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Cardiovascular Risk as a Mediator of Associations between Brain-Derived Neurotrophic Factor with Longitudinal Brain and Cognitive Trajectories in Older Adults

Jennifer Shearon
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Department of Psychological and Brain Sciences

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by

Jennifer Shearon

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Table of Contents

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ABSTRACT OF THE THESIS

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Professor Denise Head, Chair

Prior work has shown that higher levels of brain-derived neurotrophic factor (BDNF) are associated with better brain health and cognitive function. There is also evidence that BDNF is present in cardiovascular tissue and that it may be beneficial for cardiovascular function. This is evidenced by higher BDNF being associated with lower incidence of coronary heart disease and mortality, and with lower incidence of cardiovascular risk factors. The goal of the current study was to investigate the relationship between BDNF and cardiovascular function, and to assess whether there is a mediating or moderating role of cardiovascular health in the relationship between BDNF and brain and cognitive outcomes. We examined longitudinal data from 397 older adults enrolled in the Alzheimer's Disease Neuroimaging Initiative with available plasma BDNF along with medical, imaging, and cognitive assessments. We used path analysis and linear regression to estimate the mediating and moderating roles of two measures of cardiovascular health, the Framingham Risk Score (FRS) and pulse pressure, in the relationships between BDNF and longitudinal changes in brain structure and cognitive function. Analyses revealed that there was no significant association of plasma BDNF with FRS or pulse pressure, preventing us

from performing mediation analyses. Additionally, analyses did not show consistent associations between BDNF and longitudinal change in brain structural or cognitive measures. Finally, FRS and pulse pressure were not consistently associated with brain structural or cognitive outcomes. These results suggest that cardiovascular health may not play an important role in BDNF's influence on brain health. Future work is needed to resolve inconsistencies in the literature regarding effects of BDNF on both cardiovascular and brain health in older adults.

Chapter 1: Introduction

As we age, there are numerous factors that contribute to brain and cognitive decline (Gallagher et al., 2019). According to a recent World Health Organization report (2019), the number of individuals living with dementia is projected to increase from 50 million to 82 million by 2030. As the population ages (Hobbs et al., 2001), it is important to understand the myriad factors that impact brain health and, ultimately, cognitive abilities. Developing a better understanding of these relationships will allow for better prevention and treatment of cognitive decline in the coming years. Two potential factors that might combine to contribute to brain and cognitive aging are brain-derived neurotrophic factor (BDNF) and cardiovascular health. The goal of the current study was to investigate the relationship of BDNF and the cardiovascular system as a potential mechanism by which BDNF indirectly impacts brain and cognitive outcomes.

BDNF is a protein found in the brain and has been shown to have roles in neurogenesis, plasticity, and memory formation (Ding et al., 2011; Liu et al., 2018; Miranda et al., 2019). The role of BDNF in the brain has been widely studied in the animal and human literature. With its highest concentration in the hippocampus (Hofer et al., 1990), BDNF is evidenced to be a key player in the processes of learning and memory. Alonso and colleagues (2002) found that infusion of BDNF into the rat hippocampus improved memory retention, and blocking BDNF signaling with an anti-BDNF antibody impaired memory retention. Hippocampal BDNF infusion was also associated with better performance on the Morris water maze, a test of spatial memory, in rats (Cirulli et al., 2004). Additionally, a study performed in rats showed that a transient increase in BDNF was associated with a subsequent increase in hippocampal plasticity (Ding et

al., 2011). Taken together, these studies indicate the role of BDNF in the processes of learning and memory in rodent models.

A review published by Miranda and colleagues (2019) indicated that the association between BDNF and cognition, particularly in terms of causality, remains more unclear in the human literature. Erikson and colleagues (2010) found that increased age was associated with lower levels of serum BDNF and lower hippocampal volumes. In the same cohort, lower BDNF levels were associated with smaller hippocampal volumes and poorer performance on a test of spatial memory. Importantly, BDNF level mediated the associations between age and hippocampal volume, suggesting a direct role of BDNF on hippocampal and memory outcomes in older adults. Another study in older adults found supporting evidence of lower levels of serum BDNF being associated with increased age, as well as with a higher degree of cognitive impairment (Siuda et al., 2017). However, a study published by Kim and colleagues (2015) found that, in two separate cohorts of older adults, there were no cross-sectional associations between plasma BDNF and hippocampal volume or memory performance.

Longitudinal studies investigating these relationships have also yielded mixed findings. Higher serum BDNF level (Laske et al., 2011) and higher BDNF gene expression (Buchman et al., 2016) were associated with slower rates of cognitive decline in individuals with Alzheimer Disease, and in individuals with and without dementia, respectively. Two studies also found associations between BDNF genotype and rates of cognitive decline. Carriers of the Met allele of the BDNF gene, which may be indicative of lower BDNF production (Egan, 2003), were found to have steeper decline in cognitive abilities over a 7-year follow-up (Boots, 2017). Similarly, Lim and colleagues (2013) found that Met carriers had steeper decline in hippocampal volume and cognition, but this association was only present in individuals who were evidenced on a PET

scan to have high levels of amyloid beta, a protein involved in Alzheimer Disease. Conversely, a 10-year longitudinal study found no relationship between baseline serum BDNF level and cognitive trajectories in nondemented older adults (Nettiksimmons et al., 2014). Another study in clinically normal older adults found no association between plasma BDNF level and rates of cognitive decline over a nine-year follow-up (Driscoll et al., 2012). These mixed findings in the human literature could be due to variability in how BDNF was quantified, what cognitive tests were performed, or differences in sample characteristics (i.e., clinically normal vs. individuals with dementia). Nonetheless, these discrepancies suggest the need for further investigation of BDNF's impact on the brain and other candidate systems through which BDNF might influence the brain indirectly.

One potential system which might play a role in BDNF's influence on the brain is the cardiovascular system. There is a well-established relationship between cardiovascular function and brain health and cognition in older adults (Gardener et al., 2015; van der Velpen et al., 2017). Markers of poor cardiovascular health, including hypertension and arterial stiffness, have been associated with poorer cognitive performance both cross-sectionally (Elias et al., 1990; Mitchell et al., 2011) and longitudinally (Swan et al., 1998; Waldstein et al., 2008). These downstream effects of cardiovascular function on cognition have been widely studied, with a clear pathway being established between the two. As blood pressure increases and arteries stiffen, the flow of blood to distal organs becomes more pulsatile (Mitchell et al., 2004). End organs including the brain are not equipped to accommodate highly pulsatile flow and are therefore particularly susceptible to small vessel damage caused by it (Mitchell et al., 2011). Over time, this results in decreased blood supply to the brain, regional atrophy, cerebral small vessel disease, and can ultimately lead to cognitive deficits (Bown et al., 2021; Cooper et al.,

2016). Because the relationships between cardiovascular function and brain and cognitive outcomes are widely known, it is feasible as a potential pathway by which BDNF influences the brain indirectly.

Compared to the effects of BDNF on the brain, its influence on the cardiovascular system have been less explored. While it is known that BDNF is expressed in the cardiovascular tissue (Hofer et al., 1990; Kermani & Hempstead, 2019), researchers have not yet developed a complete understanding of its exact roles in the cardiovascular system. Reviews published by Pius-Sadowska (2017) and Kermani (2019) highlight roles of BDNF in the cardiovascular system, including cardiomyocyte and endothelial cell survival. Research conducted in animal models has demonstrated that increased BDNF can lead to elevated blood pressure (Choe et al., 2015; Erdos et al., 2015; Thorsdottir et al., 2021) and that this elevation is likely due to a shift in hypothalamic activity. However, this association with blood pressure has not been confirmed in humans. Prior work investigating BDNF's relationship with distal cardiovascular outcomes in humans has been limited, but there is some indication of a relationship. Higher serum BDNF level has been associated with lower risk of a future cardiovascular event (Kaess et al., 2015), and individuals with coronary heart disease have been found to have lower serum BDNF than individuals without coronary heart disease (Sustar et al., 2019). Golden and colleagues (2010) found that plasma BDNF level was associated with various cardiovascular risk factors, including diastolic blood pressure and body mass index. Based on these prior studies, it is possible that cardiovascular health plays a mediating role in the influence of BDNF on the brain and cognition. It is also possible that BDNF and cardiovascular health have an interacting relationship, such that having compromised cardiovascular function minimizes the beneficial effects of BDNF on the brain. Further investigation is needed to elucidate the specific

relationships among BDNF and measures of cardiovascular function and health, and to determine whether the cardiovascular system plays a role in the effects of BDNF on the brain.

