Published in final edited form as:

Neurobiol Aging. 2018 June ; 66: 59–67. doi:10.1016/j.neurobiolaging.2018.02.012.

Associations of Carotid Arterial Compliance and White Matter Diffusion Metrics during Midlife: Modulation by Sex

Evan P. Pasha^a, Alex C. Birdsill^b, Stephanie Oleson^b, Hirofumi Tanaka^a, and Andreana P. Haley^{b,c}

^aCardiovascular Aging Research Laboratory, Department of Kinesiology and Health Education, The University of Texas at Austin Austin, TX 78712

^bDepartment of Psychology, The University of Texas at Austin, Austin, TX 78712

^cImaging Research Center, The University of Texas at Austin, Austin, TX 78712

Abstract

Sex differences in cerebral white matter (WM) aging have been debated extensively over the past two decades without unequivocal resolution. We aimed to determine if the effects of age and arterial stiffness on white matter (WM) microstructure differ between sexes. Artery elasticity via carotid artery compliance (CAC) and WM diffusion metrics via diffusion tensor image derived fractional anisotropy (FA) and mean diffusivity (MD) were measured in 155 (87 females) middle-aged (40–62 years) adults. Males demonstrated poorer water diffusion metrics in WM than women in the corpus callosum body, cingulum, and cingulum (hippocampal). Age and CAC had greater effects on WM water diffusion in males than females in midlife independent of education and cardiovascular risk factors. Sex moderated age (cingulum FA, cingulum (hippocampal) MD, and uncinate MD, all $p < 0.05$) and CAC (cingulum FA, $p < 0.05$) related reductions in regional WM diffusion metrics. CAC mediated age-related associations in regional WM diffusion metrics (cingulum FA, cingulum MD, superior corona radiata MD, and uncinate MD, all $p < 0.05$) in males but not in females. Age and CAC were associated with WM diffusion metrics independent of cardiovascular risk factors. These associations appear to be stronger in males than in females.

Keywords

arterial compliance; vascular function; gender; MRI

Correspondence: Evan Pasha, Ph.D., ORCID#:0000-0003-0798-5844, Department of Kinesiology and Health Education, The University of Texas at Austin, 2109 San Jacinto Blvd, D3700, Austin, TX 78712, 978-505-8726 (Phone), 512-471-8194 (Fax), Evan.pasha@utexas.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Declaration: The authors declare no conflicts of interest.

1. Introduction

Coherence of water diffusion in cerebral white matter (WM) appears to peak at around the third decade in life, after which there is a steady decline before incurring some stability between the fourth and sixth decade (Westlye et al., 2010). After the sixth decade of life, an accelerated decline in diffusion properties within WM tracts ensues (Westlye et al., 2010). Whether or not sex differences exist in the aging process of WM has been debated over the past two decades with equivocal findings. Some investigations have found no water diffusion differences in WM between the sexes and no age-by-sex interactions (Hsu et al., 2008; Inano et al., 2011; Kennedy and Raz, 2009b; Sullivan et al., 2001), while others have documented such effects (Abe et al., 2010; Kochunov et al., 2012; Sullivan et al., 2010). The reason for these discrepant findings is unclear. However, these investigations vary in their methodology assessing WM (e.g. voxel based, tract-based spatial statistics, region-of-interest, etc.), sample size, and age ranges, potentially leading to inconsistencies. Importantly, few of the investigations finding a significant age-by-sex interaction have sought to explain mechanistically why the observed sexual dimorphism may exist.

Parallel to sex differences in the aging process of the brain are sex differences in arterial aging. For example, females tend to have lower carotid-femoral and brachial ankle pulse wave velocity, measures of arterial stiffness, until menopause when they begin to resemble the cardiovascular status of males (Mitchell et al., 2004; Sonesson et al., 1993; Tomiyama et al., 2003), likely attributable to a drop in estrogen (Rajkumar et al., 1997). Considering arterial stiffening has been shown to be a significant contributor to stroke (Mattace-Raso et al., 2006), cerebral microbleeds (Seo et al., 2008), white matter hyperintensities (Gustavsson et al., 2015), and age related diseases including dementia (Iturria-Medina et al., 2016), differences in arterial stiffness between sexes may play a pivotal role in age related changes in the brain.

To date, the vast majority of investigations relating arterial stiffness WM diffusion metrics have made the aortic arch and abdominal aorta their primary focus (Maillard et al., 2017; Maillard et al., 2016; Sala et al., 2016; Tarumi et al., 2015; Tjeerdema et al., 2014). Although maintaining a flexible and compliant aorta is advantageous for cerebrovascular health, observing carotid artery compliance, which determines the stretch of the vessel in response to pressure, may be more appropriate as stiffening of this vascular segment has a greater effect on cerebral hemodynamics (Pasha et al., 2017). While one study has shown carotid artery distensibility to be associated with incident cerebral microbleeds (Ding et al., 2015), a recent study found that carotid artery diameter but not carotid beta stiffness was associated with white matter hyperintensity volume, a measurement of macrostructural damage to cerebral WM (Rundek et al., 2017). To our knowledge, only one investigation has previously examined the associative relationship of carotid artery stiffness with WM diffusion metrics, where no association was found (DuBose et al., 2017). Despite these negative findings, it is quite possible that unquantified moderators as well as low statistical power in the latter study may have hindered their observations. The paucity of research assessing carotid elasticity metrics as opposed to central aortic stiffness with brain structure is concerning as carotid characteristics may play a relatively greater role in conferring

damage to cerebral WM considering their proximity to the cerebral circulation, and is thus a focus of this investigation.

The aim of the present investigation was to determine if sex differences exist in the effects of either age or carotid artery compliance on regional WM water diffusion metrics. Further, we sought to determine if carotid artery stiffness explained age related declines in WM microstructure. This study was performed in middle-aged adults as this window is a critical time period prior to accelerated age-related decreases in WM diffusion metrics (Westlye et al., 2010) when intervention strategies can be implemented. WM diffusion metrics were assessed with diffusion tensor imaging, a magnetic resonance imaging (MRI) sequence capable of characterizing the three-dimensional diffusion of water within WM tracts *in vivo*. We examined fractional anisotropy (FA), a measure of the magnitude of water diffusion in the principle direction thought to be reflective of the parallelism of water diffusion to white matter tracts with greater values indicating greater parallel diffusion (Alexander et al., 2007). Mean diffusivity (MD), a metric complimentary to but directionally opposite of FA, was also assessed. MD measures the average water diffusion rate in all three directions with greater values indicating less constrained water diffusion. Together, these metrics assess the cerebral WM at the microstructural level and thus may be more sensitive to physiological changes than white matter hyperintensities that indicate cerebral WM macrostructural changes (O'Sullivan et al., 2001b). Carotid artery stiffness was assessed with carotid artery compliance (CAC) via ultrasound and simultaneous arterial tonometry. CAC is directionally inverse to stiffness and reflects the flexibility of an artery segment measured as the change in vessel diameter in response to distending pressure. We hypothesized that males would exhibit lower FA and greater MD coefficients and a greater rate of decline in these metrics in relation to age and CAC. Further, we hypothesized that lower carotid artery compliance would explain the sexual dimorphism of age and WM water diffusion characteristics.

