Between the Head and the Heart:
Cardiovascular Risk Factors and Brain Structure and Cognitive Function

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Dedication

For my parents, my cardinal teachers, Gretchen and Doug. Because you showed us from the start the value of empathy, perseverance, and unconditional love. Because you taught us to use our head and our heart equally.

Abstract

There is growing appreciation for the relationship between cardiovascular health and brain health. Ischemic heart disease, stroke, diabetes, and hypertension rank in the top 10 leading causes of death in the United States and worldwide. The high prevalence of these chronic diseases combined with a growing aged population suggest the global prevalence of dementia will more than triple by the year 2050, relative to the 2010 prevalence. The objective of this thesis was to address gaps in our current understanding of the relationship between cardiovascular health and brain health, presented in three manuscripts.

In the first manuscript we assessed rate of annual cognitive decline in selected samples of elderly participants (mean age 71 years) in the Chicago Health and Aging Project (CHAP) who developed diabetes (n=405) and hypertension (n=837) between 1993 and 2012. We quantified annual decline in score for four neurocognitive tests during the period before and after the development of these conditions. Individuals who developed diabetes or hypertension experienced 50% to 60% greater rate of annual cognitive decline for neurocognitive tests assessing domains of general orientation and cognitive processing in the period following ascertainment relative to the period prior to ascertainment.

The second manuscript assessed the association between cognitive function and brain structure in middle adulthood and the development of diabetes in later adulthood in the Atherosclerosis Risk in Communities (ARIC) study (45 to 64 years of age at enrollment). In the 10,819 individuals with cognitive assessment at ARIC visit 2, we

identified 3,200 incident cases of diabetes over a median follow-up time of 19 years (30% cumulative incidence). Over a median of 14 years of follow-up 373 of the 1,350 participants who underwent MRI developed diabetes (28% cumulative incidence). We found that measures of cognitive function and MRI indicators of subclinical cerebrovascular disease were weakly and inconsistently associated with increased risk for diabetes.

Our final manuscript explored the association between cardiovascular health status during young adulthood, defined by the American Heart Association Life's Simple 7 metric, and measures of brain structure in middle adulthood in the Coronary Artery Risk Development in Young Adults (CARDIA) study. CARDIA participants were 18 to 30 years of age at enrollment and 43 to 55 years of age at the time of MRI. We quantified normal tissue volume of the total brain, gray, and white matter and abnormal tissue volume of white matter according to cardiovascular health score. We observed ideal levels of cardiovascular health throughout young adulthood was not consistently associated with differences in brain structure in middle age.

These findings suggest individuals who develop diabetes and hypertension experience changes in cognitive function in the areas of general orientation and processing speed, cognitive function and brain structure in midlife is not foretelling of risk for diabetes in older age, and that cardiovascular health during young adulthood is not associated with differences in brain structure in middle adulthood, but caution that differences in brain structure at this age are difficult to detect and that changes in brain structure were not assessed.

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Chapter 1. Introduction

Dementia or the loss of cognitive capacity is both a personal and a public concern. It is one of the foremost conditions of concern to older adults.[1] It affects personal autonomy, health behaviors and lifestyle, and requires increased familial care and substantial use of health-care resources. Total annual costs of health-care, long-term care, and hospice care for individuals with Alzheimer's disease and other dementias were estimated at $214 billion in 2014 in the United States, with the economic value of care provided by family and other unpaid caregivers potentially exceeding this (estimated value of unpaid care equal to $220 billion in 2013 in the US).[1] On their own, the direct and indirect costs of cardiovascular disease and stroke continue to lead all conditions in economic impact, totaling over $320 billion annually (not including cost of unpaid care).[2] The growing appreciation of the relationship between cardiovascular health and brain health offers an ample research milieu to investigate and develop cross-discipline preventive health approaches to combat these colliding chronic disease behemoths.[3]

The subsequent chapter describes the changing population structure, natural history of cognitive aging, and measurement methods of cognitive aging and concludes with a summary of the current understanding of how cognitive aging is hastened in the presence of less favorable cardiovascular health. The three manuscripts that will follow aim to address gaps in the literature. While all incorporate traditional cardiovascular health components and measures of brain health, they serve to answer unique questions regarding the relationship between cardiovascular health and the health of the brain. The first manuscript characterizes cognitive function for individuals who develop diabetes and hypertension, separately, and quantifies annual cognitive decline surrounding the

ascertainment of these two conditions. Manuscript #2 explores and develops recent research reconsidering the traditional conceptual model that elevated glycemia is upstream of cerebral damage and examine the association between cognitive function and brain structure and incident diabetes. The third and final manuscript defines and determines cardiovascular health status at three time points in young adulthood and assesses the association between cardiovascular health status during young adulthood and measures of brain structure in middle adulthood. The overall goal of this manuscript is to add to the existing body of knowledge regarding the association between cardiovascular health and brain health.

Chapter 2. Background and Rationale

2.1. An Aging Population

Over the next five decades the population of the United States (U.S.) is expected to change considerably in size and structure.[4,5] Governmental projections suggest the overall population will grow by 30% and become more racially and ethnically diverse.[4,5] In terms of age, the composition of the U.S. will become increasingly older, with greater than 20% of the population aged 65 years and older, meaning approximately 100 million U.S. residents will be ≥ 65 years of age by 2060 (Figure 2.1).[5] This aging demographic trend has been observed since 1970 when the proportion of adults aged 65 and older was roughly 10 percent.[4]

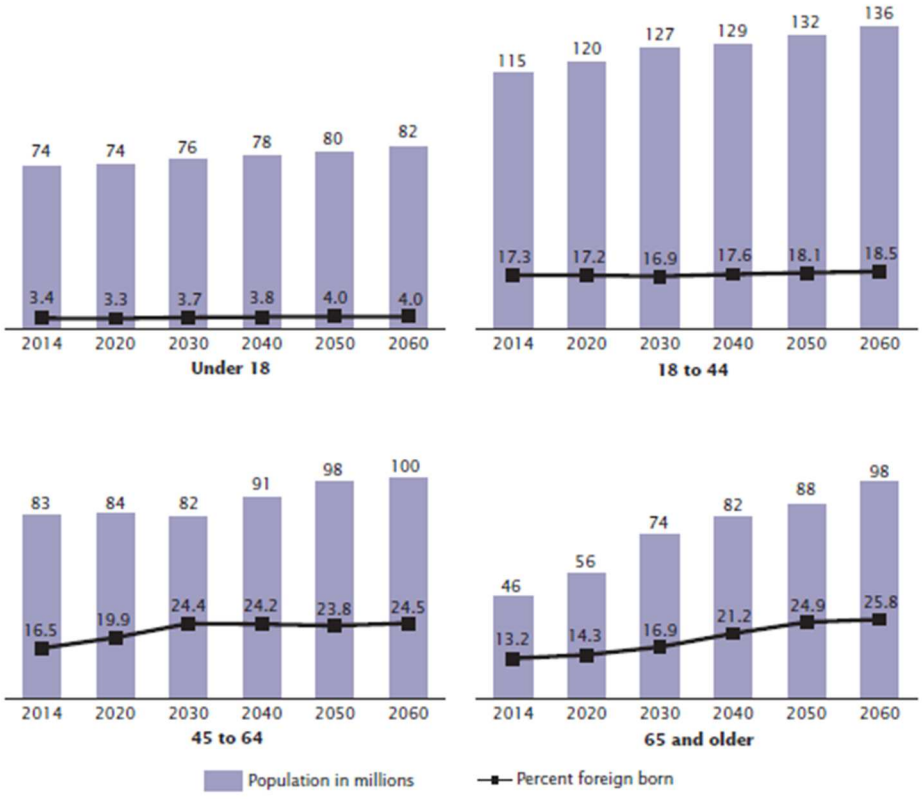


Figure 2.1. Population by selected age group and nativity: 2014 to 2060. Adapted from Colby SL and Ortman JM, *Projections of the Size and Composition of the U.S.*

Population: 2014 to 2060, Current Population Reports, P25-1143, U.S. Census Bureau, Washington, DC, 2014.

This anticipated aging is a global phenomenon, and disproportionately expected to occur in developing nations compared to developed nations.[6] This has two potentially profound global consequences by 2050: older persons will exceed the number of children (age < 18) and nearly 80% of the world's older population will live in less developed nations with limited resources.[6] There is an analogous phenomena happening in the U.S., where in addition to an aging population there is a migration of younger populations to urban areas, leaving rural areas increasingly older and with less human resources to provide the health care older populations require.[7] This creates an perilous imbalance where increasing demand for preventive health services and chronic disease treatment are augmented by lower capacity to provide the human resources and care needed.[4] This imbalance has social, economic, and public health repercussions, chiefly when there is high risk for or high prevalence of diseases considered burdensome in the aged populations, namely cardiovascular disease and its associated risk factors of high blood pressure, physical inactivity, obesity, and high fasting glucose, and neurological disorders.[8,9]

2.2. Cognitive Function and Decline

The process of aging and the development of chronic disease share similar processes, the loss of functional ability and adaptability, but it is important to differentiate the two concepts.[10] This is critical, particularly with regard to the natural aging process of the brain. Cognitive function represents the manifold mental processes including language

understanding, thought concentration and attention, memory, executive function, coordination and motor control, task planning and problem solving, and learned skills (e.g., writing, driving, and food preparation) that individuals engage in on a daily basis.[11] Typically over the lifespan cognitive function increases reaching its peak in early adulthood, levels off, and then begins decreasing in the fifth decade.[10] Known as cognitive decline, this diminution of the ability to perform mental processes occurs naturally with aging.[11,12] Healthy cognitive aging has been characterized as the ability to maintain an adequate functional level despite the age-associated structural deterioration. It has been suggested healthy cognitive aging is achieved via an adaptive process involving continual cognitive engagement and training and the development of complementary and alternative neuronal pathways used to complete the same cognitive tasks, largely facilitated with a sound prefrontal cortex, termed the scaffolding theory of aging and cognition (STAC) and conceptualized in Figure 2.2 below.[13]

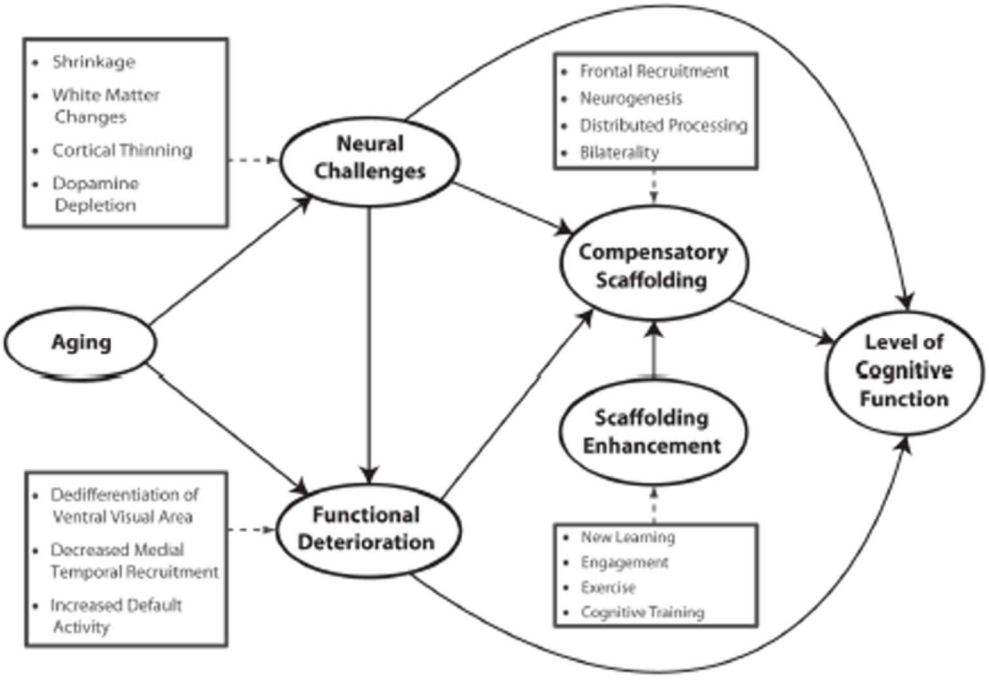


Figure 2.2. A conceptual model of the scaffolding theory of aging and cognition (STAC). From Park DC and Reuter-Lorenz P. The adaptive brain: aging and neurocognitive scaffolding. *Annu Rev Psychol*. 2009;60:173-196.

When cognitive decline begins to affect a person's ability to do things in their everyday life, it is commonly referred to as cognitive impairment (or mild cognitive impairment).[11] Clinicians will often define cognitive impairment to the point that it leads to a loss of memory and other mental abilities severe enough to interfere with and disrupt daily life as dementia, according to criteria in the *Diagnostic and Statistical Manual of Mental Disorders (DSM)*, currently in its fifth iteration.[11,14] Dementia is best to be thought of as a collection of symptoms that occur together in various combinations and severity and not as a specific disease entity.[11] Some symptoms of dementia can be mimicked by certain conditions, such as side-effects from a medication, depression, substance abuse, but it is important to make the distinction that these conditions can be reversed with treatment, while dementia is progressive and not reversible.[1]

2.3. Brain Anatomy and Aging Pathology

The central nervous system (CNS) is comprised of the brain and the spinal cord.[15] The brain is divided into two hemispheres, with each hemisphere being composed of an inner core region of white matter and an outer covering of gray matter, known as the cerebral cortex.[15] The white matter consists of bundles of myelinated nerve fibers and the gray matter of densely packed neuronal cell bodies.[15] An apt analogy is to think of the gray matter as the "computers" of the CNS and the white matter as the "wires" that connect

the computers, enabling communication. Between the brain and the skull, three distinct membranes and cerebrospinal fluid (CSF) protect and nourish the brain.[15] The main function of the CSF is to cushion the brain in the event of shocking and jarring movements that may cause the brain to hit the interior of the skull. CSF is primarily formed in the ventricular cavities within the brain, flowing out through the ventricles, circulating before being reabsorbed into the venous blood.[15] The CSF provides a good medium to detect and measure biomarkers of brain structure and function.[15]

In normal healthy individuals, various structural differences are found with aging. Increased age is associated with decreased total brain volume, global and regional gray and white matter, and corresponding increases in ventricular size and cerebrospinal fluid volume, accumulation of iron in the putamen, asymptomatic cerebral infarcts, and white matter hyperintensity volume.[16-21] While men have larger total brain volumes compared to women, adjustment for intracranial volume indicates that women have a greater percentage cerebral volume across the age spectrum.[16,22,23] Other noted sex-differences in brain structure of cognitively normal individuals include women having a higher percentage of gray matter in particular regions and certain lobe volumes, with men having greater volumes of white matter.[22-25] Longitudinal studies show men have greater brain volume decreases over time compared to women.[22,26]

The pathogenesis underlying cognitive decline, cognitive impairment, and dementia is varied. They include global and regional gray and white matter volume loss, hippocampal atrophy, neuronal loss, loss of synaptic potential and plasticity, development of neurofibrillary tangles (NFT) and amyloid plaques, development of white matter hyperintensities (WMH), clinically silent cerebral infarcts, and cerebral

microbleeds (CMB).[15,27,28] Synaptic plasticity refers to the ability of a synapse to change its own strength, either increase or decrease, as a response to its own activity or through activity of another pathway.[29] Neurofibrillary tangles and amyloid plaques, accompanied by neuronal loss, are considered the pathological hallmarks of Alzheimer's disease (contribute to, but not sufficient in clinical diagnosis).[1,28,30] Briefly, amyloid plaques are the result of the accumulation of β-amyloid (beta amyloid or Aβ) protein fragments that build up in the spaces between nerve cells and tangles are twisted fibers of another protein, tau, that build up inside nerve cells.[1,30] White matter hyperintensities (WMHs) are clinically silent indications of white matter injury with non-specific underlying pathology that includes tissue rarefaction, myelin loss and pallor, gliosis, and axonal atrophy.[27,31-33] Clinically silent cerebral infarcts are commonly referred to as "silent strokes", not severe enough to present clinically, while cerebral microbleeds are small chronic hemorrhages of small blood vessels of the brain.[34]

2.4. Neurocognitive and Imaging Assessment

As a result of various symptoms contributing to dementia, a formal diagnosis by a primary care physician requires a wide-ranging approach. The latest revision to the *DSM-5* guidelines now incorporates dementia into two diagnostic categories of neurocognitive disorders, major and minor, and advises physicians to specify the etiological origin (e.g., Alzheimer's disease, frontotemporal neurocognitive degeneration, vascular disease, Lewy bodies, traumatic brain injury, Parkinson's disease, among other medical condition, medication, substance use, or unspecified origins).[14] As the category names denote, for major neurocognitive disorder, major or significant cognitive decline must be present to

the point that it interferes with activities of daily living.[14] Cognitive impairments not reaching the level of dementia, will be classified as minor neurocognitive disorder, in which minor cognitive decline must have occurred since the last examination, but this decline, while physically and mentally taxing, does not interfere with activities of daily living.[14,35] If Alzheimer's disease is the suspected etiologic origin, physicians will also likely use criteria set by the National Institutes of Aging and the Alzheimer's Association to specify degree, preclinical Alzheimer's disease, mild cognitive impairment, and Alzheimer's disease.[36-40]

Brief validated instruments allow physicians to screen for cognitive impairment and unfavorable scores, indicative of possible cognitive impairment, cue physicians to direct more extensive mental state evaluations ranging from 1-3 hours of a battery of cognitive tests on memory, language, frontal and executive function, and visuospatial cognition by neuropsychologists with the intent for quantitative and qualitative assessment.[41-43] Potential neuropsychological tests abound for testing cognitive function and described in **Table 2.1** are those central to this manuscript, but there is no standard protocol of specific neurocognitive tests used to aid in diagnosing dementia.[42-44]

Table 2.1. Brief summary of the neurocognitive tests used to assess cognitive function of participants in the Chicago Health and Aging Project (CHAP) and the Atherosclerosis Risk in Communities (ARIC) Study, utilized in this manuscript.

Test	Brief Summary
East Boston Test (Delayed story recall)[45,46] Used in CHAP	A test of delayed episodic memory. Participants presented with oral story, three sentences in length and two idea units per sentence. Following a short period of distraction participant is asked to repeat as much of story as possible.
East Boston Test (Immediate story recall)[45,46] Used in CHAP	A test of immediate episodic memory. Participants presented with oral story, three sentences in length and two idea units per sentence. Immediately after examiner presents story the participant is asked to repeat as much of story as possible.
Delayed Word Recall[47] Used in ARIC	A test of a verbal elaborative-encoding delayed free-recall memory. Participant is presented with 10 words printed on separate 3" x 5" index cards and asked to read word and form a sentence using word. This process was repeated in similar fashion. Following a short period of distraction participant is asked to recall the words. Ample time and verbal support is offered, but no cueing is provided.
Digit Symbol Substitution[48,49] Used in ARIC	A test of executive function, incidental memory, and processing speed. Participant is presented with key with the Arabic numbers 1 through 9 in ordinal fashion, under each of which is a unique geometric symbol. Participant is allowed a short practice trial; shown a series of boxes containing numbers in top box and blank boxes directly below and asked to fill in. After this practice, the participant is asked to complete the task as a longer 90-120 second trial.
Mini-Mental State Examination[50] Used in CHAP	A test of general orientation and global cognition. In succession, participant is queried on orientation to immediate time and place, asked to name 3 objects, count backward from 100 by specific increment (7s) or spell "world" backward, asked to recall 3 previous objects, name "pencil" and "watch", repeat a short sentence, follow a 3-stage command, and read and obey a simple sentence.
Symbol Digit Modalities [51,52] Used in CHAP	A test of attention, visual scanning, and perceptual and motor speed that is inverse of the Digit Symbol Substitution Test. Participant is presented with nine different symbols corresponding to the Arabic numbers 1 through 9, and are asked to practice writing the correct number under the corresponding symbol. A second oral administration is completed and the participant is then given a blank copy of the test and asked to state the correct number for each corresponding symbol in 90 seconds.
Verbal (Word) Fluency Test [53,54] Used in ARIC	A test of executive function and expressive language. Participant is asked to generate as many unique words as possible beginning with three different letters. An alternative form may ask participants to generate categorical exemplars, for example names of animals, states, etc.

Lower scores on tests of cognitive function are associated with global and regional atrophy of brain and gray and white matter.[55-58] Increased ventricular volume, denoting brain atrophy, is associated with greater progression to dementia in cognitively normal and those with mild cognitive impairment.[59] In individuals with and without dementia, presence of, more adverse grade, and greater volume of WMHs is associated with lower cognitive functioning, lower cognitive status, and steeper cognitive decline.[55,56,60-62] Presence of clinically silent infarcts is shown to be associated with lower scores on neurocognitive tests and more abnormalities at neurological examinations.[63] Both presence and greater quantity of cerebral microbleeds have been shown to be associated with lower scores of the MMSE, however the extent to which microbleeds contribute significantly to lower levels of cognitive function is in dispute.[64-66] Presence of Alzheimer's pathology is lowest in individuals without cognitive impairment and increases incrementally across groups of individuals diagnosed with mild cognitive impairment and dementia and greater presence of neurofibrillary tangles is associated with lower memory test scores.[57,67]

Additional metrics used in specifying the etiology of dementia include tests of brain imaging and biomarkers. Magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET) are all standard brain imaging techniques each with unique advantages and disadvantages. Both MRI and CT are useful in providing insight on the structure of the brain, quantifying the shape, volume, and position of the tissue.[1,37-39,43] For example, these imaging techniques may identify a previous stroke that was undiagnosed, suggesting a vascular origin to current cognitive impairment. While also useful for evaluating structure of the brain, PET scans

also are informative to functional properties of brain tissue (i.e. how and where uptake of glucose may be abnormal).[1] A radiological-pharmaceutical intervention using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) and PET scans measures uptake of ^{18}F-FDG in the temporo–parietal cortex, measured with PET.[37] After administration of ^{18}F-FDG, PET scans distinguish how ^{18}F-FDG accumulates throughout the body; ^{18}F-FDG signifies glucose uptake in tissue and individuals with dementia show brain hypometabolism relative to normal controls.[68] Additional scanning methods include functional magnetic resonance imaging (fMRI) and single photon emission computed tomography (SPECT), but these are in the early years of use in the clinic setting, less utilized than the former scanning methods. Their utility in assessing brain health is being evaluated in research settings.

Considerable advancement in imaging has enabled the identification of biomarkers to aid in diagnosing Alzheimer's disease and detecting preclinical Alzheimer's.[37,69] For example, amyloid beta peptides ($A\beta_{40}$ and $A\beta_{42}$) are neurochemical markers measured in the cerebrospinal fluid and blood inversely correlated with concurrent and subsequent Alzheimer's pathology.[37,69-71] Levels of both tau protein and phosphorylated tau protein (p-tau) measured in the CSF as well in the blood are positively associated with Alzheimer's disease.[37,69,72]

2.4. Epidemiology of Dementia and Cognitive Decline

There are many different types of dementia and individuals can suffer from mixed types, but Alzheimer's disease and vascular dementia (their possible pathological features are presented in **Table 2.2.**) account for the majority of diagnosed cases.[1] Roughly 60%

to 80% of dementia cases are diagnosed as Alzheimer's disease and about 10% of dementia cases are diagnosed as vascular dementia.[1,73]

Table 2.2. Pathological Features of the Brain in Alzheimer's Disease and Vascular Dementia.

Alzheimer's Disease[1]	**Vascular Dementia**[74]
• Neurofibrillary tangles (tau protein buildup inside neurons) • Amyloid plaques (Amyloid β accumulation outside neurons) • Brain shrinkage (severe atrophy) • Widespread debris (dead neurons)	• Cerebral amyloid angiopathy • Vascular abnormalities • Large vessel infarction • Small vessel infarction • Abnormalities of white matter (leukoaraiosis) • Hippocampal sclerosis • Subcortical ischemia • Hemorrhage

The prevalence of dementia in the U.S. is about 1-2%, 5.5 million cases, but is 10-12% in adults 65 years of age and older and increases dramatically as age increases.[1,75] Over the last three decades, the age-adjusted incidence rates of dementia appear to be on the decline.[76] However, the greater number of individuals expected to reach older age suggests the prevalence of dementia will double in the next 25-35 years.[1,75] There are roughly half a million new cases of dementia each year; the annual incidence is estimated to rise by 100,000 cases by the year 2050.[77] In individuals with mild cognitive impairment, progression to dementia within 3 years is high (at least 20%).[73,78] The prevalence of mild cognitive impairment without dementia in the U.S. is suggested be equal to or greater than the prevalence of dementia.[79,80] However this is dependent on definition of MCI used, largely determined by the researchers. While it has been observed cognitive decline is frequent in older adulthood and the incidence of mild

cognitive impairment in the elderly may be as high as 20%, it has also been observed reversion of MCI occurs when previous impairment was limited to one domain, supporting the STAC.[13,81,82]

2.5. Intersection with Cardiovascular Health

The relationship between cardiovascular health and brain health has drawn significant attention in the past decade, particularly in the need to apply widespread and collaborative prevention strategies.[3] The potential overlap in shared risk factors for cardiovascular disease and accelerated cognitive decline offer promise to use common approaches with compounding benefit. Less favorable levels for many of the individual traditional cardiovascular risk factors have been independently shown to be associated with lower levels of cognitive function and detriment to brain structure.

Higher levels of blood pressure are associated with lower levels of cognitive function, greater cognitive decline, and increased pathologic damage (both neurodegenerative and vascular).[83-86] For example, greater visit-based measurement of and cumulative mean systolic blood pressure (SBP) was associated with greater white matter hyperintensity volume change in ARIC participants between 1993-1995 and 2004-2006 (per 20 mm HG increase in cumulative mean SBP: 2.35 cm^3; 95% CL= 1.58, 3.12 cm^3).[86] Individuals with hypertension consistently experience greater brain atrophy and odds for presence of white matter lesion volume or severity relative to individuals without hypertension.[87-89] Several studies have focused on the age at which high level of blood pressure is occurring in relation to subsequent cognitive impairment and brain pathology.[85,87,90-92] ARIC participants with hypertension at visit 2 (1990-1992)

experienced significantly greater 20-year cognitive decline compared to individuals without hypertension (additional decline 0.056 global *z* score points; 95% CI= -0.100, -0.012).[85] The association between higher mean level of blood pressure in midlife predicting greater cognitive decline is consistently observed and extends to an increase risk for adverse neurocognitive-related events.[90,91,93] In contrast, higher level of blood pressure at later stages of life is not strongly associated with cognitive decline.[85,92]

The cerebrovascular risk from diabetes is evident; overt diabetes and elevated levels of glycemia in the absence of diabetes increase the risk for stroke.[94-97] Of the traditional cardiovascular risk factors, diabetes is one of the stronger risk factors for increased cerebral infarctions, greater brain atrophy, and cognitive decline.[98,99] Individuals with diabetes have nearly twice the rate of cognitive decline compared to those without diabetes and one and a half times the risk for being diagnosed with Alzheimer's disease.[100-102] A recent meta-analysis by Cheng et al. observed the relative risk for incident vascular dementia, Alzheimer's disease, any dementia, and mild cognitive impairment for individuals with diabetes is 2.5, 1.5, 1.5, and 1.2 (all $p < 0.05$) compared to individuals without diabetes, repsectively.[102] Individuals with diabetes have greater vascular pathology and adverse changes over time (both atrophic and vascular).[84,89,103,104] Alternatively, recent research has investigated the association between cognitive function and the development of diabetes, finding increased risk for incident diabetes with lower baseline level of cognitive function.[105-107]

Research investigating the role of diet on the development of dementia is inconsistent. Studies are mixed when concluding if there is potential benefit from adherence to a diet emphasizing foods high in anti-oxidants and polyunsaturated fatty

acids (PUFAs).[108-111] The foods comprising the Mediterranean diet bear these properties, among others (high in fruits and vegetables, cereals, moderate dairy and poultry, low in saturated fats and red meat), and meta-analyses of prospective observational studies assessing the effect of the Mediterranean diet on Alzheimer's disease and cognitive impairment suggest a potential protective effect.[112,113] However, randomized clinical trials evaluating the development of cognitive impairment and Alzheimer's disease do not clearly point to long-term benefit from dietary supplementation of anti-oxidants or PUFAs.[114]

The benefit of physical activity on brain health and aging is not consistently shown in individual studies. Some observational studies have found that increased levels of physical activity are associated with lower risk for dementia and cognitive impairment, while other studies find a null association; the discrepancies may be due to differences in study population age, size, and genetic susceptibility; follow-up duration; reverse causation; determination of activity levels; or differential adjustment for confounding.[115-120] Multiple meta-analyses and systematic reviews suggest that increased levels of physical activity provide a protective effect for dementia and cognitive impairment, but also present evidence of bias in published literature resulting from an absence of null studies of smaller sample size.[120-123] Intervention studies have not assessed the relationship between an exercise regimen and the development of any dementia, but do support physical activity as an intervention to improve cognitive function.[124,125]

The risk for dementia is increased for individuals who have ever identified as participating in habitual cigarette smoking.[98,126-128] A recent met-analysis of 19 prospective studies compared individuals who have never smoked to current smokers and

found current smokers have nearly 1.8 times greater risk for incident vascular dementia (95% CI: 1.4, 2.2) and Alzheimer's disease (95% CI: 1.3, 2.5) relative to never smokers.[126] Middle-aged smokers have lower cognitive function domain-specific scores and greater decline in these same domains over time compared to nonsmokers of the same age.[129] The studies assessing smoking and brain imaging show history of cigarette use (ever use and categories of increased use) is associated with decreased total brain volume and increased Alzheimer's disease pathology.[127,130-132]

