Probing contributions of *Apolipoprotein E4* to cerebrovascular dysfunction and Alzheimer's disease

By
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Probing contributions of *Apolipoprotein E4*

to cerebrovascular dysfunction and Alzheimer's disease

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Abstract

Evidence increasingly points to an early and primary role for vascular dysfunction in the pathogenesis of late-onset Alzheimer's disease (AD). This early vascular dysfunction may be particularly impactful for people at the highest known genetic risk of AD, *Apolipoprotein E4* allele (*APOE4*) carriers. Though relatively minimal investigation has been conducted into the potential vascular mechanisms of AD pathogenesis for *APOE4* carriers, existing literature suggests *APOE4* may cause both peripheral and cerebral vascular dysfunction. Furthermore, evidence suggests the *APOE4* allele may act *synergistically* with this vascular dysfunction to promote dementia development. This highlights the need for interventions such as aerobic exercise that improve vascular health and may preferentially benefit *APOE4* carriers to delay AD onset.

In these collected works, we first examined the impact of *APOE4* carrier status on markers of cerebrovascular health. We measured cerebrovascular conductance and pulsatility at rest and found no differences for *APOE4* carriers compared to non-carriers with and without AD (Chapter 2, N = 32). Likewise, we report no significant impairment in dynamic cerebrovascular autoregulation for *APOE4* carriers with and without AD (Chapter 2, N = 29). In a separate cohort, we found no difference in the cerebrovascular response to an acute bout of exercise for cognitively-normal *APOE4* carriers (n =16) compared to non-carriers (n = 36; Chapter 3). Next, we investigated the potential synergistic relationship between vascular dysfunction and the *APOE4* allele. We found resting cerebrovascular conductance, or the amount of blood flowing to the brain per a given perfusion pressure, was inversely related to brain β-amyloid load for the *APOE4* carriers but not non-carriers (Chapter 3, N = 54). This suggests poor cerebrovascular conductance (i.e. poor brain blood flow delivery) may promote β-amyloid deposition in *APOE4*

carriers only, consistent with previous studies that have found a synergistic relationship between poor cerebrovascular health and the *APOE4* allele on increasing dementia risk. Additionally, we report *APOE4* carrier status moderates the association between pro-atherogenic cholesterol levels and the cerebrovascular response to an acute bout of exercise (Chapter 3, N = 52). Specifically, *APOE4* carriers with higher levels of pro-atherogenic cholesterol had a blunted cerebrovascular response from rest to exercise, while non-carriers exhibited the opposite relationship. These data further support the hypothesis that peripheral vascular risk factors may have a greater impact for *APOE4* carriers than non-carriers. In our final study of a randomized clinical trial (Chapter 4, N = 109), we report a year-long aerobic exercise intervention significantly improved hippocampal blood flow for *APOE4* carriers with hypertension, while there was no effect for non-carriers. Notably, we found a significant positive association between change in hippocampal blood flow and change in verbal memory performance for the intervention group, suggesting these improvements in hippocampal blood flow delivery may be clinically meaningful. Additionally, we found reductions in systolic blood pressure over one year were associated with increased hippocampal blood flow for *APOE4* carriers but not non-carriers. That is, for hypertensive *APOE4* carriers, lowering blood pressure improved blood flow delivery to the hippocampus, while this was not apparent for non-carriers, again suggesting peripheral vascular health may have a larger influence on brain health for *APOE4* carriers than non-carriers.

Taken together, the work in this dissertation supports an important role for systemic vascular health maintenance to preserve brain function in *APOE4* carriers. Interventions to improve vascular health, such as aerobic exercise, cholesterol management, and tight blood pressure control, may preferentially prevent or delay cognitive decline for people at the highest known genetic risk of late-onset AD.

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Chapter 1: Introduction

Alzheimer's Disease (AD) is the fifth leading cause of death in Americans 65 years and older (Association, 2019). While deaths due to other common causes such as heart disease and prostate cancer decreased by 7-11% from 2000 to 2015, deaths due to AD increased 123% (Association, 2019). As our population increasingly ages, the number of deaths due to AD will continue to climb since AD prevalence jumps from 10% of adults 65+ years to 32% of adults 85 years and older (Hebert et al., 2013). Therefore, there is a critical need for improvement in methods of detection, prevention and treatment of AD. Over the last three decades, the majority of AD research has centered on the amyloid hypothesis, which proposes that β-amyloid plaques in the brain cause neurodegeneration leading to cognitive decline (Hardy & Selkoe, 2002; Hardy & Higgins, 1992; Makin, 2018). However, attempts to prevent or reverse AD by targeting β-amyloid have unfortunately thus far been futile (Servick, 2019). For example, monoclonal antibody treatments have not been able to slow cognitive decline despite demonstrated efficacy in clearing brain β-amyloid in a dose-dependent manner (Sevigny et al., 2016). These disappointing clinical trial results reinforce the growing need for research that extends beyond the amyloid hypothesis into other pathophysiological mechanisms of AD.

Indeed, evidence increasingly points to a multifactorial pathogenesis of AD that incorporates vascular influences, particularly in preclinical stages of disease (Viswanathan et al., 2009). For example, a recent study using over 7,700 brain scans from the Alzheimer's Disease Neuroimaging Initiative database determined cerebrovascular dysregulation is the earliest and strongest pathological factor associated with AD progression (Iturria-Medina et al., 2016). Specifically, cerebrovascular dysregulation was found to be the earliest pathological event during AD development, followed by changes in β-amyloid deposition, metabolic dysfunction, functional impairment and structural atrophy (Iturria-Medina et al., 2016). This study and others

contributed to a proposed updated model of AD pathogenesis depicted in Figure 1-1 that places brain vascular dysfunction first in the pathological cascade, upstream of β-amyloid and tau deposition (Sweeney et al., 2018). However, this line of research into cerebrovascular mechanisms of AD pathogenesis remains in relative infancy and requires further exploration, which guided the work in this dissertation.

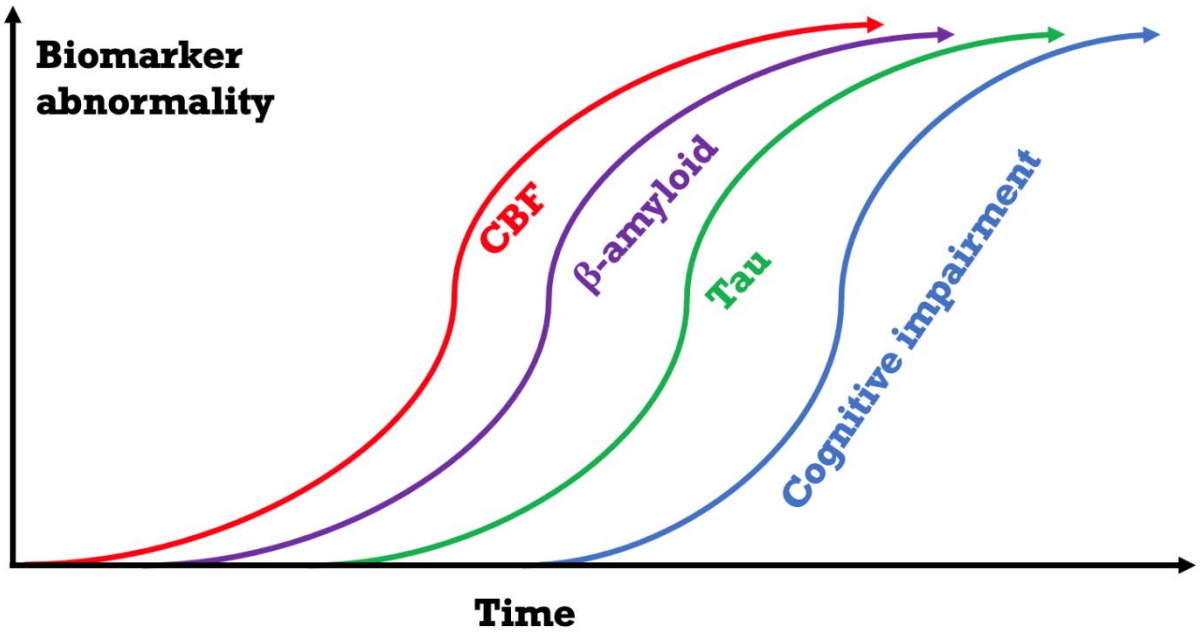

Figure 1-1: Updated model of Alzheimer's disease pathogenesis

The updated model of Alzheimer's disease pathogenesis proposed by Sweeney and colleagues (2018) places cerebral blood flow (CBF) perturbations earliest in the pathological cascade, ahead of β-amyloid and tau accumulation. All biomarker changes are posited to occur upstream of cognitive impairment and can manifest decades before dementia diagnosis. Figure 1-1 recreated by CS Kaufman.

Apolipoprotein E4 (APOE4)

The greatest known genetic risk factor for late-onset AD is the *Apolipoprotein E4* gene (*APOE4*) ("2018 Alzheimer's disease facts and figures," 2018; Strittmatter & Roses, 1996; Tai et al., 2016). One of three *APOE* allele polymorphisms is inherited from each parent - *E2, E3,* or *E4* (Heffernan et al., 2016; Mahley & Stanley C. Rall, 2000). Compared with people who have two copies of the most common form (*APOE3/APOE3*), those who inherit one copy of *APOE4* have up to 4 times the risk of developing AD while those who inherit two *APOE4* copies have a 12-fold risk (see Table 1-1) (Loy et al., 2014). In contrast, inheriting *APOE2* decreases the risk of developing AD compared with people with two copies of *APOE3* (Raber et al., 2004). The estimated percentages of the total U.S. population with either the *APOE2/APOE4, APOE3/APOE4* or *APOE4/APOE4* genotypes are 2%, 23% and 2%, respectively. Within the AD population, this prevalence increases to 65% of the population having at least one copy of the *APOE4* gene ("2018 Alzheimer's disease facts and figures," 2018).

Table 1-1: *Apolipoprotein E4* increases risk of Alzheimer's disease and lowers the average age of onset

	APOE3/APOE3	***APOE3/APOE4***	***APOE4/APOE4***
Relative lifetime risk of Alzheimer's disease	Reference Group	4x	12x
Mean age of clinical onset	84 years old	76 years old	68 years old

Although the connection between *APOE4* and Alzheimer's disease has been established for decades (Corder et al., 1993; Strittmatter & Roses, 1996), the mechanism through which *APOE4* causes Alzheimer's disease remains unclear. In fact, there is still no consensus on whether the single nucleotide change accounting for the difference between the *APOE4* and *APOE3* alleles represents a loss or gain of function (Belloy et al., 2019). This lack of clarity hinders effective therapeutic development as researchers remain undecided on such basic questions as whether the goal should be to increase or decrease *APOE* expression in order to prevent cognitive decline. Though a substantial portion of ApoE is generated peripherally by hepatocytes and macrophages, most prior research into the role of *APOE4* in Alzheimer's disease has focused on the portion of ApoE produced specifically in the brain, mainly by glial cells (Tai et al., 2016). This approach may overlook the important role of circulating ApoE, which functions primarily in systemic cholesterol transport and is not thought to cross the blood brain barrier (Belloy et al., 2019). In fact, *APOE4* has been linked to serious disease outside of the brain, with *APOE4* carriers having a significantly higher risk of coronary artery disease and myocardial infarction than non-carriers (Wei et al., 2017). It seems logical then that a single, common mechanistic pathway may explain the elevated risk associated with the *APOE4* allele for conditions ranging from stroke to myocardial infarction to multiple forms of dementia. In this dissertation, we propose vascular dysfunction may explain the heightened risk in *APOE4* carriers for these diverse conditions.

In the world of AD research, most work has focused on the impact of *APOE4* on β-amyloid metabolism or tau hyperphosphorylation, while vascular perturbations due to *APOE4* status have remained relatively unexplored (Belloy et al., 2019; DeCarli et al., 1999; Koizumi et al., 2018; Mahley & Stanley C. Rall, 2000; Raber et al., 2004; Safieh et al., 2019; Tai et al.,

2016; Ti et al., 2001; Zade et al., 2010; Zerbi et al., 2014). However, there is increasing evidence that *APOE4* may contribute to the pathogenesis of AD through cerebrovascular pathways. For example, *APOE4* transgenic mice have reduced blood flow to the cerebral cortex at rest due to decreased vascular density that causes high cerebrovascular resistance (Koizumi et al., 2018). Additionally, these mice demonstrate an attenuated cerebral blood flow (CBF) response in the somatosensory cortex after whisker stimulation, suggesting *APOE4* impairs neurovascular coupling (Koizumi et al., 2018). *APOE4* transgenic mice also fail to appropriately compensate to maintain CBF after bilateral carotid artery stenosis, suggesting impaired cerebrovascular autoregulation, and this blunted CBF causes white matter degradation leading to cognitive decline (Koizumi et al., 2018). Other studies have shown *APOE4* transgenic mice experience increased blood brain barrier breakdown (Alata et al., 2015). Importantly, these vascular defects caused by *APOE4* have been shown to *precede* neuronal dysfunction and have the ability to *initiate* neurodegenerative changes in mice (Bell et al., 2012). For example, vascular defects such as reduced CBF in *APOE4* transgenic mice reduce neuritic density and disrupt synaptic protein levels (Bell et al., 2012). Recent work has demonstrated human *APOE4* carriers also experience this accelerated blood brain barrier breakdown that is independent of β-amyloid and tau deposition and occurs prior to cognitive impairment (Montagne et al., 2020). Taken together, current evidence from both mouse and human models suggest *APOE4* may strongly influence AD development by causing cerebrovascular dysfunction.

Resting Cerebral Blood Flow (CBF)

The brain comprises only 2% of body mass yet consumes ~20% of energy produced by the body at rest (Attwell et al., 2010). Despite this elevated metabolic rate, the brain stores

relatively minimal glycogen (Tarumi & Zhang, 2018). In order to meet this disproportionate energy demand, delivery of oxygen and glucose to the brain must be tightly regulated, and CBF is thus maintained around 20% of cardiac output (Xing et al., 2017). Inadequate CBF resulting in ischemia impairs brain functioning within seconds and causes irreversible neuronal damage within minutes (Kisler et al., 2017). CBF declines with normal aging (Leenders et al., 1990) but decreases to an even greater extent in pathological aging, and studies have demonstrated significantly lower CBF in people with AD compared to age-matched healthy controls (Alsop et al., 2000; Binnewijzend et al., 2013; Bracko et al., 2021; Johnson et al., 2005; Roher et al., 2012; van de Haar et al., 2016; Wang et al., 2013; Wolters et al., 2017). In fact, scientists have recognized for decades that CBF reductions occur in AD patients, though these CBF changes have been historically investigated as a downstream biomarker of disease rather than as a contributing mechanism, in contrast to more recent years (Sweeney et al., 2018). For example, one preliminary study utilized arterial spin labeling magnetic resonance imaging (ASL-MRI) – an at the time state-of-the-art technology to measure CBF in living humans – to demonstrate qualitative brain hypoperfusion in AD patients compared to age-matched controls (Sandson et al., 1996). That same year, a separate group demonstrated hypoperfusion in the temporal and parietal lobes in people with AD using single photon emission computed tomography (SPECT) (Lehtovirta et al., 1996). These publications were followed a few years later by a larger study utilizing ASL-MRI that found significant hypoperfusion in people with AD compared to age-matched controls in the temporal, parietal, frontal and posterior cingulate cortices (Alsop et al., 2000). Since then, many studies have demonstrated hypoperfusion in people with AD compared to age-matched controls (Binnewijzend et al., 2013; Johnson et al., 2005; Roher et al., 2012; van de Haar et al., 2016; Wang et al., 2013; Wolters et al., 2017). While it is worth noting that a few

conflicting studies have found hyperperfusion in early stages of the disease (Alsop et al., 2008; Ding et al., 2014), the large majority overall report lower CBF in people with AD.

Though these CBF reductions are now relatively established to occur in people with AD, the impact of the *APOE4* allele on CBF has been less studied. However, there is some evidence that within the AD population, *APOE4* carriers have reduced CBF compared to non-carriers (Hogh et al., 2001; Lehtovirta et al., 1998; Lehtovirta et al., 1996). For example, one study found all patients with AD had bilateral temporoparietal hypoperfusion, but those with at least one *APOE4* allele also demonstrated hypoperfusion extending into the frontal and occipital lobes (Lehtovirta et al., 1996). Likewise, a different group studying AD patients found *APOE4* carriers exhibited more significantly reduced CBF than non-carriers in a variety of regions and proposed the reduced CBF in the frontal cortex might predict clinical progression specifically for *APOE4* carriers (Hogh et al., 2001). These findings suggest reductions in CBF may play an even more significant role in AD pathogenesis for *APOE4* carriers than non-carriers. Beyond the AD population, there is some evidence that even *APOE4* carriers with normal cognition display CBF perturbations. For example, young adults with an *APOE4* allele were found to have lower resting CBF in the temporal gyrus compared to non-carriers (Scarmeas et al., 2003). Additionally, one longitudinal study of cognitively-normal older adults determined *APOE4* carriers experience an accelerated CBF decline in brain regions most vulnerable to AD-related pathological changes (Thambisetty et al., 2010), suggesting more significant and rapid CBF reductions may occur during early AD pathogenesis in *APOE4* carriers. Overall, the literature suggests CBF may be blunted in *APOE4* carriers, but further studies are needed to characterize other aspects of resting cerebrovascular function such as resistance/conductance and pulsatility.

Resting cerebrovascular resistance and conductance

Our laboratory and many others in cerebrovascular physiology use middle cerebral artery velocity (MCAv) as a surrogate measure for CBF because the middle cerebral artery (MCA) is a major conduit vessel to the brain (see Figure 1-2) and is assumed to have a constant diameter, meaning dynamic changes in velocity should reflect differences in blood flow volume (Labrecque et al., 2019; Labrecque et al., 2017; Perdomo et al., 2019; Sisante et al., 2019; Witte et al., 2019).

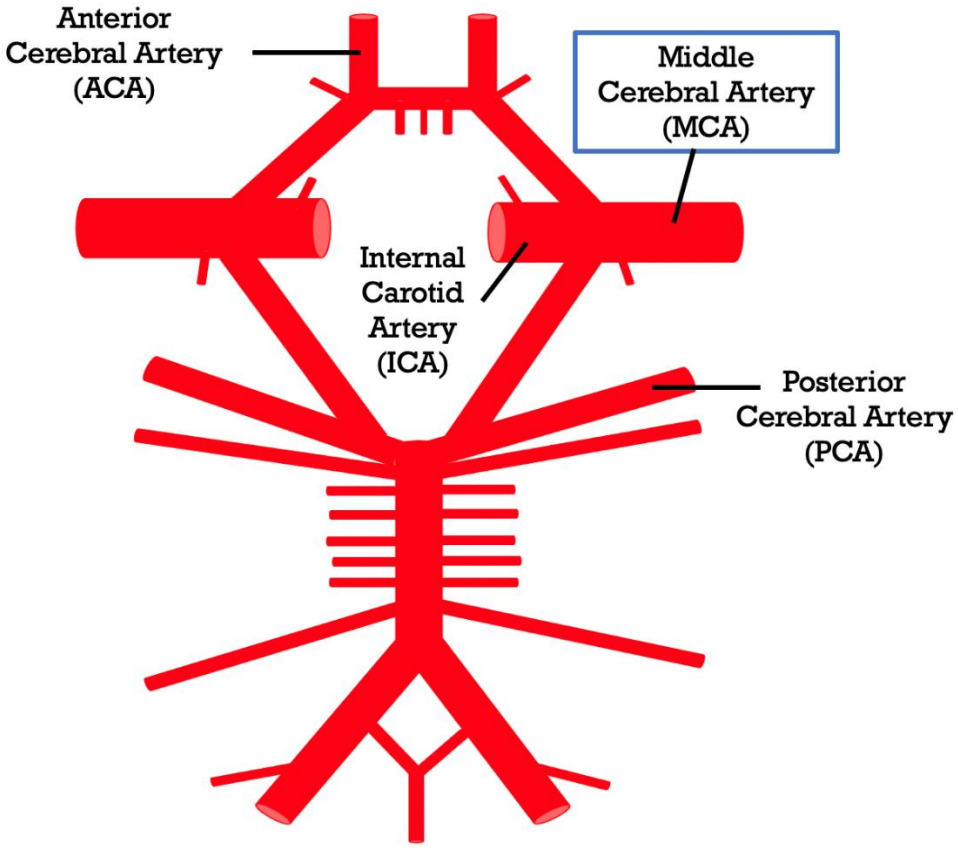

Figure 1-2: Circle of Willis representation

The Circle of Willis supplies blood to the brain and surrounding structures. In our laboratory, we use transcranial Doppler ultrasound (TCD) to insonate the middle cerebral artery velocity (MCA) in the Circle of Willis. The MCA branches from the internal carotid artery (ICA) and is the largest conduit vessel supplying the brain. The velocity of the blood traveling within the MCA (MCAv) serves as a surrogate for cerebral blood flow (CBF).

In support for the utilization of MCAv as a CBF surrogate, a study assessing changes in CBF using Gadolinium MRI, Arterial Spin Labeling (ASL) MRI, and MCAv (transcranial Doppler ultrasound, TCD) in the same individuals found a significant positive correlation between TCD and each MRI method for measuring CBF, suggesting MCAv can be a reliable surrogate for CBF (Sorond et al., 2010). Blood flow through the MCA is dictated by a combination of cerebral perfusion pressure (driven by the cardiovascular system and reflected by the mean arterial pressure, MAP) and vascular resistance/conductance, which is determined by vessel compliance and diameter of downstream arterioles, with smaller diameter and stiffened vessels resulting in higher resistance and lower conductance (Duffin et al., 2018). In order to measure cerebrovascular resistance, researchers use a ratio of MAP (representing cerebral perfusion pressure) to CBF (or MCAv when using TCD) to obtain a cerebrovascular resistance index (CVRi): CVRi = MAP/CBF (Akazawa et al., 2018; Nation et al., 2013; Yew & Nation, 2017). CVRi quantifies resistance within the cerebral vessels by accounting for the contribution of perfusion pressure to CBF (see Figure 1-4). Likewise, the reciprocal relationship provides an index of cerebrovascular conductance (CVCi = CBF/MAP) (Labrecque et al., 2019). One advantage of using CVCi and CVRi instead of MCAv alone is that alterations in cerebrovascular regulation may be detected in cases of non-significant CBF differences. For example, one study discovered more widespread changes by measuring CVRi in people with AD compared to assessing changes in CBF alone (Nation et al., 2013). Importantly, these elevations in CVRi predicted increased white matter lesions and decreased cognitive function, suggesting CVRi plays a role in cognitive decline during the pathogenesis of AD (Nation et al., 2013). In line with this hypothesis, CVRi appears to increase earlier in the natural history of AD *before* reductions in global CBF are typically observed (Yew & Nation, 2017). This increased resistance at rest

could prevent adequate blood supply from reaching the brain parenchyma and contribute to the pathogenesis of AD (Yew & Nation, 2017). That is, elevated cerebrovascular resistance appears to occur first and directly contribute to overt reductions in CBF, which then promotes disease pathogenesis in accordance with the vascular hypothesis of AD.

Resting pulsatility index

Another measurement commonly used to characterize blood flow is the pulsatility index (PI), calculated as: MCA PI = (MCA systolic velocity – MCA diastolic velocity)/mean MCA velocity (Roher et al., 2011) (see Figure 1-3). PI is most accurately understood as a reflection of pulsatile flow (Czosnyka et al., 1996; de Riva et al., 2012). Pulsatile flow originates from the contraction of the heart and is apparent in brachial blood pressure readings, with systolic pressure reflecting the peak and diastolic pressure reflecting the trough of the waveform (Zarrinkoob et al., 2016). In healthy adults, pulsatility is attenuated as it travels from the heart to the brain due to the dampening of pulsations through the Windkessel effect in elastin-rich conduit vessels like the aorta (Belz, 1995). With normal aging, stiffening of the large arteries reduces the Windkessel effect resulting in increased delivery of pulsatile flow to the fragile microvasculature of the brain, and this phenomenon is accelerated in vascular disease states (Safar, 2010; Zarrinkoob et al., 2016). Transmission of excessive pulsatility into the cerebrovasculature is associated with microvascular structural brain damage and cognitive impairment (Mitchell et al., 2011). A number of studies have convincingly shown that people with AD have both increased CVRi (Cacabelos et al., 2003; Nation et al., 2013; van Beek et al., 2012; Yew & Nation, 2017) and PI (Jin et al., 2017; Lim et al., 2017; Rivera-Rivera et al., 2017; Roher et al., 2011; Sabayan et al., 2012), but to our knowledge no one had previously evaluated

the relationship between these MCA indices (CVRi , CVCi and PI) and *APOE4* carrier status with and without AD. We thus sought to characterize this relationship in Chapter 2 of this dissertation.