2. Materials and methods

2.1 Participants

Community dwelling middle-aged (n=155, 87-f/68-m, 40–62 years) adults from the Austin, Texas area participated in this study. To qualify for this investigation, participants had to be without pre-existing diagnosed cardiovascular disease including coronary artery disease, angina pectoris, transient ischemic attack, myocardial infarction, heart failure, or cardiac surgery, neurological diseases including stroke, Parkinson's disease, and clinically significant traumatic brain injury, or contraindications to MRI as indicated by a Health Research Questionnaire. Recruited participants were minimally second generation, proficient in English, and educated in the United States. All participants provided informed consent. The University of Texas institutional review board approved this study.

Participants reported for vascular data collection in the morning following an overnight fast 8 hours having abstained from physical exercise, alcohol consumption, smoking, and caffeine for 24 hours. Height and weight were recorded with a stadiometer and digital scale respectively. Waist circumference was assessed with an elastic measuring tape placed around the trunk at the top of the iliac crest (Croft et al., 1995). A blood sample was collected by a certified phlebotomist from the antecubital vein. Blood concentrations of

triglycerides, HDL-cholesterol, and glucose were determined with standard enzymatic techniques. An automatic oscillometric method (VP-2000; Omron Healthcare, Kyoto, Japan) assessed blood pressure in the supine position following 15 minutes of comfortable rest in a temperature controlled laboratory.

2.2 Arterial Compliance Measurement

To assess CAC, an iE 33 Ultrasound System (Philips, Bothell, WA) with a high-resolution 15-MHz linear-array transducer captured a longitudinal B-mode image of the left common carotid artery. The image was acquired perpendicularly to the blood vessel 1–2 cm proximal to the carotid bulb with the near and far walls of the vessel clearly displayed. Digitized images were saved in DICOM format and later analyzed with computerized image-analysis software (Vascular Research Tool Carotid Analyzer, Medical Imaging Applications, Coralville, IA) by a single investigator. These images were paired with pulse pressure waveforms from the contralateral common carotid artery, which were simultaneously obtained with arterial applanation tonometry (VP-2000; Omron Healthcare, Kyoto, Japan). Ten consecutive pressure waveforms per participant were captured and averaged for analysis. To account for tonometer hold down pressure inherent to applanation tonometry, carotid mean arterial pressure and diastolic pressure were calibrated to oscillometric brachial mean and diastolic pressure. CAC was calculated as carotid [(Systolic diameter-Diastolic diameter)/(Systolic BP-Diastolic BP)] (O'Rourke et al., 2002).

2.3 Brain imaging

Brain imaging occurred on a day separate from vascular testing. MRI was performed using a 3T Siemens Skyra system (Siemens Medical Solutions, Malvern, PA) with a 32-channel head coil. Images were obtained in 64 directions at $b=700$ s/mm from a diffusion-weighted, spin-echo, echo planar imaging pulse sequence. Total acquisition time for DTI metrics was approximately 11 minutes with 64 slices collected. A non-diffusion weighted reference image with $b=0$ was gathered. Contiguous 2mm anterior to posterior slices were used to cover the cerebrum with the following parameters: FOV = 256mm, TR = 8300ms, TE = 84ms. Optimization of magnetic field homogeneity was accomplished with advanced shimming prior to diffusion weighted imaging, minimizing EPI distortions.

Diffusion-weighted image processing used high-dimensional normalization with the full tensor rather than tensor-derived indices such as FA (Zhang et al., 2007). Affine transformations corrected for motion and eddy current distortions in FSL (www.fmrib.ox.ac.uk/fsl/). FSL's BET (brain extraction tool) removed any Non-brain signal. Tensor fitting was performed using FSL's dtifit function (cmic.cs.ucl.ac.uk/camino/). Using iterative rigid, affine, and diffeomorphic alignments of the full tensor in DTI-TK (www.nitrc.org/projects/dtitk/) a study-specific template was created and registered to standard space using the IIT Human Brain Atlas (www.nitrc.org/projects/iit2/). FA maps were calculated from each participant's tensor map in standard space using DTI-TK. The Johns Hopkins International Consortium for Brain Mapping template was used to define ROIs in standard space. Each participant's FA map was masked by each region-of-interest (ROI). Resulting FA ROIs were thresholded at 0.2 to exclude signal from non-white matter voxels and the average FA value was extracted. MD maps were calculated in participant

space. Inverse deformation fields were calculated from standard space to participant space and applied to the ROIs to define ROIs in participant space.

Investigated ROIs were determined *a priori* from previous works. The corpus callosum (CC) has shown vulnerability to a multitude of deleterious aging processes including dementia (Head et al., 2004) and arterial stiffening (Tarumi et al., 2015). Other regions that appear negatively affected by arterial stiffening include the superior corona radiata (SCR) and anterior limb of the internal capsule (ALIC) (Maillard et al., 2016; Tarumi et al., 2015). The cingulum, cingulum (hippocampal), and uncinate fasciculus were selected because of their previously reported relationships with aging (Gazes et al., 2016; Sullivan et al., 2010).

2.4 Cognitive status

On the same day as neuroimaging, participants underwent the Mini-Mental State Examination (MMSE) to garner a picture of global cognitive health. Testing was administered by research assistants trained in administration of these tests. To foster testing standardization, the same assistants performed all scoring.

2.5 Statistical analyses

Physiological variables were evaluated with descriptive characteristics and presented as means $\pm$ SD. Frequencies are presented for categorical variables. Independent samples t-tests were performed on continuous descriptive characteristic variables. Chi-squared tests determined differences in categorical variables between sexes. Sex differences in WM diffusion metrics were determined by analysis of covariance (ANCOVA) with ROIs entered as the dependent variable, sex entered as a fixed factor, and age and years of education added as covariates. To determine the associative relationships between age, CAC, and WM diffusion metrics, the following procedures were performed. Age was regressed against WM diffusion metrics in two models, the first of which had no covariates, while the second controlled for the influence of education (Noble et al., 2013), systolic blood pressure (Gons et al., 2010; Li et al., 2015), waist circumference (Birdsill et al., 2017), glucose (Hsu et al., 2012; Reijmer et al., 2013), triglycerides (Williams et al., 2013), and total cholesterol (Williams et al., 2013). These covariates were decided upon for their relations with age, arterial stiffness, and DTI metrics. Associations were examined in the entire subject population, then data were then split by sex and the models were repeated to observe if differential associations between sexes exist. In females, post-menopausal status was not used as a covariate because of its large dependency on age. Hormone replacement therapy was included in the female cohort as an additional covariate in age associations. This same process was performed correlating CAC and WM diffusion metrics with systolic BP excluded as a covariate in the second model because of its inclusion in the calculation of CAC. Additionally, with CAC associations, post-menopausal status and hormone replacement therapy were included as additional covariates in females. Moderation analysis was performed with sex hypothesized as a moderator of the association of age or CAC with WM diffusion metrics.

