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Aortic Stiffness, increased white matter free water and altered microstructural integrity: a continuum of injury

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Abstract

Background and Purpose—Prior reports from the Framingham Heart Study have identified cross-sectional associations of arterial stiffness, as reflected by carotid-femoral pulse wave velocity (CFPWV), and systolic blood pressure (SBP) with vascular brain injury. The purpose of this study is to examine free water (FW), fractional anisotropy (FA) and white matter hyperintensities (WMH) in relation to arterial stiffness among subjects of the Framingham Offspring and 3rd generation cohorts.

Methods—In 2422 participants aged 51.3±11.6 years, FA, FW and WMH were related to CFPWV using voxel-based linear and generalized linear regressions, adjusting for relevant covariables. Mean FW, mean FA and WMH burden (log-transformed) were computed within white matter (WM) region and related to SBP and CFPWV using multiple mediation analyses.

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Disclosures

Dr. Maillard, Dr. Fletcher, Dr. Seshadri, Dr. Beiser, Dr. Satizabal, Dr. Himali and Dr. Vasan report no disclosures.

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Results—CFPWV was found to be associated with higher FW, lower FA and higher WMH incidence in WM areas covering respectively 356.1, 211.8 and 10.9cc of the WM mask. Mediation analyses revealed that the effect of SBP on FW was mediated by CFPWV (direct and indirect effects: $a=0.040$, $p<0.001$ and $a'=0.020$, $p>0.05$). Moreover, the effect of CFPWV on FA was mediated by FW (direct and indirect effects: $b=-0.092$, $p<0.001$ and $b'=0.012$, $p>0.05$), whose effect on WMH was in turn mediated by FA (direct and indirect effects: $c=0.246$, $p<0.001$ and $c'=0.116$, $p>0.05$).

Conclusions—From these data, we propose a biomechanical hypothesis designed for future research experiments to explain how hemodynamic alteration may lead to WM injury by impacting cerebral water content and more subtly WM integrity, to finally lead to WMH development.

Introduction

Hypertension and arterial stiffness are common medical conditions among individuals 60 years of age and older.¹ Besides being major risk factors for cardiovascular disease and mortality,^{2, 3} vascular alterations, including increased aortic stiffness as assessed by carotid-femoral pulse wave velocity, are also risk factors for subclinical cerebral vascular brain injury such as white matter hyperintensities (WMH), MRI infarcts, and accelerated brain atrophy.⁴⁻⁶ These imaging markers of brain aging also are associated with reduced cognitive ability⁶ and increased likelihood of incident stroke, mild cognitive impairment and dementia.⁷ Recent analyses from the Framingham Heart Study (FHS) Third Generation (average age 43) cohort suggested that hemodynamic alterations may play a central role in the early development of white matter (WM) degeneration which leads to symptomatic disease in later life^{8, 9}.

Diffusion Tensor Imaging (DTI) measures the displacement of water molecules, which in a typical brain imaging experiment displace a few tens of microns¹⁰. DTI-derived fractional anisotropy (FA) reflects how water directionality is constrained along axons and characterizes the microstructural properties and integrity of WM. Nonetheless, standard single shell DTI algorithms are contaminated by partial volume of extracellular water, reducing the sensitivity and specificity of most metrics derived from DTI, including FA¹⁰. Consequently, efforts have been recently deployed to quantify this amount of extracellular water, also referred as free water (FW). FW reflects the amount of water molecules that do not experience flow and are not restricted by their surroundings, and therefore is independent to water directionality, as opposed to FA. While interest in this new imaging metric has been increasing in the context of sensitivity improvement of DTI in the vicinity of the ventricles and for the delineation of fibers such as the fornix^{11, 12}, recent studies have also found that FW content constitutes a strong biomarker of subtle cerebral injury in individuals with various pathologies including Parkinson's disease and schizophrenia^{13, 14}.

