Vascular Determinants of Hippocampal Viscoelastic Properties in Healthy Adults Across the Lifespan

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ABSTRACT (175 words)

Arterial stiffness and cerebrovascular pulsatility are non-traditional risk factors of Alzheimer's disease. However, there is a gap in understanding the earliest mechanisms that link these vascular determinants to brain aging. Changes to mechanical tissue properties of the hippocampus (HC), a brain structure essential for memory encoding, may reflect the impact of vascular dysfunction on brain aging. We tested the hypothesis that arterial stiffness and cerebrovascular pulsatility are related to HC tissue properties in healthy adults across the lifespan. Twenty-five adults underwent measurements of brachial blood pressure (BP), large elastic artery stiffness, middle cerebral artery pulsatility index (MCAv PI), and magnetic resonance elastography (MRE), a sensitive measure of HC viscoelasticity. Individuals with higher carotid pulse pressure (PP) exhibited lower HC stiffness (β=-0.39, p=0.05), independent of age and sex. Collectively, carotid PP and MCAv PI significantly explained a large portion of the total variance in HC stiffness (adjusted R^2=0.41, p=0.005) in the absence of associations with HC volumes. These cross-sectional findings suggest that the earliest reductions in HC tissue properties are associated with alterations in vascular function.

Key Words: arterial stiffness, brain imaging, cerebrovascular pulsatility, hippocampal tissue viscoelastic properties, magnetic resonance elastography

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and one of the fastest growing causes of morbidity and mortality in the United States and other developed countries worldwide [1,2]. Many of the risk factors for AD are shared with cardiovascular diseases (CVD), including advanced age, hypertension, diabetes, and obesity [3]. In this regard, large elastic artery stiffening, which results in a reduced ability of the elastic arteries (i.e., aorta and common carotid arteries) to buffer the pulsatile pressure leaving the heart, occurs with age beginning in mid-life, and has emerged to be one of the strongest predictors of late-life cognitive impairment [4–11].

Arterial stiffening leads to an increased transmission of pulsatile energy into the more sensitive microcirculation of the brain and has been associated with cerebral microvascular damage, especially in areas of the brain related to memory formation and recall [11,12]. Most studies examining the influence of arterial stiffness on cognitive aging have focused on advanced clinical and pathological markers of dementia such as brain tissue atrophy, cerebral microbleeds, and amyloid-beta (Aβ) accumulation [11,13–20]. These end-stage effects can take decades to develop leaving a gap in understanding of the earliest mechanisms linking arterial stiffness to late-life cognitive impairment across the adult lifespan.

Non-invasive neuroimaging techniques can provide insight into the mechanisms of brain aging prior to the emergence of clinically relevant manifestations of cognitive impairment. One such technique is magnetic resonance elastography (MRE), which is used to examine the viscoelastic mechanical properties of specific brain regions [21,22] including the hippocampus (HC), a brain structure essential for memory encoding and recall [23]. Brain tissue viscoelastic properties, assessed with MRE, reflect the overall organization of neurons, axons and glial cells [24–26], thereby, providing an insight into brain tissue integrity. Moreover, acute changes to brain tissue viscoelastic properties via cerebrovascular hemodynamics may reflect changes in the brain tissue microvascular bed [27–30]. MRE measures of brain tissue viscoelastic properties are

strongly associated with normal aging [31–35] along with age-related neurodegenerative diseases such as AD [36–38] and Parkinson's disease [39,40]. Importantly, viscoelastic properties of the HC exhibit strong relationships with memory performance [41,42] and cardiometabolic risk factors [43] among healthy adults. As such, HC tissue viscoelastic properties may be better predictors of early declines in episodic memory than HC volume because an observation made by a large meta-analysis showed the presence of weak associations between memory and size of the HC in healthy aging [44]. Therefore, HC viscoelasticity may be sensitive to subtle changes caused by arterial stiffness and cerebrovascular pulsatility leading to a greater understanding of the earliest mechanisms of brain aging. In this study, we sought to determine the vascular determinants of HC tissue viscoelastic properties in healthy adults across the lifespan and whether these properties provide further information than the associations previously seen between vascular measures and traditional volumetric measures of HC tissue trophy [11,18,45]. We hypothesized that after correcting for age, elevated arterial stiffness and greater cerebrovascular pulsatility would be associated with lower HC tissue viscoelastic properties.

