# IMAGING OF RETINAL BLOOD VESSELS IN THE ASSESSMENT OF ALZHEIMER DISEASE AND DEMENTIA RISK

A thesis presented to the graduate faculty of
The New England College of Optometry in partial fulfillment
of the requirements for the degree of Master of Science.

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May, 2010

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The New England College of Optometry, 2010

**Purpose:**

Retinal imaging has revealed relatively narrow retinal venules in patients with early Alzheimer Disease (AD). The purpose of this study was to determine if this narrowing is detectable in individuals without dementia but who possess a genetic risk factor, apolipoprotein epsilon 4 (APOE4), for Alzheimer disease.

**Methods:**

For this prospective, cross-sectional study, a pool of healthy participants was recruited from another study. All had well-controlled blood pressure and HbA1c of 6.1 or less; none smoked or had dementia, diabetes, glaucoma, retinal defects, ocular inflammation, diplopia, or history of intraocular surgery other than uncomplicated cataract surgery.

An examiner blind to the APOE status of the participants conducted a clinical exam including dilation with 0.5% tropicamide, A-scan, and acquisition of two 45-degree digital fundus photographs from each eye. A reader blind to APOE status determined central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) from fundus photographs using a semi-automated, published protocol (IVAN, University of Wisconsin, Madison). Relatively high AD risk was defined by the presence of the APOE4 allele.

**Results:**

Eight of 26 participants were positive for APOE4. Neither CRVE nor CRAE was significantly associated with APOE4. Post-hoc analysis revealed CRAE to be positively associated with performance on two tests of executive function, the Stroop test (P = 0.0022) and a category fluency test, Animals (P = 0.0025). The Stroop test was also negatively associated with age (P = 0.0057); together, age and CRAE explained more variability in the Stroop scores than either alone. When the data was analyzed without one individual who had a lower score on the Mini-Mental State Examination (MMSE) consistent with cognitive impairment, the negative trend between age and Stroop score became less significant (P = 0.0073), while the associations between CRAE and these two executive function tests became more significant (Stroop P = 0.0021, Category Fluency (Animals) P = 0.0012).

**Conclusions:**

Retinal venular caliber was not associated with AD risk or cognitive function in healthy individuals. Retinal arteriolar caliber was associated with two measures of executive function, which is more typically affected early in vascular cognitive impairment. To our knowledge, this is the first study showing an association between generalized retinal vessel narrowing and cognitive function in healthy individuals without cognitive impairment. Further research is needed to determine whether generalized retinal arteriolar narrowing may be an early marker for risk of vascular dementia.

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# CHAPTER 1: GENERAL INTRODUCTION

## Epidemiology of Alzheimer disease (AD)

Alzheimer disease (AD) is the most common cause of dementia in the United States (Alzheimer's Association, 2009; Grossman, Bergmann, and Parker, 2006) and accounts for 50 to 70 % of dementia cases (Alzheimer's Association, 2007). In 2009, 5.3 million Americans were estimated to have AD (Alzheimer's Association, 2009). The incidence is strongly correlated with age, doubling every five years after age 65 (Hirtz, Thurman, Gwinn-Hardy, Mohamed, Chadhuri, and Zalutsky, 2007). Thus, as our population ages, the prevalence is expected to increase as well. By the year 2050, the number of individuals aged 75 to 84 years with AD is expected to double, while that for individuals aged 85 and older with AD is expected to more than quadruple (Hebert, Scherr, Bienas, Bennett, and Evans, 2003).

In 1999, there were 20,042 veterans in the VA system diagnosed with AD and 35,692 diagnosed with dementia (Yu, 2003). The prevalence of dementia among veterans in the VA system aged 65 years and older is 7.3% (Krishnan, Petersen, Snow, Cully, Schulz, Graham, Morgan, Braun, Moffett, Yu, and Kunik, 2005), and it is expected to increase. From 1999 to 2000 and before September 11, 2001, the number of veterans 85 years or older was increasing at a mean rate of 11% per year, and it was projected to reach 1.3 million by 2020 (Yu, Ravelo, Wagner, and Barnett, 2004). Because head injury with loss of consciousness is a risk factor for AD (Patterson, Feightner, Garcia, Hsiung, MacKnight, and Sadovnick,

2008), the high prevalence of traumatic brain injury among veterans currently returning from Operation Enduring Freedom and Operation Iraqi Freedom (Defense and Veterans Brain Injury Center, 2009) must evoke concerns of what effect, if any, these injuries will have on the prevalence of AD as this population ages.

The growing number of individuals with AD leads to significant increases in mortality: the number of annual deaths attributed to AD increased 32.8% between 2000 and 2004 (Alzheimer's Association, 2007). In 1999, while the death rate for the entire VA patient population was approximately 3%, the death rate for VA patients with AD was 16.60% (Yu, 2003).

While the emotional burden on loved ones and caregivers is no doubt immeasurable, the financial burden of the disease is substantial. Dementia is associated with high levels of medical service use among veterans (Krishnan, et al., 2005). In 2000, the average Medicare cost for a beneficiary with AD or other dementia was almost three times higher than the cost for a beneficiary without dementia (Alzheimer's Association, 2007). In the year 1999, the average veteran's inpatient cost associated with AD was $11,661 (not including medical/surgical costs), making it one of the most expensive chronic conditions in terms of average inpatient care cost, second in this category only to spinal cord injury (Yu, 2003). In the year 2000, dementia was the fourth most expensive chronic care condition in the VA, and AD was the fifth (Yu, 2003). Of the most expensive chronic care conditions in the VA, the

long term care cost of AD showed the highest percentages of total cost of condition (Yu, 2003).

**Significance**

There is no cure for Alzheimer disease, but there are several new FDA-approved drugs that can temporarily slow the worsening of symptoms of the disease (Alzheimer's Association, 2007; Grossman, et al., 2006). Despite the potential benefit of these drugs, many people with dementia, including AD, are not diagnosed (Alzheimer's Association, 2007; Boustani, Peterson, Hanson, Harris, and Lohr, 2003). Arguably, the high percentage of total cost that is allotted for long term care in AD offers the opportunity to lower costs substantially through earlier diagnosis and treatment. Early diagnosis, leading to earlier treatment, would theoretically delay institutionalization, enhance patients' quality of life, and decrease long term care costs. Identifying early markers, especially in a noninvasive manner, is of great value for timely diagnosis.

**Diagnosis of Alzheimer Disease**

At autopsy, amyloid plaques and neurofibrillary tangles are found in the brains of AD patients, particularly in the medial temporal lobe (Querfurth and LaFerla, 2003). In addition, neuronal loss, white matter atrophy, amyloid angiopathy, inflammation, and oxidative damage are also seen (Querfurth and LaFerla, 2003).

Early clinical diagnosis tends to be problematic, as AD can present heterogeneously and overlap with other forms of dementia, particularly vascular dementia (VaD). Most cases of dementia are of the "mixed" type (Querfurth and LaFerla, 2010). The four most common types of dementia and their characteristics are summarized in **Table 1**, below.

**Table 1: Diagnosis and Prevalence of Dementia, Age 65 and Older**

| | Pre-clinical | Prodrome | Dementia | Est. Prevalence /1,000 |
|---|---|---|---|---|
| Alzheimer disease | PSEN1 mutations, trisomy 21, APOE4, family history, age | Amnestic MCI | Probable AD | 67* |
| Vascular cognitive impairment | Vascular risk factors, neuro-imaging, family history, age | Non-amnestic MCI | Vascular dementia | 50† |
| Lewy body dementia | ? | ? | Dementia with Lewy bodies | 1** |
| Frontotemporal dementia | ? | ? | Frontotemporal dementia | <1†† |

Table adapted from Sonnen, Montine, Quinn, Kaye, Breitner, and Montine, 2008;
**PSEN1multation**: increases cleavage of abnormal proteins (see text page 7); **APOE4**: apolipoprotein epsilon 4 allele; **MCI**: Mild cognitive impairment;
* Hirtz, Thurman, Gwinn-Hardy, Mohamed, Chadhuri, Zalutsky, et al., 2007.
†Moorhouse and Rockwood, 2008.
**Frank, 2003.
††Ratnavalli, Brayne, Dawson, and Hodges, 2002.

**Mechanisms of Disease**

In their normal forms, the proteins thought to play a role in the disease process are found in the brain. Amyloid precursor protein (APP) is a transmembrane protein that becomes

amyloid beta through proteolytic cleavage (Patterson, et al., 2008; Querfurth and LaFerla, 2010). APP, a natural product of metabolism, is normally cleaved by α-secretase into an amyloid beta (Aβ) protein 38 to 40 molecules long, which is nonamyloidogenic (Patterson, et al., 2008; Querfurth and LaFerla, 2010). However, cleavage of APP by γ-secretase results in Aβ 1-42, which is soluble and prone to aggregation into oligomers, leading to the formation of insoluble amyloid plaques that are found extracellularly in the brains of AD patients (Patterson, et al., 2008; Querfurth and LaFerla, 2010). In a mouse model of AD, Aβ is found in the retina, and even without evidence of neuronal degeneration, accompanies reduced amplitudes of a- and b-waves on scotopic electroretinogram (Perrez, Lumayag, Kovacs, Mufson, and Xu, 2009). Intravitreal injections of Aβ induce retinal ganglion cell apoptosis in animal eyes (Cordeiro, Guo, Coxon, Duggan, Nizari, Normando, Sensi, Sillito, Fitzke, Salt, and Moss, 2010). Aβ 1-42 has also been found in the crystalline lens of patients with AD (Goldstein, Muffat, Cherny, Moir, Ericcson, Huang, Mavros, Coccia, Faget, Fitch, Masters, Tanzi, Chylack, and Bush, 2003).