The goal of the current study was to address these gaps in the literature by investigating the associations of BDNF level and cardiovascular risk with brain and cognitive outcomes using a longitudinal dataset collected in older adults. Mediating and moderating roles of cardiovascular risk were tested for the associations of BDNF with white matter hyperintensity (WMH) volume and executive function, hippocampal volume and episodic memory, and primary visual cortex (V1) volume and language abilities. We hypothesized that cardiovascular risk would mediate the associations between BDNF and WMH volume, executive function, hippocampal volume, and episodic memory. We also hypothesized that these associations would be weaker for V1 volume and language abilities. These hypotheses are based on previous work demonstrating associations of BDNF and cardiovascular risk with WMH volume and executive function (Aljondi et al., 2020; Erikson et al., 2008; Leckie et al., 2014; Taylor et al, 2008), and associations of BDNF with hippocampal volume and memory (Erikson et al., 2010). Finally, we expected that cardiovascular risk would moderate the associations of BDNF level with brain and cognitive outcomes, such that individuals with a compromised cardiovascular system would have impaired circulation and thus, less benefit of circulating BDNF on the brain.

Chapter 2: Method

2.1 Dataset and Participants

The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort was used for this project. ADNI is a multi-site study that was developed to track the progression of Alzheimer Disease with a variety of data types. ADNI data has been collected in four study phases: ADNI 1, ADNI GO, ADNI 2, and ADNI 3. The current study used data collected from participants who were enrolled in ADNI 1, because this was the only study phase which had their plasma BDNF levels tested. For the current study, we used biomarker, clinical, cognitive, and neuroimaging data to investigate the research questions. The dataset was made available through protected access, and data were downloaded from the online repository on March 27, 2023 (https://ida.loni.usc.edu/home/projectPage.jsp?project=ADNI).

The sample consisted of individuals with BDNF measured at their baseline visit and with available medical, imaging, and cognitive data from follow-up assessments. We excluded individuals with a Clinical Dementia Rating® (CDR) (Morris, 1997) score of > 0.5 at time 0, because scores greater than 0.5 represent more than very mild dementia. The final sample consisted of 397 individuals (see Table 1 for sample characteristics).

Table 1. Participant Demographics

	N=397
Baseline Age (mean (SD), range)	74.2 (7.3), 54.4-89.6
Sex (F/M)	164/233
Race	
White	374
Black	15
Asian	7
American Indian/Alaskan	1
CDR Score Screening (0/0.5)	50/347
CDR Score Change (mean (SD), range)	0.22 (0.46), -0.5-2.5
CSF ptau/Aβ42 ratio (mean (SD), range)	0.24 (0.18), 0.01-1.04

2.2 Measures

2.2.1 BDNF Measurement

BDNF data were downloaded directly from the ADNI data repository. Detailed descriptions of the assay methodology can be found online here: https://adni.loni.usc.edu/wp-content/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf. Briefly, fasted blood samples were collected in the morning and centrifuged to obtain plasma. Plasma samples were frozen within 120 minutes of collection and prepared for processing. Plasma BDNF data were obtained using a multiplex immunoassay performed by Rules Based Medicine Laboratory (Austin, TX). BDNF measurement at the baseline study visit was used for analyses.

2.2.2 Framingham Risk Score

Cardiovascular risk was estimated using the Framingham Risk Score (FRS) (D'Agostino et al., 2008). The FRS is a validated tool designed to estimate the 10-year risk for an individual to experience some cardiac event, including development of cardiovascular or coronary heart disease, cerebrovascular event, or heart failure. A higher score represents a greater risk of experiencing a cardiac event. The score is determined based on age, sex, body mass index (calculated from height in inches and weight in pounds), systolic blood pressure, current use of hypertension medication, diabetes status, and smoking status. The FRS for each participant was calculated using data obtained at the six-month follow-up visit.

Age, systolic blood pressure, hypertension medication use, and weight were collected at the six-month ADNI visit and used in the FRS calculation. Arterial blood pressure was measured using a mercury sphygmomanometer on the participant's dominant arm. Self-reported sex, height, diabetes status, and lifetime smoking history (i.e. "Do you have a clinically significant

history of smoking?") were only collected at the ADNI screening visit. Because these values were expected to be stable over a six-month time frame, they were deemed sufficient for use in FRS calculation for the six-month timepoint.

2.2.3 Pulse Pressure

Pulse pressure ($systolic\ blood\ pressure - \ diastolic\ blood\ pressure$) (Homan et al., 2018) was calculated using blood pressure measurements from the six-month visit. Pulse pressure was used in exploratory analyses as the mediator and moderator variable.

2.2.4 MRI Scan Acquisition and Analysis

All imaging data were downloaded from the ADNI data repository. Detailed descriptions of the ADNI 1 MRI acquisition and preprocessing procedures can be found online here: https://adni.loni.usc.edu/methods/mri-tool/mri-analysis. Images were acquired on 1.5T scanners, and two T1-weighted sagittal 3D MP-RAGE scans were acquired for each participant, followed by a T2-weighted dual echo scan. Outcomes of interest for the current study were WMH volume, hippocampal volume, and primary visual cortex (V1) volume. The process used to quantify WMH volumes has been described in detail previously (Schwarz et al., 2009). In short, a Bayesian Markov-Random Field approach was applied to T1 and T2 scans and used to perform binary classification of each voxel as having the presence of WMH or not. All voxels were then summed to estimate the total WMH volume. Hippocampal and V1 volumes were obtained using Freesurfer image analysis software version 4.3 (Fischl, 2012). Briefly, following motion correction and removal of nonbrain tissue, Freesurfer uses probabilistic information estimated from a manually labeled training set to assign each voxel a neuroanatomical label. More details regarding the segmentation process used by Freesurfer have been previously described (Fischl,

2012). Hippocampal and V1 volumes were summed across hemispheres and corrected for intracranial volume using the analysis of covariance approach (Buckner et al., 2004).

WMH, hippocampal, and V1 volumes were obtained for each study visit that each participant completed. Linear mixed effects models were used to calculate the linear change per year in WMH, hippocampal, and V1 volumes for each participant. The slopes estimated by these models were used as the outcome variables in the mediation and moderation analyses.

2.2.5 Cognitive Performance

Cognitive composite scores were created for executive function, episodic memory, and language. The executive function composite included the Digit Span backward test (Wechsler, 1987), Trail Making Test B (Spreen & Strauss, 1998), Digit Symbol Substitution test (Wechsler, 1981), and the Number Cancellation subtest from the Alzheimer's Disease Assessment Scale (Mohs, 1994). The episodic memory composite included immediate and delayed recall from the Logical Memory test (Wechsler, 1987), and learning, delayed recall, and delayed recognition from the Rey Auditory Verbal Learning Test (Rey, 1964). The language composite included the Boston Naming Test (Kaplan et al., 1983) and the Naming subtest from the Alzheimer's Disease Assessment Scale (Mohs, 1994).

