Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study

Pauline Maillard, PhD; Gary F. Mitchell, MD; Jayandra J. Himali, PhD; Alexa Beiser, PhD; Connie W. Tsao, MD; Matthew P. Pase, PhD; Claudia L. Satizabal, PhD; Ramachandran S. Vasan, MD; Sudha Seshadri, MD; Charles DeCarli, MD

Background and Purpose—Previous work from the Framingham Heart Study suggests that brain changes because of arterial aging may begin in young adulthood and that such changes precede cognitive deficits. The objective of this study was to determine the association of arterial stiffness with measures of white matter and gray matter (GM) integrity in young adults.

Methods—One thousand nine hundred three participants from the Framingham Heart Study Third Generation (mean age, 46±8.7 years) had complete tonometry measurements and brain magnetic resonance imaging (T1-weighted and diffusion tensor imaging). Tonometry measures included carotid-femoral pulse wave velocity, augmentation index, carotid-brachial pressure amplification, and central pulse pressure. Fractional anisotropy and GM density images were computed from diffusion tensor imaging and T1 images. Registration to a common anatomic template enabled voxel-based linear regressions relating measures of fractional anisotropy and GM to tonometry measures, adjusting for relevant covariables.

Results—Higher carotid-femoral pulse wave velocity was associated with lower regional fractional anisotropy, including the corpus callosum and the corona radiata (8.7 and 8.6 cc, respectively, \( P<0.001 \)), as well as lower GM density in the thalamus region (0.9 cc, \( P<0.001 \)). Analyses did not reveal significant associations between other tonometry measures and fractional anisotropy or GM.

Conclusions—Among young healthy adults, higher aortic stiffness was associated with measures of reduced white matter and GM integrity in areas implicated in cognitive decline and Alzheimer’s disease. Greater aortic stiffness may result in subclinical vascular brain injury at ages much younger than previously described. (Stroke. 2016;47:1030-1036. DOI: 10.1161/STROKEAHA.116.012949.)

Key Words: blood pressure ■ brain ■ diffusion tensor imaging ■ magnetic resonance imaging ■ white matter

With aging, the aorta stiffens and pressure pulsatility increases, inducing hemodynamic changes implicated in the development of cardiovascular diseases (CVD). Recent analyses from the Framingham Heart Study (FHS) and other cohorts suggest that, in older individuals, vascular remodeling may also play a central role in the development of structural brain injury, which have been associated with reduced cognitive ability and increased likelihood of incident dementia.

The earliest differences in brain structure associated with increased pulsatility, however, have yet to be determined, and it is possible that differences could be identified even earlier in life, before symptomatic disease expression, should a suitably sized cohort and sensitive magnetic resonance imaging (MRI) measures be available. The FHS Third Generation (G3) is ideal to test this hypothesis as participants were recruited at an early age and were 40 years of age on average at the time of this study. Despite the relatively young age of this cohort, sensitive measures of white-matter (WM) injury from diffusion tensor imaging (DTI) identified subtle vascular brain injury in association with elevated systolic blood pressure, suggesting that newer imaging techniques may be able to identify clinically silent structural brain differences in association with hemodynamic metrics.

Our aim was to extend our preliminary findings by exploring associations between tonometry measures and regional
white matter injury as estimated by DTI-derived fractional anisotropy (FA) and gray matter (GM) atrophy in G3 participants. We postulated that increased arterial stiffness would be associated with lower FA and GM density, which would suggest that arterial stiffness can lead to vascular brain injury as early as the fifth decade of life.

**Methods**

**Study Sample**
The design of the Framingham G3 Cohort study has been detailed previously. Of the 3519 participants who attended examination 2, 2034 underwent brain MRI between 2009 and 2013 and had successful arterial tonometry measures. Participants were excluded from the present analysis for the following reasons: prevalent stroke at the MRI evaluation, other neurological disorders that might confound the assessment of brain volumes, because of bad or incomplete tonometry data or poor MRI quality, resulting in a sample of 1903 individuals. All protocols were approved by Boston University Medical Center’s institutional review board, and participants provided written informed consent.

**Clinical Evaluation and Definitions**
Medical history, physical examination, and ECG were performed routinely at each FHS examination. Blood pressures represent the average of 2 auscultatory blood pressures obtained by the physician on seated participants at the time of each Framingham clinic examination with the use of a standardized measurement protocol.