Hyperphosphorylated tau is a major component of the intracellular neurofibrillary tangles also found in the brains of AD patients. Tau is a microtubule-associated neuronal protein normally in the brain, but when hyperphosphorylated, it becomes insoluble (Querfurth and LaFerla, 2010). While neurofibrillary tangles are not exclusive to AD, as they are also found in Lewy body disease and Parkinson's disease, tau seems to play some role in the pathology of AD: Mice with mutant amyloid beta develop amyloid plaques, but those with reduced levels of tau retain their normal behavior and cognitive abilities, while those with normal

levels of tau demonstrate reduced cognition (Ashe, 2007). However, the significance of tau's role in the development of AD is still somewhat controversial, since tau mutations do not occur in AD (Querfurth and LaFerla, 2010).

AD commonly presents with vascular injury. Age-related white matter degeneration (leukoaraiosis) is common in AD, and in many cases collagen I and III accumulate in the walls of periventricular veins, causing narrowed lumina (Brown, Moody, Thore, and Challa, 2000). Changes in the cerebral vasculature in AD have been well-documented and includes deposition of collagen XVII in the walls of cerebral blood vessels (van Horssen, Wilhelmus, Heljasvaara, Pihlajaniemi, Wesseling, de Waal, and Verbeek, 2002), disruption of the blood-brain barrier (Querfurth and LaFerla, 2010), and cerebral amyloid angiopathy, in which amyloid accumulates in the walls of blood vessels (Weller, Massey, Newman, Hutchins, Kuo, and Roher, 1998). Cerebral amyloid angiopathy affects over 90% of patients with AD (Querfurth and LaFerla, 2010). Histological studies support the theory that cerebral capillary degeneration and microvascular changes occur early in AD (de la Torre, 2002). However, these changes alone do not account for the amount of cerebral blood flow reduction seen in patients with AD (Querfurth and LaFerla, 2010).

**Genetic Risk Factors**

While most cases of AD are sporadic, studies of familial forms have helped identify a number of genetic risk factors.

Apolipoprotein E (APOE) is thought to play a role in lipid transport and metabolism. There are three alleles for the APOE gene, which resides on chromosome 19: ε2 (APOE2), ε3 (APOE3), and ε4 (APOE4). Besides age, the presence of APOE4 is the one widely established independent risk factor for AD (Coon, Myers, Craig, Webster, Pearson, Lince, Zismann, Beach, Leung, Bryden, Halperin, Marlowe, Kaleem, Walker, Ravid, Heward, Rogers, Papassotiropoulos, Reiman, Hardy, and Stephan, 2007; Payami, Grimslid, Oken, Camicioli, Sexton, Dame, Howieson, and Kaye, 1997; Corder, Saunders, Strittmatter, Schmechel, Gaskell, Small, Roses, Haines, and Pericak-Vance, 1993), while the APOE2 allele may be protective against late-onset AD and cognitive decline (Corder, et al., 1993; Deary, Whiteman, Pattie, Starr, Hayward, Wright, Visscher, Tynan, and Whalley, 2004; Wilson, Bienas, Berry-Kravis, Evans, and Bennett, 2002). The frequencies of the APOE alleles in the general Caucasian population are: 0.77 for APOE3, 0.08 for APOE2, and 0.15 for APOE4 (Liew, Shankar, Wang, Klein, Bray, Couper, Sharrett, and Wong, 2007). A majority of individuals has at least one copy of the APOE3 allele. Those individuals homozygous for APOE4 have over an 11-fold increased risk of AD compared to those homozygous for APOE3 (Patterson, et al., 2008).

Other genes known to affect AD risk play a crucial role in modulating the cleavage of APP to Aβ. APP mutations increase the activity of β-secretase, and mutations in PSEN1 and PSEN2 increase the activity of γ-secretase, promoting the cleavage of APP into insoluble Aβ 1-42 and hindering the cleavage of APP by α-secretase into the normal Aβ 1-40 protein (Patterson, et al., 2008). The gene for APP is located on chromosome 21 (Patterson, et al., 2008), which

may help explain why individuals with Down syndrome (trisomy 21) commonly suffer from early AD.

**Diagnosis Using Retinal Imaging**

As part of the central nervous system, there is mounting evidence that retinal function is affected in AD (Guo, Duggan, and Cordeiro, 2010; Cordeiro, et al., 2010). Even in the early stages of disease, visual symptoms have been reported: impairments in reading, object-finding, depth perception, color recognition, contrast sensitivity, and perceiving structure from motion (Guo, et al., 2010). Both cortical and precortical degeneration appear to play a role in the visual abnormalities in AD (Guo, et al., 2010), and *in vivo* retinal imaging techniques have demonstrated retinal ganglion cell apoptosis in a mouse model of AD (Cordeiro, et al., 2010).

Retinal imaging has demonstrated that patients with early AD have narrower retinal venule blood columns than age-matched controls (Berisha, Feke, Trempe, McMeel, and Schepens, 2007; Feke, Berisha, Trempe, McMeel, and Schepens, 2006). Berisha, Feke, and coworkers found that patients with early AD had a mean retinal venule diameter of 131.7μm, compared to a mean of 148.3μm in age-matched controls (Berisha, et al., 2007). Because the retinal vasculature and the cerebral vasculature share certain homologies (Patton, Aslam, MacGillivray, Pattie, Deary, and Dhillon, 2005), the vascular alterations in the eye may reflect those occurring in the brain. The ability of retinal imaging to detect these changes in early AD suggests that it may be a useful, noninvasive tool in the assessment of risk for AD.

Liew and coworkers examined retinal vein caliber in healthy individuals with the APOE4 allele and found that APOE alleles were "weakly and inconsistently associated with retinal microvascular signs" (Liew, et al., 2007). The authors grouped individuals with one copy of the APOE2 allele and one copy of the APOE4 allele into the "APOE4" category. However, it is important to note that individuals with this genetic profile have been shown to have lower risk for late-onset Alzheimer disease (Corder, et al., 1993) and cognitive decline (Staehelin, Perrig-Chiello, Mitrache, Miserez, and Perrig, 1999) than those without APOE2. Thus, even though the prevalence of the APOE2 allele is low, about 7% in the Western population (Bennet, DiAngelantonio, Ye, Wensley, Dahlin, Ahlbom, Keavney, Collins, Wiman, de Faire, and Danesh, 2007), it may convey a protective effect, but this has yet to be fully elucidated. Therefore, in the study by Liew and coworkers, the presence of the APOE2 allele in some individuals may have confounded the study results. The group designation was an arbitrary decision by the investigators (Tien Wong, electronic communications, 2 Aug 2007).

In our study, the effect of the APOE4 allele on retinal venous caliber was examined by recruiting subjects assigned a designation of either "high risk" for AD (APOE4/APOE4 or APOE3/APOE4), or "low risk" for AD (APOE3/APOE3).

***Purpose of Study***

The purpose of this study was to identify associations between retinal vessel caliber and risk factors for AD in patients at increased risk for, but without clinically evident dementia. Our hypothesis was that individuals at increased risk for AD, identified in the Understanding Cardiovascular and Alzheimer's Disease Risk in the Elderly Study (U-CARE) and the Claude D. Pepper Older Americans Independence Center Study (OAIC), would demonstrate retinal vessel caliber changes according to risk for AD. Specifically, we expected that retinal venule blood column diameters would be thinner in healthy individuals who were at increased risk of developing AD.

# CHAPTER 2: STUDY POPULATION AND DEMOGRAPHIC DATA

## *Setting and Overview of Study Population*

All testing took place in the eye clinic at the Jamaica Plain campus of VA Boston Healthcare System. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the VA Boston Healthcare System's Institutional Review Board and Research and Development Committee.

Participants enrolled in the U-CARE and OAIC studies in the Geriatric Neuropsychology Laboratory of the VA Boston Healthcare System were invited to participate in this prospective, cross-sectional study. The U-CARE and OAIC studies are cross-sectional studies of community dwellers, aged 50 to 70 years and 55 to 84 years, respectively. The protocols of the two studies are very similar, with the major difference being that participants in OAIC were African-American. Recruitment for the U-CARE study initially began through mailings to family members of AD patients in the Bedford VA Medical Center. While this approach was meant to obtain a high number of participants with genetic risk factors for AD, spouses of patients also participated in the study. Those who were recruited for this study gave written informed consent for the information previously collected in the U-CARE and/or OAIC studies to be used in conjunction with this study.

Participants were screened for and excluded if they had clinical dementia. All participants underwent a physical exam, basic neurological examination, blood testing (fasting glucose,

hemoglobin A1c, homocysteine level, lipid profile, APOE genetic testing), tests of executive and memory function, and many had an electrocardiogram. In addition, many participants had structural magnetic resonance imaging (MRI, including T1/T2 weighted MRI and diffusion tensilor imaging), and functional imaging (fMRI, transcranial Doppler ultrasound). A list of neuropsychological and physical examination measures appears in **Appendix A**, page 93. The participants were equally distributed amongst four categories depending on AD risk (low or high) and cerebrovascular disease (CVD) risk (low or high) as defined in the U-CARE study protocol (Milberg, et al., U-CARE protocol).

To remain consistent with the U-CARE and OAIC studies, relatively high AD risk was also defined here by the presence of the APOE4 allele. Because some studies have suggested a possible protective effect of APOE2 against late-onset AD and cognitive decline (Corder, et al., 1993; Deary, et al., 2004; Wilson, et al., 2002), and because the manifestations of this effect on the retinal vasculature and potential confounding effect on our study outcome was unknown, participants were recruited for this study if they did not have the APOE2 allele.