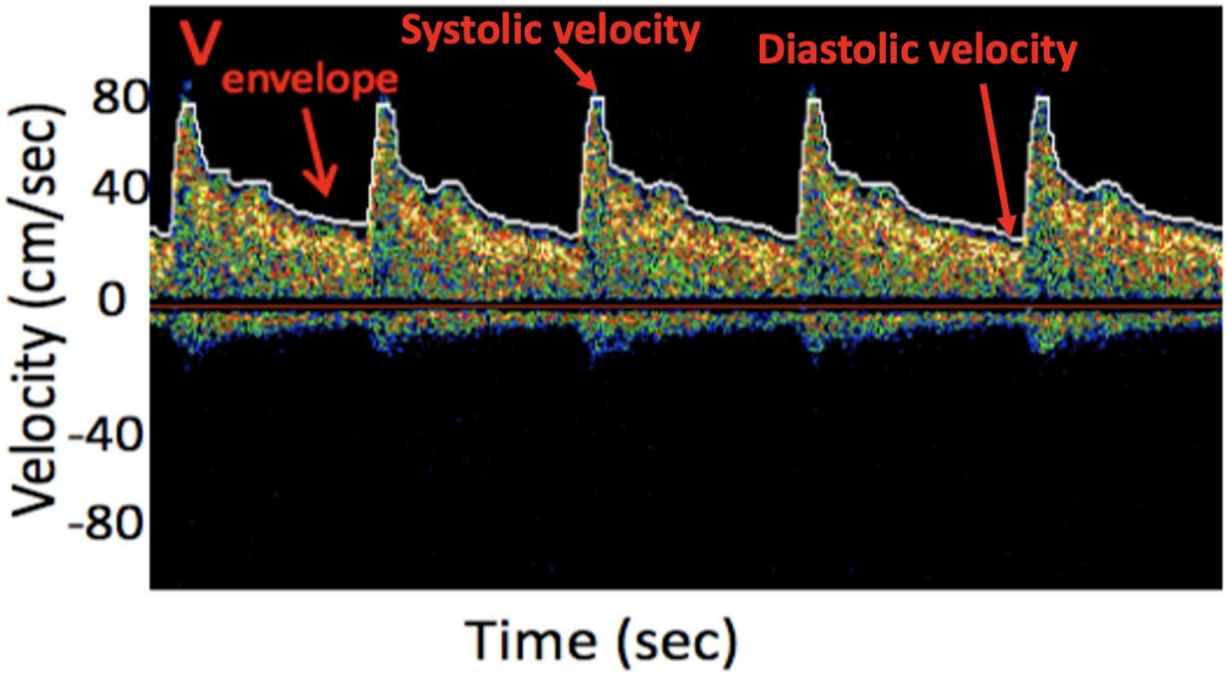

Figure 1-3: Example recording of the middle cerebral artery (MCA) using transcranial Doppler ultrasound (TCD).

The envelope velocity ($V_{envelope}$) represents the fastest velocity measured at each timepoint and is generally used to denote overall velocity for the blood vessel. Pulsatility is a function of the shape of the waveform and is calculated from systolic, diastolic and mean (area under the curve) velocities.

Dynamic CBF

Measures such as resting CBF, CVRi, CVCi and PI provide useful yet *incomplete* insight into cerebrovascular health. The cerebrovascular system undergoes a variety of physiological stressors with activities of daily living that are not captured through measurements taken at rest. An important advantage of transcranial Doppler ultrasound (TCD) is the ability to measure blood flow during *dynamic* physiological stimuli, which is not possible using other non-invasive

methods such as MRI (Aaslid et al., 1982; Billinger et al., 2017; Kim et al., 2015; Roher et al., 2011; Sorond et al., 2009). For example, TCD can examine the MCAv response upon standing (Labrecque et al., 2019; Labrecque et al., 2017; Lipsitz et al., 2000; Sorond et al., 2009) or during exercise (Billinger et al., 2017; Sisante et al., 2019; Ward et al., 2018), which may allow for detection of subtle disturbances or abnormal responses in cerebrovascular function that cannot be observed during supine rest in an MRI scanner. We capitalized on this unique ability of TCD to measure dynamic cerebrovascular function in Chapter 2 (sit-to-stand protocol to assess dynamic cerebrovascular autoregulation) and Chapter 3 (cerebrovascular response to exercise).

Dynamic Cerebrovascular Autoregulation (dCA)

After standing from seated rest, systemic blood flow return to the heart drops significantly resulting in decreased MAP (on average 21 to 26 mmHg, or 22% to 26%, within 30 seconds of standing) (Lipsitz et al., 2000). This drop in cerebral perfusion pressure would be detrimental to brain functioning if CBF decreased to an identical degree because there would be an inadequate supply of oxygen and glucose to meet the high metabolic demand of the brain (Tan & Taylor, 2014). Therefore, to counteract this decreased cerebral perfusion pressure, the cerebrovasculature must dilate to divert more blood flow into the brain (Sorond et al., 2009). This is accomplished downstream of major conduit vessels such as the MCA by vascular smooth muscle cells lining pial arterioles, the dominant mediators of cerebrovascular resistance (Nishimura et al., 2007). In response to hypotension, these vascular smooth muscle cells increase arteriole diameter and thereby decrease resistance in order to allow for higher blood flow through the upstream vessel (Duffin et al., 2018). Through this physiological mechanism, the drop in

CBF is attenuated but not fully prevented from falling below pre-standing levels (see Figure 1-5). For example, in a population of healthy adults, MAP was found to drop on average 25% upon standing while MCAv decreased 14% (Sorond et al., 2009). This phenomenon of relative CBF stability in the face of significant acute blood pressure alterations is coined dynamic cerebrovascular autoregulation (dCA) (Aaslid et al., 1989; Tiecks et al., 1995; Tzeng & Ainslie, 2014). Impaired dCA could result in exaggerated reductions in CBF during normal activities of daily living which may damage the brain over time (Aoi et al., 2012). While impairments in dCA have been demonstrated in a variety of disease states including stroke (Eames et al., 2002; Xiong et al., 2017), concussion (Kostoglou et al., 2016; Wright et al., 2018) and diabetes (Nasr et al., 2011; Vianna et al., 2015), reports on dCA in dementia have thus far proven inconclusive, with some studies finding no difference in dCA and others showing subtle changes (Claassen et al., 2009; de Heus et al., 2018; den Abeelen et al., 2014; Gommer et al., 2012; Marmarelis et al., 2017; Tarumi et al., 2014; van Beek et al., 2012). To our knowledge, autoregulation had not been previously characterized for human *APOE4* carriers, but findings from animal studies suggest autoregulation may be impaired by the *APOE4* allele. For example, *APOE4*-expressing mice experienced a more severe reduction in CBF compared to *APOE3*-expressing and wild type controls after bilateral carotid artery stenosis (Koizumi et al., 2018), suggesting reduced ability to compensate for reductions in cerebral perfusion pressure to maintain CBF. Importantly, the chronic brain hypoperfusion that resulted from the impaired cerebrovascular autoregulatory capacity in these mice caused white matter damage and subsequent cognitive decline (Koizumi et al., 2018). This suggests that if impaired dCA occurs in human *APOE4* carriers as it does in transgenic *APOE4* mice, this mechanism may directly contribute to brain damage and dementia development. We therefore sought to explore this autoregulation capacity in humans.

To this end, in Chapter 2, we report the results of a sit-to-stand experimental protocol conducted in older adults with and without an *APOE4* allele and diagnosis of early AD. This protocol assesses dynamic cerebrovascular autoregulation (dCA) by recording MCAv and MAP during the transition from sitting to standing, calculating CVCi, and plotting all three measures as a function of time (see Figures 1-4, 1-5 and 2-2). A larger change in MCAv (ΔMCAv) per change in MAP (ΔMAP) after the transition from rest to standing can indicate impaired dCA. Here, we hypothesized *APOE4* carriers would have a larger ΔMCAv per ΔMAP, as this would mean *APOE4* carriers have a blunted ability to compensate for acute reductions in perfusion pressure by minimizing CBF reductions. Additionally, time delay (TD) after standing measures the amount of time that passes before the cerebrovasculature responds to the drop in perfusion pressure with vasodilation, which causes an increase in CVCi (Labrecque et al., 2019; Labrecque et al., 2017). We hypothesized *APOE4* carriers would have a longer TD than non-carriers, which would mean the vasodilatory response is delayed and indicate impaired dCA. Finally, after the onset of regulation, the ability of the cerebrovasculature to efficiently redirect blood flow is reflected in the rate of regulation (RoR, change in CVCi per change in time divided by MAP over the first four heartbeats after regulation onset), with a faster RoR reflecting increased ability to return CBF back toward resting levels (Labrecque et al., 2019; Labrecque et al., 2017). That is, RoR reflects the efficiency of dCA (van Beek et al., 2008). As with the other dCA metrics, we expected the RoR to be blunted in *APOE4* carriers compared to non-carriers, reflecting a reduced ability of the cerebrovasculature to counteract CBF disruptions. Additionally, we hypothesized these metrics of dCA would be even more impaired in *APOE4* carriers with AD than carriers with normal cognition. Our findings on dCA in human *APOE4* carriers with and without AD are outlined in Chapter 2 of this dissertation.

Figure 1-4: Transcranial Doppler ultrasound (TCD) and Finapres device

In our laboratory, we simultaneously record middle cerebral artery velocity (MCAv), a surrogate measure of cerebral blood flow (CBF), and mean arterial pressure (MAP), a surrogate measure of brain perfusion pressure. The cerebrovascular resistance, conductance and autoregulatory responses were calculated from these recordings in Chapters 2 and 3.

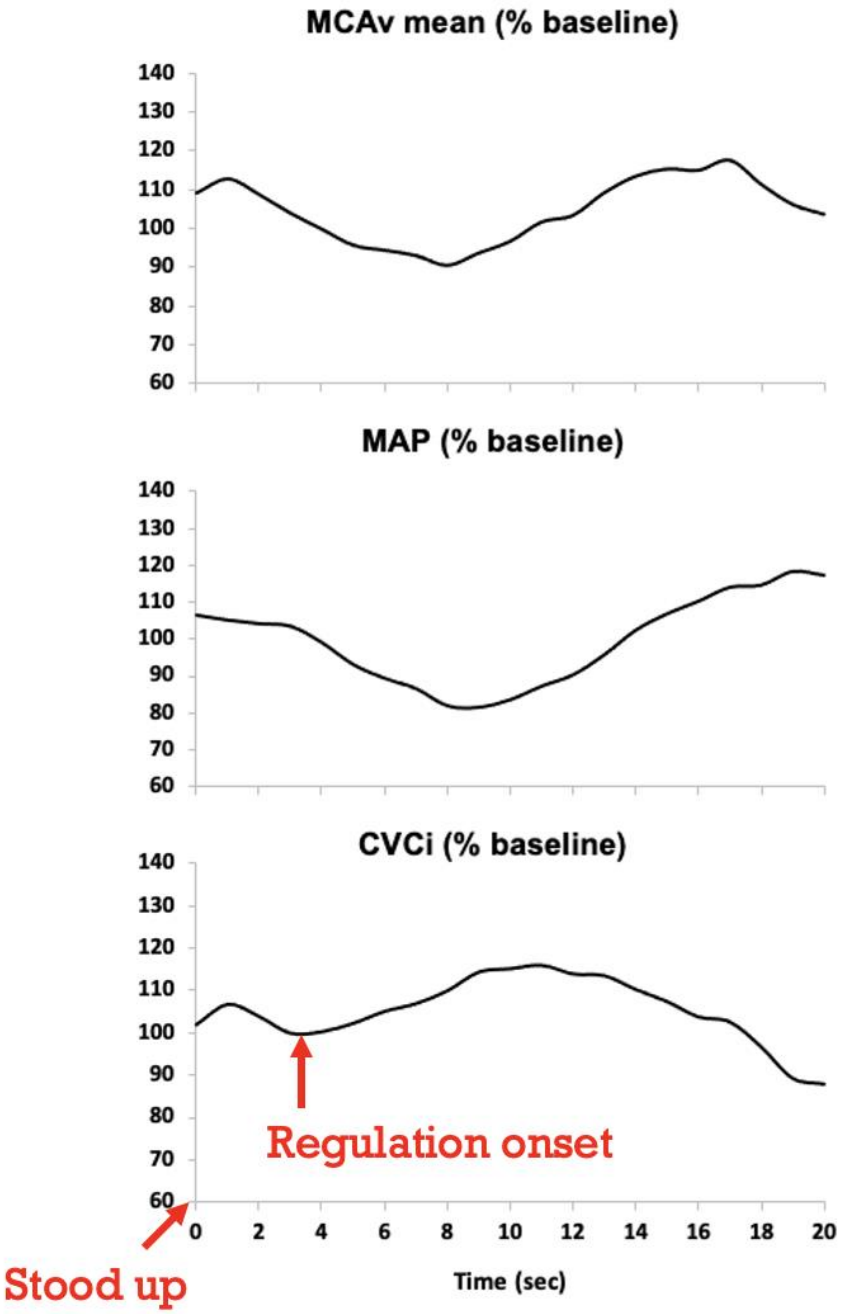

Figure 1-5: Representative data from a single participant during a sit-to-stand.

The participant stood from seated rest at 0 seconds. Regulation onset denotes the point at which cerebrovascular conductance index (CVCi) began increasing in response to the decrease in brain perfusion pressure. The number of seconds elapsed between standing and the onset of regulation is the time delay, in this case ~3 seconds. Though both middle cerebral artery velocity (MCAv) and mean arterial pressure (MAP) decrease as a result of standing, the regulatory response minimizes the MCAv decline relative to the MAP reduction.

CBF response to acute exercise

During an acute bout of moderate-intensity exercise, MCAv increases from rest and reaches a new steady-state level within a few minutes of exercise onset (Smith & Ainslie, 2017). Our lab previously established a novel protocol for measuring MCAv during exercise on a recumbent stepper (Billinger et al., 2017; Kaufman et al., 2019; Sisante et al., 2019; Ward et al., 2018). Since the moderate-intensity exercise protocol we utilize is similar to walking upstairs in terms of physiological challenge (Ferguson, 2014), perturbations in MCAv measured with this stimulus could have important implications for day-to-day functioning. We previously demonstrated a decreased cerebrovascular response (measured as ΔMCAv from rest to steady-state exercise) to moderate-intensity exercise in older compared to younger healthy adults (Ward et al., 2018). In a separate study, we found a significantly decreased cerebrovascular response to exercise in non-demented older adults with elevated brain β-amyloid compared to amyloid-negative adults, suggesting cognitively-normal people with markers of AD pathology may experience dynamic CBF abnormalities not observed during static, resting conditions (Sisante et al., 2019). The mechanisms through which aging and brain β-amyloid may contribute to a reduced cerebrovascular response to exercise remain unclear, but the blunted response likely reflects poor cerebrovascular functioning under complex physiological stimuli. That is, during an acute bout of exercise, the cerebrovasculature must respond to a range of inputs including systemic increases in pressure and carbon dioxide, elevated brain metabolism, and an acute demand for neurovascular coupling to coordinate muscle movements (Smith & Ainslie, 2017). A relative inability to increase MCAv during exercise – for example, as we previously observed in older adults with elevated brain β-amyloid illustrated in Figure 1-6 (Sisante et al., 2019) - may therefore reflect poor cerebrovascular functioning. Specifically, the observed impaired

cerebrovascular response suggests older adults with elevated brain β-amyloid may be unable to appropriately respond to inputs - including elevated circulating carbon dioxide, systemic pressure and brain metabolism - in order to meet the brain blood flow requirements of aerobic exercise.

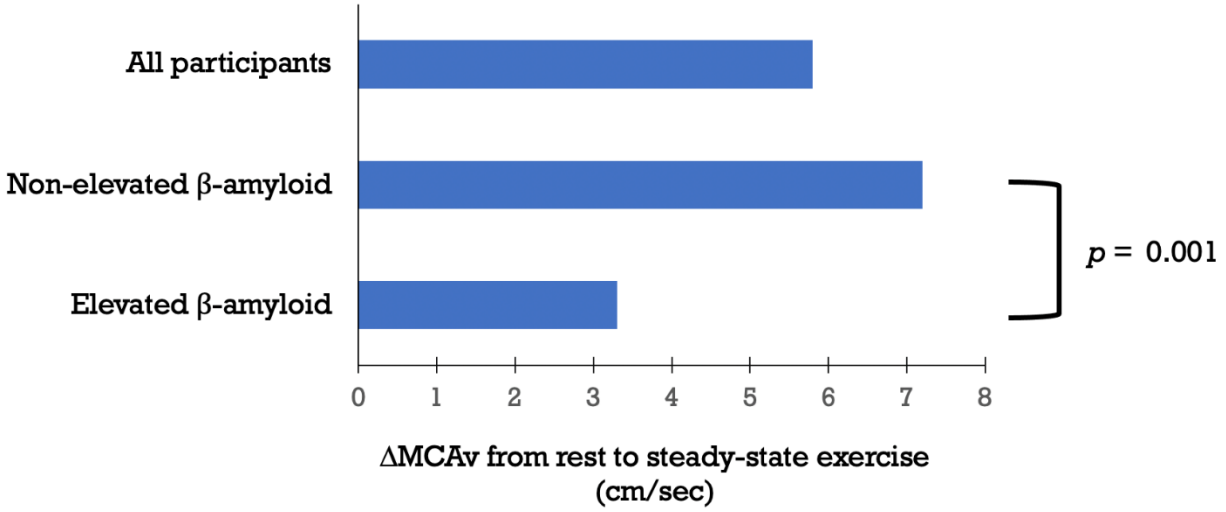

Figure 1-6: Previous data on the cerebrovascular response to exercise and brain β-amyloid

Our laboratory previously reported a significantly blunted cerebrovascular response to an acute bout of moderate-intensity exercise for older adults with elevated brain β-amyloid (Sisante et al., 2019). Specifically, the change in middle cerebral artery velocity (MCAv) from rest to exercise was 3.3 cm/s on average for those with elevated β-amyloid compared to 7.2 cm/s for participants without β-amyloid.

While this CBF response to acute exercise had not been characterized for *APOE4* carriers prior to the work in this dissertation (Chapter 3), evidence from animal studies suggests *APOE4* carriers may have an impaired cerebrovascular response to physiological stimuli. For example, one study found *APOE4*-expressing mice demonstrated impaired neurovascular coupling, or an inability to increase blood flow delivery to meet local demand (Koizumi et al., 2018). Specifically, these mice had markedly attenuated CBF response to whisker stimuation in the somatosensory cortex compared to *APOE3*-expressing and wild type mice (Koizumi et al.,

2018), suggesting dysregulation of CBF in response to neuronal activation. If a similar impairment in neurovascular coupling occurs in human *APOE4* carriers, it could contribute to a blunted cerebrovascular response to acute exercise. Additionally, human *APOE4* carriers have been shown to experience impaired cerebrovascular reactivity to carbon dioxide inhalation (Suri et al., 2015). As with neurovascular coupling, this impaired CBF response to elevated carbon dioxide could contribute to a blunted cerebrovascular response to moderate-intensity exercise, during which carbon dioxide increases systemically. Thus, in Chapter 3 we aimed to interrogate cerebrovascular function during this complex physiological stimulus in a cohort of cognitively-normal older adult participants (N = 54; distinct from the participants enrolled in the Chapter 2 study). Specifically, we measured the MCAv response to an acute bout of moderate-intensity exercise and compared this cerebrovascular response for cognitively-normal *APOE4* carriers and non-carriers (Chapter 3).

Synergy between APOE4 & vascular risk factors

Evidence increasingly suggests *APOE4* may act synergistically *with* vascular dysfunction (in addition to *causing* vascular dysfunction) to promote AD pathogenesis. For example, *APOE4* both increases the risk of stroke (Wei et al., 2017) and acts synergistically with stroke to promote cognitive impairment (Shaaban et al., 2019). That is, while both stroke and *APOE4* independently increase dementia risk, their combined effect within an individual is larger than would be expected through addition alone (Kaufman & Perales-Puchalt, 2019; Shaaban et al., 2019). In a 10-year longitudinal study including over 1,700 participants, the observed hazard ratio for dementia for an individual with both a history of stroke and an *APOE4* allele was 28.33, significantly higher than the expected additive hazard ratio of 9.31 (Shaaban et al., 2019). This

excess risk was proposed to be due to a synergistic effect between stroke history - signifying poor cerebrovascular health – and *APOE4* carrier status, which suggests cerebrovascular dysfunction is even more detrimental for *APOE4* carriers than non-carriers. Moreover, blood brain barrier breakdown more significantly predicts future cognitive decline in *APOE4* carriers than non-carriers (Montagne et al., 2020). Taken together, these findings suggest cerebrovascular dysfunction may play a larger role in promoting AD development in *APOE4* carriers than non-carriers. This proposed synergy is depicted in Figure 1-7. The mechanism through which this synergy occurs is currently unknown but may be related to vascular dysfunction playing a larger role in AD pathogenesis for *APOE4* carriers than non-carriers. That is, there is increasing evidence that the perturbations that typically occur early in the disease process are different depending on *APOE4* status (Emrani et al., 2020). Therefore, *APOE4* carriers may be primed to suffer greater downstream consequences in the face of reduced CBF, as cerebrovascular dysfunction appears to play a more significant role in their typical disease pathogenesis. Additionally, considering CBF reductions have been observed in even cognitively-normal *APOE4* carriers (Lehtovirta et al., 1998; Thambisetty et al., 2010), these genetically at-risk individuals may not have sufficient CBF reserve to compensate for cerebrovascular perturbations and thus suffer more serious effects such as β-amyloid accumulation and cognitive decline. In addition to poor *cerebro*vascular health, *peripheral* vascular risk factors have been proposed to interact with the *APOE4* allele to promote brain dysfunction. For example, hyperlipidemia and hypertension more strongly predict cognitive decline in *APOE4* carriers than non-carriers (Caselli et al., 2011a; Zade et al., 2010). Thus, in Chapters 3 and 4, we sought to further assess this potential synergistic relationship between the *APOE4* allele and both peripheral and cerebral vascular dysfunction.

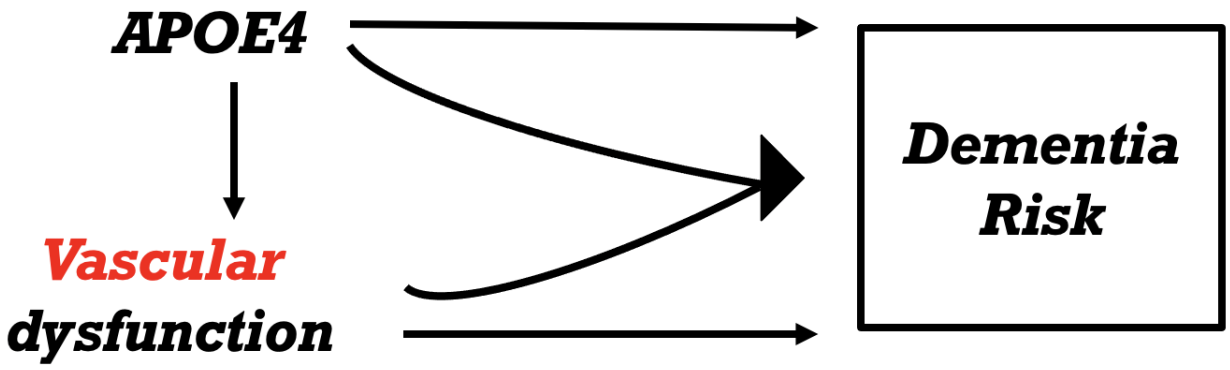

Figure 1-7. Dissertation hypotheses guide

APOE4 is the strongest known genetic risk factor for late-onset Alzheimer's disease (top horizontal arrow). Vascular dysfunction is increasingly recognized as a direct contributor to dementia pathogenesis (bottom horizontal arrow). Intriguingly, some studies suggest *APOE4* may cause both peripheral and cerebral vascular dysfunction (vertical arrow). Finally, there is some evidence for a synergistic effect between the *APOE4* allele and both peripheral and cerebral vascular dysfunction (center combined arrow), whereby poor vascular health is even more detrimental for *APOE4* carriers than non-carriers. These hypotheses drive the work in this dissertation.

CBF changes with chronic exercise

A recent systematic review found exercise improves cognitive function in older adults regardless of baseline cognitive status (Northey et al., 2018), and current guidelines recommend at least 150 minutes of moderate-to-vigorous activity per week to reduce dementia risk (Dhana et al., 2020). While aerobic exercise offers a promising avenue for preventing or slowing AD, the mechanisms through which exercise improves cognitive function in humans remain unclear. Animal models suggest a variety of potential mechanisms, including neurotrophin release leading to neurogenesis, improved synaptic plasticity, and angiogenesis leading to improved brain blood flow delivery (Gary & Brunn, 2014). However, relatively few interventional studies in humans have examined the impact of an aerobic exercise intervention on changes in CBF,

particularly in brain regions most implicated in early AD pathogenesis such as the hippocampus (Raji et al., 2009). One study found a 4-month exercise intervention significantly improved hippocampal blood flow (HBF) in older adults (Burdette et al., 2010), and at least two others have demonstrated a correlation between change in HBF and cognitive gains with exercise interventions (Chapman et al., 2013; Maass et al., 2015). However, none of these studies investigated whether these exercise-induced changes in HBF differed by *APOE* genotype, and we thus aimed to explore these potential improvements in Chapter 4 of this dissertation. Specifically, we hypothesized *APOE4* carriers would experience greater improvements in HBF with aerobic exercise compared to non-carriers.

There are a number of reasons *APOE4* carriers may be primed to benefit more substantially from exercise interventions. First, as outlined above, these is a potential synergistic effect on dementia risk between the *APOE4* allele and poor vascular health (Bender & Raz, 2012; Haan et al., 1999; Oberlin et al., 2015; Shaaban et al., 2019). For example, blood pressure has been shown to interact with *APOE4* carrier status to negatively impact cognition (de Leeuw et al., 2004; Oberlin et al., 2015; Peila et al., 2001; Zade et al., 2010). This suggests interventions that improve systemic vascular health, such as aerobic exercise, may be particularly beneficial for those at highest genetic risk of AD (Kaufman & Perales-Puchalt, 2019). Additionally, some studies have directly shown *APOE4* carriers may benefit more from exercise than non-carriers (Deeny et al., 2008; Jensen et al., 2019; Lautenschlager et al., 2008; Schuit et al., 2001; Smith et al., 2014), making aerobic exercise an especially promising intervention for improving systemic vascular function and brain health in this group. For example, one clinical trial included participants with early AD (N = 200) and found exercise improved cognitive function more significantly in *APOE4* carriers than non-carriers (Jensen et al., 2019). Additionally, an

observational study of cognitively-normal older adults (N = 97) determined physical activity reduces hippocampal atrophy in *APOE4* carriers but not non-carriers (Smith et al., 2014). However, one randomized trial found less improvement in executive function for *APOE4* carriers compared to non-carriers after an aerobic exercise intervention (Stern et al., 2019), highlighting the need for further exploration. Therefore, in Chapter 4 we assessed the impact of a year-long aerobic exercise intervention (see Figure 1-8) on hippocampal blood flow (HBF) for *APOE4* carriers compared to non-carriers. This investigation is meaningful not only because it is the first to assess HBF changes from exercise based on genetic risk, but also because improving HBF may be particularly crucial for this population, considering the previously mentioned interactions between cerebrovascular dysfunction and the *APOE4* allele on promoting AD development (Montagne et al., 2020; Shaaban et al., 2019).