**Tonometry Data Acquisition and Analysis**
Noninvasive hemodynamic data acquisition is described in the online-only Data Supplement. Mean arterial pressure (MAP) was calculated by integration of the calibrated brachial pressure waveform. Central pulse pressure (CPP) was defined as the difference between the peak and trough of the calibrated carotid pressure waveform. Augmentation index (AI) was computed from the carotid pressure waveform as described previously. Briefly, AI is the augmentation pressure divided by pulse pressure, expressed as a percentage. Carotid-femoral pulse wave velocity (CFPWV) values were calculated from tonometry waveforms and body surface transit distance, which were adjusted for parallel transmission in the brachiocephalic artery and aortic arch with the use of the supra-sternal notch as a fiducial point. The carotid-femoral transit path spans the descending aorta, making CFPWV a measure of aortic stiffness.

**Brain MRI Analysis**
We used DTI measures of FA, which is a sensitive indicator of WM integrity. FA was computed from DTI using FSL software tools. Segmentation of GM, WM, and total cranial volume were performed from T1-weighted and FLAIR images by automated procedures previously described. FA and GM maps were finally coregistered to a minimal deformation template for group statistical analyses (online-only Data Supplement).

**Statistical Analyses**

**Voxel-Based Regressions of FA and GM With Components of Hemodynamic Load**
The primary goal of the statistical analysis was to determine if, at the image voxel level, individual key components of hemodynamic load (CFPWV, AI, CPP, and MAP) were associated with greater brain injury as indicated by lower FA or GM atrophy adjusting for a set of CVD risk factors. To achieve this goal, we used a linear regression (model 1) with either measures of FA or GM density as the dependent variable and each of the 4 components as independent variables, adjusting for a reference set of standard risk factors including age, sex, use of antihypertensive therapy, total cholesterol, current smoking status, presence of diabetes mellitus, total cranial volume, and time between clinical and MRI exams. CFPWV was inverted or reduced heteroscedasticity and normalize the distribution; the value was multiplied by −1000 to convert units to milliseconds per meter and restore directionality of effects.

In a second model (model 2), we then tested whether relations of CFPWV, AI, CPP, and MAP with FA and GM may be modulated by age, sex, and antihypertensive treatment therapy. We assessed this goal by performing regressions as described in model 1, including additional interactions of the component of hemodynamic load with the 3 covariates, ie, age, sex, and antihypertensive therapy.

The T-map obtained for each comparison was evaluated for statistical significance using threshold free cluster enhancement at the P < 0.05 level (online-only Data Supplement) and corrected for multiple comparison using permutation-based correction (N=1000). We then overlaid the thresholded T-maps with the Johns Hopkins University probabilistic fiber map atlases and GM atlas, warped to the minimal deformation template space, to provide a post hoc regional description of significant voxels in terms of the WM tracts and GM structures to which they most likely belonged. For each significant region, the mean FA (respectively mean GM density) was computed for each person by superimposing the mask of the corresponding region to each individual’s FA (respectively GM) images. This enabled us to perform linear regressions as described above but at region level.

**Modulation of Hemodynamic Load Components Effects by Other Components**
To determine whether significant regional relations between 1 hemodynamic component and FA or GM were mediated in part by the other hemodynamic variables, we performed, for that component, region-based level regressions as described in model 1, including in addition the other hemodynamic variables as covariates.

**Accelerated White Matter Aging Related to Hemodynamic Load Components**
We computed the mean FA within regions identified in the voxel-based analyses as significantly associated with hemodynamic load components to be used as the dependent variable in a linear regression. The tonometry measures were categorized into 3 groups (higher, intermediate and lower tertile intervals), and used as the independent variable, adjusting for the reference set of standard risk factors. Because the mean FA measure appeared to be quadratically related to age on visual inspection (Figure 1A), we also created a model that included the quadratic effect of age.

**Results**

**Demographics**
Compared with the remainder of the G3 cohort (Table) without MRI, individuals included in this study were on average significantly younger (P=0.02) with significantly lower systolic blood pressure (P=0.001), CFPWV (P=0.008), AI (P=0.016), MAP (P=0.007), and CPP (P=0.036). In addition, they were less likely to smoke (P=0.004) to receive treatment for hypertension (P=0.007) or have diabetes mellitus (P=0.006).

**Associations of FA and GM With Components of Hemodynamic Load**

**Voxel-Based Regressions of FA and GM With Components of Hemodynamic Load**
In model 1, higher CFPWV was associated with lower FA within voxels that covered 25.5 cc of the WM (Table I in the

**Voxel-Based Regressions of FA and GM With Components of Hemodynamic Load**
In model 1, higher CFPWV was associated with lower FA within voxels that covered 25.5 cc of the WM (Table I in the
Stroke April 2016

online-only Data Supplement). WM tracts most implicated included the splenium, body, and genu of corpus callosum region (2.77, 3.78, and 2.05 cc, respectively) and the anterior, superior, and posterior part of the corona radiata (4.13, 2.55, and 1.86 cc, respectively, Figure 2A).

Higher CFPWV was also associated with lower GM density but within voxels that covered only 0.93 cc of the GM (Table I in the online-only Data Supplement), implicating the thalamic region (Figure 2B).