The present study, therefore, had two objectives. First, we aimed to evaluate the sensitivity of FW as a marker of WM injury in association with elevated blood pressure and arterial stiffness among normal adult individuals. Second, despite our cross-sectional design that does not permit conclusions based on the chronology of mechanisms that arise with

hemodynamic alteration, we used mediation analysis to propose a potential scenario of how hemodynamic alterations may first lead to increased FW content that subtly contributes to WM degeneration, as identified by reduced FA, to finally result in WMH formation. The study sample includes 2422 individuals from the Third Generation and Offspring community based cohorts of the FHS aged from 25 to 89 years old.

Methods

Study design and participants

The design of the FHS Third Generation (G3) and Offspring cohort studies has been detailed previously.¹⁵ A total of 2606 subjects underwent both brain MRI and arterial tonometry between 2009 and 2013. All subjects at assessment number 8 for the Offspring cohort and assessment number 2 for the 3rd Generation cohort were given the option to receive an MRI. As previously described¹⁶, all subjects who agreed to have MRI and were not excluded for health reasons were included in this analysis. Participants with prevalent stroke or dementia at the MRI evaluation, however, were excluded from the present analysis. Briefly, stroke was defined as an acute onset focal neurologic deficit of presumed vascular etiology, lasting 24 hours or resulting in death. Dementia was diagnosed according to the criteria of the *DSM-IV*.¹⁷ Participants were also excluded from the present analysis for the following reasons: other neurologic disorders that might confound the assessment of brain volumes, bad quality or incomplete tonometry data or with poor brain MRI quality, resulting in a sample of 2422 individuals. Demographic characteristics are summarized in Table 1.

The Institutional Review Boards at all participating institutions approved this study, and subjects or their legal representatives gave written informed consent.

Procedures

Noninvasive hemodynamic data acquisition is described in the Supplement file (please see Supplemental Data). Carotid-femoral pulse wave velocity (CFPWV) was 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 suprasternal notch as a fiducial point.¹⁸ The carotid-femoral transit path spans the descending aorta, making CFPWV a measure of descending thoracic and abdominal aortic stiffness. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured as the average of two physician recorded BPs in a seated position.

We used DTI measures of free water content (FW), of fractional anisotropy (FA), which is a sensitive indicator of WM integrity, and of WMH. FW and FA were computed from DTI using previously described algorithms.^{12, 19} Segmentation of WMH and total cranial volume (TCV) were performed from FLAIR and T1-weighted images by automated procedures previously described.^{20, 21} FW, FA and WMH maps were finally coregistered to a minimum deformation template for group statistical analyses. Each voxel in these co-registered multimodal volumetric images corresponds to the same location in the brain across all individuals. This allows corresponding imaging measures (FW, FA or WMH occurrence) to

be related to a host of independent variables. WMH volumes were log-transformed to normalize population variance.

Statistical analyses

Voxel-based regressions—The objective of the voxel-based analysis was to determine if, at the voxel level, water content as indicated by FW, subtle WM degeneration, as indicated by lower FA and most advanced stage of WM injury, as indicated with WMH occurrence, were associated with individual CFPWV. To achieve this goal, we used separate linear and generalized linear regressions with either FW (continuous measures), FA (continuous measures) or WMH (occurrence) as the dependent variables (separate models for each), and CFPWV as independent variable, adjusting for a set of standard risk factors including age, sex, use of antihypertensive therapy, total cholesterol, presence of diabetes mellitus, TCV and time between clinical and MRI exams.

Mediation analyses—Mean FW, mean FA and log-transformed WMH volume were computed for each subject by superimposing the WM mask to each individual's FW, FA or WMH images. Because FW appeared to be more sensitive to arterial stiffness (see Figure 1) than FA and WMH, in terms of both spatial extent and association's strength, we then conducted mediation analyses to investigate whether the reported association between abnormal hemodynamic measures and WMH^{9, 22} may be mediated by DTI-derived measures, including FW and FA. Briefly, mediation exists when a predictor affects a dependent variable indirectly through a third variable (the mediator).²³ Mediation analyses investigate the effect of SBP on FW with CFPWV as a mediator (Model 1), the effect of CFPWV on FA with FW as a mediator (Model 2), the effect of FW on WMH with FA as a mediator (Model 3). Each model included age, sex, use of antihypertensive therapy, total cholesterol, presence of diabetes mellitus, TCV and time between tonometry and MRI exams as covariates. In addition, Model 2 was also adjusted for SBP, and Model 3 for SBP and CFPWV. Indirect effects were examined using a 95% bootstrapped bias-corrected confidence interval established via bootstrapping with 1000 bootstrap samples.