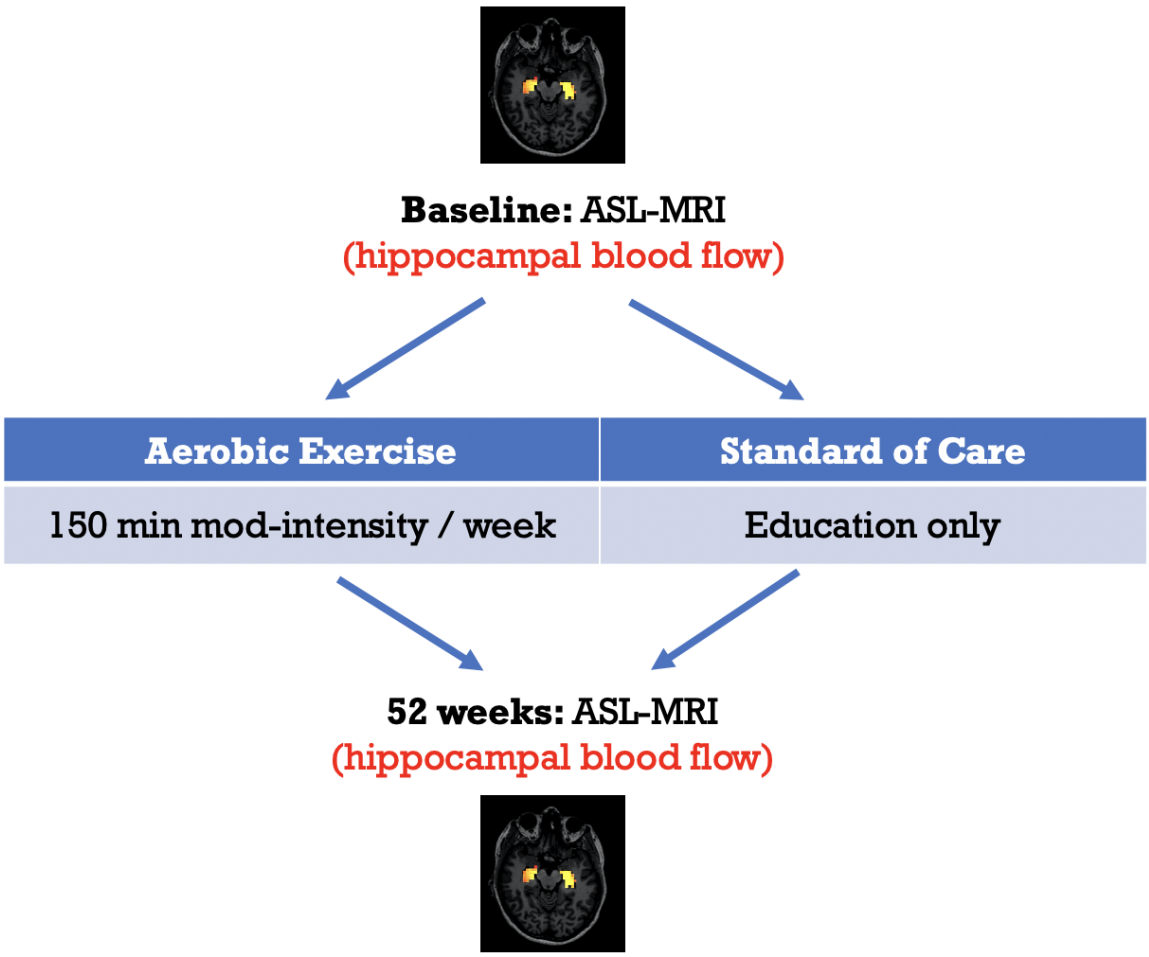

Figure 1-8: Overview of the clinical trial presented in Chapter 4

Older adults with normal cognition were randomized at a 2:1 ratio to a year-long aerobic exercise intervention or to standard of care. In this dissertation, we investigate the impact of this intervention on hippocampal blood flow measured with arterial spin labeling magnetic resonance imaging (ASL-MRI) for *APOE4* carriers compared to non-carriers.

Summary

New avenues for studying and understanding AD are desperately needed because the proportion of the population affected by AD continues to rise with relatively little progress in detection, prevention and treatment strategies (Association, 2019). As the strongest genetic risk factor for AD (Tai et al., 2016), *APOE4* provides a promising avenue for better understanding this disease, and novel observations into the mechanisms through which *APOE4* contributes to AD could benefit not only the estimated 27% of the U.S. population carrying the allele (Association, 2019) but all people affected by AD. Recent studies have suggested cerebrovascular dysregulation may play an early role in the development of AD (Iturria-Medina et al., 2016; Montagne et al., 2020; Sweeney et al., 2018), but relatively little is known about this pathophysiological mechanism and its relationship to *APOE4* carrier status. The following chapters provide insight into the pathophysiology of AD by expanding characterization of cerebrovascular function in *APOE4* carriers with and without AD. Additionally, we explore potential interactions between vascular risk factors and the *APOE4* allele, and we investigate the effects of an aerobic exercise intervention on hippocampal blood flow in *APOE4* carriers. Overall, the work in this dissertation informs understanding into the pathogenesis of late-onset AD - particularly for the population at highest genetic risk - while also inspiring new avenues for therapeutic interventions to potentially slow or prevent AD.

Chapter 2: Characterization of cerebrovascular hemodynamics for *Apolipoprotein E4* carriers with and without Alzheimer's disease

Introduction

Recent models of Alzheimer's disease (AD) pathogenesis place cerebrovascular dysfunction first in the pathological cascade, ahead of brain β-amyloid deposition (Iturria-Medina et al., 2016; Sweeney et al., 2018). Consequently, there is growing interest in understanding whether the strongest known genetic risk factor for late-onset AD, the *Apolipoprotein E4* allele (*APOE4*), acts mechanistically through cerebrovascular pathways to cause cognitive decline. Some studies suggest *APOE4* carriers have an accelerated age-related decline in cerebral blood flow (CBF) that is more pronounced in people with cognitive impairment (Lehtovirta et al., 1998; Thambisetty et al., 2010). In the present study, we sought to expand characterization of cerebrovascular function in individuals with the *APOE4* allele with and without AD with the goal of providing insight into the pathogenesis of late-onset AD.

Cerebrovascular resistance (CVRi) and conductance (CVCi)

While studies often employ CBF as a lone outcome measure for resting cerebrovascular function, more nuanced characterizations of blood flow such as cerebrovascular resistance, conductance, and pulsatility can provide unique insight and may have greater sensitivity to detect early cerebrovascular perturbations. Cerebrovascular resistance (CVRi) and the reciprocal, cerebrovascular conductance (CVCi), consider the amount of blood flow reaching the brain (CBF) *relative* to systemic perfusion pressure, typically approximated with mean arterial pressure, MAP. CVRi has been shown to significantly predict future cognitive decline and to have greater sensitivity than CBF alone (Gommer et al., 2012; Yew & Nation, 2017). For example, by using CVRi (MAP/CBF) as the outcome measure, one study found elevated CVRi in multiple brain regions in people with AD compared to controls, while these differences were

not apparent when measuring CBF alone, and elevated CVRi significantly predicted cognitive decline (Yew & Nation, 2017). Considering the potential connection between elevated CVRi and AD, here we hypothesized the *APOE4* allele may increase resistance in the cerebrovasculature of older adults. This elevated resistance would be consistent with at least one animal study showing *APOE4* transgenic mice have elevated CVRi (Koizumi et al., 2018). Likewise, the reciprocal of CVRi, CVCi, reflects vascular tone and thus provides an indication of the ability of the cerebrovasculature to transport blood to the brain parenchyma (Lautt, 1989), with higher CVCi reflecting improved conduction of oxygen and nutrients. Considering the blunted CBF observed in mice (Koizumi et al., 2018) and humans (Hogh et al., 2001) with an *APOE4* allele, we hypothesized CVCi would be lower in *APOE4* carriers than non-carriers and that this relationship would be amplified in people with early AD.

Pulsatility index (PI)

In addition to elevated CVRi, people with AD have been shown to have a higher pulsatility index (PI) compared to controls (Lim et al., 2017; Nation et al., 2013; Rivera-Rivera et al., 2017; Roher et al., 2011; Sabayan et al., 2012; van Beek et al., 2012; Yew & Nation, 2017). Elevated PI has been posited to directly damage the fragile cerebrovasculature, which subsequently promotes brain pathology and leads to cognitive decline (Mitchell et al., 2011; Suri et al., 2020). In this study, we sought to investigate whether this mechanism may be important for AD pathogenesis in *APOE4* carriers, with the hypothesis that *APOE4* carriers would have elevated PI that is exaggerated further in early AD.

Dynamic cerebrovascular autoregulation (dCA)

Characterizing cerebrovascular function during *dynamic* physiological stimuli provides complementary information to resting measures such as CVRi, CVCi and PI. Dynamic cerebrovascular autoregulation (dCA) reflects the ability of the cerebrovasculature to maintain relatively constant CBF in the face of acute fluctuations in perfusion pressure (MAP) (Aaslid et al., 1989; Labrecque et al., 2019; Tiecks et al., 1995; Tzeng & Ainslie, 2014). Unlike CVRi and PI, it remains unclear whether dCA is disrupted in people with AD (Claassen et al., 2009; de Heus et al., 2018; den Abeelen et al., 2014; Gommer et al., 2012; Marmarelis et al., 2017; Tarumi et al., 2014; van Beek et al., 2012), though this process has been shown to be impaired in other chronic diseases such as stroke (Eames et al., 2002; Xiong et al., 2017) and diabetes (Nasr et al., 2011; Vianna et al., 2015). However, a recent study in mice showed *APOE4* impairs the ability of the cerebrovasculature to compensate for reduced perfusion pressure, suggesting diminished autoregulation, and the resulting chronic hypoperfusion leads to brain parenchymal damage and cognitive decline (Koizumi et al., 2018). Thus, it seems plausible this impaired autoregulation may contribute to AD pathogenesis in humans who have an *APOE4* allele. In the present study, we sought to explore this connection by employing a sit-to-stand challenge to assess dynamic cerebrovascular autoregulation (dCA).

Study design and hypotheses

Here, we recruited older adults with and without early AD (N = 41). We continuously recorded beat-to-beat blood pressure and middle cerebral artery velocity (MCAv; a surrogate for CBF) at rest and during a sit-to-stand challenge. We hypothesized 1) *APOE4* carriers would have higher resistance (CVRi) and pulsatility (PI) and lower conductance (CVCi) at rest than non-

carriers, 2) *APOE4* carriers would have impaired dCA compared to non-carriers, as assessed through a sit-to-stand protocol, and 3) these effects would be magnified in those with early AD.

Methods

Participants

We recruited participants with and without early AD through the University of Kansas Alzheimer's Disease Center (KU ADC) clinical cohort. The following inclusion criteria applied to all participants: age 55-85 years; completion of 6+ grades of education; geriatric depression scale score < 6/15; auditory and visual acuity adequate for neuro-psychological testing; English fluency; stability of permitted medications for at least 4 weeks. The following additional inclusion criteria applied to only the early AD participants: subjective memory concerns; abnormal memory function documented by scoring below education-adjusted cutoffs on the Logical Memory II subscale (Delayed Paragraph Recall) from the Wechsler Memory Scale-Revised: a) $\leq$ 11 (out of 25) for 16 or more years of education, b) $\leq$ 9 for 8-15 years of education, and c) $\leq$ 6 for 0-7 years of education; Mini-Mental State Exam (MMSE) between 20 and 30; and Clinical Dementia Rating (CDR) of 0.5 or 1.0. Conversely, the following inclusion criteria were applied for the cognitively-normal (control) participants: MMSE score $\geq$ 26; CDR = 0; memory box score = 0; cognitively-normal based on absence of significant impairment in cognitive function and activities of daily living. The following exclusion criteria were applied for all participants: significant neurological diseases other than AD, such as Parkinson's disease, Huntington's disease, brain tumor, seizure disorder, or normal pressure hydrocephalus; major psychiatric disorders such as major depression, bipolar disorder or history of schizophrenia; history and presence of major cerebrovascular diseases such as stroke or transient ischemic

attack (TIA); severe carotid stenosis or a modified Hachinski score > 4; alcohol or substance abuse/dependence within the past two years; any significant systemic illness or unstable medical condition that could lead to difficulty participating in the study; pregnant, lactating or childbearing potential (women must be at least two years postmenopausal or surgically sterile); lab tests of clinically significant abnormalities in B12 or thyroid function; contraindications to MRI including pacemaker or metal fragments or foreign objects in the eyes, skin or body; absence of a temporal bone window to obtain reliable TCD signal; participation in clinical studies involving neuropsychological measures being collected more than one time per year which could confound the outcome measures of this study. Trained psychometricians at the KU ADC evaluated participants through neurological and neuropsychological examinations and a functional living scale examination following the National Alzheimer's Coordinating Center protocol guidelines and using the Uniform Data Set for data collection. The KU ADC holds weekly consensus meetings with clinicians (physicians, nurse practitioners) to confirm diagnosis (normal cognition or AD) based on clinical presentation and cognitive test scores. We obtained written informed consent prior to the commencement of study procedures in compliance with the Declaration of Helsinki and approved by the University of Kansas Human Subjects Committee (IRB00006196).

APOE Genotyping

DNA was isolated from whole blood for *APOE* genotyping. For most participants, genotyping was performed on National Centralized Repository for Alzheimer's Disease (NCRAD) samples by LGC Genomics (Beverly, MA) using KASP proprietary genotyping technology. Whole blood was collected into EDTA coated vacutainer tubes and sent at ambient

temperature for next-day delivery to NCRAD. For participants whose genotyping data were not available from NCRAD, in-house assessment of frozen whole blood was performed with a Taqman single nucleotide polymorphism (SNP) allelic discrimination assay (ThermoFisher). We excluded participants with an *APOE2* allele to avoid confounding due to the role of *APOE2* in reducing AD risk. Participants were then designated as *APOE4* carriers or non-carriers based on the presence of an *APOE4* allele. TCD sonographers were blinded to *APOE4* status.

Vascular laboratory visit setup

Participants were asked to abstain from food, tobacco, alcohol, caffeine, and vigorous exercise for at least 8 hours before the visit. Participants came to the laboratory within 3 months following their annual clinical evaluation. The laboratory room for the experimental protocol was kept dim and quiet to minimize external stimuli, and temperature was maintained at 22 to 24 degrees Celsius. Participants rested in the supine position for at least 10 minutes during equipment setup and prior to the recording. A five-lead ECG (Cardiocard, Nasiff Associates, Central Square, NY) continuously monitored heart rate. Continuous beat-to-beat blood pressure (BP) was acquired from the left middle finger (Finometer PRO; Finapres Medical Systems, Amsterdam, The Netherlands). Prior to the recording, an automated sphygmomanometer with a microphone (model Tango M2, Suntech, Morrisville, NC) measured brachial artery BP to ensure accurate calibration of the Finometer PRO (Billinger et al., 2017; Fisher et al., 2008). Ultrasonic gel was applied to the 2-MHz TCD probe (Multigon Industries, Yonkers, NY) which was placed over the right cranial temporal bone window using established practice standards in positioning and orienting to insonate the MCA (Alexandrov et al., 2012; Alexandrov et al., 2007). An adjustable headband was used to hold the probe in place against the temporal window. Gain, gate

and depth settings were adjusted to optimize the MCA signal. Depth remained in the 40-65 mm range to ensure insonation of the M1 segment of the MCA (Alexandrov et al., 2012; Alexandrov et al., 2007). A picture of the vascular laboratory setup can be found in Figure 2-1.

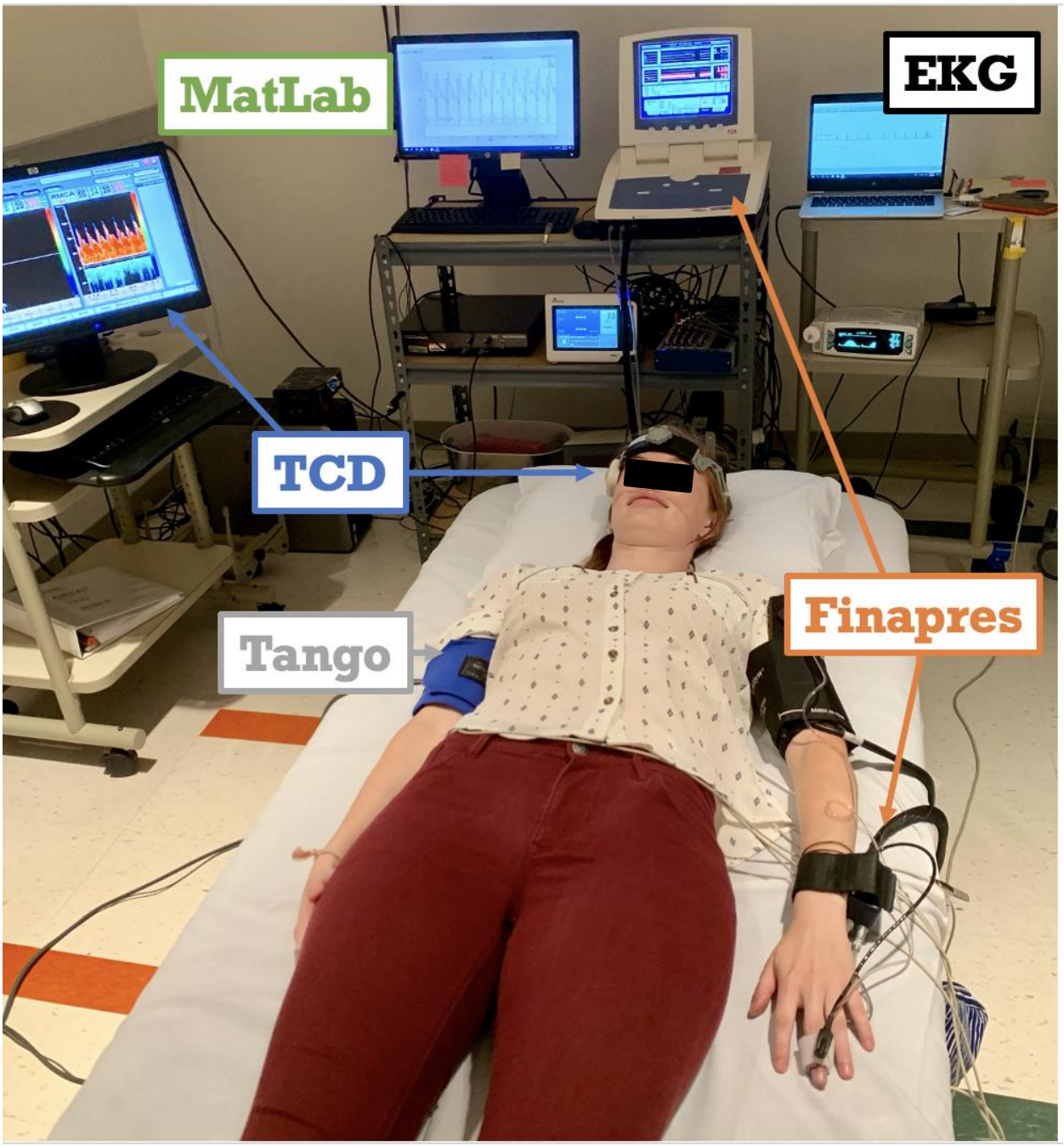

Figure 2-1: Vascular laboratory setup

For these experiments, the Finapres recorded mean arterial pressure (MAP), and the transcranial Doppler ultrasound (TCD) recorded middle cerebral artery velocity (MCAv). The Tango allowed for calibration of the Finapres. The electrocardiogram (EKG) monitored heart rate and captured R-waves. All data were collected through a continuous recording in MATLAB. The sit-to-stand recording used identical devices and setup except the participant was seated on the edge of the bed with his/her hand over the heart (as pictured in Figure 1-4) in order to transition from sitting to standing during the recording.

Vascular laboratory visit data collection

Participants were asked to keep their eyes open and abstain from talking during the recordings. Raw data were acquired through an analog-to-digital unit (NI-USB-6212, National Instruments) and custom written software operating in MATLAB (v2019a, The Mathworks Inc. Natick, MA). Sampling was performed at 500 Hz.

Supine rest recording

We first recorded from all devices for 8 minutes of supine rest. The MCAv and mean arterial pressure (MAP) were obtained as the area under the respective curve for each heartbeat. We calculated mean MCAv and mean MAP. Average resting cerebrovascular resistance index (CVRi) and cerebrovascular conductance index (CVCi) were then calculated as:

CVRi = mean MAP / mean MCAv (Yew & Nation, 2017)

CVCi = mean MCAv / mean MAP (Labrecque et al., 2019)

As there is some controversy in the field over which index is preferred (CVRi or CVCi), we have chosen to include both as outcome measures in this study. Though they are reciprocals, CVRi and CVCi are thought to provide slightly different characterization of the state of the cerebrovasculature. Specifically, while they both are thought to reflect arterial vascular tone, CVRi has been proposed to be the best index during constant flow when vascular tone changes primarily lead to differences in perfusion pressure gradient, while CVCi is considered superior in situations when vascular tone changes lead primarily to changes in flow (Lautt, 1989). In practice, both indices are regularly employed as a method of normalizing CBF (or MCAv) to

MAP (Harrell et al., 2019; Yew & Nation, 2017), and we report both measures here for completeness' sake.

We also calculated resting mean pulsatility index (PI) as:

$$PI = (\text{Mean Systolic MCAv} - \text{Mean Diastolic MCAv}) / \text{Mean MCAv}$$

(Roher et al., 2011)

Sit-to-stand recording

The participant was transitioned from supine to seated rest. The left hand was placed over the heart with elbow bent and middle finger at heart level secured by gauze tape (see Figure 1-4). Participants rested in the seated position for a minimum of 5 minutes prior to the recording to ensure resting hemodynamic equilibrium. MCAv and MAP were recorded for 5 minutes of seated rest, during the transition from sitting to standing, and for 5 minutes of standing upright. We assessed dCA using metrics (see Figure 2-2) that have been employed in previous publications by colleagues who taught us the sit-to-stand technique (Labrecque et al., 2019; Labrecque et al., 2017):

1. The absolute raw (Δ) and relative (%Δ) reductions in MCAv and MAP after standing were calculated as the difference between the baseline MCAv and MAP (averaged over the 15 seconds immediately prior to standing) and the nadir MCAv and MAP (minimum reached after standing).

2. The percent reduction in MCAv per percent reduction in MAP was calculated by dividing the %ΔMCAv by %ΔMAP from metric 1.

3. The Time Delay (TD) was determined as the number of seconds elapsed after standing before the onset of cerebrovascular regulation. Specifically, CVCi was calculated for each heartbeat and plotted as a function of time. Two observers blinded to *APOE4*-status independently identified the time at which CVCi began to increase constantly without any subsequent transient decrease (Labrecque et al., 2019; Labrecque et al., 2017). The number of seconds elapsed between time 0 (moment of standing) and this moment of continuously increasing CVCi denoted the TD.

4. The Rate of Regulation (RoR) was calculated over the 4 heartbeats immediately following individually-determined onset of regulation (TD) as:

$$RoR = (\Delta CVCi/\Delta t)/\Delta MAP2$$

where ΔMAP2 was calculated by subtracting baseline MAP from average MAP over the interval, ΔCVCi was the change in CVCi over the interval, and Δ*t* was the change in time (seconds) over the interval of regulation.

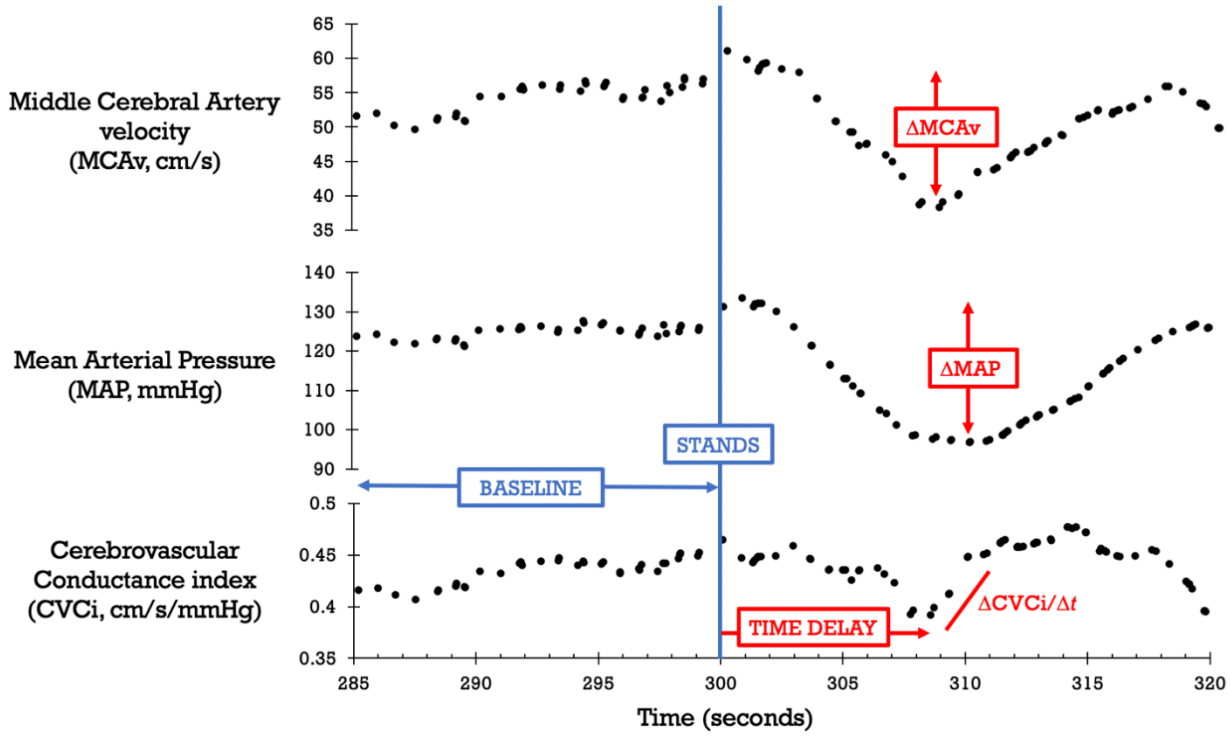

Figure 2-2: Representative sit-to-stand data from a single participant

Middle cerebral artery velocity (MCAv) and mean arterial pressure (MAP) were recorded, and cerebrovascular conductance index (CVCi) was calculated as MCAv/MAP for each heartbeat. All three measures are graphed here as beat-to-beat data on a simultaneous timeline for a single participant. We asked the participant to stand at 300 seconds as noted in the example ("STANDS"). We calculated baseline values for MCAv and MAP as the average over the 15 seconds immediately prior to standing. We defined nadir values as the minimum MCAv and MAP reached after standing. ΔMCAv and ΔMAP were then calculated as the absolute difference between baseline average and nadir. The time delay was determined as the number of seconds elapsed before the onset of regulation (here, 8.5 seconds). The $\Delta CVCi/\Delta t$ was included as the numerator in the calculation for rate of regulation (RoR), a metric that also controls for MAP over the regulatory interval relative to baseline MAP.