For each participant, data from these tasks were downloaded for as many study visits as were completed. The raw score from each task was converted to a percent of the maximum score possible. Next, the average percent per participant at a single study visit was calculated. This average percent score across tasks represented the composite score for each cognitive domain at a single timepoint, using all available test scores from that timepoint. Finally, linear mixed effects models were used to calculate the linear change per year for each cognitive

domain, represented by unique slopes for each participant. The slopes estimated by the linear mixed effects models for each participant were used as the outcome variables in the mediation and moderation analyses.

2.3 Analytic Approach

2.3.1 Mediation Analysis

Mediation analyses were in conducted in R version 4.1.1 using the lavaan package (Rosseel, 2012) with a path analysis approach to estimate the indirect effects in the models. Two mediation models were assessed. The outcomes in the first model were longitudinal change in WMH volume, hippocampal volume, and V1 volume. The outcomes in the second model were longitudinal change in executive function, episodic memory, and language. In both models, baseline BDNF was the predictor variable, and FRS at the six-month follow-up was the mediator variable. For each model, the following steps were performed. First, we verified that there were significant relationships of the mediator variable with both the predictor variable and the outcome variable. Direct effects were considered significant if the p-value was less than .05. Current recommendations do not require a significant relationship between the predictor and the outcome variable (Hayes, 2009). Next, the mediation analysis was conducted. Indirect effects were examined using 10,000 bootstrap samples and 95% percentile bootstrap confidence intervals. Full information maximum likelihood estimation was used to handle missing data.

2.3.2 Moderation Analysis

Regression analysis was conducted in R version 4.1.1 and was used to test whether cardiovascular risk moderated the association of BDNF with the outcomes. Separate models

were examined for each outcome variable (i.e., WMH volume, hippocampal volume, V1 volume, executive function, episodic memory, and language). The models included the main effects of BDNF and FRS, and the BDNF × FRS interaction.

2.3.3 Outliers

Outliers were defined as data points greater than 2.5 standard deviations from the group mean and with a Cook's D value of greater than 4/n (Cook, 1977). All results reported are with outliers removed.

2.3.4 Covariates

Covariates were age at baseline, sex and years of education collected at screening, CDR score at screening, change in CDR score from screening to each participant's last study visit, and CSF ptau/ab42 ratio at baseline. CSF ptau/ab42 ratio was included as a covariate because it is a biomarker representing the risk for Alzheimer Disease (Harari et al., 2014). Initial models were run without any covariates. Next, all covariates were added. If the observed effects differed when all covariates were added, each covariate was added to the model separately to determine which was driving the difference in effects. Results are reported from models with no covariates unless results differed between the two models.

2.3.5 Planned Exploratory Analyses

Exploratory analyses were conducted with pulse pressure as the mediator and moderator variable instead of FRS. The purpose of these exploratory analyses was to investigate the associations between a more proximal measure of current cardiovascular function, BDNF, and the brain and cognitive outcomes. Mediation and moderation models were conducted as described above, with pulse pressure in place of FRS.

Chapter 3: Results

3.1 Mediating Effects of Cardiovascular Risk

In the model assessing mediating effects of FRS on longitudinal change in brain outcomes, there was a significant effect of FRS on WMH change (β=.11, SE=.03, p=.001). This reflected a greater increase in WMH volume over time in individuals with a higher FRS. FRS was not significantly associated with changes in hippocampal volume (β=-.01, SE=.06, p=.90) or V1 volume (β=-.001, SE=.04, p=.98). Plasma BDNF was not associated with FRS (β=-.03, SE=.05, p=.65). There were no significant associations between plasma BDNF and changes in WMH volume (β=-.02, SE=.02, p=.39), hippocampal volume (β=-.03, SE=.05, p=.52), or V1 volume (β=-.03, SE=.04, p=.48). Due to the lack of significant associations between the BDNF and FRS and between FRS and outcome variables, indirect effects were not examined. See Figure 1 for full model with path coefficients.

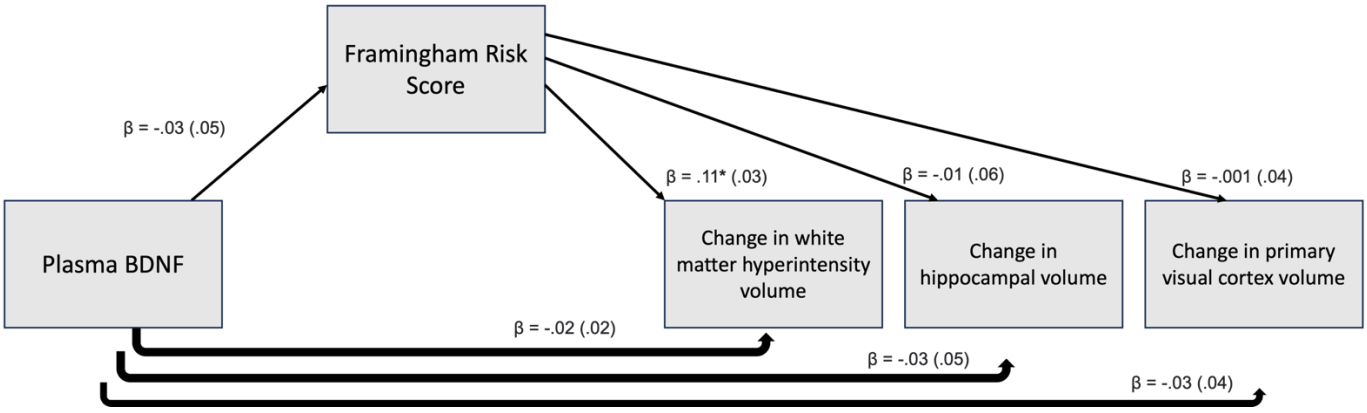

Figure 1. Path model of relationships among BDNF, FRS, and brain outcomes. Higher FRS indicates greater cardiovascular risk. Outcomes represent the slopes of longitudinal change in volume, with negative slopes indicating decrease in volume over time.

The next model assessed mediating effects of FRS on longitudinal change in cognitive outcomes. Plasma BDNF was not associated with FRS (β=-.02, SE=.05, p=.69). There were no significant associations between FRS and longitudinal change in executive function (β=.03, SE=.04, p=.42), episodic memory (β=-.03, SE=.05, p=.53), or language ability (β=-.04, SE=.04, p=.29). BDNF was not significantly associated with changes in executive function (β=.06, SE=.04, p =.19) or episodic memory (β=-.03, SE=.05, p =.57). There was a significant association of BDNF on longitudinal change in language ability (β=.08, SE=.04, p =.04), such that higher plasma BDNF was associated with less yearly decline in language ability. This association remained significant when all covariates were added to the model (β=.09, SE=.04, p =.02). Given the lack of significant associations between BDNF and FRS and between FRS and all cognitive outcomes, indirect effects of FRS were not examined. See Figure 2 for full model with path coefficients.