**Sample size calculation**

To calculate sample size for our study, the data from Feke and coworkers were used (Berisha, et al., 2007), as theirs was the only study to date to examine retinal venule blood column diameters in patients with mild AD. In their study, the difference between means for their patients vs. controls was 16.6μm, or 11.1% (Berisha, et al., 2007). We reasoned that if subjects without clinically evident AD but with early changes showed half the difference,

then the central retinal vein equivalent of our subjects should be 5.5% less than the population average of 214.0μm, which was found in a large study of adults aged 45-84 (Wong, Islam, Klein, Klein, Cotch, Castro, Sharrett, and Shahar, 2006), a population whose age range was similar to ours. Using 214μm less 5.5%, or approximately 203μm, as an estimate of the mean central retinal vein equivalent for our high-risk group, a sample size of 40 (20 in each group), was calculated to be sufficient to detect a difference between these two groups using a one-sided test, with type I error of 0.05 and power 0.80.

The goal for this study was to recruit 40 subjects with low CVD risk, equally distributed amongst two categories depending on relative AD risk: low (APOE3/APOE3) and high (APOE3/APOE4 or APOE4/APOE4). For recruitment into this study, personnel in the Geriatric Neuropsychology Laboratory screened their own study participants and re-contacted individuals who 1) had previously agreed to be contacted regarding other studies; 2) did not have dementia; 3) met the inclusion criteria for this study; and 4) were willing to return to the Jamaica Plain VA Eye Clinic and participate in this study. The examiner in this study (NC) was blinded to the APOE status of the participants, but was aware that the participants had been prescreened for certain measures.

**Inclusion Criteria**

For the purposes of consistency with the U-CARE and the OAIC studies, and in order to exclude the most common systemic conditions known to affect retinal venule diameter, low CVD risk was defined by: 1) systolic blood pressure 140 mmHg or less; 2) hemoglobin A1c

of 6.0 or less; and 3) nonsmoking. Thus, in this study, subjects had well-controlled blood pressure by self-report, were non-diabetic, and did not smoke. For the purposes of recruitment, systemic blood pressure was used as an indicator of overall cardiovascular health. As recruitment became more difficult, the cutoff for systemic blood pressure was gradually increased. Several large studies have consistently demonstrated that blood pressure has little effect on venular caliber (Wong, Islam, Klein, Klein, Cotch, Castro, Sharrett, and Shahar 2006; Leung, Wang, Rochtchina, Tan, Wong, Hubbard, Klein, Mitchell, et al., 2001; Sharrett, Hubbard, Cooper, Sortie, Brothers, Nieto, Pinsky, and Klein, 1999), providing substantial evidence that this change would not affect the outcome measures of the study.

**Exclusion Criteria**

As part of their participation in the U-CARE and OAIC studies, participants were expected to have already been screened per the criteria for those studies, in which they were excluded for: taking any CNS drugs, any medications for severe psychiatric disorders, a history of blackouts/strokes/Parkinson's disease, a history of psychological or emotional problems, a history of drug or alcohol abuse, medical problems including anemia, bleeding disorders, insulin-treated diabetes, severely hyper/hypo active thyroid, HIV, liver disease, emphysema, chronic pulmonary disease, fluid in lungs, heart attack, radiation-treated cancer, history of brain injury, worse than 20/20 vision even when corrected with glasses or contact lenses, presence of blind spots, cataracts or substantial peripheral sensory deficits, severe glaucoma, lazy eye, neurologic findings pointing to a hemispheric lesion on one side, or prior symptomatic infarct. Subjects were also excluded if they were taking medications known to

adversely affect performance on neuropsychological tests or medications to control migraine headaches (Milberg, et al., UCARE protocol).

Excluding every systemic condition known to affect retinal blood vessels would have made recruitment too restrictive to be practical. Because diabetes and current cigarette smoking have been shown to affect retinal venule caliber (Wong, et al., 2006), participants were excluded for either of these in this study. Participants were also excluded for: glaucoma or optic nerve defects, retinal detachment or retinal defect causing blind spots, history of retinal surgery or intraocular surgery besides uncomplicated cataract surgery, active ocular inflammatory disease, double vision that would make fixation difficult during ocular imaging, inability to complete ocular imaging/testing, inability to undergo pupillary dilation, or unreadable images.

***Recruitment Outcome***

None of the participants in this study was a veteran. During recruitment, the laboratory site performing the genetic testing was changed, and as a result, genetic testing was delayed for approximately 8 months. Because the recruitment protocol required serology testing in order to first determine eligibility for this study, no recruitment could be performed during the time period when genetic testing was on hold. It is not known whether this delay adversely affected the final outcome of the recruitment efforts. Every U-CARE and OAIC participant still living in the area was contacted and offered the opportunity to participate in this study, and of those, 28 people completed the protocol (see **Table 2, Study Recruitment**, below).

The mean difference in time between physical/cognitive testing and the acquisition of ocular images was 1.876 years.

**Table 2: Study Recruitment**

| | **High AD Risk** | **Low AD Risk** |
|---|---|---|
| APOE profile | E3/E4 or E4/E4 | E3/E3 |
| Goal number of participants | 20 | 20 |
| Actual number recruited | 8 (all E3/E4) | 20* |

**E3:** APOE4; **E4:** APOE4
*Includes two participants who were excluded from analysis (see text).

**Table 3** shows subject characteristics, with standard deviation denoted as SD. Gradable images were acquired from every participant in this study, 13 of whom were female, 15 were male; 2 were Black, 26 were White. The mean age was 69.9 years (range 54-81). 18 participants reported a positive family history of AD. Ten participants were on blood pressure medications: five were on one medication, three were on two medications, and two were taking three medications. Two participants were excluded from analysis, one for having a familial blood clotting disorder that might affect retinal vasculature, and one for atrial fibrillation with fluctuating Coumadin dosages; both were White women who were taking blood pressure medications. 26 sets of images were used for final analyses.

**Table 3: Subject Characteristics**

| Study No. | Age (yrs) | Gender | Race * | Eye † | Smoking History | Hyper-tension Meds†† | Family History glau-coma | Edu-cation, years | Family History AD | Years since UCARE/ OIAC |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 64 | M | W | OD | Never | B | N | 18 | Y | 2.534 |
| 2 | 66 | F | W | OD | None x 6 yrs | none | N | 17 | Y | 2.666 |
| 3 | 74 | F | W | OD | Never | none | N | 17 | Y | 2.649 |
| 4 | 76 | F | W | OD | Never | none | N | 18 | Y | 2.227 |
| 5 | 71 | F | W | OD | None x many yrs | none | N | 17 | Y | 1.112 |
| 6 | 59 | F | W | OD | None x 30 yrs | none | N | 19 | Y | 1.054 |
| 7 | 61 | F | W | OS | None x 30 yrs | none | Y | 15 | Y | 1.636 |
| 8 | 56 | F | W | OD | Never | none | Y | 12 | Y | 0.921 |
| 9 | 81 | M | W | OS | None x 50 yrs | none | N | 16 | N | 2.589 |
| 10 | 62 | F | W | OS | None x 40 yrs | none | Y | 18 | N | 1.723 |
| 11 | 80 | M | W | OD | Never | none | N | 16 | N | 1.592 |
| 12 | 76 | M | B | OS | None x 30 yrs | none | N | 13 | Y | 2.145 |
| 13 | 54 | M | W | OD | Never | none | N | 16 | Y | 1.570 |
| 14 | 73 | F | W | OS | None x 50 yrs | none | N | 18 | Y | 1.205 |
| 15 | 73 | M | W | OS | Never | B, D | N | 10 | Y | 2.718 |
| 16 | 71 | M | W | OD | Never | A,D | N | 16 | Y | 2.838 |
| 17 | 54 | M | B | OD | Never | A | N | 12 | N | 0.836 |
| 18 | 63 | M | W | OD | Never | none | N | 12 | Y | 3.299 |
| 19 | 76 | F | W | OD | None x 40 yrs | A | N | 16 | Y | 2.915 |
| 20 | 77 | F | W | OD | Never | none | N | 16 | N | 2.803 |
| 21 | 61 | F | W | OD | Never | none | N | 14 | N | 1.677 |
| 22 | 79 | M | W | OD | None x 40 yrs | A | N | 12 | N | 1.071 |
| 23 | 59 | M | W | OD | Never | A | N | 18 | N | 3.142 |
| 24** | 78 | F | W | OD | Never | A,B | N | 16 | Y | 1.211 |
| 25 | 79 | M | W | OD | None x many yrs | none | N | 12 | Y | 1.058 |
| 26 | 79 | F | W | OD | None x 50 yrs | A,B,D | N | 14 | Y | 1.058 |
| 27** | 75 | F | W | OD | Never | A,B,D | N | 14 | Y | 1.173 |
| 28 | 79 | M | W | OD | None x 45 yrs | none | N | 20 | Y | 1.121 |
| Mean (SD) | 69.9 (8.83) | | | | | | | 15.43 (2.56) | | 1.876 (0.795) |

*W: White/Caucasian, B: Black/African-American

**Excluded from final analysis.

†OD: Right Eye; OS: Left Eye

††**B**: Beta-blocker; **D**: Diuretic; **A**: Ace inhibitor

There were no significant differences between the two groups (APOE4+/-) in age, gender, years of education, or family history of AD (**Table 4**, below).

