Cardiovascular and Retinal Vascular Changes in Preclinical Alzheimer's Disease

Cláudia Yang Santos
University of Rhode Island, clausantos2910@gmail.com

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CARDIOVASCULAR AND RETINAL VASCULAR
CHANGES IN PRECLINICAL ALZHEIMER'S DISEASE

BY

CLÁUDIA YANG SANTOS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
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OF

CLÁUDIA YANG SANTOS

APPROVED:

Dissertation Committee:

Major Professor        Peter J. Snyder
Co-Major Professor     Nasser H. Zawia
                        Paula Grammas
                        Wen-Chih Wu
                        Andrea A. Rusnock
ASSOCIATE DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

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ABSTRACT

One in three adults over 85 years old suffer from Alzheimer’s disease (AD) or other forms of dementia. This already widespread condition is expected to increase further in both incidence and prevalence in coming years. As a result, the need to understand the etiology and pathogenesis of dementia becomes ever more urgent. AD, in addition to its steep cost of care, is the most common form of dementia and the sixth leading cause of death in the United States. It is a complex disease and its mechanisms are poorly understood. The more we learn about AD, the more questions are raised about our current conceptual models of disease. Despite the rapid advancement of medical technology, reliable and sensitive diagnostic markers to identify individuals at risk to AD prior onset of clinical symptoms remain in the developmental phase resulting in inefficient diagnostic procedures. Diagnosis is further hampered by the heterogeneity of behavioral presentations, cognitive impairments, and functional statuses observed in AD, all of which may be the result of varying etiologies. Furthermore, older AD patients often suffer from comorbid medical conditions that further complicate accurate disease monitoring.

In the absence of an effective AD treatment, it is prudent to apply our current knowledge of the intersection between AD, cardiovascular disease (CVD), and cerebrovascular disease to foster efforts to delay the onset of dementia more generally. The purpose of MANUSCRIPT I is to review our current understanding of the epidemiology, genetics, and pathophysiology of AD as well as the intersection between AD and vascular causes of dementia. The epidemiology and shared risk
factors and etiologies for these three disease “clusters” are explored, including shared genetic contributions and lifestyle, behavioral and environmental risk factors. In this first publication, we also explore possible mechanistic pathways of AD and the shared pathophysiology and neuropathological substrates of these three disease clusters.

CVD and cerebrovascular pathology is present for most individuals with AD, although the converse is not necessarily true. Given this relationship, it is important to address how early in the disease course those vascular changes can be observed. Such research is needed to enable early interventions to maintain quality of life in premorbid AD and reduce the burden of disease. To determine whether there is cardiovascular alteration in the early stages of AD, MANUSCRIPT II evaluated electrocardiologic measures of vagal tone for 63 adults (ages 55-75) at rest, during cognitive testing, and then again at rest. All subjects had multiple risk factors for AD, and all completed amyloid PET scans (18F-Florbetapir) to determine amyloid positivity (Aβ+). Cardiac autonomic dysfunction, specifically, an increase in sympathetic activity and a decrease in parasympathetic activity often referred to as vagal withdrawal, is prevalent among individuals with AD and is indicative of impaired autonomic function.

Preclinical AD participants (Florbetapir amyloid PET SUVr ≥ 1.1) did not consistently show changes in vagal ratio or Respiratory Sinus Arrhythmia (RSA) at any point during the experiment and they failed to demonstrate the expected response to the modest stress they experienced during cognitive task performance. Both changes are directly modulated by both muscarinic and nicotinic cholinergic autonomic neurotransmission. Because the earliest stages of AD are marked, in part,
by altered function of the basal forebrain cholinergic system, with eventual
degenerative changes including neuronal loss, this result suggests a link between Aβ
aggregation and impaired autonomic cardiovascular function, even in the preclinical
stage of AD.

Another factor that influences the lack of treatment is the absence of a reliable,
affordable, and sensitive diagnostic marker to identify individuals at risk for AD
before the onset of clinical symptoms. One possible marker may be found in the
retina, the retina shares similar embryological origins, anatomical features, and
physiological characteristics with the brain. From as early as the fourth week of
gestation, the eyes, particularly the retinal nerve fiber layer (RNFL) and the optic
nerve, are direct sensory extensions of the central nervous system as their axons
synapse directly with several brain regions. Unlike the brain, however, retinal
neuronal cell layers can be non-invasively visualized through high-resolution optical
methods such as optical coherence tomography (OCT) allowing for the precise
segmentation and measurement of these cell layers. For MANUSCRIPT III, more
than 15 years of literature with inconsistent findings on the relationship between the
morphology of retinal neuronal layers and AD were reviewed. Most papers reported
RNFL and ganglion cell layer (GCL) thickness to be significantly reduced in cross
sectional studies of patients with mild cognitive impairment (MCI) and mild to
moderate AD. For the first time, this Manuscript explores within-subjects longitudinal
change in retinal morphology during the preclinical stage of AD for all retinal
neuronal layers. For this manuscript, fifty-six older adult participants (mean age =
65.36 years) from the previous manuscript completed Spectral Domain-OCT retinal
imaging at baseline. Twenty-seven months later, they completed the same exams as well as an 18F-Florbetapir PET imaging and cognitive testing.

We observed a decrease over time in macular RNFL, outer nuclear layer and inner plexiform layer volumes in preclinical AD relative to controls. While the macular region of the retina is physiologically very active in healthy normal eyes, this region might have diminished activity in the preclinical stage of AD. This thinning could be due to either demyelination or loss of axons in the RNFL, both of which suggest the possibility of future degenerative changes to the cell bodies in the GCL followed by progression to deeper neuronal layers. Volume loss in the RNFL, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving. However, this retinal change does appear to be modestly related to relative decrements in performance on a measure of audiovisual integration efficiency that has been recently advanced as a possible early cognitive marker of mild cognitive impairment.

In order to tie together the findings of relative changes in cardiovascular function and retinal morphological changes in preclinical AD, we decided to utilize optical coherence tomography angiography (OCTA), a new technology that makes it possible to measure retinal vascular flow. Recently, a reduction in vascular bed complexity in MCI and AD relative to healthy controls was reported by using a fractal dimension (Df) approach to summarizing OCTA imaging results. In MANUSCRIPT IV, forty-eight adults (mean age = 68.76 years) from the same sample of participants had retinal OCTA images captured using an AngioVue system (Optovue, Fremont, CA, USA). The Df was measured in linearized images of the superficial vascular
plexus (SVP) to determine the space-filling linear extension of the large vessels. Our findings suggest that individuals at high-risk for preclinical AD have less density and complexity of retinal microvascular networks in the SVP of the macular region (the same region in which structural changes were observed in Manuscript III) than healthy controls. Reduced vascular density in the SVP likely leads to degradation in blood flow throughout the other parts of the retina and therefore might directly contribute to continued axonal loss as well as future GCL thinning in AD. Retinal vascular distribution and blood flow were found to be altered during the earliest stages of AD suggesting a potential cost-effective and non-invasive marker for preclinical AD.
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This dissertation was prepared in manuscript format according to the University of Rhode Island Graduate School guidelines. This dissertation consists of four manuscripts that satisfy the requirements of the department of the Interdisciplinary Neuroscience Program, University of Rhode Island.

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**Manuscript IV:** Manuscript format following submission to Journal of Neuro-Ophthalmology
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MANUSCRIPT I

“Pathophysiologic relationship between Alzheimer’s disease, cerebrovascular disease and cardiovascular risk: A review and synthesis.”

by

Cláudia Y. Santos,1,2; Peter J. Snyder, Ph.D.2,3; Wen-Chih Wu, M.D.4; Mia Zhang5; Ana Echeverria6 and Jessica Alber, Ph.D.2,7

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1. PhD Candidate at Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA. email: clausantos2910@gmail.com
2. Clinical Researcher at Lifespan Clinical Research Center, Rhode Island Hospital, Providence, RI, USA
3. Professor at Department of Neurology, Warren Alpert Medical School of Brown University, Providence, RI, USA.
4. Professor at Division of Cardiology, Department of Medicine, Warren Alpert Medical School of Brown University, Providence, RI, USA.
5. Medical Student at Griffith University School of Medicine, Goldcoast, Queensland, Australia
6. Medical Student at University of Puerto Rico School of Medicine, San Juan, Puerto Rico
7. Post-Doctoral Researcher at Department of Psychiatry and Human Behavior, Warren Alpert Medical School of Brown University, Providence, RI, USA
Abstract

As the population ages due to demographic trends and gains in life expectancy, the incidence and prevalence of dementia increases, and the need to understand the etiology and pathogenesis of dementia becomes ever more urgent. Alzheimer’s disease (AD), the most common form of dementia, is a complex disease, the mechanisms of which are poorly understood. The more we learn about AD, the more questions are raised about our current conceptual models of disease. In the absence of a cure or the means by which to slow disease progress, it may be prudent to apply our current knowledge of the intersection between AD, cardiovascular disease, and cerebrovascular disease to foster efforts to delay or slow the onset of AD. This review discusses our current understanding of the epidemiology, genetics, and pathophysiology of AD, the intersection between AD and vascular causes of dementia, and proposes future directions for research and prevention.

Keywords

Alzheimer’s disease; Cardiovascular disease; Cerebrovascular disease; Vascular contributions to cognitive impairment and dementia; VCID; Dementia; Risk factors
Research-in-Context

Systematic Review: The authors conducted online searches for all the relevant literature describing the relationship between Alzheimer’s disease (AD), cardiovascular (CVD) and cerebrovascular diseases (CBVD). A thorough review of common epidemiology, risk factors, and possible mechanic pathways that might link these three entities, is provided.

Interpretation: There is a substantial overlap in epidemiologic, genetic, and clinical literature of shared risk factors for AD and cardio- and cerebro-vascular co-morbidities. There is also very substantial overlap in shared mechanistic relationships between AD and both CVD and CBVD. We suggest that vascular/cerebrovascular pathology is present for most individuals with AD, although the converse is not necessarily true).

Future Directions: Further investigation is required to understand the mechanistic pathways for shared pathology between these two constellations of diseases. Our group and others are tracking vascular changes in preclinical AD patients. Epidemiological studies of CVD progression promise new insights on the effects of subclinical CVD on the brain. Currently ongoing cardiovascular prevention trials impacting dementia risk will provide substantial insight into the possibility of delaying the onset of AD.
List of Abbreviations

Alzheimer’s disease (AD), cardiovascular disease (CVD), cerebrovascular disease (CBVD), C-reactive protein (CRP), Late-Onset Alzheimer’s Disease (LOAD), apolipoprotein E (APOE), methylenetetrahydrofolate reductase (MTHFR), coronary heart disease (CHD), systolic blood pressure (SBP), Framingham cardiovascular risk profile (FCRP), mild cognitive impairment (MCI), blood-brain barrier (BBB), rate pressure product (RPP), amyloid-β (Aβ), blood pressure (BP), vascular contributions to cognitive impairment and dementia (VCID), diastolic blood pressure (DBP), central nervous system (CNS), Diabetes Mellitus (DM), Type 2 Diabetes Mellitus (T2DM), body mass index (BMI), brain derived neurotrophic factor (BDNF), heart rate variability (HRV), respiratory sinus arrhythmia (RSA), Cardiovascular risk factors, aging, and incidence of dementia (CAIDE), cerebral amyloid angiopathy (CAA), Atherosclerosis Risk in Communities (ARIC), positron emission tomography (PET), white matter hyper intensities (WMH), magnetic resonance imaging (MRI), National Institute of Aging (NIA), Alzheimer’s Association (AA), nitric oxide (NO), locus coeruleus (LC), Finnish geriatric intervention study to prevent cognitive impairment and disability (FINGER), National Institute of Health (NIH), molecular mechanisms of the vascular etiology of Alzheimer’s disease (M²OVE).
General Introduction

One of the greatest advancements of health in the 20th century was an increase in average life expectancy by 30 years [1]. Today, people aged 85 and older are the fastest growing segment of the population and this has led to a new set of problems for modern healthcare as the elderly are the most susceptible to disease and disability. One in three adults over 85 years old suffer from Alzheimer’s disease (AD) or other forms of dementia [2], the prevalence of which is estimated to increase dramatically over the next 40 years unless preventive measures are developed [3]. AD is currently the sixth leading cause of death in the United States and the cost of the disease is high. Approximately $236 billion will be spent on AD during 2016 calendar year overall, including patient care and caregivers’ lost wages [4].

Despite the global increase of both incidence and prevalence of AD, it is the only leading cause of death that we are currently unable to prevent or cure [5]. The remarkable heterogeneity of risk factors, etiologies, and neuropathologic processes associated with AD make it especially challenging for development of new treatments to slow disease progression [4,6]. Fortunately, a number of experimental therapies are currently in development. These are aimed at mechanisms including neurotransmission regulators, tau-based therapies, amyloid-β based strategies, intracellular signaling cascade modulators, oxidative stress reductors, mitochondrial target therapy, cellular calcium homeostasis modulators, and anti-inflammatory therapies [7,8,9,10,11]. It is possible that the heterogeneity of behavioral presentations, cognitive impairments, and functional status observed in AD is due to its potentially
varied etiology [12]. Adding to this complexity, older adults with AD typically present with comorbid medical conditions that further complicate accurate disease monitoring [13]. The current dominant AD models are insufficient to account for the complexity of biologic processes, polygenic, and epigenetic factors at work [14]. As a result, key opinion leaders have suggested that the field would benefit from the development of new conceptual models of AD [14]. The purpose of this review is to explore the complex relationship between AD, cardiovascular disease (CVD) and cerebrovascular disease (CBVD). Recent reports that question the strength of the association between these disease entities will be reviewed and recommendations will be made for additional research questions to more precisely characterize causal links between AD, CVD, and CBVD.

**Shared Genetic Contributions to AD and Cardiovascular Disease.**

The genetic contribution to AD risk is complex. Three familial autosomal dominant genes associated with early onset disease have been discovered (PSEN1, PSEN2 and APP) [15,16,17,18,19,20], and these genes may also be associated with some later onset cases; although together they likely account for less than 10% of all AD cases [21]. The most predominant type of AD is Late-Onset Alzheimer’s Disease (LOAD, referred to herein as AD), which affects adults in their sixth to eighth decade of life. While many genetic risk factors for AD have been studied, a definitive genotype that causes (late onset) AD has not yet been identified [22]. Thus far, few genetic markers have been linked to both risk of AD and CVD or CBVD risk. The ε4 allele of the Apolipoprotein E (APOE) gene is a risk factor for both AD and CVD.
Over the past two decades, numerous studies have shown that individuals who carry at least one copy of the ε4 allele have an increased risk for AD compared to those without the ε4 allele [23,24,25,26] and ε4 carriers with AD have lower blood levels of ApoE [27]. Low plasma levels of ApoE protein have been found to increase the risk of AD independent of APOE genotype [28].

Two polymorphisms (rs1801133, rs1801131) in the methylenetetrahydrofolate reductase (MTHFR) gene correlate with elevated levels of plasma homocysteine and appear to be associated with AD and vascular contributions to cognitive impairment and dementia (VCID) [29,30]. High plasma homocysteine levels have been identified as a risk factor for VCID in a Northern Irish population [29]. Mutations in the MTHFR gene were found to increase the risk of AD by 2.5 fold and VCID by 3.7 fold in an Asian population [30].

Beyond APOE and MTHFR, few other genes have been identified to significantly increase the risk of both AD and CVD. Genetic associations with smaller effects have been found but a detailed discussion of these is beyond the scope of this review. Recently, new approaches to evaluating genetic pleiotropy in complex diseases have been developed [31]. These methods are now being applied to AD [32] and one recent study demonstrated genetic overlap between AD and CVD by conditioning on CVD phenotypes including C-protein reactive (CRP) and plasma lipids [33].
Shared Risk Factors for AD and CVD

AD and CVD share important cardiometabolic and lifestyle risk factors that occur in middle-aged to elderly populations. Both AD and CVD are associated with increasing age, and both are among the leading causes of death. The primary causes of CVD are coronary heart disease (CHD), hypertension, stroke, and heart failure. These diseases are frequently interconnected and share an underlying pathology of atherosclerosis. All known risk factors for atherosclerosis have been the focus of studies to identify modifiable risk factors for AD. Researchers from the Framingham Heart Study [34,35] developed a composite measure of general cardiovascular risk, the Framingham Cardiovascular Risk Profile (FCRP), derived by evaluating one’s age, gender, diabetes, smoking, treated and untreated systolic blood pressure (SBP), total cholesterol, and high density lipoprotein (HDL) cholesterol [36]. In addition to increased risk of CVD, an elevated FCRP score on has been related to markers of abnormal brain aging, such as smaller brain volume and increased white matter hyperintensities in brain imaging exams [37]. High FCRP scores are associated with worsening of cognitive abilities, both in cognitively intact subjects and in mild cognitive impairment (MCI) patients [38] and is a reliable predictor of progression from MCI to AD [39]. Other scores developed from Framingham, the Framingham Stroke Risk Profile and the Framingham Coronary Heart Disease Risk Score, have been similarly associated with cognitive change over time, incident cognitive impairment, and dementia [35,39,40,41,43,44]. These risk models are similar to one developed specifically to assess dementia risk, the Cardiovascular Risk Factors Aging and Dementia (CAIDE) risk score [45,46,47]. Common elements across scores are:
blood pressure, cholesterol, and diabetes. Below, we review elements of these risk scores as they relate to both CVD and AD.

**Hypertension /Hypotension**

Chronic hypertension, a common risk factor for CVD, causes a thickening of vessel walls, reduced vessel elasticity and the narrowing of the lumen, especially in small vessels [48,49]. These sequelae result in reduced cerebral blood flow, a prominent step in the pathophysiology of both AD and CVD. Chronic hypertension also compromises blood-brain barrier (BBB) integrity, leading to both cerebral edema and the introduction of systemic elements into the brain parenchyma [50].

Hypertension recorded 15 years prior, has been associated with smaller brain volumes in areas typically affected by AD such as the hippocampus [51]. Our group has observed that an increased resting-state cardiac rate pressure product (RPP) as a surrogate of myocardial oxygen use, has a small to moderate correlation with neocortical amyloidosis in midlife adults with preclinical AD [52].

Epidemiological studies have shown that hypertension is risk factor for dementia [53,54, 55,65,57] but the association is complex [58]. Studies have found that the risk of dementia and AD may vary in strength and direction according to the age of onset [55,56,59,60]. Further complicating these findings, several studies have demonstrated that antihypertensive drugs can reduce the risk of AD [61,62,63,64,65]. Diuretic, angiotensin receptor-1 blocker, or angiotensin-converting enzyme inhibitor use in the Ginkgo Evaluation of Memory Study, was associated with a reduced risk for MCI and AD [62]. Among 2,197 participants from the Honolulu-Asia Aging Study
who were dementia free at baseline, beta-blocker users experienced a 31% lower risk of developing new cognitive impairments of any cause, compared to those with other antihypertensive or no antihypertensive use [63]. Taken together, these findings suggest that methods to effectively lower BP in midlife, e.g., lifestyle changes or medications, should help retain or improve cognitive function by reducing the risk of AD and/or VCID.

Conversely, there is evidence demonstrating that hypotension in late life is closely associated with a higher risk of AD. The Bronx Aging Study [66] followed a healthy cohort of older adults aged ≥75 years over a median follow up of 6.7 years. Participants with diastolic blood pressure (DBP) <70 mmHg were twice as likely to develop AD as those with DBP >90 mmHg, and this risk was even higher for subjects with persistently low DBP. Interestingly, there was no such relationship for SBP, and the association between diastolic hypotension and AD was specific; no such association existed for VCID. A pooled analysis of data from the Rotterdam Study (N = 6,668) and the Gothenburg H-70 Study (N = 317) found baseline diastolic hypotension was associated with higher risk of AD and/or VCID over an average of 2.1 years of follow-up, and that the risk was more pronounced in antihypertensive medication users [57]. Another population-based study (N = 599, mean age 83.5 years) similarly revealed that lower DBP and SBP were associated with a higher incidence of AD [67]. Extremely low DBP (≤65 mmHg) produced an adjusted relative risk of 1.7 (95% CI 1.1-2.4) for AD in a prospective study of 1,270 individuals aged 75-101 years [68]. Finally, a meta-analysis of 20 population based studies revealed
that a decline in DBP in later life may contribute to diminished cerebral perfusion, and the subsequent ischemic state may lead to increased cerebral Aβ accumulation [69].