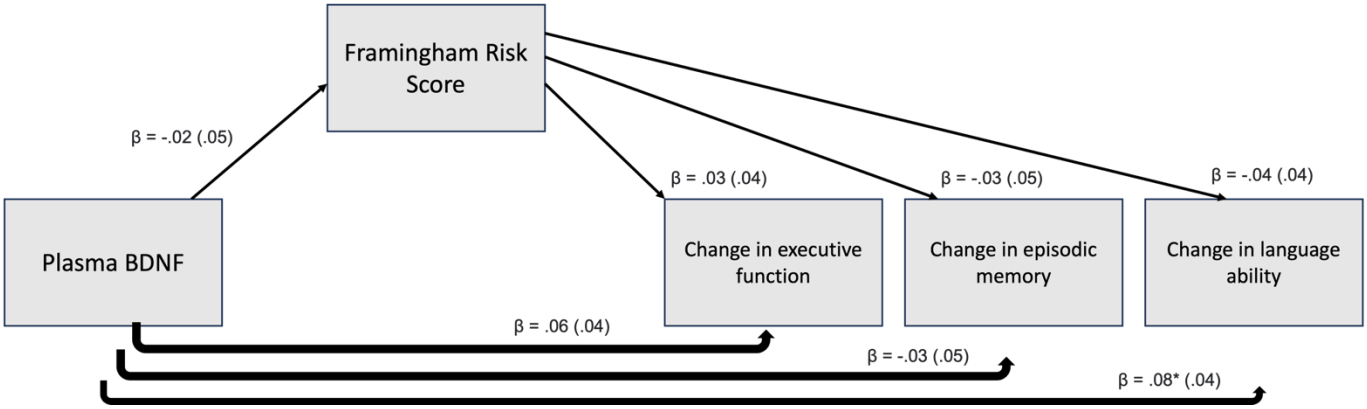

Figure 2. Path model of relationships among BDNF, FRS, and cognitive outcomes. Higher FRS indicates greater cardiovascular risk. Outcomes represent the slopes of longitudinal change in cognitive scores, with negative slopes indicating decrease in performance over time.

3.2 Moderating Effects of Cardiovascular Risk

Moderating effects of FRS on the relationships between BDNF and all six outcomes were examined with separate models. There were no significant main effects of BDNF on any of the brain or cognitive outcomes (all ps>.05). See Figure 3 and Table 2 for standardized coefficients from all regression models. The lack of a main effect of BDNF on change in language abilities was contrary to the effect observed in the mediation analyses. As such, we conducted a simple linear regression model to probe the effect of BDNF on language in the absence of other variables. This model revealed no significant effect of BDNF on language (β=.08, SE=.05, p=.10), indicating that the association observed in mediation models was likely not robust.

Moderation analysis revealed a significant association of FRS with longitudinal change in WMH volume (β=.14, SE=.05, p= .002), but this effect disappeared when covariates were added (β=.07, SE=.07, p=.30). Examining models with each covariate separately revealed that this difference in effects was driven by baseline age and ptau/ab42 ratio at baseline. We also conducted simple linear regression to further investigate the association between FRS and WHM volume, due to the discrepancy between this association in mediation and moderation analyses. This revealed a significant effect of FRS on change in WMH volume in the model with no covariates (β=.13, SE=.05, p= .01), but this effect was diminished when covariates were added to the model (β=.07, SE=.07, p= .30). In the moderation analysis, there were no BDNF × FRS interactions on any of the outcome variables (all ps>.05).

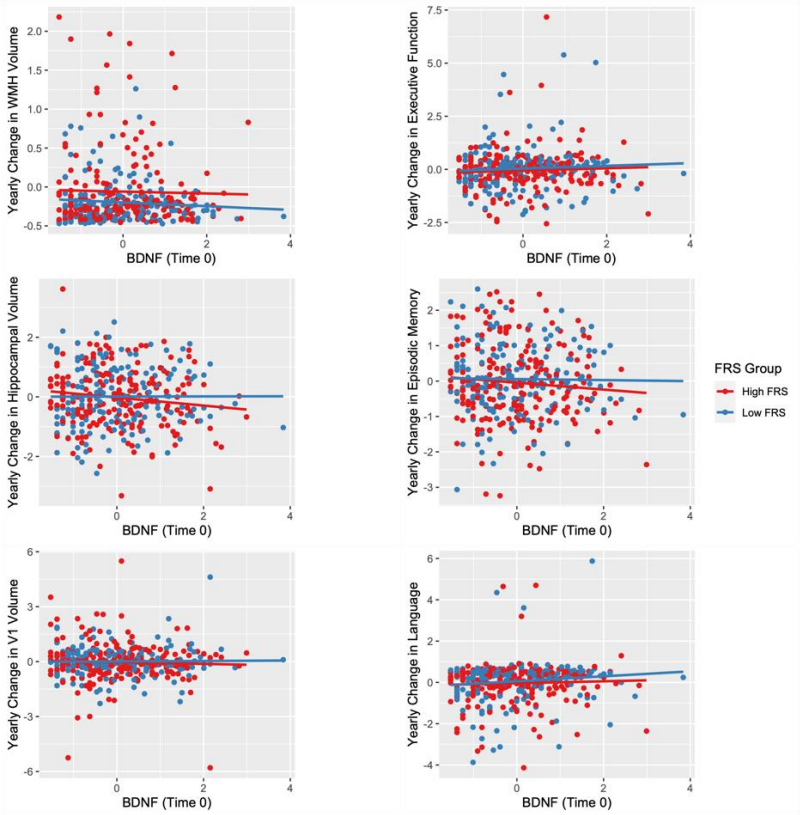

Figure 3. FRS as a moderator of BDNF on outcomes. Standardized variables plotted. Higher FRS indicates greater cardiovascular risk. Higher yearly change indicates less or no decline in brain volumes and cognitive performance over time, while lower yearly change indicates decline in brain volumes and cognitive performance over time.

Table 2. Moderating effects of Framingham Risk Score

	BDNF main effect		**FRS main effect**		**BDNF × FRS interaction**	
	No covariates	All covariates	No covariates	All covariates	No covariates	All covariates
WMH volume	.02, .05 (.70)	.001, .06 (.99)	**.14, .05 (.002)**	.07, .07 (.30)	.07, .05 (.13)	.02, .07 (.79)
Hippocampal volume	-.07, .05 (.16)	-.03, .06 (.64)	-.04, .05 (.44)	.04, .06 (.47)	-.03, .05 (.53)	-.05, .06 (.40)
V1 volume	.01, .05 (.81)	-.05, .07 (.48)	.03, .05 (.50)	-.09, .07 (.20)	.003, .05 (.95)	-.02, .07 (.80)
Executive function	.07, .05 (.20)	.03, .07 (.65)	.02, .05 (.73)	.01, .07 (.94)	.01, .05 (.82)	-.03, .07 (.70)
Episodic memory	-.02, .05 (.67)	-.08, .06 (.17)	-.05, .05 (.38)	-.04, .06 (.50)	.04, .05 (.45)	-.09, .06 (.15)
Language	.07, .05 (.17)	.12, .06 (.07)	-.08, .05 (.14)	-.12, .06 (.06)	-.03, .05 (.57)	-.03, .07 (.71)

Standardized regression coefficients, standard error (p-value). Values with p<.05 are bolded

3.3 Mediating Effects of Pulse Pressure

Exploratory mediation models assessed the mediating effects of pulse pressure on brain and cognitive outcomes. In the models assessing effects on brain outcomes, the association between plasma BDNF and pulse pressure was not significant (β=-.05, SE=.05, p=.32). There was a significant association between pulse pressure and longitudinal change in WMH volume (β=.07, SE=.03, p=.02), indicating a greater increase in WMH volume over time in individuals with higher pulse pressure. However, this effect did not remain significant when covariates were added (β=.05, SE=.03, p=.10). Adding each covariate to the model separately revealed that the difference in effects was driven by baseline age. Pulse pressure was not significantly associated with changes in hippocampal volume (β=-.07, SE=.05, p=.13) or V1 volume (β=-.03, SE=.04, p=.44). There were no significant effects of BDNF on changes in WMH volume (β=-0.02, SE=.02, p=.41), hippocampal volume (β=-.04, SE=.05, p=.48), or V1 volume (β=-.03, SE=.04, p=.47). Given the lack of associations between BDNF and pulse pressure and between pulse pressure and brain outcomes, indirect effects were not examined. See Figure 4 for full model with path coefficients.