The details of the study methods, including subject recruitment and evaluation, image acquisition and processing, and statistical analysis, are described in the Supplemental file (please see <http://stroke.ahajournals.org>).

Results

Demographics

Compared with the remainder of the G3 and Offspring cohorts (see Table 1) without MRI, individuals included in this study were on average significantly younger ($p < 0.001$), with significantly lower SBP ($p < 0.001$) and CFPWV ($p < 0.001$) and less likely to smoke, to receive treatment for hypertension or have diabetes (p values < 0.001).

Voxel-based regressions

Higher CFPWV was associated with larger FW within voxels (referred to as R_{FW}) that covered 356.1cc of the WM (see Table I in the Supplement file, please see <http://>

stroke.ahajournals.org). WM tracts most implicated as a function of volume included the superior, anterior and posterior part of the corona radiata (13.50, 12.32 and 7.03cc respectively), the body, splenium and genu of corpus callosum region (12.03, 9.30 and 6.49cc respectively) and the superior longitudinal fasciculus (11.89cc, see Figure 1).

Higher CFPWV was associated with lower FA within voxels (referred to as R_{FA}) that covered 211.8cc of the WM (see Table I in the Supplement file, please see <http://stroke.ahajournals.org>). WM tracts heavily involved, as a function of volume, also included the superior, anterior and posterior part of the corona radiata (6.85, 10.07 and 3.43cc respectively) and the body, splenium and genu of corpus callosum region (9.64, 8.65 and 6.02cc respectively, see Figure 1).

Higher CFPWV was also associated with higher WMH occurrence within voxels (referred to as R_{WMH}) that covered 10.9cc of the WM (see Table I in the Supplement file, please see <http://stroke.ahajournals.org>) implicating mostly, in term of volume, the superior and anterior part of the corona radiata (1.49 and 2.31cc respectively) and the superior longitudinal fasciculus (0.41cc, see Figure 1).

Interestingly, the overlap between R_{FW} and R_{FA} regions covered 52% of R_{FW} and 89% of R_{FA} , indicating that almost half of the voxels with a significant association between CFPWV and FW were not significant for FA while almost all voxels showing significant associations between CFPWV and FA were significant for FW.

Similarly, the overlap between R_{FA} and R_{WMH} regions covered 3% of R_{FA} and 66% of R_{WMH} , indicating that most of the voxels with a significant association between CFPWV and FA were not significant for WMH while two thirds of the voxels showing significant associations between CFPWV and WMH were significant for FA.

The overlap between R_{FW} and R_{WMH} regions covered 3% of R_{FW} and 95% of R_{WMH} , indicating that most of the voxels with a significant association between CFPWV and FW were not significant for WMH while almost all of the voxels showing significant associations between CFPWV and WMH were significant for FW.

Mediation analyses

Figure 2 shows the mediation analyses results relating SBP, CFPWV, FW, FA and WMH. In Model 1, the direct association between SBP and FW was found to be significant ($a=0.040$, $p<0.001$) but mediated by CFPWV (indirect effect: $a'=0.020$, $p>0.05$, mediation effect: $\beta=0.020$, $p<0.001$). The significant mediation effect in this model reflects that fact that the influence of SBP on CFPWV was highly significant ($\beta=0.165$, $p<0.001$), as well as the influence of CFPWV on FW ($\beta=0.122$, $p<0.001$). Similarly, Model 2 revealed that CFPWV had a direct effect on FA ($b=-0.092$, $p<0.001$), that no longer remained significant when FW was included as a mediator in the model (indirect effect: $b'=0.012$, $p>0.05$, mediation effect: $\beta=-0.104$, $p<0.001$), and whose effect on FA was found to be highly significant ($\beta=-0.851$, $p<0.001$). Finally, Model 3 revealed that FW content had a direct effect on WMH burden ($c=0.246$, $p<0.001$), but this effect was found to be mediated by FA (indirect effect: $c'=0.13$,