**Table 4: Subject Characteristics by APOE4 Status**

| | **APOE4(-)** **(n=18)** | **APOE4(+)** **(n=8)** | **P** |
|---|---|---|---|
| Age, years **(mean ± SD)** | 54-79 **(68.9 ± 9.56)** | 61-81 **(70.4 ± 7.25)** | 0.70 |
| Education, years **(mean ± SD)** | 12-20 **(15.2 ± 2.58)** | 10-19 **(16 ± 2.82)** | 0.50 |
| Gender (Number of females, % females) | 6, 44.4% | 5, 62.5% | 0.169 |
| Family History of AD (Number, %) | 14, 77.7% | 4, 50% | 0.169 |

Recruiting from participants in the U-CARE and OAIC studies offered an opportunity to collect additional ocular data from individuals about whom large amounts of systemic and cognitive information had already been collected. Staff in the Geriatric Neuropsychology Laboratory, who had already gathered physical and serologic data on their participants, prescreened potential participants for this study, thus saving non-eligible individuals the time and efforts of an additional screening procedure. However, one drawback to this approach was the reliance on genetic testing to help determine whether a potential participant was eligible for this study. The delay of genetic testing resulted in a large time difference between the original physical/cognitive testing and the acquisition of retinal images, a

potential weakness of the study. However, the time difference between cognitive testing and the acquisition of retinal photographs in our study was less than that in the ARIC study, in which the images were acquired 3 years either after or before the cognitive testing (Patton, et al., 2005). In addition, because some U-CARE and OAIC participants had moved and their contact information had changed since their original visits, not all eligible participants in the U-CARE and OAIC studies could be contacted. Many eligible participants who could be contacted were not interested in returning to the VA for another visit to have their eyes dilated for testing.

In summary, fewer participants were recruited into this study than initially anticipated, despite all recruiting efforts, including the relaxing of systolic blood pressure criteria. The mean difference in time between the physical/cognitive testing in the U-CARE/OAIC studies and acquisition of retinal photographs in this study was 1.876 years. Although this time difference was larger than desired, it was smaller than that in the ARIC study.

***Testing***

The examiner was blind to each subject's APOE4 status. Following informed consent, the following testing was performed on each eye:

- Visual acuity (Snellen equivalent, ETDRS chart)
- Pupil testing
- Abbreviated biomicroscopy (slit-lamp examination) and determination of anterior chamber angle depth and status of corneal integrity, prior to instillation of any drops

- Goldmann applanation tonometry, for determination of intraocular pressure (IOP), using 1 drop of Fluress (fluorescein sodium 0.25% and benoxinate HCl 0.4% ophthalmic solution, Bausch & Lomb)
- Autorefraction for determination of refractive error, with simultaneous auto-keratometry, using the Topcon KR 8800 auto kerato-refractometer (Topcon Medical Systems, Paramus, NJ)
- Instillation of 0.5% tropicamide ophthalmic solution (Alcon Laboratories, Fort Worth, TX), using punctal occlusion. This was used because the 0.5% preparation wears off more quickly than the stronger 1% preparation and has only a slight effect on cycloplegia (Alcon Laboratories, Inc., 2004). Maximal dilation was not required; only a 4-mm pupil was needed for optimal images (Hubbard, Brothers, King, Clegg, Klein, Cooper, Sharrett, Davis, and Cai, 1999).
- Ultrasound A-scan for determination of axial length (Sonomed, Inc., Lake Success, NY), over closed eyelids, using Aquasonic 100 ultrasound transmission gel (Parker Laboratories, Fairfield, NJ)
- 45-degree Canon CR-DGi digital posterior segment photographs: at least two per eye, centered on the optic nerve (Canon, Tokyo, Japan)

For each participant, eye drops were instilled from a new, previously unopened bottle each of Fluress and tropicamide. This was per recommendation of the VA Boston Research Pharmacist in order to enhance subject safety. At the conclusion of testing, participants were given mydriatic glasses. For each participant, total testing time took approximately 25-30

minutes or less. Images were immediately saved as tagged image file format (TIFF) files (EyeQ Lite, Digital Healthcare Ltd., UK).

A reader blind to APOE4 status determined central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) from fundus photographs using a semi-automated, published protocol (IVAN, University of Wisconsin, Madison).

***Data Analysis***

Two-sample t-tests were used to examine differences of continuous variables between the two groups (APOE4+/APOE4-). Because of the small numbers of subjects in some of the groups, Fisher exact test was used instead of chi square test to analyze gender differences, and to compare the differences between positive versus negative family history of AD. For each continuous variable of the entire sample, simple linear regression was used for analysis, and post-hoc testing with Bonferroni correction was performed to compare the blood vessel data with cognitive test results.

For continuous variables that differed significantly between the two groups, an analysis of variance (ANOVA) was first performed, followed by a one-way analysis of covariance (ANCOVA) for two independent samples (using quartiles of continuous values), in order to determine whether the difference had a significant effect on blood vessel diameter, using an online software program (Vassarstats, Lowry, R., http://faculty.vassar.edu/lowry/VassarStats.html, Poughkeepsie, NY ). This was done with CRAE, CRVE, and AVR separately.

# CHAPTER 3: MEASURING THE RETINAL VASCULATURE

## *Background*

Several methods exist for measuring retinal blood vessel diameter, and each has its advantages and disadvantages. Using a scanning laser ophthalmoscope combined with laser Doppler flowmetry (Canon Laser Doppler Blood Flowmetry, CLBF, Canon, Tokyo, Japan), blood vessels can be measured in real time over a 2-second time period, allowing multiple measurements across the different stages of the cardiac cycle (Patton, et al., 2005). This instrument uses a tracking stripe from a green 543-nm HeNe laser, whose image is analyzed using software that determines vessel width, as well as blood flow, volume, and velocity (Patton, et al., 2005). The protocol can be standardized by measuring the retinal blood vessels at a specific location, such as one optic disc diameter away from the disc margin (Berisha, et al., 2007), and once the user inputs the axial length of the eye, the device can adjust for refractive error (Patton, et al., 2005). The instrument has an automatic eye tracking system and an internal fixation target (Yoshida, Feke, Mori, Nagoka, Fujio, Ogasawara, Konno, and McMeel, 2003).

Despite these advantages, the drawbacks to the CLBF severely limit its use. The resolution is limited to vessels greater than 60 μm in diameter (Patton, et al., 2005). Arteries are not tracked during eye movements as well as veins (Berisha, et al., 2007), and while the superior or inferior blood vessels can be measured with this device, the superior vessels are technically easier to measure (Berisha, et al., 2007). This method yields measurements with

reproducibility that is variable (Patton, et al., 2005), and accessibility is severely limited, as these devices were only made during a short time and only a handful resided in the United States at the time of our study (Canon Medical Systems, Technical Support, personal communications, 2007).

Other methods of measuring retinal blood flow include fluorescein angiography, pulsatile ocular blood flowmeter (POBF, Paradigm, Inc.), and Heidelberg retinal flowmeter (HRF, Heidelberg, Germany), and none is ideal (Wang, Bower, Izatt, Tan, and Huang, 2008). Recently, Doppler Fourier-domain optical coherence tomography (FD-OCT) has been used to rapidly measure total retinal blood flow in normal human subjects and correlate it with vessel diameter (Wang, Lu, Tan, Izatt, and Huang, 2009; Wang, et al., 2008). Unfortunately, this technology was still under development and was not available at the time of our study.

Methods employing fundus photography are most commonly used for measuring retinal blood vessels. Images can be quickly acquired and read later, allowing for large numbers of research subjects and broad-based epidemiological research studies. Traditionally, this has been done by capturing images on film, which is then processed and digitized for image analysis (Patton, et al., 2005). However, the resolution of digital photography has improved such that images can be acquired using a charged coupled device with a fundus camera (Patton, et al., 2005). Once acquired, the blood vessel widths are measured from the images; today's technology allows for a computer-assisted protocol that examines the intensity profile of the vessel (Patton, et al., 2005; Wong, Knudtson, Klein, Meuer, and Hubbard, 2004).

Measuring the blood vessels in this way does not actually measure the width of the blood vessel wall, but the erythrocyte column itself. It does not measure the surrounding clear plasma zone, which compromises less than 10% of the internal diameter (Liew, et al., 2007). Because the ratio of this width to the internal vessel caliber remains constant, it appears to be proportional to the internal retinal caliber in animal studies (Liew, et al., 2007). This method allows for comparisons between subjects and, when used across studies, between those studies.

A drawback to this method is that each image is acquired at one point during the cardiac cycle. Of retinal vessels, large venules show the least amount of variation across the pulse cycle, only about 9.6 µm or 7%, while arterioles show more, with small arterioles changing about 13.8 µm or 20% and large arterioles changing about 17.2 µm or 16% across the cardiac cycle (Knudtson, Klein, Klein, Wong, Hubbard, Lee, Meuer, and Bulla, 2004). The largest source of variability in vessel measurements -- accounting for more than 50% -- is across images (Knudtson, et al., 2004). Therefore, by examining the largest venules, taking multiple photographs of the same eye, and averaging the measurements, variability across the pulse cycle can be minimized. While this is time-consuming and difficult to accomplish with large cross-sectional studies, it can be done in small studies like this one.

The images are subject to image magnification (Patton, et al., 2005; Rudnicka, Burk, Edgar, and Fitzke, 1998; Wong, Wang, Rochtchina, Klein, and Mitchell, 2004). Although

dimensionless measurements such as artery/vein ratio (AVR) are not subject to image magnification and might therefore be preferred, arterioles and venules can be affected differently under different conditions, making the AVR less useful (Cheung and Wong, 2007). Thus, for this study, all efforts were made to examine the arterioles and venules separately, adjusting for image magnification as needed.