Figure 3 illustrates regression curves relating CFPWV with the mean FA and mean GM density, within the respective significant voxels for the largest identified regions.

Voxel-based analyses did not reveal significant association between FA and GM with MAP, AI, or CPP measures.

Table. Participants’ Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Study Sample</th>
<th>Sample Without MRI</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1903</td>
<td>1485*</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>46.2 (8.7) [24; 76]</td>
<td>46.9 (8.8) [24; 78]</td>
<td>0.020</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>1005 (53)</td>
<td>790 (53)</td>
<td>0.832</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>115 (13) [80; 207]</td>
<td>117 (15) [84; 188]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive therapy, n (%)</td>
<td>328 (17)</td>
<td>309 (21)</td>
<td>0.007</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>186 (34) [78; 564]</td>
<td>188 (37) [86; 568]</td>
<td>0.081</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>170 (9)</td>
<td>178 (12)</td>
<td>0.004</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>77 (4)</td>
<td>90 (6)</td>
<td>0.006</td>
</tr>
<tr>
<td>Carotid-femoral pulse wave velocity, m/s*</td>
<td>7.1 (1.4) [4.3; 18.8]</td>
<td>7.2 (1.5) [4.5; 17.0]</td>
<td>0.008</td>
</tr>
<tr>
<td>Augmentation index, %</td>
<td>9.4 (13) [-33.6; 53.0]</td>
<td>10.6 (13.4) [-32.1; 55.4]</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>86.6 (10.8) [56; 168]</td>
<td>87.6 (11.3) [60; 134]</td>
<td>0.007</td>
</tr>
<tr>
<td>Central pulse pressure, mm Hg</td>
<td>52.6 (13.1) [19.8; 130.2]</td>
<td>53.7 (14.5) [22.1; 135.5]</td>
<td>0.036</td>
</tr>
<tr>
<td>Time between clinical and MRI exams, y</td>
<td>1.69 (0.95) [-0.68; 5.17]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean fractional anisotropy</td>
<td>0.35 (0.02) [0.20; 0.40]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean gray matter density</td>
<td>0.62 (0.02) [0.47; 0.69]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter hyperintensities volumes, cc</td>
<td>0.84 (2.2) [0.003; 67.82]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter volume, cc</td>
<td>513 (118) [303; 762]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray matter volume, cc</td>
<td>621 (134) [441; 814]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra cranial volume, cc</td>
<td>1445 (142) [1079; 1947]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean (SD) and range for continuous variables. MRI indicates magnetic resonance imaging.

* Among the 1485 individuals without MRI, 1376 have nonmissing values for carotid-femoral pulse wave velocity, 1461 for augmentation index, 1478 for mean arterial pressure, and 1461 for central pulse pressure.
Modulation of Hemodynamic Load Associations by Sex and Antihypertensive Treatment

Model 2 revealed a significant interaction between CFPWV and age on FA (Figure 4A) with increasing age being associated with a larger negative relation of CFPWV with FA measures within voxels that covered 9.22 cc of the WM. WM tracts most affected included the anterior corona radiata (3.44 cc) and the external capsule (1.19 cc, Table II in the online-only Data Supplement). The model also revealed a significant interaction of CFPWV with antihypertensive treatment therapy, indicating an attenuated relation of CFPWV with FA in individuals receiving antihypertensive treatment when compared with individuals with no treatment. This modeled interaction implicated voxels that covered 0.86 cc of the WM, mostly in the posterior corona radiata region (0.53 cc, Figure 4B and Table II in the online-only Data Supplement).

Similar analysis of relations of CFPWV with GM density at voxel-based level failed to find any significant effects.

Finally, the lack of significant associations between FA and GM with MAP, AI, and CPP was not found to be modulated by age, sex, or antihypertensive treatment.

Modulation of Hemodynamic Load Components Effects by Other Components

Adding MAP, AI, and CPP as covariates in model 1 with CFPWV as variable of interest and regional FA and GM measures as dependent variables did not significantly change the association between CFPWV and FA and GM (Table I in the online-only Data Supplement).

Accelerated WM Aging Related to Hemodynamic Load Components

CFPWV tertile intervals were T1=[4.3; 6.3], T2=[6.3; 7.3], and T3=[7.3; 18.8] (m/s). On average, WM integrity (ie, mean FA) was found to be negatively associated with CFPWV tertile groups ($P=0.016$). Post hoc analyses, corrected for multiple comparisons using Bonferroni correction, revealed that individuals of T3 group had, on average, lower FA when compared with T2 ($\beta=-2.6\times10^{-3}$, $P=0.037$) and T1 ($\beta=-3.4\times10^{-3}$, $P=0.037$).