Statistical analyses

We used SPSS Statistics (IBM) for all statistical analyses. We performed a multiple regression analysis to assess the impact of *APOE4* carrier status, diagnosis (healthy control or early AD), and the interaction between *APOE4* status and diagnosis, controlling for age and sex, on each of the resting and dCA outcome variables.

Results

We completed data collection on 41 participants. After excluding *APOE2* carriers and participants with an insufficient temporal window, the present analysis included 32 participants (28% early AD, 41% *APOE4* carriers, 47% women, 74.5 ± 6.5 years old).

Supine rest recording

We fitted three separate multiple regression models to predict mean resting CVRi, CVCi and PI from age, sex, *APOE4* carrier status, diagnosis and the interaction between *APOE4* carrier status and diagnosis. The models met assumptions of linearity, independence of residuals, homoscedasticity, normality and no outliers or multicollinearity. The multiple regression model was not significant for CVRi ($F(5, 26) = 1.260$, $p = 0.311$) or CVCi ($F(5, 26) = 0.681$, $p = 0.642$). The multiple regression model was significant for PI, $F(5, 26) = 4.543$, $p = 0.004$, adjusted $R^2 = 0.364$. This was driven by the significant effect of age on PI ($p < 0.001$). Detailed results for these regression models are found in Table 2-1.

Table 2-1: Multiple regression results for supine rest recording

	Cerebrovascular Resistance index (CVRi)		**Cerebrovascular Conductance index (CVCi)**		**Pulsatility index (PI)**	
	β	***p***	**β**	***p***	**β**	***p***
Age (years)	-0.173	0.363	0.123	0.534	0.611	< 0.001*
Sex (M)	-0.164	0.383	0.005	0.978	0.170	0.268
***APOE4* (+)**	0.171	0.444	-0.195	0.406	0.093	0.610
Diagnosis (early AD)	-0.267	0.282	0.213	0.412	-0.293	0.152
***APOE4* (+) x Diagnosis (early AD)**	-0.130	0.642	0.091	0.757	0.298	0.199

The dependent variable predicted by each regression model was resting CVRi, CVCi or PI, as indicated.
β = standardized coefficient; *significant ($p < 0.05$).

Sit-to-stand recording

The TCD signal was lost or had excessive noise during the sit-to-stand recording for three participants, leaving 29 participants for the dCA analysis. We ran separate multiple regression models to predict absolute raw (Δ) and relative (%Δ) reductions in MCAv and MAP, percent reduction in MCAv per percent reduction in MAP, TD and RoR from age, sex, *APOE4* carrier status, diagnosis and the interaction between *APOE4* carrier status and diagnosis. The models met assumptions of linearity, independence of residuals, homoscedasticity, normality and no outliers or multicollinearity. The multiple regression model was not significant for ΔMCAv, $F(5, 23) = 2.004$, $p = 0.116$, but age added significantly to the model ($p = 0.039$). The multiple regression model was not significant for %ΔMCAv ($F(5, 23) = 1.856$, $p = 0.142$), ΔMAP ($F(5, 21) = 0.644$, $p = 0.669$), or %ΔMAP ($F(5, 21) = 0.637$, $p = 0.674$). The multiple regression model significantly predicted percent reduction in MCAv per percent reduction in MAP (%ΔMCAv/%ΔMAP), $F(5, 20) = 4.413$, $p = 0.007$, adjusted $R^2 = 0.406$, with age adding significantly to the model ($p = 0.003$). The multiple regression model significantly predicted TD, $F(5, 23) = 2.933$, $p = 0.034$, adjusted $R^2 = 0.257$, with age adding significantly to the model ($p = 0.005$). The multiple regression model was not significant for RoR, $F(5, 21) = 0.286$, $p = 0.916$. Detailed results for these regression models are found in Table 2-2.

Table 2-2: Multiple regression results for sit-to-stand recording

	ΔMCAv		%ΔMCAv		ΔMAP		%ΔMAP		%ΔMCAv/ %ΔMAP		Time Delay (TD)		Rate of Regulation (RoR)	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Age (years)	-0.399	0.039*	-0.344	0.075	-0.254	0.266	0.357	0.123	-0.559	0.003*	-0.531	0.005*	0.226	0.317
Sex (M)	0.096	0.596	0.228	0.221	-0.254	0.238	0.024	0.910	0.289	0.086	0.340	0.056	0.030	0.891
***APOE4* (+)**	0.267	0.205	0.226	0.287	-0.042	0.871	0.205	0.428	0.166	0.394	0.094	0.639	0.007	0.977
Diagnosis (early AD)	-0.124	0.575	-0.244	0.282	0.131	0.617	0.224	0.393	-0.379	0.066	-0.143	0.513	0.031	0.908
***APOE4* (+) x Diagnosis (early AD)**	-0.187	0.427	-0.100	0.673	-0.149	0.594	-0.296	0.294	0.092	0.665	0.026	0.914	-0.130	0.649

The dependent variable predicted by each regression models was change in middle cerebral artery velocity (ΔMCAv), percent ΔMCAv (%ΔMCAv), change in mean arterial pressure (ΔMAP), %ΔMAP, %ΔMCAv/%ΔMAP, TD or RoR, as indicated. β = standardized coefficient; *significant ($p < 0.05$).

Discussion

In the present study, we sought to characterize cerebrovascular function in *APOE4* carriers and non-carriers with and without early AD. We found no significant differences in resting (CVRi, CVCi, and PI) or dCA (ΔMCAv, %ΔMCAv, ΔMAP, %ΔMAP, %ΔMCAv/ %ΔMAP, TD, RoR) cerebrovascular metrics based on *APOE4* carrier status. This suggests *APOE4* carriers in our sample did not have significantly impaired cerebrovascular function compared to non-carriers. Likewise, the *APOE4* allele was not found to interact with AD diagnosis for the outcome variables, suggesting the connection between the cerebrovascular measures and the *APOE4* allele was not influenced by AD diagnosis. Overall, our current results do not provide significant evidence for the role of *APOE4* in promoting cerebrovascular dysfunction, and future studies are needed to investigate this potential mechanism.

The MCA measures taken at rest in the current study (CVRi, CVCi and PI), though related, provide different information about cerebrovascular health. CVRi reflects the amount of downstream resistance, which can prevent adequate CBF (Yew & Nation, 2017), while CVCi reflects conductance in the vessel, or the amount of blood supply to the brain per a given perfusion pressure (Lautt, 1989). Meanwhile, PI reflects the pulsatile quality of flow, which can cause damage to the microvasculature and brain parenchyma (Zarrinkoob et al., 2016).

Cerebrovascular resistance (CVRi) and conductance (CVCi)

Abnormally elevated CVRi (or low CVCi) can be detrimental to brain function as it may impair delivery of required glucose and oxygen as well as clearance of harmful metabolic byproducts from the brain (Yew & Nation, 2017). In the present study, we found no difference in

CVRi or CVCi in people with AD compared to healthy controls. In fact, although not significant ($p = 0.282$), our participants with AD tended to have a lower CVRi than the control group, which contrasts with previous studies that have reported elevated CVRi for people with AD (Nation et al., 2013). The reason for this difference is unknown but may stem from protocol variances. For example, some of these prior studies utilized MRI instead of TCD to measure CBF (Nation et al., 2013; Yew & Nation, 2017). This MRI methodology for imaging CBF allows for regional analysis, and the CVRi elevations they identified occurred in specific and relatively small brain areas (Nation et al., 2013; Yew & Nation, 2017). In contrast, the approach used in the current study uses MCAv as a surrogate for overall CBF and therefore may miss aberrations that are detected through discrete regional analyses. Additionally, some studies recruited participants of a younger age (Yew & Nation, 2017), different sex distribution (van Beek et al., 2012), or at more advanced stages of AD (van Beek et al., 2012). For our question of interest, we assessed the effect of the *APOE4* allele on CVRi and CVCi. Studies in *APOE4* transgenic mice have shown the human *APOE4* allele reduces CBF with no change in MAP (Koizumi et al., 2018), which would translate to elevated CVRi and blunted CVCi. Thus, we hypothesized the same phenomenon would be observed in our human participants and that this effect would be magnified in the AD population. We did not find substantial support for this hypothesis, as there was no significant effect of the *APOE4* allele on CVRi ($p = 0.444$) or CVCi ($p = 0.406$). It is worth noting, however, that unlike the relationship observed for AD diagnosis, our participants with an *APOE4* allele did tend to have a higher CVRi and lower CVCi than non-carriers. This suggests the animal model findings may translate to the human population given a sufficiently larger sample size.

Pulsatility index (PI)

In addition to CVRi and CVCi, we measured PI to further characterize resting cerebral hemodynamics in our participants. By measuring PI in the MCA, we can estimate the degree to which potentially harmful pulsatile flow is being regularly transmitted to the brain, with higher PI reflecting a larger degree of pulsatility and increased risk of damage (Zarrinkoob et al., 2016). Multiple studies have demonstrated significantly elevated PI in people with AD compared to age-matched controls (Jin et al., 2017; Lim et al., 2017; Roher et al., 2011; Sabayan et al., 2012), and PI has been found to be negatively associated with cognitive function and positively associated with brain atrophy in older adults (Mitchell et al., 2011). As with CVRi, we found no significant effect on PI of AD diagnosis or *APOE4* carrier status, but PI tended to be unexpectedly lower in the AD group and, as hypothesized, higher in the *APOE4* carriers. The reason for the disparity in the effect of AD diagnosis on PI compared with previous studies, although not significant, is unknown but may be due to prior studies measuring PI in other vessels instead of the MCA such as the internal carotid artery and basilar artery (Jin et al., 2017) or recruiting patients with more advanced AD (Lim et al., 2017) which could cause more extreme aberrations in cerebrovascular metrics compared to early AD. PI has been consistently shown to increase with age in older adults (Safar, 2010; Zarrinkoob et al., 2016), and that relationship was readily apparent in our data ($p < 0.001$).

Dynamic Cerebrovascular Autoregulation (dCA)

In addition to resting measures, we assessed cerebrovascular response to the physiological challenge of transient hypotension induced by standing, called dynamic cerebrovascular autoregulation (dCA). Tight dCA is required to maintain relatively constant CBF

in the face of acute blood pressure fluctuations associated with activities of daily living, and failure to compensate for these changes in perfusion pressure could result in inadequate delivery of oxygen and nutrients to the brain (Lassen, 1959). Inadequate delivery of vital supplies to the brain parenchyma could then result in ongoing brain damage and a decline in cognitive function (Yew & Nation, 2017). Impaired dCA has been observed in disease states commonly associated with cerebrovascular dysfunction, including diabetes (Nasr et al., 2011; Vianna et al., 2015) and stroke (Eames et al., 2002; Xiong et al., 2017), but studies characterizing dCA in humans with AD have found conflicting results (Claassen et al., 2009; de Heus et al., 2018; den Abeelen et al., 2014; Gommer et al., 2012; Marmarelis et al., 2017; Tarumi et al., 2014; van Beek et al., 2012). Intriguingly, mouse models expressing human *APOE4* show impaired autoregulation, as evidenced by exaggerated hypoperfusion following bilateral carotid artery stenosis (Koizumi et al., 2018). Importantly, this inability to counteract the drop in perfusion by vasodilating and increasing brain blood flow led to white matter damage and cognitive decline in these mice (Koizumi et al., 2018). Thus, we hypothesized the same mechanism may occur in human *APOE4* carriers and could contribute to AD pathogenesis for this population. Although we found no significant effect of *APOE4* carrier status on the dCA outcome variables (ΔMCAv, %ΔMCAv, ΔMAP, %ΔMAP, %ΔMCAv/ %ΔMAP, TD, RoR), the general direction of the association was consistent with our hypothesis. For example, *APOE4* carriers tended to experience a slower regulatory response and greater reduction in MCAv after standing than non-carriers. This could contribute to brain dysfunction in the long-term if inadequate oxygen and nutrients are delivered to working neurons in states of transiently reduced perfusion pressure, but future studies are needed to identify a statistically significant relationship.

Interestingly, the only independent variable that significantly ($p < 0.05$) predicted multiple metrics of dCA was age. Specifically, when controlling for sex, *APOE4* carrier status and AD diagnosis, increasing age was associated with better dCA. For example, older participants had a quicker regulatory response of the cerebrovasculature after standing (decreased TD) compared to younger participants, and older age predicted an attenuated reduction in MCAv per MAP reduction. This suggests the aging cerebrovasculature not only retains the ability to maintain CBF in the face of hypoperfusion but may actually improve this ability. While this may seem counterintuitive, this phenomenon has been observed in previous studies, though it is often described in these publications as "preserved" rather than "improved" dCA with aging (Beishon et al., 2021; Lipsitz et al., 2000; Sorond et al., 2005). Thus, our current finding of improved dCA metrics with increasing age is consistent with the existing literature.

Study considerations

The present study has a number of limitations. Transcranial Doppler ultrasound is necessary to assess cerebrovascular function during dynamic stimuli such as transitioning from sitting to standing (Labrecque et al., 2017), but this method requires the assumption of constant MCA diameter in order to intepret MCAv as a surrogate for CBF, which is often listed as a limitation. However, while this assumption may not be accurate in extreme circumstances, the data suggest minimal changes in diameter occur during stimuli such as that employed in the present study (Coverdale et al., 2014; Ryan L. Hoiland & Philip N. Ainslie, 2016; Schreiber et al., 2000; Verbree et al., 2017). There are numerous methods for measuring dCA in addition to sit-to-stand such as transfer function analysis, thigh-cuff deflation and squat-to-stand protocols, and metrics from these various methods have been shown to be unrelated or show only

weak/moderate correlations (Tzeng et al., 2012). Thus, our current findings may not be generalizable to dCA under other physiological stimuli. As this was an observational, cross-sectional study, we cannot determine temporality or causality. Our sample population included individuals in only the earliest stages of AD and thus does not represent physiological status in later stages of the disease. This recruitment strategy may have also hindered the ability to identify significant interactions between AD diagnosis and the *APOE4* allele. Finally, data collection was impeded for these Chapter 2 experiments due to the COVID-19 pandemic. In the future, we hope this section topic will be expanded to include a larger sample size in order to increase power to detect potential differences among groups.

Conclusion

Overall, we found no significant effect of *APOE4* carrier status or interaction between *APOE4* carrier status and AD diagnosis on resting cerebrovascular or autoregulation measures. Older age was significantly associated with higher PI and improved metrics of dCA. Future studies with larger sample sizes are needed to further investigate cerebrovascular dysfunction as a potential mechanism of AD pathogenesis for people at highest genetic risk.

Chapter 3: *Apolipoprotein E4* moderates the association between vascular risk factors & brain pathology

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https://journals.lww.com/alzheimerjournal/Abstract/9000/Apolipoprotein_E4_Moderates_the_Association.99245.aspx

Introduction

Cerebrovascular dysfunction is increasingly recognized as an early, causal agent in the pathogenesis of late-onset Alzheimer's disease (AD) (Iturria-Medina et al., 2016; Lorius et al., 2015; Montagne et al., 2020; Sweeney et al., 2018; Wolters et al., 2017). One prominent model of AD now places brain vasculature abnormalities – a historically under-recognized mechanism of disease development - ahead of all other biomarker changes, including β-amyloid and tau deposition (Sweeney et al., 2018). Adults with the *Apolipoprotein E4* (*APOE4*) allele, the strongest known genetic risk factor for AD, demonstrate an accelerated age-related cerebral blood flow (CBF) decline compared to non-carriers (Thambisetty et al., 2010). Additionally, cognitively-normal *APOE4* carriers exhibit early blood brain barrier breakdown that occurs independently of β-amyloid and tau and predicts subsequent cognitive decline (Montagne et al., 2020).

The *APOE4* allele not only increases risk of cardiovascular disease, such as myocardial infarction and stroke (Belloy et al., 2019), but may also act synergistically with cardiovascular risk factors to promote dementia development (Caselli et al., 2011a; Gupta et al., 2015; Helzner et al., 2009; Nation et al., 2016; Shaaban et al., 2019; Zade et al., 2010). For example, cognitive dysfunction is more significantly associated with vascular risk factors like high cholesterol in *APOE4* carriers than non-carriers (Caselli et al., 2011a), suggesting elevated cholesterol (and particularly pro-atherogenic cholesterol) (Blaha et al., 2008) may preferentially promote brain pathology in *APOE4* carriers. Additionally, *APOE4* exacerbates the deleterious effects of cerebrovascular risk factors on neuropsychological performance (Zade et al., 2010), and stroke has been shown to act synergistically with the *APOE4* allele to increase dementia risk (Helzner et al., 2009; Shaaban et al., 2019).

Cerebrovascular dysfunction has been posited to contribute causally to *β*-amyloid deposition in AD pathogenesis (Kisler et al., 2017). Reduced CBF at rest occurs prior to *β*-amyloid deposition in *APOE4* carriers (Michels et al., 2016). Subtle changes in cerebrovascular resistance and conductance can be detected even earlier than global CBF reductions in early stages of disease development (Yew & Nation, 2017). Therefore, a better understanding of the relationship between resting cerebrovascular conductance and *β*-amyloid load could provide insight into the connection between cerebrovascular perturbations and AD-associated brain pathology, particularly for *APOE4* carriers. In addition to resting measures, assessing cerebrovascular function during exercise allows for a dynamic characterization of the cerebrovasculature. Changes in CBF during an acute bout of exercise reflect the cerebrovascular response to a wide variety of physiological inputs, including altered perfusion pressure, arterial blood gas, neural activity and brain metabolism (Smith & Ainslie, 2017). Our group previously demonstrated a blunted cerebrovascular response to moderate-intensity exercise with aging (Ward et al., 2018), stroke (Kaufman et al., 2019; Kempf, 2019), and *β*-amyloid deposition (Sisante et al., 2019). Importantly, our exercise stimulus provides a physiological challenge similar to common daily activities such as walking up a flight of stairs (Ferguson, 2014), suggesting these perturbations could have implications for day-to-day functioning. Still, questions remain regarding the relationship between peripheral vascular risk factors and this dynamic response of the cerebrovasculature with acute exercise, particularly for *APOE4* carriers who are at the highest known genetic risk of AD.

In the present analysis, we sought to investigate whether cerebrovascular dysfunction would more strongly predict *β*-amyloid load in *APOE4* carriers than non-carriers. Additionally, we aimed to explore whether high levels of pro-atherogenic cholesterol, one of the strongest

predictors of cardiovascular disease sequelae (including future stroke and myocardial infarction) (Di Angelantonio et al., 2009), would be more strongly associated with cerebrovascular dysfunction in *APOE4* carriers than non-carriers. To interrogate these aims, we assessed CBF velocity data collected with transcranial Doppler ultrasound (TCD) both at rest and during exercise in cognitively-normal older adults (N = 54). We measured β-amyloid load by Positron Emission Tomography (PET) (Sisante et al., 2019) and quantified pro-atherogenic blood cholesterol as non-high-density lipoprotein (non-HDL) cholesterol (Blaha et al., 2008; Di Angelantonio et al., 2009; Hawkes, 2019; Kastelein et al., 2008). We hypothesized that (1) poor resting cerebrovascular conductance (CVCi) would be more strongly associated with β-amyloid deposition in *APOE4* carriers than non-carriers, (2) the acute cerebrovascular response to exercise (ΔMCAv) would be blunted in *APOE4* carriers, and (3) this blunted ΔMCAv would be more strongly associated with elevated non-HDL cholesterol in *APOE4* carriers than non-carriers.

Methods

Participants

As noted previously (Alwatban et al., 2020; Kaufman et al., 2020; Liu et al., 2019; Perdomo et al., 2019; Sisante et al., 2019), we recruited a convenience sample of older adults from a registry of individuals interested in research. Inclusion criteria were: (1) between 65 to 90 years of age, (2) cognitively-normal/non-demented based on neuropsychological testing, (3) underactive or sedentary lifestyle, and (4) completion of a [18F-AV45] florbetapir Positron Emission Tomography (PET) scan within six months of the vascular laboratory visit. Exclusion criteria were: diagnosis of insulin-dependent diabetes, depression, congestive heart failure and

inability to exercise. The University of Kansas Institutional Review Board approved all study procedures (IRB#: STUDY00001444). The study complied with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to data collection.

Positron Emission Tomography (PET) scan for β-amyloid

Approximately 50 minutes after florbetapir (370 MBq) administration, the GE Discovery ST-16 PET/CT Scanner acquired two continuous 5-minute PET brain frames. We summed and attenuation corrected the frames. We then calculated standardized uptake value ratio to the whole cerebellum (SUVR) using a custom processing pipeline in SPM12 (http://www.fil.ion.ucl.ac.uk/spm), described previously (Liu et al., 2019).

Clinic visit

Medication information and cholesterol levels (total and high-density lipoprotein, HDL) were obtained during the clinic visit 1-2 months before the vascular laboratory visit. We chose to utilize non-HDL cholesterol ("bad" cholesterol) (Hawkes, 2019) as the predictor variable because studies have shown non-HDL cholesterol is an even more accurate marker for cardiovascular risk than LDL cholesterol (Blaha et al., 2008; Di Angelantonio et al., 2009; Kastelein et al., 2008). For example, one meta-analysis of 68 studies suggested non-HDL cholesterol was the best predictor for stroke and coronary artery disease among cholesterol measures (Di Angelantonio et al., 2009). This may be due to the fact that non-HDL cholesterol includes all pro-atherogenic particles in the blood, like very-low-density lipoprotein (VLDL) and

intermediate-density lipoprotein (IDL) cholesterol, in addition to LDL (Blaha et al., 2008). Non-HDL cholesterol was calculated as:

Non-HDL cholesterol = Total cholesterol – HDL cholesterol (Virani et al., 2011)

Apolipoprotein E (APOE) genotyping

Frozen whole blood was assessed using a Taqman single nucleotide polymorphism (SNP) allelic discrimination assay (ThermoFisher) to determine *APOE* genotype. Taqman probes to the two APOE-defining SNPs, rs429358 (C_3084793_20) and rs7412 (C_904973_10), distinguished *APOE4*, *APOE3*, and *APOE2* alleles. We excluded participants with an *APOE2* allele (whether paired with *APOE3* or *APOE4*), as this allele is associated with significantly lower risk of AD and can therefore confound the results when assessing for *APOE4* effects. There were also no *APOE4* homozygotes in our sample. Therefore, all participants were classified as either *APOE4* carriers (*APOE3/APOE4* genotype) or non-carriers (*APOE3/APOE3* genotype).

Vascular laboratory setup

Visits to our laboratory occurred in the morning (beginning between 7:30 and 9 a.m.) for each participant and have been described in detail previously (Alwatban et al., 2020; Kaufman et al., 2020; Liu et al., 2019; Perdomo et al., 2019; Sisante et al., 2019). We monitored end-tidal carbon dioxide ($P_{ET}CO_2$) with a nasal cannula and capnograph (BCI Capnocheck 9004) and heart rate (HR) with a 5-lead electrocardiogram (Cardiocard, Nasiff Associates). We continuously recorded beat-to-beat blood pressure with a finger plethysmograph (Finometer Pro, Finapres Medical Systems), from which we calculated mean arterial pressure (MAP). We insonated the

left middle cerebral artery velocity (MCAv) using a 2-MHz transcranial Doppler ultrasound (TCD) probe (RobotoC2MD, Multigon Industries) placed over the temporal window. TCD sonographers were blinded to β-amyloid status, *APOE* genotype, medication use and cholesterol profile.

Rest recording

The participant rested quietly for at least 15 minutes during setup. We then used an analog-to-digital data acquisition board (National Instruments) and custom-written script for MATLAB (v2015, Mathworks) to sample MCAv and MAP continuously at 500 Hz for 8 minutes of seated rest. MCAv and MAP were averaged over the 8 minutes, and the resting cerebrovascular conductance index (CVCi) was calculated as:

$$\text{CVCi} = \text{MCAv}/\text{MAP}$$ (Labrecque et al., 2019)

Exercise recording

Participants were instructed to maintain a rate of 90 steps per minute on the recumbent stepper (NuStep T5XR). Resistance started at 40 Watts and was increased until each participant reached his or her pre-determined target HR range for moderate-intensity exercise (40-60% of age-predicted HR reserve). Once at target HR, data collection continued until the participant completed 8 minutes of continuous exercise in the moderate-intensity HR range. We averaged MCAv over the 8 minutes of exercise and calculated the cerebrovascular response to exercise (ΔMCAv) as:

ΔMCAv = mean MCAv during exercise – mean MCAv at rest (Sisante et al., 2019)

Statistical Analyses

We used SPSS Statistics (IBM) for all statistical analyses. We performed a multiple linear regression analysis to assess the effect of *APOE4* carrier status, resting CVCi and the interaction between *APOE4* carrier status and resting CVCi on *β*-amyloid deposition, controlling for age, sex, HDL cholesterol and statin use. Likewise, we performed a multiple linear regression analysis to assess the effect of *APOE4* carrier status, non-HDL cholesterol and the interaction between non-HDL cholesterol and *APOE4* carrier status on ΔMCAv, controlling for age, sex, *β*-amyloid deposition, HDL cholesterol and statin use. Finally, as an exploratory analysis to investigate the potential influence of brain volumes on these measures, we added gray and white matter volumes to each multiple regression model.