Moderation analyses were conducted in the PROCESS macro (www.afhayes.com) in SPSS version 24 (SPSS Inc; IBM, Armonk, NY) using Model 1. In short this macro tests for a

significant interaction term based on a hypothesized moderator using bias corrected non-parametric bootstrapping of 5,000 samples. Finally, mediation analyses were performed in WM regions with age entered as the independent variable, CAC as the mediator and WM metrics entered as the dependent variable. Again, the PROCESS macro was used, this time with Model 4, where models are constructed using bias corrected non-parametric bootstrapping of 5,000 samples.

In ANCOVA and correlation analyses, to account for inflation of type-1 error due to multiple comparisons, rather than using a strict Bonferroni correction that would be too conservative in light of the intercorrelated outcomes investigated in this study, a modified Bonferroni was employed (Sankoh et al., 1997). Additionally, hypotheses were *a priori* and small effects were of interest for clinical implications, furthering our rationale for a less conservative α . Based on two degrees of freedom and mean intercorrelations of eight outcomes with $r=0.53$ and $r=0.68$ for FA and MD respectively, an $\alpha<0.019$ of $\alpha<0.026$ were determined (quantitativeskills.com). Ultimately, we rounded the more conservative of these values to select the final $\alpha<0.02$ for statistical significance. In moderation and mediation analyses, terms with 95% confidence intervals not encapsulating 0 indicated statistical significance at the conventional critical alpha $p<0.05$. All statistical analyses were performed in SPSS version 24.

3. Results

3.1 Participant characteristics

Table 1 compares descriptive characteristics between females and males. Both sexes were evenly matched in age, cognitive status, race, medications to control cardiovascular risk factors, and history of smoking. Males were taller, heavier, had greater waist circumferences, and triglycerides, but lower diastolic blood pressure, and high density lipoprotein cholesterol (all $p<0.05$). Thirty-one female participants (35.6%) reported being post-menopause. Males exhibited greater CAC than females indicating more elastic arteries (1.17 ± 0.36 vs 1.31 ± 0.32 , $p=0.012$).

3.2 Cerebral white matter water diffusion

Cingulum FA was greater in males than females ($p<0.02$) independent of age and years of education. MD was significantly greater in males than females in the CC body and cingulum (hippocampal) (both $p<0.02$) as shown in Figure 1. CC splenium ($p<0.028$) and uncinate MD ($p<0.035$) trended to be greater in males than females but did not eclipse $p<0.02$.

3.3 Age associations

The relationship of age with regional WM diffusion metrics in the whole sample, and split across sex, is shown in Table 2. When the whole sample (males and females) was analyzed, age was significantly associated with FA of the CC genu ($r=-0.285$) and MD of the ALIC ($r=0.193$), CC genu ($r=0.279$), body ($r=0.264$), and splenium ($r=0.214$) as well as the cingulum (hippocampal) ($r=0.250$) and SCR ($r=0.208$) (all $p<0.02$). When covariates were added in Model 2, age remained significantly related to CC genu FA ($r=-0.241$), and CC

genu ($r=0.236$), CC body ($r=0.270$), and cingulum (hippocampal) ($r=0.231$) MD (all $p<0.02$). All other significant Model 1 associations lost significance.

When split by sex, females demonstrated a significant age effect on regional WM diffusion metrics only in CC genu FA and MD in Model 1 (all $p<0.02$) which was abrogated in the wake of covariates. In males, age was significantly associated with CC genu ($r=-0.296$), CC splenium ($r=-0.373$), and cingulum ($r=-0.373$) FA (all $p<0.02$) as well as MD of every region ($p<0.02$). When covariates were applied to the model, age remained significantly associated with CC genu ($r=-0.324$), cingulum FA ($r=-0.374$), and CC genu ($r=0.325$), CC body ($r=0.340$), cingulum (hippocampal) ($r=0.386$), SCR ($r=0.368$), and uncinate ($r=0.330$) MD (all $p<0.02$). Although there were statistically significant differences in diastolic blood pressure between sexes in this study, controlling for diastolic pressure instead of or in addition to systolic blood pressure in age models did not significantly influence the relationship of age and DTI metrics in terms of the number of significant regions, nor the level of significance.

3.4 Arterial compliance associations

The relations of CAC with regional WM diffusion metrics are outlined in Table 3. When the sample was analyzed as a whole, ALIC ($r=0.223$) and cingulum ($r=0.227$) FA and ALIC MD ($r=-0.276$) (all $p<0.02$) were associated with CAC. ALIC ($r=0.224$) and cingulum ($r=0.201$) FA and ALIC MD ($r=-0.236$) survived the addition of covariates in Model 2. When split by sex, there were no significant associations of CAC with regional WM diffusion metrics in females (all $p>0.02$) in either model. However, in males, CAC was associated with CC splenium ($r=0.288$) and cingulum FA ($r=0.408$) (both $p<0.02$) as well as ALIC ($r=-0.364$), cingulum ($r=-0.284$), SCR ($r=-0.289$), and uncinate ($r=-0.366$) MD (all $p<0.02$) without covariates. In Model 2, CAC remained significantly independently associated with FA of the CC splenium ($r=0.367$) and cingulum ($r=0.404$), and MD of the ALIC ($r=-0.423$), SCR ($r=-0.314$), SCR ($r=-0.368$), and uncinate ($r=-0.380$) (all $p<0.02$).

3.5 Moderation

Sex was investigated as a moderator of the relationship of aging with regional WM diffusion metrics. WM regions in which a significant sex moderation occurred are shown in Figure 2a–d along with a conceptual model of the moderation effect. There was a significant interaction between age and sex when predicting cingulum FA (AGE*SEX $B=-0.0019$, S.E. $=0.0008$, 95%CI: -0.0035 to -0.0003), cingulum MD (AGE*SEX $B=0.0038$, S.E. $=0.0016$, 95%CI: 0.0005 to 0.0070), and uncinate MD (AGE*SEX $B=0.0029$, S.E. $=0.0011$, 95%CI: 0.0007 to 0.0051).

Sex was also investigated as a moderator of the relationship between CAC and regional WM diffusion metrics. Figure 3a–b shows the conceptual model of this moderation in addition to a graph of the sex moderation effect on cingulum FA. In that model, the interaction term between CAC and sex was significant (CAC*SEX $B=3.2777$, S.E. $=1.5077$, 95%CI: 0.2988 to 6.2566).