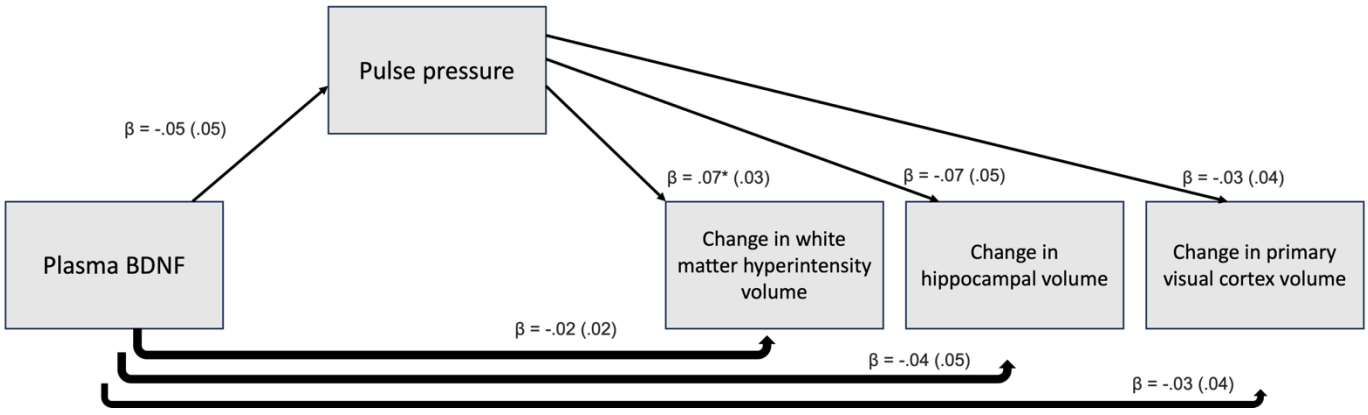

Figure 4. Path model of relationships among BDNF, pulse pressure, and brain outcomes. Outcomes represent the slopes of longitudinal change in volume, with negative slopes indicating decrease in volume over time.

In the model assessing mediating effects of pulse pressure on cognitive outcomes, there was no significant association between BDNF and pulse pressure (β=-.05, SE=.05, p=.32). There were also no significant effects of pulse pressure on changes in executive function (β=-.004, SE=.04, p=.91), episodic memory (β=-.09, SE=.05, p=.07), or language (β=-.01, SE=.04, p=.85). Plasma BDNF was not associated with change in executive function (β=.05, SE=.04, p=.14) or change in episodic memory (β=-.03, SE=.05, p=.52). There was a significant effect of BDNF on longitudinal change in language ability (β=.08, SE=.04, p=.04). This indicates that higher BDNF was associated with less yearly decline in language ability. However, due to the lack of associations between BDNF and pulse pressure and between pulse pressure and cognitive outcomes, indirect effects were not examined. See Figure 5 for full model with path coefficients.

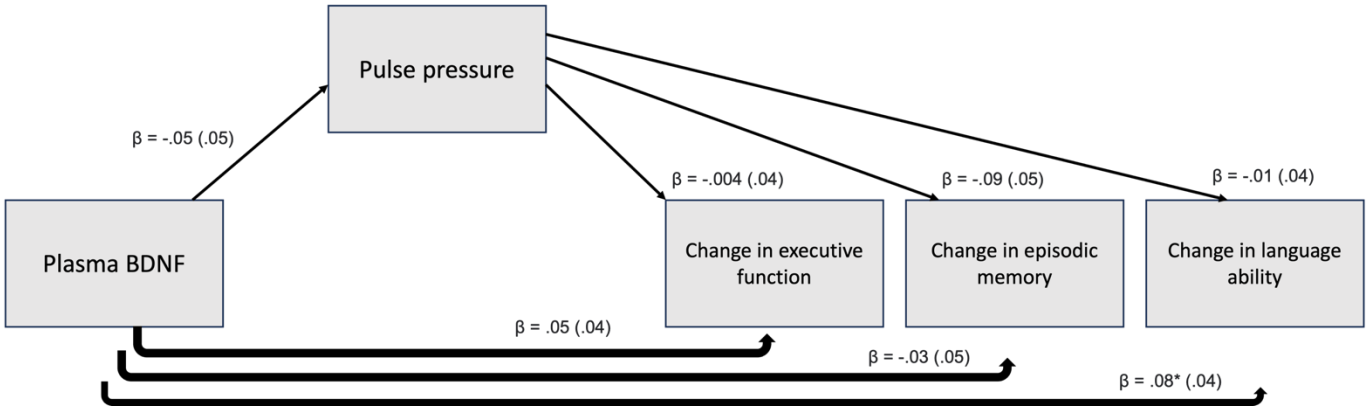

Figure 5. Path model of relationships among BDNF, pulse pressure, and cognitive outcomes. Outcomes represent the slopes of longitudinal change in cognitive scores, with negative slopes indicating decrease in performance over time.

3.4 Moderating Effects of Pulse Pressure

Moderating effects of pulse pressure on the relationships between BDNF and all six outcomes were examined with separate models. There were no significant main effects of BDNF on any of the brain or cognitive outcomes (all ps>.05; see Figure 6 and Table 3 for standardized coefficients from all models). There were also no significant main effects of pulse pressure on any outcome variable (all ps>.05). There was a significant BDNF × pulse pressure interaction on change in language abilities (β=-.11, SE=.05, p=.04), such that there was a greater positive association between BDNF and language in those with lower pulse pressure. However, this interaction did not remain significant when covariates were added (β=-.09, SE=.06, p=.14).

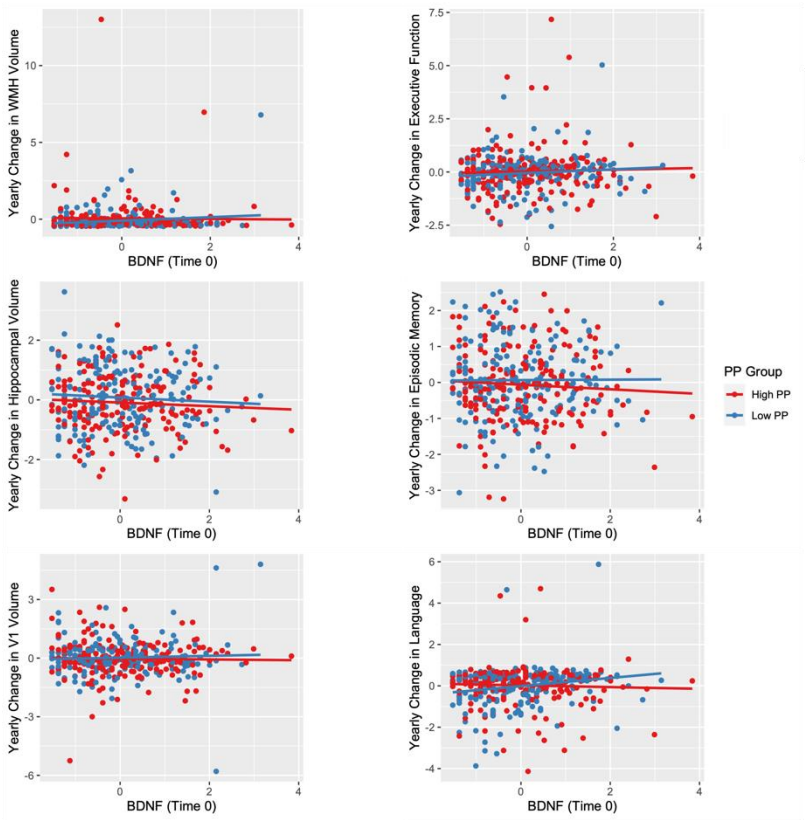

Figure 6. PP as a moderator of BDNF on outcomes. Standardized variables plotted. Higher PP indicates greater arterial stiffness. Higher yearly change indicates less or no decline in brain volumes and cognitive performance over time, while lower yearly change indicates decline in brain volumes and cognitive performance over time.