**High Cholesterol**

Cholesterol metabolism plays an important role in the central nervous system (CNS), as the brain is a cholesterol rich organ, comprising 25% of the body’s cholesterol [70]. Studies have indicated that lipoprotein lipase, an enzyme that hydrolyzes triglycerides, may be involved in the biological basis of both AD and CVD (e.g. essential hypertension, CHD) [71,72,73] through its interaction with brain lipoproteins and modulation of cholesterol homeostasis in neuronal cells [74]. Apolipoprotein E is crucial for the catabolism of triglyceride rich lipoprotein components and for cholesterol transport [75] Cholesterol supplied as a lipoprotein complex, such as HDL is critical for the maturation of synapses and the maintenance of synaptic plasticity [76,77]. Cholesterol levels influence the clearance of Aβ and the formation of neurofibrillary tangles through action at the lipid rafts located in neuronal membranes.

Outside the brain, atherosclerosis is a frequent consequence of high cholesterol and is an important risk factor for ischemic cerebrovascular disease [78]. The contribution of atherosclerosis, a frequent consequence of high cholesterol, is an important risk factor for ischemic cerebrovascular disease [78]. *APOE* E4 carrier status is both a risk marker for AD and CVD. In the Rotterdam Study, *APOE* E4 carriers with atherosclerosis frequently had co-morbid AD and more frequent co-morbid VCID [79,80].
Elevated total serum cholesterol levels have been associated with MCI and AD risk in some studies [81,56]. Others have found that the association between cholesterol and AD is complex [82,83]. Similar to hypertension, the risk of dementia associated with high cholesterol may be influenced by the timing and duration of the condition, as well as its treatment. One reason for this complexity is that plasma cholesterol levels do not reflect cholesterol concentrations inside the BBB. The association between high cholesterol and increased risk of AD has resulted in a number of studies testing the hypothesis that statins, which play a role in cholesterol reduction, might prevent the onset or progression of AD. Early epidemiological studies in this area predicted that statins could reduce the incidence of AD by as much as 70% [84,85,86]. However, whether statins and resultant reduction of cholesterol cause a significant reduction in AD pathology is still unclear. More recent results of large-scale randomized controlled trials suggest no significant clinical benefit of statins in participants at-risk for AD [87,88].

Diabetes Mellitus

Diabetes mellitus (DM) is a complex metabolic disorder that is closely associated with changes in cognition as well as other risk factors for accelerated cognitive decline and dementia, such as hypertension and atherosclerosis [89]. DM occurs when there is a prolonged period of high blood glucose levels, or hyperglycemia. There are two types of DM, Type 1 is congenital and caused by insulin deficiency, and Type 2 is acquired and caused by insulin resistance.
Although there are points of intersection between the molecular mechanisms underlying diabetes and AD, the exact mechanism of how insulin inefficiency increases the risk of AD remains unknown. The literature is currently separated into two different schools of thought [90]. One follows from Rotterdam study findings, suggesting that the excess of insulin or glucose from type 2 diabetes mellitus (T2DM) leads to AD. This is based on studies demonstrating that AD patients have significantly higher levels of insulin and glucose than healthy controls [91,92,93]. A second school of thought suggests that insulin deficiency, either due to the relative deficiency that results from insulin resistance in early stages of T2DM, or absolute deficiency that occurs when beta cell dysfunction occurs in full-blown T2DM, causes AD by impairing insulin’s ability to perform its roles in the brain [94,95,96,97].

In addition to these two theoretical approaches in the literature, some have suggested the term “Type 3 diabetes” was coined to account specifically for the underlying abnormalities associated with concurrent AD-type neurodegeneration and diabetes [98]. These researchers maintain that AD and diabetes share common pathophysiology, and therefore therapeutic regimes aimed at diabetes treatment and amelioration could be effective for treatment of AD [99].

A recent meta-analysis demonstrated a strong link between diabetes and VCID [100]. Findings from the Rotterdam study demonstrated a nearly two-fold risk of AD and suggest that DM increases the risk of dementia by 1.9 fold, and that DM patients treated with insulin were at even greater risk (4.3 fold) [101]. Multiple population-based studies have shown that patients with DM exhibit an increased risk of developing AD [102, 103, 104, 98]. The authors of one such study concluded that
39% of AD in a large sample of elderly subjects was attributable to hyperinsulinemia or DM [105].

As with other CVD risk factors, treatment for diabetes has been shown to alter the risk of AD. Metformin has been shown to reduce the risk of AD and is currently being studied in clinical trials with promising preliminary results in MCI patients [106]. A large clinical trial is currently underway to evaluate the efficacy of low dose pioglitazone as a preventive treatment for MCI due to AD [107]. Other studies have considered the use of intranasal insulin, which has been shown to exert a modest effect on memory performance in AD patients [108,109,110,111].

**Lifestyle, Behavioral, and Environmental Risk Factors**

High fat diets and sedentary lifestyles have led to a growing incidence of obesity, dyslipidemia, high blood pressure and metabolic syndromes [112, 113,114]. These conditions are precursors to, or develop along with atherosclerosis, diabetes, CVD, and an increased risk for AD [115]. Major depression has also been linked to both AD and CVD and more recently environmental exposures such as fungal pathogens and pollution have been under investigation for ties to both AD and CVD. Below, we review the literature that explores the obesity, aerobic exercise, smoking, major depression, and exposures such as fungal pathogens and air pollution, each as shared risk factors for both CVD/CBVD and AD.

**Obesity**

The mechanism by which obesity influences cognition and AD risk remains under active investigation. One mechanism may be through vascular pathologies, or
there may be hormonal, genetic, or inflammatory processes at work [116]. The Framingham Heart Study reported a marked impairment of cognitive function in patients with obesity compared with non-obese counterparts [117]. Epidemiological studies have shown associations between obesity and increased AD risk in females [118,119] although other studies have found increased risk of dementia for both genders [59]. Pooled results from 11 studies [120] demonstrated that the strength and direction of the association varies over the life course. The association between body mass index (BMI) and later onset of AD appears to be stronger when BMI is measured at midlife [121] than when BMI is measured in later life [118,122]. Although they frequently co-occur, DM and obesity are widely accepted as important independent risk factors for AD [123].

Aerobic exercise and physical fitness

In contrast to metabolic syndromes, aerobic exercise and healthy lifestyles have been shown to reduce the incidence of both CVD and AD in observational studies. Cardiovascular diseases have become very common as communities and individuals attain more wealth and pursue more sedentary lifestyles [123]. Aerobic exercise promotes brain vascularization and may reduce vascular risk factors and improve cognitive function [124,125,126,127]. Randomized controlled trials with as little as six months of exercise training lead to increased hippocampal volume and improved performance on spatial memory and executive function tasks [128,129]. Aerobic exercise also up-regulates brain derived neurotrophic factor (BDNF), which augments plasticity in the hippocampus [130,131].
Epidemiological studies have demonstrated reduced risk of cognitive decline and dementia as a function of activity levels [132,133,134] and studies have shown increased gray and white matter volume in brains of participants assigned to aerobic training [135] or those with higher levels of self-reported exercise [136]. Recent meta-analysis of clinical trials of exercise interventions in dementia patients demonstrated positive effects [137]. A recent prevention trial of an exercise intervention in sedentary older adults failed to find a significant effect on cognitive function [138], however secondary analyses found significant improvement in cognitive function among the subset of participants who were diabetic [139].

A related measure of physical fitness is heart rate variability (HRV) on continuous electrocardiography recordings. HRV is usually relatively high for those who exercise frequently [140], as well as for young and healthy individuals. Aging and poor physical fitness are associated with an impairment of cardiac vagal function [141], and HRV is lower for those with relatively diminished parasympathetic tone [142], including those with CVD [143]. Vagal tone, the ability of the vagus nerve to rapidly regulate cardiac output, accounts for a substantial portion of HRV. Vagal tone can be quantified via respiratory sinus arrhythmia (RSA), which measures the slight ebb and flow in heart rate that occurs during the respiratory cycle. RSA is higher in older adults who demonstrate better performance on tasks of verbal episodic memory, a cognitive domain that is typically impaired with the onset of AD [144].

In Table 1, we provide a listing of evidence-based interventions for the treatment of CVD, discussed in the sections above, and how these specific interventions have been explored in the treatment of AD.
Smoking

There is some evidence to suggest that long-term cigarette smoking is an independent risk factor for AD, CVD and CBVD [145,146,147]. Smoking increases total plasma homocysteine, an independent risk factor for stroke, cognitive impairment, AD and other dementias [148,149,150,151]. Smoking accelerates atherosclerosis [142] and can cause oxidative stress, which is associated with excitotoxicity, leading to neural death [152]. A dose-response relationship between smoking and dementia risk has been documented [153] and AD risk among smokers is increased in APOE ε4 carriers [101,154]. A meta-analysis of studies performed in the 1990s and early 2000s revealed that relative to non-smokers, current smokers had increased risks of 1.79 fold (95% CI 1.43–2.23) for AD and 1.78 fold (95% CI 1.28–2.47) for VCID [155]. A more recent systematic review confirmed the previous findings with increased risks of 1.59 fold (95% CI 1.15–2.20) for AD and 1.35 fold (95% CI 0.90–2.02) for VCID [156].

Major Depression

A history of major depression is another shared risk factor for AD and CVD. Late-onset depression is often associated with AD, and AD patients with episodes of major depression over their lifetimes show greater hippocampal pathology at autopsy [157]. Evidence exists to suggest that the two disorders may share common etiological substrates [158, 159]. Chronic, untreated major depressive disorder is associated with
the selective loss of noradrenergic cells in the locus coeruleus [160,161] and the loss of dorsal raphe serotonergic nuclei [162], both of which have been demonstrated in AD. Major Depression is now widely acknowledged to be a CHD risk factor, based on work demonstrating that psychosocial factors such as chronic dysphoria, anxiety, perceived loss of locus of control and perceived stress are strongly predictive of incident myocardial infarction [163]. Nearly one of five patients with CVD suffers from major depressive disorder [164]. Depression may be directly linked to cerebral ischemia secondary to reduced cerebral blood flow [164] and is associated with an increased risk of recurrent stroke in patients with VCID [165]. CVD has been proposed as a mediator of the relationship between major depression and AD [166]. Hyperhomocysteinemia has been demonstrated in both AD and major depression [167,168]. Adding to this already complex picture, heightened homocysteine levels are found in CVD [149], indicating a potential shared mechanism in AD, CVD, and major depression. The exact nature of the downstream effects of this mechanism requires further research.

Fungal pathogens

More recently, fungal macromolecules have been identified as a potential pathophysiological substrate of both AD and CVD. The presence of fungal cells in different sizes and hyphae inside capillaries and other blood vessels in some AD patients suggests that fungal infections can be detected in the neurovascular system and may, in some cases, explain the vascular pathology frequently detected in AD patients [169,170]. One study showed that Aβ peptide, a cardinal feature of AD
pathology, could exert antimicrobial activity for at least eight different microorganisms, suggesting a brain with AD could specifically induce $\text{A}\beta$-mediated inflammatory activity against *Candida albicans* [171]. Unfortunately, many macromolecules with antimicrobial activity have been shown to be cytotoxic towards vascular smooth muscle cells [172], and $\text{A}\beta$ is no exception [173]. There is also association of AD with various types of spirochetes, and *C. pneumonia* [174]. However, thus far, to our knowledge, the only documented common fungal or microbial pathogenic link between AD and CVD is *Candida albicans*. The pathophysiological link between pneumonia and acute cardiovascular events has been explained via the long-lasting infection hypothesis, which implicates microorganisms in atherosclerosis [175].

*Air pollution*

Chronic exposure to air pollution, which is associated with reduced HRV, is another environmental factor that is associated with both CVD and AD. Specifically, long-term exposure to high ozone and high particulate matter in the air leads to increased risk for obesity, metabolic syndrome [176], and a host of CVDs [177] including myocardial ischemia and infarction, heart failure, arrhythmias, stroke, and increased cardiovascular mortality [178]. Recently, a dose-response relationship was found between longitudinal exposure to high concentrations of atmospheric particulate matter <10 $\mu$m in diameter and significantly increased risk of AD and VCID in industrial regions of Taiwan [179].
Co-occurrence of AD and Cerebrovascular diseases

Cerebrovascular disease (CBVD) is a generic term from a heterogeneous set of insults to the cerebral vasculature, and such insults often lead to various cognitive impairment(s). CBVD insults include, but are not limited to, microvascular degeneration (with looping, twining and braiding vessels) [180], periventricular venous collagenases, and vascular tortuosity [181]. These microvascular changes all cause impaired cerebral perfusion [182]. Some studies have shown a correlation between capillary length per brain volume and reduction of glucose utilization [183]. Intracerebral hemorrhages due to Aβ accumulation within vessel walls can contribute to AD pathology [184]. This leads to increased incidence of infarcts in the brain tissue innervated by this system [185]. Collectively, these conditions are considered among the causes for VCID. VCID (vascular contributions to cognitive impairment and dementia) refers to a progressive worsening of cognitive functions and memory. VCID is due exclusively to vascular disease within the brain [186]. It is often very difficult to distinguish AD from VCID as they appear very similar on clinical exam. VCID patients often present with episodic memory impairments, word-finding difficulties, disorientation to time, and subtle executive deficits [187]. Thus, a differential diagnosis is aided by careful neuropsychological examination in conjunction with appropriate biomarker studies [188].

In the United States, CBVD is the leading cause of disability in adults and the third leading cause of death [189]. CVD often plays a direct causal role in cerebrovascular events, as CBVD often results from a lack of blood flow to the brain. Various CBVD pathologies are observed in 60 to 90% of AD patients, including white
matter lesions, micro-infarcts, hemorrhages, microvascular degeneration and cerebral amyloid angiopathy (CAA) [75]. CAA is the abnormal deposition of a congophilic material in meningeal and cerebral arterioles. The prevalence of CAA is high in AD and often progresses in severity causing vessel rupture [190]. Atherosclerosis, a common cause of CBVD, can lead to cerebral infarction or stroke, and disabling cognitive impairments in late life [191]. Many large population-based epidemiologic studies have provided strong support for the relationship between AD and cerebrovascular changes. One such study examining two large cohorts of AD patients found a strong relationship between AD and cerebral atherosclerosis, such that the presence of atherosclerosis was associated with worse cognitive performance in AD [192]. The Framingham Heart Study found that a lower cardiac index was associated with increased risk of AD [193]. These findings suggest that age-related changes in systemic hemodynamics may contribute to the pathogenesis or exacerbation of amyloid deposition, subsequent neuronal injury, and vascular pathology [193]. Similar findings have been reported for other large epidemiologic studies, including the Pittsburgh Cardiovascular Health Study [194] and the Prospective Population Study of Women in Gothenburg, Sweden [195]. Likewise, the Atherosclerosis Risk in Communities (ARIC) study found a high prevalence of MRI-detected cerebral abnormalities, related to cognitive functioning, that might reflect preclinical AD [196].

As described above and across a large body of published literature, VCID can manifest solely as a result of CBVD events, such as hemorrhagic or ischemic strokes, or by the accumulation of multiple ischemic events. However, it remains unclear whether AD occurs in the absence of any vascular pathology, or whether CVD and
CBVD changes are mechanistically related to the fundamental pathology of AD. Two separate imaging studies have recently cast doubt on the interdependence of AD and vascular pathologies [197,198]. The studies suggest that AD-related amyloid burden and CBVD independently affect cognitive impairment, as there was no correlation between the images comparing specific neuroanatomic coordinates of both amyloidosis (positron emission tomography (PET) PIB ligand binding) and white matter hyper intensities (WMH) with structural magnetic resonance imaging (MRI). In a study of 251 cognitively impaired subjects, Ye and colleagues found that PiB retention ratios were associated with both hippocampal atrophy and memory impairments and, conversely, the WMH imaging was more strongly associated with frontal cortex thinning and executive dysfunction. The authors concluded that the effect on cognition for individuals with both pathologies was additive and not synergistic, thus, the impact of AD and CVD pathologies on cognition are mediated through independent mechanisms [198]. Vemuri et al (2015) evaluated MRI and PET images from 393 cognitively normal participants, aged 70 to 90 from the Mayo Clinic Study of Aging and showed that for subjects with both vascular and amyloid pathologies, the effect of both pathologies on cognition was additive and not synergistic [197]. Both Ye, and Vemuri and colleagues have put forward similar arguments, but they are based on a single MRI imaging marker of CBVD. However, as we outline below, cardio- and cerebrovascular pathologies are highly complex clusters of biological processes that share many points of mechanistic links to AD. These two recent imaging studies stand as outliers within a large body of literature
suggesting that although VCID may frequently occur in the absence of AD, the converse is not necessarily true.

AD, CVD, & CBVD: Shared Pathophysiology and Neuropathological Substrates

AD, CVD and CBVD, primarily affect the same at-risk population who share many common risk factors. All three diseases may independently and/or interdependently lead to debilitating, unremitting and progressive changes in cognition. The direct causal relationship between vascular and cerebrovascular insults, and dementia or apoplexia, was described over three centuries ago by Thomas Willis [199]. Medical practitioners generally considered dementia to result from vascular insults. As early as 1833 Lobstein used the term “dementia arteriosclerotica” attributing the nature of the disease to a vascular origin [200]. A causal link between other non-vascular brain disease states and dementia was not well described until the latter half of the 19th century. In 1871, Charles Darwin received a letter from the director of England’s largest lunatic asylum, Dr. James Crichton-Browne, who observed that senile decay was the result of central nervous system disease that was linked to emotional liability [159]. Thirty-six years later, the initial case report of Alois Alzheimer’s; described the finding of senile plaques and neurofibrillary tangles found on post-mortem histopathology exam of a patient who had “ordinary dementia” and neuropsychiatric symptoms [201].

Prof. Alzheimer presaged the complexity and multi-causality of dementia by reporting atherosclerosis in the cerebral blood vessels of his 55 year-old patient, for which he coined the term Alzheimer’s sclerosis [202]. The term AD was reserved for
the diagnosis of dementia with onset between ages 40 and 90 years, if the other causal explanations (e.g., vascular causes) of dementia were absent [203]. The National Institute of Aging (NIA) and the Alzheimer’s Association (AA) revised the diagnostic and research criteria for AD in 2011, and this re-formulation of the diagnostic nosology were detailed in three publications that year [204, 205, 206]. According to the new NIA-AA criteria, a diagnosis of “pre-clinical AD” is based on the presence of relevant positive biomarkers (e.g., PET amyloid imaging) in conjunction with known risk factors for the disease (e.g., APOE e4 allele). The identification of individuals in this stage of prodromal AD is made essentially for research purposes only [204]. MCI due to AD includes patients with mild cognitive symptoms (impaired performance on measures of episodic memory function) with positive evidence of the disease from appropriate biomarker studies [205]. Ultimately, a diagnosis of AD is now based on criteria that account for recent developments in disease-specific biomarkers, which allow for confirmation of AD without the need to rely on post-mortem histopathology [206, 207,208].

Below, we briefly review the major overlapping pathophysiology between CVD, CBVD, and AD, all of which may be briefly summarized by Table 2.

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Insert Table 2 About Here

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**Reduced Cerebral Blood Flow**

Normal brain function is dependent on receiving 20% of the cardiac output of oxygenated blood and both higher and lower blood pressure may reduce this cerebral blood flow [209]. Hence, impaired cardiac function may subsequently lead to both
reduced intracranial blood flow (e.g., as measured within the Circle of Willis) and ischemia, and this is readily observed in AD patients [210]. Reduced cerebrovascular reactivity was observed in a study of 18 young adult (mean age 24 years) carriers of at least one APOE ε4 allele [211]. Suri and colleagues surmise that this lifelong relative decrease in cerebral blood flow will lead to areas of hypo-perfusion and microvascular damage; thereby, contributing to aggregation of blood products, endothelial dysfunction and impaired Aβ clearance [211]. It is of note that the sample size in this study was small, and further investigation is required to elucidate the nature of the interaction between cerebral blood flow, APOE status, and AD. A meta-analysis was conducted to investigate whether the changes in cerebral blood flow velocity and pulsatility index by Doppler ultrasonography in AD and VCID follow similar patterns. Both disease states were found to be associated with pronounced disturbances in cerebrovascular hemodynamics, with VCID patients showing significantly lower cerebral blood flow [212].

Another factor that may contribute to reduced regional cerebral blood flow is the observed decrease of endothelial Nitric oxide (NO) synthesis in AD [213]. The enzyme eNOS is responsible for NO generation, which is important for cardiovascular homeostasis and acts as a vasodilator involved in the control of vasomotor function and local blood flow [214]. Endothelial production of NO is important for the prevention of CBVD, as it mediates protection from stroke by preserving cerebral blood flow and preventing inflammation, thrombosis and apoptosis [215]. Besides the CBVD contributions, it can also increase expression of APP and BACE1 consequently increase Aβ levels [216]. The evolving opinion is that cerebrovascular dysfunction is
not only present in CBVD, but also a prominent component of neurodegenerative pathologies such as AD [217].