Results

We included 54 participants (65% female, 32% *APOE4* carriers, 71.1 ± 5.5 years old) who had complete data for *APOE* genotype, *β*-amyloid load, cholesterol levels, and resting MCAv and MAP.

Cerebrovascular function at rest

We ran a multiple linear regression to predict *β*-amyloid deposition from age, sex, statin use, *APOE4* carrier status, HDL cholesterol, non-HDL cholesterol, resting CVCi and the

interaction between resting CVCi and *APOE4* carrier status. There was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values. There was independence of residuals, as assessed by a Durbin-Watson statistic near 2. There was no evidence of multicollinearity, as assessed by tolerance values greater than 0.1. There was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values. Data met the assumption of normality, as assessed by a P-P Plot. There were no outliers, as assessed by no standardized residuals greater than ±3 standard deviations (SD).

The multiple regression model significantly predicted β-amyloid deposition, $F(8, 45) = 2.228$, $p = 0.043$, $R^2 = 0.284$, adj. $R^2 = 0.156$. Regression coefficients and standard errors can be found in Table 3-1. There was a significant interaction between resting CVCi and *APOE4* carrier status ($p = 0.026$), suggesting the relationship between CVCi and β-amyloid deposition is moderated by the *APOE4* allele. Specifically, a larger resting CVCi, which denotes better cerebrovascular conductance, predicted lower β-amyloid deposition for *APOE4* carriers but not non-carriers, while *APOE4* carriers with poor conductance tended to have higher levels of β-amyloid deposition. There was no apparent relationship between CVCi and β-amyloid deposition for the non-carriers (see Figure 3-1). There was no significant effect of *APOE4* carrier status ($p = 0.054$) or resting CVCi ($p = 0.260$) on β-amyloid deposition.

Table 3-1: Multiple regression analysis results for β-amyloid deposition

Variable	*B*	SE_B	β	*p*-value
Intercept	1.062	0.330		0.002*
Age	0.003	0.004	0.099	0.455
Sex (Male)	-0.065	0.049	-0.201	0.192
Statin use (+)	0.012	0.046	0.040	0.788
***APOE4* carrier status (+)**	0.085	0.043	0.256	0.054
HDL cholesterol	-0.001	0.001	-0.105	0.507
Non-HDL cholesterol	-0.001	0.001	-0.292	0.067
Resting CVCi	0.164	0.144	0.213	0.260
Resting CVCi x *APOE4* carrier status (+)	-0.476	0.207	-0.433	0.026*

B = unstandardized regression coefficient; SE_B = standard error of the coefficient; β = standardized coefficient; *significant ($p < 0.05$), *APOE4* = *Apolipoprotein E4*; HDL = High-Density Lipoprotein; CVCi = Cerebrovascular Conductance index

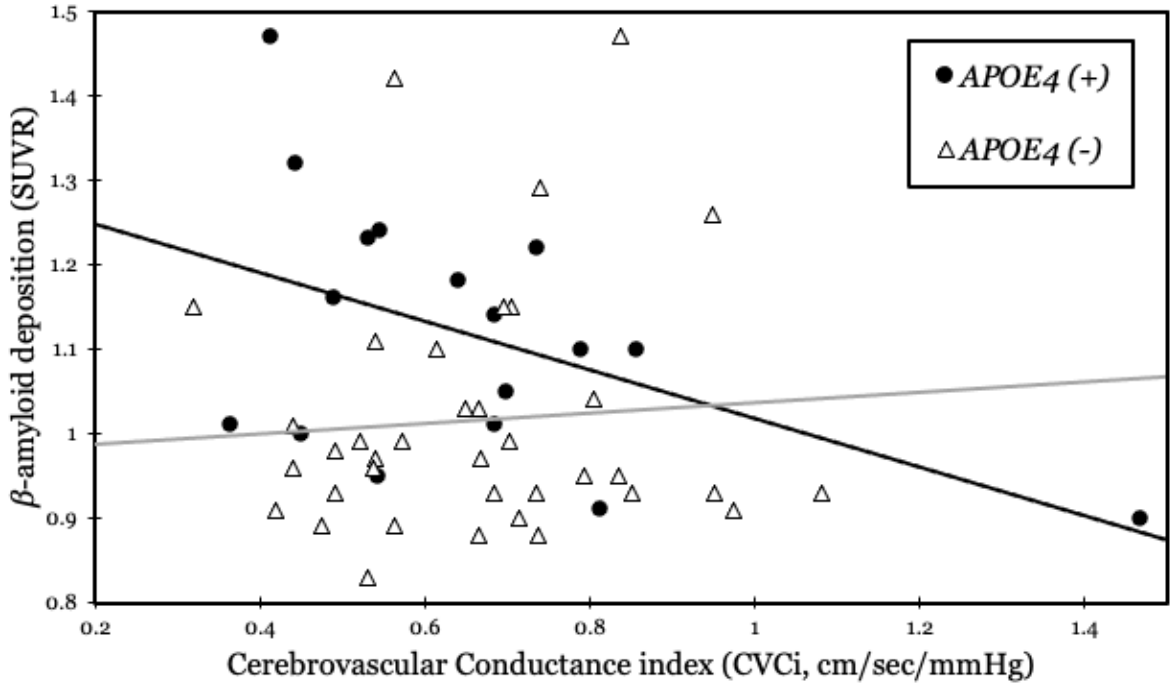

Figure 3-1: Relationship between β-amyloid deposition and resting Cerebrovascular Conductance index (CVCi) for *APOE4* carriers and non-carriers

For *APOE4* carriers, poor CVCi in the middle cerebral artery was associated with greater brain *β*-amyloid load. In contrast, *β*-amyloid load was not related to CVCi for *APOE4* non-carriers. These findings suggest cerebrovascular dysfunction may promote AD-related brain pathology preferentially in *APOE4* carriers.

To better understand the role of brain volumes on our selected variables of interest, we included gray and white matter volumes (mL) as independent variables in the multiple regression analysis. There was no significant effect of gray matter volume ($p = 0.769$) or white matter volume ($p = 0.978$) on β-amyloid deposition, and the interaction between resting CVCi and *APOE4* carrier status remained significant ($p = 0.032$). This suggests the differential relationship between CVCi and β-amyloid by *APOE4* carrier status is independent of differences in brain volume.

Cerebrovascular function during exercise

The TCD signal was lost for two participants during exercise leaving 52 participants in the exercise analysis (63% female, 31% *APOE4* carriers; 70.8 ± 5.1 years old). We ran a multiple linear regression to predict ΔMCAv to moderate-intensity exercise from age, sex, β-amyloid deposition, statin use, *APOE4* carrier status, HDL cholesterol, non-HDL cholesterol, the interaction between non-HDL cholesterol and *APOE4* carrier status. There was linearity as assessed by partial regression plots and a plot of studentized residuals against the predicted values. There was independence of residuals, as assessed by a Durbin-Watson statistic near 2. There was no evidence of multicollinearity, as assessed by tolerance values greater than 0.1. There was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals versus unstandardized predicted values. Data met the assumption of normality, as assessed by a P-P Plot. There were no outliers, as assessed by no standardized residuals greater than ±3 standard deviations (SD).

The multiple regression model significantly predicted ΔMCAv, $F(8, 43) = 2.760$, $p = 0.015$, $R^2 = 0.339$, adj. $R^2 = 0.216$. Regression coefficients and standard errors can be found in Table 3-2. There was a significant interaction ($p = 0.014$) between non-HDL cholesterol and *APOE4* carrier status, suggesting the relationship between non-HDL cholesterol and ΔMCAv is moderated by the *APOE4* allele. This relationship is shown graphically in Figure 3-2 for participants divided by *APOE4* carrier status. For *APOE4* carriers, there is a negative association between non-HDL cholesterol and ΔMCAv, with lower levels of non-HDL cholesterol predicting a more robust response to exercise in the cerebrovasculature. Conversely, the opposite relationship was apparent for *APOE4* non-carriers, with higher non-HDL cholesterol predicting a larger ΔMCAv during moderate-intensity exercise. Male participants had a significantly larger ΔMCAv than female participants ($p = 0.028$). Contrary to our hypothesis, there was no significant effect of *APOE4* carrier status on ΔMCAv ($p = 0.454$), suggesting the exercise response in the cerebrovasculature was not different between *APOE4* carriers and non-carriers.

Table 3-2: Multiple regression analysis results for the cerebrovascular response to moderate-intensity exercise (ΔMCAv)

Variable	B	SE_B	β	p-value
Intercept	8.469	11.537		0.467
Age	-0.049	0.135	-0.047	0.720
Sex (Male)	3.916	1.728	0.359	0.028*
***β*-amyloid deposition**	-6.592	4.790	-0.195	0.176
Statin use (+)	-0.665	1.518	-0.063	0.663
***APOE4* carrier status (+)**	1.183	1.567	0.104	0.454
HDL cholesterol	0.090	0.046	0.313	0.054
Non-HDL cholesterol	0.065	0.029	0.413	0.030*
Non-HDL cholesterol x *APOE4* carrier status (+)	-0.118	0.046	-0.408	0.014*

B = unstandardized regression coefficient; SE_B = standard error of the coefficient; β = standardized coefficient; *significant ($p < 0.05$), *APOE4* = *Apolipoprotein E4*; HDL = High-Density Lipoprotein

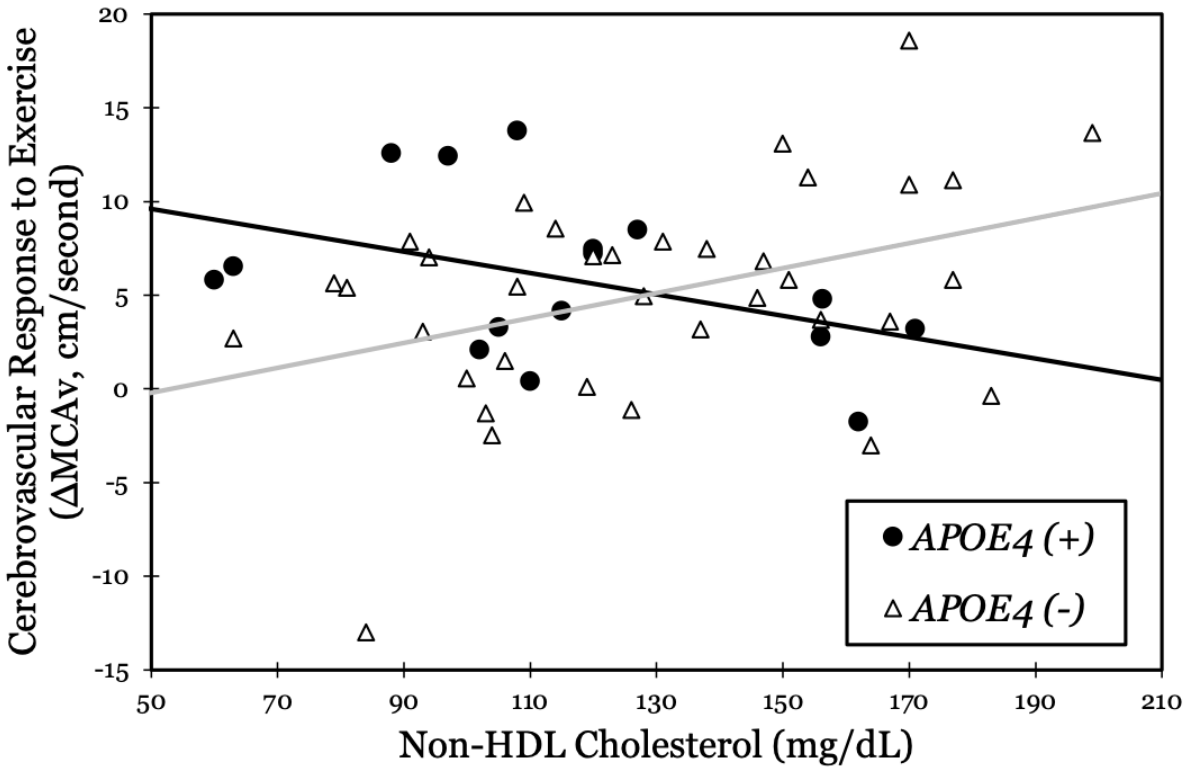

Figure 3-2: Relationship between the Cerebrovascular Response to exercise (ΔMCAv) and non-High-Density Lipoprotein (non-HDL) cholesterol levels for *APOE4* carriers and non-carriers

For *APOE4* carriers, higher non-HDL cholesterol levels were associated with a blunted ΔMCAv during an acute bout of moderate-intensity exercise. In contrast, higher non-HDL cholesterol predicted a larger ΔMCAv for *APOE4* non-carriers. These findings suggest *APOE4* moderates the association between pro-atherogenic cholesterol and cerebrovascular dysfunction.

There was no significant effect of gray matter volume ($p = 0.658$) or white matter volume ($p = 0.691$) on ΔMCAv with moderate-intensity exercise, and the interaction between non-HDL cholesterol and *APOE4* carrier status remained significant ($p = 0.015$). This suggests the observed differential relationship between non-HDL cholesterol and ΔMCAv by *APOE4* carrier status was independent of brain volume differences.

Discussion

In the current study, we found *APOE4* carrier status moderated the association between vascular risk factors and brain pathology. Specifically, poor cerebrovascular conductance (CVCi, an indication of the brain's perfusion capability) (Labrecque et al., 2019) predicted higher brain *β*-amyloid load for the *APOE4* carriers but not non-carriers. Additionally, although *APOE4* carriers and non-carriers did not differ in overall cerebrovascular response to moderate-intensity exercise (ΔMCAv), higher non-HDL cholesterol ("bad" cholesterol) (Hawkes, 2019) was associated with a blunted response of the cerebrovasculature to moderate-intensity exercise for the *APOE4* carriers, with the opposite relationship for non-carriers. Overall, these findings suggest vascular risk factors may differentially impact brain pathology for cognitively-normal *APOE4* carriers compared to non-carriers, providing insight into potential mechanisms of late-onset Alzheimer's disease (AD) pathogenesis and reinforcing the importance of maintaining vascular health specifically for people at highest genetic risk of AD.

Cerebrovascular function at rest

A recent comprehensive study including over 7,700 brain scans from the Alzheimer's Disease Neuroimaging Initiative database concluded cerebrovascular dysfunction was the strongest and earliest pathological abnormality in AD pathogenesis, followed next by *β*-amyloid deposition and later by brain metabolic dysfunction, functional MRI abnormalities and structural atrophy (Iturria-Medina et al., 2016). The relationship between cerebrovascular dysfunction and *β*-amyloid deposition is complex and likely bidirectional, with each contributing to the other at various stages of disease (Kisler et al., 2017). However, there is growing evidence that cerebrovascular dysfunction can promote *β*-amyloid deposition. For example, experimental induction of brain hypoperfusion in mice via bilateral carotid artery stenosis significantly accelerates *β*-amyloid deposition (Bannai et al., 2017; Okamoto et al., 2012; Yamada et al., 2011). The mechanisms through which hypoperfusion promotes *β*-amyloid deposition remain a matter of debate, but studies have suggested reduced blood flow may impair the dynamics of the interstitial fluid and result in congestion, which subsequently facilitates *β*-amyloid aggregation (Bannai et al., 2019). In addition to impairing clearance, low brain blood supply upregulates hypoxia-inducible factor, which increases *β*-secretase transcription leading to higher levels of *β*-amyloid production (Guglielmotto et al., 2009; Koike et al., 2010). Furthermore, the addition of vascular risk factors to mouse models of AD has been shown to facilitate *β*-amyloid deposition by enhancing amyloidogenic amyloid precursor protein processing (Faraco et al., 2016). In humans, *APOE4* carriers have been shown to have reduced CBF that precedes *β*-amyloid deposition, with lower CBF predicting increased *β*-amyloid accumulation**,** suggesting hypoperfusion may occur upstream of *β*-amyloid deposition (Michels et al., 2016).

In the present study, we found lower conductance (CVCi) in the middle cerebral artery, the largest conduit vessel in the brain, predicted elevated β-amyloid load for the *APOE4* carriers but not non-carriers. Since cerebrovascular conductance reflects the degree of cerebral blood flow (CBF) per perfusion pressure (Labrecque et al., 2019), this means *APOE4* carriers who had lower resting CBF per a given perfusion pressure had significantly greater β-amyloid load, while this was not the case for non-carriers. Considering the updated pathogenesis timeline model of AD and the increasing evidence for a direct effect of cerebrovascular dysfunction on promoting β-amyloid deposition, these findings suggest cerebrovascular dysfunction may preferentially promote AD pathology in *APOE4* carriers. This finding is in line with prior studies showing a synergistic effect between the *APOE4* allele and cerebrovascular risk factors such as stroke (Shaaban et al., 2019).

Cerebrovascular function during exercise

The product of the *APOE4* gene functions primarily in cholesterol transport throughout the body (Tai et al., 2016). *APOE4* significantly increases the risk of dyslipidemia, coronary artery disease, myocardial infarction, and ischemic/hemorrhagic stroke, in addition to AD (Belloy et al., 2019). Moreover, some studies have suggested these cardiovascular diseases are not only more common in *APOE4* carriers but may also act synergistically with the *APOE4* allele when they occur (Caselli et al., 2011a; Gupta et al., 2015; Helzner et al., 2009; Nation et al., 2016; Shaaban et al., 2019; Tai et al., 2016; Zade et al., 2010). Additionally, higher cholesterol has been associated with faster rates of cognitive decline in older adults with and without AD (Helzner et al., 2009; Lorius et al., 2015). In the present study, we sought to investigate this relationship further, specifically analyzing the interaction between non-HDL

cholesterol (colloquially known as “bad cholesterol”) (Hawkes, 2019) and the *APOE4* allele on the ΔMCAv with exercise. Additionally, considering growing evidence that the *APOE4* allele causes cerebrovascular dysfunction (Caselli et al., 2011a; Michels et al., 2016; Montagne et al., 2020; Tai et al., 2016), we hypothesized *APOE4* carriers would have a blunted cerebrovascular response to exercise (ΔMCAv) compared to non-carriers. Importantly, the inclusion of ΔMCAv in the present study expands upon the resting CVCi findings by allowing for characterization of cerebrovascular function during dynamic physiological challenge (in this instance, aerobic exercise) to the brain vasculature, which may differ from the resting state. Previous studies reported dynamic cerebrovascular dysfunction in *APOE4* carriers in response to physiological stimuli such as hypercapnia (cerebrovascular reactivity) (Suri et al., 2015) and cognitive tasks (Fleisher et al., 2009). In the present study, we utilized ΔMCAv with a moderate-intensity exercise stimulus because this reflects the cerebrovascular response to a variety of stimuli and is relevant to daily life. That is, during aerobic exercise the cerebrovasculature is challenged by simultaneous alterations in arterial blood gas, perfusion pressure, metabolism and neuronal activity (Smith & Ainslie, 2017). Characterizing the response to this dynamic physiological challenge can therefore provide novel insight into cerebrovascular dysfunction that may not be observed at rest or with other physiological stimuli. We previously demonstrated a blunted ΔMCAv during exercise with increasing age (Ward et al., 2018), after ischemic stroke (Kaufman et al., 2019; Kempf, 2019), and in people with elevated β-amyloid (Sisante et al., 2019). In the current manuscript, we sought to investigate whether the cerebrovascular response to exercise (ΔMCAv) varies as a function of *APOE4* carrier status with the hope of providing further insight into pathogenic mechanisms of AD for those at highest genetic risk.

Contrary to our hypothesis, we found no difference in ΔMCAv from rest to exercise between *APOE4* carriers and non-carriers, when controlling for age, sex, β-amyloid deposition and statin use. This may be due to the fact that our study included only participants with normal cognition and minimal history of diseases commonly associated with cardiovascular dysfunction. That is, the *APOE4* carriers in our sample may represent a group that is unusually healthy compared to the general population, which could explain why these participants had normal cognition despite significantly elevated AD risk. However, while the overall sample may be too healthy to observe group differences between *APOE4* carriers and non-carriers, the interaction term in the model allows us to investigate how certain risk factors (as they vary within the sample population) differentially relate to outcomes depending on participant genotype. To this end, we found a significant interaction between non-HDL cholesterol and *APOE4* carrier status on ΔMCAv. This suggests the relationship between non-HDL cholesterol, a comprehensive measure of pro-atherogenic particles in the blood (Blaha et al., 2008; Di Angelantonio et al., 2009; Hawkes, 2019; Kastelein et al., 2008; Virani et al., 2011), and ΔMCAv is different between *APOE4* carriers and non-carriers. For *APOE4* carriers, higher non-HDL cholesterol level was associated with a blunted ΔMCAv from rest to exercise, which could argue for a role for this vascular risk factor in promoting cerebrovascular dysfunction specifically in *APOE4* carriers. Intriguingly, the relationship between non-HDL cholesterol and ΔMCAv occurred in the opposite direction for non-carriers, with higher levels of non-HDL cholesterol predicting a more robust ΔMCAv with exercise. The mechanism behind this unexpected finding is unclear but may indicate a differential role for blood lipid components in cerebrovascular function for *APOE4* carriers and non-carriers and merits further exploration.

Our findings remained significant when controlling for brain volume (both gray and white matter) in the multiple regression models, suggesting the observed relationships exist independently of brain volume. This result is consistent with most current models of AD pathogenesis, which place CBF decline and β-amyloid deposition upstream of structural MRI changes (Iturria-Medina et al., 2016; Jack et al., 2013; Sweeney et al., 2018). For example, one large, longitudinal study determined cerebrovascular dysregulation occurs earliest in AD pathogenesis, followed by β-amyloid deposition which is then followed by brain metabolic and functional abnormalities, all of which occur before structural atrophy is observed (Iturria-Medina et al., 2016). The fact that the relationship between poor CVCi and β-amyloid deposition occurred independently of brain volume differences in our sample provides further support for this model. Likewise, the interaction between non-HDL cholesterol and ΔMCAv remained significant when controlling for white and gray matter volume, and there was no relationship between these volumes and the ΔMCAv with moderate-intensity exercise. These results parallel current models of AD that posit cerebrovascular abnormalities occur earlier in the disease process than structural atrophy (Iturria-Medina et al., 2016; Sweeney et al., 2018) and may further support the hypothesis that cerebrovascular dysregulation plays an early, causal role in AD pathogenesis.

Importantly, if vascular risk factors act synergistically with *APOE4* to cause dementia, then interventions that improve cardiovascular health may be more effective for maintaining brain health and preventing cognitive decline in *APOE4* carriers than non-carriers. Indeed, there is evidence that exercise - one of the most potent interventions to improve cardiovascular health - may preferentially benefit *APOE4* carriers. For example, physical activity and exercise have been shown to more robustly prevent cognitive decline in *APOE4* carriers than non-carriers

(Jensen et al., 2019; Pizzie et al., 2014), and physical activity may preserve hippocampal volume selectively in *APOE4* carriers (Smith et al., 2014). Our current findings provide further evidence for a potential role for improving systemic cardiovascular health in order to improve brain function and potentially prevent or delay cognitive decline in *APOE4* carriers.

Limitations

This study has important limitations. First, transcranial Doppler ultrasound (TCD) allows us to assess dynamic cerebrovascular function in ways that are not possible in an MRI scanner (Aaslid et al., 1982), but TCD requires the assumption of constant artery diameter in order for velocity to serve as a surrogate for flow, which has limitations (Brothers & Zhang, 2016; R. L. Hoiland & P. N. Ainslie, 2016). We included statin use in both regression models to control for potential impact of this lipid-lowering medication, but we treated this as a categorical variable ("yes" or "no" for statin use) which does not account for dosage or treatment duration. Cholesterol levels were obtained during a clinic visit 1-2 months before the vascular laboratory visit because we were interested in obtaining a rough measure of dyslipidemia (reflective of real-world clinical care) rather than assessing the acute effects of cholesterol on the cerebrovasculature. However, future studies could provide additional insight by including cholesterol measurements on the day of the vascular visit. Our participants were cognitively-normal older adults which may limit generalizability to patient populations. Finally, the cross-sectional design of the study makes it impossible to definitively elucidate cause and effect (for example, whether low CVCi contributes to β-amyloid deposition or vice versa), and future studies are necessary to establish directionality.

Conclusion

In the current study, we found *APOE4* carrier status moderates the association between central and peripheral vascular risk factors and brain pathology. *APOE4* carriers with poor conductance in the cerebral vasculature demonstrated a greater brain *β*-amyloid load, while this relationship was not apparent for *APOE4* non-carriers. Conversely, *APOE4* carriers with better conductance in the largest conduit vessel supplying blood to the brain had significantly lower brain *β*-amyloid deposition. Although *APOE4* carriers did not demonstrate an overall blunted response to exercise in the cerebrovasculature (lower ΔMCAv), *APOE4* was found to moderate the association between non-HDL cholesterol and ΔMCAv. Specifically, higher non-HDL cholesterol was associated with a blunted ΔMCAv in *APOE4* carriers but with a more robust ΔMCAv in non-carriers. These findings provide potential mechanistic insight into the pathogenesis of AD and reinforce the importance of maintaining both peripheral and central vascular health for people at the highest known genetic risk of late-onset AD.

Chapter 4: Aerobic exercise improves hippocampal blood flow for hypertensive *Apolipoprotein E4* carriers

This chapter was previously published as a research article in *Journal of Cerebral Blood Flow & Metabolism* and reprinted here in whole with permission from the publisher.