$p > 0.05$, mediation effect: $\beta = 0.116$, $p < 0.001$), whose effect on WMH was found to be significant ($\beta = -0.136$, $p < 0.001$).

Discussion

The present study suggests that, among a community-based sample aged 25 to 90 years, elevated blood pressure and arterial stiffness appears to trigger a pathophysiological cascade that includes an increase in free water content and subtle WM degeneration eventually leading to the development of WMH. From these data, we propose a biomechanical model whereby cerebral microvascular damage is initiated by hemodynamic alterations that have been shown to manifest in subclinical cognitive dysfunction within the FHS Offspring cohort²⁴.

Hemodynamic alteration

While there is now substantial evidence that elevated arterial stiffness is the earliest manifestation of systolic hypertension, controversy exists regarding the direction and nature of this relationship, with evidence implicating arterial stiffness in the manifestation and progression of hypertension as well as vice versa.²⁵ For example, the Framingham Heart Study, using data from Exam 7 to predict SBP and PWV in Exam 8 (7 years later on average), found that elevated PWV in Exam 7 was associated with higher SBP in Exam 8, but SBP at Exam 7 was not associated with higher PWV in Exam 8.²⁶ Conversely, findings from the Baltimore Longitudinal Study of Aging indicate that higher PWV was associated with larger increases in SBP over time but also that higher SBP was associated with accelerated increase of PWV over the same observational period. Recently, however, the SardiNIA study brought new insights in the understanding of the cross-talk trajectory of PWV and SBP over time by showing evidence of a striking dissociation in the trajectories PWV versus various measures of blood pressure with advancing age.²⁷ This finding challenges the notion of a tight coupling between blood pressure and PWV, particularly in later life. Therefore, while our mediation analyses suggest that the effect of SBP on free water content is modulated by CFPWV, and not the reverse, this cross-sectional study cannot resolve the question as to whether vascular mechanical or hemodynamic alterations initiate this process. Longitudinal assessments, emphasizing the earliest changes in SBP and CFPWV, particularly in younger individuals, are likely necessary to further examine this question.

Extracellular water

The main finding of this work is that arterial stiffness is strongly associated with the content of FW within most of the cerebral WM, particularly in the area of the middle cerebral artery WM vascular territory, suggesting that FW constitutes a more sensitive biomarker of WM injury in association with hemodynamic alteration than FA and WMH (see Figure 1). Increasing numbers of DTI studies have reported that correcting for extracellular water content improves the sensitivity of DTI in the vicinity of the ventricles and for the delineation of fibers such as the fornix.¹² However, only a few studies have examined FW content, as estimated from DTI images, specifically as a biomarker of cerebral injury, in individuals with pathologies including Parkinson's disease or schizophrenia.^{13, 14} To our

knowledge, this study is the first to investigate differences in FW content at voxel-based level among participants in a large community-based study. Using this approach, we found evidence of strong and spatially distributed associations between increased arterial stiffness and the content of FW, identifying a potentially new stage in the pathological process leading to WMH (and possibly small vessel cerebral infarction). It also raises the question of how the biological mechanisms that lead to stiffening of the arterial wall promote an increase in extracellular water content.