3.6 Mediation

Because greater effects of age and CAC existed in men, CAC was investigated as a mediator of age related reductions to WM diffusion metrics in men alone in regions with a significant age effect. In this model, age was entered as the independent variable, regional WM diffusion metrics were entered as the dependent variable, and CAC was entered as the mediator. The results of these models with significant indirect effects are shown in Table 4. Mediation is indicated by a significant indirect effect and completely abrogated or attenuated direct effect of the mediator. CAC mediated age related increases in cingulum MD (Indirect: $B=0.0004$, $S.E.=0.0003$, 95%CI: 0.0001 to 0.0012). CAC mediated the age related reduction of cingulum FA MD (Indirect: $B=-0.0004$, $S.E.=0.0002$, 95%CI: -0.0010 to -0.0001) and increase of MD in the SCR (Indirect: $B=0.0004$, $S.E.=0.0003$, 95%CI: 0.0001 to 0.0011), and uncinate (Indirect: $B=0.0005$, $S.E.=0.0003$, 95%CI: 0.0001 to 0.00012).

4. Discussion

We investigated whether the effects of age and carotid artery stiffening on regional WM diffusion metrics differ between females and males at midlife. Our principle novel contributions are threefold; 1) the negative effect of age on regional WM diffusion metrics during midlife was moderated by sex with males showing worse WM diffusion metrics and stronger negative age related changes to WM diffusion metrics than females; 2) in males, reduced midlife CAC was associated with poorer regional WM diffusion metrics independent of cardiovascular risk factors, and has a stronger negative effect on WM diffusion metrics than in females; 3) the effect of aging on regional WM diffusion metrics at midlife appears to be mediated by CAC. Poorer WM diffusion metrics with aging is a consistent finding in the field of neuroscience. The nature of that finding is less clear, with some studies reporting a global effect (Benedetti et al., 2006) while others showing regional effects with the possibility of an anterior to posterior aging gradient (Davis et al., 2009; Salat et al., 2005). A cardiovascular explanation to age related declines in WM and differences between sexes had never been explored until now.

4.1 Sex, Aging, and Cerebral WM

We found differential aging effects between sexes for cingulum FA, and MD of the CC body and cingulum (hippocampal) independent of age and education. The observed divergent age effect on WM diffusion metrics between sexes agree with two investigations but contradict others. A study of 831 individuals aged 11–90 determined males had greater average CC splenium FA than females and a steeper decline of FA with age (Kochunov et al., 2012). In a sample of 245 adults that spanned ages 21–71, steeper FA decline was observed in males in the right inferior fronto-temporal areas and anterior cingulate cortex, as well as MD increases in bilateral frontal, temporal, and parietal areas (Abe et al., 2010). Contrarily, a study found steeper age related WM diffusion changes to the CC splenium in females compared with males (Sullivan et al., 2010). Separately, a study of 145 participants aged 30–80 years, found that males showed a steeper increase in MD when compared with females (Hsu et al., 2008). This study also observed significant sex differences in right deep temporal WM and left ALIC FA, as well as in the left periventricular regions in which females displayed a greater slope. These studies had a wide age range and failed to control for the

influence of cardiovascular risk factors that influence WM diffusion metrics (Birdsill et al., 2017; Hsu et al., 2012; Wang et al., 2015). We argue that the influences of these cardiovascular risks must be accounted for in age related investigations of WM microstructure as they may confound study agreement.

4.2 Sex, Arterial Stiffness, and Cerebral WM

In addition to sex differences in age related changes in regional WM diffusion metrics, we found that sex moderated the relationship between CAC and cingulum FA such that CAC had a stronger association with WM diffusion metrics in males, despite males exhibiting slightly greater CAC. We also found that CAC was associated with CC splenium and cingulum FA, and ALIC, SCR, and uncinate MD independent of cardiovascular risk factors in males. Most previous studies employed carotid-femoral pulse wave velocity (cfPWV), a measure of aortic stiffness, when investigating arterial stiffness and WM microstructure. For example, large cross-sectional studies reported cfPWV to be associated with reduced global FA (Maillard et al., 2016), superior, anterior, and posterior corona radiata, and CC genu, body, and splenium FA (Maillard et al., 2017). Another smaller investigation similarly observed cfPWV to be related to both global FA and MD (Tarumi et al., 2015). Additionally, cfPWV was associated with poorer WM diffusion metrics in type-1 diabetics (Tjeerdema et al., 2014). More recently, an investigation assessed the relation of carotid artery stiffness to WM diffusion metrics using CAC and the beta-stiffness index in young and middle-aged/older adults, finding no associations (DuBose et al., 2017). This latter study may not have performed a sub analysis between sexes potentially revealing effects within males, or may simply have been underpowered to perform such an analysis. It also included young adults, which may have diminished the effect of arterial stiffness on WM microstructure as younger brains have had less exposure to the deleterious effects of arterial stiffening.

4.3 Potential Mechanisms

Arterial stiffness has been used as a marker for vascular aging, and males exhibit exacerbated arterial stiffening compared with females (Tomiya et al., 2003). Previous studies have shown post-menopausal status to be related to arterial stiffening (Zaydun et al., 2006), making estrogen another potential culprit for our findings. In the vasculature, estrogen stimulates vasorelaxation through promoting nitric oxide release and via calcium channels through attenuated release, influx, or increased efflux of calcium (New et al., 1997; White, 2002). In the brain, animal models suggest that estrogen is related to the maintenance of myelin sheath volume and myelinated fiber length in white matter (He et al., 2017). Human observations showed that menopause has been significantly associated with white matter hyperintensities independent of age (Seo et al., 2013). In the present study, when females alone were analyzed, post-menopausal status was not related to either CAC or regional WM diffusion metrics after controlling for the effect of age (data not shown). Stiffening of the vasculature may result in the transference of damaging pulsatile forces to the microvasculature that are incapable of buffering mechanical stressors, culminating in damage to end organs (Mitchell, 2008). This transference may result in hypoperfusion and hypoxic injury to the brain (Mitchell et al., 2011; Tarumi et al., 2011). This injury may elicit regionally reduced FA or increased MD in cerebral WM. We observed differential associations of age and CAC on cerebral WM metrics with regions differing in the metric

(e.g. FA, MD, or both) effected. However, whether fiber coherence, demyelination, or axonal damage occurs with aging or reduced CAC is difficult to infer based on DTI metrics alone.

4.4 Cognitive Implications

The importance of WM diffusion metrics for cognitive performance is controversial. Some studies have found regional DTI metrics FA and MD to be related to executive function (O'Sullivan et al., 2001a), memory (Bucur et al., 2008), and processing speed (Madden et al., 2004). Further, changes in WM diffusion metrics have mediated age related decrements in cognitive performance (Madden et al., 2009b). Regions we found vulnerable to aging (ALIC, CC Genu, CC Body, cingulum, cingulum (hippocampal), SCR and uncinata) and reduced carotid artery compliance (ALIC, cingulum, SCR, and uncinata) have shown associations with cognitive performance. For example, executive function, memory, and processing speed have all been associated with FA of the ALIC, CC genu, CC splenium, cingulum, uncinata (Madden et al., 2009a) to varying degrees. In relation to neurocognitive diseases, individuals with amnesic mild cognitive impairment exhibited reduced FA in the ALIC, CC body, CC genu, CC splenium, cingulum, and SCR (Zhuang et al., 2010). Still, these findings are not always reproducible across studies, likely due to differences in participant populations, age ranges, and methodologies (Kennedy and Raz, 2009a).