**Image Magnification**

Image magnification occurs as a result of both camera magnification and ocular magnification. Correction for magnification in retinal images is based on the Littmann formula (Littmann, 1982, as quoted in Bennett, et al., 1994), which was given as:

$$t = 1.37qs$$

where

t is the true size of a retinal feature

s is the measured size

1.37 is the magnification factor of the camera (degree/mm), also denoted as *p*

q is a factor dependent on the optical dimensions of the eye (mm/degree) (Rudnicka, et al., 1998; Garway-Heath, Rudnicka, Lowe,Foster, Fitzke, and Hitchings, 1998).

Since 1977, when Bengtsson and Krakau first described a method of correcting for magnification when taking measurements from a photographic image (Bengtsson and Krakau, 1992), a number of methods for finding q have been proposed using ametropia and keratometry; axial length only; axial length and ametropia; and axial length, anterior chamber

depth, crystalline lens thickness, keratometry, and ametropia (Garway-Heath, et al., 1998). Correction for ocular magnification has been shown to be less accurate with methods using refractive error than with methods using axial length (Rudnicka, Burk, Edgar, and Fitzke, 1998), such as Bennett's abbreviated axial length method, which is given as:

$$t = 1.37\ (0.01306\ (x - 1.82))s$$

where

x equals the axial length of the eye in millimeters (Bennett, Rudnicka, and Edgar, 1994).

But the value of p = 1.37 was specific for the particular Zeiss telecentric camera Littmann used (Rudnicka, et al., 1998). For telecentric cameras, which when focused allow rays of light to emerge parallel, p is a value determined solely by the camera and does not vary according to refractive error; for nontelecentric cameras, however, p varies according to the ocular refraction of the eye being imaged (K), such that $p = aK + b$ (Rudnicka, et al., 1998).

Rudnicka and colleagues studied several telecentric and non-telecentric fundus cameras and determined that, for the non-telecentric Canon CR4-45NM, a = 0.02 and b = 1.85 (Rudnicka, et al., 1998). Because the CR4-45NM (Canon, Tokyo) is a film camera with optics very similar to our digital Canon CR-DGi camera (Camera Medical Systems, Technical Support [Personal Communications] 27 Apr 2007), we used Rudnicka et al.'s values for a and b in our calculations, essentially duplicating their values of p for a given ocular refraction and axial length.

In other studies measuring the retinal blood vessels, ocular magnification is most commonly adjusted for using the Bengtsson formula, given as t = (1-0.017) * SER, where SER is the spherical equivalent refraction (Wong, et al., 2004; Zheng, Cheung, Aung, Mitchell, He, and Wong, 2009; Wong, et al., 2006). It should be noted, however, that Bengtsson also used a telecentric camera and derived this equation using a Gullstrand eye (Garway-Heath, et al., 1998; Bengtsson and Krakau, 1992).

**Measuring Retinal Vasculature from Fundus Photographs**

Rather than measuring retinal blood vessels at a specific point, approaches for quantifying generalized vessel narrowing may be more standardized. Methods to quantify generalized arteriolar narrowing were first developed by Parr and coworkers in 1974 (Knudtson, Lee, Hubbard, Wong, Klein, and Klein, 2003; Hubbard, Brothers, King, Clegg, Klein, Cooper, Sharrett, Davis, and Cai, 1999), and Hubbard and colleagues later developed a similar approach to quantifying retinal venular caliber (Knudtson, et al., 2003; Hubbard, et al., 1999). These formulas take into account the common trends in relationships between retinal blood vessel trunks and their branches in normotensive adults. The arteries and veins passing through the zone located 0.5 optic disc diameter to 1.0 optic disc diameter from the edge of the optic nerve ("Zone B") are measured (**Figure 1**, page 31), in order to calculate the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE), respectively.

The Parr-Hubbard formulas are:

$$\text{Arterioles } W_c = \sqrt{0.87W_a^2 + 1.01\,W_b^2 - 0.22W_aW_b - 10.76}$$

$$\text{Venules } W_c = \sqrt{0.72W_a^2 + 0.91\,W_b^2 + 450.05}$$

where $W_c$ is the caliber of the trunk vessel, $W_a$ is the caliber of the smaller branch, and $W_b$ is the caliber of the larger branch (Hubbard, et al., 1999). After all the blood vessels within the measuring zone are measured, the largest of the measured arterioles is paired with the smallest, then the next largest with the next smallest, etc., and used in the arteriole formula to obtain several new vessel equivalents (if there is an odd number of vessels, the remaining vessel is brought over to the next iteration). Of the new results, the largest is paired with the smallest, etc., and the process is repeated until there is one final value for CRAE. The process is then repeated with the venules until the value for the CRVE is obtained (Hubbard, et. al., 1999).

In order to decrease variability in these estimates, Knudtson and colleagues further refined these formulas using the six largest arterioles and six largest venules crossing through the measuring zone (Knudtson, et al., 2003). In order to compensate for the large variation in the number of bifurcations in the retinal blood vessels in an eye, a coefficient was first developed to express the relationship between vessel trunks and branches:

$$\text{Branching Coefficient} = (w_1^2 + w_2^2)/W^2$$

where $w_1$ is the width of the narrower branch, $w_2$ is the width of the wider branch, and W is the width of the parent trunk. Using data from young normotensive adults, the branching coefficients were measured, and W was solved for and used to revise the Parr-Hubbard formulas. The new formulas were:

$$\text{Arterioles:} \quad \hat{W} = 0.88 * (w_1^2 + w_2^2)^{1/2}$$

$$\text{Venules:} \quad \hat{W} = 0.95 * (w_1^2 + w_2^2)^{1/2}$$

where $w_1$ is the width of the narrower branch, $w_2$ is the width of the wider branch, and $\hat{W}$ is the estimate of the parent trunk (Knudtson, et al., 2003). The revised formulas correlate well with the previous formulas, but are more robust against the variability in the number of retinal vessels in the measuring zone (Knudtson, et al., 2003).

Using this method, correlation between right and left eyes is relatively high for both retinal arterioles and venules (Leung, Wang, Rochtchina, Tan, Wong, Hubbard, Klein, & Mitchell, 2003).

***Purpose***

The purpose of this study was to determine whether generalized retinal blood vessel narrowing, specifically retinal venule narrowing, was detectable using a standard protocol

and retinal fundus images acquired from individuals without dementia, but with a risk factor for Alzheimer disease, APOE4.

***Materials and Methods***

Images were analyzed by a single reader (NC) blinded to APOE status of the participants. The images of the right eye were analyzed (also referred to as "reading" or "grading"), although the images of the left eye were used in cases where the right eye's images were unreadable. Reading images took approximately 15-30 minutes per set. Both images of one eye were read, so that the values of each could then be averaged for that eye.

Retinal vessel diameters were determined from the fundus photographs using a semi-automated software program (IVAN, University of Wisconsin, Madison) based on a published, detailed protocol (Hubbard, et al., 1999; Knudtson, et al., 2003). The software came with an instruction manual. Reported intragrader and intergrader intraclass correlation coefficients for reproducibility of retinal vascular measurement using such a computer-assisted protocol are very good, ranging from 0.78 to 0.99 (Hubbard, et al., 1999; Wong, et al., 2006; Knudtson, et al., 2003), and in this case, the same reader analyzed all the images in this study.

The computer program uses digital photographs to measure all arterioles and venules within an area 0.5 to 1 optic nerve disc diameter from the optic disc margin ("zone B," see **Figure 1, Digital Fundus Image of a Right Eye**, next page). Through user interaction, it exports the

angles, widths, and standard deviations of the measured vessels to a Microsoft Excel spreadsheet (Microsoft Corporation).

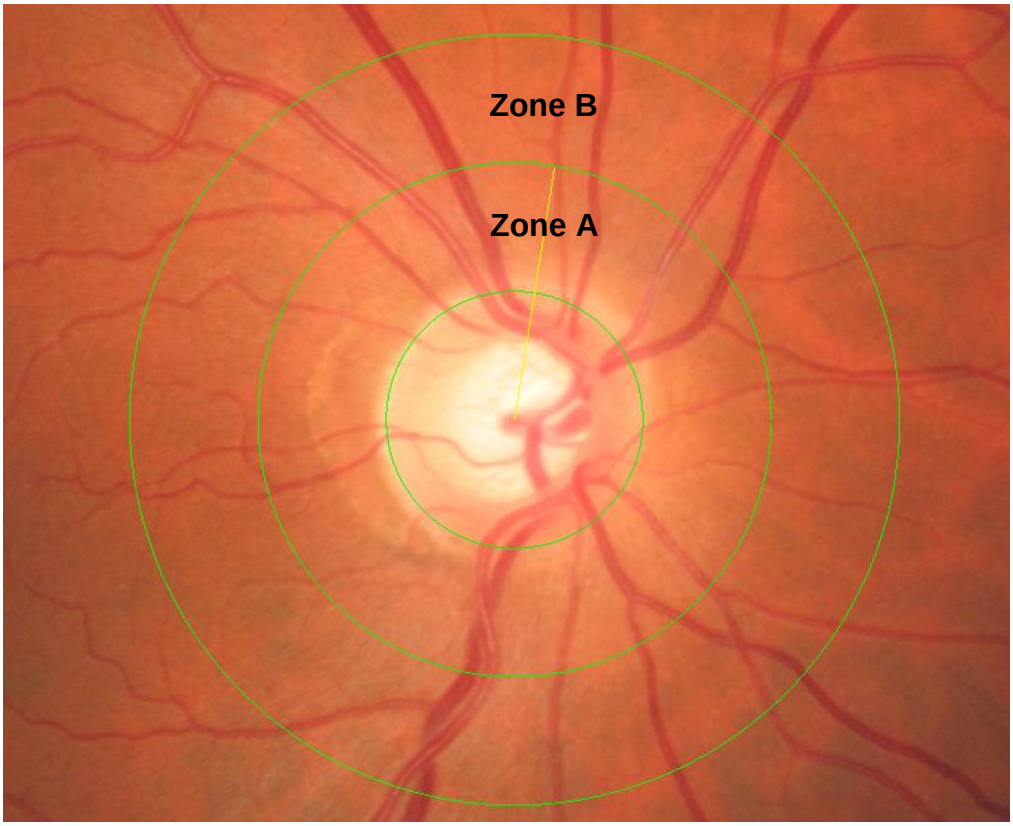



**Figure 1. Digital Fundus Image of a Right Eye.** The arteries and veins passing through the zone located 0.5 optic disc diameter to 1.0 optic disc diameter from the edge of the optic nerve ("Zone B") are measured, and the widest six arterioles and widest six venules are used for calculating the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE), respectively.