Figure 2. Regions of the cerebral white matter and gray matter (GM) in which lower fractional anisotropy (FA, A) and lower GM (B) are significantly associated with higher carotid-femoral pulse wave velocity. Color scale indicates the $P$ value.

Figure 3. Regression curves relating carotid-femoral pulse wave velocity and mean fractional anisotropy (FA) measures (A) and gray matter (GM) density (B) within the significant voxels for the respective largest identified regions (splenium of the corpus callosum and thalamus for the white and GM tissues, respectively).
arteries causes aortic systolic blood pressure to increase, which contributes to loading of stiff components of the arterial wall and subsequently further increases arterial stiffness. Structural changes that increase aortic stiffness are precipitated by a progressive increase in collagen, ground substance, and calcium deposition, coupled with the degradation and fragmentation of elastin fibers. These irregular physical forces in the arteries trigger atherogenic, hypertrophic and inflammatory responses in small vessels that may contribute to reduce microvascular reactivity. Arterial stiffness may contribute to microvascular brain injury by exposing the small vessels of the cerebral vasculature to high pressure fluctuations and flow pulsatility. Central artery stiffness may also increase short-term variability in blood pressure, which, in the presence of impaired microvascular reactivity, may impact supply of oxygen and nutrients to the brain. The impact of the reduction in microvascular reactivity on the cross-talk between large and small arteries may also play a role in brain injury, with the cross-talk between increased stiffness in large arteries and related microvascular dysfunction resulting in increased susceptibility to intermittent hypotension and relative ischemia, particularly in the periventricular WM watershed region.25

Impact of Arterial Stiffness on White Matter Integrity

Abnormal elevations in central and cerebral hemodynamic pulsatility promote the development of white matter hyper-intensities (WMH) and lacunar infarcts and increase the risk for a first CVD event.1,26 In particular, the association between central artery stiffness and WMH burden has been frequently and consistently reported among older individuals.3,4,27 In the FHS Offspring cohort of older individuals, recent findings suggest that only CFPWV and MAP are associated with WMH burden. But recent DTI studies suggest that WMH may only represent the extreme foci of a more widespread, continuous WM injury process that progresses insidiously during aging.13,28 suggesting that subtle aortic stiffness-related brain injury may occur before evident injury events, like WMH, occur, even when the overall vascular burden is low. This hypothesis is supported by the present work using DTI in a young adult sample, wherein we found continuous associations between CFPWV and microstructural WM injury in several tracts (particularly the corpus callosum) that have been reported to be affected in elderly people with Alzheimer disease.29 Our finding also extends observations from a recent tract-based spatial statistic DTI study1 that identified similar associations in a sample of 55 older individuals. Despite the fact that voxel-based and tract-based studies are 2 methods using a fairly different approach, both the prior study and the current data identified the corpus callosum, corona radiata, and internal capsule as regions that are particularly vulnerable to increased aortic stiffness.

Impact of Arterial Stiffness on GM Integrity

Our study also suggests a localized impact of CFPWV on GM integrity exclusively in the bilateral thalamic regions, covering only 0.9 cc, and may be explained by the location and blood supply of this region.30 At regional level, 1 study examined

Discussion

Results from this study indicated that, among a sample of young to middle-aged adults representative of the community, CFPWV, the reference standard noninvasive measure of aortic stiffness, was associated with injury to WM and regional GM atrophy that worsened continuously with increasing CFPWV. Such associations were found to be accentuated by age and attenuated by antihypertensive treatment. A second finding was that, in our community-based sample, CPP, AI, and MAP were not associated with measures of WM and GM integrity. These data indicate that cerebral microvascular damage and brain atrophy, associated with elevated aortic stiffness, which has been recently found to manifest in subclinical cognitive dysfunction in the FHS Offspring cohort (mean±SD age: 61±9 years old), is present and discernable even among younger individuals.

Biological Mechanisms

The biological mechanisms triggered by increased arterial stiffness are complex. With aging, stiffening of the large arteries causes aortic systolic blood pressure to increase, which contributes to loading of stiff components of the arterial wall and subsequently further increases arterial stiffness. Structural changes that increase aortic stiffness are precipitated by a progressive increase in collagen, ground substance, and calcium deposition, coupled with the degradation and fragmentation of elastin fibers. These irregular physical forces in the arteries trigger atherogenic, hypertrophic and inflammatory responses in small vessels that may contribute to reduce microvascular reactivity. Arterial stiffness may contribute to microvascular brain injury by exposing the small vessels of the cerebral vasculature to high pressure fluctuations and flow pulsatility. Central artery stiffness may also increase short-term variability in blood pressure, which, in the presence of impaired microvascular reactivity, may impact supply of oxygen and nutrients to the brain. The impact of the reduction in microvascular reactivity on the cross-talk between large and small arteries may also play a role in brain injury, with the cross-talk between increased stiffness in large arteries and related microvascular dysfunction resulting in increased susceptibility to intermittent hypotension and relative ischemia, particularly in the periventricular WM watershed region.25