**Aβ deposition**

The amyloid cascade hypothesis postulates that neurodegeneration in AD is due to an abnormal accumulation of Aβ plaques in various areas of the brain [218], and the neurodegenerative processes in AD are the consequence of the imbalance between Aβ peptide production and clearance [219]. Recently, Yau and colleagues provided clear support for targeting Aβ clearance in early AD based on their longitudinal study of 16 patients with an autosomal dominant mutation for early-onset AD. In their study, they demonstrated that amyloidosis is one of the earliest events in the neuropathology cascade leading to AD, with the majority of Aβ aggregation occurring before the progressive structural neurodegeneration and cognitive decline [220].

The abnormal aggregation of Aβ protein in the brain neuropil may lead to either diffuse plaques and/or concentrated neuritic plaques, with the latter form of deposits often present in the vicinity of the cerebral microvasculature [221]. The Aβ protein, with its crystalline molecular structure, infiltrates the vessel walls and compromises the blood-brain barrier (BBB) [221]. Deposition of Aβ protein within the walls of cerebral blood vessels also leads to CAA, increasing the risk of cerebral hemorrhage [75], which is the most common clinical presentation of CAA [222]. In an APP/PS1 transgenic mice study with induced hyperhomocysteinemia, Sudduth and colleagues showed that congophilic amyloid deposition was decreased in the parenchyma and significantly increased in the vasculature in CAA. This suggests that CBVD can
significantly impact Aβ distribution in the brain by vascular deposition, and that such deposition can induce microhemorrhages and activate neuroinflammation [223]. In an *in vivo* study of Sprague-Dawley rats, infusion of solubilized Aβ peptides enhanced constriction of cerebral and peripheral vessels, contributing to cerebral hypoperfusion and leading to decreased blood flow and increased vascular resistance [224]. The risk of both repeated hemorrhagic strokes, as well as ischemic events due to vessel wall stenosis and oligaemia, increases with continued Aβ accumulation for both CAA and AD patients. This pathologic cascade leads to medial temporal atrophy, cognitive decline and progressive brain atrophy [75]. Finally, vascular Aβ deposits observed in AD have been shown *in vitro* to induce degeneration of human and murine cerebrovascular smooth muscle and endothelial cells, resulting in vasoconstriction, intraluminal thickening, inhibiting angiogenesis, impairing vascular tone and decreasing total cerebral blood flow [225].

**Morphological Changes in the Vasculature**

Arterial stiffness can be caused by structural or cellular change within vessel walls, and amyloid deposition in the vessels leads directly to this pathophysiological process [226]. The fragmentation of elastin alters the haemodynamics of vessel walls, resulting in increased systolic pressure by increasing the speed of the arterial wave that arrives prematurely during systole rather than during diastole [227]. Atherosclerosis also accelerates arterial stiffness [228], a frequent finding in patients with CVD. Arterial stiffness is a clear risk factor for cognitive impairment in later life, as it can cause structural changes in the brain, such as white matter or cortical infarcts and cortical
brain atrophy [229, 230]. A recent systematic review of this association concludes that arterial stiffness is related to cerebral small vessel disease and decreased cognitive function [231].

The deposition of Aβ in arterial vessel walls, and subsequent impediment of perivascular drainage of Aβ due to the AD pathology, can lead to intracerebral haemorrhage and an increase of Aβ peptides [75]. These morphological and architectural changes of the cerebral vasculature, studied in APP23 tg mice, start early in life. Along with the increase in observable amyloid plaques, there is also increased atrophy and altered blood flow, suggesting that disrupted microvasculature integrity can contribute to the progression of AD [232]. However, subtle changes in cerebral microcirculation are difficult to measure with high precision for the exploration of longitudinal changes within-subjects associated with increased disease burden. These subtle morphological changes may be more easily observed in the microvasculature of the retina. Patients with AD have sparser retinal microvascular networks and other structural alterations that may mirror pathophysiological events found in the cerebral microvasculature [233]. Patients with AD show changes in retinal microvasculature, such as more tortuous retinal vessels and a narrowing of retinal venules [234]. These retinal vascular changes have been posited to precede the majority of neurodegeneration that characterizes AD progression [49].

**Alterations in Blood-Brain Barrier (BBB) Permeability**

The brain vasculature has cellular elements forming a developmental, structural and functional relationship with the brain tissue termed the neurovascular unit [226].
The neurovascular unit has a fundamental role in the broad spectrum of pathologies underlying cognitive impairment. Neural activity requires a continuous and regulated blood flow in order to activate neurons, astrocytes and vascular cells through a wide variety of molecular signals (ions, arachidonic acid, metabolites, NO, adenosine, neurotransmitters, and neuropeptides) [235]. Specific neuroimaging techniques to visualize deleterious changes to the BBB are still under exploration. Several studies have shown that plasma proteins like prothrombin, which are typically excluded from the CNS, can be found within the microvessel walls and surrounding neuropil in AD patients, showing that the leakage of the BBB may be frequent in AD [236]. The vascular abnormalities found in the AD brain, such as alteration in smooth muscle cells and pericytes, endothelial cell thinning, loss of endothelial mitochondria and thickening of the vascular basement membrane, all contribute to alterations in BBB permeability [237]. The end result is a continuous cycle of reduced cerebral perfusion leading to acceleration of the neurodegenerative process, which further reduces perfusion.

The changes in BBB permeability lead to an ionic imbalance and accumulation of toxic metabolic products. As a consequence, synaptic, neuronal and oligodendritical dysfunction occurs [238], since an intact BBB is crucial for limiting the entry of toxic products and cells into the brain. Glucose transport across the BBB is also impaired, and positron emission tomography (PET) studies show reduced regional metabolic rate in the AD brain [239]. Alterations on the (Na+/K+)-pump function of the BBB can result in fluid balance impairment, leading to deregulation of regional cerebral blood flow [240].
Aβ accumulation can contribute to the leakage of the BBB [241]. These peptides spread across a defective BBB contribute to higher oxidative and nicrosative damage, as well as increased protease activity [75]. Aβ deposition leads to microglial activation, reactive astrocytosis and a multi-protein inflammatory response [242]. Studies of WT and APOE -/- mice show that BBB permeability increases with age and a defect in the BBB is exacerbated in APOE -/- mice [243,244]. Additionally, arterial stiffness results in an uncoupling of the neurovascular unit, and this disruption of the cerebral microenvironment is likely to contribute to brain dysfunction [245]. The resulting accumulation of Aβ in the neuropil and vessel walls leads to the activation of neuroinflammatory response, which plays an important role in BBB disruption [246]. Anatomically, neuroinflammation may lead to transient increases in thickness of various cortical tissues, as well as at least one neuronal cell layer of the retina in preclinical stage disease. Snyder et al. (2016) have provided initial evidence to suggest an increase in the thickness of the retinal inner plexiform layer, in preclinical AD, and they postulate this volume increase may be partly due to a localized neuroinflammatory process and/or deposition of amyloid-containing inclusion bodies within the cell layer. They suggest that this observation may precede a gradual thinning of neuronal cell layers (e.g., the ganglion cell layer) and the retinal nerve fiber layer with continued disease progression [247].

**Cholinergic neurodegeneration**

Postmortem studies have shown reduced activity of choline acetyltransferase, decreased numbers of nicotinic acetylcholine receptors, and reduced basal forebrain
cholinergic neurons (particularly in the nucleus basalis of Mynert), contribute to the oldest model of neurobiologic dysfunction in AD – the “cholinergic hypothesis” [248,249].

This reduction in cholinergic innervation and activity may result, in part, from a reduction in noradrenaline release due to Locus coeruleus (LC) neuron loss [250]. There is a strong reciprocal connection between the LC and the prefrontal cortex, and this area is involved in the mediation of executive functioning, memory and vigilance [251]. The LC is responsible for exerting an excitatory influence on wakefulness-promoting nuclei, such as the cholinergic nuclei of the septal area, medial preoptic area and substantia innominata [252]. Pathological changes in the LC occur early in AD [253], and this reduction in LC activity likely leads to the common finding of reduced levels of arousal and alertness in AD patients. The number of LC neurons projecting to areas such as the hippocampus and the frontal cortex, decline slowly with normal aging, and this may result in some modest age-related changes in spatial learning and memory [254].

This structural and functional loss in the LC affects both efferent and afferent pathways. The LC exerts both direct (via a descending excitatory noradrenergic pathway) and indirect effects (via modulation of the activity of other premotor sympathetic nuclei) on preganglionic sympathetic neurons in the intermediolateral cell column [255]. The LC sends efferent inputs to the nucleus tratus solitarius, which is critical for the modulation of the vasomotor response to changes in blood pressure through vagal nerve stimulation and autonomic inputs to the heart. The vagal nerve

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provides cholinergic input to increase parasympathetic activity, which decreases heart rate and lowers blood pressure when needed [256].

The earliest stages of AD are marked, in part, by altered function of the basal forebrain cholinergic system, with eventual degenerative changes including neuronal loss [257,258]. We have recently reported that a down-regulation of central cholinergic neurotransmission appears to be one of the earliest neuropathological changes in preclinical AD [259], and we have also found that individuals with evidence of both decreased central cholinergic tone and amyloid aggregation within the anterior cingulate region show evidence of increased resting cardiac workload at rest [52]. The aggregation of Aβ plaques in the neocortex, within this specific region of interest that is part of the central cholinergic system, appears to be directly associated with increasing cognitive impairment as well as the higher myocardial oxygen consumption at resting state [52]. In fact, there is a growing body of literature to suggest a direct link between Aβ aggregation, basal forebrain cholinergic damage, and diminished cholinergic innervation of cortical blood vessels, leading to the microvascular pathology that has been documented in the majority of AD cases [260,261,262,263]. We are currently exploring whether indices of phasic vagal cardiac control, such as resting sinus arrhythmia (RSA) and heart rate variability (HRV) are also related to cortical amyloidosis in preclinical AD, since both RSA and HRV are directly modulated by muscarinic cholinergic and nicotinic autonomic neurotransmission.
General Discussion

This review is intended to tie together several lines of research on the shared mechanistic relationships between AD, CVD, and CBVD. The literature that binds these diseases is both large and confusing. Why is AD so tightly connected to disruption of the cerebrovasculature and to cardiologic disease? Why do these broad disease entities share so many risk factors and mechanistic relationships? One compelling answer to these larger questions may come from the field of medical anthropology and attempts to study the global distribution of \( APOE \) gene alleles across the human species. The \( \epsilon 4 \) allele is the ancestral form of \( APOE \) \[264,265\] and is associated with both higher absorption of cholesterol at the intestinal level and higher plasma cholesterol levels in carriers. The phenotypic expression of this allele would likely confer a survival benefit to humans that evolved with limited food supplies and in harsh weather conditions. Expression of the \( \epsilon 4 \) allele under contemporary/modern diet, exercise and environmental conditions, together with the relatively recent and dramatic increase in human longevity may have now led to the identification of the \( \epsilon 4 \) allele as pleiotropic, showing susceptibilities for both CVD and AD \[265\]. Although this anthropological viewpoint is not universally accepted, nor the focus of the current review, it affords us an over-arching heuristic model to explain the strong relationship between CVD, CBVD and AD.

Another question that arises from the frequent co-incidence of AD, CVD, and CBVD is whether or not they are the end result of shared etiologic mechanisms. If one supposes a direct, bi-directional causal link between these disease clusters, then all
cases of CVD and/or CBVD would also demonstrate AD pathology, and we know this not the case. Rather, we have reviewed a large literature indicating that vascular/cerebrovascular pathology is present for most individuals with AD but not all of them. In support of this notion, an autopsy study with the largest cohort to date (N = 5,715) showed increased prevalence of CBVD and vascular pathology in AD compared to healthy controls and patients with dementias of non-AD etiologies [266].

So what, then, is the causal nature of the relationships between these diseases? To answer this question, it is important to determine whether the cognitive effects of AD and CVD/CBVD are additive or synergistic. This is a topic that continues to elicit considerable scientific exploration. It is possible that AD and CVD/CBVD contribute independently to dementia, such that the severity of the dementia is a cumulative result of two separate pathologies [196]. Alternatively, these disease processes could be synergistic, such that the pathology of one accelerates the progress of the other. The majority of the literature reviewed herein supports the model of a synergistic interaction between vascular/cerebrovascular and neurodegenerative processes early in disease pathogenesis. This theory is further supported by a reciprocal relationship between Aβ accumulation and cerebrovascular insult such that Aβ deposition provokes vascular/cerebrovascular changes [75] and vice versa [267,268]. Clinical studies demonstrate that even mild cerebrovascular pathology results in reduced cognitive performance in very early AD [78, 269]. It is possible that both additive and synergistic processes are affecting cognitive decline at different stages of these disease processes. This theory is congruous with variability in observational findings based on
timing, severity, and duration of CVD risk factors. In fact, it has been suggested that later disease stages of disease could demonstrate a more additive relationship [270].

There is evidence that vascular/cerebrovascular pathology can accelerate the progression of preclinical AD and speed disease evolution [271,272]. The relationships between AD and CVD/CBVD are complex, and further investigation is required in order to discern the exact nature of these relationships at different stages of disease progression. We suggest that, because AD is so frequently accompanied by comorbid vascular and/or cerebrovascular symptoms, it is both clinically and scientifically relevant to consider these pathologies concurrently, regardless of whether their respective underlying pathologic mechanisms are independent and additive, or functionally related and synergistic.

We support the widely studied hypothesis that effectively controlling vascular risk factors serves to delay onset of AD. In fact, a recent statement by the World Dementia Council suggested that “Regular physical activity and management of cardiovascular risk factors (e.g. diabetes, obesity, smoking, and hypertension) are associated with a reduced risk of cognitive decline and may reduce the risk of dementia” [273,274]. A recent cross-sectional study concluded that the use of certain medications to treat vascular disease, especially angiotensin receptor blockers and diuretics, may decrease Aβ accumulation [275]. In a recent review, Deckers et al (2015) [276] found that the most common modifiable risk factors for AD development included hypertension, diabetes, midlife obesity, physical inactivity, hyperlipidemia, and smoking. Simple, inexpensive interventions involving diet and exercise in midlife could be very useful tools to prevent CVD, CBVD, and AD. These interventions are currently under
evaluation by several large prospective clinical trials, including the CAIDE [277] and
the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and
Disability (FINGER – [278]). Nonetheless, preliminary results indicate that the
complex multifactorial nature of AD requires interventions that simultaneously target
multiple risk factors and disease mechanisms during the preclinical stage of the
disease [277,278]. The CAIDE screening tool has been developed based on vascular
and metabolic factors shown to increase dementia risk in order to identify individuals
who are at-risk for dementia and who require preventive intervention [45] and this has
recently been developed into a freely-available smartphone application [277].

The validation of sensitive and reliable measures of dementia risk that account for
CVD susceptibility (e.g., CAIDE), paired with lifestyle intervention techniques to
control or reduce these same risk markers, will ultimately lead to a better
understanding of the relationship between these two disease clusters. The exploration
of the dynamic pathogenic relationships between AD and CVD/CBVD has the
potential to lead to a reduction and/or delay in AD incidence. In accordance with
earlier work revealing that interactions between mechanistic, genetic, and lifestyle
factors influence vascular disease, we expect that these multifactorial interventions
targeting common mechanistic and lifestyle factors in AD and CVD/CBVD will
confirm that the adoption of a heart-healthy lifestyle has the potential to contribute to a
future decline in all three disease processes.
Future Directions

Despite the rapid advancement of medical technology, we are still developing a suite of reliable and sensitive diagnostic markers to identify individuals at risk for AD prior to onset of clinical symptoms. This is an area of research that needs to be urgently addressed in order to enable the study of early interventions to maintain quality of life in premorbid AD and to reduce the individual and societal burden of the disease. There are currently several secondary prevention trials aimed at pharmacologically slowing or reducing the Aβ accumulation that occurs in preclinical AD, but as of this writing these secondary prevention trials are still in progress and we do not yet have successful therapies to prevent or slow/reduce disease progression. It is typically the case that such large prospective studies seek to exclude participants with significant cardiovascular or cerebrovascular comorbidities. Given the evidence supporting a substantial overlap of epidemiology, genetics, risk factors, and mechanistic factors in vascular and AD pathology, we argue that future secondary prevention trials should focus on a more heterogeneous and phenotypically representative population.

Aside from clinical trials and recruitment science, there continues to be an outpouring of literature aimed at untangling the mechanistic relationships between CVD, CBVD, and AD. Recently, the National Institutes of Health (NIH) launched the Molecular Mechanisms of the Vascular Etiology of Alzheimer’s Disease (M²OVE-AD) Consortium, the primary aim of which is to construct a comprehensive model of Alzheimer’s disease that more accurately reflects its complex underpinnings, and the multiple pathways of disease development. The main objectives of this initiative are to
elucidate the complex mechanisms by which cardiovascular risk factors influence the development and progression of AD, and to identify new targets for treatment and prevention. Thus far, the consortium supports five project areas addressing a wide range of topics, including: the contribution of Alzheimer’s risk genes (*APOE* E4) to AD and CVD, the contribution of DM to AD, CVD, and CBVD, the contribution of hypertension to AD development and progression, identifying metabolic signatures underlying risk factors for both AD and CVD, and investigating the mechanism of Aβ accumulation and clearance at the molecular, single-blood vessel, and whole brain level and the relationship of Aβ accumulation at all three levels in AD and CBVD. Moving forward, studies such as these that investigate the interaction of vascular biology with genetic, cardiometabolic, and lifestyle risk factors and AD pathology will be crucial to the development of therapeutic agents.

There are many questions about the relationships between AD, CBVD, and CVD that remain unanswered. One important public health question is how these diseases intersect in the oldest old. The World Health Organization has reported a world-wide dementia incidence of 47.5 million in 2015, and a projected incidence of 75.6 million in 2030. The largest risk factor for AD, CVD, and CBVD is increasing age. In the United States, the population of adults aged ≥90 is expected to grow over six-fold by 2050 [271]. As the average lifespan increases, the social and economic consequences of AD, CVD, and CBVD are expected to expand accordingly. Due to difficulties in finding, recruiting, and diagnosing the oldest-old, very little literature exists examining the relationships between AD, CBVD, and CVD in this population. Future population-based studies should aim to include this cohort in order to further
understand the nature of disease interactions over time, and to identify prevention targets to reduce cardiovascular risk (i.e. blood pressure, glycemic index, and cholesterol levels) in these specific populations. More accurate models are required for assessing prognosis and life expectancy in older adults with AD, CVD, and/or CBVD in the context of multiple chronic conditions.
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Tables

Table 1. Shared evidence-based treatments for cardiovascular disease (CVD) and Alzheimer’s disease (AD). Note: Given the breadth of this literature, all cited references are exemplars published within the past 10 years, and all are empirical reports.