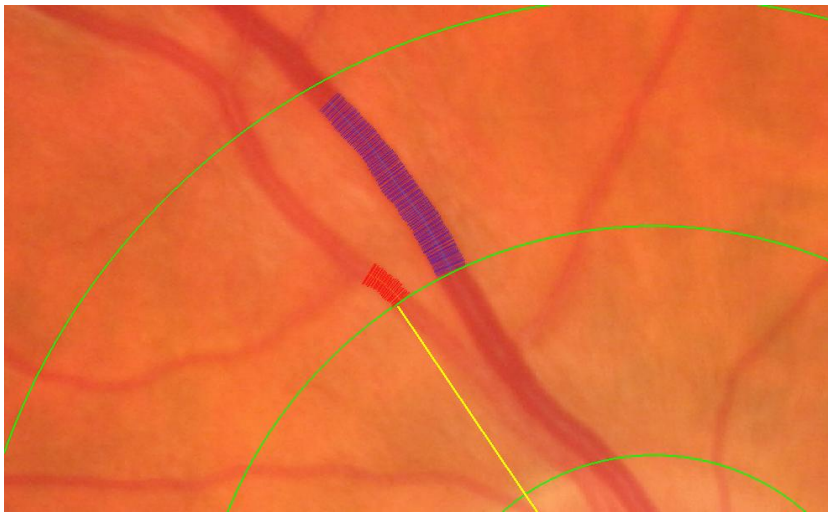


**Figure 2. Close-up View of an Arteriole and Venule During Computer-Assisted Measurements.** The IVAN program marks the measured portion of the arteriole in red and that of the venule with blue. By protocol, the vessels are measured before any bifurcations within the measurement zone (as seen with the arteriole in this image).

The diameters of the six largest arterioles and the six largest venules are then used to calculate the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively, using Parr-Hubbard formulas (Hubbard, et al., 1999), which have been revised by Knudtson (Knudtson, et al., 2003). Mean CRAE and CRVE was determined by averaging the values from both photographs of the same eye.

The measurement protocol used here is similar to that used in other published studies such as the Atherosclerotic Risk in Communities Study (ARIC) (Hubbard, et al., 1999), the Rotterdam Study (Ikram, Witteman, Vingerling, Breteler, Hofman, and de Jong, 2006), the Beaver Dam Eye Study (BDES) (Wong, Knudtson, Klein, Meuer, and Hubbard, 2004), the Blue Mountains Eye Study (BMES) (Leung, Wang, Rochtchina, Tan, Wong, Klein, Hubbard,

and Mitchell, 2003), and the Multi-Ethnic Study of Atherosclerosis (MESA) (Wong, et al., 2006).

Prior to recruitment, a method for adjusting the data to correct for the magnification effects of the camera and eye in non-emmetropes was devised. Large studies such as the ARIC study and the Cardiovascular Health Study (CHS) did not need to measure refraction and adjust for spectacle magnification in non-emmetropes (Wong, Hubbard, Klein, Marino, Kronmal, Sharrett, Siscovick Burke, and Tielsch, 2002; Wong, Klein, Couper, Cooper, Shahar, Hubbard, Wofford, and Sharrett, 2001; Wong, Wang, Rochtchina, Klein, and Mitchell, 2004; Couper, Klein, Hubbard, Wong, Sorlie, Cooper, Brothers, and Nieto, 2002; Sharrett, Hubbard, Cooper, Sortie, Brothers, Kieto, Pinksly, and Klein, 1999; Wong, Klein, Sharrett, Duncan, Couper, Tielsch, Klein, and Hubbard, 2002; Wong, Klein, Sharrett, Schmidt, Pankow, Couper, Klein, Hubbard, and Duncan, 2002; Cheung and Wong, 2007), because these studies used artery/vein ratio (AVR) to indicate generalized arteriolar narrowing, and the AVR is not affected by refraction (Wong, et al., 2004). However, the AVR has its limitations: When trying to measure veins as well as arteries, the AVR could mask variability in venules if there is variability in arterioles (Cheung and Wong, 2007). For this reason, separate measures of arteries and veins were used in this study.

We first considered whether analyzing CRAE and CRVE instead of AVR would necessitate an adjustment for refractive error to correct for magnification effects of the camera and the eye in non-emmetropes. (Wong, et al., 2004). When refraction is corrected for, measurement

error is decreased, and the statistical power to detect associations of retinal vessel diameters is increased (Wong, et al., 2004). This becomes especially important in studies with smaller sample sizes (Wong, et al., 2004). As mentioned above, the properties of a non-telecentric Canon camera were described by Rudnicka and coworkers (Rudnicka, et al., 1998), and a non-telecentric digital Canon CR-DGi digital camera (Canon Medical Systems, Irvine, CA) was used in this study. Therefore, we duplicated their methods for adjusting for camera magnification, while we duplicated Bennett's use of the Littmann formula when adjusting for ocular magnification, such that:

$$t = (0.02K+1.85)* (0.01306(x-1.82)) * s$$

where

t is the true size of a retinal feature

K is the ocular refraction

x is the axial length in millimeters

s is the measured size of the retinal feature (Rudnicka, et al., 1998).

The retinal vascular data were expected to follow a normal distribution (Wong, et al., 2006). After data were gathered from the first 20 participants, the measurements were analyzed to determine whether camera and eye magnification had a significant effect. After data were collected from all participants, separate linear regression analyses were used to analyze the effect of spherical equivalent refraction on CRVE, CRAE, and AVR.

Images were successfully acquired from all participants, although in six cases, the left eye was graded. The most common reason for using the left eye was poor image quality in the right eye (poor dilation, poor media, etc.), but one participant (#12) had a large scar in the right eye that affected the blood vessels in the reading field. In one case (#10) the a-scan readings were unreliable, so the left eye was used. When comparing the values derived from the two different images acquired from the same eye, the mean difference in CRAE was 1.13 µm (range 0.08-17.69 µm, standard deviation 7.7 µm), and the mean difference in CRVE between the two images was 0.31 µm (range 0.18-15.9 µm, standard deviation 6.4 µm).

No subjects withdrew from the study. **Table 5,** next page, shows subject and select eye characteristics for all participants. Spherical equivalent refraction ranged from -5.50 to +4.25 diopters, with a mean of +1 diopter. Axial length ranged from 22.62 to 26.29mm, with a mean of 25.0mm; corneal curvature ranged from 39.75 to 47.75 diopters with a mean of 43.75 diopters.

**Table 6**, page 37, shows the ocular measurement and blood vessel data from the participants used in final data analysis. These values were adjusted and then compared to literature values. Without adjusting the data for spherical equivalent refraction, spherical equivalent refraction did not correlate significantly with CRAE ($y = -0.5957x + 150.05$, $R^2 = 0.011709$, $P = 0.599$) (**Figure 3**, page 38), or CRVE ($y = 0.1201 + 212$, $R^2 = 0.000124$, $P = 0.957$) (**Figure 4**, page 38), nor did it correlate with AVR ($y = -0.00356x + 0.712554$, $R^2 = 0.0171$,

P = 0.524). Camera magnification (p), which was used to adjust the data, ranged from 1.75 to 1.94 degree/mm, with a mean of 1.86 degree/mm and standard deviation ±0.04 (not shown).