The atherogenic effect of cholesterol and potential association with amyloid deposition would suggest cholesterol to have a potential contribution to the development of dementia (both vascular and Alzheimer's) and cognitive decline.[133-136] However, results from epidemiological studies are mixed, suggesting higher levels of cholesterol are associated with Alzheimer's disease but not vascular dementia.[135] Further, it appears that high levels of cholesterol at mid-life are more strongly associated with Alzheimer's disease and cognitive decline and high cholesterol in late-life is not consistently associated with dementia and cognitive decline.[135] The lack of association between cholesterol level and vascular dementia may be due to a lack of power, as vascular dementia is less prevalent than Alzheimer's disease and few studies assess vascular dementia alone.[135,137] Important to this potential association is the apolipoprotein ε (*APOE* ε) gene.[138] Polymorphisms of this gene are associated with both cholesterol levels and Alzheimer's disease, specifically those individuals with ε4 polymorphisms have higher cholesterol levels and are at significantly increased risk for Alzheimer's disease relative to ε3 carriers (heterozygous ε4: AD_{RR}=3.0; homozygous ε4: AD_{RR} =14.0).[138] The relative frequencies for the ε2, ε3, and ε4 alleles differ by race in U.S. populations, but ε3

is the most common (65-85%) with ε4 (13-20%) and ε2 (2-13%) being less prevalent.[138,139] Individuals with the ε4 polymorphism of the *APOE* ε allele are at increased risk for developing Alzheimer's disease, relative to having ε2 and/or ε3 alleles.[138] Depending on the population, this gene is estimated to be responsible for 30-70% of the Alzheimer's disease cases (population attributable risk; higher PAR found in the more homogeneous populations).[114,140] The association between *APOE* ε and vascular dementia is suggestive of an association similar to that of Alzheimer's disease, but is in need of clarification.[74]

The relationship between body weight and dementia and cognitive decline is complex, likely because body weight decreases during senescence and end-of-life.[141,142] Evidence suggests that body weight changes over the course of dementia development.[101,143,144] A meta-analysis on the association between obesity and incident Alzheimer's disease reported an overall relative risk of 1.59 (95% confidence limits= 1.02, 2.48).[101] Of the eight studies assessed, six had estimates that included the null and two were suggestive of obesity being protective (non-significant) in regard to incident Alzheimer's disease.[101] This meta-analysis, in addition to recent studies, suggest weight loss in older age is greater in individuals who end up being diagnosed with dementia.[101,143-145] Recent work by Tolppanen et al. and Gu et al. report higher baseline level of BMI was found to be associated with incident dementia and a steeper decrease in BMI over time was associated with incidence of dementia, in support of the notion that this association may depend on stage of life at which BMI is measured.[143-145]

In addition to the major modifiable CVD risk factors, other characteristics may also influence the association between cardiovascular health and brain health. Women are

more likely to develop dementia relative to men.[1] At the age of 65, 1 in 5 women will develop Alzheimer's disease before death and the lifetime risk for men at this age is slightly lower, roughly 17%.[146] This slight sex difference is small and may reflect an actual difference or may be an artifact due to other circumstances, such as differences in life-span due to competing risks or diagnosis in a clinical setting.[147] For example, relative to men with similar levels of Alzheimer's disease pathology, women have significantly higher odds of clinical diagnosis for Alzheimer's disease.[148] However, it is widely recognized woman "lag" men in age at coronary mortality, and have lower mean values of blood pressure and cholesterol concentration compared to men of equal age.[133] In the U.S., compared to older whites, African Americans have twice the risk and Hispanics have 1.5 times the risk of developing dementia, possibly due to less favorable levels of cardiovascular risk factors and differences in healthcare access.[1,133] Similar to findings from epidemiological studies of cardiovascular outcomes, moderate alcohol consumption may be beneficial relative to no alcohol consumption and high levels of alcohol consumption in regard to cognitive outcomes.[149-152] Individuals with greater education, continuing education, and engagement in cognitive activities have more favorable levels of cognitive functioning, later development of impairment and dementia, and shorter life-periods with impaired cognition.[114,153-155] Two factors of support, lower social economic status (financial support) and less social support (social-personal network) are also associated with increased risk for cognitive impairment and dementia.[156,157] Related to cognitive activity and social support, mental health may also play a role in the development of dementia; two meta-analyses and a large prospective cohort of Danish citizens suggest individuals with depression are at greater risk for developing dementia

compared to individuals without depression.[158-160] There is a breadth of literature suggesting psychosocial factors are rooted in the development of cardiovascular disease and deterioration of traditional cardiovascular health components.[133,161] Prospective work evaluating sleep disorders is limited in scope and population diversity, but suggests compared to individuals without sleep conditions, sleep-disordered breathing may increase the risk for developing mild cognitive impairment or dementia (OR=1.85, 95% CI: 1.11, 3.08) and may hasten cognitive decline.[162,163] However an association between sleep-disordered breathing and cognitive decline is not consistently observed.[164] Taken together, this highlights that dementia is a complex disease condition, with biological, physiological, behavioral, social, and psychological correlations. A simplified directed acyclic graph (DAG) illustrates the complexity of the potential factors underlying accelerated cognitive decline and dementia and their corollaries in Figure 2.3. The three papers of this manuscript will look to develop and contest aspects of this DAG.

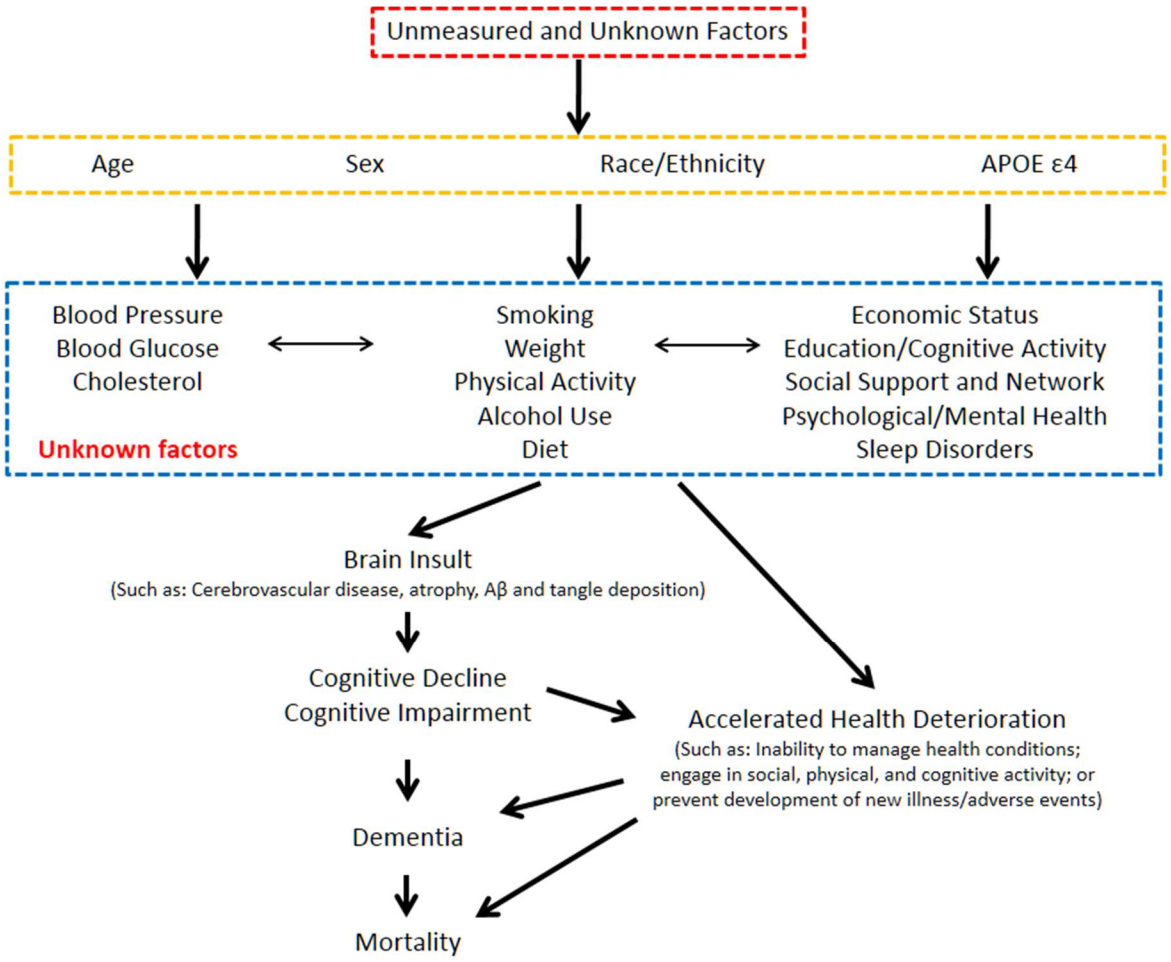


Figure 2.3. Directed acyclic graph of potential factors leading to the cascade of brain insult, accelerated cognitive decline, general health deterioration, dementia, and early mortality.

The release of the American Heart Association's Strategic Impact Goal for 2020 and Beyond and the introduction of the construct "ideal cardiovascular health" and the Life's Simple 7 metric has encouraged research to explore the relationship between a broader cardiovascular health profile and the health of the brain.[3,165] Early findings suggest an association between less favorable cardiovascular profile and lower level of cognitive function and increased risk for cognitive impairment, but are missing structural

assessment.[166-168] A comparison of the Framingham cardiovascular risk score and the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) risk score found scores for each, measured in midlife, predict cognitive decline and that the Framingham score is a stronger predictor for 10-year cognitive decline compared to the dementia risk score.[169] In both community- and population-level studies, the percent of Alzheimer's disease and other dementias attributable to cardiovascular risk factors is noteworthy.[170-172] Norton et al estimated the combined population attributable risk for Alzheimer's due to low education, depression, physical inactivity, diabetes, midlife hypertension, midlife obesity, and smoking was approximately 30%.[170] Moreover, in developed nations such as the US, countries of Europe, and the United Kingdom, physical inactivity was the major risk factor attributable to Alzheimer's disease. Taken together, this would suggest a strong modifiable cardiovascular component in cognitive deterioration. In sum, our growing aging population and improving knowledge of the relationship between modifiable cardiovascular health and the health of the brain supports research that identifies and elaborates on gaps in our understanding.

Chapter 3. Manuscript 1: Cognitive Function Trajectory in Relation to New Diagnosis of Diabetes and Hypertension; The Chicago Health and Aging Project (CHAP)

3.1. Overview

Background: An abundance of research has shown individuals with diabetes or hypertension experience significantly greater cognitive decline relative to individuals without these cardiovascular risk factors. Comparisons of individuals with diabetes or hypertension to those without these conditions does not address the possible individual changes in cognitive function and decline following the development of diabetes or hypertension.

Methods: We assessed cognitive function according to scores from four neurocognitive tests in selected samples of CHAP participants who developed diabetes (n=405) and hypertension (n=837) between 1993 and 2012. At in-home assessments, participants completed 4 neurocognitive tests including the Mini-Mental Status Exam (general orientation, attention, and global cognition), Symbol Digit Modalities Test (perceptual speed), and an Immediate and Delayed Recall (derivations of the East Boston Memory Test). We calculated rate of annual decline for each test during the periods prior to and following the ascertainment of each condition, taking into account potential confounding factors.

Results: At baseline, the mean age (SD; range) for individuals who developed diabetes was 70.5 years (SD: 4.6; range: 64 – 91 years) and for individuals who developed hypertension was 71.0 years (SD: 5.1; range 65 – 93 years). The mean time of observation before and after the ascertainment of diabetes was 6.8 years (SD=3.7) and 4.1

years (SD=3.9), while for hypertension these mean times were 5.9 years (SD=3.4) and 5.0 years (SD=4.1). Individuals who developed diabetes or hypertension experienced 50% to 60% greater rate of annual cognitive decline for neurocognitive tests assessing domains of general orientation and cognitive processing in the period following ascertainment relative to the period prior to ascertainment. Rate of annual cognitive decline for score on the Mini-Mental Status Exam increased from -0.2 before to -0.3 after ascertainment of diabetes (deviation slope: -0.1; 95% CL: -0.2, -0.04; $p<0.005$) and from -0.2 before to -0.4 after ascertainment of hypertension (deviation slope: -0.2; 95% CL: -0.2, -0.1; $p<0.0001$). For the Symbol Digit Modalities Test, rate of annual cognitive decline increased from -0.5 before to -0.8 after the ascertainment of diabetes (deviation slope: -0.3; 95% CL: -0.5, -0.1; $p<0.05$) and there was a similar magnitude of change in rate of annual decline before (-0.5) and after (-0.8) the ascertainment of hypertension (deviation slope: -0.3; 95% CL: -0.4, -0.1; $p<0.01$). We did not observe significant differences between pre- and post-ascertainment annual cognitive decline in scores on neurocognitive assessment of immediate or delayed memory for either cases of diabetes or hypertension or in global cognition for diabetes cases.

Conclusion: Individuals identified as newly diabetic or hypertensive should be informed of potential cognitive changes in functional domains of general orientation and cognitive processing that may occur in the period after the ascertainment of diabetes and hypertension. Future research should consider including neurocognitive tests assessing a greater variety of cognitive domains and more frequent assessment of cognitive function to determine the timing of these changes.

3.2. Introduction

With an aging global population the development of cardiovascular disease risk factors and accelerated cognitive decline pose a large public health concern.[3,4,6] The corollaries of accelerated cognitive decline are varied and range from disruptions of activities of daily living to catastrophic events. Individuals with lower cognitive function show lower medication adherence and greater cognitive decline is associated with impaired mobility, increased hospitalization, increased risk for cardiovascular events and early mortality.[173-178] The capacity to minimize cognitive decline may depend on the underlying structural health of the brain, continual engagement in cognitive activities and recruitment of new neuronal pathways, and exposure to factors that accelerate the brain aging process.[13]

Elevated levels of blood glucose and blood pressure are among the strongest cardiovascular risk factors associated with accelerated cognitive decline; individuals with diabetes or hypertension experience greater cognitive decline compared to their healthy contemporaries.[85,93,99,179] Most research on the effect of diabetes and hypertension on cognitive decline compare individuals with diabetes and hypertension (or elevated normal levels of blood glucose and blood pressure) to those without these conditions at baseline and compare cognitive function over time. This design ignores the rate of cognitive decline in the period before the development of diabetes or hypertension. Additionally, rather than comparing cognitive decline of distinct groups of individuals, an approach that looks at cognitive function surrounding the development of diabetes or hypertension permits inference about subject-specific changes in cognitive function that may be a direct result of disease development. For example, research assessing cognitive function before and after stroke events has proven beneficial in identifying individual changes in

cognitive function before and after stroke occurence.[180,181] Stroke is a definite event with noticeable physical and cognitive effects while diabetes and hypertension are diagnosed according to reaching a cut-point on a continuum. However there is limited research on individual changes in cognitive function surrounding the development of these management intensive conditions. Therefore, research is needed estimating cognitive decline before and after ascertainment of diabetes and hypertension and quantifying the changes in cognitive function during this period.

We had two aims. Our first aim was to quantify annual cognitive decline before and after the diagnosis of diabetes and hypertension. The second aim was to quantify the change in decline occurring between these two periods. We were determined to characterize this association with four domain-specific neurocognitive tests and a global measure of cognitive function. We hypothesize that the rate of annual cognitive decline will be significantly greater in the period after the ascertainment of both diabetes and hypertension relative to the rate of annual cognitive decline in the period before ascertainment for all measures of cognitive function.

3.3. Methods

The CHAP Design and Population

The Chicago Health and Aging Project (CHAP) is a prospective cohort designed to study common chronic health problems of older individuals.[182] The study design and population has been previously described in detail.[182,183] Briefly, the geographic parameters of the study population are three contiguous neighborhoods in the south side of Chicago, Illinois, including Morgan Park, Washington Heights, and Beverly. Between

1993 and 1996, study staff conducted a census and determined the racial composition was largely African American and non-Hispanic white individuals. Of the 7,813 residents who were 65 years of age or older, 6,158 (78.9%) enrolled during 1993 and 1996 for the population cohort. Follow-up interviews were conducted in 3-year cycles for this cohort. Beginning in 2001, community residents who attained the age of 65 were enrolled as successive age cohorts with baseline exams occurring for these individuals in 2001-2003, 2003-2006, 2006-2009, and 2009-2011, continuing on the same 3-year cycles of interviews. A total of 10,802 individuals were enrolled in CHAP with 1,133 individuals completing 6 exams. All participants provided written informed consent and the study was approved by the Rush University Medical Center Institutional Review Board.

In-Home Assessment of Population Cohort

Assessment of participants occurred in their own home with trained staff conducting interviews with structured questions assessing sociodemographic factors, lifestyle characteristics, psychosocial factors, medical history and medication use, and a brief cognitive battery in addition to a standardized assessment of blood pressure.[92,182,184] Age was determined from self-report of date of birth. Education was assessed as the highest grade or year of regular school completed (up to 30 years). Total household income at the time of the interview was measured using a color-coded card with 10 income categories ranging from less than $5,000/year to greater than $75,000/year.

Participants were asked to self-report history of medical conditions diagnosed by a doctor, nurse, or therapist. Participants were requested to present all prescription and over-the-counter medications at the time of the in-home interview to staff who recorded

each by name and dosage. Medications were classified using the MediSpan drug database system.[185] This system enables grouping medications by their biologically active agents and physician adjudication of all medications that may have glucose lowering or antihypertensive effects was done to create separate variables for diabetes medications and antihypertensive medication use. These variables were created without knowledge on the specific medical condition for which each medication was prescribed.

Individuals were queried on current status and history of cigarette use. Past and current regular alcohol consumption was assessed with questions inquiring the amount and duration of alcoholic beverages of any kind (entire life) and specific types of beverage (i.e., beer, wine, and liquor) for the past 12 months. The physical activity portion of the questionnaire assessed the frequency and duration of 10 activities over the past 14 days including walking for exercise; jogging or running; gardening or yard work; dancing; calisthenics or general exercise; golfing; bowling; biking (including stationary bike); swimming or water exercise; other exercise, sports, or physically active hobbies not listed (asked to specify). Weight was measured using a portable digital scale, zeroed and placed on a hard flat surface without participants wearing shoes. Height was reported by participants in feet and inches. Body mass index was calculated in kg/m^2 after converting height and weight to the metric system. Individuals were queried on multiple areas of psychosocial health. A modified short-form 10-item scale of the Center for Epidemiological Studies (CES-D) was used to capture depressive symptoms with scores ranging from 0 to 10, increasing in score with depressive symptoms.[186,187] Individuals were asked about engagement in cognitive activities such as watching television;

listening to the radio; reading newspapers, books, and magazines; playing games such as cards, checkers, crosswords, or other puzzles; and visiting museums.[188]

At the end of cycle 2, a blood draw was obtained on a stratified random sample of roughly one-sixth of all participants and again for a sub-set of individuals during the cycle 5 visit and all participants during cycle 6. Apolipoprotein ε (*APOE* ε) genotyping from the original cohort and successive cohorts was described previously.[189] Briefly, methods for *APOE* ε genotyping were adapted from Hixson and Vernier using primers based on Wenham et al.[190,191] The hME Sequenom MassARRAY® platform was used to genotype the two single nucleotide polymorphisms (SNPs) rs7412 and rs429358, which indicate presence of the *APOE*-ε2 and *APOE*-ε4 alleles, respectively. These SNPs had high genotyping success rates (both ≥99.8%) and were in Hardy-Weinberg equilibrium (significance tests for deviation from HWE, both $p > 0.05$).[189]

Definition of Hypertension and Diabetes

Resting seated blood pressure was measured from 1993 to 2005 with mercury sphygmomanometers and by digital sphygmomanometers from 2006 through 2011. Following a 5-minute resting period, blood pressure was measured in the non-dominant arm in duplicate, with measurements occurring 1 minute apart and the average of the two values used as measures of systolic and diastolic blood pressure. Hypertension was defined as having any of the following: systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive medications according to the medication inventory. Diabetes was determined as self-report of previous diagnosis or use of diabetes medications according to the medication inventory.

Neurocognitive Testing

Four neurocognitive tests were given to participants during the in-home visit. They included the Mini-Mental Status Examination, which assesses general orientation, attention, and global cognition.[50] There were two tests of memory, immediate and delayed recall, which are derivations of the East Boston Test.[45] The last test included was the Symbol Digits Modalities Test, which assesses perceptual speed.[51] A summary measure of global cognitive function was previously constructed based on *z*-scores from the individual tests during each wave.[188] Briefly, during each wave of assessment, *z*-scores for each test were constructed by subtracting the overall mean test score from each individual's test score and dividing by the standard deviation (SD) of the test. The global measure of cognitive function was created as an average of the four z-scores divided by the standard deviation of the averages, with a higher score indicating higher cognitive function.[188]

Statistical Analysis

To be included in the analytic sample, individuals had to develop the condition of interest (diabetes or hypertension) during CHAP participation and have a cognitive assessment before, at the time of, and subsequent to the exam at which the condition was determined as incident. Of the 4,978 CHAP participants who had at least 3 visits, 405 were identified as not having diabetes or previous stroke at baseline (and did not develop stroke over follow-up) and met the aforementioned criteria, while 837 individuals were identified as not having hypertension or previous stroke at baseline (and did not develop

stroke over follow-up). We used Markov Chain Monte Carlo (MCMC) methods for multiple imputation of missing covariate data drawing from the full CHAP cohort, assuming an arbitrary missing data pattern, and created 10 data sets which were analyzed separately. Parameter estimates were combined according to Rubin's Rules.[192] MCMC methods use data from available cases as initial estimates and assume that data come from a multivariate normal distribution, shown to be a reliable approach given sufficient sample size.[193] We imputed 7% of baseline BMI values for each analysis and 3% of systolic blood pressure values for the hypertension analysis, otherwise missing data for any given variable was < 1%.

We used piecewise linear mixed models to assess the annual rate of change in cognitive function before and after the ascertainment of diabetes and hypertension, separately (including only individuals identified as a case for each). For the purpose of this analysis, the exam at which the ascertainment of diabetes or hypertension occurred is considered the breakpoint.[194] For each analysis, we classified time into two periods, from baseline to the breakpoint and from the breakpoint to the end of follow-up. We included random effects for the intercept and pre-breakpoint slope. This allows for person-specific deviation from the population mean for intercept and slope. The advantages of the piecewise model method used here compared to a traditional two regression ordinary least squares include accounting for the correlated nature of the data, ensuring the pre-breakpoint slope and post-breakpoint slope meet at the breakpoint time, and allowing for individual variation relative to the population averages.[194,195] We assessed cognitive function as a global measure and also assessed domain-specific cognitive test associations.

A continuous physical activity score, expressed as hours per week, was derived from average time spent on the 6 activities of walking, yard-work, calisthenics, biking, swimming, and other activities of exercise. We created categories for formal education (≤12 years, 13-15 years, ≥16 years), current smoking status (never, former, current), regular alcohol consumption (none, moderate consumption: any up to 1 drink daily for women/2 drinks daily for men, or heavy consumption: greater than 1 drink daily for women/2 drinks daily for men), and CES-D score (0, 1, ≥2). In multivariable models, we adjusted for age at the time of diabetes or hypertension ascertainment, sex, race, enrollment cohort, and baseline levels of education, BMI, physical activity, smoking status, alcohol consumption, measures of depression and cognitive activity, and *APOE* ε4. Our final model included adjustment for time-varying systolic blood pressure because it is recognized as a strong predictor of concomitant and future cognitive function. Specific to each case analysis we included adjustment for use of diabetes medications or antihypertensive medications. We separately assessed effect modification of the associations by sex, race, and *APOE* ε4 status on the additive scale by including a product term between the annual slope and each characteristic. Individuals who have lower baseline cognitive function and greater decline and those who develop diabetes and hypertension may be more likely to be lost to follow-up. We ran sensitivity analyses employing inverse probability of attrition weighting (IPAW). IPAW is a statistical method used to address differential dropout.[196] Using logistic regression, we predicted the probability of attrition due to drop-out and death at each exam separately. Due to the nature of enrollment in CHAP, this was done separately for each enrollment wave. For example, weights were calculated for those enrolled in the original cohort separately from

those enrolled at wave 3 and wave 4. To increase prediction ability, we included additional characteristics not used in the primary aim, specifically income level, diastolic blood pressure, current marriage status, and pack-years of smoking. All IPAW models included the same covariates: baseline age, sex, race, income level, education, systolic and diastolic blood pressure, regular alcohol consumption, current smoking status and pack-years, BMI, physical activity, currently married (yes/no), antihypertensive and diabetes medication use, and scores from psychosocial tests of depression and cognitive activities. We created stabilized weights by re-running these prediction models with a subset of covariates, multiplying the probabilities from the reduced models by the inverse of the full models ($1/P$). Lastly, we ran a linear mixed model assessing the potential of a quadratic association between cognitive function and time, to understand if any association identified by the piecewise modeling could be explained by this phenomenon. SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC) was used for statistical analysis.

3.4. Results

Baseline characteristics for the entire CHAP cohort and the 405 cases of diabetes and 837 cases of hypertension are presented in **Table 3.1**. Compared to the full cohort, the cases were on average younger and had higher scores on all four neurocognitive tests at their enrollment visit. Individuals included in the diabetes analysis were more likely to be black and had a higher BMI at their enrollment visit compared to the full cohort while those included in the hypertension analysis were on average more physically active and had lower systolic and diastolic blood pressure at enrollment.

For each analysis, the mean number of examinations was 4.3. For the diabetes case analysis, the mean time of observation before ascertainment of diabetes was 6.8 years (SD=3.7) with a mean time of observation after ascertainment of diabetes of 4.1 years (SD=3.9). The mean time of observation before and after ascertainment of hypertension was 5.9 years (SD=3.4) and 5.0 years (SD=4.1), respectively. The mean age at ascertainment of diabetes and hypertension was 77 years (age at ascertainment of diabetes=77.3 years and hypertension=76.9 years).

Diabetes Case Analysis and Global Cognitive Function

In the diabetes case only analysis, global cognitive function declined at a rate of -0.02 units per year prior to the ascertainment of diabetes, before adjustment (95% CL= -0.03, -0.01). This rate of decline hastened to -0.04 (95% CL=-0.05, -0.03) units per year in the period after the ascertainment of diabetes (deviation slope= -0.02; 95% CL= -0.03, -0.003; p=0.01 for test of null hypothesis of no difference in slope) and is illustrated in **Figure 3.1**. The rate of annual decline for global cognitive function prior to the ascertainment of diabetes was unchanged with adjustment for age at diagnosis of diabetes; however the rate of decline for global cognitive function in the period after ascertainment of diabetes was attenuated, no longer statistically significantly different from the decline in the pre-breakpoint phase (deviation slope= -0.01; 95% CL= -0.03, 0.001; p=0.08). Adjustment for sex, race, enrollment cohort, and baseline levels of education, BMI, physical activity, smoking status, alcohol consumption, psychosocial measures, *APOE* ε4 status, and time-varying systolic blood pressure (and antihypertensive medication use) and diabetes medications did not alter these estimates

appreciably and can be compared to the annual decline associated with an increase in one year of age in **Table 3.2**.

Diabetes Case Analysis and Specific Domains

Figures 3.2 to 3.5 show the unadjusted annual cognitive function in the pre- and post-breakpoint periods for the four domain-specific neurocognitive tests. After adjustment, the rate of annual decline was significantly greater in the post-breakpoint period (post-breakpoint rate= -0.3; 95% CL= -0.4, -0.2) compared to the pre-breakpoint period (pre-breakpoint rate= -0.2; 95% CL= -0.2, -0.1) for scores of the Mini-Mental Status Exam (fully adjusted deviation slope: -0.1; 95% CL= -0.2, 0.0; $p<0.005$). The pre-breakpoint rate of annual decline on the Symbol-Digit Modalities Test was -0.5 (95% CL= -0.7, -0.4) while the post-breakpoint rate was -0.8 (95% CL= -1.0, -0.7). The post-breakpoint rate of annual decline on the Symbol-Digit Modalities Test was significantly greater compared to the pre-breakpoint rate of decline (fully adjusted deviation slope: -0.3; 95% CL= -0.5, -0.1; $p<0.05$). There was not an appreciable difference between the rate of annual decline in the pre- and post-breakpoint period observed for either derivations of the East Boston Memory Tests (Immediate and Delayed Recall).

The pre-breakpoint annual cognitive decline and post-breakpoint annual cognitive decline did not differ by sex, race, or *APOE* ε4 status for diabetes cases. Accounting for selective attrition did not alter estimates from the final adjusted model (data not shown). When this association was modeled as a quadratic association between time and annual cognitive decline, both beta estimates for time and quadratic time were negative (p for quadratic term: $p<0.05$).

Hypertension Case Analysis and Global Cognitive Function

For the hypertension case only analysis, the annual unadjusted rate of global cognitive decline prior to the ascertainment of hypertension was -0.02 units per year (95% CL= -0.03, -0.01) shown in **Table 3.3**. This unadjusted rate of annual decline was -0.05 (95% CL=-0.05, -0.04) units per year after the ascertainment of hypertension, accelerated by -0.03 units (95% CL= -0.04, -0.02; $p<0.0001$), illustrated in **Figure 3.6**. These results were unaltered by adjustment for potential confounding factors including the adjustment for time-varying systolic blood pressure. For comparison, a 1-year increment in age was associated with a rate of annual cognitive decline equal to -0.03 (95% CL= -0.05, -0.01).

Hypertension Case Analysis and Specific Domains

The rate of annual decline in score for the Mini-Mental Status Exam during the pre-breakpoint period was -0.2 (95% CL= -0.2, -0.1) points per year. In the post-breakpoint period, this rate was -0.4 (95% CL= -0.4, -0.3) points per year. This was a change in rate of decline equal to -0.2 points (95% CL= -0.2, -0.1). For the Symbol Digit Modalities Test, the rate of annual decline during the pre-breakpoint period was -0.5 (95% CL= -0.7, -0.4) symbols per year. After the breakpoint, the rate of annual decline was -0.8 (95% CL= -0.9, -0.7) symbols per year (deviation slope: -0.3; 95% CL = -0.4, -0.1). No appreciable difference in pre- and post-breakpoint rate of annual decline was observed for either of the memory tests (**Figures 3.7 to 3.10**).