Original article: Kaufman CS, Honea RA, Pleen J, Lepping RJ, Watts A, Morris JK, Billinger SA, Burns JM, Vidoni ED. Aerobic exercise improves hippocampal blood flow for hypertensive *Apolipoprotein E4* carriers. *Journal of Cerebral Blood Flow & Metabolism*. January 2021. doi:10.1177/0271678X21990342

Introduction

Evidence increasingly points to an early, primary role of cerebrovascular dysfunction in the pathogenesis of late-onset Alzheimer's disease (AD) (Iturria-Medina et al., 2016; Montagne et al., 2020; Sweeney et al., 2018; Wolters et al., 2017). Considering the well-established benefits of aerobic exercise on vascular health (Green & Smith, 2018), exercise may act mechanistically through improvements in vascular function to reduce dementia risk. Indeed, intervention trials have shown exercise may improve cognitive function at least partially through increasing cerebral blood flow (CBF) (Espeland et al., 2018; Guadagni et al., 2020; Kleinloog et al., 2019; Thomas et al., 2020), particularly in the hippocampus (HBF) (Burdette et al., 2010; Chapman et al., 2013; Maass et al., 2015).

The strongest known genetic risk factor for AD, the *APOE4* allele, may act synergistically with poor vascular health to increase dementia risk (Bender & Raz, 2012; Caselli et al., 2011b; Haan et al., 1999; Shaaban et al., 2019; Zade et al., 2010). This suggests interventions that improve systemic vascular health may be particularly beneficial for *APOE4* carriers (Kaufman & Perales-Puchalt, 2019). Some studies have shown *APOE4* carriers benefit more from exercise (Deeny et al., 2008; Jensen et al., 2019; Schuit et al., 2001; Smith et al., 2014), but others have suggested less improvement in *APOE4* carriers (Lautenschlager et al., 2008; Stern et al., 2019), meriting further exploration.

We performed an analysis of a secondary outcome from a randomized controlled trial in which cognitively-normal older adults were assigned to a 52-week aerobic exercise intervention or to education only (Vidoni et al., 2020). For the present analysis, we selected only participants with hypertensive blood pressure at the time of enrollment, defined as systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 80 mmHg (Whelton et al., 2018). We

hypothesized that 1) the exercise intervention would be more effective in increasing HBF in *APOE4* carriers than non-carriers, and 2) reductions in SBP would have a greater impact on improving HBF for *APOE4* carriers than for non-carriers.

Methods

Study design

The Alzheimer's Prevention through Exercise study (APEx) was a 52-week clinical trial of aerobic exercise in cognitively-normal older adults. The primary outcome was change in β-amyloid deposition, and these results have been published previously (Vidoni et al., 2020). The present study is an analysis of the secondary outcome of regional arterial spin labeling (ASL) MRI data, specifically HBF, for the participants in the APEx clinical trial (NCT02000583).

Participants

Participants were required to meet the following inclusion criteria: 65 years and older, sedentary or underactive as defined by the Telephone Assessment of Physical Activity, on stable medications for at least 30 days, willingness to undergo an 18F-AV45 PET scan for cerebral β-amyloid load and learn the result (elevated or non-elevated), willingness to perform prescribed exercise (or not) for 52 weeks at a community fitness center, and ability to complete graded maximal exercise testing with a respiratory exchange ratio >=1.0. Exclusion criteria included insulin-dependence, significant hearing or vision problems, clinically evident stroke, cancer in the previous 5 years (except for localized skin or cervical carcinomas or prostate cancer), change in blood pressure medication within the last 30 days, or recent history (<2 years) of major cardiorespiratory, musculoskeletal or neuropsychiatric impairment. During in-person screening,

a clinician of the University of Kansas Alzheimer's Disease Center performed a clinical assessment that included a Clinical Dementia Rating, the Uniform Data Set neuropsychiatric battery, and other supplementary tests.

Neuroimaging assessments

Individuals who consented to screening underwent florbetapir 18F-AV45 (370 MBq) PET scans. β-amyloid status was disclosed to all participants (Burns et al., 2017). We enrolled those participants with cortical-to-cerebellar β-amyloid burden greater than 1 because these participants may have accelerated β-amyloid deposition and memory decline (Landau et al., 2018). At baseline, enrollees had a T1-weighted MRI of the brain (3T Siemens Skyra scanner; MP-RAGE 1x1x1.2mm voxels, TR = 2300ms, TE = 2.98ms, TI = 900ms, FOV 256x256mm, 9° flip angle; Pulsed ASL single-shot EPI 3.8x3.8x4.0mm,TR = 3400ms, TE = 13ms, TI = 700ms, FOV 240x240mm, 90° flip angle) with regional volumes parcellated and extracted using the CAT12 toolbox (http://www.neuro.uni-jena.de/cat/) and the Automatic Anatomic Labeling atlas. ASL-MRI data were processed using the ASLTbx for SPM12 (Hu et al., 2010). All neuroimaging assessments were repeated at 52 weeks.

Physiological assessments

At the baseline study visit, the participant sat at rest for 5 minutes before BP was measured twice with one minute of rest between measurements (Axia TRIA Touch Screen Patient Monitor, Association for the Advancement of Medical Instrumentation/American National Standards Institute performance standards SP10:2002). We averaged the two resting SBP and two resting DBP to determine one average baseline SBP/DBP, which was used for

grouping into hypertensive and normotensive categories based on the most recent guidelines published by the American College of Cardiology and American Heart Association (ACC/AHA) (Whelton et al., 2018). Before beginning the study, participants performed graded maximal exercise testing on a treadmill to maximal capacity or volitional termination to quantify cardiorespiratory fitness (VO_2max). The BP measurement and graded maximal exercise test were repeated at 52 weeks.

Cognitive assessments

A trained psychometrist performed a comprehensive cognitive test battery at baseline and 52 weeks, employing validated, alternate versions of tests every other visit. We created composite scores for three cognitive domains (executive function, verbal memory, visuospatial processing) using Confirmatory Factor Analysis. Scores were standardized to baseline so subsequent scores could be interpreted relative to baseline. The executive function composite score was made up of verbal fluency, Trailmaking Test B, Digit Symbol Substitution test, and the interference portion of the Stroop test. The verbal memory composite score was made up of the Logical Memory Test immediate and delayed, and the Selective Reminding Test. The visuospatial composite score was made up of scores from Block Design, space relations, the paper folding test, hidden pictures, and identical pictures. Missing data in the factor analysis were accounted for using full information maximum likelihood algorithm.

Intervention

Participants were randomized in a 2:1 ratio to either 150 minutes per week of supported moderate intensity aerobic exercise or standard of care education. The education control group

was provided standard exercise public health information but was otherwise not supported nor prohibited from exercise. For those randomized to the aerobic exercise group, the intervention was conducted at their nearest study-certified exercise facility with the support of certified personal trainers. The intervention group was asked to refrain from changing their regular physical activities other than those prescribed by the study team. Participants exercised 3–5 days a week at an intensity that began at 40 – 55% of Heart Rate Reserve (% of the difference between maximal and resting) and was increased by 10% every 3 months.

APOE genotype determination

Whole blood was collected and stored at -80C until genetic analyses could be conducted. To determine *APOE* genotype, frozen whole blood was assessed using a Taqman single nucleotide polymorphism (SNP) allelic discrimination assay (ThermoFisher). *APOE4*, *APOE3*, and *APOE2* alleles were distinguished using Taqman probes to the two *APOE*-defining SNPs, rs429358 (C_3084793_20) and rs7412 (C_904973_10). The term "*APOE4* carrier" was used to describe the presence of 1 or 2 *APOE4* alleles. Since *APOE2* is associated with reduced AD risk, all *APOE2* carriers were excluded from the analysis (whether homozygous or paired with a different *APOE* allele).

Standard protocol approvals, registrations, and patient consents

All study (ClinicalTrials.gov, NCT02000583; trial active between 11/1/2013–11/6/2019) procedures were approved by the University of Kansas Institutional Review Board (HSC #13376) and complied with the World Medical Association Declaration of Helsinki. Written

informed consent was obtained from all participants.

Statistical approach

All statistical analyses were performed using SPSS Statistics (IBM). Normality was assessed before each analysis by Shapiro-Wilk's test ($p > 0.05$) and Q-Q Plot. Baseline group differences were assessed by independent t-test, chi-square test for homogeneity, Mann Whitney U, Kruskal-Wallis H test, or one-way ANOVA, as appropriate. A multiple linear regression further characterized the relationship between SBP, DBP and baseline HBF. A two-way ANCOVA was conducted to examine the effects of *APOE4* carrier status and Treatment Group (exercise or control) on ΔHBF, after controlling for age and sex. The significant interaction term was followed up by an analysis of simple main effects using a Bonferroni adjustment ($p < 0.025$). The same two-way ANCOVA analysis was performed with outcome measure percent ΔHBF (%ΔHBF = (HBF at 52 weeks – HBF at baseline)/HBF at baseline) instead of ΔHBF, in order to control for baseline HBF. A multiple regression was run to predict ΔHBF from sex, *APOE4* carrier status, ΔSBP, and the interaction between ΔSBP and *APOE4* carrier status. Age, ΔDBP and the interaction term for ΔDBP and *APOE4* carrier status worsened the predictive value and were therefore excluded from the model. Two-way ANCOVAs were utilized to examine the effects of *APOE4* carrier status and Treatment Group (exercise or control) on change in cognitive functioning (visuospatial, executive and memory), ΔSBP, ΔDBP, change in hippocampal volume, change in body mass index (BMI), and change in VO2max (ΔVO2mx), after controlling for age and sex. A Pearson's Product-Moment Correlation assessed the relationship between ΔHBF and change in verbal memory function (ΔVM) for the *APOE4* carriers who underwent the exercise intervention.

Data availability policy

This trial was prospectively registered with ClinicalTrials.gov (NCT02000583). Anonymized data will be shared by request from any qualified investigator.

Results

A total of 109 participants (93%: control n = 34, aerobic exercise n = 75) completed the study (Figure 4-1). Genotyping was not completed for 3 participants. *APOE2* carriers (n = 14) and participants with incomplete ASL MRI data (n = 4) were excluded from the present analysis. Of the remaining 88 participants, 44 had hypertension at the baseline visit, classified due to having SBP ≥ 130 mmHg (n = 22), DBP ≥ 80 mmHg (n = 5), or both (n = 17) (Whelton et al., 2018). Sixteen participants completed less than 80% of the prescribed exercise, but all participants were included in the analyses regardless of the percent exercise intervention prescription completed.

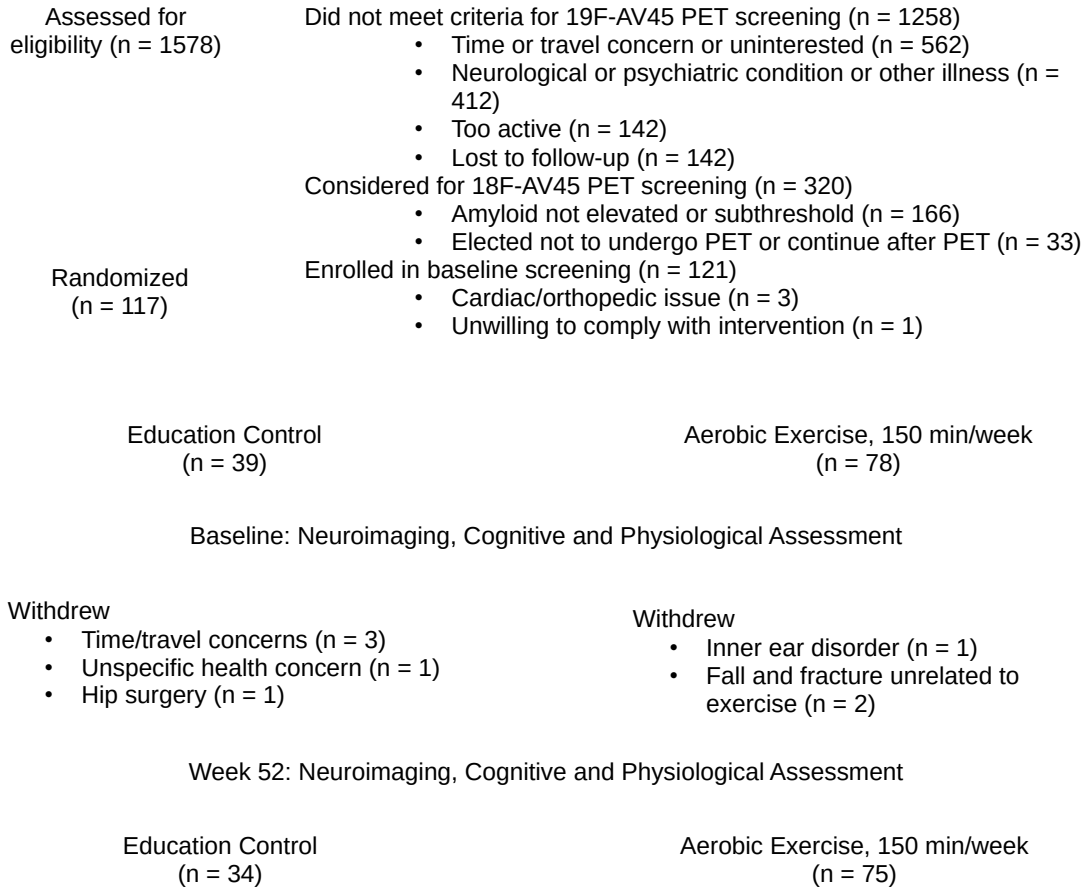

Figure 4-1 Flowchart of Alzheimer's Prevention through Exercise (APEx) study participants

Baseline characteristics of hypertensive and normotensive participants

At baseline, HBF was significantly lower in the hypertensive participants (30.17 ± 6.06 mL/100g/min) than the normotensive participants (34.00 ± 9.54 mL/100g/min) ($p = 0.028$). A multiple linear regression showed this difference was driven by baseline SBP. Specifically, the regression analysis, controlling for age, sex and *APOE4* carrier status, significantly predicted HBF ($F(5,82) = 2.657$, $p = 0.028$) with SBP contributing significantly to the model ($B = -0.157$, $p = 0.035$) and DBP having a non-significant effect ($B = 0.086$, $p = 0.461$). There were no significant differences between the hypertensive and normotensive participants in age, sex, education, VO_2max or hippocampal volume (see Table 4-1).

Table 4-1 Baseline characteristics by group

	A) Non-carrier, Control (n = 7)	B) Non-carrier, Exercise (n = 15)	C) *APOE4* Carrier, Control (n = 8)	D) *APOE4* Carrier, Exercise (n = 14)	*p*-value Groups A-D	E) HTN Total (A+B+C+D) (n = 44)	F) NTN (n = 44)	*p*-value Groups E&F
Age, years	72.4 (5.1)	70.9 (4.5)	72.9 (6.8)	71.9 (6.0)	0.895	71.8 (5.4)	71.2 (4.8)	0.602
Males, n (%)	3 (43%)	7 (47%)	2 (25%)	5 (36%)	0.768	17 (39%)	12 (27%)	0.257
Education, years	16.0 (2.3)	16.0 (2.8)	16.5 (1.7)	16.1 (1.4)	0.851	16.1 (2.1)	16.0 (2.4)	0.706
Anti-HTN med, n (%)	4 (57%)	6 (40%)	3 (38%)	7 (50%)	0.861	20 (46%)	16 (36%)	0.516
APOE4 carriers, n (%)	0 (0%)	0 (0%)	8 (100%)	14 (100%)	-	22 (50%)	22 (50%)	1.000
Baseline SBP, mmHg	144.1 (13.5)	138.4 (10.3)	133.5 (5.4)	141.0 (14.0)	0.254	**139.2 (11.6)**	**118.1 (8.5)**	**<0.001***
Baseline DBP, mmHg	79.9 (8.4)	81.0 (9.5)	78.0 (7.7)	81.1 (8.6)	0.920	**80.3 (8.5)**	**70.0 (5.9)**	**<0.001***
Baseline HBF, mL/100g/min	30.40 (4.94)	30.96 (6.36)	33.02 (5.67)	27.59 (6.04)	0.207	**30.17 (6.06)**	**34.00 (9.54)**	**0.028***
Baseline VO_2max, mL/kg/min	19.76 (3.34)	23.33 (6.56)	22.58 (4.08)	21.56 (4.65)	0.482	22.06 (5.13)	23.15 (5.20)	0.325
Baseline HV, mL	7.43 (0.94)	7.34 (0.89)	7.19 (1.17)	7.15 (0.87)	0.906	7.27 (0.92)	7.57 (0.78)	0.096

***significant (p < 0.05)**

Values are shown as mean (standard deviation) unless otherwise indicated. HTN Total (E) combines groups A-D. NTN (F) is a separate group. Statistical tests were applied to compare baseline characteristics among groups A-D (*p*-value Groups A-D) or between groups E&F (*p*-value Groups E&F). NTN = normotensive (participants with normal blood pressure at baseline), HTN = hypertensive (participants with hypertensive blood pressure at baseline), Anti-HTN med = taking anti-hypertension medication, SBP = systolic blood pressure, DBP = diastolic blood pressure, HBF = hippocampal blood flow, VO_2max = maximal oxygen uptake, HV = hippocampal volume

Baseline characteristics by intervention and APOE4 carrier status

Only the participants with hypertension at the baseline visit (n = 44) were included in further analyses. Half of these participants were *APOE4* carriers (*APOE3/APOE4*, n = 20; *APOE4/APOE4*, n = 2). At baseline, there were no significant differences among *APOE4* non-carriers assigned to the control group (n = 7), *APOE4* non-carriers assigned to exercise (n = 15), *APOE4* carriers assigned to the control group (n = 8), and *APOE4* carriers assigned to exercise (n = 14) in age, sex, education, anti-hypertensive medication use, SBP, DBP, HBF, maximal oxygen uptake (VO_2max), or hippocampal volume (Table 4-1).

ΔHBF with exercise intervention

The two-way ANCOVA met the assumptions of homoscedasticity and homogeneity. There were no outliers in the data, as assessed by no cases with studentized residuals greater than ±3 SD. Studentized residuals were normally distributed, as assessed by Shapiro-Wilk's test (p > .05). Means, adjusted means (for age and sex), SD and standard errors are presented in Table 4-2 and shown graphically in Figure 4-2. There was a significant two-way interaction between *APOE4* carrier status and Treatment Group on ΔHBF, while controlling for age and sex, $F(1, 38) = 4.504$, $p = 0.040$, partial $\eta^2 = 0.106$. Therefore, an analysis of simple main effects for *APOE4* carrier status and Treatment Group was performed with statistical significance receiving a Bonferroni adjustment and being accepted at the $p < 0.025$ level.

Table 4-2 Mean change in hippocampal blood flow (HBF) from baseline to 52 weeks

ΔHBF (%ΔHBF) mL/100g/min (%)	**Non-carrier Control (n = 7)**	**Non-carrier Exercise (n = 15)**	***APOE4* Carrier Control (n = 8)**	***APOE4* Carrier Exercise (n = 14)**
Mean	-0.19 (-0.2%)	-0.48 (-1.8%)	-2.22 (-5.5%)	+4.05 (+17.1%)
(SD)	2.14	4.06	4.32	6.21
$Mean_{adjusted}$	-0.32 (-0.8%)	**-0.54* (-2.1%)$^{¥}$**	**-2.08^{+} (-4.8%)‡**	**+4.09$^{*,+}$ (+17.3%)$^{¥,‡}$**
(SE)	1.79	1.23	1.69	1.26

SD = Standard Deviation; SE = Standard Error; $^{*}p = 0.013$; $^{+}p = 0.006$; $^{¥}p = 0.005$; $^{‡}p = 0.007$

The mean change value (ΔHBF in mL/100g/min) is followed by mean percent ΔHBF (%ΔHBF) for each group. $Mean_{adjusted}$ represents the group means, adjusted for age and sex, for participants divided by *APOE4* carrier status and clinical trial arm (exercise intervention or control). Significant results of the simple main effects analysis that followed up the significant two-way ANCOVA interaction are indicated by superscripts ($^{*,+,¥,‡}$). All other pairwise comparisons were not significant.

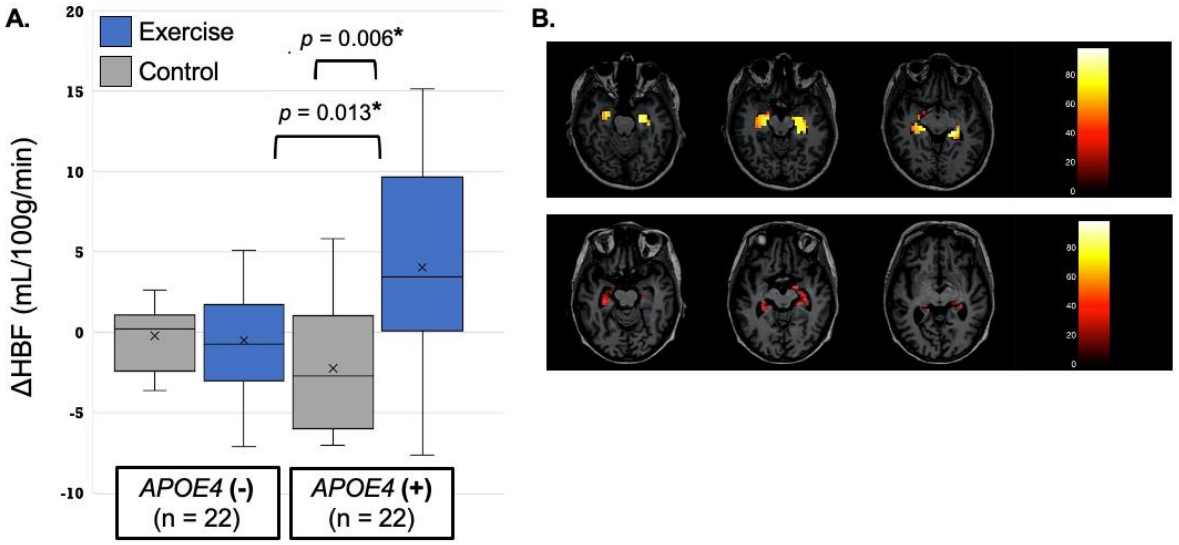

Figure 4-2 Mean change in hippocampal blood flow (ΔHBF) from baseline to 52 weeks

- **A)** Among *APOE4* carriers, those who underwent the exercise intervention had a significantly larger ΔHBF (increased HBF) over the 52 weeks than the control group ($p = 0.006$). Additionally, within the exercise intervention arm, ΔHBF was significantly larger for the *APOE4* carriers than the non-carriers ($p = 0.013$). X = mean; horizontal line = median
- **B)** Representative arterial spin labeling MRI (ASL-MRI) scans from a participant with high (top panel) and a participant with low (bottom panel) HBF.

The effect of Treatment Group on ΔHBF was significant for the *APOE4* carriers, $F(1, 38) = 8.597$, $p = 0.006$, partial $\eta^2 = 0.184$. Specifically, for the *APOE4* carriers, adjusted mean ΔHBF was higher for participants who underwent the exercise intervention (4.09 mL/100g/min) than for the control group (-2.08 mL/100g/min), a significant difference of 6.17 (95% CI, 1.91 to 10.44) mL/100g/min. The effect of Treatment Group was not significant in the *APOE4* non-carriers, $F(1, 38) = 0.011$, $p = 0.918$, partial $\eta^2 < 0.0001$.

The effect of *APOE4* carrier status on ΔHBF in the exercise group was significant, $F(1, 38) = 6.853$, $p = 0.013$, partial $\eta^2 = 0.153$. Specifically, for participants who underwent the exercise intervention, adjusted mean ΔHBF was higher for the *APOE4* carriers (4.09 mL/100g/min) than the non-carriers (-0.54 mL/100g/min), a significant difference of 4.63 (95% CI, 1.05 to 8.22) mL/100g/min. In the control group, the effect of *APOE4* carrier status on ΔHBF was not significant, $F(1, 38) = 0.514$, $p = 0.478$, partial $\eta^2 = 0.013$.

Overall, the 52-week exercise intervention improved mean HBF for the *APOE4* carriers from 27.59 to 31.64 mL/100g/min. Over this same time period, the *APOE4* carriers in the control group experienced a decline in HBF from 33.02 to 30.80 mL/100g/min, and the *APOE4* non-carriers remained relatively stable in both the control (30.40 to 30.22 mL/100g/min) and exercise intervention (30.96 to 30.48 mL/100g/min) groups. It is thus noteworthy that the *APOE4* carriers in the exercise intervention had the lowest mean HBF at baseline but the highest HBF after the intervention, although these groups differences were not significant at baseline ($p = 0.207$) or post-intervention ($p = 0.961$).

%ΔHBF with exercise intervention

There was a significant two-way interaction between *APOE4* carrier status and Treatment Group on %ΔHBF, while controlling for age and sex, $F(1, 38) = 4.392$, $p = 0.043$, partial $\eta^2 = 0.104$. The effect of Treatment Group on %ΔHBF was significant for the *APOE4* carriers, $F(1, 38) = 8.002$, $p = 0.007$, partial $\eta^2 = 0.174$. Specifically, for the *APOE4* carriers, adjusted mean %ΔHBF was higher for participants who underwent the exercise intervention (+17.3%) than for the control group (-4.8%), a significant difference of 22.1% (95% CI, 6.3% to 38.0%). The effect of Treatment Group was not significant in the *APOE4* non-carriers, $F(1, 38) = 0.028$, $p = 0.869$, partial $\eta^2 = 0.001$. The effect of *APOE4* carrier status on %ΔHBF in the exercise group was significant, $F(1, 38) = 8.723$, $p = 0.005$, partial $\eta^2 = 0.187$. Specifically, for participants who underwent the exercise intervention, adjusted mean %ΔHBF was higher for the *APOE4* carriers (+17.3%) than the non-carriers (-2.1%), a significant difference of 19.4% (95% CI, 6.1% to 32.7%). In the control group, the effect of *APOE4* carrier status on %ΔHBF was not significant, $F(1, 38) = 0.197$, $p = 0.660$, partial $\eta^2 = 0.005$.

