction in carotid compliance. Taken together, the data still remain unclear whether transient and chronic elevations in SNS activity modulate the mechanical properties of the aorta and carotid arteries. Thus, additional studies are needed, taking into consideration the method of measuring stiffness (eg, CFPWV versus local compliance), whether elevated baseline SNS activity is present, and differences in age, sex, and other clinical characteristics of the cohort.

Mäki-Petäjä et al explored the role of both the parasympathetic nervous system (PNS) and SNS on arterial stiffness.
Using heart rate variability obtained from resting ECG in a subset of adults from the Enigma cohort study, participants were first stratified into tertiles of high frequency power, an indirect index of PNS activity. They found that CFPWV was significantly lower with increasing levels of high frequency power (PNS activity), but the differences were abolished after adjustment for mean arterial BP. Consistent with this, stepwise regression models, age, sex, BP, and heart rate, but not high frequency power, entered the models for CFPWV, suggesting that PNS activity does not modulate aortic stiffness. Furthermore, there was no difference in CFPWV or mean BP if the participants were stratified by low frequency power (index of SNS activity), suggesting that SNS activity does not appreciably modulate CFPWV in young adults. Next, to assess the contribution of the acute changes in SNS and PNS on aortic stiffness, a ganglionic blocker was administered to inhibit SNS and PNS activity, but this resulted in no change in CFPWV despite a paradoxical increase in BP and heart rate. In another study, acute sympathoinhibition of SNS with an intravenous ganglionic blocker resulted in reduced CFPWV despite a paradoxical increase in BP and heart rate.

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**Klotho and Sirtuin-1**

Klotho gene is an antiaging gene expressed mostly in the kidney and lesser extent in the brain that when mutated results in myriad premature aging phenotypes and early death. In contrast, overexpression of Klotho reverses many of the age-related impairments in physiological function and extends lifespan in mice. Klotho gene also results in a secreted protein, whereby circulating concentrations are reduced with aging and hypertension, and inversely associated with carotid artery stiffness in humans. Therefore, several recent studies investigated whether reduction in Klotho could be a putative mechanism mediating aortic stiffness with aging. Chen et al demonstrated that mice heterogenous for Klotho gene exhibited elevated aortic PWV compared with wild-type littersmates. Importantly, aortic PWV appeared to be elevated at least 2 weeks before the increase in BP, suggesting that aortic stiffness related to loss of one Klotho allele may be causal rather than a result of the elevated BP. Klotho haplodeficient mice also exhibited increased collagen I and reduced elastin in media of aortas (but not smaller carotid and femoral arteries), with concomitant elevated aortic expression of matrix metalloproteinase-2 and -9, transforming growth factor-β1, and myofibroblast differentiation (indicated by α-smooth muscle cell positive cells). Furthermore, a novel finding was that Klotho-deficient mice demonstrated elevated circulating aldosterone concentrations and blockade with the mineralocorticoid receptor (MR) antagonist eplerenone for 3 weeks completely abolished the Klotho deficiency-mediated increase in aortic stiffness and aforementioned cellular vascular alterations.

A follow-up study indicated that heterozygous Klotho-deficient mice fed a high-fat diet (HFD) exhibited increased aortic PWV and glucose within 5 weeks but not in the wild-type mice, suggesting that Klotho deficiency exacerbates HFD-mediated aortic stiffening and impaired glucose metabolism. However, it should be noted that systolic BP was also elevated about week 5, making it difficult to determine if the aortic stiffening was a cause or a consequence of the raised BP induced by HFD. In addition to upregulation of collagen I and transforming growth factor-β1, Klotho-deficient mice on HFD also demonstrated reduced endothelial nitric oxide synthase serine 1177 and adenosine monophosphate-activated protein kinase α phosphorylation. Weekly injection of an adenosine monophosphate-activated protein kinase activator abrogated the elevation in aortic PWV, BP, and glucose, suggesting that adenosine monophosphate-activated protein kinase may be a key mechanism in promoting the HFD-mediated stiffening and hypertension in the presence of Klotho deficiency. In addition, overnight fasting-induced reduction in aortic PWV in high fat/high sucrose (HFS) diet-fed mice was prevented in SIRT1 (sirtuin-1) vascular smooth muscle cell (VSMC) knockout mice. Similarly, overexpression of VSMC SIRT1, an NAD+-dependent deacetylase, or 1 week treatment with SIRT1 activator SRT1720 prevented the HFS diet-related elevation in aortic PWV. However, corresponding BPs were not reported, so it is unclear whether changes in BP mediated the change in PWV with the fasting, diet-, and SIRT1 activation interventions.