The pre-breakpoint annual cognitive decline and post-breakpoint annual cognitive decline were consistent across sex, race, and *APOE* ε4 status for hypertension cases.

Accounting for selective attrition did not alter estimates compared to the final un-weighted adjusted model (IPAW data not shown). When we modeled this association with a quadratic term for time, without piecewise methods, we observed a decreasing quadratic association between time and annual cognitive decline (estimates for time and quadratic time both negative; p for quadratic term: $p<0.05$).

3.5. Discussion

In this prospective population-based biracial cohort of elderly individuals, participants who developed diabetes or hypertension experienced significantly greater decline for specific cognitive domains in the period following their case ascertainment relative to the period preceding case ascertainment. We found annual decline increased 50% to 60% in the period after the ascertainment of diabetes and hypertension relative to the period before ascertainment, primarily for scores from the Mini-Mental Status Exam and the Symbol Digit Modalities Test, which reflect alterations to general orientation and cognitive processing during this stage in disease progression. However, individuals who developed diabetes and hypertension did not experience change in annual declines in immediate and delayed memory. Our primary results were unchanged after accounting for attrition. These results suggest individuals who develop diabetes or hypertension experience significant changes in specific cognitive domains during the time period surrounding the development of each of these diseases.

Findings from research assessing the differences in cognitive function between individuals with and without diabetes and hypertension may help with understanding our results. Comparing individuals with known diabetes to those without, Rawlings et al

found significantly greater decline in scores from tests of global cognition, executive function, and processing speed, but not memory over 20 years of follow-up in the Atherosclerosis Risk in Communities study.[99] Additionally, individuals with prediabetic levels of HbA1c or undiagnosed diabetes had an increased rate of decline in global cognition compared to individuals with normal glycemia.[99] In contrast, Tuligenga et al observed greater decline over 10 years in cognitive domains of memory, reasoning, and global cognition in individuals with known diabetes (and worse control of diabetes) relative to their normoglycemic counterparts in Whitehall II, but did not observe a difference in cognitive decline in these areas when comparing individuals with normal glycemia to those identified with prediabetes or newly diagnosed diabetes.[179] Interventions intended to achieve glycemic control or weight loss in individuals with diabetes have found that cognitive scores do not differ between treatment arms. In the Look AHEAD (Action for Health in Diabetes), while the intensive lifestyle intervention group lost significantly more weight over 8 years of follow-up, scores from cognitive tests on verbal fluency and memory, attention, executive function, and processing speed did not differ between groups at this time.[197] While achieving significantly lower median HbA1c than the control arm, the ACCORD MIND (Action to Control Cardiovascular Risk in Diabetes – Memory in Diabetes) intervention group not differ from the control group in average scores on tests of verbal memory, processing speed, and executive function at 20 and 40 months of follow-up.[198] Taken together this may suggest that intervening after the occurrence of diabetes is too late in the natural history of the disease to slow cognitive decline and controlling blood glucose levels earlier in life is critical to minimizing cognitive decline later in life.

Similarly, maintaining normal blood pressure levels in early adulthood and midlife may be more important to later cognitive function and decline than maintaining normal blood pressure levels later in life. Compared to individuals with normal blood pressure in midlife, ARIC participants with hypertension in midlife had significantly greater 20-year cognitive decline on neurocognitive tests assessing executive and verbal function, sustained attention, psychomotor speed and global cognition.[85] In this same study, individuals defined as pre-hypertensive at baseline did not experience significantly greater cognitive decline relative to individuals with normal blood pressure.[85] This cognitive decline associated with overt hypertension in midlife was shown across race/ethnicities and can manifest as clinical events in later adulthood; individuals with hypertension in midlife are more likely to be hospitalized for dementia in the future compared to individuals without hypertension.[90,93] However, high blood pressure as determined in later adulthood is not consistently shown to be associated with increased cognitive decline compared to with normal blood pressure.[85,92] This is possibly due to the effects of blood pressure already being borne out by this time or due to use of antihypertensive medications modifying cognitive effects of hypertension. In this current study, we did not observe significant change in our estimates after accounting for level of systolic blood pressure in later life.

Both diabetes and hypertension are best thought of as cut-points on a continuum of disease development. The reason for a change in the trajectory of cognitive decline at this cut-point is unclear, but may be explained by the limited number of exams and duration between assessments, roughly 3 years. For example, three years is sufficient time to progress from mild impairment to Alzheimer's.[73,78] However, it may be the

experience of developing the conditions acting through a psychosocial pathway, exacerbating decline. For example, individuals who develop diabetes are at increased risk for depression and depression is associated with greater cognitive decline and incidence of dementia.[158,159,199] This may suggest the changes observed in cognitive decline are mediated by depressive symptoms though this is untested in our data. It is unclear why we observed differential results by cognitive test for each condition. For instance, it has been suggested that in addition to processing speed and executive function, memory is one the cognitive domains most negatively affected by diabetes.[200] However, as noted above, studies reporting differences in scores on cognitive tests of memory between individuals with and without diabetes are mixed in their findings.[99,179] The Mini-Mental Status Exam and the Symbol Digit Modalities Test assess higher order mental functions including substitution tasks, attention, visual scanning, and motor speed associated with the frontal lobe.[15,50-52] The brain is an adaptive organ, receptive to stimulation and training, and these differential results may be explained in the context of cognitive scaffolding.[13] Cognitive scaffolding is theorized as a compensatory process facilitated by a healthy prefrontal cortex by which the brain maintains functional capacity during aging and neuronal challenges by developing new neuronal pathways and building on strong existing neuronal connections.[13] These tests may be capturing the functional deterioration of the prefrontal cortex occurring with the development of diabetes and hypertension. It may also be that the Mini-Mental Status Exam and the Symbol Digit Modalities Test are more sensitive to changes in cognition in general than the East Boston memory tests. However, this is unconfirmed speculation.

Limitations of this work should be noted. First, piece-wise models are ideal for quantifying a characteristic surrounding an event of interest that has a demarcated occurrence (e.g., myocardial infarction or implementation of a new law). It is likely the effects of diabetes and hypertension on the brain are heterogeneous for individuals across the spectrum of these conditions. In an attempt to allow for these individual differences, we included random effects into our model for both intercept and slope, allowing individual deviation from the population means for baseline cognitive function and annual decline over time. Second, assessments occurred, on average, every three years limiting the ascertainment of diabetes and hypertension and the measurement of cognitive function to large time windows which may not capture the sensitive changes in cognition related to disease status. The actual time of onset of diabetes and hypertension is unknown. Third, case ascertainment of diabetes included data from self-report of diabetes diagnosis by physician in addition to medication inventory. Self-report of diabetes diagnosis would be a concern if lower cognitive functioning individuals misreport diabetes status. In this analysis all individuals identified as having diabetes via self-report were also determined to use diabetes medications (either concurrently or at a subsequent point). Fourth, the design of this analysis required observing individuals at a time preceding and subsequent to determination of diabetes and hypertension. This has become a selected sample, subject to length time bias and attrition. We attempted to account for attrition using IPAW methods and did not observe an impact on our estimates. We did not use methods to account for selection (i.e., length time) bias. However, all individuals over the age of 65 years living in this community were invited to participate, and initial recruitment of the eligible population was nearly 80%.

Nevertheless, our results may not generalize to individuals who develop diabetes and hypertension at ages younger than this cohort. Strengths of this analysis include the large number of biracial participants from a geographically defined population and repeated measurement of cognitive assessment up to 6 occasions including 4 unique cognitive domains.

Overall this work suggests older individuals who develop diabetes and hypertension are susceptible to acceleration in rate of cognitive decline in domains of general orientation, attention, and cognitive processing surrounding the ascertainment of these conditions. From a clinical perspective, it may be important for clinicians to identify changes in patients' cognitive function, particularly in individuals with comorbid conditions which require an understanding of and ability to manage complications due to these diseases and accompanying prescription medications. Clinicians may use these findings to prepare patients for experiencing these changes and incorporate this knowledge into their management plan of diabetes and hypertension, possibly offering resources and actions to minimize these unfavorable cognitive changes. This research can be elaborated upon and improved with more frequent assessment of both cognitive function and diabetes and hypertension, furthermore assessing additional unique cognitive domains and using comprehensive and clinical methods of diabetes and hypertension ascertainment.

3.6. Tables

Table 3.1. Participant characteristics at the time of enrollment for the full cohort, the diabetes case analysis, and the hypertension case analysis, the Chicago Health and Aging Project (1993-2011)

Characteristic	Full Cohort	Diabetes Cases	Hypertension Cases
N	10,802	405	837
Age, years	73.4 ± 7.1	70.5 ± 4.6	71.0 ± 5.1
Age at diagnosis, years	NA	77.3 ± 6.0	76.9 ± 6.1
Men, %	38	40	37
Black, %	63	75	60
> High school education, %	39	40	46
BMI, kg/m^2	27.8 ± 6.0	30.2 ± 5.8	27.1 ± 5.1
Alcohol none daily, %	69	72	62
Alcohol moderate daily, %	24	21	32
Former smoker, %	39	43	36
Current smoker, %	14	14	16
Physical activity, hours/week	3.0 ± 5.0	3.1 ± 5.3	3.8 ± 5.8
Systolic blood pressure, mm Hg	138 ± 20	139 ±17	125 ±10
Diastolic blood pressure, mm Hg	78 ± 11	79 ± 11	74 ± 8
Hypertensive, %	74	80	0
Diabetes, %	16	0	8
APOE ε4 status (≥1 ε4 allele), %	33	34	32
Mini-mental status exam, (0-30)	25.9 ± 5.3	27.1 ± 3.6	27.4 ± 3.2
Symbol digit modalities test	29.0 ±14.4	31.5 ± 12.7	33.7 ± 13.8
East Boston immediate recall, (0-12)	8.4 ± 2.8	8.8 ± 2.5	9.0 ± 2.4
East Boston delayed recall, (0-12)	7.9 ± 3.2	8.2 ± 2.5	8.5 ± 2.7

Values are means ± standard deviations for continuous and percentages for categorical. Moderate alcohol consumption is any up to 1 drink daily for women and any up to 2

drinks daily for men. NA is not applicable. Physical activity is hours per week of walking, yard-work, calisthenics, biking, swimming, and other activities of exercise.

Table 3.2. Adjusted mean cognitive function scores at the time of diabetes ascertainment and annual rate of change in cognitive function before and after this time (n=405), the Chicago Health and Aging Project (1993-2011)

			Neurocognitive Test			
		Global *Z-score*	Mini-Mental Status Exam	Symbol Digit Modalities Test	East Boston Immediate Recall	East Boston Delayed Recall
Mean at diabetes ascertainment		0.19	26.2	28.4	8.6	8.1
	95% CL	(0.12, 0.26)	(25.8, 26.7)	(27.3, 29.6)	(8.4, 8.8)	(7.8, 8.3)
Model		Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)
Unadjusted	Before	-0.02 (-0.03, -0.01)	-0.1 (-0.2, -0.1)	-0.5 (-0.6, -0.4)	-0.04 (-0.07, 0.00)	-0.03 (-0.07, 0.00)
	After	-0.04 (-0.05, -0.03)	-0.3 (-0.4, -0.2)	-0.8 (-1.0, -0.6)	-0.04 (-0.09, 0.00)	-0.07 (-0.12, -0.01)
	Deviation	-0.02 (-0.03, 0.00)	-0.2 (-0.3, -0.1)	-0.3 (-0.6, -0.1)	-0.01 (-0.07, 0.05)	-0.03 (-0.10, 0.04)
P for test of deviation slope		0.01	<0.0001	<0.005	0.84	0.35
Model 1	Before	-0.03 (-0.03, -0.02)	-0.2 (-0.2, -0.1)	-0.5 (-0.7, -0.4)	-0.06 (-0.09, -0.03)	-0.06 (-0.10, -0.02)
	After	-0.04 (-0.05, -0.03)	-0.3 (-0.4, -0.2)	-0.8 (-1.0, -0.7)	-0.04 (-0.08, 0.01)	-0.06 (-0.11, -0.01)
	Deviation	-0.01 (-0.03, 0.00)	-0.2 (-0.2, -0.1)	-0.3 (-0.5, -0.1)	0.02 (-0.04, 0.08)	0.00 (-0.07, 0.07)
P for test of deviation slope		0.08	<0.001	<0.05	0.48	0.94

Model 2	Before	-0.03 (-0.04, -0.02)	-0.2 (-0.2, -0.1)	-0.6 (-0.7, -0.4)	-0.07 (-0.10, -0.03)	-0.07 (-0.11, -0.03)
	After	-0.04 (-0.05, -0.03)	-0.3 (-0.4, -0.2)	-0.8 (-1.0, -0.7)	-0.04 (-0.09, 0.00)	-0.06 (-0.12, -0.01)
	Deviation	-0.01 (-0.03, 0.00)	-0.1 (-0.2, 0.00)	-0.3 (-0.5, -0.1)	0.03 (-0.03, 0.09)	0.01 (-0.06, 0.08)
P for test of deviation slope		0.10	<0.01	<0.05	0.39	0.86
Model 3	Before	-0.03 (-0.04, -0.02)	-0.2 (-0.2, -0.1)	-0.5 (-0.7, -0.4)	-0.07 (-0.11, -0.03)	-0.06 (-0.11, -0.02)
	After	-0.04 (-0.05, -0.03)	-0.3 (-0.4, -0.2)	-0.8 (-1.0, -0.7)	-0.04 (-0.09, 0.00)	-0.06 (-0.12, -0.01)
	Deviation	-0.01 (-0.03, 0.01)	-0.1 (-0.2, 0.0)	-0.3 (-0.5, -0.1)	0.03 (-0.04, 0.09)	0.00 (-0.07, 0.08)
P for test of deviation slope		0.09	<0.005	<0.05	0.39	0.99
Beta estimate for age, 1 year		-0.02	-0.1	-0.6	-0.05	-0.07

Model 1 is adjusted for age at ascertainment of diabetes, sex, race, education, and enrollment cohort. Model 2 is adjusted for model 1 covariates and baseline level of BMI, physical activity, smoking status, regular alcohol consumptions, and time-varying systolic blood pressure (and antihypertensive medication use). Model 3 is adjusted for model 2 covariates and psychosocial measures of depression and cognitive activities, *APOE* ε4 status, and time-varying diabetes medications use. The difference between "before" and "after" mathematically equals the deviation. Instances where this does not appear so is due to rounding. "P for test of deviation slope" tests the null hypothesis the deviation slope = 0. Beta estimate for age provides a comparison of estimates to an increase in 1 year of age.

Table 3.3. Adjusted mean cognitive function scores at the time of hypertension ascertainment and annual rate of change in cognitive function before and after this time (n=837), the Chicago Health and Aging Project (1993-2011)

		Neurocognitive Test				
		Global *Z-score*	Mini-Mental Status Exam	Symbol Digit Modalities Test	East Boston Immediate Recall	East Boston Delayed Recall
Mean at hypertension ascertainment		0.29	26.6	31.0	8.8	8.4
	95% CL	(0.24, 0.34)	(26.3, 26.9)	(30.1, 31.9)	(8.7, 9.0)	(8.2, 8.6)
Model		Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)
Unadjusted	Before	-0.02 (-0.03, -0.01)	-0.1 (-0.2, -0.1)	-0.5 (-0.6, -0.3)	-0.02 (-0.05, 0.00)	-0.03 (-0.06, 0.00)
	After	-0.05 (-0.05, -0.04)	-0.4 (-0.4, -0.3)	-0.8 (-0.9, -0.7)	-0.07 (-0.10, -0.04)	-0.10 (-0.14, -0.07)
	Deviation	-0.03 (-0.04, -0.02)	-0.2 (-0.3, -0.2)	-0.3 (-0.5, -0.2)	-0.05 (-0.09, -0.01)	-0.08 (-0.13, -0.03)
P for test of deviation slope		<0.0001	<0.0001	<0.001	<0.05	<0.005
Model 1	Before	-0.02 (-0.03, -0.02)	-0.1 (-0.2, -0.1)	-0.5 (-0.6, -0.4)	-0.04 (-0.07, -0.01)	-0.05 (-0.08, -0.02)
	After	-0.05 (-0.05, -0.04)	-0.4 (-0.4, -0.3)	-0.8 (-0.9, -0.7)	-0.06 (-0.09, -0.04)	-0.10 (-0.13, -0.06)
	Deviation	-0.02 (-0.03, -0.01)	-0.2 (-0.3, -0.1)	-0.3 (-0.5, -0.1)	-0.03 (-0.07, 0.01)	-0.05 (-0.10, 0.00)
P for test of deviation slope		<0.0001	<0.0001	<0.01	0.18	<0.05
Model 2	Before	-0.03 (-0.04, -0.02)	-0.2 (-0.2, -0.1)	-0.5 (-0.7, -0.4)	-0.07 (-0.10, -0.03)	-0.08 (-0.12, -0.05)

	After	-0.05 (-0.06, -0.04)	-0.4 (-0.4, -0.3)	-0.8 (-0.9, -0.7)	-0.08 (-0.10, -0.05)	-0.10 (-0.14, -0.08)
	Deviation	-0.02 (-0.03, -0.01)	-0.2 (-0.2, -0.1)	-0.3 (-0.4, -0.1)	-0.01 (-0.05, 0.04)	-0.03 (-0.08, 0.02)
P for test of deviation slope		<0.0001	<0.0001	<0.01	0.70	0.25
Model 3	Before	-0.03 (-0.04, -0.02)	-0.2 (-0.2, -0.1)	-0.5 (-0.7, -0.4)	-0.07 (-0.10, -0.03)	-0.08 (-0.12, -0.05)
	After	-0.05 (-0.06, -0.04)	-0.4 (-0.4, -0.3)	-0.8 (-0.9, -0.7)	-0.08 (-0.10, -0.05)	-0.10 (-0.14, -0.08)
	Deviation	-0.02 (-0.03, -0.01)	-0.2 (-0.2, -0.1)	-0.3 (-0.4, -0.1)	-0.01 (-0.05, 0.04)	-0.03 (-0.08, 0.02)
P for test of deviation slope		<0.0001	<0.0001	<0.01	0.70	0.25
Beta estimate for age, 1 year		-0.03	-0.1	-0.7	-0.07	-0.09

Model 1 is adjusted for age at ascertainment of hypertension, sex, race, education, and enrollment cohort. Model 2 is adjusted for model 1 covariates and baseline level of BMI, physical activity, smoking status, regular alcohol consumptions, and time-varying systolic blood pressure (and antihypertensive medication use). Model 3 is adjusted for model 2 covariates and psychosocial measures of depression and cognitive activities, *APOE* ε4 status, and time-varying diabetes medications use. The difference between "before" and "after" mathematically equals the deviation. Instances where this does not appear so is due to rounding. "P for test of deviation slope" tests the null hypothesis the deviation slope = 0. Beta estimate for age provides a comparison of estimates to an increase in 1 year of age.

3.7. Figures

Figure 3.1. Unadjusted mean annual cognitive function and 95% confidence limits (blue dash) according to global *z*-score before and after the ascertainment of diabetes (n=405), the Chicago Health and Aging Project (1993-2011)

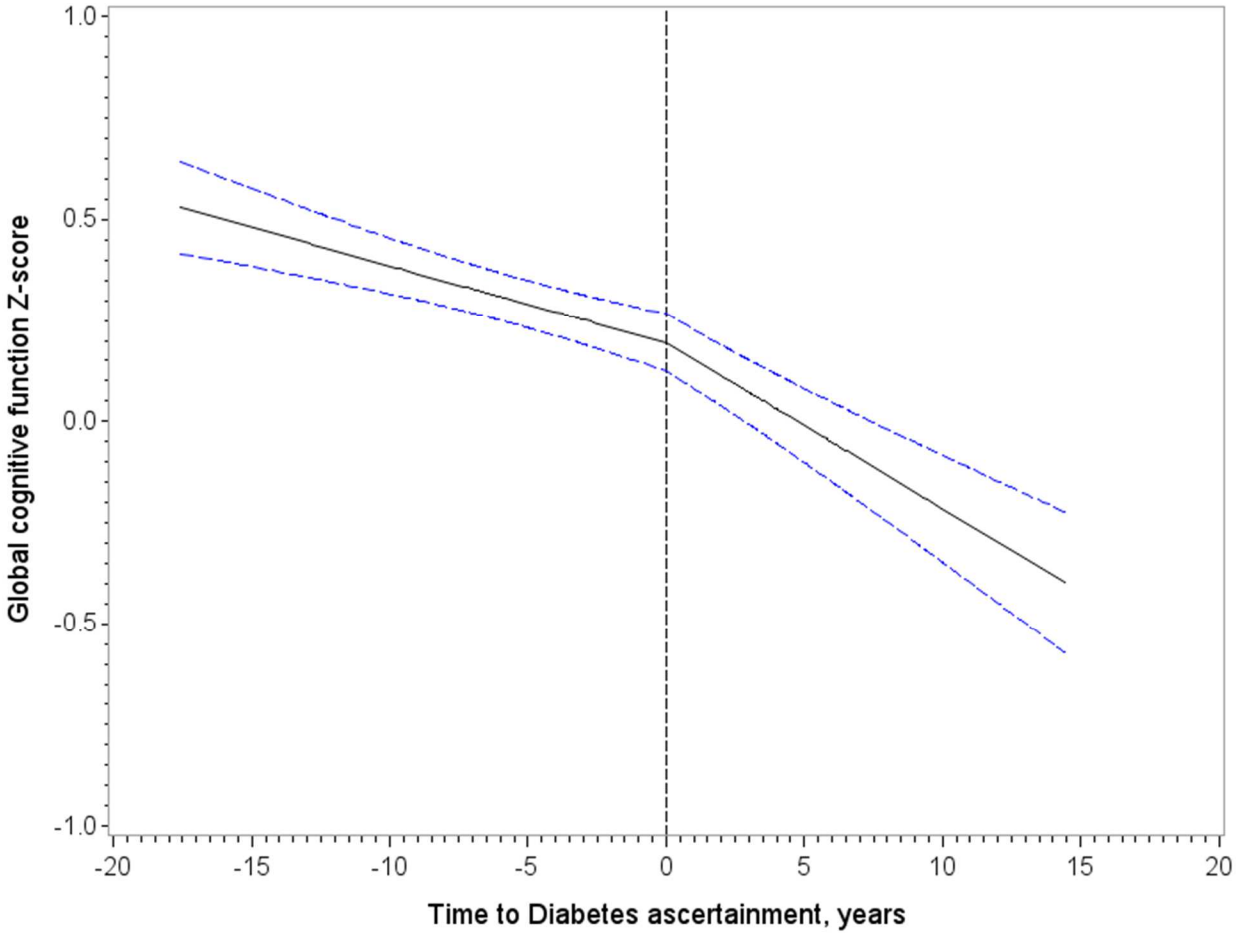


Figure 3.2. Unadjusted mean annual score and 95% confidence limits (blue dash) on the mini-mental status exam before and after the ascertainment of diabetes (n=405), the Chicago Health and Aging Project (1993-2011)

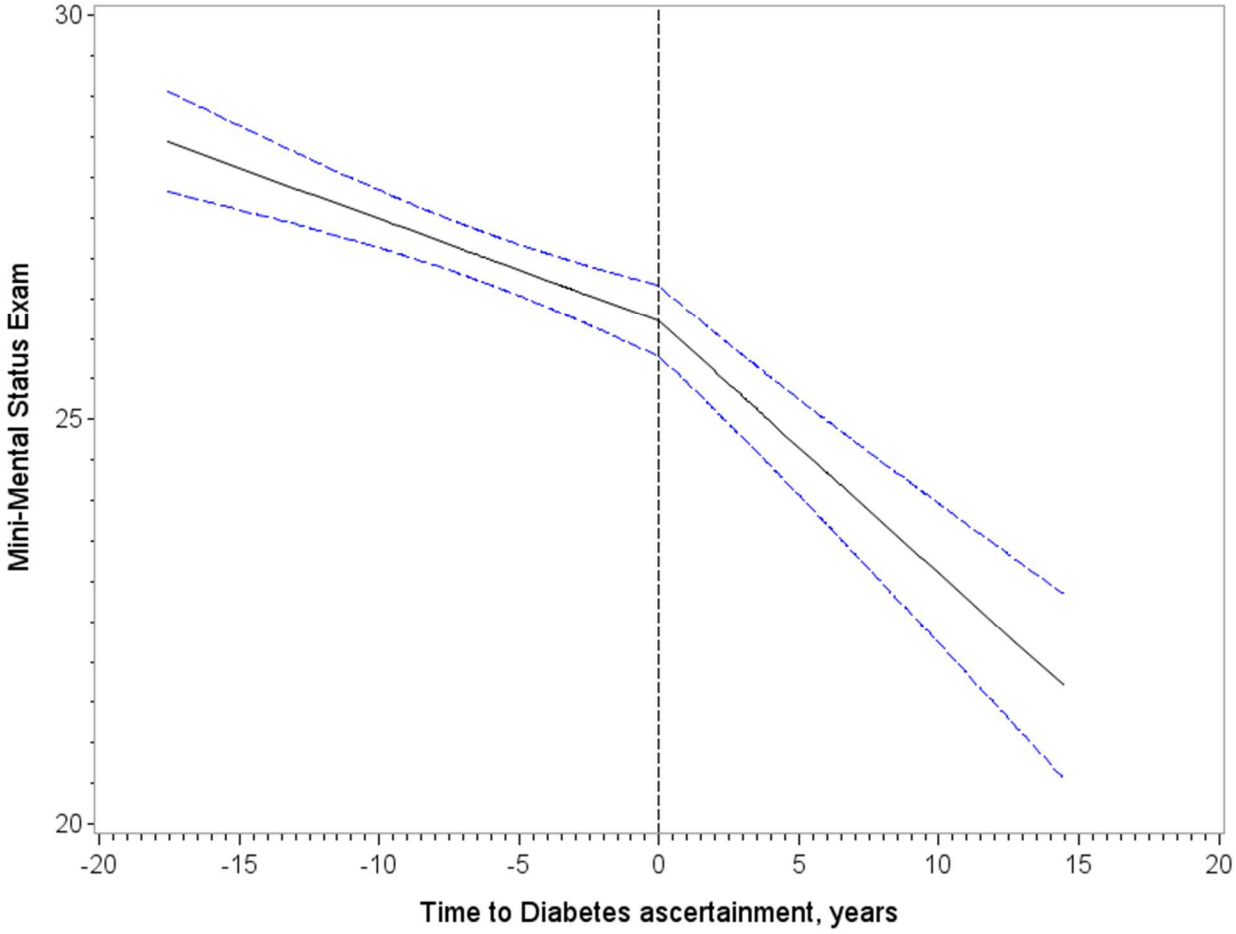


Figure 3.3. Unadjusted mean annual score and 95% confidence limits (blue dash) on the symbol digit modalities test before and after the ascertainment of diabetes (n=405), the Chicago Health and Aging Project (1993-2011)

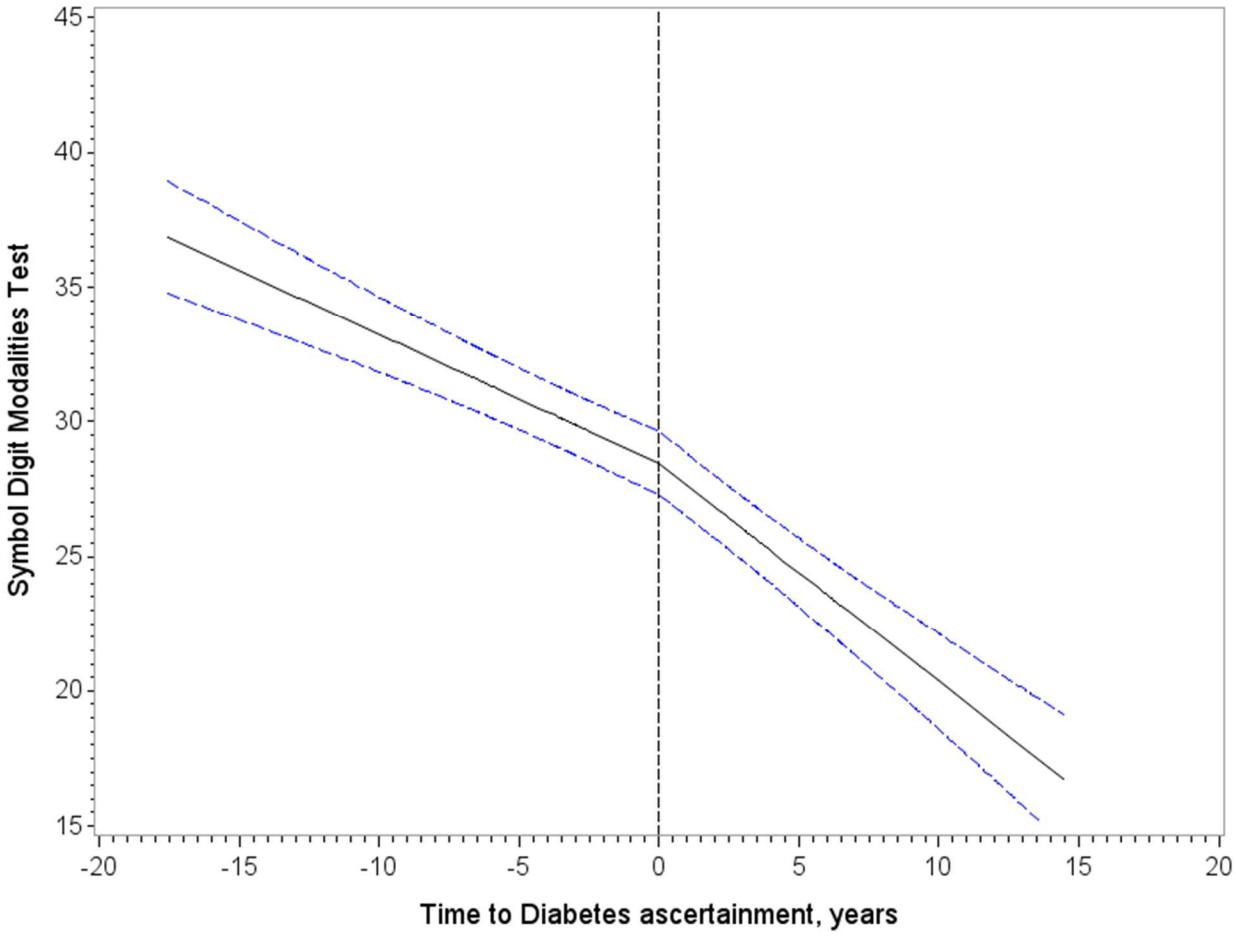


Figure 3.4. Unadjusted mean annual score and 95% confidence limits (blue dash) on the East Boston immediate recall test before and after the ascertainment of diabetes (n=405), the Chicago Health and Aging Project (1993-2011)