One hypothesis is that stiffening of the aorta increases transmission of pulsatile energy into small vessels that are not accustomed to high levels of pulsatility. High levels of pulsatility could lead to changes in pre-capillary pressure gradients thereby resulting in greater flow of solute into the interstitium until interstitial pressure equilibrates with intravascular pressure and a new equilibrium is achieved, albeit with an expanded extravascular FW content. A second hypothesis is that arterial stiffening and elevated blood pressure may result in subtle blood-brain barrier dysfunction, shifting the equilibrium point between arterial and osmotic pressure, which in turn may allow extra water, toxins, proteases, or other substances in the blood to enter the brain interstitial space and disturb the composition of the interstitial fluid within the WM milieu. Additional studies relating arterial stiffness to measures derived from additional MRI sequences such as dynamic contrast-enhanced imaging²⁸ could be used to directly test this hypothesis.

Free water excess and white matter integrity

A second important finding is that while, by their nature, WMH are expected to be associated with the amount of FW content, in our analysis this association was mediated by differences in FA. The underlying process by which this occurs, however, is unclear. One hypothesis could be that alterations in the composition of interstitial fluids, through the increase of FW, may contribute to subtle demyelination and axonal destruction over time. Indeed, previous studies have reported associations between reduced FA and higher level of interstitial proteins, including beta-amyloid protein.²⁹ The design of the present study, however, does not enable identification of the components within FW that may be implicated in the degeneration process of demyelination or axon loss in neural fiber tracts.

A second hypothesis is that, rather than demyelination, an increase in FW content may result in axonal dispersion, lessening the constraint of water directionality along axons, as reflected by reduced FA. Again, our cross sectional design does not allow us to conclude that FW increase precedes or is concurrent to a decrease in FA. Longitudinal studies using probabilistic tractography, which measures voxel-based connectivity index along WM pathways that reflects fiber organization such as tract geometry and length, may help to answer this question.

White matter hyperintensities as the peak of WM injury

The present study showed, for the first time, voxel-based associations between arterial stiffness and occurrence of WMH, extending findings of previous studies reporting such associations, but at the regional level^{9, 22}. WMH burden was also found to be associated with FA. These results confirm, within a larger cohort, previous DTI studies which show that

WM degeneration is an insidious and continuous process, with WMH constituting the most advanced stage of injury.³⁰ Recent studies have extended this finding using different quantitative imaging techniques. For example, larger burden of WMH was found to be associated with reduced cerebral blood flow, cerebrovascular reactivity and with increased blood-brain barrier permeability.^{31, 32} Importantly, WMH constitute the end-stage of demyelination and axonal damage, as reported by pathology studies,³³ and while the excess of FW content might be reversible, much of WMH, as they appear on FLAIR images, likely reflect permanent damage, as supported by longitudinal MRI studies that tracked individual lesions over time.³⁰ This evidence, in addition to the evidence that WMH burden is associated with trajectories of significant cognitive decline⁷ emphasizes the importance of early detection of change in WM properties as a preventive strategy against white matter injury and subsequent cognitive decline. In the present study, the association between elevated arterial stiffness and excess of FW content involved almost the entire cerebral WM, with associations between CFPWV and FW being strongest in regions where WMH occur (Figure 1). This finding suggests that FW may constitute a sensitive biomarker to assess and monitor therapies aimed at achieving the goal of reduced vascular brain injury, although the potential reversibility of increased interstitial FW has yet to be determined.

Strengths and limitations

The strengths of this study are the large, population-based setting with quantitative and sensitive measurements of WM integrity. Original features of this study are the wide age range of the study cohort as compared to previous publications. There, however, are limitations including the cross-sectional design that limits our ability to establish causal relations between arterial stiffness and brain aging measures. Additional longitudinal studies are needed to support the proposed order of cerebral mechanisms arising from differences in CFPWV. The present study also did not aim to decipher the conundrum of SBP and CFPWV as briefly described above and more fully addressed in other publications²⁵ but instead proposes a hypothesis to explain how their combination contributes to WM degeneration. Finally, the FHS cohort is mostly of white descent and therefore does not fully represent the general population of the USA for whom the impact of hypertension and vascular stiffness may be even more relevant.