ΔHBF with change in systolic blood pressure (ΔSBP)

A multiple regression was run to predict ΔHBF from sex, *APOE4* carrier status, ΔSBP, and the interaction between ΔSBP and *APOE4* carrier status. The regression analysis met the assumptions of linearity, normality, homoscedasticity, and independence of residuals. There was no multicollinearity. There were no outliers, as assessed by no studentized deleted residuals greater than ±3 SD. The multiple linear regression model significantly predicted ΔHBF, $F(4, 39) = 3.134$, $p = 0.025$, $R^2 = 0.243$, adjusted $R^2 = 0.166$. Regression coefficients and standard errors can be found in Table 4-3.

Table 4-3 Regression model for 52-week change in hippocampal blood flow (ΔHBF)

Variable	*B*	SE_B	β	*p*-value
Intercept	-1.529	1.232		0.222
Sex (Male)	2.369	1.478	0.226	0.117
ΔSBP	-0.015	0.065	-0.041	0.815
***APOE4* carrier status (+)**	2.479	1.458	0.243	0.097
ΔSBP x *APOE4* carrier status (+)	-0.243	0.111	-0.376	0.035*

*significant ($p < 0.05$), ΔSBP = change in systolic blood pressure

The multiple linear regression model for participants with baseline hypertension (N = 44) significantly predicted ΔHBF (mL/100 g/min), $F(4, 39) = 3.134$, $p = 0.025$, $R^2 = 0.243$, adjusted $R^2 = 0.166$.

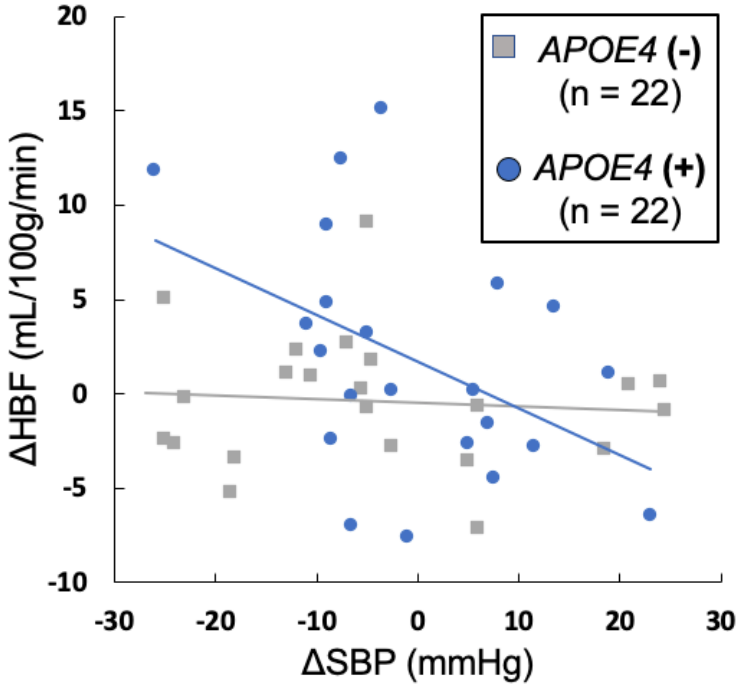

Figure 4-3 Relationship between change in systolic blood pressure (ΔSBP) and hippocampal blood flow (ΔHBF)

There was a significant interaction between ΔSBP and *APOE4* carrier status on ΔHBF (p = 0.035). The relationship between ΔSBP and ΔHBF is shown graphically for *APOE4* carriers and non-carriers in Figure 4-3. For *APOE4* carriers, reductions in SBP over the year of the study resulted in higher HBF, while increases in SBP (reflecting further elevation of already elevated blood pressure) resulted in decreased HBF. In contrast, there was no relationship between changes in SBP and HBF for the *APOE4* non-carriers.

Cognitive function changes

There was no significant two-way interaction between *APOE4* carrier status and Treatment Group on change in visuospatial functioning (p = 0.755), executive functioning (p = 0.841), or verbal memory (ΔVM, p = 0.434), suggesting no group differences in mean change in cognitive scores from baseline to post-intervention. However, there was a significant positive correlation between ΔHBF and ΔVM for the *APOE4* carriers who underwent the exercise intervention (r = 0.561, p = 0.037). That is, improvements in HBF from the exercise intervention correlated with improved verbal memory performance in *APOE4* carriers.

Other physiological changes

There was no significant two-way interaction between *APOE4* carrier status and Treatment Group for ΔSBP (p = 0.058), ΔDBP (p = 0.260), or change in hippocampal volume (p = 0.767). There was no significant two-way interaction between *APOE4* carrier status and Treatment Group on change in BMI (p = 0.921). However, the main effect of Treatment Group on change in BMI was significant (p = 0.015). Specifically, participants who exercised reduced their BMI on average over the year (mean change of -0.540 kg/m^2) while participants in the

control group gained weight (mean change of +0.185 kg/m^2). Likewise, there was no significant two-way interaction between *APOE4* carrier status and Treatment Group on ΔVO_2max (p = 0.116) but the main effect of Treatment Group on ΔVO_2max was significant (p = 0.002). Participants who underwent the exercise intervention had a mean ΔVO_2max of 2.73 mL/kg/min, which was significantly higher than the control group mean ΔVO_2max of 0.41 mL/kg/min. These findings show the exercise intervention improved cardiorespiratory fitness and reduced BMI and that these effects were not different between *APOE4* carriers and non-carriers.

Discussion

In this analysis of a secondary outcome from a randomized controlled trial, we report that an aerobic exercise intervention selectively improved hippocampal blood flow (HBF) for hypertensive *APOE4* carriers. Additionally, we demonstrate that reductions in systolic blood pressure (SBP) for hypertensive individuals were tied to improvements in HBF for *APOE4* carriers only. Finally, we found that these improvements in HBF were correlated with improved verbal memory performance.

People with Alzheimer's disease (AD) have lower cerebral blood flow (CBF) than age-matched controls (Roher et al., 2012; Wolters et al., 2017). A recent study involving over 7,700 scans from 1,171 people in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database found cerebrovascular dysregulation was the earliest pathological event during AD development, followed by changes in β-amyloid deposition, metabolic dysfunction, functional impairment and structural atrophy (Iturria-Medina et al., 2016). Therefore, interventions that maintain or improve CBF with aging may prevent or delay dementia development, and this may be particularly true for regions closely involved in the disease process, such as the hippocampus (Asllani et al., 2008;

Raji et al., 2009). Indeed, one recent study of almost 250 participants found *APOE4* carriers exhibited increased blood brain barrier breakdown specifically in the hippocampus and medial temporal lobe (Montagne et al., 2020). This hippocampal cerebrovascular dysfunction was present even in *APOE4* carriers who had normal cognition, manifested independently of β-amyloid and tau pathology, and showed increasing severity with cognitive decline (Montagne et al., 2020). Additionally, higher blood brain barrier breakdown at baseline predicted future cognitive decline for the *APOE4* carriers only (Montagne et al., 2020). This blood brain barrier breakdown has been associated with reduced CBF (Bell et al., 2012), suggesting impaired hippocampal blood flow (HBF) could be an important mechanism early in the pathogenesis of AD for *APOE4* carriers. This mechanism may therefore provide a novel therapeutic target to prevent or treat AD for those at highest genetic risk.

In the present study, we employed aerobic exercise as a therapeutic approach to prevent AD, and we report that aerobic exercise improved blood delivery to the hippocampus (HBF) for hypertensive *APOE4* carriers. Specifically, we found a mean HBF increase (+17.3%) for *APOE4* carriers who exercised compared to a mean HBF decline (-4.8%) for *APOE4* carriers in the control group over one year. This HBF improvement could prevent or delay AD for these individuals at highest known genetic risk, considering the growing evidence that CBF reductions precede measurable cognitive decline (Iturria-Medina et al., 2016; Yew & Nation, 2017) and likely contribute causally to dementia pathogenesis (Bell et al., 2012; Gorelick et al., 2011; Koizumi et al., 2018; Montagne et al., 2020; Sweeney et al., 2018), particularly for *APOE4* carriers (Hays et al., 2020; Koizumi et al., 2018; Shaaban et al., 2019). Indeed, *APOE4* carriers have been shown to experience an accelerated age-related CBF decline (Michels et al., 2016; Thambisetty et al., 2010), and some studies suggest cerebrovascular dysfunction may act

synergistically with the *APOE4* allele to promote cognitive decline (Hays et al., 2020; Montagne et al., 2020; Shaaban et al., 2019). If true, this would mean CBF maintenance is even more important for *APOE4* carriers than non-carriers in order to prevent dementia, strengthening the clinical relevance of our current findings.

An observational study published earlier this year combined data from the Chicago Health and Aging Project and the Memory and Aging Project and concluded that adults should participate in at least 150 minutes of moderate-to-vigorous physical activity per week to reduce dementia risk (Dhana et al., 2020), which is notably the same exercise prescription utilized in the intervention arm of our trial. Previous randomized controlled trials have shown promising results for exercise in preventing cognitive decline (Blumenthal et al., 2019; Guadagni et al., 2020; Jensen et al., 2019; Lautenschlager et al., 2008), and a recent systematic review concluded exercise improves cognitive function in people 50+ years of age, independent of baseline cognitive status (Northey et al., 2018). A better understanding of the mechanisms through which this occurs would allow tailoring and targeting of exercise interventions for populations most likely to benefit. One important mechanism may be through improvements in CBF, particularly in the hippocampus. Indeed, aerobic exercise has been shown to increase HBF both acutely (Steventon et al., 2020) and chronically (Burdette et al., 2010; Pereira et al., 2007; Thomas et al., 2020), with increased HBF correlating with cognitive gains (Chapman et al., 2013; Maass et al., 2015). Here, we add to the literature by reporting a selective increase in HBF with chronic exercise for *APOE4* carriers. The positive correlation between ΔHBF and ΔVM for our *APOE4* intervention group suggests these improvements in HBF may play a role in preventing cognitive decline.

There have been conflicting reports on the influence of the *APOE4* allele in exercise-induced changes in cognitive function and brain health. One randomized trial involving 170 older adults with self-reported memory problems suggested less cognitive benefit from exercise for *APOE4* carriers (Lautenschlager et al., 2008). However, a more recent trial of 200 patients with mild AD found *APOE4* carriers experienced more improvement in cognitive function after an exercise intervention than non-carriers (Jensen et al., 2019). Observational studies have likewise produced conflicting findings. For example, one study of cognitively-normal older adults concluded high physical activity level may preserve hippocampal volume in *APOE4* carriers selectively (Smith et al., 2014). However, a different cross-sectional study including older adults with and without AD found *APOE4* carrier status did not influence the relationship between cardiorespiratory fitness and brain atrophy (Honea et al., 2009). Our current findings expand upon these conflicting results by providing further evidence for a preferential benefit of exercise for *APOE4* carriers, specifically in HBF improvement. We likely observed this preferential benefit for *APOE4* carriers in the current study due to our selected outcome measure (HBF). There is increasing evidence that AD manifests differently for *APOE4* carriers and non-carriers, with *APOE4* carriers experiencing early pathology in the hippocampus associated with verbal memory impairment, while non-carriers with AD tend to have early pathology in the frontal and parietal lobes associated with impaired visuospatial and executive functioning (Emrani et al., 2020). Additionally, *APOE4* carriers with normal cognition have greater blood brain barrier breakdown in the hippocampus than non-carriers, and this cerebrovascular dysfunction is more predictive of future cognitive decline in *APOE4* carriers than non-carriers (Montagne et al., 2020). This suggests that distinct mechanisms may be involved in the pathogenesis of AD depending on *APOE4* genotype. Thus, we may have detected a benefit in

HBF from the exercise intervention specifically for *APOE4* carriers because cerebrovascular dysfunction in this region plays a more important role for participants with this genotype, who are therefore primed to show the greatest benefit. Additionally, hypertension has been repeatedly shown to be more detrimental for *APOE4* carriers than non-carriers (Caselli et al., 2011b; Zade et al., 2010), which means the hypertensive *APOE4* carriers in our study were likely at greater risk of brain pathology than the hypertensive *APOE4* non-carriers.

Hypertension may act mechanistically through cerebrovascular dysfunction to cause brain pathology (Wiesmann et al., 2013). Unlike family history, hypertension is a modifiable risk factor for dementia, and lowering blood pressure is thus an enticing intervention strategy to slow or prevent cognitive decline. The landmark Systolic Blood Pressure Intervention Trial Memory and Cognition in Decreased Hypertension (SPRINT-MIND) trial published in 2019 demonstrated that aggressive BP lowering (SBP < 120 mmHg) through medication significantly reduced the risk of mild cognitive impairment (MCI) in 9,361 older adults (Williamson et al., 2019). Notably, this was the first instance of an intervention of any type effectively reducing MCI incidence in a large population (Williamson et al., 2019). Although the mechanism through which aggressive SBP lowering prevented cognitive decline was beyond the scope of the SPRINT-MIND trial, other studies have suggested reducing BP in people with hypertension may act mechanistically by augmenting CBF. For example, one group found intensive BP lowering in cognitively-normal older adults significantly increased gray matter CBF (Tryambake et al., 2013). More recently, the Nilvadipine in AD (NILVAD) trial reported that reducing SBP significantly increased HBF in adults with mild-to-moderate AD, suggesting SBP reductions may provide benefit by increasing blood flow to the hippocampus specifically (de Jong et al., 2019). In the current study, baseline assessment of the entire population (hypertensive and

normotensive participants, n = 88) showed higher SBP was related to lower HBF, even when controlling for age, sex and *APOE4* carrier status. This finding provides further evidence for the connection between hypertension and reduced HBF in older adults.

Notably, it is unclear whether lowering BP provides the same benefit regardless of the intervention (i.e. medication, exercise, dietary changes). The SPRINT-MIND trial allowed a variety of medications to reach target SBP, including thiazide-type diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin receptors blockers and more (Williamson et al., 2019). Therefore, the observed effect of decreased MCI incidence was unlikely to have been tied to a specific mechanism of a single medication and more likely to have resulted directly from reduced SBP. Additionally, in the present study we found a significant relationship between reductions in SBP and improved HBF with aerobic exercise, while the NILVAD trial found reductions in SBP associated with improved HBF with a specific medication, nilvadipine (de Jong et al., 2019). We thus propose that the common mechanism may be reduced SBP, which results in cerebrovascular remodeling and improved blood flow delivery. Still, it is likely that both exercise and medications have unique effects throughout the body beyond BP reduction, and further exploration is needed to identify the ideal method for lowering BP in order to improve brain health.

By selecting only individuals with hypertension at baseline (defined using the ACC/AHA Clinical Practice Guidelines (Whelton et al., 2018)), the present analysis included older adults at high baseline vascular risk. We found that reductions in SBP for these participants with initially elevated BP were tied to improvements in HBF for the *APOE4* carriers only. This is in line with previous literature showing elevated blood pressure acts synergistically with *APOE4* carrier status to impair cognitive function (Caselli et al., 2011b) and promote brain pathology (Zade et

al., 2010). Furthermore, while the NILVAD sub-study did not assess *APOE4* carrier status (de Jong et al., 2019), it seems plausible that the observed improvement in HBF from SBP reduction could have been driven by a large proportion of *APOE4* carriers in the study population, considering the high prevalence of *APOE4* in AD (~65% of people with AD compared to ~25% of the general population) ("2018 Alzheimer's disease facts and figures," 2018). Regardless, the existing literature combined with our current data provide strong evidence for a synergistic relationship between the *APOE4* allele and peripheral BP on promoting brain pathology and cognitive dysfunction, suggesting interventions to lower BP are particularly important for this patient population.

It is important to note that although we observed a significant improvement in HBF only for *APOE4* carriers with baseline hypertension, this does not imply that exercise lacks beneficial value for *APOE4* carriers without hypertension. Clinical trials such as the present study cover a relatively short, defined time period during which it can be challenging to elicit significant measurable change. For this reason, clinical trials often select participants at elevated risk of illness to increase the likelihood of observing an effect of the intervention. Thus, in the present analysis we selected *APOE4* carriers with baseline hypertension, which places them at elevated vascular risk, because we sought to assess a vascular outcome, HBF. Therefore, it is likely that we observed significant differences with the intervention specifically in the hypertensive group because this was a particularly at-risk population for cerebrovascular dysfunction. That is, these participants with poor vascular health were primed to have the greatest (and therefore, most obvious) cerebrovascular benefits from aerobic exercise, as this intervention is known to induce profound, beneficial vascular adaptations (Green & Smith, 2018). Additionally, it is possible that *APOE4* carriers with normotension at baseline experienced significant improvements in other

areas that were not captured through an analysis of HBF. For example, aerobic exercise has been shown to reduce hippocampal inflammation in rats (Barrientos et al., 2011; Mokhtari-Zaer et al., 2020), an effect that would not have been captured through ASL-MRI utilized in the current study. Therefore, future studies are needed to assess changes in domains beyond brain blood flow that may contribute to cognitive improvements observed with aerobic exercise.

Our study has a number of limitations. This was an analysis of a secondary outcome of a clinical trial with different primary aims (Vidoni et al., 2020), and the findings should therefore be interpreted with caution. All participants were healthy and cognitively-normal, which may complicate the generalizability of our data to patient populations. Although changes in verbal memory did correlate with changes in HBF over the 52-week period for the *APOE4* carriers who exercised, there were no differences among intervention groups in mean change in cognitive functioning for the three domains, which means we cannot state that exercise improved cognition. The ACC/AHA guidelines recommend BP measurements taken on at least two separate occasions in order to diagnose hypertension. For the current study, we obtained two BP measurements on a single occasion. Therefore, some participants may have been included in our hypertensive group who would not have met the criteria if given a second reading on a separate occasion. We analyzed data from all participants regardless of the amount of exercise completed, which does not account for the potential effect of real exercise dose on HBF (only prescribed dose). In future studies, implementation of wearable activity monitors in both the intervention and control arms could allow for better characterization of this relationship. As we did not perform a lipid panel or arterial stiffness assessment on these participants, we could not assess the impact of the aerobic exercise intervention on changes in arterial stiffness or cholesterol levels, both of which likely played a role in mediating some of the observed exercise effects.

Finally, although our trial included a longer intervention than the majority of previously-published exercise intervention trials (Blumenthal et al., 2019; Chapman et al., 2013; Guadagni et al., 2020; Jensen et al., 2019; Kleinloog et al., 2019; Lautenschlager et al., 2008), the follow-up period of 52 weeks may still be too short to sufficiently characterize long-term clinical relevance of the exercise intervention. Future studies that follow participants for many years post-intervention would be better suited to assess clinical implications such as dementia risk.

In the current study, we report an aerobic exercise intervention improved HBF for cognitively-normal hypertensive *APOE4* carriers (but not non-carriers) and that greater HBF improvement over the 52-week intervention period related to better verbal memory performance for this group. Additionally, we show reductions in SBP may improve HBF for *APOE4* carriers only. These findings suggest aerobic exercise interventions, especially those that lower SBP, may be particularly beneficial for *APOE4* carriers with baseline hypertension. This knowledge could inform the design and execution of future interventional trials.

Chapter 5: Conclusions

Through the work in this dissertation, we sought to investigate contributions of vascular dysfunction to the pathogenesis of late-onset Alzheimer's disease (AD), specifically for *APOE4* carriers who are at highest known genetic risk. First, we characterized cerebrovascular function in *APOE4* carriers compared to non-carriers both with and without AD (Chapters 2 & 3). Next, we investigated the potential synergistic relationship between both peripheral and cerebral vascular risk factors and the *APOE4* allele on promoting brain pathology and dementia development (Chapters 3 & 4). And finally, we assessed whether an aerobic exercise intervention could improve cerebrovascular health and cognitive function for this at-risk population (Chapter 4).

APOE4 and cerebrovascular health

Cerebrovascular dysfunction has been proposed to be the earliest event in the pathogenesis of AD (Iturria-Medina et al., 2016; Sweeney et al., 2018), and recent evidence suggests *APOE4* carriers may experience cerebrovascular dysfunction that directly promotes AD pathology and cognitive decline (Koizumi et al., 2018; Montagne et al., 2020; Tai et al., 2016). In mouse models, *APOE4* has been shown to cause decreased vascularization which leads to elevated cerebrovascular resistance (CVRi), and the resulting chronic cerebral hypoperfusion causes downstream brain damage and cognitive decline (Koizumi et al., 2018). These mice have also shown an inability to compensate for reduced perfusion pressure after bilateral carotid artery stenosis, suggesting impaired cerebrovascular autoregulation, and they demonstrate a blunted cerebral blood flow (CBF) response to stimuli, suggesting impaired neurovascular coupling (Koizumi et al., 2018). Thus, we sought to characterize these metrics of cerebrovascular function in human *APOE4* carriers (Chapters 2 & 3). However, we did not find sufficient evidence that

these animal model findings translate to humans through our studies. Specifically, the *APOE4* allele was not associated with elevated cerebrovascular resistance or pulsatility (Chapter 2), and *APOE4* carriers did not demonstrate significantly impaired autoregulation (Chapter 2) or a blunted cerebrovascular response to exercise (Chapter 3) compared to non-carriers. Nonetheless, these studies should be repeated in larger and more diverse populations and employ alternative approaches, such as those outlined below in Considerations for Future Research.

APOE4 **moderates the relationship between vascular dysfunction and brain pathology**

Vascular risk factors are increasingly recognized to play an important role in cognitive decline and AD pathogenesis (Larsson & Markus, 2018). However, most studies investigating the connection between vascular health and brain function have not considered how this relationship may differ by *APOE4* carrier status. We thus sought to interrogate these differences in this dissertation work. In Chapters 3 and 4, we reported *APOE4* carrier status moderates the association between both *peripheral* vascular dysfunction (high pro-atherogenic cholesterol, elevated blood pressure) and *cerebral* vascular dysfunction (poor cerebrovascular conductance) and brain pathology.

APOE4 interacts with poor peripheral vascular health

Prospective studies have found an association between medications controlling common peripheral vascular risk factors - specifically, anti-hypertensive and statin use - and reduced AD risk (Forette et al., 2002; Jick et al., 2000; Larsson & Markus, 2018), suggesting hyperlipidemia and hypertension may promote cognitive decline. Additionally, our laboratory previously

reported impaired endothelial function, measured by brachial artery flow mediated dilation, predicts brain β-amyloid burden (Liu et al., 2019). Intriguingly, some studies have found evidence for an even stronger association between these peripheral vascular risk factors and cognitive dysfunction for *APOE4* carriers (Caselli et al., 2011a; Nation et al., 2016; Zade et al., 2010). Expanding upon this work, in Chapter 3 we described an interaction between *APOE4* carrier status and pro-atherogenic cholesterol level on the cerebrovascular response to an acute bout of exercise in cognitively-normal older adults. Specifically, higher non-high-density lipoprotein (non-HDL) cholesterol was associated with a blunted cerebrovascular response to exercise for the *APOE4* carriers only, while the opposite relationship was observed for non-carriers. Evidence from our laboratory suggests a blunted cerebrovascular response (lower ΔMCAv from rest to steady-state exercise) represents cerebrovascular dysfunction during this physiological stimulus. For example, we previously demonstrated a blunted cerebrovascular response to exercise in older adults with stroke (Kaufman et al., 2019; Kempf, 2019) and elevated brain β-amyloid load (Sisante et al., 2019). Thus, we propose the lower cerebrovascular response observed in *APOE4* carriers with elevated non-HDL cholesterol - controlling for β-amyloid load - reflects cerebrovascular pathology. In Chapter 3 we concluded high pro-atherogenic cholesterol may preferentially promote cerebrovascular dysfunction during physiological stressors such as acute exercise in *APOE4* carriers. In Chapter 4, we analyzed data from a randomized controlled trial that enrolled sedentary, cognitively-normal older adults. We found *APOE4* moderated the association between the change in systolic blood pressure (SBP) and hippocampal blood flow (HBF) over the year-long trial period for participants with baseline hypertension. Specifically, improving hypertension over the year bolstered HBF only in *APOE4* carriers. Likewise, *APOE4* carriers with worsening hypertension experienced additional

reductions in HBF, while this was not the case for non-carriers. These data expand on previous studies showing a synergistic relationship between hypertension and the *APOE4* allele on cognitive decline (Caselli et al., 2011a; Oberlin et al., 2015; Peila et al., 2001; Zade et al., 2010) and provide further evidence for tight blood pressure control particularly in *APOE4* carriers.

APOE4 interacts with poor cerebrovascular health

In addition to peripheral vascular dysfunction, *cerebro*vascular dysfunction may act synergistically with the *APOE4* allele to promote brain pathology and cognitive decline. For example, one large, longitudinal study (N = 1701; 10-year follow-up) found stroke and the *APOE4* allele act synergistically to increase dementia risk (Shaaban et al., 2019). Interpreting stroke history as an extreme indication of cerebrovascular dysfunction (and poor cerebrovascular health in general), this suggests cerebrovascular dysfunction may be even more detrimental in *APOE4* carriers than non-carriers in terms of promoting cognitive decline (Kaufman & Perales-Puchalt, 2019). Consistent with this idea, a recent study found blood brain barrier breakdown preferentially predicts future cognitive decline for *APOE4* carriers (Montagne et al., 2020). In Chapter 3, we sought to further probe this potential relationship. We found *APOE4* carrier status moderated the association between resting cerebrovascular conductance (CVCi) and brain β-amyloid load in cognitively-normal older adults. Specifically, poor CVCi was associated with higher β-amyloid load for the *APOE4* carriers only. This provides further evidence that cerebrovascular dysfunction may be more detrimental for *APOE4* carriers than non-carriers. That is, while the cross-sectional nature of this study does not allow for determination of directionality, animal studies have demonstrated that experimentally reducing cerebral blood flow and, therefore, CVCi, can promote β-amyloid deposition by both increasing β-amyloid

production and impairing β-amyloid clearance (Bannai et al., 2019; Bannai et al., 2017; Guglielmotto et al., 2009; Koike et al., 2010; Okamoto et al., 2012; Yamada et al., 2011). We thus propose based on our findings in Chapter 3 that low CVCi may cause increased β-amyloid deposition preferentially in *APOE4* carriers. This suggests that in addition to *peripheral* vascular function, maintaining *cerebral* vascular function is particularly important for people at highest genetic risk of late-onset AD.