Impact of Arterial Stiffness on White Matter Integrity

Abnormal elevations in central and cerebral hemodynamic pulsatility promote the development of white matter hyper-intensities (WMH) and lacunar infarcts and increase the risk for a first CVD event.1,26 In particular, the association between central artery stiffness and WMH burden has been frequently and consistently reported among older individuals.3,4,27 In the FHS Offspring cohort of older individuals, recent findings suggest that only CFPWV and MAP are associated with WMH burden. But recent DTI studies suggest that WMH may only represent the extreme foci of a more widespread, continuous WM injury process that progresses insidiously during aging,13,28 suggesting that subtle aortic stiffness-related brain injury may occur before evident injury events, like WMH, occur, even when the overall vascular burden is low. This hypothesis is supported by the present work using DTI in a young adult sample, wherein we found continuous associations between CFPWV and microstructural WM injury in several tracts (particularly the corpus callosum) that have been reported to be affected in elderly people with Alzheimer disease.29 Our finding also extends observations from a recent tract-based spatial statistic DTI study1 that identified similar associations in a sample of 55 older individuals. Despite the fact that voxel-based and tract-based studies are 2 methods using a fairly different approach, both the prior study and the current data identified the corpus callosum, corona radiata, and internal capsule as regions that are particularly vulnerable to increased aortic stiffness.

Impact of Arterial Stiffness on GM Integrity

Our study also suggests a localized impact of CFPWV on GM integrity exclusively in the bilateral thalamic regions, covering only 0.9 cc, and may be explained by the location and blood supply of this region.30 At regional level, 1 study examined
correlation between CFPWV and regional cerebral perfusion, using arterial spin labeling, within GM regions including hippocampus, thalamus, and caudate nucleus in 35 middle-aged adults but did not find evidence of significant associations of CFPWV with any of the GM structures. Although the ability of this study to detect associations may have been hindered by low statistical power, a potential hypothesis explaining the lack of associations is that the impact of arterial stiffness on GM integrity may be slow, subtle, and hardly perceptible during early and midadulthood life, but exacerbated later in the aging process, suggesting that cortical atrophy may be the end stage of advanced vascular disease. Longitudinal analysis of the effect of CFPWV on GM integrity may clarify this issue.

**Implications of Hypertension Treatment**

There is now substantial evidence that elevated arterial stiffness is the earliest manifestation of systolic hypertension. For example, in the FHS, the baseline measure of CFPWV was found to be strongly associated with incident hypertension. However, after controlling for baseline measure of CFPWV, no blood pressure component (systolic, diastolic, or mean) entered the model for future stiffness, supporting the hypothesis that aortic stiffness may antedate and contribute to the development of hypertension. This hypothesis is also supported by recent basic and clinical studies that find associations between altered CFPWV, through the disruption of elastin in the aortic wall, and subsequent development of hypertension. Finally, it should be noted that results from studies that seek to show that elevation in blood pressure precedes increase in CFPWV, are more controversial. Arterial stiffness, which is easily measurable, may therefore serve to identify individuals at greatest risk of hypertension progression. Our results find that treated hypertension is associated with more normal CFPWV values. This finding suggests that the elastic nature of the aortic vasculature may be more resilient and amenable at younger ages indicating that early life treatment of hypertension may be substantially more beneficial. Such a hypothesis deserves further testing.

**Absence of Association Between Brain Integrity Measures and AI, CPP, and MAP**

Overall, our findings do not suggest significant relations of AI and CPP with WM and GM integrity. Previous work from the FHS has identified CFPWV as a significant predictor of systolic hypertension. In contrast, neither carotid-radial pulse wave velocity, AI, nor CPP were related to CVD events in risk factor–adjusted models that included peripheral systolic blood pressure. Thus, CFPWV may be superior to other hemodynamic measures as a marker of risk that is distinct from standard vascular risk factors; the present analysis suggests that attention should be focused on aortic pulse wave velocity as a biomarker of microstructural WM integrity, even in younger individuals.

**Strengths and Limitations**

The cross-sectional design limits our ability to establish causal relations between arterial stiffness and brain aging measures. In addition, the FHS cohort is mostly of white descent and therefore does not fully represent the general population of the USA. Third, we referred to our sample as an adult healthy population although 73 participants were 60 years old and older. Nonetheless, excluding these older participants from the analyses did not change significantly the findings reported in the present study. Fourth, we did not include systolic blood pressure in our regression analyses to avoid introducing collinearity in the models. Similarly, we did not include measures of dietary or exercise, which both have been found to have beneficial effects on cognition, as recent studies have reported a significant impact of these nondrug interventions on arterial stiffness by several mechanisms.