4.5 Study Strengths and Limitations

The present study benefited from having a large, diverse (36% minority) study population that was evenly distributed across sex with well-matched participant characteristics observed at midlife. Studying the neurocognitive health of midlife populations is critical as it is within this window when cardiovascular related neuropathology likely begins to snowball. Using DTI to monitor WM microstructure status is another significant strength as DTI is well suited to detect subtle changes to WM (O'Sullivan et al., 2001b). Although we observed CAC rather than cPWV, the gold standard for arterial stiffness, compliance of this vascular segment is immediately pertinent to the brain as it directly precedes the cerebrovasculature. Other indices of carotid artery stiffness and elasticity such as the beta stiffness index and arterial distensibility demonstrated associations within similar regions of similar magnitude within our data set but were omitted to avoid redundancy (data not shown).

Limitations to this investigation must also be acknowledged. Although we were able to demonstrate moderation and mediation effects, these effects are derived from statistical analyses in a cross-sectional design. Therefore, our findings are implicitly limited and cause and effect between aging, carotid artery stiffening, and changes to WM diffusion metrics cannot be claimed. Further, it is quite possible that the observed relationships were the product of factors outside of arterial stiffening. For example, carotid plaques, that were uncharacterized in the present study, are associated with arterial stiffening (Zureik et al., 2002) and increased white matter hyperintensities (Pico et al., 2002) may have been greater in the male subject population than females, potentially explaining the greater stiffening effects on cerebral WM. Females may also experience stronger age effects post menopause (Table S1). Menopausal status was not controlled in this study resulting in female subjects in pre-, peri-, and post-menopausal states due to the age range of interest, potentially confounding our results. Additionally, effects were only present in some WM regions but not

others, suggesting either a preferential effect of aging and carotid artery stiffening, or effects may have yet to present themselves in this middle-aged cohort. Finally, investigating regional rather than whole brain WM microstructure limits our ability to determine the global effects of aging, carotid artery stiffening, and sex, and thus effects may extend beyond our investigated regions.

5. Conclusion

We found a stronger negative association of age and carotid artery stiffening on WM diffusion metrics in males than females during midlife. The association of age with regional WM diffusion metrics in males was mediated by carotid artery stiffening. We are the first to report these findings in a cohort of middle-aged adults, a critical time period before major age related changes to WM microstructure. This evidence is the first to demonstrate that WM integrity is negatively associated with carotid artery stiffness independent of cardiovascular risk factors. Future investigations should track individuals' cardiovascular and WM diffusion metrics over decades in a prospective study design to fully elucidate the complex interplay between age, sex, vascular health, and WM microstructure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was made possible by funding provided by the National Institute of Neurological Disorders and Stroke (R01 NS075565; to A.P.H.) and the National Science Foundation (GRFP; to A.B.).

References

- Abe O, Yamasue H, Yamada H, Masutani Y, Kabasawa H, Sasaki H, Takei K, Suga M, Kasai K, Aoki S, Ohtomo K. Sex dimorphism in gray/white matter volume and diffusion tensor during normal aging. *NMR in biomedicine*. 2010; 23(5):446–458. [PubMed: 20310078]
- Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2007; 4(3):316–329. [PubMed: 17599699]
- Benedetti B, Charil A, Rovaris M, Judica E, Valsasina P, Sormani MP, Filippi M. Influence of aging on brain gray and white matter changes assessed by conventional, MT, and DT MRI. *Neurology*. 2006; 66(4):535–539. [PubMed: 16505308]
- Birdsill AC, Oleson S, Kaur S, Pasha E, Ireton A, Tanaka H, Haley A. Abdominal obesity and white matter microstructure in midlife. *Human brain mapping*. 2017
- Bucur B, Madden DJ, Spaniol J, Provenzale JM, Cabeza R, White LE, Huettel SA. Age-related slowing of memory retrieval: contributions of perceptual speed and cerebral white matter integrity. *Neurobiology of aging*. 2008; 29(7):1070–1079. [PubMed: 17383774]
- Croft JB, Keenan NL, Sheridan DP, Wheeler FC, Speers MA. Waist-to-hip ratio in a biracial population: measurement, implications, and cautions for using guidelines to define high risk for cardiovascular disease. *Journal of the American Dietetic Association*. 1995; 95(1):60–64. [PubMed: 7798582]
- Davis SW, Dennis NA, Buchler NG, White LE, Madden DJ, Cabeza R. Assessing the effects of age on long white matter tracts using diffusion tensor tractography. *NeuroImage*. 2009; 46(2):530–541. [PubMed: 19385018]