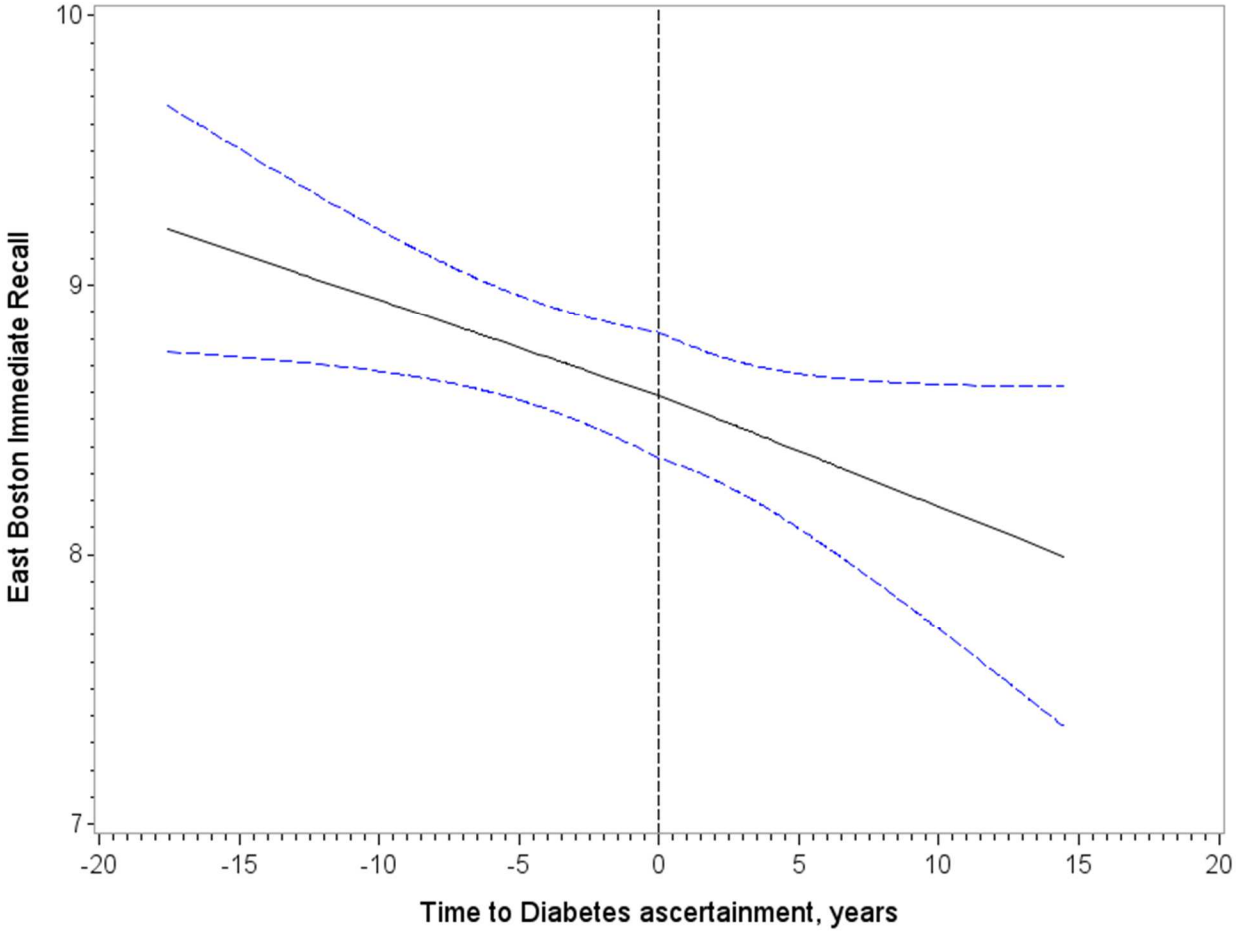


Figure 3.5. Unadjusted mean annual score and 95% confidence limits (blue dash) on the East Boston delayed recall test before and after the ascertainment of diabetes (n=405), the Chicago Health and Aging Project (1993-2011)

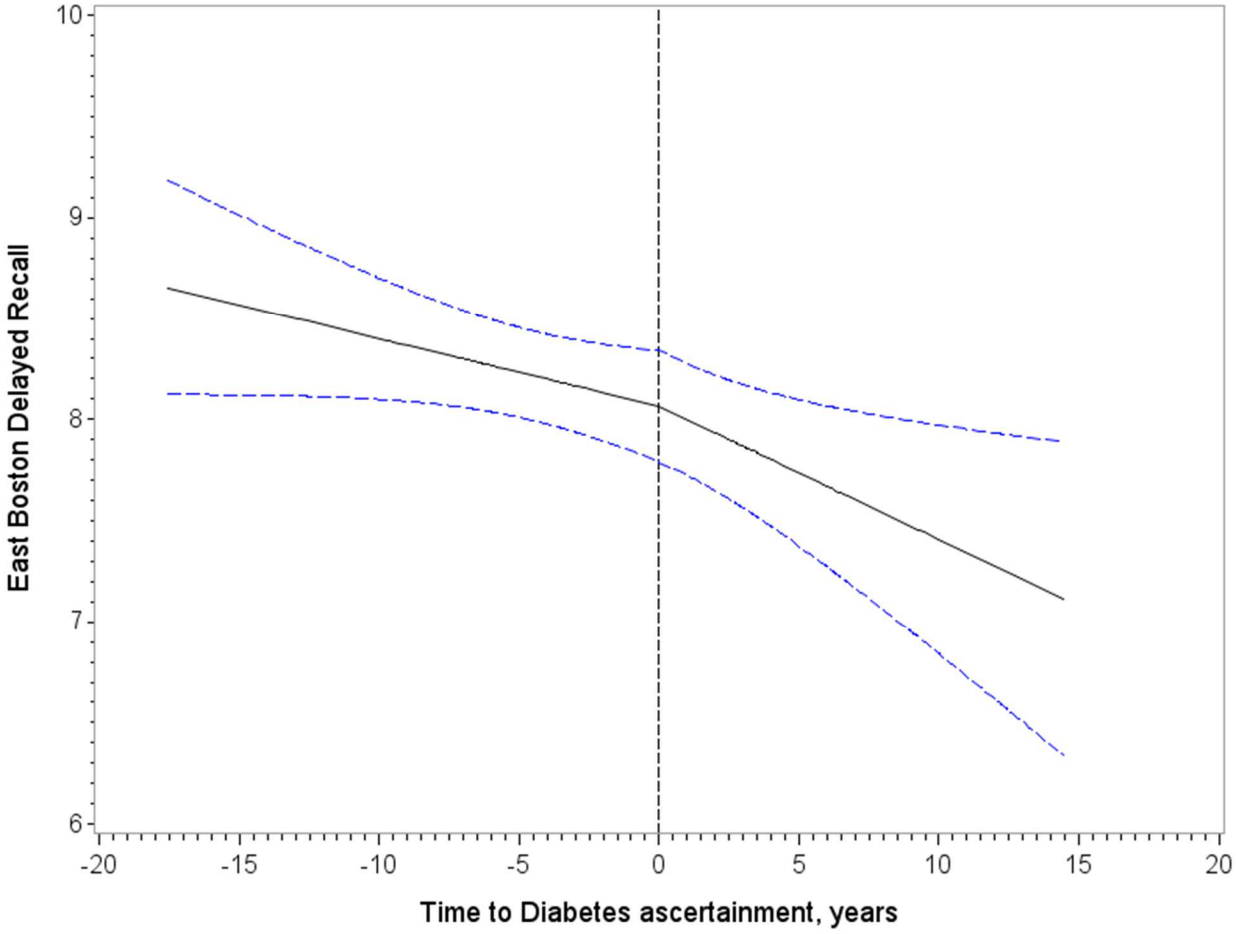


Figure 3.6. Unadjusted mean annual cognitive function and 95% confidence limits (blue dash) according to global *z*-score before and after the ascertainment of hypertension (n=837), the Chicago Health and Aging Project (1993-2011)

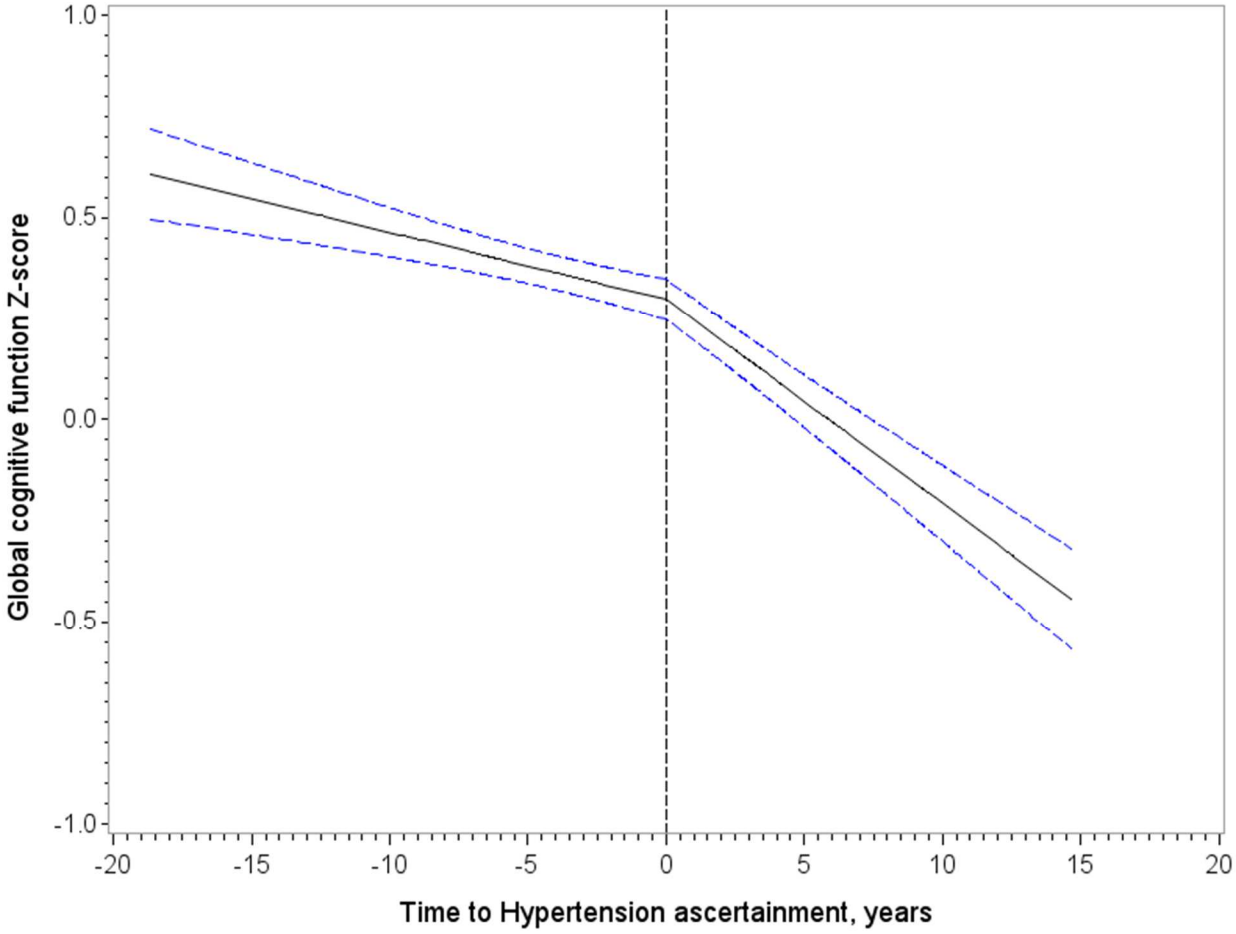


Figure 3.7. Unadjusted mean annual score and 95% confidence limits (blue dash) on the mini-mental status exam before and after the ascertainment of hypertension (n=837), the Chicago Health and Aging Project (1993-2011)

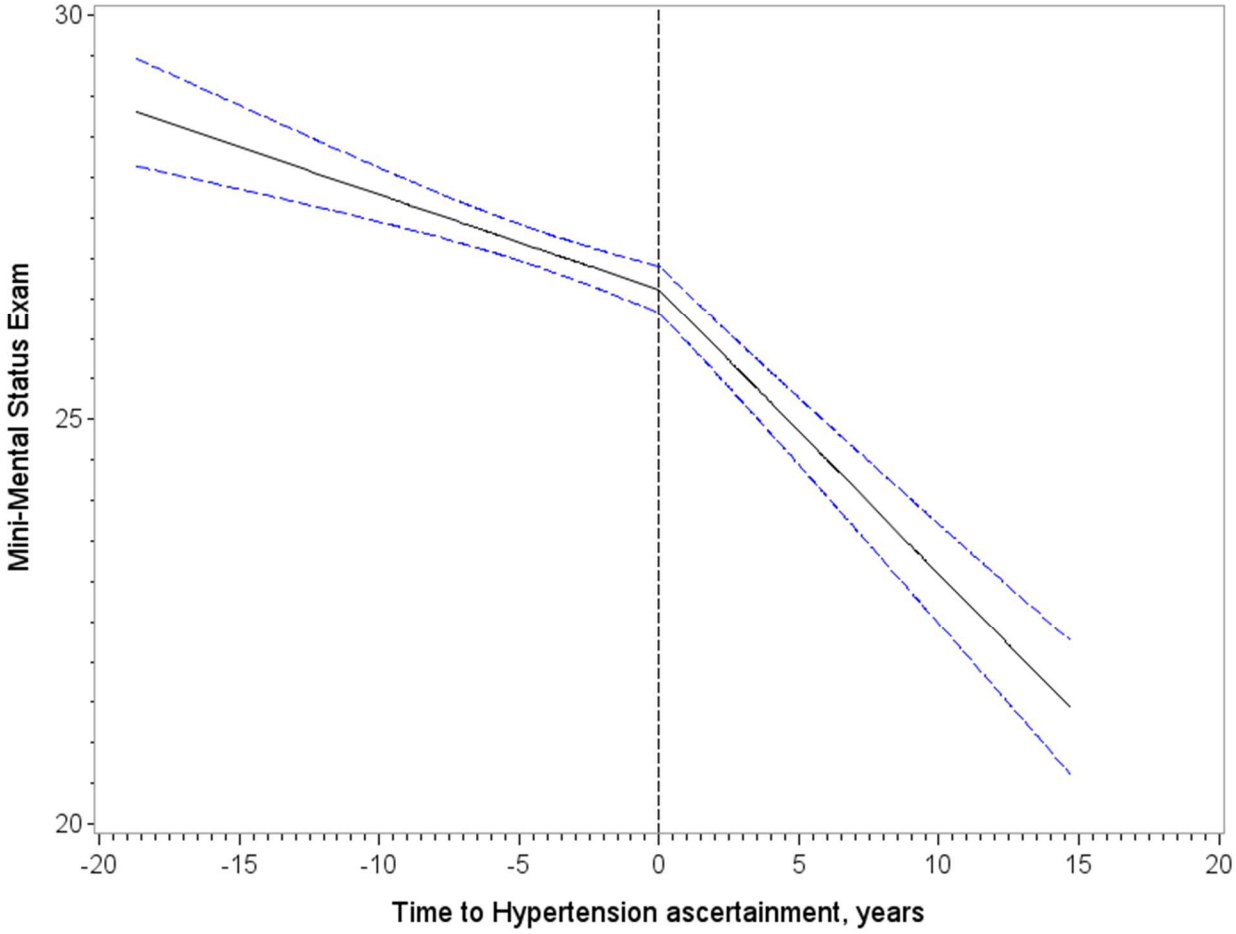


Figure 3.8. Unadjusted mean annual score and 95% confidence limits (blue dash) on the symbol digit modalities test before and after the ascertainment of hypertension (n=837), the Chicago Health and Aging Project (1993-2011)

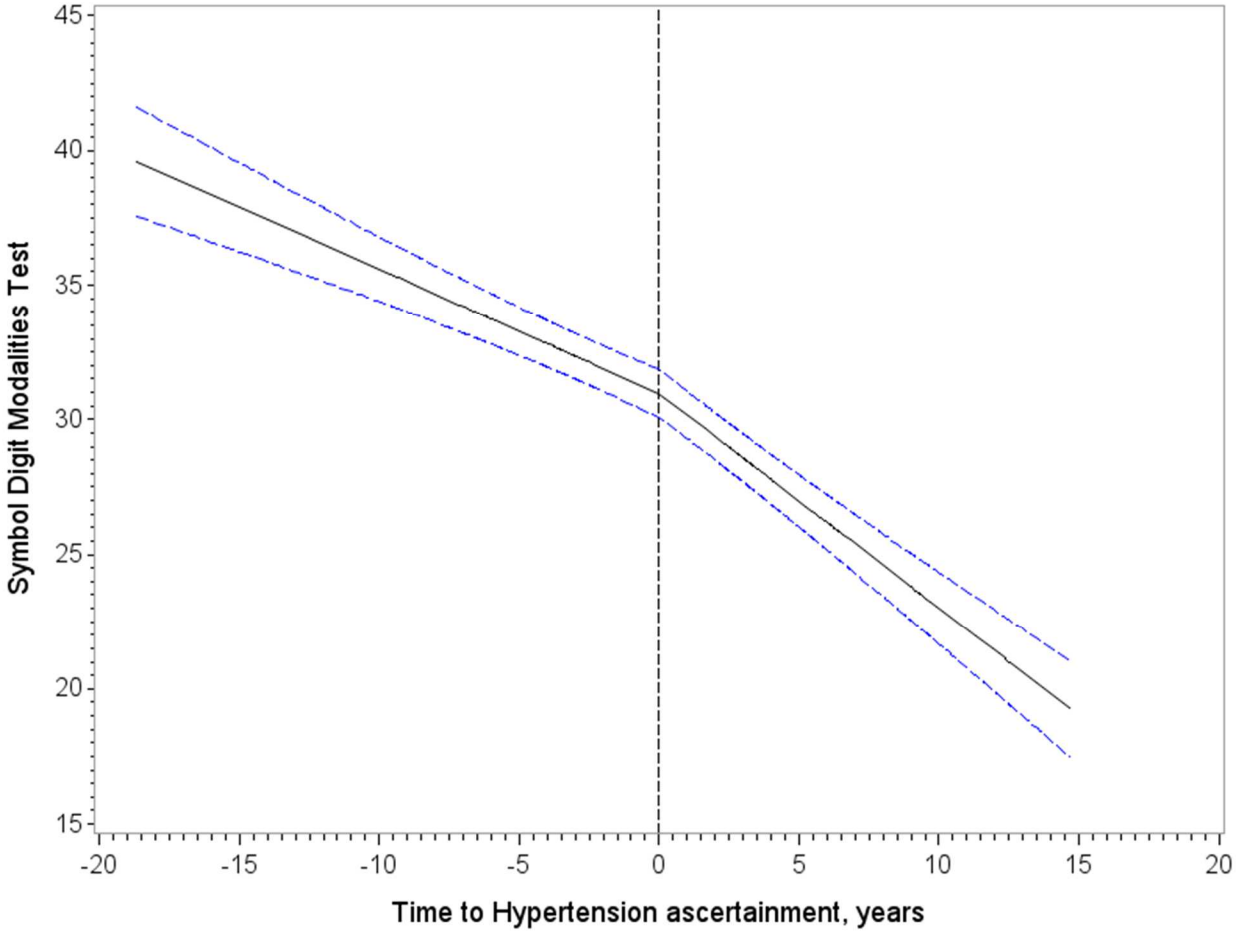


Figure 3.9. Unadjusted mean annual score and 95% confidence limits (blue dash) on the East Boston immediate recall test before and after the ascertainment of hypertension (n=837), the Chicago Health and Aging Project (1993-2011)

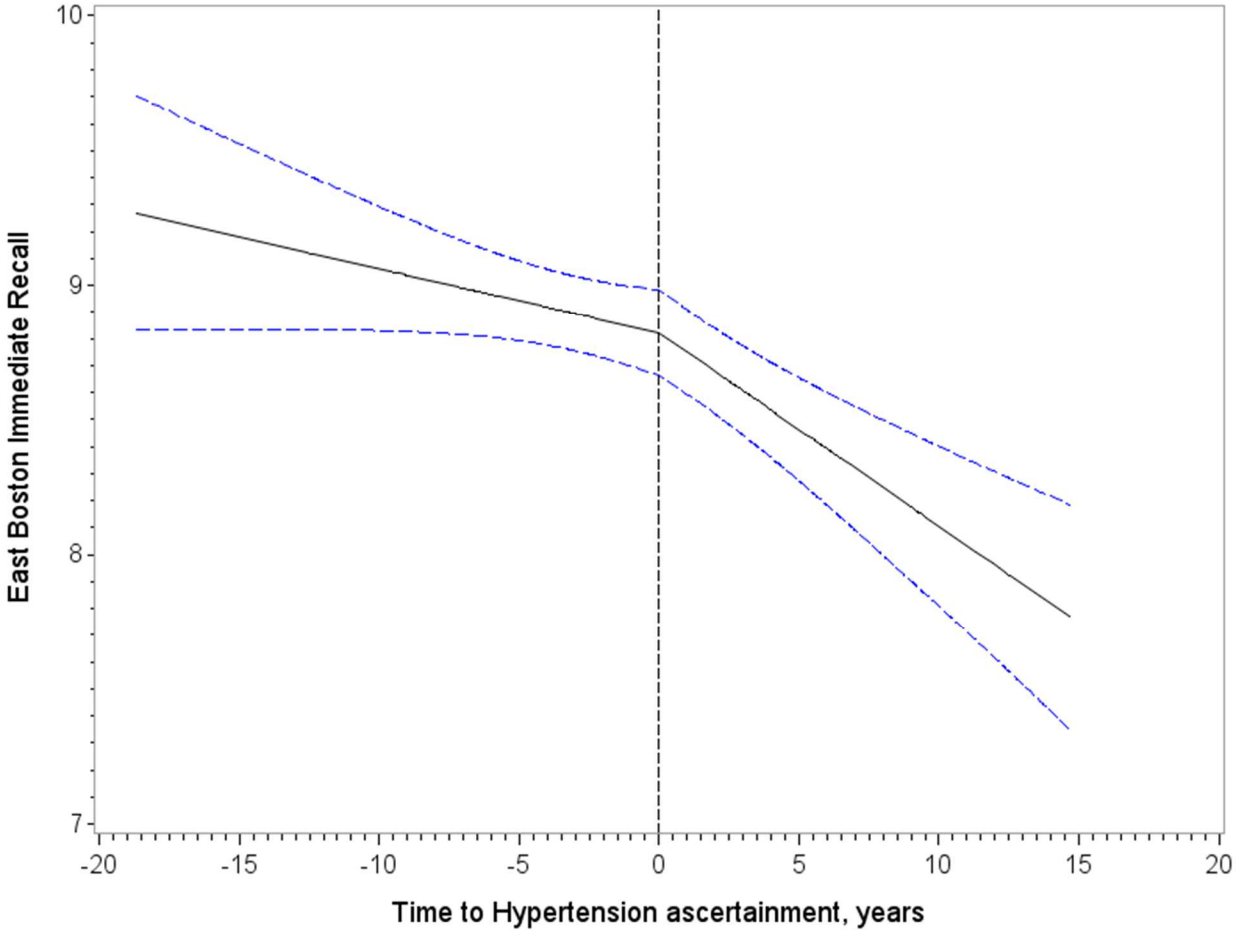
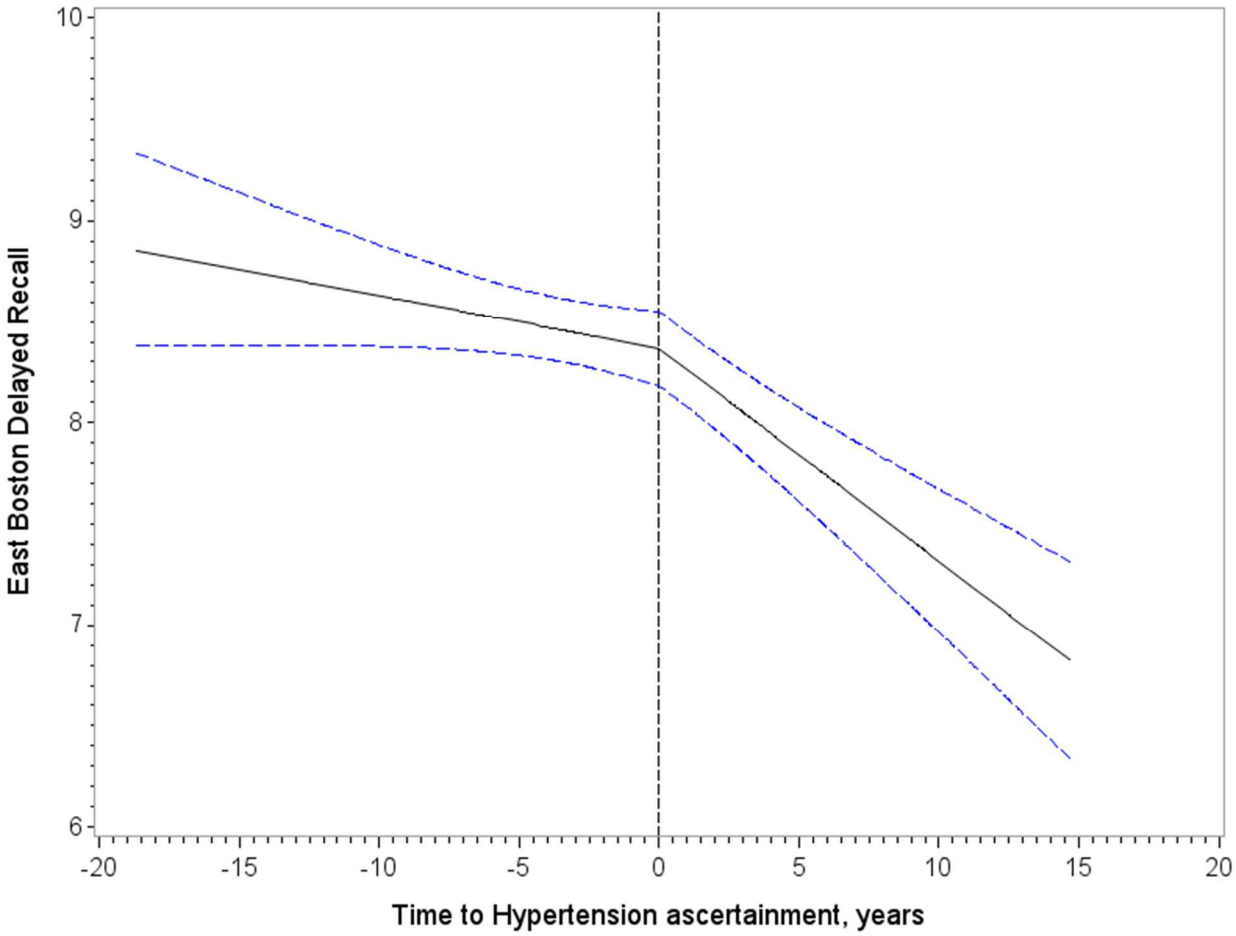


Figure 3.10. Unadjusted mean annual score and 95% confidence limits (blue dash) on the East Boston delayed recall test before and after the ascertainment of hypertension (n=837), the Chicago Health and Aging Project (1993-2011)



Chapter 4. Manuscript 2: Brain Function and Structure and Risk for Incident Diabetes; The Atherosclerosis Risk in Communities (ARIC) Study

4.1. Overview

Background: Overt diabetes is a risk factor for cognitive decline. Recent research suggests this association may extend in the opposite direction, where lower level of cognitive function may increase the risk for developing diabetes. Lack of adjustment for potential confounders, selective attrition, and behaviors downstream of baseline cognitive function complicate interpretation.

Methods: We used data from the prospective ARIC cohort to assess cognitive function and brain structure, including data from three neuropsychological tests (1990-1992) and brain magnetic resonance imaging (1993-1994). We estimated hazard ratios for developing diabetes after taking into account traditional diabetes risk factors, selective attrition, and time-varying level of diabetes risk factors according to level of cognitive function and grade of brain structure.

Results: After accounting for traditional diabetes risk factors, higher level of cognitive function (per 1 SD global *z*-score, HR: 0.95; 95% CI: 0.90, 1.00) and greater burden of white matter hyperintensities (per 1 grade increment, HR: 1.13; 95% CI: 1.01, 1.26) and ventricular size (per 1 grade increment, HR: 1.11; 95% CI: 1.00, 1.23) were marginally associated with increased risk for developing diabetes. Sulcal size was not associated with incidence of diabetes (per 1 grade increment, HR: 1.01; 95% CI: 0.90, 1.14). These

results were consistent in sensitivity analyses accounting for selective attrition and time-dependent levels of diabetes risk factors.

Conclusion: In this prospective study of middle aged adults, measures of cognitive function and MRI indicators of subclinical cerebrovascular disease were weakly and inconsistently associated with increased risk for diabetes. Brain health in middle age is not predictive of glucose metabolism in older adulthood.