METHODS

Participant characteristics

Twenty-five adult men and women (16M/9F) between the ages of 22-69 years, without a history of chronic clinical disease (cardiovascular or cerebrovascular disease, chronic kidney disease, diabetes) including any major psychological (schizophrenia, bipolar disorder, major depressive disorder) or neurological diseases (Alzheimer's disease or other form of dementia, Parkinson's disease, epilepsy, multiple sclerosis, head trauma with loss of consciousness for over 30 minutes) were included in this study. The presence of these exclusionary diseases was assessed with a medical history questionnaire, blood sampling, and participant self-report. The study protocol was approved by the University of Delaware's Institutional Review Board and conformed to the standards outlined in the Declaration of Helsinki, and all participants provided

written informed consent prior to participation. Participants were excluded if they were smokers or if they had any MRI contraindications (e.g., metal implants). Female participants of child-bearing age were confirmed to be non-pregnant and their vascular measures were obtained during the early follicular phase of their menstrual cycle (days 1-4) or during the placebo period if they were on oral contraceptives, to control for the effects of estrogen on vascular function [46]. Participants refrained from food, caffeine, and beverages other than water for 12 h prior to vascular measures, and avoided non-prescription medications for 48 h and aerobic exercise and alcohol for 24 h prior to all measures.

Participant height and body mass were measured using a stadiometer and electronic scale from which body mass index (BMI) was calculated in kilograms per meters squared. Physical activity was acquired using the Modifiable Activity Questionnaire and calculated as metabolic equivalents (METs) in hours per week of leisure activity [47]. Serum lipids (triglycerides, total cholesterol, high- and low- density lipoproteins) and serum glucose was measured using standard lipid and metabolic panels (Quest Diagnostics, USA), respectively during the time of initial screening following a 12 h overnight fast.

Blood pressure assessment

Resting blood pressure of the brachial artery was measured in a seated position after 10 minutes of quiet rest using a semi-automated blood pressure device (ADView 2®, American Diagnostic Corporation, Hauppauge, NY). Repeat measurements were made from the non-dominant arm with 2 min of quiet rest between each measurement until three blood pressure values were within 5 mmHg of each other. These three values were averaged to determine resting systolic and diastolic blood pressure (SBP, DBP).

Arterial stiffness

Aortic stiffness was assessed non-invasively using carotid-to-femoral pulse wave velocity (PWV), which is the gold standard measure of large elastic artery stiffness in humans [48]. Carotid and femoral pulse pressure waveforms were captured simultaneously by applanation tonometry (SphygmoCor XCEL, AtCor Medical, Sydney, Australia) of the right common carotid artery and volumetric displacement of a cuff placed on the participants' upper thigh. A measuring tape was used to measure distances from 1) the suprasternal notch to the carotid tonometry site, 2) the femoral artery in the inguinal ligament region to the proximal end of the thigh cuff and 3) the suprasternal notch to the proximal end of the thigh cuff. The first two distances were subtracted from the third distance to obtain the corrected transit distance as described by Hwang et al. (2014) [49]. The aortic PWV was then determined by dividing the corrected distance over the time delay between the carotid and femoral pulse waves, automatically calculated by the tonometry device.

Common carotid artery (CCA) stiffness and compliance were acquired using duplex ultrasound (Logiq e, GE Healthcare, Chicago, IL) and applanation tonometry of the carotid artery. A 1-min video of the right common carotid artery, imaged 1 cm proximal to the carotid bulb using a 4.2- to 13.0-MHz linear-array transducer in B-mode, was recorded. The carotid artery video image was analyzed with an offline wall-tracking software (Cardiovascular Suite, Quipu srl, Pisa, Italy) that is well validated based on a contour tracking algorithm [50]. Carotid systolic and diastolic blood pressures were obtained from carotid pressure waveforms using applanation tonometry (Sphygmocor CvMS, AtCor Medical, Sydney, Australia). Carotid waveforms were calibrated to the estimated carotid SBP and DBP produced by using the generalized transfer function of the Sphygmocor CvMS device. It is important to note that blood pressures obtained by this method of carotid waveform calibration are prone to calibration errors [51,52]. Carotid pulse pressure (PP) was calculated as the difference between the carotid SBP and DBP. Carotid stiffness was computed by the software from the Bramwell-Hill equation:

$$Stiffness = \sqrt{\frac{D_d^2.(P_s - P_d)}{\rho.(D_s^2 - D_d^2)}}$$

Carotid compliance was computed using the following equation:

$$Compliance = \frac{\pi}{4}\left(\frac{D_s^2 - D_d^2}{P_s - P_d}\right)$$

where ρ = blood density assumed to equal 1.06 g/cm^3; P_s= carotid systolic pressure; P_d= carotid diastolic pressure; D_s= external systolic diameter; D_d= external diastolic diameter between the media-adventitia interfaces.