Table 3. Moderating effects of pulse pressure

	BDNF main effect		PP main effect		BDNF × PP interaction	
	No covariates	All covariates	No covariates	All covariates	No covariates	All covariates
WMH volume	.03, .05 (.55)	.0001, .07 (1.0)	.07, .05 (.11)	.07, .07 (.34)	-.003, .05 (.95)	-.04, .07 (.53)
Hippocampal volume	-.07, .05 (.19)	-.03, .05 (.53)	-.06, .05 (.29)	-.01, .05 (.79)	.01, .05 (.83)	.02, .06 (.78)
V1 volume	.01, .05 (.82)	-.05, .07 (.47)	-.03, .05 (.59)	-.07, .07 (.29)	.02, .05 (.77)	.01, .07 (.84)
Executive function	.07, .05 (.17)	.02, .07 (.71)	.04, .05 (.37)	.02, .07 (.72)	.01, .05 (.80)	-.03, .07 (.70)
Episodic memory	-.03, .05 (.50)	-.07, .06 (.22)	-.09, .05 (.09)	-.08, .06 (.16)	-.05, .05 (.39)	-.08, .06 (.17)
Language	.06, .05 (.19)	.11, .06 (.08)	-.05, .05 (.35)	-.06, .06 (.33)	**-.11, .05 (.04)**	-.09, .06 (.14)

Standardized regression coefficients, standard error (p-value). Values with p<.05 are bolded

3.5 Post-hoc Analyses

3.5.1 Association of BDNF with Cardiovascular Measures

We used simple linear regression to test the associations of BDNF on FRS and pulse pulse pressure. There was no significant association between BDNF and FRS (β=-.02, SE=.05, p=.73) or between BDNF and pulse pressure (β=-.03, SE=.05, p=.54). These relationships are plotted in figure 7.

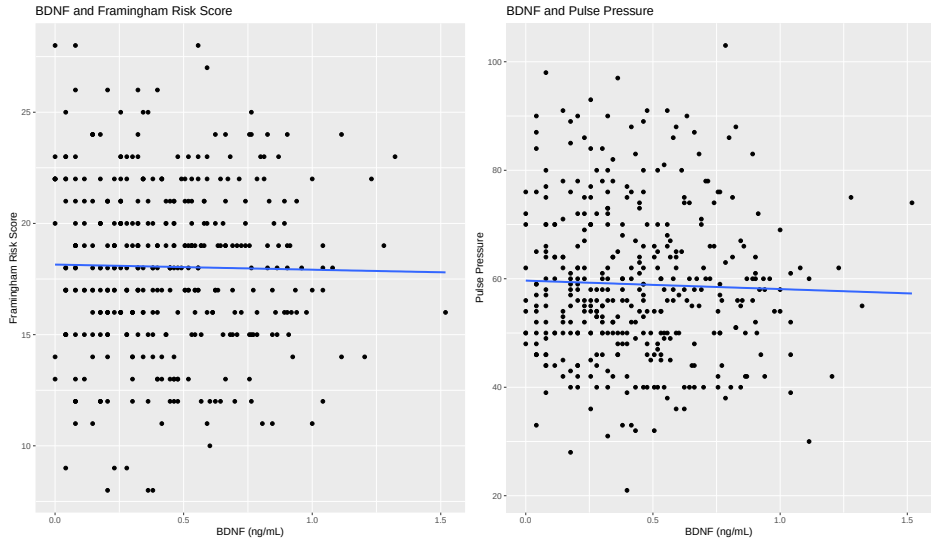

Figure 7. BDNF and cardiovascular measures**.** Unstandardized variables plotted. Higher FRS indicates greater cardiovascular risk. Higher pulse pressure indicates greater arterial stiffness.

3.5.2 Associations with Quadratic Change

Because expected associations were not found with brain and cognitive outcome variables derived from the linear mixed effects models, we conducted post-hoc analyses to investigate whether change over time in outcome variables would be better modeled by non-linear change. Quadratic terms were added to the mixed effects models to account for non-linear change over time in brain volumes and cognitive performance, and a likelihood ratio rest was performed to compare the model fits. Based on chi-square test statistics and corresponding p-values, model comparisons indicated that the mixed effect models which included the quadratic term captured the data better than the linear model for all six outcome variables (all ps < 0.001). Thus, we next examined whether FRS or BDNF were associated with quadratic change in the outcomes.

To test whether baseline BDNF was associated quadratic change, we added time × BDNF interactions to the separate mixed effects models of with quadratic terms for each of the six outcomes. Variables for these analyses were not standardized in the effort to maintain interpretability; unstandardized regression coefficients are reported. We observed a significant interaction between time and BDNF for WMH volume (B=1.02, SE=.48, p=.03), such that increases in WMH volume over time were greater in those with higher baseline BDNF compared to those with lower baseline BDNF. This association was opposite from what we expected, based on prior literature. There were no other significant time × BDNF interactions for the other brain and cognitive outcomes (all ps>.05; see table 4 for regression coefficients for each model).

We conducted the same analyses with FRS to assess for associations between cardiovascular risk and quadratic change. There was a significant time x FRS interaction effect on V1 volume (B=.95, SE=.46, p=.04), such that the longitudinal change in V1 volume over time

was more positive in individuals with higher FRS compared to those with lower FRS. There were no other significant time × BDNF interactions on the other brain and cognitive outcomes (all ps>.05; see table 4 for regression coefficients for each model).

Table 4. Moderating effects of BDNF and FRS on quadratic change in outcomes

	Time × BDNF interaction	Time × FRS interaction
WMH volume	**1.02, .48 (.03)**	.01, .04 (.81)
Hippocampal volume	9.01, 5.01 (.07)	.51, .34 (.14)
V1 volume	-2.44, 6.69 (.71)	**.95, .46 (.04)**
Executive function	.76, 1.00 (.45)	.03, .07 (.71)
Episodic memory	.56, .52 (.28)	-.02, .04 (.53)
Language	-.73, .75 (.33)	.01, .05 (.86)

Unstandardized regression coefficients, standard error (p-value). Values with p<.05 are bolded

3.5.3 Sex Differences

Next, we examined possible sex differences in longitudinal change in outcomes and their relation to BDNF and FRS. These analyses were performed based on prior work indicating that changes in brain structure and cognition may differ between sexes (McCarey et al., 2016) and these differences are moderated by cardiovascular risk factors (Armstrong et al., 2019). Additionally, there may be sex differences in BDNF level (Shimada et al., 2014), associations between BDNF and episodic memory (Komulainen et al., 2008), and cardiovascular aging (Appelman et al., 2015; Merz & Cheng, 2016). We first performed comparisons between growth models accounting only for linear change and those modeling quadratic change for each of the six outcome variables, for males and females separately. Table 5 displays the better-fitting model for each outcome, in males and in females. The model accounting for quadratic change over time fit the data better than the linear model for all outcomes except hippocampal volume in males and for all outcomes except WMH volume in females.

Table 5. Model fit for longitudinal change in outcome variables, split by sex

Outcome variable	Males better fit	Females better fit
WMH volume	Quadratic	Linear
Hippocampal volume	Linear	Quadratic
V1 volume	Quadratic	Quadratic
Executive function	Quadratic	Quadratic
Episodic memory	Quadratic	Quadratic
Language	Quadratic	Quadratic

We next added the time × BDNF interaction to the linear or quadratic model of longitudinal change according to which was a better fit in the model comparison described above, separately for males and females. There was a significant time × BDNF interaction effect on hippocampal volume in females (B=19.58, SE=.19, p=.01), and this was not present in males. There were no significant time × BDNF interactions on any other outcomes in males or females (see table 6 for regression coefficients from all models).