4.2. Introduction

Diabetes is an established risk factor for cerebrovascular disease, wherein individuals with diabetes have significantly greater risk for incident stroke relative to individuals without diabetes.[2,201] Mounting evidence over the last decade suggests overt diabetes has detrimental consequences for the brain in regard to cognitive function and tissue structure.[102] In the absence of dementia, individuals with diabetes experience greater cognitive decline and have a greater future risk for developing dementia compared to individuals without diabetes.[100,202,203] Research has also shown diabetes to be associated with greater current brain pathology and subsequent neurodegeneration and progression of ischemic leukoaraiosis.[84,89,103] This current physiological model assumes hyperglycemia is upstream of brain damage, but it is important to note that diabetes and dementia and neurodegeneration are chronic diseases generally affecting older adults. The processes share similar risk factors (e.g., obesity, insulin resistance, lower socio-economic status and educational attainment, depression, social support, and poor diet and low physical activity) and may have long overlapping preclinical phases.[98,115,199,204,205]

This study investigates the association of brain health with glucose metabolism, focusing on how brain structure and cognitive function influence metabolic processes over time. This research departs from how this relationship is typically studied, in the opposite direction, and uses the function of the central nervous system (CNS) in regulating glucose homeostasis as its framework. The central nervous system is comprised of the brain and spinal cord and short-term homeostasis of energy expenditure and blood glucose is regulated by the CNS via afferent-efferent interchange. Within the brain, the hypothalamus integrates the nervous system and the endocrine system and prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis and CNS are shown to have metabolic and hemodynamic consequences.[12,15,206-208] The role of the CNS in metabolic homeostasis over the long-term is unclear. There are few prospective studies of brain health and subsequent metabolic health and they are limited to cognitive assessment only, missing evaluation of cerebral structure.[105-107] Research that elaborates on these previous studies is merited and may help identify brain-related factors that disrupt metabolic homeostasis and help clarify the etiology of diabetes.

Recent epidemiological evidence suggests a potential association between baseline level of cognitive function and the future development of impaired fasting glucose and diabetes.[105-107] However, lack of adjustment for potential confounders, selective attrition, and behaviors downstream of baseline cognitive function complicate interpretation of the results. The Atherosclerosis Risk in Communities (ARIC) Study affords a unique opportunity to assess brain health with more breadth than previous studies in this area, detailing both cognitive function and brain structure.

Our first objective was to assess the association between cognitive function in middle adulthood and the development of diabetes in later adulthood. The second objective was to incorporate a structural assessment of brain health and evaluate the association between three unique measures of brain structure in middle adulthood and the development of diabetes in later adulthood. We hypothesized lower cognitive function and worse brain structure would be associated with an increased incidence of diabetes.

4.3. Methods

The ARIC Study Design

The ARIC study was initiated in 1987, designed to investigate the etiology and natural history of atherosclerosis and its clinical outcomes, and variation in cardiovascular risk factors, medical care, and disease by race, gender, location, and date, utilizing two different population methods: a prospective cohort and a community surveillance component.[209] For the purpose of this analysis, we utilized the data from the ongoing prospective cohort. Probability sampling was used to select, recruit, and enroll individuals drawn from four U.S. communities: Forsyth County, NC (including the city of Winston-Salem); the city of Jackson, MS; seven suburbs of Minneapolis, MN (Brooklyn Center, Brooklyn Park, Crystal, Golden Valley, New Hope, Plymouth, and Robbinsdale); and Washington County, MD (including the city of Hagerstown). Each field center enrolled men and women aged 45-64 reflecting the racial/ethnic makeup of the community, with the exception of the Jackson cohort which only enrolled blacks (black residents recruited at the Washington County and Minneapolis field centers were

small in number), for a cohort total of 15,792 study participants. Participants have been invited to participate in 5 clinic examinations (the fifth exam was part of the ARIC Neurocognitive Study and a sixth exam will begin in June 2016), with annual follow-up telephone calls to update health-related developments occurring since the last contact. Clinic exams occurred in 1987-1989, 1990-1992, 1993-1995, 1996-1998, and 2011-2013. Participants gave written informed consent and the ARIC study procedures were reviewed and approved by each institution's review board.

Assessment of Cognitive Function

Cognitive function was assessed on all ARIC participants present at visits 2, 4, and 5. At visit 3, a sub-set participants were invited to participate in cognitive assessment and complete brain MRI scans and were invited to have a follow-up exam for each in 2006-2008. A sub-set of ARIC participants had cognitive assessment done as part of an ancillary study in 2004-2005. All cognitive assessment data for the first objective of this manuscript reflect that collected at visit 2. Cognitive function was measured with three neuropsychological tests: Delayed Word Recall (DWR), Digit Symbol Substitution (DSS) (a subtest of the Wechsler Adult Intelligence Scale-Revised), and first-letter Word Fluency Test (WFT). The DWR assesses memory and is composed of a set of ten common nouns presented to participants and asked to recall after a 5-minute interval. The test shows fair test re-test reliability over 6 months (r=0.75), with high specificity and sensitivity for dementia at a cut-off of 3 or more correct words recalled (specificity=0.98; sensitivity=0.89).[47] The DSS is a test requiring the subject to associate numbers with

unique symbols, testing sustained attention and psychomotor speed.[48,210] Test-retest reliability in middle-aged adults is 0.82.[48] The WFT requires participants to produce as many words as possible that begin with three different letters of the alphabet.[211] This test measures verbal function and mental agility in retrieving words and is the sum of all three trials.[212,213] Forms of this test have high test-retest reliability over 19-42 days in normal middle–aged adults (r=0.81 to 0.88).[214] A *z*-score for each test was calculated by subtracting the overall mean test score from each individuals' test score and dividing by the standard deviation. Then a global cognitive function *z*-score for each individual was calculated by averaging the *z*-scores of the three tests, standardizing to the global *z* mean (SD). This global *z*-score can be interpreted as the cognitive performance per *z*-standard deviations above/below the mean score.[215]

Brain Magnetic Resonance Imaging

Basic details of MRI measurement and imaging analysis have been described previously.[216-218] Briefly, during the first two years of the ARIC visit 3 wave (1993 and 1994), 1.5-Tesla MRI scanners (GE Signa or Picker) were used to capture brain images for selected participants at the Jackson, MS and Forsyth County, NC field centers. ARIC participants at the Forsyth County and Jackson field centers who were 55 years of age or older at the time of their Visit 3 examination were eligible for the cerebral MRI examination. Participants were screened for the MRI evaluation and were ineligible for MRI for any of the following: previous surgery for an aneurysm in the brain; metal fragments in the eyes, brain, or spinal cord; valvular prosthesis, cardiac pacemaker,

cochlear implant, spinal cord stimulator, or other internal electrical device; pregnancy; and occupations associated with exposure to metal fragments. A total of 2,891 participants were screened for eligibility. Of these 2% of women and 6% of men were ruled ineligible and a further 25% of women and 21% of men declined participation in the MRI procedure, leaving 1,949 participants who underwent cerebral MRI.

Scans were interpreted at the ARIC MRI Reading Center at Johns Hopkins Medical Institutions using a validated scoring protocol and assigned a grade.[216,217] Each image had a primary and secondary interpretation adjudicated by different individuals, blinded to participant's age, gender, race, previous imaging findings, or vascular risk factors. All primary readers were board-certified radiologists with specialty training in neuroradiology. Secondary readers were draw from the same radiologists in addition to experienced neuroimaging technicians.

Brain MRI measures included ventricular size, sulcal size, and white matter hyperintensities. Ventricular and sulcal size are indicators of brain volume, with larger volumes of each measure signifying lower brain volume.[217] White matter hyperintensities, also termed leukoariosis, are suspected ischemia that have resulted in tissue rarefaction, myelin loss and pallor, gliosis, or axonal atrophy.[32] All three measures are associated with lower levels of cognitive function.[55] Each MRI measure was evaluated according to a semi-quantitative 10-point scale with visual pattern matching. For example, each image for ventricle size was compared with a series of eight images of successively increasing ventricular size ranging from "small and presumably normal" (grade 1) to "severe atrophy" (grade 8). Images with ventricles smaller than those in

grade 1 received a grade of 0 and those worse than a grade 8 received grade 9. In a similar fashion, sulcal size was assessed by comparison with eight images with successively increasing sulcal size, using grades 0 to 9 as with ventricular size. White matter hyperintensities were estimated as the total volume of periventricular and subcortical white matter signal abnormality, similarly by visual comparison with eight images that successively increased from "barely detectable white matter changes" (grade 1) to "extensive, confluent changes" (grade 8). Images interpreted as no white matter change received grade 0 and those with changes more significant that grade 8 received grade 9. Abnormalities suspected to represent cerebral infarcts or hemorrhages were coded separately by size and location. This grading system is shown to have high intra-reader reliability for ventricular size (kappa=0.89) and white matter hyperintensities (kappa=0.81) and moderate reliability for sulcal size (kappa=0.66).[218]

Determination of Diabetes

Diabetes status was determined at each ARIC visit as fasting glucose ≥ 126 mg/dL (≥ 7.0 mmol/L), non-fasting glucose ≥ 200 mg/dL (≥ 11.1 mmol/L), self-reported use of diabetes medication in the past 2 weeks, or self-report of a physician diagnosis, and at annual telephone follow-up (AFU) calls by self-report of physician diagnosis or self-report of diabetes medication use. Percent glycated hemoglobin (HbA1c) was measured at ARIC visit 2 and values were used to exclude any additional potential undetected diabetes cases with HbA1c ≥ 6.5% that were not identified by the above criteria. Glucose was measured with the hexokinase/glucose-6-phosphate dehydrogenase method. HbA1c

was measured in stored whole blood samples using high-performance liquid chromatography with instruments standardized to the Diabetes Control and Complications Trial assay (Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer and Tosoh G7).

Ascertainment of Other Characteristics

Interviewer-administered questionnaires were used to collect information on demographics, education, cigarette-years smoking and current smoking status, regular alcohol consumption, and medical and reproductive history. Individuals were requested to fast for 12 hours prior to the examination and bring all prescription and nonprescription medications used in the two weeks prior to the clinical examination date. Sitting blood pressure was measured with a random-zero sphygmomanometer in triplicate with 5-minute rest intervals; the mean of the last two measurements was used for analysis. Self-reported anti-hypertensive medication use was defined as reporting to have taken an anti-hypertensive medication within the last two weeks or taking a medication which is classified as an anti-hypertensive medication. Participants, wearing light clothing, were asked to remove their shoes and height and weight were measured and body mass index was calculated as the weight, in kilograms, divided by height, in meters, squared. Waist circumference was measured at the level of the umbilicus and hip girth was measured at the level of maximal protrusion of the gluteal muscles. At ARIC visits 1 and 3, physical activity (PA) in the past year was assessed with a modified Baecke questionnaire determining PA in sports, leisure-time, and at work, and converted to sports

index score ranging 1 to 5.[219,220] When compared to accelerometer readings, peak oxygen consumption determination, and percent body fat, this questionnaire is shown to have high reliability and good ability to distinguish heavy intensity physical activity in adults (r=0.73 for men and r=0.63 for women); results for light intensity showed lower agreement (r=0.39 for men and r=0.23 for women).[221] At visit 2, psychosocial measures of social support, anger, and depression were collected, described previously.[222] The 40-item Interpersonal Support Evaluation List was designed to assess perceived social support. This instrument has a six-week test-retest correlation of 0.63 to 0.70 with an in internal consistency of 0.88 to 0.90.[223] We summarized the response from 6 of the 40 questions that specifically asked whether social support was available when needed, for a range of 0 to 18 with a higher score indicating a lower level of perceived social support. The 10-item Spielberger Trait Anger Scale was used to assess anger proneness, a greater score indicating greater trait anger. This is a modified version of the 15-item instrument, which has an internal consistency ranging from 0.81 to 0.92 and correlates with the Buss–Durkee Hostility Inventory (correlation range from 0.66 to 0.73).[224] A 21-item Vital Exhaustion Questionnaire was developed to measure a state of exhaustion, which is shown to have high correlation with depressive symptoms (r= 0.62).[225] The questions cover vegetative depressive symptoms (fatigue, sleep pattern, energy and concentration), non-vegetative depressive symptoms (crying spells, hopelessness, irritability, decreased libido and suicidality), and functional depressive symptoms (coping and productivity). However, the test-retest correlation of this questionnaire over 4-years was reported as 0.30.[226]

Blood was drawn from an antecubital vein of seated participants, centrifuged at 3000 x *g* for 10 minutes at 4°C, serum separated, and store at -70°C. Blood chemistries and measurements have been described previously.[209] Total cholesterol and triglycerides were measured with enzymatic methods.[227,228] High density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation and the Friedewald formula was used to determine low density lipoprotein cholesterol (LDL).[229,230] Apolipoprotein ε (*APOE ε*) genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, CA), described previously.[231] The standard TaqMan assay can detect only two alleles per reaction and the *APOE* variants at codons 112 and 158 were detected separately. The data from these two codons were combined to generate the six *APOE* genotypes (ε2/2, ε2/3, ε2/4, ε3/3, ε3/4, and ε4/4). The ABI 7900 and Sequence Detection System software (Applied Biosystems) were used for allele detection and genotype calling.

Statistical Analysis

Our first aim was to assess the association between level of cognitive function (assessed at visit 2) and the incidence of diabetes. Our second aim was to assess the association between three different measures of brain imaging (assessed at visit 3 in a sub-set of participants) and the incidence of diabetes. We used multiple imputation to impute missing covariate data. Briefly, we assumed an arbitrary missing data pattern and used data from available cases to impute missing data assuming an origin from a multivariate normal distribution. We created 10 data-sets which were analyzed separately and

combined according to Rubin's Rules.[192] The maximum number of observations imputed for any covariate totaled < 5% of the analytic sample. Individuals were excluded from aim 1 if they did not attend ARIC visit 2 (aim 1 baseline visit) or were missing information from neurocognitive testing. Individuals were excluded from aim 2 if they did not participate in the brain MRI at visit 3 (aim 2 baseline visit) or were missing information for MRI grading. Common exclusions further applied for each aim included excluding individuals who did not return after their baseline visit, were determined to have prevalent diabetes at their baseline visit, self-reported prevalent stroke at their baseline visit. Because of small numbers, participants at all centers who were neither black nor white and blacks from MD and MN were excluded. These exclusions are enumerated in **figure 4.1**. Summary characteristics were calculated according to quartile of global cognitive function *z*-score. Categorical characteristics included race-field center (black-MS; black-NC; white-MD; white-MN; and white-NC), current smoking status (never; former; current), current alcohol consumption (zero; any up to 1 drink daily for women/up to 2 drinks daily for men; and greater than 1 drink daily for females/ greater than 2 drinks daily for males), educational attainment (less than high school degree; high school graduate or vocational school; college degree or graduate school/professional school), parental history of diabetes (yes/no to maternal or paternal), and *APOE* ε4 genotype (0; 1; or 2 alleles). All other characteristics were quantified and modeled continuously.

Cox proportional hazards were used to estimate hazard ratios for incident diabetes (and 95% confidence limits) with each individual's contributed time to the analysis

concluding with the date of exam or telephone call at which diabetes was first ascertained or administrative censoring on the date of their last contact. We tested for violations to the proportional hazards assumption by including a product term between each exposure of interest and natural log of contributed person-time.

For aim 1, we ran four unique sets of models assessing the association between cognitive function and incident diabetes, where cognitive function was modeled as the global cognitive function *z-score,* and then for each domain specific *z-score* of the delayed word recall, digit symbol substitution, and word fluency tests. For analyses of aim 1, HRs for incident diabetes were calculated per 1 standard deviation unit increment in cognitive function *z-score* before and after adjustment for age, sex, race-field center, educational attainment, smoking (cigarette-years and current status), alcohol consumption, physical activity sports index, BMI, waist circumference, fasting glucose and insulin, family history of diabetes, *APOE* ε4 genotype, and psychosocial measures of anger, depression, and social support. Because hypertension is a major risk factor for cognitive decline, white matter hyperintensities, and other general brain pathology, we included systolic blood pressure and self-reported use of hypertension medication in our final primary model. All values reflect those measured at ARIC visit 2 except for education, cigarette-years smoking, fasting insulin, paternal history of diabetes, and physical activity which were measured at visit 1 (on average 3 years prior).

For aim 2, the distribution of each MRI measure was right-skewed. Categorical variables consisting of 4 levels were constructed for each MRI measure. Ventricular size and sulcal size grade categories were defined as: grades 0-1 (reference), grade 2, grade 3,

and grades ≥4. White matter hyperintensity grade categories were defined as grade 0 (reference), grade 1, grade 2, and grades ≥3. These categories were chosen according to physiological interpretation (normal healthy, slightly abnormal, increasing abnormality/severity) and to ensure adequate numbers for events and comparison. HRs for incident diabetes were calculated according to categorical grade and according to a 1-grade increment for each MRI measure before and after adjustment for characteristics similar to aim 1: age, sex, race-field center, educational attainment, smoking (cigarette-years and current status), alcohol consumption, physical activity sports index, BMI, waist circumference, fasting glucose and insulin, paternal history of diabetes, *APOE* ε4 genotype, psychosocial measures of anger, depression, and social support, systolic blood pressure, and anti-hypertensive medication use. All values reflect those measured at visit 3, except for cigarette-years smoking, family history of diabetes, and fasting insulin which were measured at visit 1 (on average 6 years prior) and the psychosocial measures of anger, depression, and social support which were measured at visit 2 (on average 3 years prior). Tests of linear trend across categories of each MRI measure were performed by assigning participants the median of their MRI measure category and entering this new variable into a separate Cox proportional-hazards regression model. For each exposure of brain imaging, we assessed effect modification by age, sex, and race by including a product term between the imaging measure and each potential effect modifier to the model.

We conducted several sensitivity analyses to address potential bias and limitations in prior studies. First, because individuals with lower baseline cognitive function or who

develop diabetes are more likely to be lost to follow-up, we used inverse probability of attrition weighting (IPAW) to examine the influence of selective attrition on our estimates.[196] Briefly, we used two logistic models to predict the probability of loss to follow-up due to dropout and loss to follow-up due to death at each exam at which diabetes was ascertained (not including annual follow-up calls). All IPAW models used the same covariates of age, sex, race-field center, education, smoking status (and history) and regular alcohol consumption, BMI, fasting glucose, hypertension status, total cholesterol, LDL-C, HDL-C, triglycerides, physical activity index, cognitive scores, *APOE* ε4 genotype, and lipid-lowering and blood-pressure lowering medication. To create stabilized weights, we re-ran these models with a sub-set of covariates and multiplied the probabilities from these models by the weight from the respective previous model. The purpose of IPAW is to create a pseudo-population representative of the original population, by ascribing larger weights to individuals who are followed completely who have characteristics similar to those of individuals who are lost to follow-up.[196] Second, we ran analyses stratified by baseline glycemic status (normal glycemia versus prediabetes levels of glycemia) to identify if any observed association was driven (or masked) by individuals with underlying elevated normal glucose metabolism. Third, baseline cognitive function may lead to changes in behaviors and diabetes risk factors that influence subsequent diabetes risk. To address this phenomenon, we ran analyses with time-varying covariates for BMI, waist circumference, physical activity, and fasting glucose for a sub-set with these values available at visit 3 for aim 1 and visit 4 for aim 2. Lastly, we performed sensitivity analyses when excluding the first 3

years of observation, to mitigate the potential for reverse causation or underlying disease explaining our results. A combination of SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC) and STATA 13.0 software (College Station, TX) was used for statistical analysis.

4.4. Results

Among the 10,819 individuals meeting inclusion criteria for aim 1 at ARIC visit 2, female sex, white race, greater educational attainment, greater physical activity level, and higher perceived social support were associated with higher *z-scores* of global cognitive function (**Table 4.1.**). In contrast, older age, current smoking, higher BMI, waist circumference, systolic blood pressure, and fasting insulin, and greater cigarette-years of smoking and depressive responses were associated with lower *z-scores* of global cognitive function.

In regard to aim 2, only older age, male sex, higher systolic blood pressure, and greater cigarette-years of smoking were consistently associated with worse grade for each MRI measure at visit 3. **Tables 4.2. to 4.4.** present summary statistics according grade category for each MRI measure. A majority of participants were graded as slightly abnormal imaging (**Figure 4.2.**).

Cognitive Function and Incident Diabetes

For aim 1, during a median follow-up time of 19.1 years (maximum=25 years), there were 3,200 newly identified cases of diabetes (30% cumulative incidence). Before

adjustment, a 1-SD unit increment in global cognitive function *z-score* was significantly associated with 23% lower rate of incident diabetes (HR=0.77 and 95% CI=0.74, 0.80). Adjustment for basic demographics greatly attenuated the association of cognitive function and incident diabetes (HR=0.90 and 95% CI=0.86, 0.95) (**Table 4.5.**). After adjustment for age, sex, race-field center combination, educational attainment, smoking (cigarette-years and current status), alcohol consumption, physical activity sports index, BMI, waist circumference, fasting glucose and insulin, family history of diabetes, *APOE* ε4 genotype, and psychosocial measures of anger, depression, and social support, and systolic blood pressure and anti-hypertension medication use (primary final model) cognitive function was inversely associated with incidence of diabetes (HR=0.95; 95% CI=0.90, 1.00; p=0.048), albeit at a marginally statistically significant level. During model adjustments, race-field center and education level accounted for the greatest attenuation to the observed association. After employing IPAW, the HR became positive and the confidence limits widened (HR=1.10; 95% CI=0.90, 1.35). Global cognitive *z*-scores were not associated with diabetes risk for individuals with normal glycemia at baseline (per 1 SD unit increment: HR=0.99; 95% CI=0.89, 1.09) and were inversely associated with diabetes risk for individuals with prediabetic levels of glycemia at baseline (HR=0.93; 95% CI=0.87, 0.98). The interaction test between cognitive function and baseline glycemic status was not statistically significant (p>0.1). We examined if changes in diabetes risk factors subsequent to cognitive assessment would alter HR estimates for incident diabetes. Compared to the primary final model, there was slight attenuation to the HRs for incident diabetes when adjusting for time-varying diabetes risk

factors in the subset of individuals who had measurements for BMI, waist circumference, physical activity, and fasting glucose at visit 3. Excluding the first 3 years of observation had minimal impact on the diabetes HR estimates. Associations of specific domains of cognitive function and incident diabetes were not significant, with the exception of the word fluency test which was inversely association with incident diabetes in fully adjusted models (**Table 4.5.**). We did not find evidence of a quadratic association between any measure of cognitive function and incidence of diabetes nor did we find evidence for violations of the proportional hazards assumption. All tests for effect modification were not significant (all $p>0.10$).

Brain MRI Measures and Incident Diabetes

Median follow-up time for aim 2 was 14.1 years (maximum=22 years), during which 373 new cases of diabetes were identified (28% cumulative incidence). In separate unadjusted analyses, worse grade of both white matter hyperintensities and ventricular size were associated with increased risk for incident diabetes, regardless of modeling exposure as categorical or continuous (**Tables 4.6. and 4.7.**). By contrast, sulcal size was not associated with incidence of diabetes. Adjustment for fasting glucose resulted in slight strengthening of the categorical and continuous HRs in the association between white matter hyperintensities and incident diabetes, while adjustment for systolic blood pressure resulted in the greatest attenuation in this association. Adjustment for baseline level of fasting glucose resulted in the greatest attenuation of the HRs for ventricular size and incident diabetes. In the fully adjusted model, both worse grade of white matter

hyperintensities and ventricular size were significantly associated with increased risk for incident diabetes. The HR estimates for linear grade increment were not meaningfully altered with IPAW modeling. The linear trend across HRs for categories of ventricular size was significant with IPAW modeling (p=0.05) while white matter hyperintensities was no longer significant with IPAW. In individuals with normal glucose, both worse grade of white matter hyperintensities and worse grade of sulcal size was associated with increased HRs for incident diabetes. In individuals with prediabetes, neither white matter hyperintensities nor sulcal size was associated with incident diabetes. A test of interaction between prediabetes status and each MRI measure was done to investigate this difference further and was not significant (p>0.1 for both interaction tests). Compared to the final primary model, updating BMI, waist circumference, and fasting glucose with values measured at visit 4 had no appreciable impact on HRs for any MRI measure, nor did excluding the first 3 years of observation. All tests for effect modification were not significant (all p>0.10).

4.5. Discussion

This study does not provide strong support for our hypothesis that lower level of cognitive function and worse brain imaging in middle-age is associated with developing diabetes in later adulthood. We found a significant inverse association between baseline level of cognitive function and incident diabetes, but the magnitude of this inverse association was weak. After separately taking into account selective attrition and subsequent changes to diabetes risk factors HR estimates shifted to a non-statistically

significant positive association and confidence limits widened considerably suggesting a null association. Results from our second aim were inconsistent across MRI measures; worse grade of white matter hyperintensities and ventricular size were associated with significantly increased risk for developing diabetes, while sulcal size was not. The results from the sensitivity analyses were informative for both aims, suggesting it may be important to consider selective attrition, changes to diabetes risk factors, and stratifying by prediabetes status when evaluating potential associations between cognitive function and brain structure and incidence of diabetes.

The results from this analysis are important because they expand on the body of research from two cohorts previously assessing the association between cognitive function and glucose metabolism.[105-107] In Metabolic, Lifestyle and Nutrition Assessment in Young Adults (MELANY) study, individuals with the lowest level of cognitive function had two-fold increased risk for developing diabetes and impaired fasting glucose, separately, compared to individuals with the highest level of cognitive function after adjustment for diabetes risk factors.[105,107] Data from Elderly Nutrition and Health Survey in Taiwan (NAHSIT Elderly) found women with cognitive impairment had nearly 2.5 times significantly greater risk for developing diabetes compared to those without cognitive impairment, while in men this association was more modest and not significant.[106]

Several factors may have contributed to our finding that cognitive function was not associated with incident diabetes in the same magnitude as previously reported. The MELANY cohort is considerably younger than the ARIC population. The factors that

lead to onset of diabetes in early adulthood may differ in their strength of association with developing diabetes in older age.[232] The difference in findings between ARIC and NAHSIT could be due to adjustment schemes or reverse-causation. The results reported in NAHSIT data are minimally adjusted and individuals had, on average, fasting glucose levels reflecting prediabetes.[106] We observed a slight inverse association between cognitive function and incidence of diabetes in individuals with prediabetic levels of glucose, but did not observe an association between cognitive function and incidence of diabetes in individuals with normal glucose. We also showed accounting for attrition with IPAW reversed the direction of the observed HR (albeit an overall null association) in the association between cognitive function and incidence of diabetes.

The results from our second aim are novel. We found that worse grade of white matter hyperintensities and ventricular size were associated with a marginally greater risk for developing diabetes, but sulcal size was not associated with incidence of diabetes. Previous analysis in ARIC has shown diabetes to be associated with worsening sulcal grade and incident vascular infarcts, but not with changes to ventricular size or development of white matter hyperintensities.[84] Assessment of these associations in the opposite direction (baseline brain health on subsequent metabolic health) merits investigation as it is plausible this association could extend both ways. Diabetes is shown to have complex associations with other conditions of the brain; diabetes and depression are shown to independently predict the incidence of the other.[199,233]

The brain is highly dependent on glucose to function and home to a large concentration of insulin receptors, principally in the hypothalamus, hippocampus,

cerebral cortex, and cerebellum.[234,235] Some of these receptors play a role in glucose transport within the CNS while others have a role in the process by which the CNS, mainly the hypothalamus, regulates the body's homeostatic controls.[206,235,236] Relatedly, the hypothalamic-pituitary-adrenal (HPA) axis, a stress response pathway with feedback capability also works to counteract threats to homeostasis of the body's systems and stress-induced activation of the HPA axis is associated with less favorable levels of cardiovascular and diabetes risk factors.[208,237] This suggests dysfunction or abnormalities to the hypothalamus or HPA axis could have an impact on metabolic homeostasis. Insulin resistance rising from excess adiposity may also explain our results. We did not observe noticeable differences in fasting insulin levels, a marker of insulin resistance, across grade for any of the MRI measures; however these were measured, on average, 6 years prior, and we did observe greater mean fasting glucose levels, strongly correlated with insulin, with worsening ventricular grade. Brains of patients with Alzheimer's disease show impaired insulin signaling and reduced expression of genes encoding insulin signaling compared to controls.[238] This brain-limited form of impaired insulin signaling and insulin resistance has been proposed as a neuroendocrine condition similar to, yet unique from, type 2 diabetes mellitus, termed "Type 3 diabetes".[238,239] This phenomenon has been proposed to play an important role in the pathogenesis of Alzheimer's disease.[239] In addition to insulin resistance, diabetes and Alzhiemer's disease share other risk factors. This may suggest that common genetic and environmental antecedents are responsible for the association between diabetes and Alzheimer's disease. The idea that

diseases previously thought distinct spring from a "common soil" is not unique and was proposed for the connection between cardiovascular disease and diabetes.[240]

There are several limitations to our study that warrant consideration. First, the technology used in the brain MRIs and the visual pattern matching methods used to determine and assign pathology are dated compared to current methods, which may lead to greater misclassification of exposure. Second, it is possible that reverse-causation may explain our results; it has been shown diabetes is associated with subsequent cognitive decline and brain pathology and these are two disease processes with long concurrent preclinical phases. Determining temporality is difficult, particularly in an older population where these processes have likely begun. We excluded from analysis individuals with glycemic levels indicative of diabetes and we performed analyses stratified by baseline glycemic level to identify if any observed association could be explained by elevated yet non-diabetic glucose levels. We did not observe results to suggest any of the associations were driven by individuals with elevated normal glucose levels. Third, MRI was only performed on a select group of individuals in the North Carolina and Mississippi field centers; these findings may not generalize to other geographic and socioeconomic populations with different risk factor profiles. Fourth, historically (and evident in our data) education is strongly associated with cognitive function and individuals with lower education may have less familiarity with testing or greater test anxiety resulting in lower scores on measures of neurocognitive performance. If our measure of cognitive function is simply another proxy for education and not an actual impairment in cognitive processes, models that adjusted for education may have

diminished any association between cognitive function and incidence of diabetes. Lastly, this is an observational epidemiological study and it is possible that unmeasured confounding present. Our study also has some notable strengths, including using statistical techniques to address selective attrition and changes to time-dependent diabetes risk factors and extensive assessment of potential confounders in a large biracial cohort. In view of the limitations and strengths of this work the results should be interpreted cautiously.