Cerebrovascular pulsatility

Cerebral blood velocity pulsatility was measured at the MCA using Transcranial Doppler (TCD; Spencer Technologies, Redmond, WA). A 2-MHz TCD probe was insonated over the right temporal window to assess MCA velocity (MCAv) [53,54]. The probe was secured with an adjustable headpiece for optimal M-mode imaging (M600 bilateral headframe, Spencer Technologies). MCAv for pulsatility calculation was collected from participants after at least 10 min of quiet rest in a semi-recumbent position for 2 min. Raw measures of MCAv were sampled at 1,000 Hz using an analog-to-digital converter (Powerlab) and exported to LabChart data acquisition software (ADInstruments, Colorado, CO) for offline analysis. MCAv pulsatility was calculated using Gosling's pulsatility index (PI) equation:

$$PI = \frac{Pulse\ Amplitude\ (Peak\ systolic\ velocity - End\ diastolic\ velocity)}{Mean\ velocity}$$

Magnetic resonance elastography (MRE)

A 3T Siemens Prisma MRI scanner with a 64-channel head coil (Siemens, Erlangen, Germany) was used for all scans. As the participants lay in the scanner, a pneumatic actuator and soft pillow driver (Resoundant Inc, Rochester, MN) were used to generate vibrations of 50

Hz to the participants' heads. MRE images, synchronized with the vibrations, were acquired using a 3D multiband, multishot spiral sequence at 1.25 mm^3 isotropic resolution (240 x 240 mm^2 field of view (FOV), 96 slices, repetition time (TR)/echo time (TE) = 3360/70 ms) with an acquisition time of 10 minutes and 45 seconds [55]. An external vibration of 50 Hz was applied to participants' heads using a pneumatic actuator and soft pillow driver (Resoundant Inc, Rochester, MN), and flow-compensated motion encoding gradients were used to capture resulting brain deformations, as previously described by our collaborators [56].

T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) scans were acquired for each subject with the following parameters: TR/TE=2300/2.32ms, 192 slices, 240 x 240 FOV (0.9mm^3 isotropic voxel size). FreeSurfer 6.0 was used to acquire bilateral HC mask segmentations, and were registered to MRE space using FLIRT tool in FSL (FMBIRB Software Library v.6.0.0). We also obtained bilateral HC volumes from the Freesurfer outputs, which were normalized to total intracranial volume [57].

After data quality assessment using methods previously described [58], a nonlinear inversion algorithm (NLI), combined with soft prior regularization (SPR) was used to estimate the HC viscoelastic complex shear modulus ($G = G' + iG''$)[59,60]. Maps of the complex shear modulus were used to calculate viscoelastic parameters, shear stiffness ($\mu = 2(|G|^2)/(G' + |G|)$) and damping ratio ($\xi = G''/2G'$), which are commonly reported in brain MRE literature [41,42,61–63]. Shear stiffness, μ, is a viscoelastic tissue property that describes resistance to deformation due to applied loading, and is often considered related to tissue composition [24]. Damping ratio, ξ, is a dimensionless quantity that refers to the relative attenuation level in a material; higher ξ indicates that the material exhibits more viscous behavior, while lower ξ indicates a more elastic solid. Higher ξ also suggests a less densely connected solid phase, allowing for more viscous and frictional losses, which is an indicator of poorer tissue integrity [24].

Statistical analyses

Normality of distribution of each variable was assessed using the Shapiro-Wilk test [64] and by examining Q-Q plots. Partial Pearson's correlations were performed to identify whether measures of brachial BP, arterial stiffness, and cerebrovascular pulsatility were associated with measures of HC tissue viscoelastic properties and traditional measures of HC structure such as volume. The Spearman rank correlation test was performed on variables that were not normally distributed (carotid stiffness, carotid compliance, MCAv PI and BMI). Because we recruited participants across the adult lifespan and it is known that advancing age is a strong contributing factor to the decline in both vascular function [11,65–68] and brain viscoelasticity [31–35,61], we adjusted our partial correlation analyses for age and included age as a covariate. We also adjusted correlation analyses for sex and included it as a covariate because of its association with both vascular measures and brain viscoelastic properties [31,35,65,68]. BMI and habitual physical activity were used as independent variables as they are known to be associated with vascular function as well as with brain viscoelastic properties [31,62,65,68–71]. Multiple linear regression was performed to identify the association between HC tissue viscoelastic properties and each significant independent variable identified from the partial correlation analyses (carotid PP and MCAv PI), adjusting for age and sex. Multicollinearity among the independent variables included in the multiple regression model was screened using the variance inflation factor (VIF). A VIF of 2.5 or greater was considered indicative of multicollinearity among the independent variables based on our prior work [72] and previous literature [73]. Normality of residuals of the variables used in the multiple regression model was verified using the Shapiro-Wilk test and with Q-Q plots. Statistical significance for all analyses was determined at $p \leq 0.05$. All analyses were performed with RStudio v.1.2.1355 (RStudio Inc., Boston, MA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA).