Conclusion

The present study finds that FW is a sensitive measure of subtle brain injury in association with increased CFPWV and proposes a biomechanical hypothesis to explain how hemodynamic alteration may lead to late clinically relevant WM degeneration (i.e. WMH). Our findings suggest that elevated carotid-femoral pulse wave velocity impacts the content of cerebral FW and WM integrity, as reflected by FA, finally leading to WMH development. Longitudinal studies are needed to assess the exact chronological order of these mechanisms that arise with hemodynamic alteration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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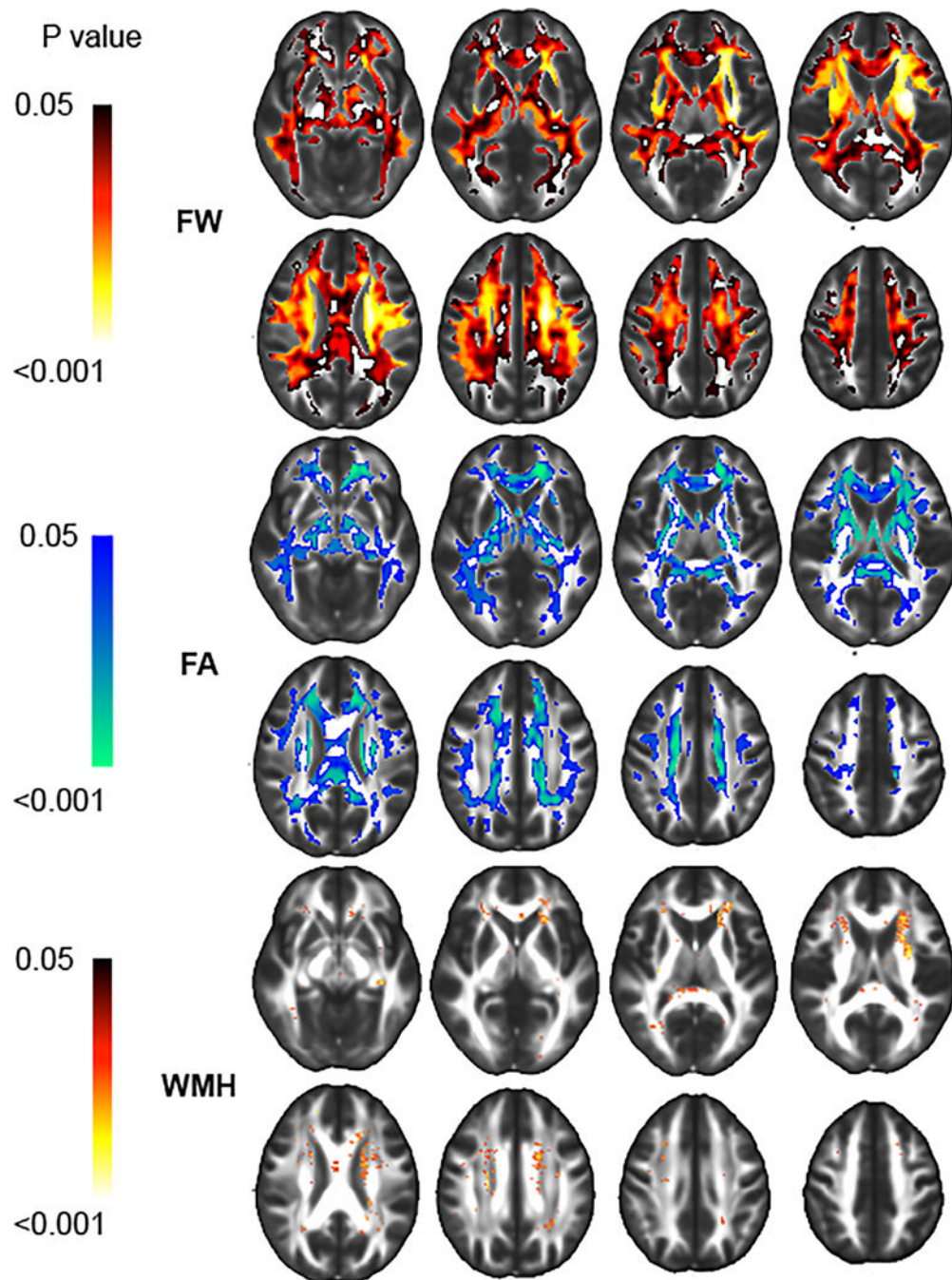