Table 6. Moderating effects of BDNF on longitudinal change by sex

	Time × BDNF interaction	
	Males	Females
WMH volume	1.56, .80 (.05)	.14, .19 (.46)
Hippocampal volume	-37.95, 29.47 (.20)	**19.58, 7.28 (.01)**
V1 volume	-3.58, 7.64 (.64)	-6.53, 10.34, .53
Executive function	1.83, 1.28 (.16)	-.33, 1.57 (.83)
Episodic memory	1.16, .69 (.09)	-.44, .80 (.58)
Language	.05, 1.19 (.97)	-.60, .73 (.41)

Unstandardized regression coefficients, standard error (p-value). Values with p<.05 are bolded

We conducted the same analysis as described above, with time × FRS interactions added to the growth models. These revealed no significant time × FRS interactions for any outcome variable in males or in females (see table 7 for regression coefficients from all models).

Table 7. Moderating effects of FRS on longitudinal change by sex

	Time × FRS interaction	
	Males	Females
WMH volume	.02, .07 (.79)	.006, .01 (.66)
Hippocampal volume	-.32, 2.26 (.89)	.79, .50 (.12)
V1 volume	.96, .53 (.08)	.95, .69 (.17)
Executive function	-.02, .10 (.86)	.08, .11 (.47)
Episodic memory	.004, .05 (.95)	-.04, .05 (.43)
Language	-.06, .09 (.52)	.03, .05 (.54)

Unstandardized regression coefficients, standard error (p-value). Values with p<.05 are bolded

We also investigated the association of BDNF with FRS in males and females separately. Regression analyses were performed with standardized variables to assess these relationships. BDNF did not have a significant association with FRS in males (B=-.10, SE=.06, p=.11) or females (B=.11, SE=.08, p=.19), though the direction of the relationship was opposite in the two sexes.

3.5.4 CDR Group Differences

Because prior work has shown that individuals with dementia have higher rates of cognitive decline (Adak et al., 2004; Boyle et al., 2006) and may have different levels of circulating BDNF (Laske et al., 2006; Yasutake et al., 2006), we also conducted post-hoc analyses to investigate associations separately for individuals who were CDR=0 and those who were CDR=0.5 at the study outset. We conducted a one-way ANCOVA to test the difference in BDNF level for different CDR groups, controlling for age. This revealed no significant difference in plasma BDNF level for the CDR=0 group compared to the CDR=0.5 gorup, when controlling for age ($F_{1, 394} = 1.18$, $p = 0.28$). We also performed model comparisons between growth models accounting only for linear change and those modeling quadratic change for each of the six outcome variables, for the CDR=0 group and the CDR=0.5 group separately. Table 8

displays the better-fitting model for each outcome in the two groups. Modeling quadratic change was better for four out of six outcomes in the CDR=0 group, while quadratic change was a better fit for all six outcomes in the CDR=0.5 group.

Table 8. Model fit for longitudinal change in outcome variables, split by CDR score

Outcome variable	**CDR=0 better fit**	**CDR=0.5 better fit**
WMH volume	Quadratic	Quadratic
Hippocampal volume	Linear	Quadratic
V1 volume	Quadratic	Quadratic
Executive function	Quadratic	Quadratic
Episodic memory	Linear	Quadratic
Language	Quadratic	Quadratic

We then added the time × BDNF interaction to growth models, based on which was a better fit for each particular outcome in each CDR group. Table 9 shows the coefficients for the interaction effect from each model. No significant interaction effects were detected for any outcome variable in either of the CDR groups.

Table 9. Moderating effects of BDNF on longitudinal change by CDR score

	Time × BDNF interaction	
	CDR=0	CDR=0.5
WMH volume	.49, .29 (.09)	1.13, .57 (.05)
Hippocampal volume	31.14, 29.03 (.29)	10.45, 5.83 (.08)
V1 volume	13.11, 16.04 (.42)	-5.91, 7.38 (.42)
Executive function	-.17, 1.24 (.89)	.83, 1.16 (.48)
Episodic memory	-2.49, 1.95 (.21)	.41, .56 (.46)
Language	-.40, .71 (.57)	-.72, .90 (.43)

Unstandardized regression coefficients presented. Bolded values are significant effects, with $p<.05$

We also probed the time × FRS interaction effects on change in outcomes for separate CDR groups. These analyses revealed a significant time × FRS interaction on V1 volume (B=1.09, SE=.52, p=.04) in the CDR=0.5 group, and this effect was not present in the CDR=0

group. Neither CDR group showed time × FRS interaction effects for the other brain or cognitive outcome variables (see table 10 for coefficients from all models).

Table 10. Moderating effects of FRS on longitudinal change by CDR score

	Time × FRS interaction	
	CDR=0	CDR=0.5
WMH volume	.02, .02 (.33)	.01, .04 (.77)
Hippocampal volume	.74, 1.85 (.69)	.30, .41 (.47)
V1 volume	1.32, 1.01 (.20)	**1.09, .52 (.04)**
Executive function	-.03, .08 (.71)	.04, .09 (.61)
Episodic memory	.06, .13 (.63)	-.04, .04 (.40)
Language	-.07, .04 (.12)	.02, .07 (.79)

Unstandardized regression coefficients presented. Bolded values are significant effects, with $p<.05$

Finally, the association of BDNF with FRS was investigated in each CDR subgroup. Regression analyses were performed with standardized variables to assess these relationships. There was not a significant association of BDNF with FRS in the CDR=0 group (B=.02, SE=.16, p=.88) or the CDR=0.5 group (B=-.02, SE=.05, p=.77).

Chapter 4: Discussion

Prior research investigating the relationships among BDNF and brain and cognitive health in older adults has led to mixed findings (Erikson et al., 2010; Kim et al., 2015; Laske et al., 2011; Nettiksimmons et al., 2014). The current study aimed to probe the potential intermediate role of cardiovascular health in the associations between plasma BDNF and longitudinal change in selected neural and cognitive outcomes. Overall, our results indicate that cardiovascular health may not be a meaningful link in these relationships. Additionally, results support some prior evidence that circulating BDNF may be limited as a predictor of longitudinal trajectories in brain structure and cognitive function (Driscoll et al., 2012; Nettiksimmons et al., 2014).

We found that plasma BDNF level at baseline was not significantly associated with cardiovascular disease risk, estimated by the Framingham Risk Score. Planned exploratory analyses also revealed a lack of an association between BDNF and pulse pressure, a marker of arterial stiffness. Previous studies have found that higher serum BDNF is associated with lower risk of developing cardiovascular disease (Kaess et al., 2015), and that individuals with coronary heart disease have lower plasma BDNF levels (Sustar et al., 2019). While prior research on these relationships has been limited, the available evidence is contrary to the results of the current study. It is possible that this difference in findings is due, in part, to the difference in how cardiovascular health was measured. Kaess and colleagues (2015) had data on which individuals experienced a cardiac event in the years following their BDNF measurement, while the current study relied on a 10-year risk profile for a cardiac event rather than actual incidence of these events. Prior research investigating the accuracy of cardiovascular risk calculators has

demonstrated decent predictive accuracy (Hemann et al., 2007; Marquez-Vidal et al., 2009), though there are certainly limitations including overestimation in populations with low risk and underestimation of risk in other populations including those with low socioeconomic status (Brindle et al., 2003; Brindle et al., 2005; Ko et al., 2020). Had our study incorporated actual cardiac event incidence rather than a risk score, it is possible that we would have detected a relationship with BDNF. The lack of associations between BDNF and either cardiovascular measure indicates minimal connection between plasma BDNF and heart health in our sample. Because there is prior evidence of plasma BDNF's association with specific cardiovascular risk factors (i.e., diastolic blood pressure, cholesterol, body mass index) (Golden et al., 2010), future work should consider these factors separately in addition to a calculated risk score.