<table>
<thead>
<tr>
<th>Specific Medication Interventions</th>
<th>Clinical Effects in Treatment of CVD</th>
<th>Clinical Effects in Treatment of AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuretics</td>
<td>Thiazide diuretics lead to lowering of blood pressure [279,280].</td>
<td>Long-term use of diuretics may be associated with decreased incidence of AD [61,62].</td>
</tr>
<tr>
<td>Angiotensin receptor - 1 blocker (ARB) or angiotensin-converting enzyme (ACE) inhibitor</td>
<td>Reduce risk of cardiovascular events [281,282].</td>
<td>ARB and ACE inhibitors may slow progression of symptoms in mild-moderate AD [62,64].</td>
</tr>
<tr>
<td>β-blockers</td>
<td>β-blockers can prevent cardiovascular events in patients at increased cardiovascular risk [283,284].</td>
<td>β-blocker use is associated with a lowered risk of developing cognitive impairment in older adults without dementia [63,65].</td>
</tr>
<tr>
<td>Statins</td>
<td>Reduce risk of cardiovascular events [285,286].</td>
<td>Mixed literature, with no consistent evidence that statins reduce the incidence of AD or slow cognitive decline [87,88].</td>
</tr>
<tr>
<td>Anti-Inflammatory Drugs</td>
<td>Mixed literature on use of Non-aspirin anti-Inflammatory drugs to reduce cardiovascular risk [287,288]. Low-dose aspirin is commonly used as an anti-platelet agent for secondary CVD prevention.</td>
<td>Mixed literature, suggesting that NSAIDs may confer modest protective effects [10,11]. Low-dose aspirin is commonly used as an anti-platelet agent for stroke prevention.</td>
</tr>
<tr>
<td>Insulin Treatment</td>
<td>Effective diabetes treatment confers long-term beneficial effects on risk of CVD [289,290].</td>
<td>Intranasal insulin appears to improve cognition and modulates Aβ aggregation in early AD [111, 94].</td>
</tr>
<tr>
<td><strong>Specific Behavioral Interventions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic Exercise &amp; Physical Fitness</td>
<td>Long-term protective effects on risk of cardiovascular disorders [291,292].</td>
<td>Aerobic exercise promotes brain vascularization and may reduce vascular risk factors as well as to improve cognitive function [125,127].</td>
</tr>
<tr>
<td>Healthy Diets (e.g., MIND diet)</td>
<td>Healthy diets may protect against CVD [293,294].</td>
<td>High-fat diets have shown an increased risk for AD [112,115]. Conversely, carefully designed diets have been shown to confer protective effects [113,114].</td>
</tr>
</tbody>
</table>
Table 2. Domains of shared patient characteristics and pathophysiology between cardio- and cerebrovascular disease, and Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>AD</th>
<th>CVD/CBVD</th>
<th>Key References numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td>Yes</td>
<td>Yes</td>
<td>2, 34,35,189</td>
</tr>
<tr>
<td>Genetic risk factor</td>
<td>Yes</td>
<td>Yes</td>
<td>23-33, 264-266</td>
</tr>
<tr>
<td>Hypertension/ Hypotension</td>
<td>Yes</td>
<td>Yes</td>
<td>34,35,38,39-44,51-69</td>
</tr>
<tr>
<td>High Cholesterol</td>
<td>Yes</td>
<td>Yes</td>
<td>36,71-86</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Yes</td>
<td>Yes</td>
<td>34,35,38-44,89-110</td>
</tr>
<tr>
<td>Obesity</td>
<td>Yes</td>
<td>Yes</td>
<td>111-121</td>
</tr>
<tr>
<td>Poor physical fitness</td>
<td>Yes</td>
<td>Yes</td>
<td>124-137</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>Yes</td>
<td>34,35,39-45,143-154</td>
</tr>
<tr>
<td>History of depression</td>
<td>Yes</td>
<td>Yes</td>
<td>157-168</td>
</tr>
<tr>
<td>Pathogens (e.g., fungal)</td>
<td>Yes</td>
<td>Yes</td>
<td>169,170</td>
</tr>
<tr>
<td>Air pollution</td>
<td>Yes</td>
<td>Yes</td>
<td>176-179</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>AD</th>
<th>CVD/CBVD</th>
<th>Key References numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced cerebral blood flow</td>
<td>Yes</td>
<td>Yes</td>
<td>182,209-217</td>
</tr>
<tr>
<td>Aβ deposition</td>
<td>Yes</td>
<td>No</td>
<td>184,218-225</td>
</tr>
<tr>
<td>Morphological changes in vasculature</td>
<td>Yes</td>
<td>Yes</td>
<td>49,180,181,226-234</td>
</tr>
<tr>
<td>Alterations in BBB permeability</td>
<td>Yes</td>
<td>Yes</td>
<td>235-247</td>
</tr>
<tr>
<td>Cholinergic neurodegeneration</td>
<td>Yes</td>
<td>Yes</td>
<td>248-263</td>
</tr>
</tbody>
</table>
MANUSCRIPT II

“Autonomic Cardiac Function in preclinical Alzheimer’s disease”

by

Cláudia Y. Santos¹,²; Jason T. Machan, PhD³; Wen-Chih Wu, MD⁴; Peter J. Snyder, PhD²,⁵


1. PhD Candidate at Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA, email: clausantos2910@gmail.com
2. Clinical Researcher at Lifespan Clinical Research Center, Rhode Island Hospital, Providence, RI, USA
3. Director of Lifespan Biostatistics Core, Lifespan Hospital System, Providence, RI, USA
4. Professor at Division of Cardiology, Providence VA Medical Center and Alpert Medical School of Brown University, Providence, RI, USA
5. Professor at Department of Neurology, Alpert Medical School of Brown University, Providence, RI, USA
Abstract

To explore early autonomic cardiac changes in pre-clinical AD, we have evaluated electrocardiologic measures of vagal tone for 63 adults (ages 55-75) at rest, during cognitive testing, and then again at rest. All subjects had multiple risk factors for AD, and all completed amyloid PET scans ($^{18}$F-Florbetapir) to determine amyloid positivity (Aβ+).

No change in ECG measures were observed for Aβ+ participants under each testing condition, whereas Aβ- subjects showed an expected increase in vagal tone during the cognitive stress condition. These findings suggest an early relationship between cortical Aβ accumulation, a precursor to AD development, and autonomic cardiac function.

Keywords

Alzheimer’s disease, cardiac, vagal tone, resting sinus arrhythmia, aging, heart rate variability
Introduction

A recent review of the complex relationship between Alzheimer’s disease (AD), cardiovascular disease (CVD), and cerebrovascular disease (CBVD) [1] highlights the many shared risk factors and pathophysiologic mechanisms across these diseases and, as a result, the preclinical stages of these diseases may often concurrently affect the same at-risk population [1]. Clinical studies demonstrate that even mild cerebrovascular pathology results in reduced cognitive performance in very early AD [2,3]. Additionally, there is evidence that vascular/cerebrovascular pathology can accelerate the progression of AD [4,5]. Moreover, the blood-brain barrier seems to be disrupted early in AD, causing dysfunction in brain perfusion [6] and reduced cerebral blood flow [7]. It remains unclear just how early in the disease course the shared pathological relationships between AD, CVD, and CBVD can be observed. The exploration of the mechanistic relationships between these disease clusters in a preclinical population is important to better understand the complicated co-occurrence of these three diseases.

We recently examined the relationship between CNS amyloid beta (Aβ) burden, and a simple measure of myocardial oxygen consumption: rate pressure product (RPP), a well-known cardiac workload marker that reflects myocardial stress based on the number of times that the heart needs to beat per minute in relation to the arterial blood pressure that it is pumping against. In cognitively normal participants at high-risk for the development of AD [8]. This work supports a relationship between elevated neocortical Aβ aggregation and inefficient myocardial oxygen consumption.
in the absence of significant metabolic demands (after accounting for expected effects of age).

In the current study, we sought to explore if the changes in cardiac workload that we see in preclinical AD during cognitive testing are due to centrally-mediated changes in vagal tone measured by vagal ratio and the resting sinus arrhythmia (RSA). Both of these indices become less reactive and they decrease in amplitude with normal aging, due to declines in parasympathetic tone [9,10]. The vagal ratio is the ratio between low and high frequency components of the Heart Rate Variability (HRV) and the RSA is a measure of the HRV in synchrony with respiration. In this study, rapid changes in these two indices of vagal tone were measured before, during, and immediately after the administration of a cognitive stressor, to explore the adaptive flexibility of parasympathetic autonomic function in response to a mild cognitive stressor (performance on a hidden maze learning task).
Methods

Participants: Sixty-three adults aged between 55 and 75 years (mean age = 62.79 years), all at some risk for development of Alzheimer’s disease (AD) based on the presence of two risk factors for the disease (a first degree family history and subjective memory complaints), were recruited for a longitudinal study of individuals with possible preclinical AD [11]. From this larger sample, we identified 15 participants who were categorized as presenting with preclinical stage disease based on evidence of both 1) elevated neocortical beta-amyloid burden as determined by PET amyloid imaging [12,13]; and 2) relative cognitive impairments in response to a challenge with a very low-dose muscarinic anticholinergics [11,14]. Potential participants were excluded from this study if they presented with diagnoses of either mild cognitive impairment (MCI) or AD, following NIA-AA diagnostic criteria [13,15], diabetes, or histories of any neurological or psychiatric disorders. With respect to cardiovascular risk, four of 15 subjects in the preclinical AD group (26.7%) and 12 of 48 subjects in the control group (25%) presented with hypercholesterolemia that was under medication control. Two individuals in the preclinical AD group (13.3%) and 8 persons in the control group (16.6%) were under treatment for hypertension. For all subjects, a score on the Mini Mental State Examination (MMSE) >27 was required for study entry.

With respect to genetic risk for AD, eight of 15 individuals (53%) in the preclinical AD group (Florbetapir PET SUVr scores ≥1.10) had at least one copy of the APOE ε4 allele, whereas 20 of 45 individuals (45%) in the control group
(Florbetapir PET SUVr scores < 1.10) had at least one copy of the APOE ε4 allele. Hence, roughly half of the subjects in each group presented with this additional risk factor for AD, but due to small sample sizes we were not able to further evaluate the specific effect of APOE genetic risk on the relationship between vagal tone and cognitive performance. Subject demographic information, for both groups, are provided in Table 1.

**Neuroimaging:** PET scans were performed at baseline, with 370MBq (10 mCi +/- 10%) bolus injection of 18F-florbetapir administered intravenously. PET standardized uptake value (SUV) data were summed and normalized to the whole cerebellum SUV, resulting in a region-to-cerebellum ratio termed SUV ratio (SUVr). We defined amyloid positivity (Aβ+) as individuals with elevated ligand binding (SUVr≥1.1) in the anterior cingulate (ACC). Consistent with Lim et al. (2015) [14], this specific region of interest was selected because: (1) the young age of participants enrolled in the study [16,17], and as such, we would not expect widespread neocortical amyloidosis in this very early preclinical disease stage, (2) the relationship between the ACC and early changes in cholinergic tone [18,19], and (3) emerging research that suggests that increased Aβ burden, in the ACC specifically, is related to memory changes in early AD [20,21]. The SUVr calculation was performed using MIMneuro software [22]. For all cases, Aβ positivity was confirmed by consensus over-read by two board-certified radiologists who were also board-certified in Nuclear Medicine [14].
**Cardiac measures:** Electrocardiographic (ECG) measures of resting-sinus arrhythmia (RSA) and vagal ratio (described above) were recorded by BioPac Systems [23] for each participant: 1) at rest, sitting quietly in a semi-supine position (120 seconds); 2) during the performance of the Groton Maze Learning Test (GMLT; approx. 190 seconds); and 3) post-test at rest in a semi-supine position (120 seconds).

Both measures were calculated using the Heart Rate Variability (HRV) analysis of fixed-width intervals around events, which follows the European Heart Journal guidelines [24] for the spectral method with the Multi-epoch HRV processing [25].

RSA is computed using the peak-valley method, which uses both a recorded ECG signal and a respiration signal. By using respiration information, this analysis method can provide breath-to-breath analysis that does not require parameter tweaking for individual subjects [23,26].

The vagal ratio measure accounts for the fact that parasympathetic control via the vagus nerve is believed to primarily modulate the R-R intervals at a rate of between 0.15 and 0.4 cycles per second. Frequency based analysis of HRV in AcqKnowledge computes vagal ratio as the amount of power in the parasympathetic band normalized to an approximation of the total power in the signal [25].

**Cognitive Assessment:** All participants completed the Groton Maze Learning Test (GMLT; http://www.cogstate.com) as a mild cognitive stressor, during the ECG recording. The GMLT is a computerized hidden spatial maze learning task, developed by one of the authors (P.J.S.) as a measure of working memory, learning efficiency and reasoning/problem solving [27]. This GMLT composite score was used to explore
group differences in performance. All subjects completed a practice trial of the GMLT, to allow for initial task familiarity; and after a 10-min break, all subjects repeated the GMLT as a baseline assessment (at which time ECG recordings were obtained, as described above).

Additionally, all subjects completed a test of verbal episodic memory: International Shopping List Test (ISLT) [28], the Mini-Mental State Examination (MMSE) as a measure of general cognitive function, the 15-item Geriatric Depression Scale and the Depression, Anxiety and Stress Scale (DASS). Subjective memory impairment was determined using the Memory Complaint Questionnaire (MAC-Q). All measures were performed by trained staff supervised by a licensed clinical neuropsychologist.

**Statistical Treatment of Data:** Generalized estimating equations (GEEs) were used to compare within-subject changes in vagal ratio and RSA across the three time points (rest à cognitive task à rest) between preclinical AD subjects (N=15) and the control subjects (N=48). GEEs are generalized linear models wherein the within-subjects (or other) nesting is accounted for in the residual error of the model (R-side), as contrasted with constructing random effects separate from the residual (G-side as in random, mixed, or hierarchical models) [29]. In our case, a compound symmetry variance-covariance matrix was used across testing phases, which assumes a common variance for each phase as well as a common covariance between all phases. Despite best intentions and approach, there can be non-obvious imprecision in these specification and so classical sandwich estimation [30] was used to adjust for any misspecification in this structure by subsequently adjusting the variances and
covariances in the structure based on the actual distribution of the data (inverse of the empirical variance “sandwiched” between model variance(s)). The Shaffer step-down adjustment was used to maintain alpha at 0.05 across all comparisons within each model. Figures 2 and 3 were designed to simultaneously illustrate between- and within-participant variation.

This study was approved by and complied with the regulations of Rhode Island Hospital’s Institutional Review Board, and all participants provided written informed consent.
Results

There were no statistically significant differences between the two groups for any demographic or clinical outcome, with the exception of the expected elevation in cortical amyloid aggregation as demonstrated on florbetapir PET scan (Table 1). There were also no between-groups differences in at-rest vagal ratio or RSA, with respect to main effects for APOE ε4 carrier status, presence of hypercholesterolemia and hypertension (p > .05 for all analyses).

Insert Table 1 About Here

With respect to performance on the GMLT, no between-groups differences on the composite score [14], were found between those with Aβ+ PET scans vs. those in the Aβ- healthy control group.

Insert Figure 1 About Here

However, subjects in the healthy control group (those with Aβ- PET scans) showed an increase in vagal ratio during the cognitive task condition, relative to either of the two at-rest conditions (Pre-GMLT: p=0.0061; adj. p=0.0304; Post-GMLT: p<.0001; adj. p=0.0007), with post-GMLT vagal ratio not differing significantly from pre-GMLT levels (p=0.2283; adj. p=0.9185). In contrast, the preclinical AD (Aβ+) group did not consistently show any change in vagal ratio at any point during testing (pre- vs. GMLT p=0.1837, GMLT vs. post- p=0.2221, post- vs. pre-GMLT p=0.7883; adj. p =0.9985 for all). Further, the increased vagal ratio during the cognitive stress
condition (p=0.0099; adj. p=0.0304) and subsequent decreases (p=0.0013; adj. p=0.0007) for the Aβ- group differed significantly from the Aβ+ group.

Similarly, to vagal ratio, the Aβ- subjects showed a significant increase in RSA during the cognitive task condition, relative to either of the two at-rest conditions (Pre-GMLT: p<.0001; adj. p<.0001; Post-GMLT: p=<.0001; adj. p=0.0003), with post-GMLT RSA not differing significantly from pre-GMLT levels (p=0.2471; adj. p=1.0). As with the vagal ratio measure, those in the Aβ+ group did not consistently show any change in RSA at any point during testing (pre- vs. GMLT p=0.6321, GMLT vs. post-p=0.7719, post- vs. pre-GMLT p=0.8510; adj. p =1.0 for all). Further, the increased RSA scores during the cognitive stress condition (p=0.0021; adj. p=0.0104) were significantly larger for the Aβ- group compared to the Aβ+ group. The subsequent decrease in RSA for the Aβ+ group after the cognitive stress were significantly greater than for the Aβ- group prior to adjustment, but not after multiplicity adjustment (p=0.0304; adj. p=0.0913).
Discussion

Participants in this preclinical AD (Aβ+) group demonstrated statistically significant attenuation in the reactivity of two separate measures (vagal ratio and RSA) of parasympathetic response to a mild cognitive stress condition. Not only was the magnitude of their response to the cognitive stressor less than those without elevated neocortical Aβ, but no significant differences found for these measures, within the Aβ+ group, across any of the three ECG time points. In order to further explore the relationship between neocortical Aβ aggregation and inefficient myocardial oxygen consumption in the absence of significant metabolic demands [8], it is essential to rely on metrics that accurately reflect the autonomic processes that control cardiac function. Cardiac autonomic dysfunction is prevalent among individuals with AD, and this generally reflects a decrease in parasympathetic activity [31,32]. It has been previously shown that individuals with MCI demonstrate significant parasympathetic deficits [33]. The parasympathetic nervous system (PNS) influences heart rate by the release of acetylcholine (ACh) via the vagus nerve, which causes the heart rate activity to slowdown. This reduction in PNS activity is often referred to as vagal withdrawal, and is indicative of impaired autonomic function [34].

The ratio between low and high frequency components of the HRV (the LF/HF ratio, or vagal ratio) has long been relied on as a measure of sympatho-vagal balance [35,36,37]. Healthy individuals under mental stress while completing a cognitive task showed a reduced HF HRV component compared to a control group who were monitored whilst awake and at rest [38,39]. The clinical reliability of this vagal ratio
as marker of autonomic activity has been nonetheless controversial [40,41]. Therefore, we have chosen to supplement this measure of autonomic cardiac function in preclinical AD by computing the respiratory sinus arrhythmia (RSA), which considers the variation in heart rate during the breathing cycle and is commonly relied on as a measure of parasympathetic nervous system activity. Typically, both vagal ratio and RSA decrease with normal aging due to decline in parasympathetic tone [9,10].

Disruption of cholinergic neurotransmission with low-dose scopolamine has been previously shown to exacerbate subtle cognitive deficits that are otherwise not (yet) detectable for subjects in the Aβ+ group [14], whereas standard baseline testing with the GMLT does not reveal any group differences. We believe that the subjects enrolled in the Aβ+ group, for this study, are in the preclinical stage of the disease and that it might be too early to observe clear impairments in learning efficiency and working memory/executive function unless such defects are unmasked by intentionally down-regulating cholinergic tone [14].

Participants in this Aβ+ group, however, did not consistently show changes in vagal ratio or RSA at any point during the experiment, failing to demonstrate the expected response to modest stress elicited during cognitive task performance. Both measures were expected to show modest increases, reflecting heightened autonomic arousal, during completion of a neuropsychological test, even after adjusting for age effects [42]. Enhanced vagal ratio is characterized by greater vagal reactivity and faster vagal recovery from psychological stressors, and it has been linked with greater ANS flexibility and an improved response selection in the face of stress [43,44] and return to homeostasis [45]. Conversely, in a study of healthy male sailors who
completed a fitness training program, those with higher vagal ratio scores
demonstrated improved performance on measures of working memory, reaction time
and attentional controls [46]. In our study, the Aβ- group demonstrated an expected
increase in vagal ratio in response to the cognitive stressor, whereas the Aβ+ group
demonstrated no change in vagal ratio across all three testing conditions.

The same pattern of results was observed for the related RSA measure, with
participants in the Aβ- group showing an expected increase in RSA during GMLT
performance. RSA is a composite of integrated respiratory and cardiovascular
responses [47] that are responsive not only to metabolic demands but also to levels of
alertness and, in humans at least, different types of emotion, mental activity and
arousal metabolism [48]. Typically, expression of RSA decreases with age [49];
however, adults in excellent cardiovascular health, such as athletes, are likely to have
a higher RSA [50]. Higher RSA is also usually associated with fewer errors in a
complex memory task, on measures of attentional control and response selection and
on cognitive function tasks that are associated with the integrity of the ACC [51].
Aβ+ participants showed decreased RSA compared to Aβ- during the cognitive test,
and this pattern was present in participants with cognition impairments or with
diabetes and cardiovascular disease [10].