Klotho-deficient mice also demonstrated reduced expression of SIRT1 that was associated with antioxidant, anti-inflammatory, and antisenescence phenotypes and downregulation of endothelial nitric oxide synthase. Because Klotho deficiency downregulated aortic SIRT1 of the mice and activation of SIRT1 by SRT1720 did not alter circulating Klotho concentrations, this suggests that Klotho signaling is upstream from SIRT1 and acts as a hormone given that Klotho gene is expressed in the kidney and brain and not aorta. These findings are clinically important because older adults demonstrate lower SIRT1 expression in vascular endothelial cells that is associated with an age-related reduction in endothelial function. Importantly, because stiffening precedes the elevation of BP in the Klotho-deficient mice and several large
prospective studies suggest that aortic stiffness antecedes development of hypertension, further investigation is warranted to determine whether boosting Klotho is a novel target for treatment of aortic stiffness–related hypertension.

**Parental and Early Life Determinants**

The heritability of high CFPWV and related hemodynamic variables (eg, forward and reflected wave amplitude, augmentation index) is estimated to be between 0.21 and 0.48, which is in the same range as hypertension heritability. In this regard, Andersson et al investigated whether normotensive young/middle-aged offspring of parents with hypertension exhibited greater aortic stiffness and hemodynamic variables before the onset of clinical hypertension with the idea that aortic stiffness is causal in the pathogenesis of the development of hypertension. Indeed, offspring with 1 or 2 parents with hypertension demonstrated higher CFPWV, forward pressure wave amplitude, augmentation index, and mean arterial BP after adjustments for age, sex, and height. However, in fully adjusted models, only mean arterial BP and forward wave amplitude remained associated with parental hypertension status. Because aortic forward pressure wave amplitude, determined from pressure and flow-related characteristic impedance in proximal aorta, is a robust predictor of CVD risk in risk factor–adjusted models that include CFPWV, this suggests that parental hypertension could be an important predictor of this understudied hemodynamic biomarker.

In addition to heritable influences on aortic stiffness, early life social and psychological factors in childhood may also contribute to higher arterial stiffness and CVD risk in adulthood. Consistent with this, adverse childhood experiences, such as physical and sexual abuse, neglect, and household dysfunction, seem to have a strong influence on increases in BP in young adulthood after age 30 years, that are not explained by CVD risk factors or socioeconomic status. In addition, children and young adults exposed to moderate or severe adverse childhood experiences demonstrate higher diastolic BP, total peripheral resistance, and carotid-radial PWV (ie, peripheral muscular artery stiffness) after adjustment for demographic factors, education, and socioeconomic status. However, the comparisons for PWV were not adjusted for BP, and CFPWV was not assessed. Thus, future studies investigating whether a BP-independent increase in CFPWV is present in youth exposed to adverse childhood experiences are still needed. Taken together, these studies underscore that traumatic psychological experiences in childhood may be an underappreciated critical risk factor for CVD that requires additional investigation.

**Calcification**

Calcification of the arterial media is proposed to be involved with stiffening of the aorta in addition to alterations in load-bearing extracellular matrix (ECM) proteins elastin and collagen. However, whether progression of calcification parallels increases in aortic stiffening in humans was unknown until recently. As such, Guo et al assessed aortic calcification via chest computed tomography from aortic arch to iliac bifurcation and brachial-ankle PWV (baPWV) prospectively in middle-aged men at baseline and 4 to 7 years later. As predicted, annual change in aortic calcification was significantly associated with increases in baPWV in multivariate-adjusted models. In men without calcification at baseline, baPWV was associated with incident aortic calcification at follow-up, and baPWV also was associated with progression of aortic calcification in participants with baseline aortic calcification, suggesting that arterial stiffness and calcification are causally linked. However, the direction of the association could not be determined in this study, and the relationship could be bidirectional. Furthermore, because baPWV includes both peripheral and central arterial stiffness, additional studies are needed to confirm these findings with CFPWV.