RESULTS

Participant characteristics

Twenty-five healthy participants between the ages of 22-69 (mean age: 44 ±16 y) were included in this study. Basic participant characteristics and clinical laboratory values are presented in Table 1. Participants were free of overt cardiovascular diseases or other exclusionary chronic conditions and the group mean laboratory values were within normal clinical ranges.

Partial correlation among brachial blood pressures, arterial stiffness, cerebrovascular pulsatility, and hippocampal tissue viscoelastic properties and volume

Partial correlation coefficients among vascular measures and hippocampal tissue viscoelastic properties and volume, adjusted for age and sex were examined and are presented in Table 2. HC stiffness (μ) was lower in participants with higher carotid PP ($r=-0.57$, $p=0.004$) and MCAv PI ($r_s=-0.52$, $p=0.01$). There were no significant associations between HC μ and other vascular measures. HC damping ratio (ξ) and HC volume were not significantly associated with any vascular measures and other independent variables. Although we observed that carotid PP was significantly correlated to MCAv PI ($r_s=0.61$, $p=0.002$), these variables were assessed for multicollinearity in the subsequent multiple regression model.

Independent associations among, arterial stiffness, cerebrovascular pulsatility, and hippocampal tissue viscoelastic properties

Multiple linear regression was performed next to determine the independent associations among the vascular measures that were significantly correlated with HC tissue viscoelastic properties from Table 2, while correcting for age and sex (Table 3). There were no significant associations between vascular measures and HC ξ and volume in the age- and sex-adjusted

partial correlations; therefore, these HC properties were not included in this model. Vascular predictors included in this model were CCA PP and MCAv PI as they were associated with the dependent variable which was HC μ in the partial correlation analyses. Age and sex were included as covariates in the model. CCA PP was inversely associated with HC μ (β=-0.39; r=-0.41, p=0.05) after correcting for other covariates (Figure 1C). Representative images of a participant with higher CCA PP and lower HC μ (Figure 2A) compared to a participant with lower CCA PP and higher HC μ (Figure 2B) are presented.

There were no significant independent associations between other predictors or covariates and HC μ. Collectively, age, sex, CCA PP and MCAv PI explained 41% of the variance in HC μ (adjusted R^2=0.41, p=0.005) with a major independent contribution from CCA PP. We confirmed that the observed variance in HC μ was not due to partial volume effects by examining associations between original HC volumes (not normalized to total intracranial volume) and HC μ (r=-0.007, p=0.97) and ξ (r=-0.14, p=0.50), which were not statistically significant (Supplementary Figure S1).

All predictors and covariates included in the model had a VIF lower than 2.5 suggesting that there was no multicollinearity among CCA PP, MCAv PI, age and sex. Additionally, age was not significantly associated with HC μ (r=-0.30, p=0.14; Figure 1A) or CCA PP (r=0.09, p=0.65; Figure 1B).

DISCUSSION

This study examined the vascular determinants of hippocampal (HC) tissue viscoelastic properties in healthy humans across the adult lifespan. We observed that a higher carotid pulse pressure (PP) has a significant independent association with lower HC stiffness and collectively, carotid PP and MCA velocity pulsatility index (MCAv PI) explain a considerable portion of the variance in HC stiffness, independent of age and sex. Importantly, we did not observe similar

associations among vascular function and HC volume. These findings suggest that vascular factors may play an early role in brain aging through alterations in HC tissue viscoelastic properties, prior to volume loss. However, we acknowledge that our study is largely observational and we cannot confirm a causal role of vascular determinants on HC tissue viscoelastic properties from our cohort. Further longitudinal studies are needed to prove that altered vascular function is associated with reductions in HC tissue properties and that these reductions precede clinical markers of HC tissue atrophy.