**Table 5: Subject Eye Characteristics**

| Study No. | Eye* | Spherical Equivalent Refraction (Diopters) | Axial length (mm) | Corneal Curvature, Diopters | Phakic† |
|---|---|---|---|---|---|
| 1 | OD | +1.50 | 25.74 | 43.25 | Y |
| 2 | OD | +1.25 | 24.93 | 45.75 | Y |
| 3 | OD | +3.50 | 24.5 | 42 | Y |
| 4 | OD | +0.25 | 25.39 | 42.5 | IOL |
| 5 | OD | +1.25 | 23.4 | 45.25 | Y |
| 6 | OD | -5.50 | 25.62 | 44.75 | Y |
| 7 | OS | 0 | 25.54 | 44.5 | Y |
| 8 | OD | +0.50 | 26.21 | 39.75 | Y |
| 9 | OS | -0.25 | 26.37 | 42.75 | IOL |
| 10 | OS | -0.25 | 24.17 | 47.75 | Y |
| 11 | OD | +0.75 | 24.96 | 44.75 | Y |
| 12 | OS | -0.25 | 25.82 | 45.25 | Y |
| 13 | OD | -2.50 | 25.3 | 42.75 | Y |
| 14 | OS | +1.25 | 24.96 | 44.75 | IOL |
| 15 | OS | +4.00 | 24.29 | 43.5 | Y |
| 16 | OD | -0.75 | 23.33 | 43.75 | IOL |
| 17 | OD | 0 | 24.6 | 44.25 | Y |
| 18 | OD | -0.12 | 25.5 | 42.87 | Y |
| 19 | OD | +4.00 | 22.62 | 44.87 | Y |
| 20 | OD | 0 | 25.26 | 44.12 | Y |
| 21 | OD | +3.00 | 23.94 | 43.25 | Y |
| 22 | OD | +3.75 | 26.29 | 41.75 | Y |
| 23 | OD | +0.75 | 25.57 | 44.25 | Y |
| 24** | OD | +3.75 | 24.97 | 43 | Y |
| 25 | OD | +1.50 | 25.46 | 42 | Y |
| 26 | OD | +4.25 | 24.23 | 44.5 | Y |
| 27** | OD | +2.00 | 24.72 | 44.5 | Y |
| 28 | OD | +1.00 | 25.43 | 42.75 | Y |
| Mean (SD) | | +1.00 (2.11) | 24.97 (0.9) | 43.75 (1.54) | |

*__OD__: Right Eye; **OS:** Left Eye
†Crystalline lens status; **Y**: natural; **IOL**: intraocular lens implant
**Excluded from final data analysis (see text)

## Table 6: Ocular Measurement and Blood Vessel Data*

| No.** | CRAE (μm) | CRVE (μm) | AVR | A-scan (mm) | AXIAL AMETROPIA (q) | Corneal Curvature (diopters) | Littmann CRAE (μm) | Littmann CRVE (μm) | Littmann AVR | Bengtsson CRAE (μm) | Bengtsson CRVE (μm) | Bengtsson AVR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 143.41 | 197.58 | 0.73 | 25.74 | 0.3124 | 43.25 | 84.25 | 116.08 | 0.73 | 139.75 | 192.54 | 0.73 |
| 2 | 155.52 | 216.83 | 0.72 | 24.93 | 0.3018 | 45.75 | 88.02 | 122.72 | 0.72 | 152.21 | 212.22 | 0.72 |
| 3 | 152.32 | 204.52 | 0.75 | 24.5 | 0.2962 | 42 | 86.76 | 116.50 | 0.74 | 143.25 | 192.35 | 0.74 |
| 4 | 141.45 | 185.18 | 0.77 | 25.39 | 0.3078 | 42.5 | 80.76 | 105.73 | 0.76 | 140.85 | 184.39 | 0.76 |
| 5 | 162.04 | 226.34 | 0.72 | 23.4 | 0.2818 | 45.25 | 85.64 | 119.62 | 0.72 | 158.60 | 221.53 | 0.72 |
| 6 | 140.76 | 188.42 | 0.75 | 25.62 | 0.3108 | 44.75 | 76.42 | 102.29 | 0.75 | 153.92 | 206.04 | 0.75 |
| 7 | 165.16 | 245.22 | 0.68 | 25.54 | 0.3098 | 44.5 | 94.66 | 140.54 | 0.67 | 165.16 | 245.22 | 0.67 |
| 8 | 147.39 | 186.51 | 0.79 | 26.21 | 0.3185 | 39.75 | 87.32 | 110.49 | 0.79 | 146.14 | 184.92 | 0.79 |
| 9 | 140.83 | 196.60 | 0.72 | 26.37 | 0.3206 | 42.75 | 83.30 | 116.29 | 0.72 | 141.43 | 197.43 | 0.72 |
| 10 | 178.02 | 229.37 | 0.78 | 24.17 | 0.2919 | 47.75 | 95.87 | 123.53 | 0.78 | 178.78 | 230.34 | 0.78 |
| 11 | 150.97 | 219.97 | 0.69 | 24.96 | 0.3022 | 44.75 | 85.09 | 123.98 | 0.69 | 149.04 | 217.17 | 0.69 |
| 12 | 154.75 | 247.56 | 0.63 | 25.82 | 0.3134 | 45.25 | 89.48 | 143.15 | 0.63 | 155.40 | 248.61 | 0.62 |
| 13 | 170.61 | 228.18 | 0.75 | 25.3 | 0.3066 | 42.75 | 94.23 | 126.03 | 0.75 | 177.86 | 237.88 | 0.75 |
| 14 | 166.03 | 237.50 | 0.70 | 24.96 | 0.3022 | 44.75 | 94.10 | 134.60 | 0.70 | 162.50 | 232.45 | 0.70 |
| 15 | 149.26 | 227.82 | 0.66 | 24.29 | 0.2934 | 43.5 | 84.70 | 129.28 | 0.66 | 139.11 | 212.32 | 0.66 |
| 16 | 135.78 | 190.96 | 0.71 | 23.33 | 0.2809 | 43.75 | 69.99 | 98.44 | 0.71 | 137.51 | 193.39 | 0.71 |
| 17 | 145.87 | 257.19 | 0.57 | 24.6 | 0.2975 | 44.25 | 80.28 | 141.55 | 0.57 | 145.87 | 257.19 | 0.57 |
| 18 | 143.32 | 191.72 | 0.75 | 25.5 | 0.3093 | 42.87 | 81.90 | 109.56 | 0.75 | 143.61 | 192.11 | 0.75 |
| 19 | 134.61 | 191.07 | 0.71 | 22.62 | 0.2716 | 44.87 | 70.71 | 100.37 | 0.70 | 125.46 | 178.08 | 0.70 |
| 20 | 148.21 | 190.66 | 0.78 | 25.26 | 0.3061 | 44.12 | 83.93 | 107.97 | 0.78 | 148.22 | 190.65 | 0.78 |
| 21 | 145.51 | 180.42 | 0.81 | 23.94 | 0.2889 | 43.25 | 80.38 | 99.67 | 0.81 | 138.09 | 171.22 | 0.81 |
| 22 | 148.89 | 218.24 | 0.68 | 26.29 | 0.3196 | 41.75 | 91.76 | 134.50 | 0.68 | 139.40 | 204.32 | 0.68 |
| 23 | 149.75 | 248.04 | 0.60 | 25.57 | 0.3102 | 44.25 | 89.58 | 148.37 | 0.60 | 140.20 | 232.23 | 0.60 |
| 25 | 124.36 | 194.76 | 0.64 | 25.46 | 0.3087 | 42 | 72.20 | 113.08 | 0.64 | 121.19 | 189.79 | 0.64 |
| 26 | 152.08 | 219.40 | 0.69 | 24.23 | 0.2927 | 44.5 | 86.33 | 124.55 | 0.69 | 141.09 | 203.55 | 0.69 |
| 28 | 140.87 | 194.68 | 0.72 | 25.43 | 0.3083 | 42.75 | 81.24 | 112.27 | 0.72 | 138.48 | 191.37 | 0.72 |
| Mean (SD) | 149.53 (11.76) | 212.11 (23.09) | 0.71 (0.06) | 24.98 (0.95) | 0.30 (0.01) | 43.75 (1.58) | 84.57 (7.04) | 120.04 (14.29) | 0.71 (0.06) | 138.10 (8.2) | 196.96 (17.51) | 0.71 (0.07) |

*__CRAE:__ Central retinal artery equivalent using Knudtson formulae (Knudtson, et al., 2003); __CRVE:__ Central retinal vein equivalent using Knudtson formulae (Knudtson, et al., 2003); AVR__:__ artery/vein ratio; __K:__ keratometry

__Littmann:__ adjusted with Bennett's abbreviated Littmann formula (Garway-Health, Rudnicka, Lowe, Foster, Fitzke, and Hitchings, 1998; Bennett, Rudnicka, and Edgar, 1994)

__Bengtsson:__ adjusted using Bengtsson's method (Bengtsson and Krakau, 1992).

*CRAE, CRVE, and AVR were averaged from two images of the same eye*.