Our measures of cardiovascular health, FRS and pulse pressure, were also not significantly associated with longitudinal changes in the brain and cognitive outcomes as we hypothesized, other than WMH volume. In mediation models, FRS and pulse pressure were both significantly associated with change in WMH volume. These associations indicated that individuals with a higher cardiovascular risk score had greater increases in WMH volume over time and that individuals with higher pulse pressure also had greater increases in WMH volume over time. These results were consistent with expectations, given evidence from many previous studies illustrating the relationship between poorer cardiovascular health and worse cerebrovascular outcomes, including WMHs (Bown et al., 2021, Cooper et al., 2016; Mitchell et al., 2011). However, the main effect of pulse pressure was not significant in the moderation analysis, and the main effect of FRS in moderation analysis did not remain significant when covariates were controlled for. These results indicate that although there were expected effects of

cardiovascular health on WMH volume in the mediation models, these associations may not be robust.

The lack of associations between cardiovascular risk and other outcomes was contrary to our hypotheses as well as previous findings. Cross-sectional studies have revealed that higher FRS is associated with worse performance on tests of executive function (Joosten et al., 2013) and overall cognitive function, as well as performance in other cognitive domains including attention, memory, and language (Torres et al., 2020). While the current study used longitudinal change in cognitive performance as outcomes, we still expected to see similar associations between FRS and cognitive performance. Similarly, pulse pressure has also been shown to be related to cognition. A cross-sectional study published by Mitchell and colleagues (2011) found that higher central pulse pressure was associated with lower memory scores. Additionally, a longitudinal study revealed that higher pulse pressure at baseline was predictive of greater decline in scores of verbal learning, working memory, and nonverbal memory over time (Waldstein et al., 2008).

The discrepancy in findings could be due to a few factors, including the makeup of the study cohort. The mean age of our sample was 74.2, while that of the studies cited above was generally younger (mean ages: 54-75). Additionally, 87% of participants in the current study had a CDR score of 0.5 at their initial study visit. A CDR score of 0.5 is said to represent "very mild dementia" and is associated with a histological diagnosis of Alzheimer Disease (Morris, 1997). Because our sample did not consist wholly of clinically normal individuals, it is possible that other factors (i.e., Alzheimer pathology) were more influential on brain and cognitive changes throughout the study period than cardiovascular health or risk. However, post-hoc analyses comparing effects of FRS on longitudinal change in outcomes did not reveal larger effects of

FRS in the CDR=0 group compared to the CDR=0.5 group. Future studies could investigate the effects of FRS in low and high Alzheimer Disease pathology groups instead of CDR subgroups to further probe this speculation.

Finally, results from the current study indicate that plasma BDNF was not associated with longitudinal change in any of the selected outcomes, except language. There was a significant relationship between BDNF and longitudinal change in language ability, such that higher baseline BDNF was associated with less decline in language ability over time. These findings were contrary to our hypotheses; however, the effect is likely not robust, given that it was detected only in mediation but not moderation analyses. We expected that executive function and episodic memory would be more strongly associated with BDNF than language ability, based on previous findings linking serum BDNF to executive function and memory performance in older adults (Erikson et al., 2010; Leckie et al., 2014). Our results showing that BDNF had no significant associations with changes in brain structure were also unexpected, based on prior literature demonstrating relationships between serum BDNF and WMH volume (Pikula et al., 2013) and between serum BDNF and hippocampal volume (Erikson et al., 2010). Sample size should be considered as a potential source of differences in findings. Numerous prior longitudinal studies which detected an association of BDNF with cognitive decline had smaller sample sizes than that of the current study (sample sizes: 40-165) (Erikson et al., 2008; Laske et al., 2011; Lim et al., 2013), though there have been associations detected in larger sample sizes, at least in terms of the BDNF genotype if not circulating BDNF (Boots et al., 2017). Overall, our results align with prior work which failed to detect cross-sectional or longitudinal associations between circulating BDNF and brain and cognitive outcomes (Driscoll et al., 2012; Kim et al., 2015; Nettiksimmons et al., 2014).

It is also possible that the makeup of the sample influenced the relationships among BDNF and these outcomes, as there are indications that BDNF levels change in individuals with early dementia and that the effects of BDNF on the brain may interact with AD pathology (Lim et al., 2013). Some studies have shown that individuals with dementia have elevated serum BDNF (Angelucci et al., 2010; Laske et al., 2006), and others have shown opposite associations with circulating BDNF and BDNF in postmortem brains (Hock et al., 2000; Peng et al., 2005; Yasutake et al., 2006). Although we did not observe a difference in BDNF between individuals with a CDR score of 0 and a score of 0.5 in our sample, we conducted post-hoc analyses to further tackle this limitation. However, there were only 50 individuals in our study sample with a baseline CDR score of 0, resulting in decreased power to examine these relationships in that subgroup. Splitting the sample by CDR score did not reveal quantitative difference in the effects of BDNF or FRS on the time course of brain and cognitive outcomes. Additionally, effect sizes did not reveal consistently stronger effects of BDNF or FRS on the trajectories of outcomes in either CDR group compared to the other. A large study cohort consisting solely of clinically normal individuals could have had different BDNF levels and potentially different relationships with the brain and cognitive outcomes.

In addition to the CDR breakdown of the current sample, there are a couple other limitations to consider. While the research questions were generally about relationships among BDNF, brain and cognitive outcomes, and cardiovascular health, the mediating and moderating variable in the primary analyses was a cardiovascular risk score. This score represents an individual's risk of experiencing a cardiovascular event in the next 10 years (D'Agostino et al., 2008) but does not necessarily represent the overall cardiovascular health or function of the individual at the time of measurement. Pulse pressure was used instead of FRS for planned

exploratory analyses to combat this limitation, though future research could incorporate more measures of current function to better characterize the relationships with BDNF. Additionally, we used plasma BDNF in our analyses, which has been shown to be correlated with the amount of BDNF in brain tissue (Klein et al., 2011). However, many of the previous studies which found associations between BDNF and cognition used other measures of BDNF, including serum BDNF (Erikson et al., 2010; Laske et al., 2011) and the BDNF gene polymorphism (Boots et al., 2017; Buchman et al., 2016; Lim et al., 2013). Research into the correlation between plasma and serum measures of BDNF has been mixed, with some studies showing a close correlation (Yoshimura et al., 2010) and others showing no correlation between the two measures (Bocchio-Chiavetto et al., 2010). There is also evidence that measurement of serum BDNF is relatively reliable and stable over time (Naegelin et al., 2018), while plasma BDNF measures are more dependent upon timing and storage conditions of the samples (Tsuchimine et al., 2014). Thus, it is possible that measurement error of plasma BDNF in the current study could have impacted our results. Additionally, it is unclear if BDNF levels in plasma and in the brain correlate with BDNF levels in cardiovascular tissue. This is an important clarification to make in the context of the specific research questions, because cardiovascular and brain BDNF levels may relate differently to brain structural and cognitive outcomes.

Overall, the current study sought to investigate the associations among BDNF, cardiovascular health, and the brain and cognition. Strengths of our study include a large sample size with repeated assessment, which allowed us to investigate change over time in outcomes of interest. We also used sequential timepoints for our predictor variable, mediating variable, and outcome variables in mediation models, affording us the ability to test the mechanistic nature of the relationships among variables of interest. Results suggest that cardiovascular risk and pulse

pressure might not be important factors in the relationship between BDNF and the brain. The effects of BDNF on the cardiovascular system should continue to be studied, with a focus on proximal measures of current cardiovascular function. Additionally, findings from the current study support some previous work demonstrating that plasma BDNF might not be predictive of longitudinal changes in brain structure and cognitive performance. Researchers should aim to determine an optimal measure of circulating BDNF, which will allow for more consistent measurement and comparison of these associations across studies. Future work should also focus on longitudinal associations in cognitively normal (i.e., CDR=0) older adults to confirm these relationships outside the context of Alzheimer Disease pathology or abnormal cognitive decline. More research is needed to fully understand the degree to which BDNF impacts the brain in older adults and the mechanisms by which it does.

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