**Conclusions**

In conclusion, CFPWV, a noninvasive measure of arterial stiffness, was associated with deterioration of WM integrity in young to middle-aged adults likely years before overt WMH seem. With the accumulating evidence that arterial stiffness impacts the trajectory of cognition later in life, the public health implications of our results emphasize the need for primary and secondary prevention of vascular stiffness and remodeling as early as the fifth decade. Use of tonometric measures may provide a relatively simple measure to identify higher risk young individuals who may most benefit from hypertension or other vascular risk factor treatment.

**Sources of Funding**

The study was supported by NIH grants to C.D. (R01 AG033040 and P30 AG010129), by NIH to C.W.T. (K23 LH118529) and by the NHLBI, Framingham Heart Study, NHLBI/NIH Contract #N01-HC-25195 and HHSN2682015000011 (R.S.V.) and the Boston University School of Medicine and by HL076784, G028321, HO70100, HL060040, HL080124, HL071039, HL077447, HL107385, and 2-K24-HL04334.

**Disclosures**

Drs Maillard, Seshadri, Beiser, Satizabal, Himmel, and Vasan report no disclosures. Dr DeCarli is a consultant to Novartis, Pharmaceuticals. Dr Pase is funded by an Australian National Health and Medical Research Council Early Career Fellowship (APP1089698). Dr Tsao is partially supported by an award from the American Heart Association (11CRP4930004). Dr Mitchell is owner of Cardiovascular Engineering Inc (a company that develops and manufactures devices to measure vascular stiffness) and is a consultant to Novartis, Merck and Servier.

**References**


Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study

*Stroke*. 2016;47:1030-1036; originally published online March 10, 2016; doi: 10.1161/STROKEAHA.116.012949

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/4/1030

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2016/03/10/STROKEAHA.116.012949.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Effects Of Arterial Stiffness On Brain Integrity In Young Adults From The Framingham Heart Study
Pauline Maillard1,2, PhD; Gary F. Mitchell3, MD; Jayandra J. Himali4,5, PhD; Alexa Beiser4,5,6, PhD; Connie W. Tsao7, MD; Matthew P. Pase4,5,8, PhD; Claudia L. Satizabal4,5, PhD; Ramachandran S. Vasan5, MD; Sudha Seshadri4,5, MD; Charles DeCarli, MD

1 Imaging of Dementia and Aging (IDeA) Laboratory, Davis, CA
2 Department of Neurology and Center for Neurosciences, University of California, Davis, CA
3 Cardiovascular Engineering, Inc, Norwood, MA
4 The Framingham Heart Study, Framingham, MA
5 Boston University School of Medicine, Boston, MA
6 Department of Biostatistics, Boston University School of Public Health, Boston, MA
7 Cardiovascular Division, Beth Israel Deaconess Medical Center and Harvard Medical School
8 Centre for Human Psychopharmacology, Swinburne University of Technology, Hawthorn, Australia
Supplemental Methods

Noninvasive Hemodynamic Data Acquisition

Participants were studied in the supine position after resting for ≈5 minutes. Supine brachial systolic and diastolic blood pressures were obtained with the use of a semiautomatic auscultatory device. Arterial tonometry with simultaneous ECG was obtained from brachial, radial, femoral, and carotid arteries with the use of a custom tonometer (Cardiovascular Engineering, Inc., Norwood, MA). All of the recordings were performed on the right side of the body. Transit distances were assessed by body surface measurements from the suprasternal notch to each pulse-recording site; a caliper was used for the femoral site. Tonometry and ECG data were digitized (1000 Hz) during the primary acquisition and transferred to the core laboratory (Cardiovascular Engineering, Inc, Norwood, MA) for analyses that were performed blinded to clinical data. Tonometry waveforms were signal averaged with the ECG R wave used as a fiducial point. Systolic and diastolic cuff BP obtained at the time of the tonometry acquisition were used to calibrate the peak and trough of the signal-averaged brachial pressure waveform. Diastolic and integrated mean brachial pressures were used to calibrate carotid pressure tracings. Calibrated carotid pressure was used as a surrogate for central pressure.