Although the link between arterial stiffness, cerebrovascular pulsatility, and brain tissue structure and function has been investigated in large epidemiological cohorts [11,45,74,75], our study is the first to cross-sectionally relate these measures of vascular function to important markers of HC tissue viscoelastic properties using high-resolution MRE. HC tissue viscoelastic properties assessed by MRE, an advanced brain imaging modality, is indicative of the microstructural integrity of brain tissue and provides two important indices of tissue properties – stiffness and damping ratio [24]. Brain tissue stiffness is believed to reflect neuronal density, myelin content, and neural network strength based on evidence in animal models [24,76,77]. Hence, a higher HC stiffness is suggestive of a greater number of neurons in a stronger neuronal matrix, while a lower HC stiffness represents a softer HC tissue and weakened neuronal matrix [24]. Alternatively, brain tissue damping ratio represents the relative viscous-to-elastic behavior and organization of neuronal tissue [25,26]. As such, a higher HC damping ratio is thought to indicate a more viscous tissue with fewer neuronal connections, while a lower HC damping ratio indicates a more elastic tissue with denser neuronal connections [24]. Collectively, a lower HC stiffness and higher HC damping ratio are suggestive of poorer HC tissue integrity and have been associated with advancing age [33], memory impairment [41,42], and possible transition to AD [78]. Previous studies have observed that acute manipulation of cerebrovascular hemodynamics, induced by increased cerebral blood flow via hypercapnia [28,29] and increased flux rate positively [27] influences MRE measures of brain viscoelastic properties, whereas systolic cerebral arterial

pulsation [30] negatively influences brain viscoelastic properties; however, these findings do not provide insight into the long-term effects of vascular factors on the brain. In contrast, our cross-sectional data may reflect the chronic additive impact of vascular factors on HC tissue properties. If confirmed in longitudinal studies, it may be observed that altered vascular properties induce a more permanent and potentially damaging effect on HC tissue properties.

Despite strong evidence that brain tissue viscoelastic properties change with age and predict cognitive decline, there is limited understanding of the underlying mechanisms driving these changes. Our findings suggest the presence of an important association between vascular factors, specifically carotid PP and HC tissue stiffness. Large elastic arteries such as the carotid arteries, play an important role in damping the large oscillations in blood pressure that occur during ejection of blood from the left ventricle, and thereby protect more sensitive downstream vascular networks from large fluctuations in pressure and flow [79,80]. Age-related stiffening of the elastic arteries results in an increase in central PP that cannot be effectively buffered, thus leading to increased pressure wave transmission into the brain microcirculation [67]. Changes in arterial stiffness and central PP are also believed to underlie increases in cerebrovascular pulsatility [11,81] and carotid PP, specifically, has been associated with greater cerebrovascular pulsatility in older adults [82]. While the exact mechanisms linking increased pulsatility to reduced brain tissue properties are not completely known, our finding is consistent with previous animal literature demonstrating that chronic exposure to higher pulsatile pressure leads to vascular remodeling of cerebral arteries and cortical pial vessels [83]. The intracranial cerebral arteries are structurally fragile, with a thin internal media and lack of an external elastic lamina [84], rendering them susceptible to remodeling or damage from abnormal pressure or blood flow pulsatility [85]. The deep cortical regions of the brain are highly perfused by these penetrating branch vessels and are less able to damp increased pulsatility [10] which makes the neuronal tissue along with the microvasculature of the HC (a deep cortical structure) particularly susceptible to damage from excessive pulsatility. Additionally, direct manipulation of arterial

stiffness and carotid PP in rodents have been shown to be associated with increased endothelial dysfunction and oxidative stress, and reduced perfusion in the cerebral arteries, along with increased neuroinflammation and neurodegeneration [86] - all of which can provide mechanistic explanations of the contribution of vascular hemodynamics on brain tissue viscoelasticity in humans.

It should be noted that we also observed correlations among several vascular factors including brachial BP, arterial stiffness, and MCAv PI in our partial correlation matrix; however, we did not find evidence of multicollinearity among the specific vascular factors included in our multiple regression model. This suggests that each measure of vascular function (carotid PP and MCAv PI), contribute to the overall variance in HC tissue stiffness but collectively play an important role in dictating overall tissue stiffness.