In conclusion, our results do not provide strong support to the hypothesis that lower cognitive function or adverse brain structure in middle age is associated with increased risk for developing diabetes in older adulthood independent of traditional diabetes risk factors. Future research assessing the association between cognitive function and brain structure and the incidence of diabetes should evaluate and characterize brain health with multi-faceted approach in younger metabolically healthy populations.

4.6. Tables

Table 4.1. Participant characteristics (n=10,819) according to quartile of global cognitive function *z-score*, the Atherosclerosis Risk in Communities Study, 1990-1992

		Global Cognitive Function Quartiles, *z-score*				
Characteristic	*z-score* range	Q1 (-4.4, -0.49)	Q2 (-0.5, 0.19)	Q3 (0.2, 0.79)	Q4 (0.8, 3.3)	**Overall** (-4.4, 3.3)
N		2704	2705	2703	2707	10,819
Incident diabetes cases over follow-up		917	805	758	720	3200
Age, years		58.2 ± 5.7	57.0 ± 5.6	56.3 ± 5.6	55.1 ± 5.4	56.7 ± 5.7
Women, %		44	53	60	68	56
Race, white, %		58	82	88	93	80
Education, ≥ some college, %		15	34	47	61	40
Current smoking, %		27	22	20	16	21
Cigarette-years smoking		349 ± 453	297 ± 401	267 ± 380	231 ± 346	286 ± 399
Alcohol, none daily, %		72	65	61	56	64
Alcohol, moderate daily, %		22	28	31	34	28
Sports index (range 1-5)		2.3 ± 0.7	2.5 ± 0.8	2.6 ± 0.8	2.6 ± 0.8	2.5 ± 0.8
Systolic blood pressure, mm Hg		124 ± 19	119 ± 17	119 ± 17	116 ± 17	120 ± 18
Anti-hypertension medications, %		36	28	25	21	27

BMI, kg/m^2	28.4 ± 5.5	27.4 ± 4.8	27.1 ± 4.8	26.6 ± 4.8	27.4 ± 5.0
Waist circumference, cm	99 ± 13	97 ± 13	96 ± 14	93 ± 14	96 ± 14
Fasting glucose, mmol/L	5.7 ± 0.5	5.6 ± 0.5	5.6 ± 0.5	5.6 ± 0.5	5.6 ± 0.5
Fasting insulin, μU/mL	12 ± 8	11 ± 8	10 ± 7	9 ± 6	10 ± 8
Total cholesterol, mmol/L	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.0
HDL Cholesterol, mmol/L	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.5	1.3 ± 0.4
LDL Cholesterol, mmol/L	3.5 ± 0.9	3.4 ± 0.9	3.4 ± 0.9	3.4 ± 0.9	3.4 ± 0.9
Triglycerides, mmol/L	1.4 ± 0.9	1.5 ± 0.8	1.5 ± 0.9	1.4 ± 0.8	1.4 ± 0.9
Cholesterol lowering medications, %	5	6	6	5	6
Parental history of diabetes, %	23	23	21	20	22
APOE ε4 genotype, 0 allele, %	67	70	70	72	70
APOE ε4 genotype, 1 allele, %	30	27	28	26	28
Anger (range 0 – 20; higher indicative of more anger)	7 ± 3	7 ± 3	7 ± 3	7 ± 3	7 ± 3
Social support (range 0-18; higher indicative of less support)	3 ± 3	3 ± 3	3 ± 3	3 ± 3	3 ± 3
Depression (range 0-42; higher indicative of more depressive)	12 ± 9	10 ± 9	9 ± 8	9 ± 8	10 ± 8
Delayed word recall, total words (range 0-10)	5.4 ± 1.3	6.5 ± 1.1	7.1 ± 1.0	8.0 ± 1.1	6.7 ± 1.5
Digit symbol substitution, correct symbols (no upper limit)	31.6 ± 10.5	44.2 ± 8.3	50.7 ± 8.2	59.3 ± 8.8	46.4 ± 13.5
Word fluency test, words listed (no upper limit)	22.2 ± 8.6	30.9 ± 7.8	37.0 ± 8.3	46.3 ± 9.7	34.1 ± 12.3

Values are means ± standard deviations for continuous and percentages for categorical. Moderate alcohol consumption is up to 1 drink daily for women and up to 2 drinks daily for men. Cigarette-years smoking, education, physical activity sports index, parental history of diabetes, and fasting insulin were measured at ARIC visit 1, 1987-1989

Table 4.2. Participant characteristics (n=1,350) according to category of white matter hyperintensities, the Atherosclerosis Risk in Communities Study, 1993-1994

	White matter hyperintensities grade, range 0 - 9			
	0	1	2	≥ 3
n (incident diabetes cases over follow-up)	204 (59)	706 (191)	308 (82)	132 (41)
Age, years	60.4 ± 4.1	61.8 ± 4.4	63.7 ± 4.2	65.1 ± 4.1
Women, %	63	61	56	58
Race, white, %	35	53	75	55
Education, ≥ some college, %	44	41	42	39
Current smoking, %	15	15	19	23
Cigarette-years smoking	203 ± 340	248 ± 390	271 ± 430	317 ± 464
Alcohol, none daily, %	73	74	66	73
Alcohol, moderate daily, %	23	21	25	17
Sports index (range 1-5)	2.5 ± 0.8	2.6 ± 0.8	2.6 ± 0.8	2.5 ± 0.8
Systolic blood pressure, mm Hg	124 ± 17	125 ± 18	127 ± 20	133 ± 24
Anti-hypertension medications, %	34	36	35	52
BMI, kg/m^2	28.2 ± 5.4	27.4 ± 4.8	26.6 ± 4.4	27.0 ± 4.7
Waist circumference, cm	98 ± 13	97 ± 13	97 ± 12	97 ± 12
Fasting glucose, mmol/L	5.5 ± 0.5	5.5 ± 0.5	5.5 ± 0.5	5.4 ± 0.5

Fasting insulin, μU/mL	10 ± 7	11 ± 8	9 ± 6	11 ± 7
Total cholesterol, mmol/L	5.5 ± 1.0	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.1
HDL Cholesterol, mmol/L	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.5	1.5 ± 0.6
LDL Cholesterol, mmol/L	3.4 ± 0.9	3.3 ± 0.9	3.3 ± 0.9	3.2 ± 0.9
Triglycerides, mmol/L	1.4 ± 0.8	1.4 ± 1.0	1.5 ± 0.9	1.4 ± 0.9
Cholesterol lowering medications, %	8	7	10	7
Parental history of diabetes, %	24	20	17	20
APOE ε4 genotype, 0 allele, %	70	67	73	66
APOE ε4 genotype, 1 allele, %	28	30	24	27
Anger (range 0 – 20; higher indicative of more anger)	6 ± 3	7 ± 3	7 ± 3	7 ± 3
Social support (range 0-18; higher indicative of less support)	2 ± 3	2 ± 2	3 ± 3	2 ± 3
Depression (range 0-42; higher indicative of more depressive)	10 ± 8	10 ± 9	10 ± 8	11 ± 8
Global cognitive function, *z-score*	-0.26 ± 1.01	-0.13 ± .98	-0.05 ± 1.02	-0.41 ± 1.11

Values are means ± standard deviations for continuous and percentages for categorical. Moderate alcohol consumption is up to 1 drink daily for women and up to 2 drinks daily for men. Cigarette-years smoking, education, parental history of diabetes, and fasting insulin were measured at ARIC visit 1, 1987-1989, while anger, social support, and depressive symptoms were measured at ARIC visit 2, 1990-1992

Table 4.3. Participant characteristics (n=1,350) according to category of sulcal size, the Atherosclerosis Risk in Communities Study, 1993-1994

	Sulcal size grade, range 0 - 9			
	0 - 1	2	3	≥ 4
n (incident diabetes cases over follow-up)	320 (84)	685 (203)	265 (66)	80 (20)
Age, years	60.9 ± 4.3	62.5 ± 4.4	63.1 ± 4.5	64.3 ± 4.5
Women, %	73	59	51	44
Race, white, %	56	56	58	44
Education, ≥ some college, %	37	41	48	43
Current smoking, %	15	17	20	16
Cigarette-years smoking	196 ± 309	255 ± 410	274 ± 400	400 ± 577
Alcohol, none daily, %	75	73	69	71
Alcohol, moderate daily, %	21	21	25	22
Sports index (range 1-5)	2.5 ± 0.8	2.6 ± 0.8	2.6 ± 0.8	2.4 ± 0.8
Systolic blood pressure, mm Hg	126 ± 19	127 ± 19	125 ± 20	129 ± 16
Anti-hypertension medications, %	36	40	30	39
BMI, kg/m^2	27.7 ± 5.1	27.2 ± 4.7	26.8 ± 4.7	28.1 ± 5.5
Waist circumference, cm	97 ± 13	97 ± 12	97 ± 13	99 ± 14
Fasting glucose, mmol/L	5.4 ± 0.5	5.5 ± 0.5	5.5 ± 0.6	5.6 ± 0.6

Fasting insulin, μU/mL	11 ± 8	10 ± 7	10 ± 8	11 ± 7
Total cholesterol, mmol/L	5.5 ± 1.0	5.4 ± 1.0	5.4 ± 1.0	5.2 ± 1.0
HDL Cholesterol, mmol/L	1.5 ± 0.5	1.4 ± 0.5	1.5 ± 0.5	1.5 ± 0.5
LDL Cholesterol, mmol/L	3.3 ± 0.9	3.3 ± 0.9	3.3 ± 0.9	3.1 ± 1.0
Triglycerides, mmol/L	1.5 ± 0.9	1.4 ± 0.9	1.5 ± 1.2	1.2 ± 0.7
Cholesterol lowering medications, %	8	8	7	5
Parental history of diabetes, %	23	20	16	19
APOE ε4 genotype, 0 allele, %	64	70	70	73
APOE ε4 genotype, 1 allele, %	32	28	24	26
Anger (range 0 – 20; higher indicative of more anger)	7 ± 3	7 ± 3	7 ± 3	6 ± 2
Social support (range 0-18; higher indicative of less support)	11 ± 8	10 ± 9	10 ± 8	11 ± 9
Depression (range 0-42; higher indicative of more depressive)	2 ± 2	2 ± 3	3 ± 3	3 ± 3
Global cognitive function, *z-score*	-0.12 ± 0.99	-0.15 ± .98	-0.20 ± 1.07	-0.27 ± 1.09

Values are means ± standard deviations for continuous and percentages for categorical. Moderate alcohol consumption is up to 1 drink daily for women and up to 2 drinks daily for men. Cigarette-years smoking, education, parental history of diabetes, and fasting insulin were measured at ARIC visit 1, 1987-1989, while anger, social support, and depressive symptoms were measured at ARIC visit 2, 1990-1992

Table 4.4. Participant characteristics (n=1,350) according to category of ventricular size, the Atherosclerosis Risk in Communities Study, 1993-1994

	Ventricular size grade, range 0 - 9			
	0 - 1	2	3	≥ 4
n (incident diabetes cases over follow-up)	234 (57)	591 (166)	339 (99)	186 (51)
Age, years	60.6 ± 4.2	61.8 ± 4.3	63.3 ± 4.4	64.4 ± 4.3
Women, %	74	67	47	42
Race, white, %	47	57	56	62
Education, ≥ some college, %	32	43	41	50
Current smoking, %	18	17	16	16
Cigarette-years smoking	197 ± 308	228 ± 358	280 ± 443	356 ± 519
Alcohol, none daily, %	74	73	72	65
Alcohol, moderate daily, %	22	21	19	27
Sports index (range 1-5)	2.5 ± 0.8	2.5 ± 0.8	2.6 ± 0.8	2.6 ± 0.9
Systolic blood pressure, mm Hg	125 ± 19	125 ± 18	128 ± 19	129 ± 23
Anti-hypertension medications, %	39	37	34	40
BMI, kg/m^2	27.3 ± 4.8	27.3 ± 5.0	27.4 ± 4.7	26.9 ± 4.7
Waist circumference, cm	96 ± 12	97 ± 13	98 ± 12	97 ± 12
Fasting glucose, mmol/L	5.4 ± 0.5	5.5 ± 0.5	5.5 ± 0.6	5.5 ± 0.6

Fasting insulin, μU/mL	11 ± 8	10 ± 7	10 ± 8	11 ± 7
Total cholesterol, mmol/L	5.5 ± 1.0	5.5 ± 1.0	5.3 ± 0.9	5.4 ± 1.1
HDL Cholesterol, mmol/L	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.5	1.5 ± 0.5
LDL Cholesterol, mmol/L	3.4 ± 0.9	3.3 ± 0.9	3.3 ± 0.8	3.3 ± 1.0
Triglycerides, mmol/L	1.4 ± 0.9	1.4 ± 1.0	1.4 ± 0.9	1.5 ± 0.8
Cholesterol lowering medications, %	7	9	6	7
Parental history of diabetes, %	24	19	19	19
APOE ε4 genotype, 0 allele, %	64	69	70	70
APOE ε4 genotype, 1 allele, %	32	27	28	27
Anger (range 0 – 20; higher indicative of more anger)	7 ± 3	7 ± 3	7 ± 3	7 ± 3
Social support (range 0-18; higher indicative of less support)	11 ± 9	10 ± 8	10 ± 8	10 ± 9
Depression (range 0-42; higher indicative of more depressive)	2 ± 2	2 ± 3	2 ± 3	3 ± 3
Global cognitive function, *z-score*	-0.23 ± 0.92	-0.03 ± .99	-0.24 ± 1.05	-0.35 ± 1.07

Values are means ± standard deviations for continuous and percentages for categorical. Moderate alcohol consumption is up to 1 drink daily for women and up to 2 drinks daily for men. Cigarette-years smoking, education, parental history of diabetes, and fasting insulin were measured at ARIC visit 1, 1987-1989, while anger, social support, and depressive symptoms were measured at ARIC visit 2, 1990-1992

Table 4.5. Hazard ratios (HR) and 95% confidence intervals (95% CI) for incident diabetes according to domain of cognitive function *z*-score in 10,819 participants, the Atherosclerosis Risk in Communities Study, 1990-1992 through 2014

	HR (95% CI) per 1 SD cognitive function *z*-score			
Model Adjustments	Global	DWR	DSS	WFT
Unadjusted	0.77 (0.75, 0.81)	0.88 (0.85, 0.91)	0.77 (0.74, 0.80)	0.82 (0.79, 0.85)
Model 1	0.90 (0.86, 0.95)	0.97 (0.94, 1.01)	0.93 (0.90, 1.00)	0.92 (0.88, 0.96)
Model 2	0.93 (0.88, 0.98)	0.98 (0.95, 1.02)	0.95 (0.90, 1.00)	0.93 (0.89, 0.97)
Model 3	0.94 (0.89, 0.98)	0.98 (0.95, 1.02)	0.95 (0.90, 1.01)	0.94 (0.90, 0.98)
Model 4	0.94 (0.89, 0.99)	0.98 (0.94, 1.02)	0.96 (0.91, 1.01)	0.94 (0.90, 0.98)
Model 5	0.94 (0.90, 0.99)	0.99 (0.95, 1.03)	0.97 (0.91, 1.02)	0.94 (0.90, 0.98)
Primary final model	0.95 (0.90, 1.00)	0.99 (0.95, 1.03)	0.98 (0.93, 1.04)	0.94 (0.91, 0.98)
Bias Models				
IPAW	1.10 (0.90, 1.35)	1.08 (0.92, 1.27)	1.06 (0.87, 1.29)	1.00 (0.89, 1.13)
Stratified				
Glucose < 5.6 mmol/l and HbA1c < 5.7	0.99 (0.89, 1.09)	1.02 (0.94, 1.10)	1.00 (0.90, 1.11)	0.96 (0.88, 0.98)

Glucose ≥ 5.6 mmol/l or HbA1c ≥ 5.7	0.93 (0.87, 0.98)	0.97 (0.93, 1.02)	0.96 (0.90, 1.02)	0.93 (0.89, 0.98)
Time-varying: BMI, WC, PA, and FG	0.95 (0.90, 1.00)	0.99 (0.95, 1.03)	0.97 (0.92, 1.03)	0.94 (0.91, 0.98)
Exclude first 3 years of observation	0.95 (0.90, 1.00)	0.98 (0.94, 1.02)	0.97 (0.92, 1.03)	0.94 (0.90, 0.98)

z-scores: Global: global cognitive function, 1 SD=0.96; DWR: delayed word recall,1 SD=0.97; DSS: digit symbol substitution, 1 SD=0.95; WFT: word fluency test, 1 SD=0.99. Model adjustments include all previous models, except the bias models, which are performed on the primary final model. All values reflect those measured at visit 2 unless noted. Model 1: age, sex, race-field center, and education (visit 1). Model 2: smoking (status and cigarette-years [visit 1]), regular alcohol consumption, and PA sports index (visit 1). Model 3: BMI, waist circumference, fasting glucose, and fasting insulin (visit 1). Model 4: parental history of diabetes (visit 1) and *APOE* ε4. Model 5: psychosocial measures of trait anger, social support, and depressive symptoms. Primary final model: Systolic blood pressure and anti-hypertension medication use. Sample size for time-varying analyses is 9,174.

Table 4.6. Hazard ratios (HR) and 95% confidence intervals (95% CI) for incident diabetes per 1 grade increment of white matter hyperintensities, ventricular size, and sulcal size in 1,350 participants, the Atherosclerosis Risk in Communities Study, 1993-1994 through 2014

	HR (95% CI) per 1 grade increment in MRI measure		
Model Adjustments	White Matter Hyperintensities	Ventricular Size	Sulcal Size
Unadjusted	1.15 (1.04, 1.27)	1.15 (1.04, 1.26)	1.04 (0.92, 1.16)
Model 1	1.13 (1.02, 1.25)	1.11 (1.01, 1.23)	1.01 (0.90, 1.14)
Model 2	1.13 (1.02, 1.25)	1.11 (1.01, 1.23)	1.00 (0.88, 1.13)
Model 3	1.16 (1.05, 1.29)	1.11 (1.01, 1.23)	0.99 (0.88, 1.12)
Model 4	1.16 (1.04, 1.29)	1.11 (1.00, 1.23)	0.99 (0.88, 1.12)
Model 5	1.17 (1.05, 1.30)	1.11 (1.00, 1.23)	0.99 (0.88, 1.13)
Primary final model	1.13 (1.01, 1.26)	1.11 (1.00, 1.23)	1.01 (0.90, 1.14)
Bias Models			
IPAW	1.13 (0.98, 1.30)	1.14 (1.01, 1.28)	1.06 (0.91, 1.23)
Stratified			

Glucose < 5.6 mmol/l and HbA1c < 5.7	1.23 (1.04, 1.45)	1.20 (1.02, 1.41)	1.11 (0.91, 1.38)
Glucose ≥ 5.6 mmol/l or HbA1c ≥ 5.7	1.06 (0.91, 1.24)	1.08 (0.94, 1.23)	0.94 (0.81, 1.10)
Time-varying: BMI, WC, PA, and FG	1.14 (1.02, 1.27)	1.11 (1.00, 1.24)	0.99 (0.87, 1.12)
Exclude first 3 years of observation	1.16 (1.03, 1.30)	1.15 (1.03, 1.28)	1.01 (0.88, 1.15)

Model adjustments include all previous models, except the bias models, which are performed on the primary final model. All values reflect those measured at visit 3 unless noted. Model 1: age, sex, race-field center, and education (visit 1). Model 2: smoking (status and cigarette-years [visit 1]), regular alcohol consumption, and PA sports index. Model 3: BMI, waist circumference, fasting glucose, and fasting insulin (visit 1). Model 4: parental history of diabetes (visit 1) and *APOE* ε4. Model 5: psychosocial measures of trait anger, social support, and depressive symptoms (visit 2). Primary final model: Systolic blood pressure and anti-hypertension medication use. Sample size for time-varying analyses is 9,174.

Table 4.7. Hazard ratios (HR) and 95% confidence intervals (95% CI) for according to category of white matter hyperintensities, ventricular size, and sulcal size in 1,350 participants, the Atherosclerosis Risk in Communities Study, 1994-1995 through 2014

		HR (95% CI) for incident T2DM according to grade category for selected models					
	Grade	Unadjusted	Model 1	Model 2	Primary Final	IPAW	Time-Varying
White Matter Hyperintensities	0	Reference	Reference	Reference	Reference	Reference	Reference
	1	0.99 (0.74, 1.33)	1.00 (0.75, 1.34)	0.94 (0.69, 1.27)	0.95 (0.70, 1.29)	0.96 (0.64, 1.44)	0.89 (0.65, 1.20)
	2	1.12 (0.80, 1.57)	1.25 (0.88, 1.77)	1.16 (0.81, 1.67)	1.10 (0.77, 1.59)	0.97 (0.61, 1.55)	1.06 (0.73, 1.53)
	≥3	1.76 (1.18, 2.62)	1.68 (1.11, 2.53)	1.88 (1.23, 2.87)	1.68 (1.09, 2.59)	1.73 (0.99, 3.00)	1.67 (1.08, 2.56)
P for trend		0.008	0.006	0.003	0.02	0.14	0.02
Ventricular Size	0-1	Reference	Reference	Reference	Reference	Reference	Reference
	2	1.20 (0.89, 1.62)	1.21 (0.89, 1.64)	1.11 (0.82, 1.51)	1.20 (0.88, 1.63)	1.46 (0.95, 2.25)	1.09 (0.80, 1.49)
	3	1.46 (1.05, 2.02)	1.36 (0.97, 1.91)	1.24 (0.88, 1.76)	1.29 (0.91, 1.83)	1.58 (0.96, 2.58)	1.18 (0.83, 1.68)
	≥4	1.60 (1.10, 2.34)	1.45 (0.97, 2.15)	1.42 (0.95, 2.12)	1.42 (0.95, 2.13)	1.68 (0.98, 2.88)	1.41 (0.94, 2.12)
P for trend		0.004	0.04	0.06	0.08	0.05	0.1
Sulcal Size	0-1	Reference	Reference	Reference	Reference	Reference	Reference

	2	1.20 (0.93, 1.55)	1.13 (0.87, 1.47)	1.13 (0.87, 1.48)	1.15 (0.88, 1.50)	1.30 (0.92, 1.82)	1.08 (0.82, 1.41)
	3	1.03 (0.75, 1.42)	0.99 (0.71, 1.38)	0.98 (0.70, 1.37)	1.06 (0.76, 1.49)	1.30 (0.86, 1.96)	1.03 (0.73, 1.44)
	≥4	1.32 (0.81, 2.15)	1.20 (0.73, 1.98)	1.09 (0.66, 1.80)	1.09 (0.66, 1.82)	1.13 (0.57, 2.27)	0.97 (0.58, 1.61)
P for trend		0.49	0.76	0.99	0.74	0.38	0.95

Model adjustments include all previous models, except for "IPAW" and "Time-Varying" models which are performed on the primary final model. All values reflect those measured at visit 3 unless noted. Model 1: age, sex, race-field center, and education (visit 1). Model 2: smoking (status and cigarette-years [visit 1]), regular alcohol consumption, physical activity sports index, BMI, waist circumference, fasting glucose, and fasting insulin (visit 1). Primary final: parental history of diabetes (visit 1), *APOE* ε4, psychosocial measures of trait anger, social support, and depressive symptoms (visit 2), systolic blood pressure, and anti-hypertension medication use. Sample size for time-varying analyses is 1,073.

4.7. Figures

Figure 4.1. Participant exclusions for aim 1 (1990-1992 through 2014) and aim 2 (1993-1994 through 2014), the Atherosclerosis Risk in Communities Study

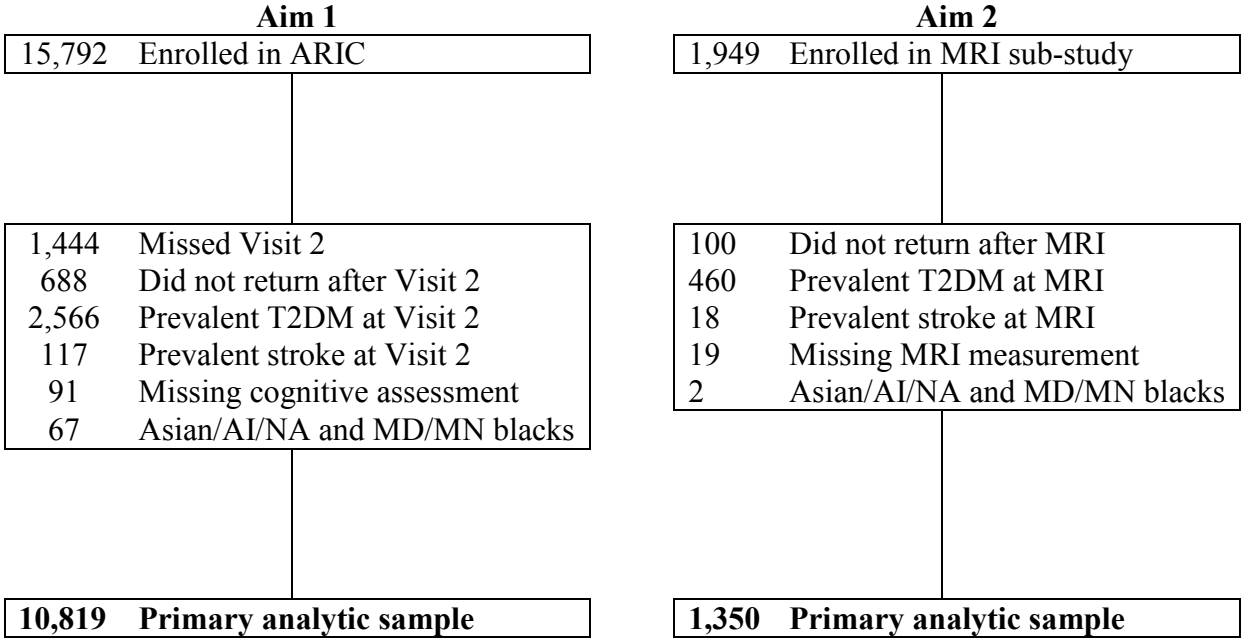
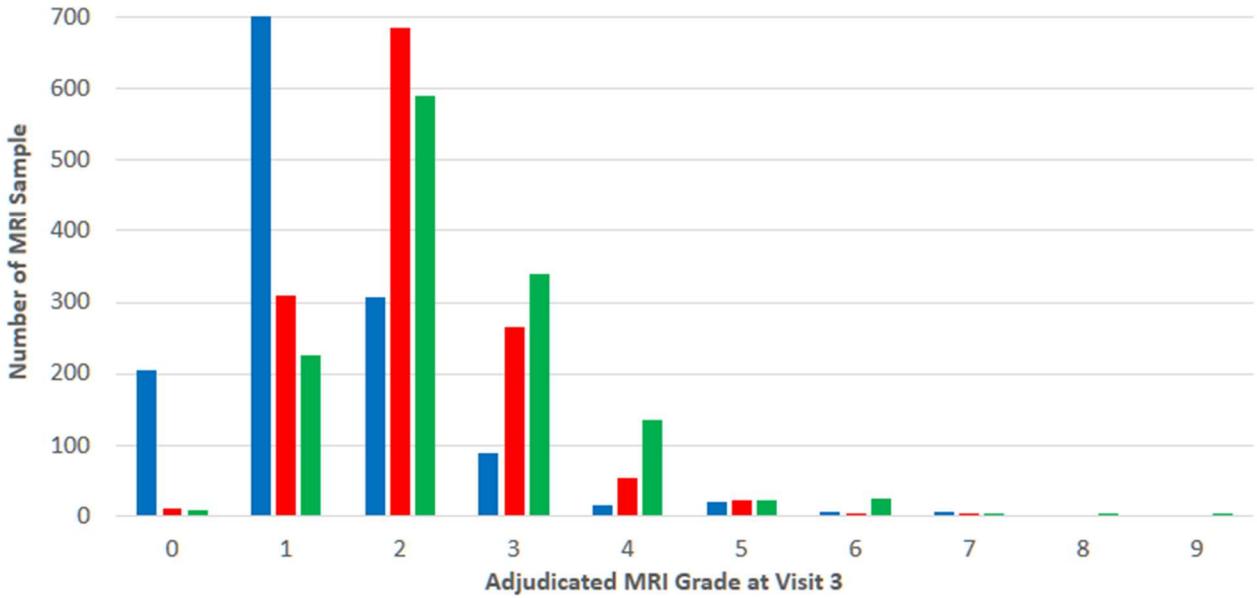


Figure 4.2. Distribution of adjudicated grade for white matter hyperintensities, sulcal size, and ventricular size at visit 3 MRI sub-sample (1993-1994), the Atherosclerosis Risk in Communities Study



White Matter Hyperintensities	204	706	308	88	14	19	6	5	0	0
Sulcal Size	11	309	685	265	55	22	2	1	0	0
Ventricular Size	8	226	591	339	135	22	24	2	2	1

Chapter 5. Manuscript 3: Cardiovascular Health Status in Young Adulthood and Brain Structural Imaging in Midlife; The Coronary Artery Risk Development in Young Adults (CARDIA) Study

5.1. Overview

Background: The American Heart Association (AHA) Life's Simple 7 (LS7) is a summary of 7 major modifiable cardiovascular health behaviors and factors, equipping individuals, clinicians, and researchers with a resource to encourage, measure, and track individual and population-level improvements in cardiovascular health. The LS7 is associated with level of cognitive function, cognitive decline, and predict incidence of cognitive impairment. The association between the LS7 and brain structure is unclear.