Brain MRI analysis

MRIs were performed on a 1.5T Siemens Avanto scanner (version syngo MR B15). Three sequences were used: a 3-dimensional T1-weighted coronal spoiled gradient-recalled echo (SPGR) acquisition, a fluid attenuated inversion recovery (FLAIR) sequence, and a diffusion tensor imaging (DTI) sequence. DTI was performed using the following parameters: repetition time (TR)=3600 ms, echo time (TE)=94 ms, 25 slices total, FOV=25 cm, acquisition matrix = 128 × 128, slice thickness = 5 mm with 5 mm gap. Diffusion weighted images were generated using 30 gradients directions with total gradient diffusion sensitivity of b=1000 s/mm², and one image with b=0 s/mm². Centralized reading of all images was performed using in-house designed imaging, visualization and analysis software (Quanta 2). The segmentation and quantification of WMH was performed using a semi-automated procedure that has been previously described² and which demonstrates high inter-rater reliability⁴. Segmentation of GM was performed on native T1-weighted images using an in-house implementation of a Bayesian maximum-likelihood expectation-maximization algorithm method⁵. FA maps were calculated from DTI⁶ and linearly aligned to the corresponding T1-weighted scan, which in turn was deformed to a minimal deformation template⁶,⁷ (MDT) with voxel dimensions of 0.98 x 1.5 x 0.98 mm³. This allowed transfer of GM and FA maps to the MDT space. A map of mean FA in the MDT space was created by averaging individual FA images across the population. Thresholding this mean FA map provided a binary WM mask in the MDT space. An FA threshold of 0.3 was chosen to select voxels in highly organized tracts, while minimizing inclusion of voxels with a higher degree of partial volume contamination.

Total cranial volume based on FLAIR was quantified using the Quanta 2 package of software routines according to a previously reported analysis protocol² and was used to correct for differences in head size. WMH volumes were log-transformed to normalize population variance.

Threshold free cluster enhancement

The T-maps obtained were evaluated for statistical significance using threshold free cluster enhancement (TFCE)⁸. In short, this methodology combines cluster size and significance
into a single parameter, the TCFE-score, by integrating the cluster size over a range of significance thresholds. A TFCE image was computed for each T map. The distribution of maximum TFCE scores under the null hypothesis was investigated for each independent variable using random permutation analysis, with 1000 iterations. Once the 95\textsuperscript{th} percentile in the null distribution was found then the TFCE images were thresholded at this level to allow inference at the $p < 0.05$ level.\textsuperscript{8}
Supplemental Tables
Table I: Associations between decreasing fractional anisotropy and gray matter density with carotid-femoral pulse wave velocity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Region</th>
<th>Model 1 Volume (cc)</th>
<th>Beta (×10^3)</th>
<th>P value</th>
<th>Model 3 Volume (cc)</th>
<th>Beta (×10^3)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter</td>
<td>Anterior corona radiata</td>
<td>4.13</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td>-0.19</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body of corpus callosum</td>
<td>3.78</td>
<td>-0.19</td>
<td>&lt;0.001</td>
<td>-0.21</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Splenium of corpus callosum</td>
<td>2.77</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td>-0.14</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superior corona radiata</td>
<td>2.55</td>
<td>-0.13</td>
<td>&lt;0.001</td>
<td>-0.16</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genu of corpus callosum</td>
<td>2.05</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td>-0.18</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posterior corona radiata</td>
<td>1.86</td>
<td>-0.13</td>
<td>&lt;0.001</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posterior thalamic radiation</td>
<td>1.84</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posterior limb of internal capsule</td>
<td>1.74</td>
<td>-0.08</td>
<td>&lt;0.001</td>
<td>-0.10</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anterior limb of internal capsule</td>
<td>1.06</td>
<td>-0.09</td>
<td>&lt;0.001</td>
<td>-0.09</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerebral peduncle</td>
<td>0.95</td>
<td>-0.09</td>
<td>&lt;0.001</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>External capsule</td>
<td>0.62</td>
<td>-0.09</td>
<td>&lt;0.001</td>
<td>-0.09</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superior longitudinal fasciculus</td>
<td>0.60</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td>-0.12</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superior fronto-occipital fasciculus</td>
<td>0.43</td>
<td>-0.12</td>
<td>&lt;0.001</td>
<td>-0.11</td>
<td>0.0028</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fornix (cres) / Stria terminalis</td>
<td>0.30</td>
<td>-0.12</td>
<td>&lt;0.001</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fornix (column and body of fornix)</td>
<td>0.26</td>
<td>-0.19</td>
<td>0.0046</td>
<td>-0.19</td>
<td>0.0155</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cingulum (cingulate gyrus)</td>
<td>0.25</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td>-0.12</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sagittal stratum</td>
<td>0.14</td>
<td>-0.11</td>
<td>0.0056</td>
<td>-0.14</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retrolenticular part of internal capsule</td>
<td>0.11</td>
<td>-0.10</td>
<td>&lt;0.001</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cingulum (hippocampus)</td>
<td>0.01</td>
<td>-0.08</td>
<td>0.0197</td>
<td>-0.10</td>
<td>0.0122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tapetum</td>
<td>0.01</td>
<td>-0.11</td>
<td>0.041</td>
<td>-0.15</td>
<td>0.0172</td>
<td></td>
</tr>
<tr>
<td>Gray matter</td>
<td>Thalamus</td>
<td>0.93</td>
<td>-0.81</td>
<td>&lt;0.001</td>
<td>-0.88</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Model 1 refers to the linear regression including fractional anisotropy or gray matter density as the dependent variable and carotid-femoral pulse wave velocity as the independent variable, adjusting for age, gender, use of antihypertensive therapy, total cholesterol, current smoking status and presence of diabetes mellitus, ICV and time between clinical and MRI exams. Model 3 corresponds to Model 1 with central pulse pressure mean arterial pressure and augmentation index included as additional adjusting variables.
### Table II Interaction between Age and Hypertension with carotid-femoral pulse wave velocity on fractional anisotropy