Cecelja et al measured expression of genes previously identified to be associated with arterial stiffness in lymphoblastoid cells in the Twins UK cohort in a cross-sectional study and after 4 years follow-up. Of the 52 genes analyzed, they found that CFPWV was associated with higher expression of ektocutis phosphate pyrophosphate/phosphodiesterase (ENPP1), a gene transcript associated with calcification. Importantly, they discovered that CFPWV and ENPP1 were more strongly correlated among monozygotic than dizygotic twins consistent with a significant genetic effect of ENPP1. In the prospective analysis, baseline ENPP1 expression was also related to progression of CFPWV during the 4-year follow-up in addition to collagen type IV α1 (COL4A1), a gene involved in type IV collagen protein expression. Interestingly, progression of decreased carotid distensibility was most closely associated with endothelial nitric oxide synthase (nitric oxide synthase 3) and angiotensin-converting enzyme genes but not ENPP1. Thus, these data suggest that genes involved in regulation of hypertensive and carotid stiffness progression may differ because of anatomic variances in ECM composition of arterial wall of aorta and carotids or differences in measurement techniques of arterial stiffness between the two arterial sites.

Matrix Gla-protein (MGP) is a strong inhibitor of soft tissue calcification that is fully activated when it undergoes glutamate carboxylation and serine phosphorylation. The carboxylation of MGP is a vitamin K–dependent process. Because these post-translational steps are not fully accomplished, the inactive form of MGP, dephospho-uncarboxylated MGP would be predicted to be associated with higher aortic stiffness in part from accelerated vascular calcification. Whereas several studies measuring total or uncarboxylated MGP without phosphorylation status assessed have failed to identify a correlation with aortic stiffness, Pivin et al reported a positive association between circulating dephospho-uncarboxylated MGP and CFPWV in a large sample of middle-aged adults after adjustment for BP and CVD risk factors. Given that active MGP resides in the vascular media and inhibits medical calcification of elastin, and that inactive dephospho-uncarboxylated MGP could be activated by vitamin K administration, vitamin K supplementation may be a novel intervention to attenuate vascular calcification–mediated aortic stiffness, but this has not been tested in clinical studies to date.

**Vascular Smooth Muscle and Endothelial Cell Intrinsic Stiffness**

Alterations in the mechanical properties of the large elastic arteries with aging and or hypertension are typically attributed
to reductions in elastin and increases in collagen ECM content, as well as collagen cross-linking in the vascular media, with additional contributions from impaired nitric oxide–mediated vascular tone. However, a series of studies have recently identified increased intrinsic stiffness of aortic VSMCs in animal models of hypertension and aging using novel in vitro biomechanical/chemical techniques. Using a reconstituted aortic tissue model and atomic force microscopy in isolated primary aortic VSMCs from monkeys, Qiu et al demonstrated that VSMCs exhibited increased stiffness in the aortic tissue and primary isolated VSMCs higher exhibited greater intrinsic elastic modulus (stiffness) in old compared with young monkeys. Similarly, Sehgel et al found that cellular stiffness from reconstituted aortic tissue was increased 4-fold, and the intrinsic stiffness of individual VSMCs was increased by 2-fold, in spontaneously hypertensive rats compared with Wistar-Kyoto normotensive rats. A novel finding was that hypertension-mediated increases in individual VSMC stiffness, adhesion to fibronectin, and medial layer thickening were all exacerbated with aging. In contrast, the ECM composition of elastin and collagen in the vascular media was not altered in the aorta of young spontaneously hypertensive rat, but collagen was increased and elastin reduced in the aged spontaneously hypertensive rats. These findings suggest that mechanical properties of VSMCs and perhaps other medial layer remodeling processes (eg, glycation-mediated cross-linking collagen) play a more significant role in hypertension than the ECM proteins elastin and collagen. Moreover, VSMCs and ECM seem to contribute synergistically when aging is superimposed with hypertension; however, these results do not provide evidence that age-related changes in VSMC stiffness, adhesion, or ECM remodeling contribute to hypertension because BP was not elevated in the aged Wistar-Kyoto rats. Endothelial dysfunction may also contribute, at least in part, to the development of aortic stiffness through chronic increases in VSMC tone from reduced nitric oxide bioavailability. However, much less is known about the potential contribution of intrinsic cortical stiffness of individual VSMCs. In this regard, DeMarco et al demonstrated that mice fed a HFS diet for 4 months exhibited a 5-fold increase in endothelial cell and VSMC surface stiffness using atomic force microscopy from aortic explants. This was paralleled by elevation in aortic PWV, endothelial dysfunction, oxidative stress, and a proinflammatory vascular phenotype despite no increase in BP. Interestingly, treatment with spironolactone, an MR antagonist, completely abolished the increase in aortic PWV and cortical stiffness of endothelial cells and VSMCs, as well as the pro-oxidative stress and proinflammatory phenotype. MR antagonism also reversed HFS diet-induced elevation in femoral artery stiffness in the absence of any reduction in BP. This is important because destiffening effects of MR antagonism occurred in a BP-independent manner, suggesting a direct effect of MR signaling on the vascular remodeling of aorta and femoral arteries making the MR a potential novel target for treatment of HFS-induced aortic stiffness. A follow-up study in this model demonstrated that mice fed the HFS diet and given running wheels for 16 weeks exhibited reversal of endothelial cell cortical stiffness in aortic tissue in the absence of changes in body weight. Surprisingly, exercise did not reduce aortic PWV despite reductions in endothelial and medial nitrotyrosine and decreased endothelial transforming growth factor-β and adventitial fibronectin. In contrast, wheel running reversed femoral artery stiffness, and this was associated with increases in elastin and number of fenestrae in the internal elastic lamina. These data suggest that the improvements in the endothelial cell surface stiffness and fenestrae with exercise may precede changes in aortic stiffness, or alternatively, that exercise training was not long enough or stiffness adaptations are specific to the arteries perfusing the exercising muscle in this model.