- Ding J, Mitchell GF, Bots ML, Sigurdsson S, Harris TB, Garcia M, Eiriksdottir G, van Buchem MA, Gudnason V, Launer LJ. Carotid arterial stiffness and risk of incident cerebral microbleeds in older people: the Age, Gene/Environment Susceptibility (AGES)-Reykjavik study. *Arteriosclerosis, thrombosis, and vascular biology*. 2015; 35(8):1889–1895.
- DuBose LE, Voss MW, Weng TB, Kent JD, Dubishar KM, Lane-Cordova A, Sigurdsson G, Schmid P, Barlow PB, Pierce GL. Carotid beta-stiffness index is associated with slower processing speed but not working memory or white matter integrity in healthy middle-aged/older adults. *Journal of applied physiology*. 2017; 122(4):868–876. [PubMed: 28126907]
- Gazes Y, Bowman FD, Razlighi QR, O'Shea D, Stern Y, Habeck C. White matter tract covariance patterns predict age-declining cognitive abilities. *NeuroImage*. 2016; 125:53–60. [PubMed: 26477658]
- Gons RA, de Laat KF, van Norden AG, van Oudheusden LJ, van Uden IW, Norris DG, Zwiers MP, de Leeuw FE. Hypertension and cerebral diffusion tensor imaging in small vessel disease. *Stroke; a journal of cerebral circulation*. 2010; 41(12):2801–2806.
- Gustavsson AM, Stomrud E, Abul-Kasim K, Minthon L, Nilsson PM, Hansson O, Nagga K. Cerebral Microbleeds and White Matter Hyperintensities in Cognitively Healthy Elderly: A Cross-Sectional Cohort Study Evaluating the Effect of Arterial Stiffness. *Cerebrovascular diseases extra*. 2015; 5(2):41–51. [PubMed: 26120319]
- He Q, Luo Y, Lv F, Xiao Q, Chao F, Qiu X, Zhang L, Gao Y, Xiu Y, Huang C, Tang Y. Effects of estrogen replacement therapy on the myelin sheath ultrastructure of myelinated fibers in the white matter of middle-aged ovariectomized rats. *J Comp Neurol*. 2017
- Head D, Buckner RL, Shimony JS, Williams LE, Akbudak E, Conturo TE, McAvoy M, Morris JC, Snyder AZ. Differential vulnerability of anterior white matter in nondemented aging with minimal acceleration in dementia of the Alzheimer type: evidence from diffusion tensor imaging. *Cerebral cortex*. 2004; 14(4):410–423. [PubMed: 15028645]
- Hsu JL, Chen YL, Leu JG, Jaw FS, Lee CH, Tsai YF, Hsu CY, Bai CH, Leemans A. Microstructural white matter abnormalities in type 2 diabetes mellitus: a diffusion tensor imaging study. *NeuroImage*. 2012; 59(2):1098–1105. [PubMed: 21967726]
- Hsu JL, Leemans A, Bai CH, Lee CH, Tsai YF, Chiu HC, Chen WH. Gender differences and age-related white matter changes of the human brain: a diffusion tensor imaging study. *NeuroImage*. 2008; 39(2):566–577. [PubMed: 17951075]
- Inano S, Takao H, Hayashi N, Abe O, Ohtomo K. Effects of age and gender on white matter integrity. *AJNR. American journal of neuroradiology*. 2011; 32(11):2103–2109. [PubMed: 21998104]
- Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC. Alzheimer's Disease Neuroimaging, I. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nature communications*. 2016; 7:11934.
- Kennedy KM, Raz N. Aging white matter and cognition: differential effects of regional variations in diffusion properties on memory, executive functions, and speed. *Neuropsychologia*. 2009a; 47(3): 916–927. [PubMed: 19166865]
- Kennedy KM, Raz N. Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk. *Brain research*. 2009b; 1297:41–56. [PubMed: 19712671]
- Kochunov P, Williamson DE, Lancaster J, Fox P, Cornell J, Blangero J, Glahn DC. Fractional anisotropy of water diffusion in cerebral white matter across the lifespan. *Neurobiology of aging*. 2012; 33(1):9–20. [PubMed: 20122755]
- Li X, Liang Y, Chen Y, Zhang J, Wei D, Chen K, Shu N, Reiman EM, Zhang Z. Disrupted Frontoparietal Network Mediates White Matter Structure Dysfunction Associated with Cognitive Decline in Hypertension Patients. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2015; 35(27):10015–10024. [PubMed: 26157001]
- Madden DJ, Bennett IJ, Song AW. Cerebral white matter integrity and cognitive aging: contributions from diffusion tensor imaging. *Neuropsychology review*. 2009a; 19(4):415–435. [PubMed: 19705281]
- Madden DJ, Spaniol J, Costello MC, Bucur B, White LE, Cabeza R, Davis SW, Dennis NA, Provenzale JM, Huettel SA. Cerebral white matter integrity mediates adult age differences in

- cognitive performance. *Journal of cognitive neuroscience*. 2009b; 21(2):289–302. [PubMed: 18564054]
- Madden DJ, Whiting WL, Huettel SA, White LE, MacFall JR, Provenzale JM. Diffusion tensor imaging of adult age differences in cerebral white matter: relation to response time. *NeuroImage*. 2004; 21(3):1174–1181. [PubMed: 15006684]
- Maillard P, Mitchell GF, Himali JJ, Beiser A, Fletcher E, Tsao CW, Pase MP, Satizabal CL, Vasan RS, Seshadri S, DeCarli C. Aortic Stiffness, Increased White Matter Free Water, and Altered Microstructural Integrity: A Continuum of Injury. *Stroke; a journal of cerebral circulation*. 2017; 48(6):1567–1573.
- Maillard P, Mitchell GF, Himali JJ, Beiser A, Tsao CW, Pase MP, Satizabal CL, Vasan RS, Seshadri S, DeCarli C. Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study. *Stroke; a journal of cerebral circulation*. 2016; 47(4):1030–1036.
- Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation*. 2006; 113(5):657–663. [PubMed: 16461838]
- Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *Journal of applied physiology*. 2008; 105(5): 1652–1660. [PubMed: 18772322]
- Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension*. 2004; 43(6):1239–1245. [PubMed: 15123572]
- Mitchell GF, van Buchem MA, Sigurdsson S, Gotal JD, Jonsdottir MK, Kjartansson O, Garcia M, Aspelund T, Harris TB, Gudnason V, Launer LJ. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility--Reykjavik study. *Brain : a journal of neurology*. 2011; 134(Pt 11):3398–3407. [PubMed: 22075523]
- New G, Timmins KL, Duffy SJ, Tran BT, O'Brien RC, Harper RW, Meredith IT. Long-term estrogen therapy improves vascular function in male to female transsexuals. *Journal of the American College of Cardiology*. 1997; 29(7):1437–1444. [PubMed: 9180101]
- Noble KG, Korgaonkar MS, Grieve SM, Brickman AM. Higher education is an age-independent predictor of white matter integrity and cognitive control in late adolescence. *Dev Sci*. 2013; 16(5): 653–664. [PubMed: 24033571]
- O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *American journal of hypertension*. 2002; 15(5):426–444. [PubMed: 12022246]
- O'Sullivan M, Jones DK, Summers PE, Morris RG, Williams SC, Markus HS. Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline. *Neurology*. 2001a; 57(4):632–638. [PubMed: 11524471]
- O'Sullivan M, Summers PE, Jones DK, Jarosz JM, Williams SC, Markus HS. Normal-appearing white matter in ischemic leukoaraiosis: a diffusion tensor MRI study. *Neurology*. 2001b; 57(12):2307–2310. [PubMed: 11756617]
- Pasha EP, Birdsill AC, Oleson S, Haley AP, Tanaka H. Impacts of Metabolic Syndrome Scores on Cerebrovascular Conductance Are Mediated by Arterial Stiffening. *American journal of hypertension*, hpx132. 2017
- Pico F, Dufouil C, Levy C, Besancon V, de Kersaint-Gilly A, Bonithon-Kopp C, Ducimetiere P, Tzourio C, Alperovitch A. Longitudinal study of carotid atherosclerosis and white matter hyperintensities: the EVA-MRI cohort. *Cerebrovascular diseases*. 2002; 14(2):109–115. [PubMed: 12187015]
- Rajkumar C, Kingwell BA, Cameron JD, Waddell T, Mehra R, Christophidis N, Komesaroff PA, McGrath B, Jennings GL, Sudhir K, Dart AM. Hormonal therapy increases arterial compliance in postmenopausal women. *Journal of the American College of Cardiology*. 1997; 30(2):350–356. [PubMed: 9247504]
- Reijmer YD, Brundel M, de Bresser J, Kappelle LJ, Leemans A, Biessels GJ. Utrecht Vascular Cognitive Impairment Study, G. Microstructural white matter abnormalities and cognitive