<table>
<thead>
<tr>
<th>Covariate</th>
<th>White matter tract</th>
<th>Volume (cc)</th>
<th>Beta (×103)</th>
<th>P value</th>
<th>Interaction (×103)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior corona radiata</td>
<td>3.44</td>
<td>-0.05</td>
<td>0.062</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>External capsule</td>
<td>1.20</td>
<td>-0.04</td>
<td>0.042</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Superior corona radiata</td>
<td>0.89</td>
<td>-0.05</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Superior longitudinal fasciculus</td>
<td>0.82</td>
<td>0.00</td>
<td>0.99</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Posterior limb of internal capsule</td>
<td>0.55</td>
<td>-0.06</td>
<td>0.043</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cerebral peduncle</td>
<td>0.43</td>
<td>-0.05</td>
<td>0.011</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Anterior limb of internal capsule</td>
<td>0.29</td>
<td>-0.02</td>
<td>0.088</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Posterior corona radiata</td>
<td>0.19</td>
<td>-0.05</td>
<td>0.0040</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Superior cerebellar peduncle</td>
<td>0.19</td>
<td>-0.03</td>
<td>0.018</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fornix (cres) / Stria terminalis</td>
<td>0.13</td>
<td>-0.07</td>
<td>0.005</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fornix (column and body of fornix)</td>
<td>0.13</td>
<td>-0.11</td>
<td>0.017</td>
<td>-0.02</td>
<td>0.0013</td>
<td></td>
</tr>
<tr>
<td>Retrolenticular part of internal capsule</td>
<td>0.08</td>
<td>-0.04</td>
<td>0.029</td>
<td>-0.01</td>
<td>0.0030</td>
<td></td>
</tr>
<tr>
<td>Inferior cerebellar peduncle</td>
<td>0.08</td>
<td>-0.09</td>
<td>0.004</td>
<td>-0.02</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Superior fronto-occipital fasciculus</td>
<td>0.02</td>
<td>-0.06</td>
<td>0.003</td>
<td>-0.01</td>
<td>0.0046</td>
<td></td>
</tr>
<tr>
<td>Splenium of corpus callosum</td>
<td>0.01</td>
<td>-0.02</td>
<td>0.19</td>
<td>-0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Genu of corpus callosum</td>
<td>0.00</td>
<td>0.03</td>
<td>0.99</td>
<td>-0.01</td>
<td>0.0053</td>
<td></td>
</tr>
<tr>
<td>Posterior corona radiata</td>
<td>0.77</td>
<td>-0.07</td>
<td>0.50</td>
<td>0.29</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

| Age                        | Superior corona radiata      | 0.53        | -0.09       | 0.15    | 0.34               | <0.001             |
|                           | Posterior thalamic radiation | 0.12        | -0.07       | 0.93    | 0.33               | <0.001             |
|                           | Retrolenticular part of internal capsule | 0.07     | -0.04       | 0.73    | 0.28               | <0.001             |
|                           | Superior longitudinal fasciculus | 0.04       | -0.12       | 0.63    | 0.38               | 0.0021             |
|                           | Tapetum                      | 0.04        | -0.04       | 0.77    | 0.26               | 0.0012             |
|                           | Body of corpus callosum       | 0.03        | -0.19       | 0.015   | 0.34               | 0.0073             |
|                           | Splenium of corpus callosum   | 0.02        | -0.14       | 0.029   | 0.27               | 0.0032             |
|                           | Posterior limb of internal capsule | 0.01     | -0.04       | 0.26    | 0.27               | 0.0021             |

Analysis refers to the linear regression including fractional anisotropy as the dependent variable and carotid-femoral pulse wave velocity (CFPWV) as the independent variable including the interaction of CFPWV with age, gender and use of antihypertensive therapy and adjusting for age, gender, use of antihypertensive therapy, total cholesterol, current smoking status and presence of diabetes mellitus, ICV and time between clinical and MRI exams.
References