Our study is limited by its cross-sectional design and the inclusion of a fairly small sample of participants from across the adult lifespan. Hence, we are not able to determine any causal links between increased arterial stiffness, cerebrovascular pulsatility, and reduced HC tissue viscoelastic properties. Previous studies have identified mid-life as a critical timeframe during which most changes to central hemodynamic properties begin to occur, including stiffening of the elastic arteries and a widening of the pulse pressure [66,68]. However, because we enrolled a broad age range that included younger adults, it is possible that these age-related changes in brachial BP and arterial stiffness did not yet fully converge to cause increased cerebrovascular pulsatility in a substantial subset of our study cohort, thus leading to an underestimation of the contribution of pulsatility to HC stiffness. In fact, Fico et al. (2022) reported a significant association between aortic stiffness and cerebrovascular pulsatility in older adults that was absent in younger adults, supporting the hypothesis that stiffness-mediated cerebrovascular pulsatility occurs as a function of advancing age [87].

Currently, few longitudinal analyses of arterial stiffness and cerebrovascular pulsatility exist and have either studied older individuals [5,8,11,15,88], where significant loss of brain health

and cognitive function likely obscure sensitive mechanistic relationships, or have assessed changes in arterial stiffness, cerebrovascular pulsatility, and markers of brain health spanning multiple decades [45,65,68,75], where relationships had been confounded by general aging effects. These limitations can be largely overcome in future studies by assessing longitudinal changes in arterial stiffness, cerebrovascular pulsatility, and brain tissue properties within the narrower window of mid-life, when early changes in these properties are most likely to occur.

In addition, we are limited in our small sample size to observe any effect of sex on our cohort's vascular measures and HC tissue properties. Previous studies have reported increased cerebrovascular pulsatility in females [65,68] , which could have important implications for brain tissue properties, and thus neurodegenerative disease risk, among women. However, in previous studies, females were observed to have stiffer occipital and temporal lobes compared with males of the same age [31], suggesting higher tissue integrity of females in these regions. These discrepancies may exist because of the cross-sectional nature of prior studies and differences in age-ranges that were tested [31,65,68]. Thus, there is a need for longitudinal studies in adults with a tight age range so that the effect of sex on arterial stiffness, cerebrovascular pulsatility, and brain tissue properties can be fully elucidated.

In conclusion, our data provide important insight into the vascular determinants of brain tissue viscoelastic properties in healthy adults and indicate that increased carotid PP is independently associated with a reduction in HC stiffness, an important brain region that is affected by cognitive aging and AD. Moreover, our study suggests that carotid PP and MCAv PI collectively explain a significant portion of the total variance in HC stiffness, independent of age and sex and in absence of changes in HC volume. This observation may provide insight into the earliest mechanisms of brain aging in humans and identify large elastic artery stiffness as a therapeutic target for delaying cognitive aging. Future studies with a longitudinal design in mid-life adults are needed to elucidate the earliest changes in vascular function and brain tissue properties.

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DISCLOSURES

We declare no potential conflicts of interest, with respect to the research, authorship and/or publication of this article.

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AUTHOR CONTRIBUTIONS

CRM and CLJ conceived and designed the study; FS, PLD, and TMD performed the study procedures; FS, PLD, and LVH analyzed the data; FS and PLD prepared the figures; FS, RTP, CLJ, and CRM interpreted the results; FS drafted the manuscript; all authors critically revised and approved the final version of the manuscript.

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FIGURES

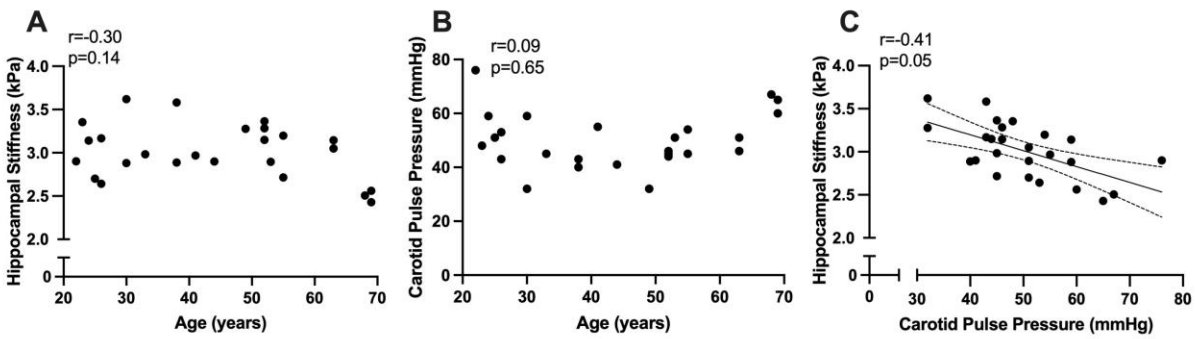


Figure 1. Age, carotid pulse pressure and hippocampal stiffness. Association between age and (**A**) hippocampal stiffness and (**B**) carotid pulse pressure; (**C**) Association between carotid pulse pressure and hippocampal stiffness (controlling for age, sex, and MCA pulsatility index). Dashed lines represent 95% confidence interval; N=25 in all panels; *p ≤ 0.05.