Methods: We determined cardiovascular health (CVH) according to the AHA LS7 at baseline (aged 18-30 years in 1985-1986), year 7 follow-up examination (Y7), and year 25 follow examination (Y25) for 710 participants of the CARDIA Brain MRI sub-study at Y25. CVH at each visit and cumulative exposure was assessed in relation to normal tissue volume (cm^3) of the total brain, gray matter, and white matter, and abnormal tissue volume of white matter at year 25.

Results: The proportion of individuals meeting more favorable levels of CVH was low and decreased with age. Mean (± SD) normal tissue volume of the total brain, gray matter, and white matter was 982 (± 107) cm^3, 517 (± 54) cm^3, and 465 (± 59) cm^3. Greater CVH score at baseline (per 1 unit CVH score: 1.3 cm^3, 95% CL: 0.03, 2.5) and greater cumulative exposure to favorable CVH throughout young adulthood (to Y25) was

associated with greater normal tissue volume of the total brain (per 1 SD in cumulative CVH exposure to Y25: 3.3 cm^3, 95% CL: 0.5, 6.1). In a fully adjusted model, greater visit-based CVH score at Y7 was associated with greater normal tissue volume of white matter at Y25. Otherwise, visit-based or cumulative exposure to favorable CVH was not significantly associated with normal gray or white matter volume or abnormal white matter volume.

Conclusion: Maintaining ideal levels of cardiovascular health throughout young adulthood is not associated with differences in brain structure in middle age. The lack of consistent associations across multiple brain tissues warrants further longitudinal investigation of the LS7 and brain structure.

5.2. Introduction

With the 2020 Strategic Goals, the American Heart Association (AHA) introduced a construct, termed the Life's Simple 7 (LS7), which framed cardiovascular health (CVH) according to meeting more favorable levels of 7 modifiable behaviors and factors.[165] These 7 behaviors and factors include current smoking, body mass index, regular physical activity, diet, total cholesterol, blood pressure, and fasting glucose.[165] The LS7 provide a simple and versatile summary of cardiovascular health, applicable for measurement and tracking at the clinical and population level and several studies have shown that a small number of Americans achieve ideal levels for all 7, a trend for at least the last few decades.[166,241-243] Considering the LS7 was based on the primordial prevention of cardiovascular disease risk factors, it comes as no surprise that research has

shown lower accumulation of the 7 components strongly predict atherosclerotic and hypertensive cardiovascular disease events.[241,244-248] Harmonious to the 2020 Strategic Goals, research has shown the utility of the LS7 for predicting incidence of several chronic diseases, including diabetes, various cancers, respiratory diseases, and all-cause and cause-specific mortality.[243,249-254] This demonstrates the importance of these behaviors and factors in successful aging in general and for potential cross-discipline preventive health collaboration and intervention.

The relationship between favorable cardiovascular health and successful aging of the brain, both cognitive function and brain structure and physiological function, has been emphasized.[3] Dementia and other neurological conditions are a growing global public health concern, particularly in countries with aging populations and less favorable cardiovascular health profiles.[137,255] Investigation on maintenance of ideal cardiovascular health and improvement of less than ideal cardiovascular health is imperative to understanding prevention or delay of early cognitive decline and brain pathology.[255] Recently, research has shown meeting more favorable levels of the 7 AHA cardiovascular health components is associated with better concurrent and subsequent scores on neurocognitive tests and with lower incidence of developing cognitive impairment.[166-168] The cardiovascular health profile integral to maintaining healthy brain structure is less clear and merits investigation. Magnetic resonance imaging (MRI) provides a non-invasive method to measure potential structural changes of the brain (i.e. atrophy or shrinkage), before clinical onset of dementia, other neurological disease, and clinically significant cerebrovascular damage. Additionally, detecting subclinical changes

in the brain associated with modifiable cardiovascular behaviors and factors in early adulthood affords the opportunity to identify periods of intervention and cessation or mitigation of damage and to understand the pathophysiological processes in the early stages of disease.[132] Lastly, identifying an association between the LS7 and healthy brain structure reinforces the importance of the LS7 in preventive health approaches for numerous health outcomes.

We had multiple objectives with this work. First, we sought to define and determine ideal cardiovascular health at three time points during young adulthood, according to a score and cumulative exposure according to criteria set forth by the AHA Life's Simple 7 and based on the data available in CARDIA. Our second objective was to assess these measures of cardiovascular health in relation to outcomes of brain structure in middle adulthood. Our hypothesis was that meeting more favorable levels of cardiovascular health at distinct junctures and through young adulthood would be associated with greater volume of normal tissues of the brain and lower volume of abnormal tissue of the brain.

5.3. Methods

The CARDIA Study Design

The CARDIA Study is an ongoing longitudinal cohort study of 5,115 black and white men and women from four metropolitan populations (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). CARDIA participants were 18-30 years of age at enrollment (1985-1986, exam year 0; Y0). Participants were free

from cardiovascular disease and the cohort was balanced across categories of age (18 – 24 and 25 – 30 years of age), race (black and white), sex (men and women), and education (≤ high school graduation and > high school education).[256] Since enrollment participants have been contacted by telephone annually and invited to participate in 7 in-person follow-up examinations at roughly 2 (91% retention of surviving cohort), 5 (86%), 7 (81%), 10 (79%), 15 (74%), 20 (72%), and 25 (72%) years (Y2-Y25), respectively, succeeding enrollment (an 8th in-person follow-up, year 30 exam, is currently in progress).[256] Participants were given an explanation of the study at their initial visit and have provided written informed consent at each follow-up and each field center/coordinating center has been granted institutional review board (IRB) approval for all exams.

Assessment of Demographics, Health Behaviors, and Clinical Examination

For each clinic examination (Y0-Y25), subjects were instructed to arrive on the morning of their examination having completed an overnight fast (fasting at least 12 hours, excluding water). Additionally, subjects were asked to forgo smoking or participating in heavy physical activity for 2 hours prior to the examination. Self- and interviewer-administered questionnaires were used to collect information on sociodemographic factors (race, educational attainment, and residence), medical and family history, use of medications, and individual lifestyle characteristics including, tobacco use (status and frequency), regular alcohol consumption, illicit drug use, and moderate and strenuous

physical activity. Venous blood was drawn, plasma and serum separation was performed before aliquots were stored at -70°C and shipped on dry ice to a central laboratory.

Lipid measurement has been detailed previously.[257] Total cholesterol was measured enzymatically within 6 weeks of collection.[258] Serum glucose measurement across exams has been previously described.[259] At Y0, serum glucose was measured using the hexokinase ultraviolet method by American Bio-Science Laboratories (VanNuys, CA) and at subsequent examinations using hexokinase coupled to glucose-6-phosphate dehydrogenase by Linco Research (St. Louis, MO). Apolipoprotein ε phenotype was determined from plasma samples collected at Y7 using a modification of the method detailed by Kamboh et al.[260] Kataoka et al refined this procedure, which involves isoelectric focusing and immunoblotting techniques, showing high concordance with genetic phenotyping (98% concordance) and reliability in blinded duplicates (96% agreement) in 431 samples.[261]

At exam years 0-15, seated blood pressure was measured in triplicate after 5 minutes of rest using a random-zero sphygmomanometer. At exam Y20 and Y25, blood pressure was measured with an Omron (Omron Corp., Schaumburg, IL) HEM907XL oscillometer and calibrated to the random-zero readings for comparability across exams. Omron values were recalibrated to corresponding random zero values based on a study of both measurement techniques in 903 participants at year 20, as estimated random zero systolic value = 3.74 + (0.96 X Omron systolic value), and estimated random zero diastolic value = 1.30 + (0.97 X Omron diastolic value).[262] The average of the last two measurements was used for this analysis. Body weight was measured using a calibrated

balance beam scale to the nearest 0.2 kg with participants in light clothing and height (without shoes) was measured to the nearest 0.5 cm using a vertical ruler.

An interviewer-administered dietary questionnaire designed specifically for CARDIA was used to assess dietary intake for the previous 28 days and was employed at CARDIA Y0, Y7, and Y20 exams.[263] Interviewers asked open-ended questions about food consumption in the previous month with 100 food categories, referencing 1,609 unique food items. Individuals were also queried on frequency of fast food consumption, and frequency of regular meals (breakfast, lunch, dinner, and morning, afternoon, and evening snacks). This diet history instrument shows better validity and stronger reliability for habitual food intake in whites compared to blacks.[264] Self-reported sports-related physical activity was assessed with an interviewer-administered questionnaire of frequency of participation over the previous 12 months for 13 categories of sports and exercise, 8 of which were considered vigorous-intensity and 5 considered moderate-intensity. Vigorous-intensity activities included running or jogging; racquet sports; biking; swimming; exercise or dance class; job lifting, carrying, or digging; shoveling or lifting during leisure; and strenuous sports while moderate-intensity activities included non-strenuous sports, walking and hiking, golfing and bowling, home exercises or calisthenics, and home maintenance or gardening. Participants were queried on the frequency and duration of participation in these activities and an intensity score ranging from 2 to 5 (most physically active) was determined for each activity.[265] An overall physical activity score was derived by multiplying the sum of months of infrequent activity plus 3 times the months of frequent activity by intensity of the activity, and

summing over all activities. The score was expressed in "exercise units" (EU), with a score of 300 EU corresponding to 30 minutes of moderate physical activity 5 times per week.[266] The physical activity questionnaire used in CARDIA has high test-retest reliability (0.77-0.84) over a two-week period.[265]

Determination of *Ideal, Intermediate, and Poor* Cardiovascular Health (CVH) Status and Overall CVH Score

We determined CVH status for all participants at CARDIA exam Y0, Y7, and Y25 according to the 7 CVH behaviors and factors introduced by the AHA (definitions provided in **Table 5.1.**).[165] These three exam years were chosen because of the completeness of Life's Simple 7 components measured or for relation of concurrent measurement of brain imaging, illustrated in **Figure 5.1**.

Slight modifications were made for diet and physical activity based on the data available in CARDIA. For example, we used diet data from Y20 to determine diet status for Y25 and we used physical activity equivalents to the AHA Simple 7 guidelines. The American College of Sports Medicine suggests 5 sessions of 1260 kJ [300 kcal] of energy expenditure weekly is sufficient to support weight loss and 300 EU is approximately equivalent.[266]

Brain Magnetic Resonance Imaging (MRI) Sub-Study Methods

The CARDIA Brain MRI sub-study, conducted at exam Y25 (2010-2011), was limited to a defined subset of CARDIA participants (n=719) from three of the field centers,

Birmingham, Minneapolis, and Oakland (CARDIA manuals and protocols publicly available: http://www.cardia.dopm.uab.edu/images/more/pdf/mooy25/chapter12.pdf and http://www.cardia.dopm.uab.edu/images/more/CARDIA_Y25_Exam_Protocol_v2012-03-01.pdf).[267] Participants were recruited at the time when Y25 appointments were scheduled. Individuals were excluded from participation in the Brain MRI sub-study if they had a contra-indication to MRI (claustrophobia, pacemaker, defibrillator, neuro-stimulator, ferro-magnetic or unknown aneurysm clip, 3T MR incompatible metal implant of any kind, or potentially-dangerous foreign metal objects in the body such as bullets, shrapnel, metal slivers, etc.), possible pregnancy, or a body size that was too large for the MRI tube bore (BMI>40 kg/m^2; waist or hips > 50 inches in diameter). This selective inclusion resulted in enrolling individuals in the Brain MRI sub-study who were healthier than those from the overall cohort in regard to body size (and weight) and general health issues such prior surgeries. Previous studies have described MRI acquisition and processing.[268] Briefly, 3T magnetic resonance scanners were used to acquire structural imaging, standardized across machines using a common machine head phantom (CA: Siemens 3T Tim Trio/VB 15 platform; MN: Siemens 3T Tim Trio/VB 15 platform; and AB: Philips 3T Achieva/2.6.3.6 platform).[268] Using sagittal 3D T1 sequence, total intracranial volume (TICV) was estimated as the sum of gray matter (GM), white matter (WM), and cerebral spinal fluid (CSF) volumes, and total brain tissue volume (TBV) as the sum of GM and WM volumes. Abnormal white matter tissue was estimated from the sagittal 3D FLAIR, T1, and T2 sequences. Structural image processing was based on an automated multispectral computer algorithm that classified

brain tissue into GM, WM, and CSF. GM and WM were further characterized as normal and abnormal and into specific regions of interest.[268] All images were subject to quality control inspection for motion artifacts or other quality issues before being processed by the Section of Biomedical Image Analysis, Department of Radiology, University of Pennsylvania. Quality assurance protocols, developed for the Functional Bioinformatics Research Network (FBIRN), and the Alzheimer's disease Neuroimaging Initiative (ADNI), included the evaluation of scanner stability and image distortion prior to each site's acceptance and subsequent quarterly quality control evaluations.

Statistical Analysis

Our analytic sample for this analysis draws from the 719 individuals who underwent brain MRI at Y25. Nine of these individuals were excluded due to missing imaging data (n=710). Multiple imputation was used to impute all other missing data, detailed in **Figure 5.2**. We created 10 imputed datasets (m=10), assuming an arbitrary missing data pattern and using participants with full data to impute missing data. At the modeling stage, the datasets were analyzed individually and combined according to Rubin's Rules.[192] We calculated CVH scores at each of the three exams, assigning points (0, 1, or 2) for meeting poor, intermediate, or ideal criteria for each of the 7 components. For each exam, scores could range from 0 to 14, with a CVH score of 0 interpreted as meeting poor criteria for all 7 components and a CVH score of 14 interpreted as meeting ideal criteria for all 7 components, as previously modeled in other studies.[167,245,248] In contrast to creating a CVH score based solely on meeting ideal criteria or not (a sum total of the 7

components; 0-7), this CVH score incorporates intermediate status into the cardiovascular profile, providing a more nuanced evaluation of CVH benefit across each component. This score was further categorized into 3 groups according to cut points of 0-7, 8-11, 12-14. We chose our cut points for consistency of score definition across time and for adequate sample sizes for comparison groups. We also calculated a cumulative exposure to CVH for each participant over the duration of observation. This was done by calculating the cumulative exposure separately for two periods, from Y0 to Y7 and then from Y7 to Y25. Cumulative exposure was calculated as the product of cardiovascular health score and year duration between reassessment of cardiovascular health. We then summed the cumulative scores from the two periods for an overall cumulative exposure. This method assumes a constant value of CVH until the next assessment of CVH. For example, cumulative exposure to CVH from baseline to Y7 for an individual with a CVH score of 10 at their baseline exam would be 7 x 10 = 70. Cumulative exposure to CVH between Y7 and Y25 would be calculated similarly using the CVH score at Y7, for example a score of 8, multiplied by the duration until Y25 (18 years) or 8 x 18 = 144. The aggregate score of these two periods reflecting cumulative exposure to CVH over the complete 25 year duration of observation, in this instance 214 (70 + 144).

Our outcomes were volume (cm^3) of normal tissue of the total brain, gray matter, white matter, and abnormal white matter. A larger volume of normal tissue after accounting for intracranial volume would suggest more optimal brain structure (i.e., less atrophy) while a larger volume of abnormal tissue would indicate more pathology. Tissue classified as abnormal white matter results from small vessel infarcts or leukoaraiosis

(myelin rarefaction, demyelination, and axonal destruction). While 80% of the analytic sample exhibited presence of any abnormal white matter, the individual volumes were small and distribution was right-skewed. We created tertiles of abnormal white matter and tested for violations to the proportional odds assumption with the Brandt test of parallel regression and the likelihood-ratio test of proportionality of odds and did not find occurrence of violation for any model (all $p>0.05$). Therefore, we assessed the odds of being in the top tertile of abnormal white matter versus being in the lower 2 tertiles using multivariable logistic regression in the multiply imputed data. This cut point was not based on clinical significance, but for adequate sample size of cases, and is arbitrary in nature. For all normal tissues we assessed associations between CVH and tissue volumes with separate multivariable linear regression models in the multiply imputed data. For each outcome, we modeled CVH in three fashions: continuous CVH score (0-14) at each exam; categorical CVH at each exam; and cumulative exposure to CVH to Y25. We included adjustment for TICV, age, sex, race, field center, educational attainment (any up to high school graduate, any college, or more than 4 years of college), alcohol consumption (no regular, moderate consumption: up to 1 drink daily for women/2 drinks daily for men, or heavy consumption: greater than 1 drink daily for women/2 drinks daily for men), and apolipoprotein ε4 (*APOE* ε4) phenotype (0 ε4 alleles or ≥1 ε4 alleles). When models included adjustment for time-dependent characteristics (e.g., age, education, alcohol, etc.), values reflecting those measured concurrently with CVH were used. We assessed effect modification by sex and race by including a product term between CVH and sex and race, separately, for each measure of brain imaging. We

assessed the sensitivity of our estimates to the variability introduced with imputation with analyses in the reduced sample of non-imputed data. Lastly, we examined the association between cumulative CVH score for to each of the 7 components and each of the brain structures. This was done to identify if any component was individually strongly associated with each outcome. A combination of SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC) and STATA 13.0 software (College Station, TX) was used for statistical analysis.

5.4. Results

Of the 710 individuals with complete MRI data, 53% were female and 60% were white. Men had larger total intracranial volumes (TICV) relative to women, as whites had larger TICV compared to blacks. After adjustment for TICV and age, women had significantly ($p<0.001$) larger volumes of normal tissue of the total brain and gray matter compared to men (**Table 5.2**). Adjusted tissue volume of normal gray matter was significantly larger in whites compared to blacks while volume of normal white matter was significantly larger in blacks compared to whites ($p<0.001$). **Table 5.3** presents the participant characteristics at year 25 averaged over the 10 imputed datasets according to category of cardiovascular score. Individuals who had a higher CVH score at Y25 were more likely to be white, highly educated, moderate consumers of alcohol, and have zero *APOE* ε4 alleles. Over the course of observation, the number of individuals attaining a higher CVH score declined (**Figure 5.3**).

Brain Imaging Outcomes

Tables 5.4 to 5.7 present results for multivariable adjusted mean differences in normal tissue volumes of the total brain, gray matter, and white matter, separately, and odds ratios for being in the top tertile of abnormal white matter compared to being in the lower 2 tertiles according to modeling technique of CVH. None of the associations varied significantly by race or sex (*p* for interaction > 0.1, for all).

Normal Tissue Volume of the Total Brain

When CVH score for each individual visit was modeled continuously, a greater CVH score at Y0 was associated with significantly greater normal tissue volume of the total brain at Y25 after final adjustment (**Table 5.4**: per 1 unit in score: 1.3 cm^3; 95% CL=0.03, 2.5). Categorical CVH at Y0 was not associated with normal tissue volume of the total brain at Y25. Alternatively, individuals in the upper categories of CVH at Y7 had significantly greater normal tissue volume of the total brain at Y25 compared to individuals in the lowest category of CVH at Y7, while continuous CVH at Y7 was not significantly associated with greater normal tissue volume of the total brain at Y25. Neither continuous nor categorical CVH score at Y25 was associated with normal tissue volume of the total brain at Y25. However, greater cumulative CVH to Y25 was associated with significantly greater normal tissue volume of the total brain at Y25 (per 1 SD increment in cumulative CVH to Y25: 3.3 cm^3; 95% CL= 0.5, 6.1). For reference, a 1 year increase in age was associated with 1.4 cm^3 less normal tissue volume of the total brain (95% CL= -0.7, -2.0).

Normal Tissue Volume of Gray Matter

After adjustment for TICV, age, sex, race, field center, education, alcohol consumption, and *APOE* ε4 phenotype, continuous CVH score at Y0, Y7, and Y25 was not associated with normal gray matter volume at Y25 (**Table 5.5**). No association was observed for categorical CVH score at any exam or cumulative CVH and normal gray matter volume at Y25 after final adjustment.

Normal Tissue Volume of White Matter

Continuous CVH score at Y0, and Y25 was not associated with normal white matter volume at Y25, before or after adjustment shown in **Table 5.6**. However, a 1 unit increment in CVH score at Y7 was associated with 1.0 cm^3 more normal white matter volume (95% CL= 0.2, 2.0. Individuals in the upper categories of CVH at Y7 had significantly greater normal white matter volume at Y25 compared to individuals in the lowest category of CVH at Y7, and this association was significant for a linear trend across categories (p=0.03). Category of CVH score at Y0 and Y25 was not associated with normal white matter volume at Y25. Cumulative CVH was not significantly associated with normal white matter volume at Y25.

Abnormal Tissue Volume of White Matter

Continuous CVH score at Y0, Y7, and Y25 was not associated with being in the top tertile of abnormal white matter volume at Y25 before or after adjustment for TICV, age,

sex, race, field center, education, alcohol consumption, *APOE* ε4 phenotype, and previous CVH (where applicable) (**Table 5.7**). No significant association was observed for categorical CVH score or cumulative CVH at each of these years and abnormal white matter volume at Y25 after final adjustment.

Results in Case-Only Analyses

Beta-estimates from sensitivity analyses in the reduced sample of non-imputed data were consistently stronger, indicating larger absolute differences in the normal tissue volumes and lower odds ratios for being in the top tertile of abnormal white matter volume when comparing the upper categories of CVH at each respective examination year to the lowest category of CVH at the corresponding examination year and the cumulative exposure to CVH (shown in bottom rows of **Table**s **5.4 to 5.7**). For example in **Table 5.4**, the increase in normal tissue of the total brain due to 1 SD increment in cumulative CVH in the imputed data (per 1 SD increment in cumulative CVH: 3.3 cm^3; 95% CL=0.5, 6.1) was 48% larger in the non-imputed data (per 1 SD increment in cumulative CVH: 4.9 cm^3; 95% CL= 1.9, 8.0).

We assessed the association between cumulative exposure to each of the 7 components and each of the brain structure outcomes. Cumulative CVH exposure to any of the individual 7 metrics was not associated with any of the brain structures (all $p > 0.05$; data not shown**)**.

5.5. Discussion

These results do not support an association between cardiovascular health through young adulthood, defined by the Life's Simple 7 components, and brain structure in middle adulthood. In this sub-sample of CARDIA participants who underwent brain magnetic resonance imaging, individuals with better cardiovascular health scores at baseline and 7-year follow-up exam had marginally greater total volumes of normal tissue of the total brain at the year 25 follow-up exam. Additionally, individuals with greater cumulative exposure to favorable cardiovascular health throughout young adulthood had greater normal tissue volume of the total brain and marginally greater volume of normal white matter at examination year 25. These potentially beneficial associations were not consistently observed for greater normal tissue volumes of gray and white matter or for lower burden of abnormal white matter. The clinical significance of our observed associations is unclear. For example, the observed differences in volume for normal tissue of the total brain were roughly equivalent to an increase in age of 2 years. However, the increment of cumulative exposure was equivalent to a difference in CVH score of nearly 2 units per year over the 25 years.

Brain health can be assessed functionally and structurally and assessing brain structure and pathology in relation to cardiovascular health is an important addition to previous work of this theme. By adding structural assessment, this work complements previous longitudinal assessment of the LS7 components and cognitive function in the CARDIA cohort and REasons for Geograhic And Racial Differences in Stroke (REGARDS) Study.[166,167] Reis et al reported CARDIA participants meeting more

favorable criteria for the LS7 in young adulthood and middle age exhibited better scores for multiple measures of cognitive function in middle adulthood.[166] More recently, Thacker et al reported individuals in the REGARDS study who were meeting intermediate or ideal levels of cardiovascular health had significantly lower risk for incidence of cognitive impairment compared to those with the poorest cardiovascular health profiles.[167]

The structure and integrity of the brain is important to its own physiological function (e.g., cerebral blood flow, oxygen and glucose supply, neuronal signaling) and cognitive function. Adequate perfusion of the brain is vital; the brain depends on a continuous circulation of blood to supply oxygen and glucose to its tissues.[15] Greater brain atrophy is associated with lower cerebral blood flow and may explain the association between lower cerebral blood flow and worse cognitive function.[269] The presence of clinically silent infarcts is shown to be associated with lower scores of cognitive function and more abnormalities at neurological examinations.[63] Greater brain atrophy is associated with lower scores on tests of cognitive functioning, possibly reflecting underlying neuronal loss.[55] Presence and progression of white matter hyperintensity volume is associated with greater cognitive decline.[61] These causes and corollaries of structural assessment of the brain traditionally come from cohorts older than CARDIA and the differences in normal and abnormal tissue volumes in our sample may not have clinical significance at this point in CARDIA. We did observe a majority of our sample had presence of abnormal white matter, though the individual volumes were small. This is consistent with what has been observed in other healthy populations, where

age-related differences white matter hyperintensity volume were not pronounced until the 7th decade.[16] Sowell et al observed a non-linear association between normal white matter volume and age, with an increase in volume until the age of 43, leveling, and then a decline in volume.[270] Data from the Framingham Heart Study observed a curvilinear association for age-related differences in brain volume, showing little difference in brain volumes before the age of 55 years with a pronounced steepening after the age of 55.[16] Individuals in our cohort were all under the age of 55 and we observed comparable age-related differences in brain volume to the Framingham participants under the age of 55. We did observe low prevalence of individuals meeting ideal criteria for 0 or all 7 of the components, consistent with other community-based studies and the full CARDIA cohort.[166,167,241,271] However, the CARDIA Brain MRI sub-study population was a relatively young healthy population at the time of MRI which may limit the ability to detect early differences in brain structure due to cardiovascular health factors.

In regard to the structural health of the brain, the individual components of hypertension, diabetes, overweight/obesity, and cigarette smoking have consistently been shown to be independently associated with less favorable structural brain imaging due to vascular and neuronal damage and general atrophy.[84,87,127,132,272] When looking at the impact of the aggregation of some less favorable cardiovascular health metrics, research exploring the metabolic syndrome may offer the best insight. In a relatively healthy sample of Korean adults, researchers found that individuals with a greater number of components for the metabolic syndrome had a significantly greater number of silent brain infarcts.[273] A separate study of Korean adults found a dose-dependent increase in the

prevalence of white matter hyperintensity with increase in the number of components of the metabolic syndrome.[274] However, this association was observed only in women, not in men, and this study population was a highly selective group of adults seeking routine visits to the health care system.[274] A study of Japanese adults with no history of stroke who visited a health care facility for routine care reported increased odds of the metabolic syndrome in individuals with leukoaraiosis compared to those without leukoaraiosis.[275] Also important to note, all three studies were cross-sectional in design, limiting inference and generalizability.[273-275] In contrast, results from an elderly population of Austrian men and women free from stroke and dementia did not show cross-sectional differences in white matter hyperintensity volume, total brain volume, or presence of lacunes or silent infarcts between individuals with the metabolic syndrome and those without.[276]

A value of the Life's Simple 7 construct is the ability to summarize and monitor major modifiable cardiovascular health determinants, providing goals and targets for clinicians, patients, and populations.[165] Originally constructed to aid in achieving the American Heart Association's 2020 Impact Goals, the utility of this measure extends beyond the walls of cardiovascular health showing strong capacity to predict overall health and susceptibility to various chronic diseases of non-cardiovascular origin such as diabetes, various cancers, respiratory illnesses of pneumonia and chronic obstructive pulmonary disease, and hip-fracture.[249,250,253] In recent years the importance of maintaining ideal cardiovascular health to prevent risk to the brain beyond stroke, including early cognitive decline, dementia, and tissue damage and pathology has been

noted.[3] Our results do not provide strong evidence that maintaining ideal cardiovascular health through young adulthood is beneficially associated with brain structure in midlife.

It is important to consider the limitations of this study. First, brain imaging was assessed at only a single time in midlife. Therefore, we are unable to determine if ideal cardiovascular health through young adulthood is associated with change in brain volume or development of pathology. We are unable to ascertain if early development of abnormal tissue or reduced brain volume leads to less favorable lifestyle and health behavior changes, which would be considered reverse causation. Second, we assume each component of cardiovascular health can be weighted equally in constructing the score. High blood pressure and levels of fasting glucose are the cardiovascular-related characteristics that typically most strongly predict change in cerebral tissue.[84] In our data, cumulative exposure over the 25 years of observation for any of the components was not associated with of the brain volume outcomes and we show in our results that average levels of all 7 components become more favorable with increasing cardiovascular health score, so the concern that one component alone is driving any association may not be a major limitation. To that point, the objective of this work is not to identify any individual predictor of brain pathology, but to determine the application and association of the Life's Simple 7 as a whole with regard to brain health. Third, while retention in CARDIA is noteworthy, individuals may miss an exam over the course of follow-up and we used multiple imputation to minimize the influence of missing data. This added variation due to imputation may have attenuated estimates compared to analyses including only participants with complete information on all study variables. However, the general

inference is not different between the two analytic approaches. Fourth, participants of the Brain MRI sub-study were somewhat more likely at baseline to have more favorable cardiovascular health than the full CARDIA cohort, specifically lower smoking, BMI and waist circumference, and negligible cardiovascular disease events over the 25 years of observation. Lastly, this is an observational epidemiological study subject to residual confounding.

In summary, the current study showed cumulative exposure to more ideal level of cardiovascular health through young adulthood was associated with greater volume of normal tissue of the total brain in middle adulthood. Cardiovascular health score at individual visits in young adulthood was marginally associated with greater volume of normal tissue of the total brain in middle adulthood. Cumulative cardiovascular health exposure or score at individual visits during young adulthood was not significantly associated with volume of normal tissue of gray or white matter or volume of abnormal white matter at midlife. These results do not suggest that maintaining ideal levels of cardiovascular health throughout young adulthood is associated with early differences in multiple structural areas of the brain in middle age. This association warrants longitudinal investigation.