Cardiac autonomic dysfunction is prevalent among individuals with AD, as
reflected by exacerbated sympathetic nervous activity and decreased parasympathetic
nervous activity [31,52,53,54]. The results of the current study suggest that for
individuals identified as falling within the preclinical stage of AD (our Aβ+ group),
there is a muted response on two related indices of phasic vagal cardiac control, RSA
and vagal ratio, when presented with a modest cognitive stressor. Both RSA and vagal ratio are directly modulated by both muscarinic and nicotinic cholinergic autonomic neurotransmission, and the earliest stages of AD are marked, in part, by altered function of the basal forebrain cholinergic system, with eventual degenerative changes including neuronal loss [54]. We have recently reported that a down-regulation of central cholinergic neurotransmission appears to be one of the earliest neuropathological changes in preclinical AD [14] and we have also found that individuals with evidence of both decreased central cholinergic tone and amyloid aggregation within the ACC region show evidence of increased resting cardiac workload [8]. The aggregation of Aβ plaques in the neocortex, within this specific region of interest that is part of the central cholinergic system, appears to be directly associated with higher cognitive impairment as well as myocardial oxygen consumption [8]. These results add to a growing body of literature that suggests a direct link between Aβ aggregation, basal forebrain cholinergic damage, and impaired cardiovascular function, even in the preclinical stage of Alzheimer’s disease [1].
References


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### Table 1. Demographic Characteristics

<table>
<thead>
<tr>
<th>Main Outcome</th>
<th>Full sample (n = 63)</th>
<th>Preclinical AD (n = 15)</th>
<th>Healthy Controls (n = 48)</th>
<th>p</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>No. of female</td>
<td>39 (61.9%)</td>
<td>11 (73.3%)</td>
<td>28 (58.3%)</td>
<td>.296</td>
</tr>
<tr>
<td></td>
<td>APOE</td>
<td>29 (46%)</td>
<td>8 (53.3%)</td>
<td>21 (43.8%)</td>
<td>.516</td>
</tr>
<tr>
<td></td>
<td>Number of ε4 carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>Number of participants</td>
<td>10 (15.9%)</td>
<td>2 (13.3%)</td>
<td>8 (16.6%)</td>
<td>.758</td>
</tr>
<tr>
<td><strong>Hypercholesterolemia</strong></td>
<td>Number of participants</td>
<td>16 (25.4%)</td>
<td>4 (26.7%)</td>
<td>12 (25%)</td>
<td>.897</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>No. of years</td>
<td>62.79 (5.35)</td>
<td>63.93 (6.31)</td>
<td>62.44 (5.04)</td>
<td>.349</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td>No. of years</td>
<td>17.21 (2.77)</td>
<td>17.47 (3.46)</td>
<td>17.14 (2.55)</td>
<td>.689</td>
</tr>
<tr>
<td><strong>Florbetapir PET SUVr (ACC region of interest)</strong></td>
<td>Standardized Uptake Value ratio</td>
<td>1.04 (0.19)</td>
<td>1.30 (0.20)</td>
<td>0.95 (0.08)</td>
<td>.000</td>
</tr>
<tr>
<td><strong>GDS</strong></td>
<td>Total Score</td>
<td>1.86 (2.16)</td>
<td>1.60 (1.45)</td>
<td>1.94 (2.35)</td>
<td>.602</td>
</tr>
<tr>
<td><strong>DASS Depression Subscale</strong></td>
<td>Total Depression Subscale Score</td>
<td>3.56 (6.70)</td>
<td>2.60 (2.59)</td>
<td>3.87 (7.56)</td>
<td>.526</td>
</tr>
<tr>
<td><strong>DASS Anxiety Subscale</strong></td>
<td>Total Anxiety Subscale Score</td>
<td>2.73 (4.53)</td>
<td>2.40 (3.58)</td>
<td>2.83 (4.83)</td>
<td>.752</td>
</tr>
<tr>
<td><strong>DASS Stress Subscale</strong></td>
<td>Total Stress Subscale Score</td>
<td>6.73 (6.77)</td>
<td>6.67 (4.55)</td>
<td>6.74 (7.38)</td>
<td>.969</td>
</tr>
<tr>
<td><strong>Body Mass Index</strong></td>
<td>Body Mass Index</td>
<td>26.69 (5.50)</td>
<td>28.66 (7.95)</td>
<td>26.07 (4.41)</td>
<td>.113</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>Total Score</td>
<td>29.05 (1.02)</td>
<td>28.93 (1.16)</td>
<td>29.08 (0.99)</td>
<td>.624</td>
</tr>
<tr>
<td><strong>ISLT Total Recall</strong></td>
<td>Total words recalled</td>
<td>25.59 (4.22)</td>
<td>24.73 (4.46)</td>
<td>25.85 (4.16)</td>
<td>.374</td>
</tr>
</tbody>
</table>

*Note: SUVr = standardized uptake value ratio; ACC = Anterior Cingulate Cortex; GDS = Geriatric Depression Scale; DASS = Depression, Anxiety, and Stress Scale; MMSE = Mini Mental State Examination; ISLT = International Shopping List Test; bolded value is significant at the p<.001 level*
Figures Legends

Figure 1. GMTL composite z-scores for participants with low (left) versus high (right) for Aβ aggregation in the anterior cingulate region of interest (PET amyloid imaging) (p > 0.05).

Figure 2. Plots of vagal ratio in patients testing negative (blue, left) and positive (red, right) for Aβ (ACC) as a function of pre-, during, and post-GMLT testing. Least squares means (large circles) and 95% confidence intervals were adjusted for age. Small circles indicate the raw individual patient values for each phase of testing in order to convey the variability in values between patients. Light gray lines illustrate the individual changes relative to pre-GMLT testing (normalized to pre-GMLT by subtraction) in order to convey the consistency of within-subject changes. Aβ- group showed an increase in vagal ratio during the cognitive task condition, relative to either of the two at-rest conditions (Pre-GMLT: p=0.0061; adj. p=0.0304; Post-GMLT: p<.0001; adj. p=0.0007), with post-GMLT vagal ratio not differing significantly from pre-GMLT levels (p=0.2283; adj. p=0.9185). Aβ+ group did not consistently show any change in vagal ratio at any point during testing (pre- vs. GMLT p=0.1837, GMLT vs. post- p=0.2221, post- vs. pre-GMLT p=0.7883; adj. p =0.9985 for all). Further, the increased vagal ratio during the cognitive stress condition (p=0.0099; adj. p=0.0304) and subsequent decreases (p=0.0013; adj. p=0.0007) for the Aβ- group differed significantly from the Aβ+ group.

Figure 3. Plots of RSA in participants testing negative (blue, left) and positive (red, right) for Aβ (ACC) as a function of pre-, during, and post-GMLT testing. Least squares means (large circles) and 95% confidence intervals were adjusted for age. Small circles indicate the raw individual patient values for each phase of testing in order to convey the variability in values between patients. Light gray lines illustrate the individual changes relative to pre-GMLT testing (normalized to pre-GMLT by subtraction) in order to convey the consistency of within-subject changes. Aβ- subjects showed a significant increase in RSA during the cognitive task condition, relative to either of the two at-rest conditions (Pre-GMLT: p<.0001; adj. p<.0001; Post-GMLT: p=0.2471; adj. p=1.0). As with the vagal ratio measure, those in the Aβ+ group did not consistently show any change in RSA at any point during testing (pre- vs. GMLT p=0.6321, GMLT vs. post- p=0.7719, post- vs. pre-GMLT p=0.8510; adj. p =1.0 for all). Further, the increased RSA scores during the cognitive stress condition (p=0.0021; adj. p=0.0104) were significantly larger for the Aβ- group compared to the Aβ+ group. The subsequent decrease in RSA for the Aβ+ group after the cognitive stress were significantly greater than for the Aβ- group prior to adjustment, but not after multiplicity adjustment (p=0.0304; adj. p=0.0913).
Figures:

Figure 1.
MANUSCRIPT III

“Change in retinal structural anatomy during the preclinical stage of Alzheimer’s disease”

by

Cláudia Y. Santos, MS\textsuperscript{1,2}; Lenworth N. Johnson, MD\textsuperscript{2,3}; Stuart E. Sinoff, MD\textsuperscript{4}; Elena K. Festa, PhD\textsuperscript{5}; William C. Heindel, PhD\textsuperscript{5}; Peter J. Snyder, PhD\textsuperscript{1,2,6}

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1. PhD Candidate at Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA, email: clausantos2910@gmail.com

2. Clinical Researcher at Lifespan Clinical Research Center, Rhode Island Hospital, Providence, RI, USA

3. Professor at Department of Ophthalmology, Rhode Island Hospital & Alpert Medical School of Brown University, Providence, RI, USA

4. Clinician at Department of Ophthalmology, BayCare Medical Group, Clearwater, FL, USA

5. Professor at Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI, USA

6. Professor at Ryan Institute for Neuroscience, University of Rhode Island, Kingston, RI, USA
Structured Abstract

Introduction: We conducted a 27-month longitudinal study of mid-life adults with preclinical Alzheimer’s disease (AD), using Spectral domain Optical Coherence Tomography (SD-OCT) to compare changes in volume and thickness in all retinal neuronal layers to those of age-matched healthy control subjects.

Methods: Fifty-six older adults (mean age = 65.36 years) with multiple risk factors for AD completed SD-OCT retinal imaging and cognitive testing at baseline. Twenty-seven months later they completed the same exams as well as an ¹⁸F-Florbetapir PET imaging study.

Results: Compared to healthy control subjects, those in the preclinical stage of AD showed a significant decrease in macular retinal nerve fibre layer (mRNFL) volume, over a 27-month follow-up interval period, as well as a decrease in outer nuclear layer (ONL) and inner plexiform layer (IPL) volumes and thickness in the inferior quadrant. However, only the mRNFL volume was linearly related to neocortical PET amyloid SUVr after controlling for any main effects of age ($R^2=0.103; \rho = 0.017$). Furthermore, the magnitude of mRNFL volume reduction was significantly correlated with performance on a task of participants’ abilities to efficiently integrate visual and auditory speech information (McGurk effect).

Discussion: We observed a decrease in mRNFL, ONL and IPL volumes, in preclinical AD relative to controls. Moreover, the largely myelinated axonal loss in the RNFL is related to increased neocortical Aβ accumulation after controlling for age. Volume loss in the RNFL, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving. However, this retinal change does appear to be modestly related to relative decrements in performance on a measure of audiovisual integration efficiency that has been recently advanced as a possible early cognitive marker of mild cognitive impairment.

Keywords
Pre-clinical, Alzheimer’s disease, retinal, cognition, RNFL, OCT, Optical Coherence Tomography, McGurk Effect, Amyloid
Research in Context

Systematic Review
We reviewed 15+ years of literature on the relationship between the morphology of retinal neuronal layers and Alzheimer’s disease (AD), and the evidence of disease-related retinal change over the progression of AD. This literature is mixed, with inconsistent findings, and it consists mostly of cross-sectional studies in patients with symptomatic disease. We were unable to identify prior publications that have sought to explore within-subjects longitudinal change in retinal morphology during the preclinical stage of AD for all retinal neuronal layers.

Interpretation
After controlling for expected effects of normal aging, we observed a decrease in the macular region of the RNFL (mRNFL) that is moderately correlated with PET imaging evidence of amyloid aggregation. Volume loss in the mRNFL, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving, but is modestly related to relative decrements in performance on a measure of visual-auditory integration of new information. This may be a first report of neuronal retinal layer volumetric changes in a within-subjects, prospective and longitudinal study of preclinical AD.

Future directions
Larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), should be followed for an even longer time interval in order to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging methods could improve our understanding of the mechanisms of disease-related retinal change, such as OCT Angiography and scanning polarimetry.
Background

The formation of the eye begins in the third week of human embryologic development, with the retina being a crucial component of the ocular globe and the central nervous system (CNS). The retina is derived from pluripotent neuroectodermal cells that migrate from the diencephalic invagination of the neural tube [1], and so it is structurally, physiologically, and functionally brain tissue — with the retina sometimes described as a “protrusion” from the brain. As is the case throughout the neocortex, the retina consists of discrete neuronal cell layers, with multiple types of neurons and neurotransmitter systems, glial cells and microvasculature. Unlike the rest of the CNS, however, the retinal neuronal cell layers can be non-invasively visualized through high-resolution optical methods such as spectral domain optical coherence tomography (SD-OCT) [2].

A recent review [3] cites the wide variety of retinal biomarkers that have been explored in patients with Alzheimer's disease (AD), ranging from retinal anatomical and vascular markers, to curcumin binding studies [4], retinal oxymetry and electroretinogram (ERG) studies. Clinically, it is reasonable to suspect the presence of early ocular involvement in AD because visual system changes such as decreased vision, abnormal pupillary reaction, visual field changes, motion detection abnormalities, impaired color vision, and decreased contrast sensitivity have been identified in mild-to-moderate AD [3,5].

SD-OCT allows for the precise segmentation and measurement of the retinal cell layers, thereby promoting exploration of neuronal changes that might be directly related to specific neurodegenerative diseases. Over the past two decades there have been over 70 peer-reviewed publications exploring retinal
optical coherence tomography (OCT) correlates of Alzheimer’s disease, in humans, non-human primates, and other animal models of AD disease. With respect to humans, most prior reports have consisted of cross-sectional studies comparing groups of AD patients to groups of seemingly “healthy controls”. When compared with healthy age-matched controls, patients with AD have reduced numbers of ganglion cell axons, and are three times more likely to have an increased optic nerve cup-to-disc ratio, a potential consequence of ganglion cell and nerve fiber loss [6]. Such reports have confirmed an earlier report of substantial loss of ganglion cells in AD patients, based on histopathology of autopsy materials [7]. Further, peripapillary retinal nerve fiber layer (pRNFL) thickness has been found to be significantly thinner, suggesting the presence of optic atrophy in patients with mild cognitive impairment (MCI) and mild-to-moderate AD when compared with age-matched controls [8]. Reduction of macular RNFL volume (mRNFL) also has been identified in AD [9]. The loss of RNFL tissue in the retina may constitute an early biomarker of AD. If so, a reduction in RNFL thickness or volume may be observed prior to widespread damage to the mesiotemporal CNS memory system that is characteristic of AD [10,11,12].

With respect to the RNFL we have surveyed all available literature, including those studies relying on other imaging approaches, such as 2D fundus photography and histological analyses, and we have found variable reports of decreased RNFL and/or ganglion cell layer (GCL) thicknesses in AD and MCI. A search on Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) was performed (25 August, 2017) to find all published articles, using the search terms “Alzheimer’s” and “retinal layer”. This search led to 134 papers identified and, of these, only 34
papers consisted of human studies that compare retinal layer morphology changes in Alzheimer’s patients to healthy individuals (see Table 1).

Nearly all of these 34 publications resulted from cross-sectional studies based on comparisons of cases to putatively ‘healthy controls’. Most often the healthy controls had no biomarker confirmation to indicate that they did not fall within the preclinical stage of AD. One study did search for thickness differences in all 10 retinal layers [20], with the remaining 33 studies concentrating on measurement differences for the mRNFL, pRNFL and the GCL. Of note, the GCL was only once reported as a single layer [20], being most often considered in conjunction with an adjacent cell layer, either as the ganglion cell – inner plexiform layer complex (GC-IPL complex) or as the retinal nerve fiber layer – GCL complex (RGCL or GCC).

From the 33 papers seeking to compare group differences for the RNFL, 29 found RNFL thinning in AD compared with age-matched controls (see Table 1). Only seven of these 29 published reports appear to have accounted for participant age as a statistical co-variate in their analyses. This is important because there is normal age-related thinning of the RNFL and other retinal layers [44]. Of these seven publications that did account for effects of aging, one determined that the observed thinning was due primarily to the main effect of aging rather than disease burden [26]; one study found RNFL thinning in MCI but not in AD [30]; two studies reported RNFL thinning solely in one or two specific quadrants [13, 32]; and three studies reported robust disease-burden after accounting for age [15, 17, 18]. Only two of the 33 reviewed studies reported within-subjects longitudinal results (both in symptomatic AD patients vs. controls), and they both reported increased RNFL thinning compared with controls [18, 27].
Alzheimer’s disease-related amyloid-beta (Aβ) plaques can start to accumulate abnormally in the brain up to 20 years before symptom onset, and this stage is classified as pre-clinical AD [45,46]. Only two of the studies reviewed above, both relying solely on cross-sectional data, recruited individuals in the pre-clinical stage of the disease. One from the same larger study that we draw from in the current report [16], found a thicker inner plexiform layer (IPL) in preclinical AD compared with healthy controls. Another study [14] found no difference in the RNFL thickness between AD, preclinical AD and healthy controls. Golzan and colleagues reported a significant difference in the RGCL thickness across the three groups, and yet they found no association between retinal structural measurements and PET Aβ binding in the neocortex. Aside from these two cross-sectional studies of preclinical AD [14, 16], none of the other published reports compared PET imaging evidence of neocortical amyloidosis with the retinal OCT measurements for this earliest stage of the disease.

In this current report, we followed the same cohort of subjects at high-risk for preclinical AD over 27 months to explore changes in all retinal neuronal layers and we relate these findings to PET imaging evidence of cortical amyloid aggregation in the same participants.

In our current study, we compared morphological changes in retina with cognitive performance. Previous studies examining this relationship have relied on the Mini-Mental State Exam (MMSE) as a screening measure of general cognitive
function, and these studies have led to conflicting results. Some studies have found no relationship between RNFL thinning and MMSE in AD [41, 47] and MCI [39], while one study found a correlation between RNFL thickness and MMSE scores in MCI [29]. Another study reported a correlation between macular volume thinning (whole retina) and MMSE scores in patients with AD [9]. For individuals in the pre-clinical stage of disease severity, the MMSE would be a poor choice as a cognitive marker, due to several psychometric limitations for this test [48]. With respect to the MCI stage of disease severity, two prior investigators have reported surprisingly inverse relationships between performance on measures of verbal episodic memory and RNFL thickness [49, 50]. Importantly, both of these studies reported cross-sectional data, and it is unclear as to whether this inverse relationship (that is, relatively enhanced performance on verbal episodic memory tests associated with relatively diminished RNFL thicknesses) would persist if participants were followed longitudinally and as the disease progresses.

Our intent was to explore this very question, that of the relationship between cognitive functioning and evidence of morphologic change in the retina, over the course of several years, and within the very early, pre-clinical stage of the disease. Based on prior literature in this specific patient population, we chose the Groton Maze Learning Test (GMLT) as a measure of learning efficiency, problem solving and working memory [51,52], and the International Shopping List Test (ISLT) to measure verbal episodic memory [53, 54]. Finally, we administered an audiovisual McGurk task, a speech processing paradigm that relies on the integrity of white matter tracts that underlie corticocortical connectivity, as prior work has shown disruption of functional integration for posterior sensory regions in AD [55, 56]. Because alterations in white matter integrity may occur in early stages of the
disease [57, 58, 59], similar early changes in the RNFL - which is comprised mostly of myelinated axons from the cell bodies in the GCL - might be directly related to the loss of white matter tracts in the cortex, and hence correlate with performance on this cognitive task that specifically assesses corticocortical connectivity and has recently been advanced as a potential biomarker of early stage AD pathology [60].

Methods

2.1. Participants

A total of Fifty-six adults aged between age 55 and 75 years (mean age = 65.36 years old) with two well-established risk factors for AD, namely, a self-reported first-degree family history of the disease and self-identification of subjective memory concerns, were recruited using a selection process described previously [61]. All participants underwent a detailed medical screening interview. Exclusion criteria included a diagnosis of MCI or AD following the National Institute on Aging - Alzheimer’s Association (NIA-AA) diagnostic criteria [45, 62], history of neurological or psychiatric disorder, any significant systemic illness or unstable medical condition (e.g., active cardiovascular disease), and current use of any medications known to affect cognition (e.g., use of sedative narcotics). Subjects with histories of cataract surgery, corneal (LASIK) surgery, AMD, or subjects with known ophthalmic pathology were excluded. Inclusion criteria included having an MMSE score of ≥ 27 and performance within normal limits on a battery of cognitive tests described previously (listed in Table 2) [63,64]. Since RNFL thinning is a characteristic of glaucoma, subjects with
glaucoma were excluded. Participants were also excluded if they had a history of optic neuritis, intraocular surgery apart from cataract extraction, or a history of visual loss apart from refractive error as these conditions may affect the RNFL thickness [65, 66, 67]. Additionally, upon examining the OCTs, we excluded any eye OCT that had evidence of epiretinal membrane [68].

All participants live independently, most were engaged in full-time or part time employment, and many were caretakers for a parent with AD. The amount of neocortical beta-amyloid protein aggregation (Aβ) and apolipoprotein E (APOE) genotype were unknown at the time of assessment and were not used to determine enrollment. Although Aβ status and APOE genotyping were conducted as a part of the study protocol, researchers remained blinded to these results throughout testing.

From this larger sample, we identified 15 participants who were categorized as presenting with preclinical stage disease based on evidence of both 1) elevated neocortical beta-amyloid burden as determined by PET amyloid imaging [45,46]; and 2) relative cognitive impairments in response to a challenge with a very low-dose muscarinic anticholinergics [59, 63].

The study was approved by and complied with the regulations of Rhode Island Hospital’s Institutional Review Board, and all participants provided written informed consent in accordance with the Declaration of Helsinki. The study complied with (HIPAA) regulations.

2.2. Aβ PET imaging
To assess neocortical amyloid burden, all participants had an Aβ PET scan at baseline and another at 27-month visit. A 370MBq (10 mCi 1/2 10%) bolus injection of 18F-florbetapir was administered intravenously. Approximately 50 minutes’ post-injection, a 20-minute PET scan was performed with head CT scan for attenuation correction purposes. Images were obtained using a 128X128 matrix and reconstructed using iterative or row action maximization likelihood algorithms. PET standardized uptake value (SUV) data were summed and normalized to the whole cerebellum SUV, resulting in a region-to-cerebellum ratio termed SUV ratio (SUVr).