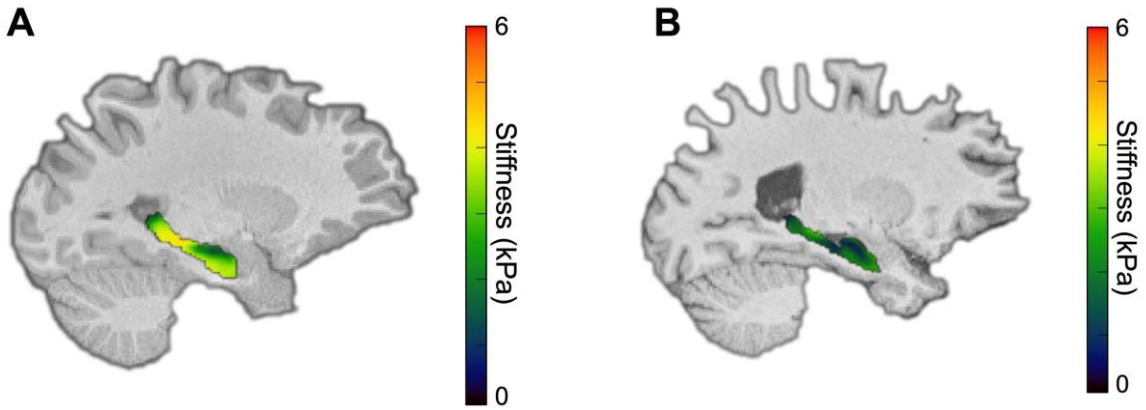


Figure 2. Representative images. (**A**) Representative image of participant with low carotid pulse pressure (32 mmHg), and high hippocampal stiffness (3.6 kPa); (**B**) Representative image of participant with high carotid pulse pressure (65 mmHg) and low hippocampal stiffness (2.4 kPa).

Table 1. Participant characteristics and clinical values

Characteristic	Mean	Range
Sex (M/F)	16/9	N/A
Age (years)	44 ± 16	22-69
Body Mass (kg)	79 ± 17	55-110
BMI (kg/m^2)	27 ± 5	21.2-38.9
SBP (mmHg)	115 ± 11	90-142
DBP (mmHg)	71 ± 10	48-93
Heart Rate (bpm)	62 ± 9	45-78
Total Cholesterol (mg/dl)	186 ± 30	132-256
HDL-C (mg/dl)	62 ± 17	34-105
LDL-C (mg/dl)	104 ± 26	54-175
Triglycerides (mg/dl)	108 ± 53	39-230
Blood Glucose (mg/dl)	91 ± 10	72-112
Statin use, n (%)	3 (12)	N/A
Antihypertensive use, n (%)	5 (20)	N/A

Abbreviations: BMi: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol. Data are mean ± SD

Table 2. Partial correlation matrix

Variable		HC μ	HC ξ	HC Vol	SBP	DBP	Ao PWV	CCA PWV (r_s)	CC (r_s)	CCA PP	MCAv PI (r_s)	BMI (r_s)	MET
HC μ	r	---											
	p	---											
HC ξ	r	-0.35	---										
	p	0.10	---										
HC	r	0.13	0.10	---									
Vol	p	0.56	0.65	---									
SBP	r	-0.08	0.20	0.25	---								
	p	0.73	0.35	0.25	---								
DBP	r	0.37	-0.04	0.16	0.38	---							
	p	0.08	0.85	0.48	0.08	---							
Ao	r	-0.09	0.22	0.18	0.29	**0.43**	---						
PWV	p	0.69	0.31	0.42	0.17	**0.04**	---						
CCA	r	-0.03	0.33	-0.14	-0.006	0.16	0.14	---					
PWV	p	0.90	0.12	0.53	0.98	0.45	0.52	---					
(r_s)													
CC	r	-0.02	-0.24	-0.07	-0.07	-0.04	-0.15	**-0.77**	---				
(r_s)	p	0.92	0.28	0.73	0.76	0.87	0.49	**<0.001**	---				
CCA	r	**-0.57**	0.36	0.14	**0.44**	**-0.56**	-0.13	0.02	-0.12	---			
PP	p	**0.004**	0.09	0.53	**0.03**	**0.006**	0.57	0.92	0.58	---			
MCAv	r	**-0.52**	0.01	-0.04	0.13	**-0.72**	-0.18	-0.07	0.04	**0.61**	---		
PI (r_s)	p	**0.01**	0.95	0.86	0.54	**<0.001**	0.42	0.75	0.87	**0.002**	---		
BMI	r	-0.13	0.18	0.21	0.38	0.28	0.18	0.12	0.08	0.17	-0.18	---	
(r_s)	p	0.56	0.40	0.34	0.07	0.20	0.41	0.58	0.72	0.43	0.40	---	
MET	r	0.18	0.23	-0.07	0.04	0.004	-0.27	0.01	-0.11	0.23	-0.08	-0.02	---
	p	0.42	0.30	0.74	0.85	0.99	0.21	0.96	0.60	0.29	0.72	0.94	---