5.6. Tables

Table 5.1. Adapted definitions of *ideal, intermediate,* and *poor* cardiovascular health for each component for adults (age ≥ 18 years), the Coronary Artery Risk Development in Young Adults Study

Component	Ideal Health	Intermediate Health	Poor Health
Tobacco Smoking (Use)	Never **OR** quit ≥ 12 months	Former ≤ 12 months	Current use
Weight Status (BMI)	< 25 kg/m^2	25 - 29.9 kg/m^2	≥ 30 kg/m^2
Regular Physical Activity (Exercise Units)	≥ 300 Exercise Units	100-299 Exercise Units	< 100 Exercise Units
Total Cholesterol Level (mg/dL)	< 200 mg/dL (untreated)	200 - 239 mg/dL **OR** treated to goal	≥ 240 mg/dL
Resting Blood Pressure Level (mm HG)	SBP < 120 mm Hg **AND** DBP < 80 mm Hg (untreated)	SBP 120 – 139 mm Hg **OR** DBP 80 - 89 mm Hg **OR** treated to goal	SBP ≥ 140 mm Hg **OR** DBP ≥ 90 mm Hg
Fasting Glucose Level (mg/dL)	< 100 mg/dL (untreated)	100-125 mg/dL **OR** treated to goal	≥ 126 mg/dL
Usual Diet	4 - 5 components	2 - 3 components	0 - 1 components

Treated to goal may mean an individual meets ideal criteria for a given component, but are attaining this level via pharmacotherapy. Dietary components: Fruits & Vegetables ≥ 4.5 cups per day; Fish ≥ 7 ounces per week; Fiber-Rich Whole Grains ≥ three 1-oz-servings per day; Sodium < 1500 mg per day; Sugar-Sweetened Beverages ≤ 36 fluid ounces per week (≤ 450 kcal)

Table 5.2. Overall mean, standard deviation, and range and adjusted means and standard errors for volumes (cm^3) of normal tissues of the total brain, gray matter, and white matter and abnormal tissue volume of white matter according to race and sex for 710 individuals, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (2010-2011)

			Brain MRI sub-sample strata					
Imaging Measure		Overall	Women	Men	*P* sex	White	Black	*P* race
Normal Tissue Volumes	n	710	375	335		424	286	
Total Brain, mean ± SE*		982 ± 107	984 ± 1.6	979 ± 1.7	<0.001	982 ± 1.4	981 ± 1.8	NS
	Range	670 - 1349	670 - 1175	763 - 1349		738 - 1349	670 - 1261	
Gray Matter, mean ± SE*		517 ± 54	520 ± 1.2	514 ± 1.3	<0.001	521 ± 1.1	511 ± 1.3	<0.001
	Range	381 - 677	381 – 635	386 – 677		418 – 677	381 – 640	
White Matter, mean ± SE*		465 ± 59	465 ± 1.4	465 ± 1.5	NS	462 ± 1.2	470 ± 1.5	<0.001
	Range	290 - 672	290 - 595	343 - 672		312 - 672	290 - 650	
Abnormal Tissue Volume								
White Matter, mean ± SE*		0.51 ± 1.12	0.58 ± 0.06	0.42 ± 0.07	NS	0.45 ± 0.06	0.60 ± 0.07	NS
	Median	0.3	0.3	0.2		0.3	0.2	
	Range	0 - 23	0 - 9	0 - 23		0 - 9	0 - 23	

*The overall estimates for each imaging measure are unadjusted, presented as mean ± standard deviation. Adjusted normal tissue volumes are adjusted for TICV and age and presented as adjusted mean ± standard error of adjusted mean. Adjusted abnormal white

matter is adjusted for TICV and age and the median values are presented in addition to the adjusted mean ± standard error. *P* is comparison of adjusted means between sex and race groups. NS: $p > 0.05$

Table 5.3. Participant characteristics (n=710) at examination year 25 according to category of cardiovascular health score (range 0-14) averaged over 10 imputed datasets, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (2010-2011)

	Category of cardiovascular health (CVH) score at Year 25				
Characteristic	CVH (0-7)	CVH (8-11)	CVH (12-14)	*p* for trend	**Overall**
N (% sample)	184 (25%)	412 (58%)	114 (16%)		**710**
Age, years (mean)	51	51	51	0.94	**51**
Women, %	56	47	70	0.13	**53**
White, %	42	61	85	<0.001	**60**
>4 years college, %	11	19	38	0.001	**20**
Moderate alcohol, %	33	45	49	0.004	**42**
APOE ε4 (≥1 allele), %	35	31	22	0.03	**30**
Individual cardiovascular health score components					
BMI, kg/m^2	33	29	23	<0.001	**29**
Never smoked/quit > 1 year, %	59	88	96	<0.001	**82**
Physical activity, EU	175	391	544	<0.001	**360**
Total cholesterol, mg/dL	197	193	186	0.02	**193**
Cholesterol lowering medications, %	26	12	2	<0.001	**14**
Systolic blood pressure, mm HG	126	118	108	<0.001	**118**
Diastolic blood pressure, mm HG	80	73	65	<0.001	**74**

Blood pressure medications, %	44	18	4	<0.001	**23**
Fasting glucose, mg/dL	105	95	88	<0.001	**97**
Diabetes medications, %	12	4	0	<0.001	**6**
Number of ideal diet components	1.2	1.7	2.2	<0.001	**1.6**
Fruits & Vegetables, cups/day	4.5	5.7	7.9	<0.001	**5.8**
Fish, oz/week	6.4	6.2	5.5	0.41	**6.1**
Fiber-rich whole grains, oz/day	1.3	1.8	2.3	<0.001	**1.7**
Sodium, mg/day	3021	2984	2846	0.07	**2970**
Sugar-sweetened beverages, s/wk	9.5	5.9	3.5	<0.001	**6.5**

Values are means for continuous and percentages for categorical. Moderate alcohol consumption is any up to 1 drink daily for women and any up to 2 drinks daily for men. Sugar-sweetened beverages are servings/week (1 serving = 8 fl. oz; *ideal* ≤ 4.5 s/wk). *P* for trend is testing for linear trend across cardiovascular health categories.

Table 5.4. Multivariable adjusted mean difference and 95% confidence limits in normal tissue volume (cm^3) of the total brain for 710 individuals according to cardiovascular health (CVH) score, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (1985-1986 to 2010-2011)

		Mean Difference (95% CL) in Normal Tissue Volume (cm^3), Total Brain		
Model	CVH Score	Year 0 CVH	Year 7 CVH	Year 25 CVH
M1	0-7	Reference	Reference	Reference
	8-11	5.6 (-3.4, 14.7)	**9.3 (2.3, 16.2)**	0.4 -5.0, 5.8)
	12-14	6.8 (-2.7, 16.3)	**11.3 (3.3, 19.2)**	0.9 (-6.4, 8.1)
	p for trend	0.25	**0.02**	0.81
	Per 1 unit CVH score	1.2 (-0.04, 2.4)	**1.4 (0.2, 2.7)**	0.4 (-0.6, 1.4)
Per 1 SD increment in cumulative CVH		NA	NA	2.5 (-0.4, 5.0)
M2	0-7	Reference	Reference	Reference
	8-11	6.0 (-3.1, 15.0)	**9.2 (2.2, 16.1)**	0.9 (-4.6, 6.3)
	12-14	7.4 (-2.3, 17.1)	**11.7 (3.5, 19.9)**	1.8 (-5.7, 9.3)
	p for trend	0.21	**0.012**	0.64
	Per 1 unit CVH score	**1.3 (0.03, 2.5)**	**1.6 (0.2, 2.9)**	0.06 (-0.5, 1.6)
Per 1 SD increment in cumulative CVH		NA	NA	**3.3 (0.5, 6.1)**

Non-imputed data				
M2	0-7	Reference	Reference	Reference
	8-11	8.3 (-1.4, 18.0)	**10.8 (3.6, 18.1)**	3.7 (-2.6, 10.10
	12-14	9.5 (-1.0, 19.9)	**14.7 (6.2, 23.2)**	5.6 (-3.4, 14.5)
	p for trend	0.19	**0.002**	0.194
	Per 1 unit CVH score	**1.7 (0.4, 3.1)**	**6.5 (2.4, 10.6)**	2.9 (-1.5, 7.3)
Per 1 SD increment in cumulative CVH		NA	NA	**4.9 (1.9, 8.0)**

M1 adjusted for total intracranial volume, age, sex, and race. M2 further adjusted for field center, educational attainment, regular alcohol consumption, and *APOE* ε4. Linear CVH per 1 score unit is volume difference according to 1 unit increment in linear score (0-14). For cumulative CVH, 1 SD unit of cumulative CVH years at Y25 is 45.2. Sample sizes for non-imputed data reduce to 606, 595, and 501 for Y0, Y7, and Y25 analyses.

Table 5.5. Multivariable adjusted mean difference and 95% confidence limits in normal tissue volume (cm^3) of gray matter for 710 individuals according to cardiovascular health (CVH) score, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (1985-1986 to 2010-2011)

		Mean Difference (95% CL) in Normal Tissue Volume (cm^3), Gray Matter		
Model	CVH Score	Year 0 CVH	Year 7 CVH	Year 25 CVH
M1	0-7	Reference	Reference	Reference
	8-11	3.2 (-3.8, 10.1)	2.4 (-2.9, 7.7)	1.7 (-2.3, 5.7)
	12-14	4.4 (-2.9, 11.7)	4.0 (-2.2, 10.1)	3.0 (-2.5, 8.5)
	p for trend	0.26	0.22	0.27
	Per 1 unit CVH score	0.9 (-0.04, 1.8)	0.8 (-0.2, 1.7)	0.6 (-0.2, 1.3)
Per 1 SD increment in cumulative CVH		NA	NA	**2.0 (0.2, 3.8)**
M2	0-7	Reference	Reference	Reference
	8-11	1.9 (-4.9, 8.8)	1.3 (-4.0, 6.5)	0.9 (-3.1, 4.9)
	12-14	2.3 (-5.1, 9.6)	2.3 (-3.9, 8.5)	1.3 (-4.2, 6.9)
	p for trend	0.63	0.46	0.61
	Per 1 unit CVH score	0.6 (-0.4, 1.5)	0.5 (-0.4, 1.5)	0.4 (-0.4, 1.2)
Per 1 SD increment in cumulative CVH		NA	NA	1.5 (-0.5, 3.4)

Non-imputed data				
M2	0-7	Reference	Reference	Reference
	8-11	3.1 (-4.2, 10.3)	1.8 (-3.8, 7.4)	2.2 (-2.5, 6.8)
	12-14	2.7 (-5.0, 10.5)	3.2 (-3.3, 9.8)	1.9 (-4.7, 8.4)
	p for trend	0.75	0.33	0.50
	Per 1 unit CVH score	0.7 (-0.3, 1.8)	1.6 (-1.6, 4.7)	1.1 (-2.1, 4.3)
Per 1 SD increment in cumulative CVH		NA	NA	**2.2 (0.0, 4.4)**

M1 adjusted for total intracranial volume, age, sex, and race. M2 further adjusted for field center, educational attainment, regular alcohol consumption, and *APOE* ε4. Linear CVH per 1 score unit is volume difference according to 1 unit increment in linear score (0-14). For cumulative CVH, 1 SD unit of cumulative CVH years at Y25 is 45.2. Sample sizes for non-imputed data reduce to 606, 595, and 501 for Y0, Y7, and Y25 analyses.

Table 5.6. Multivariable adjusted mean difference and 95% confidence limits in normal tissue volume (cm^3) of white matter for 710 individuals according to cardiovascular health (CVH) score, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (1985-1986 to 2010-2011)

		Mean Difference (95% CL) in Normal Tissue Volume (cm^3), White Matter		
Model	CVH Score	Year 0 CVH	Year 7 CVH	Year 25 CVH
M1	0-7	Reference	Reference	Reference
	8-11	2.5 (-5.1, 10.0)	**6.9 (1.0, 12.8)**	-1.3 (-6.0, 3.4)
	12-14	2.4 (-5.5, 10.4)	**7.3 (0.5, 14.1)**	-2.1 (-8.1, 3.9)
	p for trend	0.72	0.09	0.74
	Per 1 unit CVH score	0.3 (-0.7, 1.3)	0.7 (-0.3, 1.6)	-0.2 (-1.0, 0.7)
Per 1 SD increment in cumulative CVH		NA	NA	0.5 (-1.6, 2.6)
M2	0-7	Reference	Reference	Reference
	8-11	4.0 (-3.5, 11.6)	**7.9 (1.9, 13.8)**	-0.1 (-4.8, 4.7)
	12-14	5.1 (-3.0, 13.3)	**9.4 (2.3, 16.5)**	0.4 (-5.9, 6.7)
	p for trend	0.29	**0.03**	0.92
	Per 1 unit CVH score	0.7 (-0.3, 1.8)	**1.0 (0.2, 2.0)**	0.2 (-0.7, 1.1)
Per 1 SD increment in cumulative CVH		NA	NA	1.8 (-0.4, 4.1)

Non-imputed data				
M2	0-7	Reference	Reference	Reference
	8-11	5.2 (-3.1, 13.6)	**9.0 (2.7, 15.3)**	1.6 (-3.7, 6.8)
	12-14	6.7 (-2.3, 15.7)	**11.5 (4.1, 18.8)**	3.7 (-3.7, 11.1)
	p for trend	0.21	**0.01**	0.33
	Per 1 unit CVH score	1.0 (-0.2, 2.2)	**5.0 (1.4, 8.5)**	1.8 (-1.8, 5.5)
Per 1 SD increment in cumulative CVH		NA	NA	**2.7 (0.2, 5.2)**

M1 adjusted for total intracranial volume, age, sex, and race. M2 further adjusted for field center, educational attainment, regular alcohol consumption, and *APOE* ε4. Linear CVH per 1 score unit is volume difference according to 1 unit increment in linear score (0-14). For cumulative CVH, 1 SD unit of cumulative CVH years at Y25 is 45.2. Sample sizes for non-imputed data reduce to 606, 595, and 501 for Y0, Y7, and Y25 analyses.

Table 5.7. Multivariable adjusted odds ratios (OR) and 95% confidence limits for being in the top tertile of abnormal white matter compared to being in the lower 2 tertiles for 710 individuals according to cardiovascular health (CVH) score, the Coronary Artery Risk Development in Young Adults Brain MRI sub-study (1985-1986 to 2010-2011)

		Odds Ratios (95% CL) for being in the Top Tertile of Abnormal White Matter		
Model	CVH Score	Year 0 CVH	Year 7 CVH	Year 25 CVH
M1	0-7	Reference	Reference	Reference
	8-11	0.88 (0.45, 1.73)	0.97 (0.56, 1.66)	0.69 (0.46, 1.04)
	12-14	0.73 (0.36, 1.49)	0.66 (0.35, 1.24)	0.62 (0.35, 1.08)
	p for trend	0.26	0.11	0.06
	Per 1 unit CVH score	0.94 (0.85, 1.03)	0.93 (0.85, 1.02)	0.93 (0.86, 1.01)
Per 1 SD increment in cumulative CVH		NA	NA	**0.80 (0.67, 0.96)**
M2	0-7	Reference	Reference	Reference
	8-11	0.98 (0.49, 1.97)	0.96 (0.55, 1.69)	0.74 (0.47, 1.16)
	12-14	0.94 (0.44, 2.00)	0.73 (0.37, 1.41)	0.76 (0.41, 1.41)
	p for trend	0.82	0.26	0.29
	Per 1 unit CVH score	0.97 (0.88, 1.08)	0.95 (0.86, 1.05)	0.97 (0.89, 1.05)
Per 1 SD increment in cumulative CVH		NA	NA	0.87 (0.71, 1.07)

Non-imputed data				
M2	0-7	Reference	Reference	Reference
	8-11	0.92 (0.44, 1.93)	1.06 (0.59, 1.90)	0.70 (0.43, 1.14)
	12-14	0.87 (0.39, 1.93)	0.76 (0.38, 1.51)	0.74 (0.36, 1.49)
	p for trend	0.70	0.29	0.29
	Per 1 unit CVH score	0.97 (0.87, 1.08)	0.84 (0.60, 1.17)	0.83 (0.58, 1.18)
Per 1 SD increment in cumulative CVH		NA	NA	0.85 (0.67, 1.08)

M1 adjusted for total intracranial volume, age, sex, and race. M2 further adjusted for field center, educational attainment, regular alcohol consumption, and *APOE* ε4. Linear CVH per 1 score unit is odds ratio according to 1 unit increment in linear score (0-14). For cumulative CVH, 1 SD unit of cumulative CVH years at Y25 is 45.2. Sample sizes for non-imputed data reduce to 606, 595, and 501 for Y0, Y7, and Y25 analyses.

5.7. Figures

Figure 5.1. Analytic sample timeline and data measurement periods, the Coronary Artery Risk Development in Young Adults Study

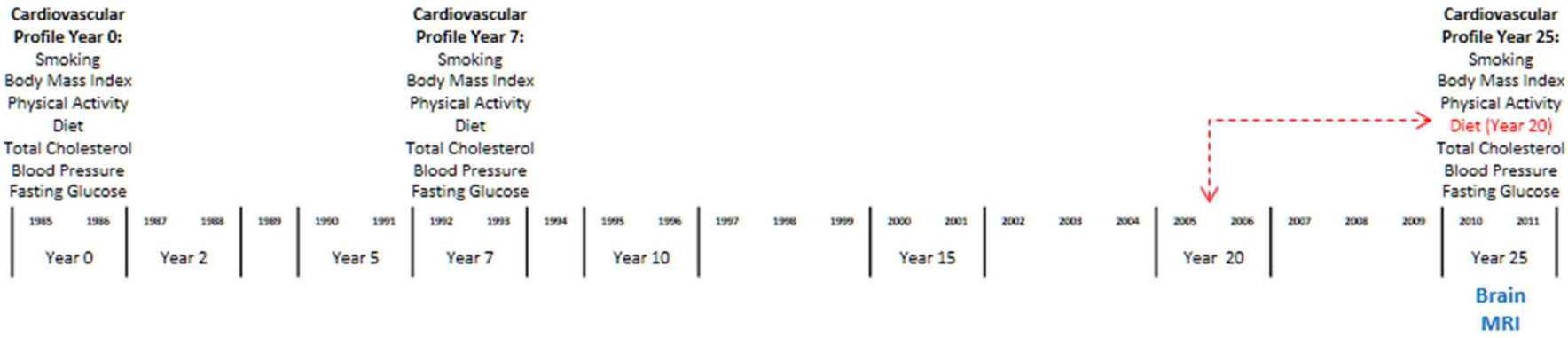


Figure 5.2. Participant exclusions addressed with multiple imputation, the Coronary Artery Risk Development in Young Adults Study. All missing data except for missing imaging data was imputed.

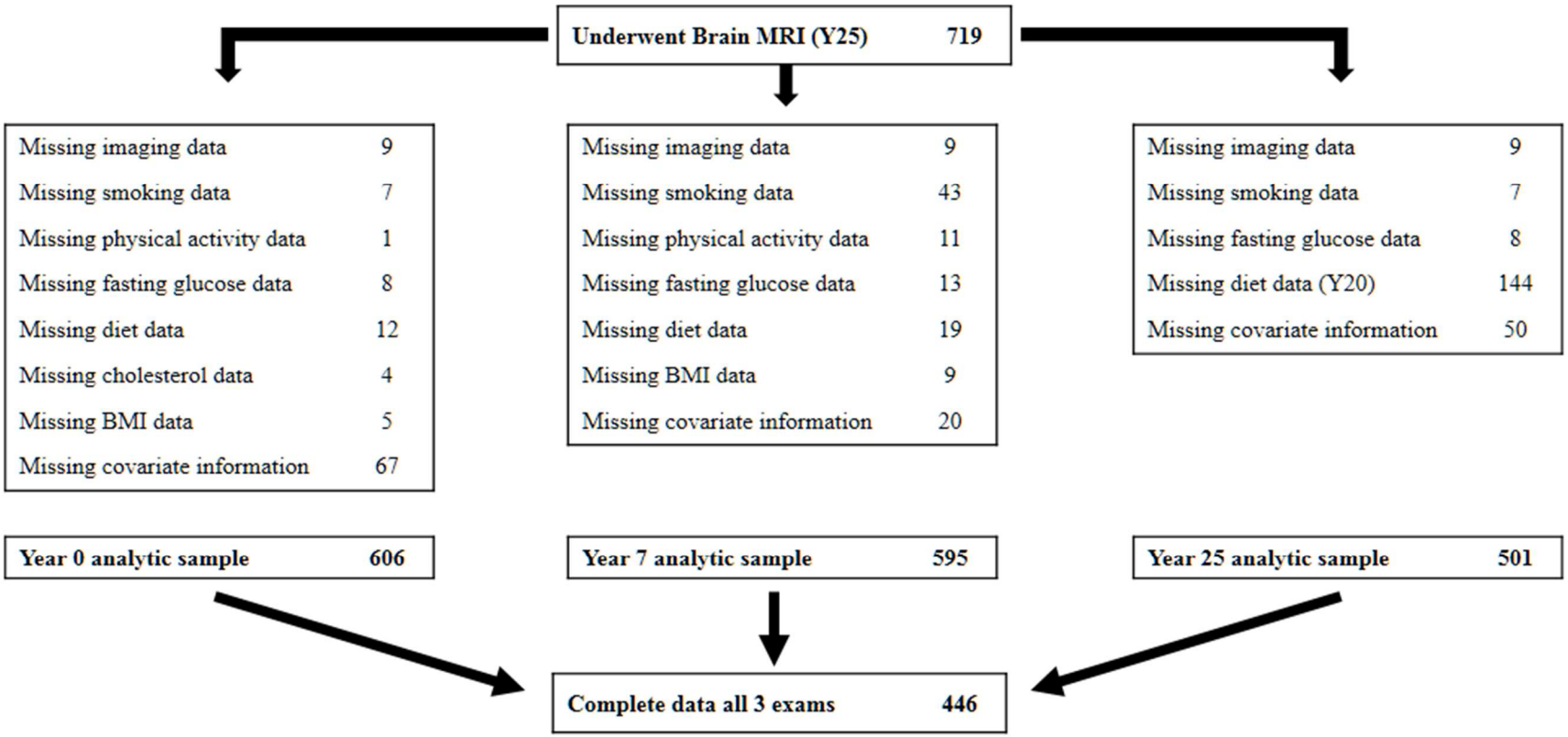
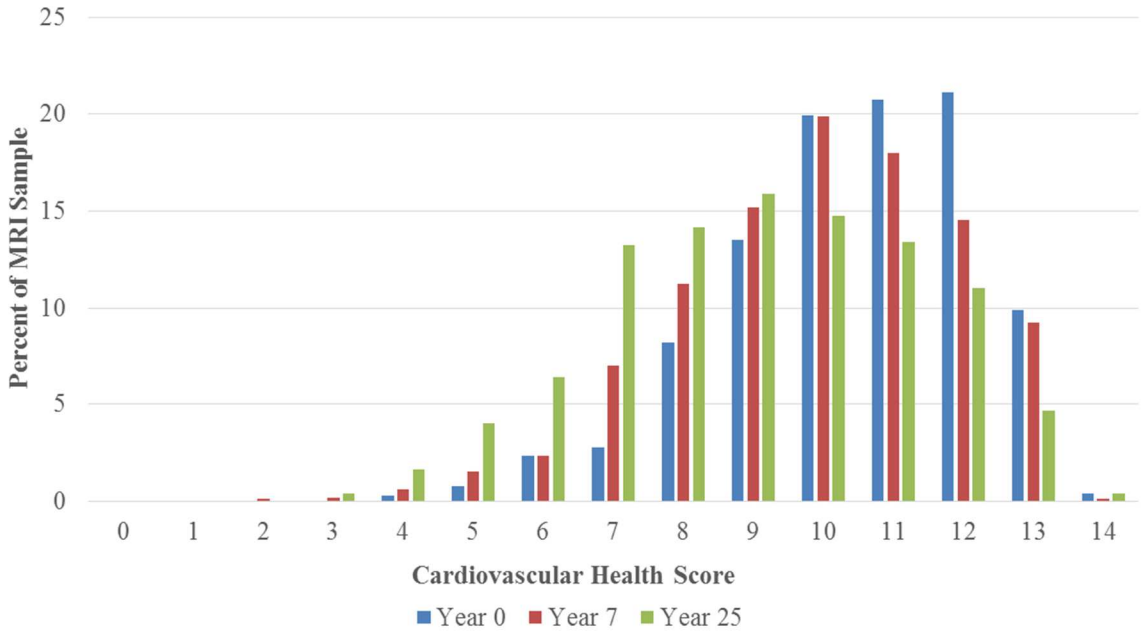


Figure 5.3. Distribution of cardiovascular health score (CVH range 0-14) for 710 individuals at examination year 0, year 7, and year 25, the Coronary Artery Risk Development in Young Adults Study. Cardiovascular health score point attribution according to criteria: 0=poor; 1=intermediate; 2=ideal.



Chapter 6. Conclusions

There is a growing appreciation for the association between cardiovascular health and the health of the brain. The objective of this dissertation was to deconstruct this complex relationship and address three current gaps in the literature. This dissertation set out to characterize annual cognitive decline in the time periods before and after the development of diabetes and hypertension, separately. Our hypothesis was that the rate of annual cognitive decline would be greater in the period after the development of both of these conditions. This research also sought to assess the association between cognitive function and brain structure and the development of diabetes. We hypothesized both lower level of cognitive function and worse brain structure would be associated with increased incidence of diabetes. Lastly, we aimed to define and determine cardiovascular health status at separate junctures in young adulthood and assess the association between cardiovascular health status during young adulthood and brain structure in midlife. Our hypothesis was that better cardiovascular health in young adulthood would be associated with healthier brain structure in middle age.

In our first manuscript we assessed cognitive function before and after the development of diabetes and hypertension in a biracial cohort (CHAP) of adults age 65 and older. Annual cognitive decline is greater in individuals with diabetes and hypertension compared to those without, however the individual change in cognitive function that occurs with the development of these conditions is less clear. We found that the rate of annual cognitive decline was greater in the period after the development of diabetes and hypertension compared to the period before for tests of general orientation

and processing speed, but not for tests of immediate or delayed recall. This highlights that there are potential changes in cognition that occur surrounding the development of these conditions and that these changes may be specific to particular cognitive domains. Future studies could extend this research by assessing cognitive function using a greater breadth and number of neurocognitive tests and with more frequent and clinically-based assessment of diabetes and hypertension to improve validity of ascertainment. This may help identify periods in the continuum of developing these conditions at which individuals are most sensitive to cognitive changes.

Recent research has found lower level of cognitive function may predict the subsequent development of diabetes, suggesting this association may also extend in the opposite direction than traditionally thought in which diabetes increases the risk for cognitive decline. In our second manuscript we evaluated the prospective association between baseline level of cognitive function and brain structure and subsequent development of diabetes in the ARIC cohort. We observed a weak inverse relationship between global level of cognitive function and incidence of diabetes. This association was mainly driven by scores from a test assessing verbal function and mental agility. Additionally, we observed that greater burden of white matter hyperintensities and greater ventricular volume were associated with increased incidence of diabetes, but sulcal size was not. Our observed associations were weak and inconsistent, suggesting cognitive function and brain structure in midlife are not strong factors in the development of diabetes in later adulthood. This may not be surprising, as in middle and older age people accumulate traditional diabetes risk factors which may overwhelm any association

between weak risk factors and the incidence of diabetes. Our work could be improved by assessing the association between cognitive function and brain structure and incidence of diabetes using more specific classifications of brain structure and incorporating brain functional imaging. It is important that any future research assessing the association between brain structure and function and the incidence of diabetes be done in individuals with normal metabolism, to minimize potential for reverse causation.

A better cardiovascular health profile in young adulthood is associated with a higher level of cognitive function in middle adulthood, but the relationship between cardiovascular health in young adulthood and brain structure at midlife is unclear. For our third and final manuscript we defined and assessed cardiovascular health in young adulthood and its association with brain structure in middle age in the CARDIA cohort. We observed that better cardiovascular health during young adulthood was associated with greater volume of the total brain in middle adulthood, but, contrary to our hypothesis, not associated with normal volumes of gray or white matter or abnormal white matter volume. This inconsistent finding suggests that a better cardiovascular profile during young adulthood is not associated with early differences in brain structure. This may not be unexpected given the literature on the natural history of brain structure in the absence of clinical disease shows minimal differences in brain structure before the age of 55. Our study population was relatively healthy, we suspect this limited the ability to detect early differences in brain structure. Our study could be improved by assessing cardiovascular health and brain structure in a population that has greater levels of cardiovascular risk factors than CARDIA; however it may be more fruitful to continue

investigation in CARDIA evaluating the association between cardiovascular health profile and longitudinal changes in brain structure into older adulthood. This may help identify when differences in brain structure occur and what changes in brain structure occur first and the cardiovascular profile that best lends itself to maintaining the integrity of the brain.

In conclusion, this dissertation has focused on addressing three gaps in our understanding of the relationship between cardiovascular health and the health of the brain. We have presented our results and identified future directions for each research question. Specifically, these findings suggest individuals who develop diabetes and hypertension experience changes in cognitive function in the areas of general orientation and processing speed and clinicians should take this into account during disease management and may see fit to inform patients and prepare them for these changes. These findings also suggest cognitive function and brain structure in midlife is not predictive of risk for diabetes in older age and diabetes prevention measures in this age group should focus on preventing or managing traditional diabetes risk factors such as elevated blood glucose, insulin resistance, and excess adiposity. Lastly, our results indicate cardiovascular health during young adulthood is not associated with early differences in brain structure, but caution that differences in brain structure at this age are difficult to detect in healthy populations such as ours and that changes in brain structure were not assessed. In general, successful aging is an important personal and public health challenge. Despite our findings, a body of evidence suggests a large percentage of dementia cases are attributable to the cardiovascular risk factors of low educational

attainment, depression, diabetes, midlife hypertension, midlife obesity, physical inactivity, and smoking.[170-172] In developed countries such as the US, these vascular risk factors may account for as much as 30% of all Alzheimer's disease cases, with physical inactivity the greatest contributing factor.[170] This is a complex problem, with global significance, and should be a priority for all nations projected to experience an increase in their older populations. Nations currently with high prevalence of these cardiovascular risk factors would benefit to focus interventions to reduce the prevalence of these risk factors and emphasize primordial prevention in future generations to improve both cardiovascular health and brain health. Nations with low prevalence of these risk factors should continue public health strategies focused on promoting and increasing physical activity while reducing and preventing smoking, thereby helping prevent development of diabetes, obesity, and hypertension.

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