An SUVr threshold of 1.1 or greater was used to discriminate between Aβ+ and Aβ-. These SUVr calculations were performed using the MIMneuro software, with a normative database of 74 healthy normal individuals (48 males, 26 females), aged between the ages of 18–50 years, who all had negative amyloid scans on visual assessment [69]. For all cases, Aβ positivity was confirmed by consensus over-read by two board-certified radiologists who were also board certified in nuclear medicine.

2.3. SD-OCT imaging

Participants were administered two drops of tropicamide (Mydriacyl 1%) per eye for pupil dilation before OCT imaging. The Heidelberg SPECTRALIS SD-OCT was used to acquire retinal OCT scans of the optic nerve head and the macula for the right and left eyes of all participants at baseline and 27 months. Heidelberg SPECTRALIS has automatic segmentation and quantification of retinal layers, uses the eye-tracking image alignment for repeated measures (TruTrack; Heidelberg Engineering, Inc). Outcome measures for the SD-OCT imaging,
following the Anatomic Positioning System (APS) protocol sequences, included pRNFL, mRNFL, GCL, inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL). For each individual participant, the volume and thickness of all retinal neuronal layers were measured and averaged for both eyes.

The mean macular thickness of each retinal layer was measured at 4 sectors (superior, inferior, nasal, and temporal) leading to a macular volume for each retinal layer (see Fig.1). For the pRNFL, the mean thickness also was calculated in 4 sectors (superior, inferior, nasal, and temporal) centered in the optic nerve and the thickness of all areas were averaged to result in the average thickness.

The volumes for all layers were obtained separately, across the entire macular region extending 3.45 mm from the center of the fovea. Mean volumes (mm$^3$) for each layer and thickness (μm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. The difference between these two exam time points was obtained for each subject and for each layer. Multicolor imaging, which uses individual laser wavelengths [70] to characterize anatomic and pathologic detail at different retinal depths, served to identify and exclude individuals with background diabetic retinopathy and retinal microbleeds within the region of interest.

2.4. Cognitive Assessment
The Groton Maze Learning Test (GMLT; http://www.cogstate.com) is a computer administered hidden maze test, designed by one of the authors (P.J.S.) to measure spatial working memory and problem-solving functions [71]. The GMLT has been described previously both in terms of performance within the context of studies with healthy elderly adults [51, 72,73], as well as in MCI [74].

Additionally, all subjects completed the International Shopping List Test (ISLT; www.cogstate.com) as a measure of verbal episodic memory [75], the MMSE as a measure of general cognitive function, and the Memory Complaint Questionnaire (MAC-Q) [76] as a measure of subjective memory complaints.

As noted above, we also administered an audiovisual McGurk Task that has recently been described as a potential new marker of early-stage AD [60]. The McGurk effect is a compelling misperception in which discrepant visual and auditory speech information presented simultaneously results in the listener hearing a fused audiovisual speech sound, rather than the veridical auditory sound (e.g., /ba/ auditory + /ga/ visual is heard as /da/ rather than /ba/) [77]. The integrity and efficiency of the audiovisual integration process can be measured by comparing accuracy and response times to identify the auditory information under Congruent (matching audio and visual components) and Incongruent (conflicting audio and visual components) conditions. For the purpose of this study, we focused on a measure of integration efficiency, defined as the proportional increase in response time from Congruent to Incongruent conditions for the weaker McGurk stimulus in this task. This stimulus condition was particularly sensitive at discriminating patients diagnosed with amnestic mild cognitive impairment (MCI) from healthy elderly [60]. Higher efficiency scores reflect greater sensitivity to binding strength.
All cognitive measures were performed by trained staff supervised by a licensed clinical neuropsychologist.

2.5. Statistical Methods

For the demographic variables, t-tests were performed for each variable between the preclinical AD and healthy control groups. For the layers RNFL (macular and peripapillary regions), GCL, ONL, outer plexiform layer, inner nuclear layer and IPL paired 1-sided significance level of 0.05 t-tests were performed, and the effects sizes for each comparison were computed with the Cohen’s $d$ statistic. For each layer, the differences between baseline and 27-month measurements, and between Aβ+ and Aβ- subjects for the total volume, and the thickness of the average and the quadrants (N3.45, T3.45, S3.45, I3.45) were computed. Linear regression models were performed with neocortical PET imaging SUVr entered as a response variable, and retinal layer measures as the explanatory variables, and covarying for effects of normal aging. Although age was not significantly different between groups, it is a key predictor of volumetric loss over time in the macula. In order to assess whether a correlation exists between retinal layer thickness and any of the cognitive tests, linear regression analysis (Pearson’s test) was adopted, a $p$ value less than 0.05 was considered significant, and the magnitude of differences was quantified using Cohen’s $d$.

Results

3.1. Sample Demographics

Of the 56 participants enrolled, 35 were female and 11 of them were amyloid positive on PET imaging. There were no significant differences between Aβ+ and
Aβ- groups with respect to sex (p=0.311). Likewise, there were no group differences in the proportion of individuals with the APOE ε4 genetic risk marker for AD. The mean age for the total sample was 65.36 years old and this sample had an average of 17.31 years of education. All relevant demographic information, for both groups, is provided in Table 2. There were no group differences with respect to body mass index, subjective memory complaints, cognitive performance at baseline exam (on any of the tests described above). By definition, both groups significantly differed with respect to neocortical amyloid aggregation as measured via PET imaging (p = 0.000).

With respect to genetic risk for AD, eight of 15 individuals (53%) in the preclinical AD group (Florbetapir PET SUVr scores ≥1.10) had at least one copy of the APOE ε4 allele, whereas 20 of 45 individuals (45%) in the control group (Florbetapir PET SUVr scores < 1.10) had at least one copy of the APOE ε4 allele. Hence, roughly half of the subjects in each group presented with this additional risk factor for AD, but due to small sample sizes we were not able to further evaluate the specific effect of APOE genetic risk on retinal layers measurements. Subject demographic information, for both groups, is provided in Table 2.

3.2. Retinal Layer Measurements

At baseline exam, no group differences were found with respect to either the thicknesses of any macular quadrant for any neuronal layer, nor were there any group differences observed with respect to total volumes for each layer in the same region (Table 3).
Change over the 27-month follow-up period, for each of these measures, was calculated by subtracting the volumes (mm$^3$) and thickness (μm) obtained at the baseline visit from the same measurements obtained at the 27-month visit. As shown in Table 4, a significant group difference was found for the mRNFL (p=0.050), ONL (p=0.026) and IPL volumes (p=0.020). In all cases, the pre-clinical AD group showed a larger reduction in volume over this time period, compared to the healthy control group, and in all cases these group differences were of a moderate effect size. The only significant changes observed in thickness after controlling for effects of age were in the inferior quadrant of the ONL (0.026) and IPL (p=0.028), with a larger reduction in the preclinical group, and in the temporal quadrant of the OPL (0.040), with a larger increase in the preclinical group compared to the control.

Considering each subject group separately, the change from baseline to 27 months in the total volume (mm$^3$) of the mRNFL was significantly decreased for the pre-clinical AD group (-0.032±0.003, p=0.002) as well as for the healthy control group (-0.0190±0.003, p=0.03). With respect to the pRNFL, although we found an overall non-significant difference in the magnitude of the thinning between the two groups, over the 27-month period, we observed substantially greater ranges of variance of measurements for both groups, as reflected by markedly larger standard deviations (SD) of measurement (Table 3). This region of the pRNFL is thought to have greater within-subject and between-subject variability because it contains a multitude of larger diameter blood vessels, particularly with respect to vascular innervation within the regions of the superior and inferior arcuate bundles, individual differences in optic canal sizes, the
presence or absence of space-occupying nerve head drusen, and other individual differences in this region [78].

3.3. **Retinal Layer Change in Relation to Neocortical Amyloid Aggregation**

A multivariate linear regression model, controlling for participant age, with total neocortical PET amyloid ligand binding (SUVr) entered as the dependent measure was conducted. Macular RNFL (mRNFL) volume change, over the 27-month study interval, accounted for 10% of the variance in PET amyloid neocortical binding at the end of the study (Adj $R^2 = 0.106$, $\rho = 0.017$; Fig 2). By comparison, change in the GCL over the same time was related to participant age ($\rho=0.05$), but not significantly related to PET amyloid SUVr ($\rho=0.78$). For all neuronal cell layers, other than the mRNFL, the observed change over time was not related to Neocortical PET Amyloid SUVr.

3.4. **Retinal Layer Change in Relation to Cognitive Performance**

There were no significant relationships found between volume reductions for any of the retinal layers over 27 months and performance on measures of spatial working memory and learning efficiency (GMLT) or on a measure of word-list learning and episodic verbal memory (ISLT) ($p>0.005$).

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Insert Table 4 About Here

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However, an interesting correlation was found between the magnitude of mRNFL volume reductions and increasing difficulties on the McGurk task of efficiency for audiovisual integration. That is, individuals with greater volume
reduction in mRNFL showed reduced sensitivity to the binding strength of the audiovisual stimulus ($\rho = 0.037$, 1-tailed; Fig 3).

**Discussion**

The average RNFL thicknesses for both groups in our study are similar to the control groups reported by others [5, 39] as well as to the measurements obtained by Golzan and colleagues in their preclinical AD [14]. Likewise, our OCT measurements of the GCL and IPL layers are quite similar to those reported by others [22].

Of the prior studies we reviewed and described above, Garcia-Martin and colleagues reported retinal layer thickness values that differed substantially from both our measurements as well as from most other published reports [20].

Most published research on this topic have relied on cross-sectional study designs, with measurements of the thickness and volume of various retinal layers at a single point in time. Such studies are likely to be limited in their ability to identify retinal markers of the preclinical stage of AD, as such individuals are still relatively young and such between-groups comparisons have not generally accounted for a variety of confounding variables including, but not limited to, effects of sex differences, ethnicity, axial length, optic disc area, and refractive status on OCT measurements [44, 79, 80, 81].
Hence, we performed this within-subjects longitudinal study to measure individuals’ structural parameters that can be reliably re-measured at different time points using the *Eye Tracker* software for SPECTRALIS SD-OCT [82]; we then calculated the difference after 27 months of all measures collected (Table 4) for the preclinical AD and the healthy control group. To our knowledge, this is the first attempt to both explore within-subject change in retinal neuronal layer structures, in the preclinical stage of AD, and to relate any such observed changes to performance on a cognitive assay that has been shown to be sensitive to detection of early-stage disease burden. Our findings suggest that a decrease in mRNFL volume is the earliest detectable structural retinal change associated with AD. Moreover, this change in mRNFL volume is related with neocortical Aβ accumulation in very early AD (Fig. 1), even after correcting for expected age-related decline [83] (Table 5). By comparison, the decrease in GCL volume observed over the same time period was principally related to the effects of aging (p=0.05) rather than due to cortical beta-amyloid aggregation (p=0.78), at least during this earliest detectable stage of disease progression.

The macular region of the retina is physiologically very active in healthy normal eyes [7], and this “hyperexcitation” might be diminishing in the preclinical stage of AD. In support of this hypothesis, postmortem histological studies have found prominent pathological alteration of retinal ganglion cells (RGCs) in the macular region in AD patients [26, 84] as well as a preferential loss of larger axons, suggesting early involvement of the magnocellular RGCs in AD as they contribute large caliber fibers to the optic nerve [85]. The RNFL is
adjacent to the GCL and it is composed largely of ganglion cell axons that are
organized in superior and inferior arcuate bundles, and the papillomacular bundle
(PMB), that lead to the optic nerve. Early in multiple sclerosis there is
demyelination without axonal loss. Likewise, the mRNFL may be thinned due to
demyelination, and possibly only when there is axonal loss will we see loss of
ganglion cells (GCL). Our results suggest early demyelination in the RNFL in the
preclinical stage of AD, and our data confirm at least one other report of mRNFL
changes occurring prior to pRNFL changes in AD [86]. Moreover, there is some
suggestion that this might be most readily observed in the superior quadrant of the
mRNFL [86] and this would make sense in light of at least one clinical report of
preferential inferior visual field loss in AD [87]. However, in a meta-analysis of
17 studies comparing AD patients with healthy controls and five studies
comparing individuals with mild cognitive impairment (MCI) with controls, there
were significant decreases in all four quadrants compared to controls, thus
suggesting that the degenerative process affects the entire macular region [8].

There have now been ample work showing that there is GCL thinning
observed in the symptomatic stage of AD [5,15, 22]. Moreover, Martin et al. [20]
studied 150 patients with AD and 75 age-matched controls to model how changes
in RNFL and other neuronal cell layers’ thicknesses are associated with disease
duration and severity. This work suggests that there is axonal degeneration in the
RNFL early in the disease followed by degenerative changes to the cell bodies in
the GCL and then progression to deeper neuronal layers [20]. This progressive
pattern of mRNFL loss prior to GCL loss is further confirmed by our results
reported above. Additionally, we found that only loss of mRNFL tissue, and not
loss in any other neuronal layer, was correlated with beta-amyloid protein
aggregation in the cerebral cortex. These results fit nicely with data from a large population-based epidemiological study (Rotterdam Study, 2007-2012) showing that having a thinner RNFL at a baseline exam was significantly associated with increased risk of later developing dementia [88].

4.1. Relationship of RNFL Thinning to Cognitive Function in Preclinical AD

Contrary to a few prior reports [49, 50, 89], we did not observe any correlation between any retinal layer changes and change in either episodic verbal memory (ISLT) or for performance on a working memory and reasoning task for visuospatial information (GMLT). However, because white matter degeneration is readily observable in the early stages of AD [90], and the cerebral white matter is principally composed of myelinated axons and glial cells – as is the retinal RNFL – we chose to administer a cognitive task that is putatively sensitive to functional disruption of corticocortical connections caused by disruption of white matter integrity [60]. Both prodromal and preclinical AD patients with high beta-amyloid burden have been previously found to display changes in audiovisual integration efficiency that are likely due to AD-related disruptions in functional connectivity between posterior sensory regions. Using McGurk-like audiovisual speech stimuli (i.e., video clips of an individual mouthing a speech sound while the audio channel provides either consistent or inconsistent auditory speech information), we found that greater volume reduction in mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus (Fig. 2). While healthy individuals typically show faster response times to consistent stimuli than inconsistent stimuli (i.e., response times are faster when the auditory and visual components provide the same compared to conflicting speech information), we
found that this response time difference decreased with greater RNFL volume reduction. In fact, greater volume reduction in mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus, suggesting that mRNFL volume reduction is related to white matter loss. Consistent with previous findings [60], this reduced sensitivity may reflect subtle disruptions of corticocortical projections within unimodal and heteromodal sensory cortices that indicate individual risk of AD.

4.2. Change in Retinal Neuronal Layers, Other than the RNFL, in Preclinical AD

As noted above, we observed significant changes in ONL (p=0.026) and IPL volumes (p=.002), and inferior quadrant thicknesses for these same layers, respectively (p=.026; p=0.028), in the preclinical AD group compared to the controls, over 27 months. However, neither of these changes were related to PET imaging severity of neocortical amyloidosis at the end of the 27-month interval (Table 5). We have previously reported, with the same cohort of subjects at their baseline examinations, an increase in the IPL volume in preclinical AD that may be related to either Aβ deposition and/or an inflammatory process [16]. Twenty-seven months later we have now found evidence of tissue loss in the IPL, within our preclinical group relative to controls, suggesting that although there may be an initial early stage of IPL volume increase due to an inflammatory process, there is nonetheless some volume loss in this structure over continued disease progression.

Finally, the ONL consists of photoreceptor cell bodies and thinning of this layer has previously been shown in patients with age-related macular degeneration in association with tears in the retinal pigment epithelium [91]. In the context of
early AD, we believe that our observation of tissue loss in the ONL could suggest retrograde transsynaptic degeneration, but future analysis would be necessary.

**Conclusion and Future Directions**

To our knowledge this is a first report of a within-subjects, prospective, longitudinal study of retinal anatomic changes in the preclinical stage of AD. *We have found that thinning of the mRNFL may be the earliest anatomic marker of retinal neuronal loss in the preclinical stage of AD, and such loss appears to account for 10% of the variance in observed PET imaging measurement of neocortical amyloidosis.* Whereas most reports have relied on clinical examinations for determining disease burden, our study used PET imaging biomarker of disease burden. Our study is limited by a small sample size, and so these findings require replication in a larger patient population. Secondly, we chose to recruit participants who were at high risk for the very early stages of the disease, that is, during a prodromal stage that is defined by a relative absence of readily identifiable cognitive and/or functional impairments, as well as by biomarker evidence of disease burden that is subtle and for which clear diagnostic criteria have not yet been established [46]. Third, retinal imaging is not only being studied to identify novel biomarkers for this neurodegenerative disease, but changes in the GCL and RNFL thickness have also been reported in Parkinson’s disease and multiple sclerosis [92, 93, 94, 95].

Future work on this topic will require the recruitment of larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), and ideally such a large cohort should be followed for an even longer...
time interval in order to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging modalities should be included in such a study (e.g., OCT Angiography), as retinal blood flow appears to be reduced in MCI and AD [19,40], and the disease has been associated with a retinal venular stenosis, reduced complexity of the branching pattern and geometry, and reduced tortuous venules [96]. We also would recommend inclusion of scanning laser polarimetry (SLP) methods to measure RNFL retardance in preclinical AD because, in glaucoma, RNFL retardance seems to occur before actual RNFL thinning [97]. The mechanisms of glaucomatous injury appear to be related to damage the integrity of axonal cytoskeletal ultrastructure prior to the point of RNFL thinning detectable by OCT [97,98,99]. The axonal cytoskeleton disruption that causes RNFL retardance [97] may possibly be similar to the effects of Aβ plaques and tau tangles in the AD brain.