**Subclinical Consequences of Aortic Stiffness**

There is a growing body of evidence that the high blood flow, low resistance organs, such as the brain and kidney, suffer the hemodynamic consequences (ie, elevated pulsatile flow and pulse pressure) of increased large elastic artery stiffness with aging and hypertension. Indeed, higher CFPWV was associated with lower memory scores and subcortical white matter hyperintensities (but not processing speed or executive function) in older adults age 70 to 80 years. The association between aortic stiffness and memory was mediated, in part, from greater cerebral vascular resistance and subcortical white matter damage. In a study among middle-aged adults (age 45-65 years), higher CFPWV was associated with slower processing speed and executive function and white matter hyperintensities, and the change in CFPWV over 4 years was related to the longitudinal decline in executive function, memory scores, and working memory even after adjustment for BP and the presence of hypertension. Importantly, persons with hypertension and high CFPWV (>7 m/s) had the largest decline in executive function for 4 years, suggesting an additive effect of hypertension and aortic stiffness on selective domains of cognition. Furthermore, in older adults without dementia at baseline, CFPWV predicted the 10-year incidence of diagnosed mild cognitive impairment in all older adults, and with dementia in nondiabetic aged persons. In addition to the brain, previous studies have also linked aortic stiffness and excess flow pulsatility with reduced renal glomerular filtration rate in patients with hypertension and chronic kidney disease. An elegant study by Hashimoto and Ito extended these findings and revealed that aortic flow reversal, quantified by increased retrograde thoracic aortic flow during diastole as a result of aortic stiffness, explained the link between aortic stiffness and decline in renal function by impairing antegrade aortic and renal flow in patients at risk for or with kidney disease. Taken together, these studies support the idea that interventions targeting aortic stiffness may be a novel approach to starve off the adverse effects of stiffness-associated subclinical damage to these highly vulnerable organs.

**Summary and Future Directions**

Aortic stiffness continues to be a robust area of investigation spanning the translational continuum of epidemiology, clinical and basic mechanistic studies. Given that most antihypertensive drugs do not target stiffness of the large elastic arteries, these and other recent discoveries may provide initial guidance in discovering potential new therapeutic targets to treat or prevent the development of aortic stiffness and...
subsequent subclinical target organ damage. The major hurdle is that promising interventions (eg, both exercise or pharmacological) in rodent models of aging and hypertension do not always translate to effective treatments in human clinical studies. Nonetheless, this should not diminish efforts to move basic studies to small, randomized, or experimental integrative studies in humans in order to inform additional basic studies and guide larger clinical trials. However, perhaps equally important than treating aortic stiffness once it is established is maintaining aortic health in early life through the middle-age adult years likely making this the most effective strategy for preventing an onslaught of aortic stiffness–associated CVD, kidney disease, and cognitive impairment in our aging population.

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