Figure 1. Significant associations between increasing carotid-femoral pulse wave velocity (CFPWV) with higher free water (FW), lower fractional anisotropy (FA) and higher white matter hyperintensities (WMH) occurrence. Color intensity indicates strength of association.

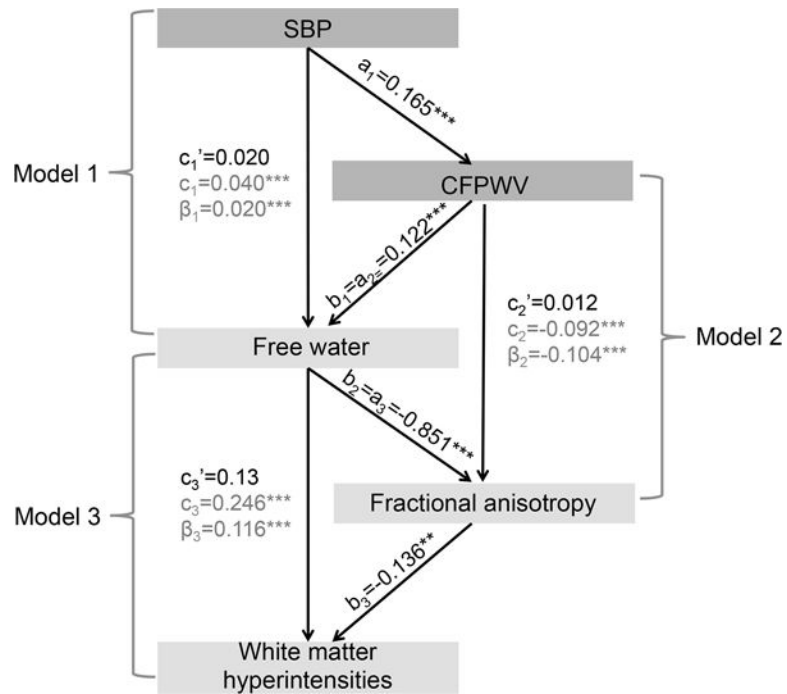


Figure 2.

For each mediation Model i , (e.g. a_i , b_i , c_i) indicate the direct effects, whereas c_i' indicates the indirect effect and β_i the mediation effect. Mediation analyses investigate the effect of systolic blood pressure (SBP) on free water, with carotid-femoral pulse wave velocity (CFPWV) as mediator (Model 1), the effect of CFPWV on fractional anisotropy with free water as mediator (Model 2), the effect of free water on white matter hyperintensities burden with fractional anisotropy as mediator (Model 3). **: $p < 0.01$; ***: $p < 0.001$

Table 1

Subject's characteristics

	Tonometry only N=2272	Tonometry with MRI N=2422	P-value
Age, years	56.38 ± 13.64	51.28 ± 11.67	<0.001
Male	1043 (45.91)	1102 (45.50)	0.78
Systolic blood pressure, mmHG	122.73 ± 17.37	118.25 ± 14.75	<0.001
Total Cholesterol, mg/dL	186.66 ± 36.91	187.02 ± 34.71	0.73
Carotid-femoral pulse wave, m/s, median (Q1, Q3)	7.90 (6.70, 10.00)	7.20 (6.30, 8.60)	<0.001
History of diabetes	238 (10.76)	150 (6.22)	<0.001
Current smoker	259 (11.41)	196 (8.10)	<0.001
Treatment for hypertension	836 (36.86)	620 (25.60)	<0.001

Data presented as mean (SD) or n (%), unless otherwise stated