These results point to the first retinal anatomic changes occurring in the early stages of AD. Characterizing retinal changes at this stage with a non-invasive method will facilitate the search for treatments that can delay or stop the progression of AD. This study also demonstrates that, although many different approaches have already been taken in this field, there is still much more to explore and much that we do not understand with respect to the effects of Alzheimer’s disease on the retina.
Acknowledgements/Conflicts/Funding Sources

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None of the authors have any conflicts of interest to disclose. This article was first-authored by CYS in partial fulfillment of her Ph.D. dissertation project
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Table 1. Review of Papers published until 25 July 2017, using the search terms “Alzheimer’s” and “retinal layer”.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Layers</th>
<th>Results</th>
<th>Cross sectional</th>
<th>Subjects</th>
<th>Age matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunha et al., 2017 [13]</td>
<td>pRNFL and retinal</td>
<td>thinner pRNFL, and superior pericentral and peripheral retinal sectors</td>
<td>Yes</td>
<td>AD</td>
<td>Yes, age as covariate</td>
</tr>
<tr>
<td>Golzan et al., 2017 [14]</td>
<td>RNFL and GC-IPL</td>
<td>RGCL thinner, no difference in RNFL</td>
<td>Yes</td>
<td>preclinical AD</td>
<td>Yes, age as covariate</td>
</tr>
<tr>
<td>Ferrari et al., 2017 [15]</td>
<td>pRNFL and GC-IPL</td>
<td>thinning</td>
<td>Yes</td>
<td>MCI and AD</td>
<td>Yes, age as covariate</td>
</tr>
<tr>
<td>Snyder et al., 2016 [16]</td>
<td>IPL</td>
<td>thicker in preclinical</td>
<td>Yes</td>
<td>preclinical AD</td>
<td>Yes</td>
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<tr>
<td>Choi et al., 2016 [17]</td>
<td>pRNFL and GC-IPL</td>
<td>thinner in the temporal quadrant</td>
<td>Yes</td>
<td>MCI and AD</td>
<td>Yes, age as covariate</td>
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<tr>
<td>Trebbastoni et al., 2016 [18]</td>
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<td>thinner</td>
<td>No, 1 year</td>
<td>AD</td>
<td>Yes, age as covariate</td>
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<tr>
<td>Feke et al., 2015 [19]</td>
<td>pRNFL</td>
<td>no difference</td>
<td>Yes</td>
<td>MCI and AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Cunha et al., 2016 [5]</td>
<td>Macular and GCL+ (GC-IPL)</td>
<td>thinner</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Garcia-Martin et al., 2016 [20]</td>
<td>pRNFL, GCL, INL, IPL, ONL, OPL</td>
<td>RNFL, GCL and IPL thinner</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Pillai et al., 2016 [21]</td>
<td>pRNFL, macular and GC-IPL</td>
<td>no difference</td>
<td>Yes</td>
<td>MCI and AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Eraslan et al., 2015 [22]</td>
<td>pRNFL and GCC (RGCL)</td>
<td>thinner</td>
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<td>Yes</td>
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<td>Güneş et al., 2015 [23]</td>
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<td>thinner</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
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<tr>
<td>Cesareo et al., 2015 [24]</td>
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<td>thinner</td>
<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>Study</td>
<td>Outcome Measure</td>
<td>Findings</td>
<td>Comparison</td>
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<td></td>
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<td>-----------------------------</td>
<td>----------------------------------</td>
<td>-----------------------------------------------</td>
<td>------------</td>
<td></td>
<td></td>
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<tr>
<td>Salobrar-Garcia et al., 2015</td>
<td>pRNFL and macular thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Morgia et al., 2016</td>
<td>pRNFL age-related thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shi et al., 2016</td>
<td>pRNFL thinner in the inferior quadrant</td>
<td>No, 27 months</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liu et al., 2015</td>
<td>pRNFL thinner in the superior and superior quadrant</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oktem et al., 2015</td>
<td>pRNFL thinner, but no differences between MCI and AD</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gao et al., 2015</td>
<td>pRNFL and macula lutea thinner specially in MCI</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheung et al., 2015</td>
<td>pRNFL and GC-IPL thinner superior quadrant, MCI had thinner GC-IPL</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kromer et al., 2014</td>
<td>pRNFL thinner in nasal superior</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bambo et al., 2014</td>
<td>pRNFL thinner in the inferior and inferiortemporal</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marziani et al., 2013</td>
<td>pRNFL +GCL thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kirbas et al., 2013</td>
<td>pRNFL thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moschos et al., 2012</td>
<td>pRNFL and macular thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kesler et al., 2011</td>
<td>pRNFL thinner</td>
<td>Yes</td>
<td>MCI AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lu et al., 2010</td>
<td>pRNFL thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi et al., 2010</td>
<td>pRNFL thinner</td>
<td>Yes</td>
<td>AD Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Region/Morphology</td>
<td>Findings</td>
<td>MCI Yes</td>
<td>AD Yes</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------</td>
<td>-----------------------------------</td>
<td>---------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Paquet et al., 2007 [39]</td>
<td>pRNFL</td>
<td>Thinner, but no differences in MCI and AD</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Berisha et al., 2007 [40]</td>
<td>pRNFL</td>
<td>Thinner in the superior quadrant</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Iseri et al., 2006 [9]</td>
<td>pRNFL and macula</td>
<td>Thinner</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Parisi et al., 2001 [41]</td>
<td>pRNFL</td>
<td>Thinner</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Kergoat et al., 2001 [42]</td>
<td>pRNFL</td>
<td>No differences</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
<tr>
<td>Hedges et al., 1996 [43]</td>
<td>pRNFL</td>
<td>Thinner in the superior quadrants</td>
<td>Yes</td>
<td>AD</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer’s disease; GCL, ganglion cell layer; GC-IPL, ganglion cell–inner plexiform layer complex; INL, inner nuclear layer; IPL, inner plexiform layer; MCI, mild cognitive impairment; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer; RGCL (or GCC), retinal nerve fiber layer–GCL complex; RNFL, retinal nerve fiber layer.
<table>
<thead>
<tr>
<th>Main Outcome</th>
<th>Full sample (n = 56)</th>
<th>Aβ+ (n=15)</th>
<th>Aβ- (n=41)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(%)</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of female</td>
<td>35 (62.5)</td>
<td>11 (73.3)</td>
<td>24 (58.5)</td>
<td>.311</td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Ɛ4 carriers</td>
<td>27 (4.2)</td>
<td>8 (53.3)</td>
<td>19 (46.3)</td>
<td>.643</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of years</td>
<td>65.36 (5.55)</td>
<td>68.25 (5.81)</td>
<td>64.56 (5.26)</td>
<td>.06</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of years</td>
<td>17.31 (2.77)</td>
<td>17.75 (3.91)</td>
<td>17.19 (2.42)</td>
<td>.54</td>
</tr>
<tr>
<td>Florbetapir PET SUVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized Uptake Value ratio</td>
<td>1.02 (0.2)</td>
<td>1.32 (0.18)</td>
<td>0.94 (0.09)</td>
<td>.000</td>
</tr>
<tr>
<td>GDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Score</td>
<td>1.4 (1.87)</td>
<td>0.91 (0.94)</td>
<td>1.52 (2.02)</td>
<td>.34</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>26.69</td>
<td>26.58 (4.36)</td>
<td>26.86 (6.05)</td>
<td>.879</td>
</tr>
<tr>
<td>MAC-Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Score</td>
<td>21.90 (3.08)</td>
<td>21.97 (2.81)</td>
<td>21.90 (3.08)</td>
<td>.527</td>
</tr>
<tr>
<td>MMSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Score</td>
<td>29.25 (1.29)</td>
<td>28.72 (1.85)</td>
<td>29.38 (1.10)</td>
<td>.132</td>
</tr>
<tr>
<td>ISLT Total Recall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total words recalled</td>
<td>26.07 (3.79)</td>
<td>26.00 (3.52)</td>
<td>26.09 (3.90)</td>
<td>.944</td>
</tr>
<tr>
<td>GMTL Total Errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of errors</td>
<td>8.40 (5.31)</td>
<td>8.27 (3.53)</td>
<td>8.44 (5.71)</td>
<td>.92</td>
</tr>
</tbody>
</table>

Table 2: Demographic Characteristics
Abbreviations: APOE, apolipoprotein E; GDS, Geriatric Depression Scale; GMLT, GrotonMaze Learning Test; ISLT, International Shopping List Test; MAC-Q, Memory Complaint Questionnaire; MMSE, Mini–Mental State Examination; PET, positron emission tomography; SUVr, standardized uptake value ratio. Bold values denote significant group differences.
<table>
<thead>
<tr>
<th>Retinal Measures</th>
<th>Preclinical (n=15)</th>
<th>Control (n=41)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mRNFL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm$^3$)</td>
<td>0.227±0.021</td>
<td>0.231±0.025</td>
<td>0.283</td>
</tr>
<tr>
<td>average thickness</td>
<td>28.58±3.08</td>
<td>28.92±3.57</td>
<td>0.383</td>
</tr>
<tr>
<td>Inferior</td>
<td>33.04±3.53</td>
<td>32.69±4.39</td>
<td>0.599</td>
</tr>
<tr>
<td>Nasal</td>
<td>28.87±3.87</td>
<td>29.12±4.70</td>
<td>0.433</td>
</tr>
<tr>
<td>Superior</td>
<td>32.12±4.42</td>
<td>32.74±5.10</td>
<td>0.353</td>
</tr>
<tr>
<td>Temporal</td>
<td>20.29±1.20</td>
<td>21.12±2.76</td>
<td>0.157</td>
</tr>
<tr>
<td><strong>pRNFL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>average thickness</td>
<td>103.36±5.36</td>
<td>101.48±9.76</td>
<td>0.726</td>
</tr>
<tr>
<td>Inferior</td>
<td>134.25±8.15</td>
<td>129.05±13.49</td>
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</tr>
<tr>
<td>Nasal</td>
<td>83.04±17.36</td>
<td>85.3±19.88</td>
<td>0.368</td>
</tr>
<tr>
<td>Superior</td>
<td>127.45±10.32</td>
<td>124.17±13.72</td>
<td>0.765</td>
</tr>
<tr>
<td>Temporal</td>
<td>67.86±11.07</td>
<td>67.71±11.68</td>
<td>0.612</td>
</tr>
<tr>
<td><strong>GCL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm$^3$)</td>
<td>0.444±0.018</td>
<td>0.428±0.029</td>
<td>0.942</td>
</tr>
<tr>
<td>average thickness</td>
<td>51.15±2.20</td>
<td>48.94±3.61</td>
<td>0.964</td>
</tr>
<tr>
<td>Nasal</td>
<td>53.60±2.03</td>
<td>51.23±3.70</td>
<td>0.970</td>
</tr>
<tr>
<td>Temporal</td>
<td>50.90±3.77</td>
<td>49.01±4.41</td>
<td>0.889</td>
</tr>
<tr>
<td>Superior</td>
<td>48.75±4.01</td>
<td>48.08±3.88</td>
<td>0.683</td>
</tr>
<tr>
<td>Inferior</td>
<td>51.35±1.94</td>
<td>47.42±4.18</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>IPL</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm$^3$)</td>
<td>0.353±0.030</td>
<td>0.357±0.027</td>
<td>0.339</td>
</tr>
<tr>
<td>average thickness</td>
<td>39.40±3.67</td>
<td>39.18±3.27</td>
<td>0.578</td>
</tr>
<tr>
<td>Inferior</td>
<td>39.00±4.92</td>
<td>37.54±3.78</td>
<td>0.862</td>
</tr>
<tr>
<td>Nasal</td>
<td>40.37±3.37</td>
<td>40.66±3.66</td>
<td>0.403</td>
</tr>
<tr>
<td>Superior</td>
<td>37.50±3.63</td>
<td>37.39±3.68</td>
<td>0.534</td>
</tr>
<tr>
<td>Temporal</td>
<td>40.71±4.73</td>
<td>41.10±3.28</td>
<td>0.369</td>
</tr>
<tr>
<td><strong>OPL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm$^3$)</td>
<td>0.294±0.373</td>
<td>0.301±0.037</td>
<td>0.031</td>
</tr>
<tr>
<td>average thickness</td>
<td>31.32±3.40</td>
<td>32.10±3.89</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>Inferior</td>
<td>Nasal</td>
<td>Superior</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>mRNFL</td>
<td>30.91±3.38</td>
<td>33.87±6.19</td>
<td>30.16±3.36</td>
</tr>
<tr>
<td></td>
<td>31.51±4.68</td>
<td>35.33±7.42</td>
<td>30.65±3.42</td>
</tr>
<tr>
<td></td>
<td>0.410</td>
<td>0.620</td>
<td>0.442</td>
</tr>
<tr>
<td>INL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm³)</td>
<td>0.338±0.020</td>
<td>0.348±0.028</td>
<td>0.338±0.020</td>
</tr>
<tr>
<td>average thickness</td>
<td>38.08±1.39</td>
<td>38.54±3.13</td>
<td>38.08±1.23</td>
</tr>
<tr>
<td>Inferior</td>
<td>37.54±1.23</td>
<td>38.45±3.04</td>
<td>37.70±1.55</td>
</tr>
<tr>
<td>Nasal</td>
<td>39.70±1.55</td>
<td>39.67±4.66</td>
<td>38.08±1.81</td>
</tr>
<tr>
<td>Superior</td>
<td>38.08±1.81</td>
<td>38.70±3.18</td>
<td>37.00±2.83</td>
</tr>
<tr>
<td>Temporal</td>
<td>37.00±2.83</td>
<td>37.34±3.37</td>
<td>37.00±2.83</td>
</tr>
<tr>
<td></td>
<td>0.645</td>
<td>0.645</td>
<td>0.372</td>
</tr>
<tr>
<td>ONL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total volume (mm³)</td>
<td>0.679±0.066</td>
<td>0.671±0.067</td>
<td>0.679±0.066</td>
</tr>
<tr>
<td>average thickness</td>
<td>67.09±7.02</td>
<td>66.48±7.04</td>
<td>64.16±7.38</td>
</tr>
<tr>
<td>Inferior</td>
<td>67.92±8.66</td>
<td>65.18±8.97</td>
<td>68.08±7.14</td>
</tr>
<tr>
<td>Nasal</td>
<td>67.92±8.66</td>
<td>64.51±8.97</td>
<td>68.08±7.14</td>
</tr>
<tr>
<td>Superior</td>
<td>68.20±7.17</td>
<td>68.34±6.63</td>
<td>68.34±6.63</td>
</tr>
<tr>
<td>Temporal</td>
<td>68.20±7.17</td>
<td>69.09±7.68</td>
<td>68.34±6.63</td>
</tr>
<tr>
<td></td>
<td>0.644</td>
<td>0.644</td>
<td>0.453</td>
</tr>
</tbody>
</table>

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

NOTE. All measurements, for each layer, are acquired from all radial quadrants from the ETDRS circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD. All values are in micrometer (mm), unless otherwise indicated.
Table 4. Change over 27 Months by Groups

<table>
<thead>
<tr>
<th>Location</th>
<th>Pre-Clinical AD (N=15)</th>
<th>Healthy Controls (N=41)</th>
<th>p-value</th>
<th>Effect Size (Cohen’s d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNFL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>-0.032±0.003</td>
<td>-0.019±0.003</td>
<td>0.050</td>
<td>0.550</td>
</tr>
<tr>
<td>Average</td>
<td>-4.45±3.68</td>
<td>-3.26±3.94</td>
<td>0.448</td>
<td>0.3072</td>
</tr>
<tr>
<td>Inferior</td>
<td>-4.45±3.314</td>
<td>-3.09±4.323</td>
<td>0.656</td>
<td>0.3352</td>
</tr>
<tr>
<td>Superior</td>
<td>-5.10±3.671</td>
<td>-4.50±4.845</td>
<td>0.348</td>
<td>0.1318</td>
</tr>
<tr>
<td>Nasal</td>
<td>-4.50±3.801</td>
<td>-2.41±4.416</td>
<td>0.196</td>
<td>0.4917</td>
</tr>
<tr>
<td>Temporal</td>
<td>-3.75±3.936</td>
<td>-3.04±2.212</td>
<td>0.77</td>
<td>0.2528</td>
</tr>
<tr>
<td>pRNFL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>-3.389±2.058</td>
<td>-1.181±4.057</td>
<td>0.060</td>
<td>0.605</td>
</tr>
<tr>
<td>Inferior</td>
<td>-1.39±6.22</td>
<td>1.63±9.10</td>
<td>0.386</td>
<td>0.357</td>
</tr>
<tr>
<td>Superior</td>
<td>-3.33±11.92</td>
<td>-1.41±14.10</td>
<td>0.631</td>
<td>0.141</td>
</tr>
<tr>
<td>Nasal</td>
<td>1.67±5.97</td>
<td>3.81±7.79</td>
<td>0.381</td>
<td>0.290</td>
</tr>
<tr>
<td>Temporal</td>
<td>-1.04±4.13</td>
<td>0.18±4.51</td>
<td>0.400</td>
<td>0.276</td>
</tr>
<tr>
<td>GCL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>-0.016±0.016</td>
<td>-0.011±0.019</td>
<td>0.206</td>
<td>0.29</td>
</tr>
<tr>
<td>Average</td>
<td>-1.96±2.59</td>
<td>-1.34±3.03</td>
<td>0.590</td>
<td>0.209</td>
</tr>
<tr>
<td>Inferior</td>
<td>-2.4±2.97</td>
<td>-0.86±3.38</td>
<td>0.091</td>
<td>0.464</td>
</tr>
<tr>
<td>Superior</td>
<td>-1.0±3.51</td>
<td>-1.43±4.18</td>
<td>0.614</td>
<td>0.107</td>
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<tr>
<td>Nasal</td>
<td>-0.65±3.54</td>
<td>-0.05±3.54</td>
<td>0.321</td>
<td>0.166</td>
</tr>
<tr>
<td>Temporal</td>
<td>-3.8±2.87</td>
<td>-3.02±3.83</td>
<td>0.590</td>
<td>0.210</td>
</tr>
<tr>
<td>OPL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>0.017±0.04</td>
<td>-0.003±0.032</td>
<td>0.965</td>
<td>0.603</td>
</tr>
<tr>
<td>Average</td>
<td>0.843±3.05</td>
<td>-0.605±2.89</td>
<td>0.932</td>
<td>0.495</td>
</tr>
<tr>
<td>Inferior</td>
<td>0.75±3.51</td>
<td>-0.84±2.59</td>
<td>0.956</td>
<td>0.566</td>
</tr>
<tr>
<td>Superior</td>
<td>0.5±3.37</td>
<td>-0.23±3.57</td>
<td>0.262</td>
<td>0.208</td>
</tr>
<tr>
<td>Nasal</td>
<td>-0.83±4.88</td>
<td>-1.77±6.85</td>
<td>0.329</td>
<td>0.144</td>
</tr>
<tr>
<td>Temporal</td>
<td>2.95±4.31</td>
<td>0.43±4.53</td>
<td>0.040</td>
<td>0.562</td>
</tr>
<tr>
<td>ONL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>-0.029±0.030</td>
<td>-0.007±0.035</td>
<td>0.026</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>Inferior</td>
<td>Superior</td>
<td>Nasal</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>-1.61±2.86</td>
<td>-2.25±5.66</td>
<td>-1.70±2.60</td>
<td>0.87±3.58</td>
</tr>
<tr>
<td></td>
<td>-0.068±3.39</td>
<td>0.84±4.54</td>
<td>-1.43±3.73</td>
<td>2.92±7.27</td>
</tr>
<tr>
<td></td>
<td>0.077</td>
<td>0.026</td>
<td>0.405</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>0.469</td>
<td>0.644</td>
<td>0.078</td>
<td>0.305</td>
</tr>
</tbody>
</table>

**IP**

<table>
<thead>
<tr>
<th>Volume (mm³)</th>
<th>-0.014±0.015</th>
<th>-0.006±0.011</th>
<th>0.020</th>
<th>0.686</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>-1.80±1.91</td>
<td>-2.41±3.32</td>
<td>-1.06±1.66</td>
<td>0.099</td>
</tr>
<tr>
<td>Inferior</td>
<td>-0.84±2.17</td>
<td>-1.06±2.49</td>
<td>0.133</td>
<td>0.368</td>
</tr>
<tr>
<td>Superior</td>
<td>-0.95±2.23</td>
<td>-1.91±1.50</td>
<td>-1.07±2.011</td>
<td>0.567</td>
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<tr>
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<td>-0.95±2.23</td>
<td>-1.29±2.57</td>
<td>0.22</td>
</tr>
<tr>
<td>Temporal</td>
<td>-1.91±2.11</td>
<td>-1.91±2.11</td>
<td>-1.29±2.57</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**INL**

<table>
<thead>
<tr>
<th>Volume (mm³)</th>
<th>0.004±0.019</th>
<th>0.001±0.022</th>
<th>0.64</th>
<th>0.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>2.04±2.14</td>
<td>2.30±5.50</td>
<td>0.43</td>
<td>0.276</td>
</tr>
<tr>
<td>Inferior</td>
<td>2.04±2.32</td>
<td>0.19±2.67</td>
<td>0.98</td>
<td>0.708</td>
</tr>
<tr>
<td>Superior</td>
<td>0.33±1.91</td>
<td>0.011±2.38</td>
<td>0.665</td>
<td>0.140</td>
</tr>
<tr>
<td>Nasal</td>
<td>0±1.97</td>
<td>0.47±3.94</td>
<td>0.34</td>
<td>0.131</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.70±2.75</td>
<td>0.39±2.25</td>
<td>0.655</td>
<td>0.131</td>
</tr>
</tbody>
</table>

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

**NOTE.** All measurements, for each layer, are acquired from all radial quadrants from the (ETDRS) circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD, unless otherwise indicated. All values are in micrometer (mm), unless otherwise indicated.
Table 5. Multivariate linear regression model of volume (mm$^3$), for different locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Coef. Neocortex</th>
<th>Neocortex SUVr</th>
<th>Coef. Age</th>
<th>Age P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUVr P-Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNFL</td>
<td>-0.228</td>
<td>0.041</td>
<td>0.00009</td>
<td>0.024</td>
</tr>
<tr>
<td>GCL</td>
<td>-0.003</td>
<td>0.78</td>
<td>-0.0008</td>
<td>0.05</td>
</tr>
<tr>
<td>OPL</td>
<td>2.05</td>
<td>0.84</td>
<td>-0.034</td>
<td>0.691</td>
</tr>
<tr>
<td>ONL</td>
<td>-0.0267</td>
<td>0.423</td>
<td>0.00026</td>
<td>0.834</td>
</tr>
<tr>
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<td>-0.014</td>
<td>0.144</td>
<td>-0.0034</td>
<td>0.196</td>
</tr>
<tr>
<td>INL</td>
<td>0.026</td>
<td>0.08</td>
<td>-0.005</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; PET, positron emission tomography; pRNFL, peripapillary retinal nerve fiber layer; SUVr, standardized uptake value ratio.

NOTE. Values are in cubic millimeter (mm$^3$) for the coefficients. Controlling for participants’ age, with total neocortical PETamyloid ligand binding (SUVr) entered as the dependent measure.
Fig. 1. Representative cross-sectional OCT image through macular region, with the fovea in the center (green line). Labels are shown for the RNFL, GCL, and IPL. The mean macular thickness of each retinal layer was measured within four sectors (superior, inferior, nasal, and temporal), extending 3.45 mm from the center of the fovea, leading to a macular volume measurement for each retinal layer. Mean volumes (mm3) for each layer and thickness (mm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; OCT, optical coherence tomography; RNFL, retinal nerve fiber layer.