- functioning in type 2 diabetes: a diffusion tensor imaging study. *Diabetes care*. 2013; 36(1):137–144. [PubMed: 22961577]
- Rundek T, Della-Morte D, Gardener H, Dong C, Markert MS, Gutierrez J, Roberts E, Elkind MSV, DeCarli C, Sacco RL, Wright CB. Relationship between carotid arterial properties and cerebral white matter hyperintensities. *Neurology*. 2017; 88(21):2036–2042. [PubMed: 28446647]
- Sala M, van den Berg-Huysmans A, van der Grond J, Huisman M, Brandts A, Westenberg JJ, de Roos A. Aortic Arch Stiffness Is Associated With Incipient Brain Injury in Patients With Hypertension. *American journal of hypertension*. 2016; 29(6):705–712. [PubMed: 26404903]
- Salat DH, Tuch DS, Greve DN, van der Kouwe AJ, Hevelone ND, Zaleta AK, Rosen BR, Fischl B, Corkin S, Rosas HD, Dale AM. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. *Neurobiology of aging*. 2005; 26(8):1215–1227. [PubMed: 15917106]
- Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Statistics in medicine*. 1997; 16(22):2529–2542. [PubMed: 9403954]
- Seo SK, Jung I, Lee SM, Cho S, Choi YS, Chung TS, Lee BS. Relationship between leukoaraiosis and menopause in healthy middle-aged women. *Fertil Steril*. 2013; 100(2):500–504. [PubMed: 23622889]
- Seo WK, Lee JM, Park MH, Park KW, Lee DH. Cerebral microbleeds are independently associated with arterial stiffness in stroke patients. *Cerebrovascular diseases*. 2008; 26(6):618–623. [PubMed: 18984946]
- Sonesson B, Hansen F, Stale H, Lanne T. Compliance and diameter in the human abdominal aorta--the influence of age and sex. *European journal of vascular surgery*. 1993; 7(6):690–697. [PubMed: 8270073]
- Sullivan EV, Adalsteinsson E, Hedehus M, Ju C, Moseley M, Lim KO, Pfefferbaum A. Equivalent disruption of regional white matter microstructure in ageing healthy men and women. *Neuroreport*. 2001; 12(1):99–104. [PubMed: 11201100]
- Sullivan EV, Rohlfing T, Pfefferbaum A. Quantitative fiber tracking of lateral and interhemispheric white matter systems in normal aging: relations to timed performance. *Neurobiology of aging*. 2010; 31(3):464–481. [PubMed: 18495300]
- Tarumi T, de Jong DL, Zhu DC, Tseng BY, Liu J, Hill C, Riley J, Womack KB, Kerwin DR, Lu H, Munro Cullum C, Zhang R. Central artery stiffness, baroreflex sensitivity, and brain white matter neuronal fiber integrity in older adults. *NeuroImage*. 2015; 110:162–170. [PubMed: 25623500]
- Tarumi T, Shah F, Tanaka H, Haley AP. Association between central elastic artery stiffness and cerebral perfusion in deep subcortical gray and white matter. *American journal of hypertension*. 2011; 24(10):1108–1113. [PubMed: 21654859]
- Tjeerdema N, Van Schinkel LD, Westenberg JJ, Van Elderen SG, Van Buchem MA, Smit JW, Van der Grond J, De Roos A. Aortic stiffness is associated with white matter integrity in patients with type 1 diabetes. *Eur Radiol*. 2014; 24(9):2031–2037. [PubMed: 24828536]
- Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N, Hinohara S. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement--a survey of 12517 subjects. *Atherosclerosis*. 2003; 166(2):303–309. [PubMed: 12535743]
- Wang R, Fratiglioni L, Laukka EJ, Lovden M, Kalpouzos G, Keller L, Graff C, Salami A, Backman L, Qiu C. Effects of vascular risk factors and APOE epsilon4 on white matter integrity and cognitive decline. *Neurology*. 2015; 84(11):1128–1135. [PubMed: 25672924]
- Westlye LT, Walhovd KB, Dale AM, Bjornerud A, Due-Tønnessen P, Engvig A, Grydeland H, Tamnes CK, Ostby Y, Fjell AM. Life-span changes of the human brain white matter: diffusion tensor imaging (DTI) and volumetry. *Cerebral cortex*. 2010; 20(9):2055–2068. [PubMed: 20032062]
- White RE. Estrogen and vascular function. *Vascular pharmacology*. 2002; 38(2):73–80.
- Williams VJ, Leritz EC, Shepel J, McGlinchey RE, Milberg WP, Rudolph JL, Lipsitz LA, Salat DH. Interindividual variation in serum cholesterol is associated with regional white matter tissue integrity in older adults. *Human brain mapping*. 2013; 34(8):1826–1841. [PubMed: 22438182]

- Zaydun G, Tomiyama H, Hashimoto H, Arai T, Koji Y, Yambe M, Motobe K, Hori S, Yamashina A. Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. *Atherosclerosis*. 2006; 184(1):137–142. [PubMed: 15913634]
- Zhang Y, Schuff N, Jahng GH, Bayne W, Mori S, Schad L, Mueller S, Du AT, Kramer JH, Yaffe K, Chui H, Jagust WJ, Miller BL, Weiner MW. Diffusion tensor imaging of cingulum fibers in mild cognitive impairment and Alzheimer disease. *Neurology*. 2007; 68(1):13–19. [PubMed: 17200485]
- Zhuang L, Wen W, Zhu W, Trollor J, Kochan N, Crawford J, Reppermund S, Brodaty H, Sachdev P. White matter integrity in mild cognitive impairment: a tract-based spatial statistics study. *NeuroImage*. 2010; 53(1):16–25. [PubMed: 20595067]
- Zureik M, Temmar M, Adamopoulos C, Bureau JM, Courbon D, Thomas F, Bean K, Touboul PJ, Ducimetiere P, Benetos A. Carotid plaques, but not common carotid intima-media thickness, are independently associated with aortic stiffness. *Journal of hypertension*. 2002; 20(1):85–93. [PubMed: 11791030]

Highlights

- Age and carotid artery compliance affect cerebral DTI metrics at midlife
- Carotid artery compliance mediates the effect of age on DTI metrics in males
- Carotid stiffness adversely affects cerebral white matter particularly in men

Figure 1. Differences in WM diffusion metrics between females and males at midlife adjusted for age and years of education. Data are estimated marginal means $\pm$ standard error. * $p < 0.02$

Figure 2.
a–d. Conceptual model of sex moderating the relationship of age with regional WM diffusion metrics with corresponding scatter plots demonstrating a greater effect of age on regional WM integrity in males than females.

Figure 3.
a–b. Conceptual model of sex moderating the relationship of CAC with regional WM FA with corresponding scatter plot demonstrating a greater effect of CAC on regional WM FA in males than females.