Aerobic exercise preferentially improves cerebrovascular function in hypertensive *APOE4* carriers

There has been increasing emphasis on following a healthy lifestyle in order to reduce dementia risk (Dhana et al., 2020). Aerobic exercise in particular has become recognized as one of the most effective tools for preventing cognitive decline (Dhana et al., 2020). Considering the profound systemic vascular adaptations that occur with regular exercise - such as enhanced endothelial functioning and angiogenesis, improved lipid profiles and reduced vascular stiffness and pressure (Green & Smith, 2018) - as well as the link between cardiovascular disease risk and cognitive decline in older adults (Song et al., 2020), it seems plausible that the cognitive benefits of aerobic exercise stem from improvements in vascular health. *APOE4* carriers may be especially primed to benefit from exercise, as these individuals tend to have poor systemic vascular health (Belloy et al., 2019) as well as early cerebrovascular dysfunction that predicts cognitive decline (Montagne et al., 2020). Thus, in Chapter 4, we investigated whether *APOE4* carriers experienced greater vascular improvement from an aerobic exercise intervention.

We assessed cerebral blood flow (CBF) with Arterial Spin Labeling MRI (ASL-MRI) before and after a year-long aerobic exercise intervention (150 minutes per week) in cognitively-normal but sedentary older adults. We chose to focus on hippocampal blood flow (HBF) in particular because *APOE4* carriers have early blood brain barrier breakdown specifically in this region that occurs prior to β-amyloid or tau accumulation (Montagne et al., 2020) and can impair blood flow (Bell et al., 2012). We found the aerobic exercise intervention significantly improved HBF only for *APOE4* carriers with baseline hypertension. Specifically, the *APOE4* carriers in the control arm experienced an average loss of 2.08 mL/100g/min (-4.8%) while the *APOE4* carriers who underwent the exercise intervention improved HBF by 4.09 mL/100g/min (+17.3%). Importantly, these improvements significantly correlated with better verbal memory performance, which is the cognitive domain impaired earliest in *APOE4* carriers and most closely associated with hippocampal function (Emrani et al., 2020). These findings suggest aerobic exercise may be an effective intervention to prevent or delay cognitive decline in people at highest genetic risk of AD. Additionally, the observed HBF changes (and associated improved verbal memory performance) suggest aerobic exercise may mechanistically reduce dementia risk through cerebrovascular pathways.

Limitations

Here, we discuss general considerations for all studies included in this dissertation. Study-specific limitations are covered as relevant in the Discussion sections of Chapters 2-4.

Our study population

Recruitment for all three studies (Chapters 2-4) was conducted in collaboration with the Alzheimer's Disease Center (ADC) at the University of Kansas. This allowed for rapid and reliable recruitment of well-characterized participants. However, this study registry is somewhat homogeneous in terms of education (predominantly college educated), ethnicity (predominantly White) and socioeconomic status (predominantly upper-middle class), which means our findings are less generalizable to the entire population. Future studies should aim to recruit a more diverse and representative sample population.

Human subjects research

While human subjects research provides an unparalleled translational quality to data, it inherently does not allow for the same level of control and manipulation as research conducted at the bench. That is, the humans recruited for our studies enroll with many potentially confounding attributes that are not as prevalent in mouse or cell culture experiments. We attempted to account for potential confounders by including these variables in our multiple regression analyses, but there likely remains some variability unaccounted for due to unknown or unmeasured attributes. Additionally, human subjects research does not allow for the same level of experimental manipulation as that employed in animal research (for example, potentially harmful interventions such as bilateral carotid artery stenosis). Thus, in Chapters 2 and 3 we make conclusions about directionality from cross-sectional data based on evidence from the animal literature, with the caveat that directionality cannot be definitively determined. The question of interest in Chapter 4 could be answered ethically through a clinical trial (exercise intervention) and allows for a more robust determination of directionality. Despite these limitations, human subject studies remain

the most powerful tool for translational research and provide vital insight into human physiology and disease.

Methods utilized to image cerebral blood flow (CBF)

There are trade-offs involved with each method of imaging the cerebrovasculature employed in this dissertation work. Transcranial Doppler ultrasound (TCD, Chapters 2 & 3) does not allow for regional analysis but permits assessment of the cerebrovascular response during physiological stimuli such as standing and exercising, which is not possible with MRI. Conversely, Arterial Spin Labeling MRI (ASL-MRI, Chapter 4) provides regional specificity, which allowed us to study the hippocampus specifically, but lacks the temporal resolution of TCD. Overall, we hope the use of both imaging modalities in this dissertation provides a complementary characterization of cerebrovascular function in *APOE4* carriers.

COVID-19 pandemic

The coronavirus disease 2019 (COVID-19) pandemic produced unique challenges for this dissertation work. While in-person data collection for Chapter 4 had been completed prior to pandemic-related shutdowns, data collection was ongoing for the studies in Chapters 2 and 3. We are therefore grateful to the University of Kansas Medical Center and Alzheimer's Disease Center for helping our laboratory devise new methods to safely acquire data after only a brief period of inactivity. We were able to quickly switch the consent process for genotyping to an entirely electronic format to reduce face-to-face contact with participants. We also learned to maintain proper cleaning techniques and wear the appropriate personal protective equipment for

in-person visits in the REACH Laboratory. Through these measures we have been able to protect the laboratory members and our participants while collecting data that meets the same high-quality standards. Unfortunately, our recruitment efforts for Chapter 2 have suffered as a result of the pandemic, and we therefore report data in this dissertation for fewer participants than originally anticipated (N = 41 instead of 50). We continue to work to recruit participants for this study but realize it can be challenging for Alzheimer's patients and their study partners to choose to come to the medical center at this time. We hope the coming years will bring brighter days and allow for full recruitment for this study.

Considerations for Future Research

The work in this dissertation supports a role for systemic vascular dysfunction in AD pathogenesis for *APOE4* carriers. Additionally, the data highlight potentially efficacious interventions such as tight blood pressure control and aerobic exercise in order to prevent or delay cognitive decline in this at-risk population. We hope the findings presented in the present dissertation will inspire new research avenues and alternative approaches, some of which are proposed in the following section.

Recruitment age for participants

First, future studies should aim to recruit a wider age range and include participants in midlife in addition to older adults. Vascular dysfunction likely begins years before brain pathological changes such as β-amyloid and tau (Iturria-Medina et al., 2016; Sweeney et al., 2018). For example, *APOE4* carriers may demonstrate impaired cerebrovascular reactivity by the

third to fourth decade of life (Suri et al., 2015). Additionally, studies have found mid-life cardiovascular risk predicts future cognitive decline (Gupta et al., 2015; Virta et al., 2013), and this association may be even stronger than that observed for late-life cardiovascular disease (Olaya et al., 2019). Research into vascular health and interventions for middle-aged *APOE4* carriers could therefore prove even more fruitful than in older adults. Future studies characterizing cerebrovascular function in *APOE4* carriers in both middle and late adulthood could allow for identification of the ideal window for interventions such as aerobic exercise and blood pressure treatment (Chapter 4) or cholesterol control (Chapter 3). Additionally, evaluating a younger population may allow for detection of cerebrovascular dysfunction such as elevated CVRi or PI that is not apparent in older age groups (Chapter 2).

Second, I have come to wonder whether the simple age-matching performed by most of us studying human *APOE4* carriers (for example, comparing an 80-year-old *APOE4* carrier to an 80-year-old non-carrier) obscures an important piece of the puzzle. As noted in Table 1-1, the average age of AD onset is 68 years for *APOE4* homozygotes and 76 years for *APOE4* heterozygotes, compared to 84 years for non-carriers ("2018 Alzheimer's disease facts and figures," 2018). It could be proposed, then, that a cognitively-normal *APOE4* carrier who is 80 years of age (multiple years *older* than the average age of onset) may have overcome his/her genetic susceptibility and is less likely to develop AD, while the cognitively-normal non-carrier who is also 80 years of age (multiple years *younger* than average age of onset) has not demonstrated that feat. Likewise, it seems plausible that an 80-year-old *APOE4* carrier could have retained normal cognition through cerebrovascular compensation. In this case, the compensation could obscure differences when comparing the two participants or could even result in the *APOE4* carrier appearing to have *better* cerebrovascular function than the non-

carrier. To illustrate this point, consider one of the participants enrolled in the Chapter 3 study. This participant was an *APOE4* heterozygote but was entirely cognitively-normal at 86 years of age. Remarkably, her cerebrovascular response to exercise was 12.42 cm/sec, which falls in the top 10% of responses for the study (compare to mean of 5.41 cm/sec for all participants). This may suggest that this individual does not represent the typical pathology contributing to dementia development in *APOE4* carriers. That is, we hypothesized in Chapter 3 that the *APOE4* carriers would have a lower cerebrovascular response than non-carriers due to the *APOE4* allele acting mechanistically through cerebrovascular dysfunction to cause AD development. However, considering this 86-year-old had no cognitive impairment at the time of data collection despite carrying the strongest known genetic risk factor for AD, it would seem she does not represent typical physiology for an *APOE4* carrier who is on his/her way to dementia development. That is, this individual's cerebrovascular measures are likely better interpreted as representing compensatory or resiliency mechanisms of cerebrovascular function rather than pathological changes associated with the *APOE4* allele. If true, we may have combined both compensatory and pathological cerebrovascular changes when considering outcome measures for *APOE4* carriers in our sample. To avoid this issue for future studies, perhaps we should modify our definition of "age-matched" to adjust for genetic susceptibility. For example, it may be more informative to compare a 72-year-old *APOE4* heterozygote (4 years prior to average AD onset for heterozygotes) to an 80-year-old non-carrier (4 years prior to average AD onset for non-carriers). Though this would not be a straightforward problem to solve nor likely readily accepted in the field, a new approach to recruitment and/or data analysis that accounts for this issue could have profound impacts on the way we conduct and interpret *APOE4* research.

Cerebrovascular resistance and conductance

Future studies should repeat the cerebrovascular assessments performed in Chapters 2 and 3 over multiple years to assess longitudinal changes. While we did not detect differences in CVRi or CVCi between *APOE4* carriers and non-carriers in cross-sectional data (Chapter 2), we may observe important differences in the trajectory of these metrics over time based on genotype. For example, one longitudinal study with an 8-year follow-up period found *APOE4* carriers had a significantly greater rate of decline in CBF over time than the non-carriers (Thambisetty et al., 2010). Similarly, *APOE4* carriers may experience more significant changes in CVRi and CVCi over time. This longitudinal approach has other important advantages when paired with repeated studies of other outcome measures such as cognitive testing or biomarker scanning. For example, in Chapter 3 we found an inverse association between CVCi and brain *β*-amyloid load for the *APOE4* carriers based on cross-sectional data. We propose this may reflect poor conductance causing *β*-amyloid deposition specifically in those at genetic risk of AD, but we cannot rule out the opposite mechanistic direction, with elevated *β*-amyloid causing reduced cerebrovascular conductance more significantly in the *APOE4* carriers – and in fact, both mechanisms are likely at play. If future studies obtain multiple scans over time, we could better assess temporality and assert causality. For example, an observation that blunted CVCi precedes *β*-amyloid deposition by a few years would provide evidence that low conductance may increase *β*-amyloid load rather than vice versa.

Clinical trials to increase conductance or decrease resistance in the cerebrovasculature could provide even more compelling evidence. Vasodilators such as nilvadipine have been shown to increase regional CBF while reducing mean arterial pressure (MAP) (de Jong et al., 2019). Considering the equations used to calculate CVCi (CBF/MAP) and CVRi (MAP/CBF),

these changes therefore result in elevated CVCi and reduced CVRi. One potential future clinical trial, then, could involve administration of vasodilators to manipulate these cerebrovascular measures and observe downstream effects such as β-amyloid accumulation. If we were able to show that improving CVCi could prevent or slow subsequent β-amyloid accumulation in *APOE4* carriers, this would provide strong evidence for this mechanism as well as offer a new therapeutic avenue.

Pulsatility

Future studies should characterize pulsatile flow in vessels throughout the vascular system in addition to the middle cerebral artery (MCA) measured in Chapter 2. Pulsatile flow originates in the heart and is therefore most extreme in proximal vessels and attenuated distally. For example, Figure 5-1 illustrates the average attenuation in pulsatile flow for older and younger adults according to data from a previously published study (Zarrinkoob et al., 2016). Intriguingly, these authors demonstrated that pulsatility not only varied among intracranial arteries, but the ability to dampen pulsatile flow in each successively more distal vessel was significantly attenuated in older compared to younger adults. For example, younger adults on average had 86% of the pulsatility in the MCA compared to the immediately upstream internal carotid artery (ICA) while older adults had 94% of the pulsatility still present in the MCA (see Figure 5-1). Thus, it could be informative in future studies to record pulsatility (PI) in multiple vessels in addition to the MCA and assess percent attenuation from upstream vessels. With this approach, we may detect differences in dampening ability between *APOE4* carriers and non-carriers with and without AD. Additionally, measuring PI in other vessels could allow us to identify aberrations not apparent in the MCA. For example, one group found elevated PI in

people with AD compared to controls in the ICA and basal artery (BA) (Jin et al., 2017), but we did not record PI for these vessels in the Chapter 2 study. Future studies assessing PI in multiple intracranial vessels could provide a more comprehensive picture of cerebrovascular health for *APOE4* carriers.

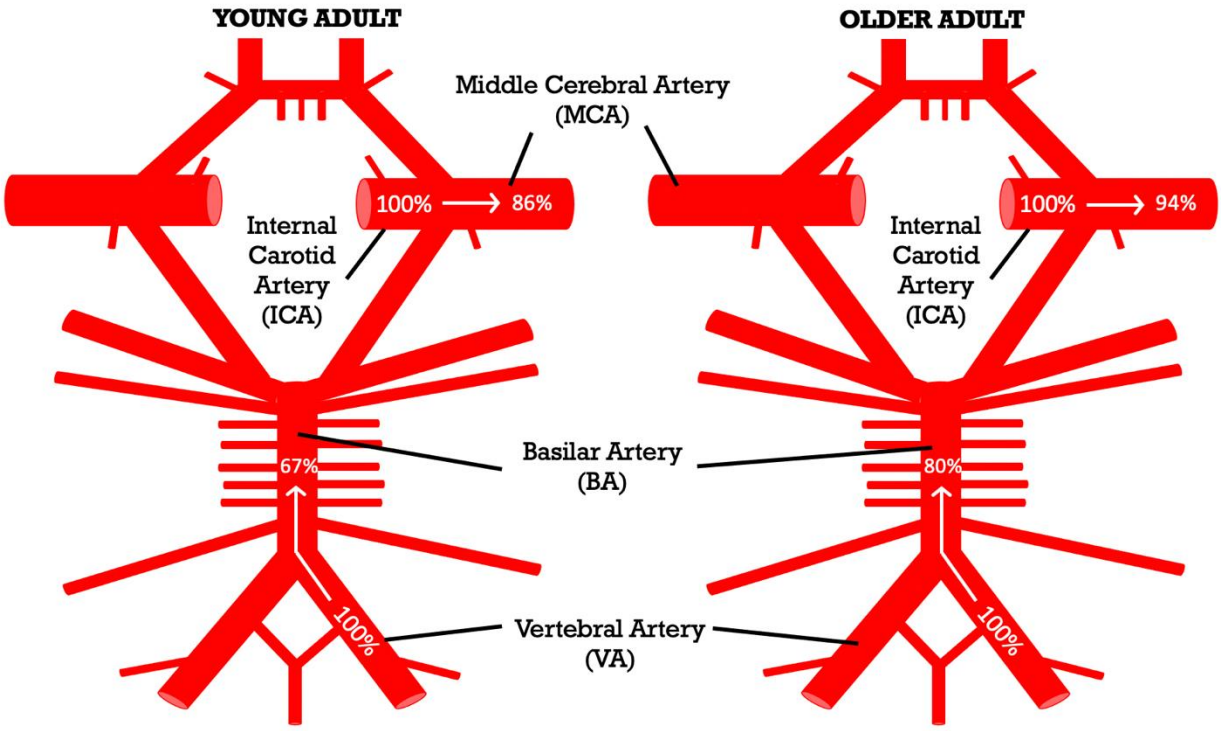

Figure 5-1: Attenuation of pulsatility in the cerebrovasculature

In the anterior circulation, the pulsatility of blood flow is attenuated on average by 14% in young and 6% in older adults as it travels from the internal carotid artery (ICA) to the middle cerebral artery (MCA). In the posterior circulation, pulsatility is attenuated 33% in younger adults and 20% in older adults as it travels from the vertebral arteries (VA) to the basilar artery (BA). Data presented in this illustration were derived from a study by Zarrinkoob and colleagues (2016). We present pulsatility in only four major vessels for simplicity.

Autoregulation

We found no difference between *APOE4* carriers and non-carriers in metrics of dynamic cerebrovascular autoregulation (dCA) as measured with the sit-to-stand protocol (Chapter 2). Future studies should assess dCA using other methods such as transfer function analysis (Zhang et al., 1998) and squat-stand maneuvers to confirm this null result. Squat-stand maneuvers have been shown to improve coherence and therefore strengthen the reliability of the dCA analysis (Barnes et al., 2017), though they can be physically challenging for older adults. However, even with these repeated studies and larger sample sizes, it seems possible dCA will not be found to be significantly disturbed in *APOE4* carriers. This may be due to a true lack of relationship and/or to the limitations of human studies. For example, in the mouse study that demonstrated reduced autoregulation in *APOE4* transgenic animals, the experimental protocol involved bilateral carotid artery stenosis (Koizumi et al., 2018). This methodology severely and chronically reduces blood flow delivery to the brain. Not only would this be unethical and undesirable in humans, but the resulting reductions in flow differ from what is induced in dCA protocols such as sit-to-stand. That is, in our sit-to-stand protocol (Chapter 2), we cause only a rapid and transient (seconds) reduction in perfusion pressure by asking the participant to stand. In contrast, the mouse studies that found impaired autoregulation with the *APOE4* allele induced severe reductions in CBF along a chronic timeline, over days to weeks (Koizumi et al., 2018). Regardless, future studies are warranted to investigate dCA in human *APOE4* carriers.

Acute and chronic cerebrovascular response to exercise

Future studies on the acute cerebrovascular response to exercise should measure MCAv during different types of aerobic exercise. For instance, other groups have recorded the MCAv

response to exercise on a treadmill (Parfitt et al., 2017) and stationary bicycle (Pott et al., 1996). Additionally, there is increasing interest in interval training exercise but little existing knowledge on how interventions such as high-intensity interval training acutely affect the cerebrovasculature (Whitaker et al., 2020). Characterizing the cerebrovascular response during a variety of exercise modalities, in addition to the moderate-intensity exercise stimulus on the recumbent stepper (employed in Chapter 3), would provide a more comprehensive picture of cerebrovascular function for *APOE4* carriers. Likewise, assessing the cerebrovascular response during diverse exercises may allow us to identify the ideal intervention modalities for this at-risk population. Further, future clinical trials involving exercise interventions should measure the acute cerebrovascular response during exercise before and after the intervention period. For example, in Chapter 4 we found *APOE4* carriers experienced greater improvements in hippocampal blood flow (HBF) after a year-long aerobic exercise intervention compared to non-carriers. It would be fascinating to measure the cerebrovascular response to an acute bout of exercise before and after this intervention to observe how the acute changes in MCAv *during* an acute training intervention may connect to the long-term changes in CBF observed *after* a chronic intervention. Specifically, I would hypothesize that the exercise intervention (such as that employed in Chapter 4) would improve the acute cerebrovascular response to exercise (measured in a different cohort in Chapter 3) at 52 weeks compared to baseline. Additionally, this protocol could investigate the hypothesis that aerobic exercise mediates beneficial adaptations in the cerebrovasculature through acute shear stress (Parfitt et al., 2017). If true, we may find participants with a more robust *acute* cerebrovascular response to exercise (larger ΔMCAv) – suggesting greater shear stress in the cerebrovasculature during exercise – would experience more significant improvements in CBF from the exercise intervention.

Clinical applicability

Moving forward, I hope to help drive a better understanding and recognition of cerebrovascular contributions to Alzheimer's disease and cognitive function in not only the scientific but also clinical realms. There are a variety of physiological assessments such as those performed in the chapters presented in this dissertation that could inform clinical care in the future. TCD is a remarkably affordable technology (compared to MRI or PET scans) that can be employed quickly and easily at the bedside. I can envision a future in which we use TCD to regularly assess cerebrovascular health (for example, measuring CVRi, CVCi, PI, dCA, ΔMCAv with exercise) in a manner similar to how blood pressure measurements and lipid panels are currently employed. As we continue to provide insight into how to interpret these measures through scientific studies, we will hopefully be able to use single measurements as well as changing measurements within an individual over time (for example, increasing PI in an older adult) as a screening tool to identify people in need of further evaluation or treatment. For example, our data presented in Chapter 3 suggest *APOE4* carriers with low CVCi are more likely to have elevated brain *β*-amyloid, suggesting they may be at an even further increased risk of developing AD. Additionally, we previously showed older adults with elevated brain *β*-amyloid have a significantly blunted cerebrovascular response (ΔMCA) to exercise. Could these affordable, painless and non-invasive measures be utilized in clinics in the future in order to identify patients at elevated risk of cognitive decline, without needing to employ expensive biomarker testing such as brain *β*-amyloid PET scans? Before we reach that reality, we will need more data from older adults such as that currently being collected in our laboratory and others. Still, TCD holds significant promise in terms of future clinical application.

Conclusion

This dissertation provides an extensive characterization of the relationship between vascular health and brain pathology in *APOE4* carriers (see Figure 5-2). First, we described cerebrovascular resistance, conductance, pulsatility and autoregulation for *APOE4* carriers with and without AD in order to investigate the impact of *APOE4* on measures of cerebrovascular function (Chapter 2). Next, we measured the cerebrovascular response to an acute bout of exercise for a separate cohort of cognitively-normal *APOE4* carriers and non-carriers (Chapter 3). Additionally, we discovered that the *APOE4* allele moderates the association between 1) pro-atherogenic cholesterol and the cerebrovascular response to exercise and 2) cerebrovascular conductance and brain β-amyloid load (Chapter 3). We found further evidence for this moderating effect in a third cohort, when we described an inverse relationship between change in blood pressure and hippocampal blood flow for *APOE4* carriers only (Chapter 4). Finally, we presented results from a randomized clinical trial that found aerobic exercise improves hippocampal blood flow for *APOE4* carriers with baseline hypertension, and this improved hippocampal blood flow was associated with better verbal memory (Chapter 4). Overall, our data support an important role for maintaining systemic cardiovascular health in order to enhance cerebrovascular function and potentially delay or prevent cognitive decline in *APOE4* carriers who are at highest known genetic risk of late-onset AD.

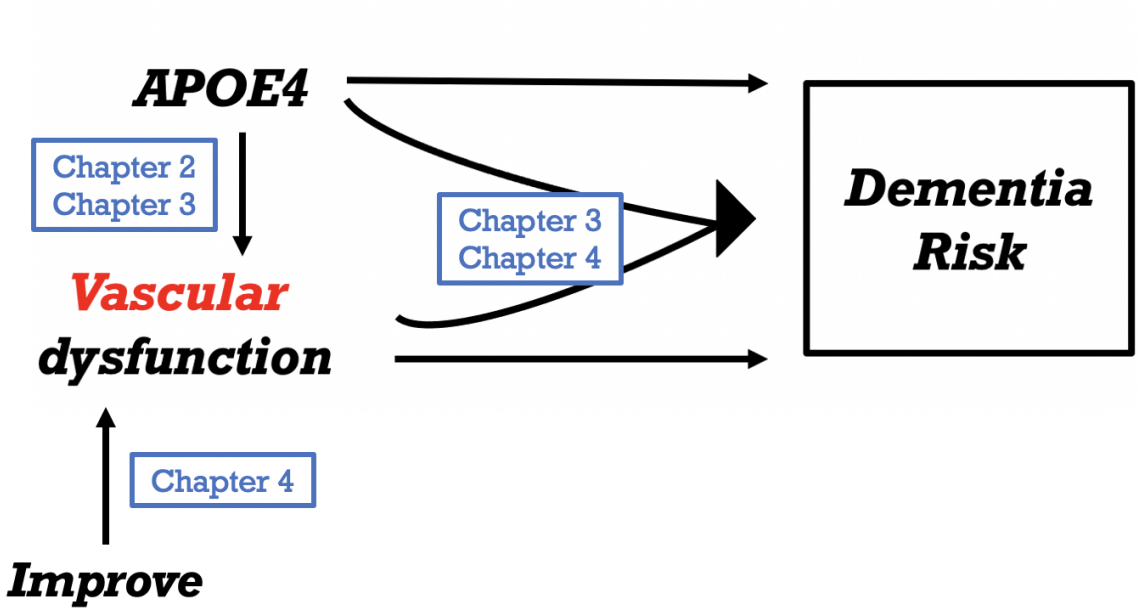

Figure 5-2: Dissertation summary

We first investigated the impact of *APOE4* on cerebrovascular measures (Chapter 2: CVRi, CVCi, PI, dCA; Chapter 3: ΔMCA with exercise). We then explored the synergistic relationship between the *APOE4* allele and poor vascular health on brain health (Chapter 3: low CVCi & high *β*-amyloid, high pro-atherogenic cholesterol & blunted ΔMCA with exercise; Chapter 4: high systolic blood pressure & low hippocampal blood flow). Finally, we assessed the impact of an aerobic exercise intervention on improving vascular health for *APOE4* carriers (Chapter 4: aerobic exercise improves hippocampal blood flow).

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