Linear Fit: Adj $R^2 = 0.106$, $\rho < 0.017$

Fig. 2. Relationship between macular RNFL volume change over 27 months and total neurocortical amyloid aggregation (18F-florbetapir PET SUVr) at end of study. Abbreviations: PET, positron emission tomography; RNFL, retinal nerve fiber layer; SUVr, standard uptake value ratio.
Linear Fit: Adj $R^2 = 0.098$, $p < 0.037$ (1-tailed)

Fig. 3. Relationship between macular RNFL volume change over 27 months and performance on a measure of audiovisual integration efficiency (“McGurk Task”[60]). Abbreviation: RNFL, retinal nerve fiber layer.
MANUSCRIPT IV

“Decreased density and complexity of the retinal vasculature in the preclinical stage of Alzheimer’s disease”

by

Cláudia Y. Santos, MS1, 2; Kimberly Hernandez 2; Stuart E. Sinoff, MD3; Peter J. Snyder, PhD1, 4, 5*

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1. PhD Candidate at Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA, email: clausantos2910@gmail.com
2. Clinical Researcher at Lifespan Clinical Research Center, Rhode Island Hospital, Providence, RI, USA
3. Clinician at Department of Ophthalmology, BayCare Medical Group, Clearwater, FL, USA
4. Professor at Ryan Institute for Neuroscience, University of Rhode Island, Kingston, RI, USA
5. Professor at Department of Neurology, Alpert Medical School of Brown University, Providence, RI, USA
Abstract

**Introduction:** Retinal and cerebral microvasculature share similar embryological origins, anatomical features and physiological properties; and reduced retinal blood flow has been reported previously in mild cognitive impairment (MCI) and Alzheimer’s disease (AD), relying on both Doppler ultrasonography and optical coherence tomography angiography (OCTA) imaging technologies. The present study relies on OCTA to explore this same question in the preclinical AD stage of the disease.

**Methods:** Forty-eight adults (mean age = 68.76 years) with well-established risk factors for AD were recruited. Florbetapir amyloid PET scans were obtained and neocortical PET and standardized uptake value ratios (SUVr) threshold of 1.1 or greater was used to identify individuals with preclinical AD. Retinal OCTA images were captured using an AngioVue system (Optovue, Fremont, CA, USA), and the Df was measured in linearized superficial microvascular plexus images to measure the space-filling linear extension of the large vessels.

**Results:** The mean Df of preclinical AD subjects (N = 10) was significantly lower (ρ=0.005), with substantially greater variability, than that of healthy controls in the macular region (N = 28) (heteroscedastic Student’s t-test, 2-tailed). For the peripapillary region there was no significant difference (ρ=0.12) between both groups.

**Conclusion:** Our findings suggest that individuals at high-risk for preclinical AD have less density and complexity of retinal microvascular networks in the superficial vascular plexus in the macular region than healthy controls. Retinal vascular distribution and blood flow are already altered during the very earliest stages of AD.

**Keywords:** angio-OCT; retinal; blood flow; preclinical; Alzheimer’s; fractal dimension
Introduction

The co-occurrence of various cerebrovascular changes and Alzheimer’s disease (AD) is both well established and interdependent (1, 2). Whereas unimpaired cerebral blood flow (CBF) supports healthy neural activity and function, reduced CBF has been repeatedly observed in mild cognitive impairment (MCI) (3-9). Moreover, vascular density is significantly reduced, specifically in the basal forebrain region and the hippocampus of AD brains (10), and the capillary ultrastructure in the limbic cingulate cortex is severely compromised (11). Such cerebral capillary damage can lead to pathophysiological consequences, such as compromised nutrient transport, insufficient neuronal metabolism and subsequent cognitive disturbances; it should be considered a significant factor in the development of AD (11-14). AD has been associated with decreased anatomic complexity (e.g., tortuosity) of the cerebral vasculature, with venous collagenosis, string vessels (capillary remnants), decreased vascular density, and with concomitant microembolic brain injuries (15).

Diminished or altered CBF has not been conclusively shown to occur in the preclinical stage of AD following National Institute on Aging – Alzheimer’s Association (NIA-AA) criteria (16), and AD-related perfusion studies are largely restricted to correlating CBF with genetic and cognitive factors (17). For example, Okonkwo and colleagues (18) reported decreased CBF in right superior and middle frontal cortices for middle-aged adults with familial history of AD compared to age-matched cognitively normal subjects. Moreover, relative decrements in CBF were associated with modest impairments in memory function (18, 19) and general cognitive performance (20,21). Several recent publications have suggested specific
cerebral perfusion markers as potential indicators for amyloidosis and early disease detection (22-24).

The measurement of blood flow in the retina is likely to be very closely related to, and indicative of, neocortical blood flow. Retinal angiographic imaging allows for non-invasive access to the microcirculation, and the retinal vasculature shares similar embryological origin, anatomical features, and physiology with the cerebral vasculature (25-30). Reduced retinal blood flow has been reported in MCI and AD patients using Doppler methodology (31,32), and such retinal abnormalities as vascular attenuation, increased variability in vessel widths, reduced complexity of the branching pattern and less tortuous venules have been found in AD (33).

With very recent advances in angiographic optical coherence tomography imaging (OCTA), the retinal microvasculature can now be visualized without the need for intravenous administration of a contrast dye agent (34), to provide a range of measures such as the width of the lumen of vessels as small as 6-8 µm, vessel tortuosity and bifurcations, and these images can be subjected to a broad array of analytic approaches. OCTA has shown potential efficacy in the evaluation of common ophthalmologic diseases (35). In diabetic retinopathy, the foveal avascular zone (FAZ) and parafoveal vessel density provide a detailed view of the retinal vasculature and have been measured with high reproducibility by OCTA in both superficial and deep plexus (36). OCTA can also detect changes in choroidal blood vessel flow in age related macular degeneration. Narrowing of central retinal venous column diameter has been reported in AD and MCI (31,32).
Although there are many potential approaches to the extraction of important data from highly complex OCTA retinal images, one approach that relies of fractal geometry and the computation of fractal dimension (Df) values to objectively score the complexity and density of vascular branching, has already been used with apparent success. Using this very approach, Jiang and colleagues reported microvascular loss, beyond what is seen as a consequence of normal aging, in patients with MCI and AD (37). Since there remains a clear need for reliable, non-invasive techniques for screening potential high-risk individuals who may be in the earliest stages of AD, we decided to adopt this same analytic approach to explore retinal blood flow changes in the preclinical stage of AD. We have not been able to identify any other published literature that has addressed this question at such an early stage of the disease process.
Methods

Participants

A total of Fifty-six adults aged between 55 and 75 years (mean age = 65.36 years old) with two well-established risk factors for AD, namely, a self-reported first-degree family history of the disease and self-identification of subjective memory concerns, were recruited using a selection process described previously (38). All participants underwent a detailed medical screening interview. Exclusion criteria included a diagnosis of MCI or AD following NIA-AA diagnostic criteria (39,40), history of neurological or psychiatric disorder, any significant systemic illness or unstable medical condition (e.g., active cardiovascular disease), and current use of any medications known to affect cognition (e.g., use of sedative narcotics). Subjects with histories of cataract surgery, corneal LASIK surgery, age-related macular degeneration, glaucoma, diabetic retinopathy or subjects with known ophthalmic pathology were excluded.

Inclusion criteria included a score on the mini-mental state examination (MMSE) ≥ 27 and performance within normal limits on the International Shopping List Test (ISLT; www.cogstate.com) as a measure of verbal episodic memory; and the Memory Complaint Questionnaire (MAC-Q) (41,42). All participants live independently, most were engaged in full-time or part time employment, and many were caretakers for a parent with AD.

From this larger sample, we identified 15 participants who were categorized as presenting with preclinical stage disease based on evidence of elevated neocortical
beta-amyloid burden as determined by PET amyloid imaging (16, 39). The study was approved by and complied with the regulations of Rhode Island Hospital’s Institutional Review Board, and all participants provided written informed consent in accordance with the Declaration of Helsinki, and this study complied with HIPAA regulations.

**Aβ PET imaging**

To assess neocortical amyloid burden, all participants had an Aβ PET scan. A 370MBq (10 mCi 1/2 10%) bolus injection of 18F-florbetapir was administered intravenously. Approximately 50 minutes’ post-injection, a 20-minute PET scan was performed with head CT scan for attenuation correction purposes. Images were obtained using a 128X128 matrix and reconstructed using iterative or row action maximization likelihood algorithms. PET standardized uptake value (SUV) data were summed and normalized to the whole cerebellum SUV, resulting in a region-to-cerebellum ratio termed SUV ratio (SUVr).

A SUVr threshold of 1.1 or greater was used to discriminate between Aβ+ and Aβ-. These SUVr calculations were performed using the MIMneuro software, with a normative database of 74 healthy normal individuals (48 males, 26 females), aged between 18–50 years, who all had negative amyloid scans on visual assessment (43). For all cases, Aβ positivity was confirmed by consensus over-read by two board-certified radiologists who were also board certified in nuclear medicine.
Angio-OCT

OCTA images were captured using AngioVue (Optovue, Fremont, CA, USA). OCTA volume scans were obtained horizontally and vertically to decrease motion artifacts and fixation changes. Split-spectrum amplitude-decorrelation angiography was used to detect flow and produce OCTA images and en face sections. A 3x3-volume scan centered on the fovea and in the optic nerve were obtained with an A-scan rate of 70kHz. Each volume scan consists of 304x304 A-scans with 2 consecutive B-scans at each position. Two right-angled OCT-A volume scans are performed for orthogonal registration to correct for motion artefacts. Split-spectrum amplitude-decorrelation angiography was used to detect flow and produce OCTA images and en face sections. We selected the right eye OCTA of each participant for the fovea and optic nerve images to realize the Df analysis, using the superficial microvascular plexus images (3 µm below internal limiting membrane and 15 µm below inner boundary of inner plexiform layer).

The Df was measured in linearized vascular images in order to measure the space-filling linear extension of the vessels. Df was estimated with a computer program implementing the method of boxcounting. With this method, the image is overlaid with series of square boxes of decreasing size (s = 512, 256, 128, . . . 1, where s denotes a single pixel), and the number of boxes [N(s)] containing at least one black pixel is counted. The negative value of the least squares regression slope of the plot of log N(s) versus log s yields Df (44). The images were analyzed removing the small vessels, keeping only vessels with a diameter of more or equal to 25 µm (37).
Statistical Analysis

Demographic characteristics of the preclinical AD and healthy controls subjects were compared (Tables 1 and 2) for those individuals with good quality OCTA images in the macular region (N=38) and peripapillary region (N=48). To compare the variables Sex and APOE, the freq procedure was done in SAS and the Fisher’s exact test was used due to the small sample sizes. For all the other demographic and cognitive variables: Age, Education, Florbetapir PET SUVr, MAC-Q, MMSE and ISLT Total recall, the means ± SD were computed for each group and t-tests were used to identify group differences.

For each individual, the Df (with a grid size of 64 pixels) for the superficial vascular plexi, in the macular (centered on the fovea) and peripapillary (centered on optic nerve head) regions of the right eye retinas, were computed (mean and standard deviation (SD) of the measurements). Only good quality images were included (that is, those that were free of substantial imaging artefacts), resulting in 10 participants for the preclinical AD group and 28 healthy controls for the macular region analyses, and an additional 10 healthy control cases for the peripapillary region analyses. The normality of the Df measurement distributions, for both groups, were verified with the Shapiro-Wilks test, with normally distributed data then analyzed using the t-test pooled method and non-normally distributed data analyzed using the Satterthwaite method.
Results

Of the 38 participants with high-quality macular OCTA images, there were no significant differences between Aβ+ and Aβ- groups with respect to sex ($\rho =0.69$). Likewise, there were no group differences in the proportion of individuals with the APOE Ɛ4 genetic risk marker for AD ($\rho =0.269$). The mean age of the total sample was 69.36 years old and this sample had an average of 17.25 years of education with no education difference between groups ($\rho =0.64$). All relevant demographic information, for both groups, are provided below in Table 1. There were no group differences with respect to general cognitive function or subjective memory complaints (MMSE and MAC-Q) nor on a performance measure of episodic verbal memory (ISLT). By definition, both groups significantly differed with respect to neocortical amyloid aggregation as measured via PET imaging ($\rho = 0.000$).

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Insert Table 1 About Here
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Of the 48 participants with high-quality peripapillary OCTA images (Table 2), there were similarly no significant differences between Aβ+ and Aβ- groups with respect to sex (sex ($\rho =0.281$). Likewise, there were no group differences in the proportion of individuals with the APOE Ɛ4 genetic risk marker for AD ($\rho =0.151$). The mean age of the total sample was 69.76 years old and this sample had an average of 17.45 years of education with no education difference between groups ($\rho =0.789$). There were no group differences with respect to general cognitive function or subjective memory complaints (MMSE and MAC-Q) nor on a performance measure of episodic verbal memory (ISLT). By definition, both groups significantly differed
with respect to neocortical amyloid aggregation as measured via PET imaging ($\rho = 0.000$).

Results of the region-based fractal analysis (Df values) of linearized vascular patterns extracted from OCTA of preclinical AD and healthy controls, are summarized in Figure 1 for the macular region and Figure 2 for the peripapillary region. In the macular region, mean ± SD of healthy controls was 1.5896± 0.0999 and of preclinical AD was 1.3860±0.1799. The mean Df in the macula of preclinical subjects (N=10) is both significantly lower ($\rho=0.005$), and there is clearly greater variability in these measurements, as compared to the healthy controls (N=28).

In the peripapillary region, mean ± SD of healthy controls was 1.4290± 0.0975 and of preclinical AD was 1.3744±0.1064. The mean Df of preclinical subjects (N=10) in the peripapillary is not significantly different ($\rho=0.12$) than that of healthy controls (N=38).
Conclusion

The observed decrement in vascular bed density and complexity, as measured by the computation of the Df for each image of the superficial and/or deep vascular plexi, was reported to be reduced by 0.05 in AD and 0.03 in MCI, compared to elderly controls (37). We have found that at any even early stage of disease progression (in preclinical AD) the microvascular network loss in the superficial capillary plexus for the macular region was similarly reduced compared to healthy controls ($p=0.005$) (see examples in Figure 3).

These results fit nicely with our recent report that a volume reduction in the macular retinal nerve fiber layer (RNFL) appears to be the location of the earliest observable structural (retinal cell layer) change in the preclinical stage of AD (45). As we previously described, the macular region of the retina is physiologically very active in healthy normal eyes (46), and this “hyperexcitation” might be diminishing in the preclinical stage of AD. Postmortem histological studies have revealed prominent pathological alteration of retinal ganglion cells (RGCs) in the macular region in clinical AD patients (47,48). However, in the preclinical disease stage we may be observing either demyelination or loss of axons in the RNFL, prior to any loss of cell bodies in the ganglion cell layer (GCL) (45).

The superficial microvascular plexus (SVP) is supplied by the central retinal artery and composed of larger arteries, arterioles, capillaries, venules and veins that primarily
course through the GCL (49). Primate histology demonstrated that the SVP supplies all other vascular plexi through vertical pre-capillary arterial segments, which typically ascend to the nerve fiber layers and descend to the deeper layers (50,51). Reduced vascular density in the SVP likely leads to degradation in blood flow throughout the retina and, hence, might directly contribute to continued axonal loss as well as GCL thinning in AD.

This is a small experimental study with a limited sample size, but we believe that these results fit nicely with our emerging understanding of early retinal changes in the preclinical stage of AD, and we are now planning to replicate this work in a much larger longitudinal natural history study, with a participant cohort that will span the entire spectrum of the AD severity range.
References


30. Patton N, Aslam T, MacGillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular


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Table 1. Demographic Characteristics for Subjects with Acceptable Quality OCTA Imaging in the Macular Region

<table>
<thead>
<tr>
<th>Main Outcome</th>
<th>Full sample (n = 38)</th>
<th>Aβ+ (n=10)</th>
<th>Aβ- (n=28)</th>
<th>ρ</th>
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<tr>
<td></td>
<td>N(%)</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number of female</td>
<td>27 (71.34)</td>
<td>8 (80)</td>
<td>19 (67.86)</td>
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<td>APOE</td>
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<td></td>
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<tr>
<td>Number of Ɛ4 carriers</td>
<td>19 (50)</td>
<td>7 (70)</td>
<td>12 (42.86)</td>
<td>.269</td>
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<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>ρ</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of years</td>
<td>69.36 (5.20)</td>
<td>71.4 (5.66)</td>
<td>68.64 (4.93)</td>
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<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of years</td>
<td>17.25 (2.71)</td>
<td>17.12 (2.47)</td>
<td>17.6 (3.43)</td>
<td>.64</td>
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<tr>
<td>Florbetapir PET SUVr</td>
<td>1.03 (0.17)</td>
<td>1.29 (0.13)</td>
<td>0.94 (0.07)</td>
<td>.000</td>
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<tr>
<td>MAC-Q</td>
<td></td>
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<td></td>
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<tr>
<td>Total Score</td>
<td>25.5 (3.20)</td>
<td>26.6 (2.36)</td>
<td>25.10 (3.40)</td>
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<td>MMSE</td>
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<tr>
<td>Total Score</td>
<td>29.10 (1.07)</td>
<td>28.7 (1.25)</td>
<td>29.25 (0.98)</td>
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<td>ISLT Total Recall</td>
<td>25.92 (4.23)</td>
<td>24.10 (4.7)</td>
<td>26.78 (3.84)</td>
<td>.080</td>
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*Note: SUVr = standardized uptake value ratio; MAC-Q= Memory Complaint Questionnaire; MMSE = Mini Mental State Examination; ISLT = International Shopping List Test
Table 2. Demographic Characteristics for Subjects with Acceptable Quality OCTA Imaging in the Peripapillary Region

<table>
<thead>
<tr>
<th>Main Outcome</th>
<th>Full sample (n = 48)</th>
<th>Aβ+ (n=10)</th>
<th>Aβ- (n=38)</th>
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<tr>
<td></td>
<td>N(%)</td>
<td>N(%)</td>
<td>N(%)</td>
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<tr>
<td>Sex</td>
<td>Number of female</td>
<td>30 (62.5)</td>
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<td>APOE</td>
<td>Number of ε4 carriers</td>
<td>24 (50)</td>
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<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
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<tr>
<td>Age</td>
<td>Number of years</td>
<td>68.76 (5.30)</td>
<td>71.4 (5.66)</td>
<td>68.07 (4.95)</td>
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<tr>
<td>Education</td>
<td>Number of years</td>
<td>17.45 (2.82)</td>
<td>17.12 (2.47)</td>
<td>17.32 (2.52)</td>
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<td>Florbetapir PET SUVr</td>
<td>Standardized Uptake Value ratio</td>
<td><strong>1.01 (0.16)</strong></td>
<td><strong>1.29 (0.13)</strong></td>
<td><strong>0.94 (0.07)</strong></td>
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<td>MAC-Q</td>
<td>Total Score</td>
<td>26.32 (3.85)</td>
<td>26.6 (2.36)</td>
<td>25.97 (4.01)</td>
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<tr>
<td>MMSE</td>
<td>Total Score</td>
<td>29.16 (1.0)</td>
<td>28.7 (1.25)</td>
<td>29.27 (0.93)</td>
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<tr>
<td>ISLT Total Recall</td>
<td>Total words recalled</td>
<td>25.78 (4.31)</td>
<td>24.10 (4.7)</td>
<td>25.89 (4.04)</td>
</tr>
</tbody>
</table>

*Note: SUVr = standardized uptake value ratio; MAC-Q= Memory Complaint Questionnaire; MMSE = Mini Mental State Examination; ISLT = International Shopping List Test*
Figure 1. Mean, range and standard deviation’s (SD) for fractal dimension (Df) measurements, in the superficial vascular plexus within the macular region, for individuals with elevated (preclinical AD, N = 10) vs. normal neocortical amyloid aggregation (healthy controls, N = 28).
Figure 2. Mean, range and standard deviation’s (SD) for fractal dimension (Df) measurements, in the superficial vascular plexus within the peripapillary region, for individuals with elevated (preclinical AD, N = 10) vs. normal neocortical amyloid aggregation (healthy controls, N = 38).
Figure 3(a). Example of a linearized OCTA image for the superficial vascular plexus from an individual who meets amyloid PET imaging criteria for preclinical AD.

Figure 3(b). Example of a linearized OCTA image for the superficial vascular plexus from a healthy control individual.