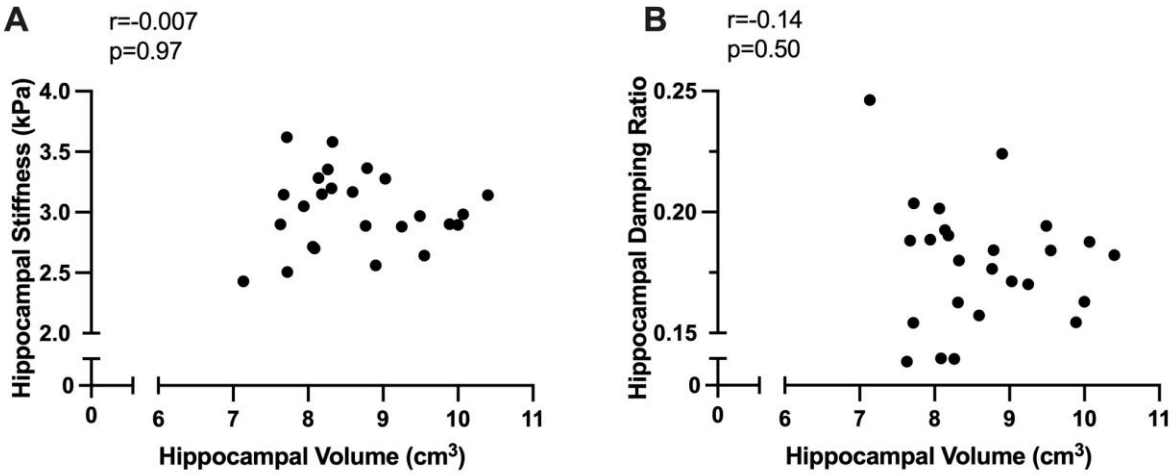
Abbreviations: HC μ: hippocampal stiffness, HC ξ: hippocampal damping ratio, HC Vol: hippocampal volume, SBP: systolic blood pressure, DBP: diastolic blood pressure, Ao PWV: aortic pulse wave velocity/stiffness, CCA PWV: common carotid artery stiffness, CCA PP: common carotid artery pulse pressure, CC: carotid compliance, MCAv PI: middle cerebral artery velocity pulsatility index, BMI: body mass index, MET: leisure physical activity metabolic equivalents hours/week; r_s represents variables for which Spearman rank correlation test was run in which the r values are Spearman correlation coefficients; rest of the r values represent Pearson correlation coefficients; $p \leq 0.05$ (significant associations indicated by boldface); correlations adjusted for age and sex.

Table 3. Multiple regression model

Hippocampal Stiffness			
	β	**p-value**	**VIF**
Age	-0.29	0.09	1.09
Sex	-0.08	0.61	1.16
CCA PP	**-0.39**	**0.05**	1.50
MCAv PI	-0.32	0.09	1.49
Model Variance (adjusted R^2)	***0.41***	***0.005***	

Abbreviations: CCA PP: common carotid artery pulse pressure, MCAv PI: middle cerebral artery pulsatility index; VIF: variance inflation factor; Standardized regression coefficient (β) represent association between each independent variable and the dependent variable while holding the other independent variables constant; $p \leq 0.05$ (significant associations indicated by boldface).

SUPPLEMENTARY MATERIAL



Supplementary Figure S1. Original hippocampal volumes (not normalized to intracranial volume) and hippocampal viscoelastic properties. Association between hippocampal volume and (**A**) hippocampal stiffness; (**B**) hippocampal damping ratio. N=25 in all panels; *p ≤ 0.05.