Table 1

Descriptive characteristics

		Female (n=87)	Male (n=68)	p-value
Descriptive	Age, years	48.6 ± 6.6	49.6 ± 6.3	0.347
	Education, years	16.1 ± 2.2	16.3 ± 2.5	0.700
	MMSE, score	28.6 ± 1.6	28.7 ± 1.5	0.689
	BDI-II, score	7.8 ± 6.7	6.6 ± 6.4	0.264
	Height, m	1.64 ± 0.07	1.75 ± 0.07	<0.001 *
	Weight, kg	77.1 ± 17.1	85.6 ± 15	0.001 *
	BMI, kg/m ²	28.7 ± 6.1	28.0 ± 5.1	0.477
	Waist Circumference, cm	92.4 ± 14	97.9 ± 13	0.012 *
	Systolic BP, mm Hg	121 ± 14	121 ± 10	0.654
	Diastolic BP, mm Hg	75 ± 9	71 ± 10	0.006 *
	Glucose, mg/dL	95 ± 26	101 ± 24	0.105
	Total-C, mg/dL	202 ± 41	204 ± 46	0.809
	HDL-C, mg/dL	60 ± 16	45 ± 16	<0.001 *
	LDL-C, mg/dL	123 ± 35	135 ± 41	0.073
	Triglycerides, mg/dL	104 ± 53	138 ± 92	0.007 *
Arterial Stiffness	CACx100, cm/mmHg	1.17 ± 0.36	1.31 ± 0.32	0.012 *
	Smoking, n (%)	17, (19.5)	7, (10.3)	0.114
	Post-menopause, n (%)	31, (35.6)	0, (0)	<0.001 *
Race	Caucasian, n (%)	55, (63.2)	44, (64.7)	0.848
	African American, n (%)	8, (9.2)	4, (5.9)	0.444
	Latino, n (%)	16, (18.4)	15, (22.1)	0.571
	Asian, n (%)	3, (3.4)	2, (2.9)	0.859
	Other, n (%)	5, (5.7)	3, (4.4)	0.709
Medication	Anti-hypertensive, n (%)	17, (19.5)	13, (19.1)	0.947
	Anti-lipid, n (%)	12, (13.8)	6, (8.8)	0.338
	Insulin, n (%)	1, (1.1)	3, (4.1)	0.204
	HRT, n (%)	10, (11.5)	0, (0)	0.004 *
	Birth control, n (%)	5, (5.7)	0(0)	0.043 *

MMSE=mini mental status exam; BDI=Beck depression inventory; BMI=body mass index; BP=blood pressure; C=cholesterol; CAC=carotid artery compliance; HRT=hormone replacement therapy

Data are means ± SD

* p<0.05

Table 2

Pearson correlations of age on regional white matter integrity split across sex

Region										
		Metric	ALIC	CC Genu	CC Body	CC Splenium	Cingulum	Cingulum (hippocampal)	SCR	Uncinate
Total (n=155)	Model 1	FA	-0.079	-0.285*	-0.173	-0.165	-0.197*	0.043	-0.028	-0.090
		MD	0.193*	0.279*	0.264*	0.214*	0.140	0.250*	0.208*	0.117
	Model 2	FA	-0.032	-0.241*	-0.096	-0.087	-0.135	0.058	-0.063	-0.094
		MD	0.173	0.236*	0.270*	0.182	0.127	0.231*	0.203*	0.109
Female (n=87)	Model 1	FA	-0.054	-0.296*	-0.103	-0.121	-0.097	0.090	-0.041	-0.118
		MD	0.136	0.241*	0.145	0.123	0.052	0.117	0.127	-0.055
	Model 2	FA	0.039	-0.236	0.007	-0.021	-0.027	0.069	0.080	-0.093
		MD	0.086	0.224	0.106	0.083	0.028	0.063	0.061	-0.065
Male (n=68)	Model 1	FA	-0.149	-0.296*	-0.272*	-0.213	-0.373*	-0.035	-0.021	-0.074
		MD	0.257*	0.314*	0.379*	0.307*	0.247*	0.369*	0.299*	0.357*
	Model 2	FA	-0.144	-0.324*	-0.244	-0.148	-0.376*	-0.028	0.010	-0.118
		MD	0.253	0.325*	0.427*	0.290	0.247	0.386*	0.368*	0.355*

FA=fractional anisotropy; MD=mean diffusivity; ALIC=anterior limb of internal capsule; CC=corpus callosum; SCR=superior corona radiata

Model 1: No covariates

Model 2: Controlling education, systolic blood pressure, waist circumference, glucose, triglycerides, and total cholesterol

*
p<0.02

Table 3
Bivariate and partial correlations of carotid artery compliance on regional white matter integrity split across sex

Region										
Metric		ALIC	CC Genu	CC Body	CC Splenium	Cingulum	Cingulum (hippocampal)	SCR	Uncinate	
Total (n=155)	Model 1	FA	0.223[*]	0.098	0.104	0.136	0.227[*]	0.115	0.078	0.148
		MD	-0.276[*]	-0.001	-0.011	-0.043	-0.148	-0.109	-0.169	-0.159
	Model 2	FA	0.224[*]	0.085	0.061	0.108	0.201[*]	0.118	0.071	0.164
		MD	-0.236[*]	0.043	0.025	-0.028	-0.140	-0.114	-0.141	-0.134
Female [^] (n=87)	Model 1	FA	0.176	0.009	0.015	0.078	0.066	0.099	-0.005	0.128
		MD	-0.258	-0.024	-0.041	-0.071	-0.111	-0.179	-0.135	-0.113
	Model 2	FA	0.160	-0.023	-0.078	0.027	0.015	0.139	-0.019	0.066
		MD	-0.163	0.067	0.032	-0.031	-0.115	-0.166	-0.080	-0.104
Male (n=68)	Model 1	FA	0.232	0.180	0.262	0.288[*]	0.408[*]	0.121	0.188	0.135
		MD	-0.364[*]	-0.034	-0.095	-0.104	-0.284[*]	-0.131	-0.289[*]	-0.366[*]
	Model 2	FA	0.223	0.161	0.286	0.367[*]	0.404[*]	0.068	0.137	0.176
		MD	-0.423[*]	-0.055	-0.149	-0.164	-0.314[*]	-0.178	-0.368[*]	-0.380[*]

FA=fractional anisotropy; MD=mean diffusivity; ALIC=anterior limb of internal capsule; CC=corpus callosum; SCR=superior corona radiata

Model 1: No covariates

Model 2: Controlling age, education, waist circumference, glucose, triglycerides, and total cholesterol

[^] Menopausal status and hormone replacement therapy included as additional covariates in Model 2

* p<0.02

Table 4

Summary of bias corrected non-parametric bootstrapped models of arterial stiffness mediating the negative effect of age on regional WM integrity

95% CI					
Measure	Effect	B	S.E.	Lower Limit	Upper Limit
Cingulum FA	Direct	-0.0015	0.0005	-0.0026	-0.0005
	Indirect	-0.0004	0.0002	-0.0010	-0.0001
Cingulum MD	Direct	0.0017	0.0008	-0.0001	0.0032
	Indirect	0.0004	0.0003	0.0001	0.0012
SCR MD	Direct	0.0020	0.0008	0.0004	0.0035
	Indirect	0.0004	0.0003	0.0001	0.0011
Uncinate MD	Direct	0.0018	0.0007	0.0005	0.0032
	Indirect	0.0005	0.0003	0.0001	0.0012

FA=fractional anisotropy; MD=mean diffusivity; SCR=superior corona radiata; B=unstandardized regression coefficient