**Subjects 24 and 27 were excluded from data analysis (see text).

**Figure 3: Central Retinal Artery Equivalent vs. Spherical Equivalent Refraction**

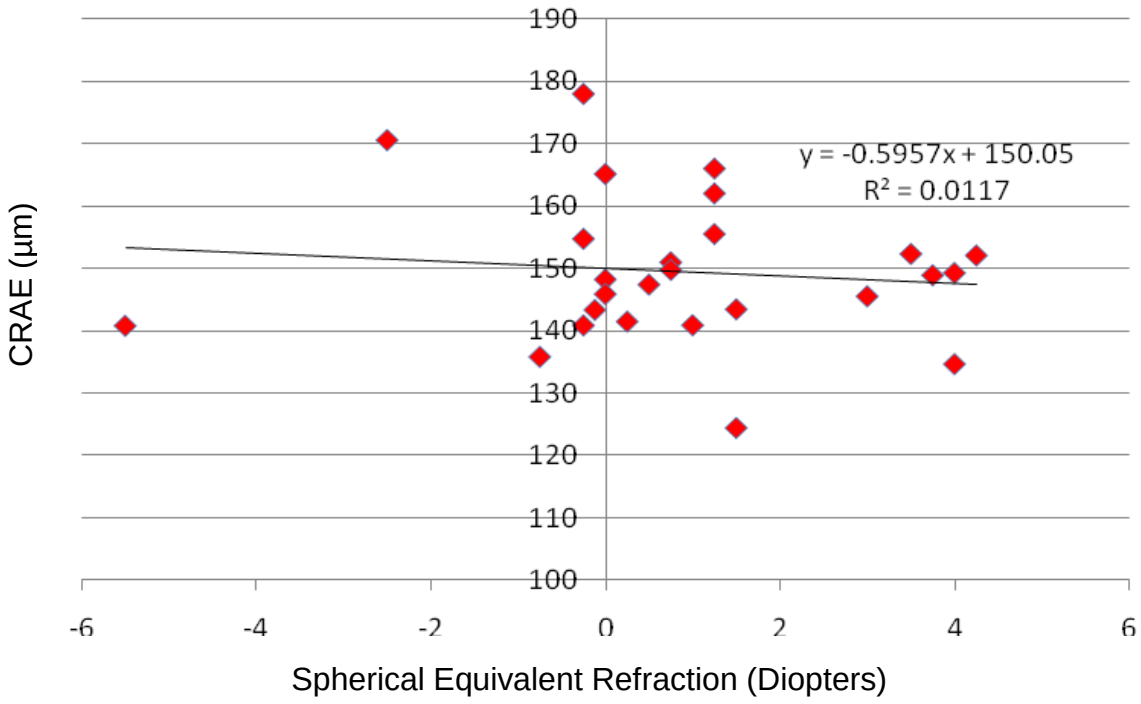
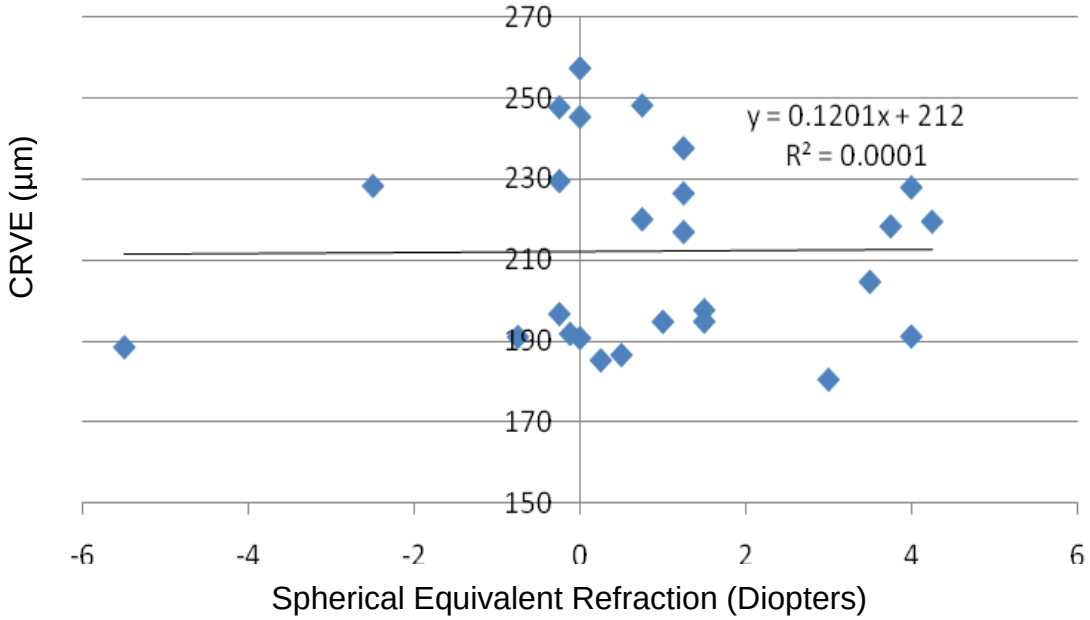



**Figure 4: Central Retinal Vein Equivalent vs. Spherical Equivalent Refraction**



***Discussion***

As the data were collected, it became clear that the IVAN software uses an internal formula by which it corrects for much of the camera magnification. When the program is first configured by the administrator, the number of pixels per average micron must be calculated using a sample set of twenty images and Photoshop CS4 (Adobe Systems, Inc., San Jose, CA), as detailed in the manual supplied by the developers. The distance between the center of the optic disc to the center of the macula in a sample set of twenty images from the first ten participants was measured in pixels, then the number of pixels was averaged and converted into microns, using the average distance of 1800 μm as found in the Atherosclerosis Risk in Communities Study (Wong et al., 2002). The administrator must then input this ratio into a configuration file used by the IVAN program. While there is generally some residual effect of refractive errors on vessel measurements, the effect is small, about 3-4% difference across a range of +2 to -6 diopters, and does not affect the association between vessel measurements and systemic conditions (Cheung N, electronic communications, 17 May 2007).

As seen in **Table 7: Comparison of Computed Values, Littmann-Adjusted Values, Bengtsson-Adjusted Values, and Selected Literature Values,** next page, our raw values were closest to those of Wong et al., 2006, and Ikram et al., 2004. This is not surprising, since these two studies also used the formulas modified by Knudtson (Knudtson, et al., 2003), referred to as "Big6" in the table.

**Table 7: Comparison of Computed Values, Littmann-Adjusted Values, Bengtsson-Adjusted Values, and Selected Literature Values***

| | Mean CRAE (µm) (SD) | Mean CRVE (µm) (SD) | Mean AVR (SD) | Camera type | Formulae | Population |
|---|---|---|---|---|---|---|
| **Raw data, this study (Big6)†** | **149.53 (11.76)** | **212.10 (23.09)** | **0.71 (0.06)** | **45° Canon CR-DGi (N)**** | **Big6†** | **54-81-year-old non-diabetics without cardiovascular disease** |
| **Big6, Littmann-Adjusted** | **84.57 (7.04)** | **120.04 (14.29)** | **0.71** | | | |
| **Big6, Bengtsson-Adjusted** | **138.10 (8.20)** | **196.96 (17.51)** | **0.71** | | | |
| **P-H Raw data, this study††** | **169.42 (14.72)** | **310.80 (16.45)** | **0.55 (0.04)** | | **P-H††** | |
| **P-H, Littmann-Adjusted** | **95.71 (8.7)** | **175.64 (11.9)** | **0.55** | | | |
| **P-H, Bengtsson-Adjusted** | **169.97 (23.99)** | **311.93 (40.11)** | **0.55** | | | |
| Wong, et al., 2006 (Multi-Ethnic Study of Atherosclerosis) | 144.1 (14.4) | 214.0 (22.2) | 0.67 | 45° 6.3 megapixel digital non-mydriatic | Big6, Bengtsson-adjusted | 45-84-year-olds without cardiovascular disease |
| Wong, et al., 2004 (Blue Mountain Eye Study), right eye, emmetropes | 193.2 (SE 0.5)*** | 224.4 (SE 0.5)*** | 0.86 (SE 0.002)*** | Zeiss FF3, digitized for grading (T)** | P-H | Non-diabetics aged 49 years and older |
| Wong, et al., 2004 (Blue Mountain Eye Study), right eye, emmetropes, adjusted for refraction)** | 192.5 (SE 0.5)*** | 223.6 (SE 0.5)*** | 0.86 | | P-H, Bengtsson-adjusted | |
| Leung, et al., 2003 (Blue Mountain Eye Study | | | | | P-H | |
| Males | 191.2 (21.3) | 225.5 (20.6) | 0.85 | | | |
| Females | 194.6 (20.4) | 224.6 (20.8) | 0.87 | | | |
| Ikram, et al., 2006 (Rotterdam Study) | 149.5 (14.0) | 223.8 (19.9) | 0.67 (0.05) | 20° Topcon, digitized for grading | Big6, Littmann-adjusted | 56-74-year-old community dwellers |
| Wong, et. al., 2003 (Beaver Dam Eye Study), right eye | 194.2 (21.2) | 225.0 (20.5) | 0.865 (0.08) | Unknown type, 30° digitized for grading | P-H | Non-diabetic adults |
| Hubbard, et. al., 1999; Liew, Sharrett, Wang, Klein, Klein, Mitchell, & Wong, 2008 (Atherosclerosis Risk in Communities Study) | 162.1 (1.3) | 192.2 (1.6) | 0.84 | Canon CR-45UAF (N)**, slides digitized for grading | P-H | Non-diabetics aged 48-73 years |

***CRAE:** Central retinal artery equivalent; **CRVE:** Central retinal vein equivalent; **AVR:** artery/vein ratio; **Littmann-Adjusted:** adjusted using Littmann method, modified for camera magnification (Rudnicka, Burk, Habil, Edgar, and Fitzke, 1997); **Bengtsson-Adjusted:** adjusted using Bengtsson method (Bengtsson and Krakau, 1992)

****T**: Telecentric; **N**: Non-telecentric

*****SE: Standard error

**†Big6:** Knudtson-modified Parr-Hubbard formulas (Knudtson, Lee, Hubbard, Wong, Klein, & Klein, 2003);

**††P-H:** original Parr-Hubbard formulas (Hubbard, Brothers, King, Clegg, Klein, Cooper, Sharrett, Davis, & Cai, 1999)

The reasons for the differences in our own values -- even between the Littmann and Bengtsson formulas -- and the adjusted values found in the literature are not immediately clear. Our raw CRVE values obtained using the Parr-Hubbard formulas (Hubbard, et al., 1999) are much larger than those in any other compared study. Applying the Bengtsson adjustment did not make the CRVE more comparable, but the Littmann adjustment made the CRAE much smaller than that of any compared study. The amount of difference was unexpected, since previous evidence has shown the Bengtsson and Krakau's formula was a reasonable approximation of Littmann's formula (Spencer, Sadiq, Pawson, and Vernon, 1995). Monsour compared Bengtsson and Krakau's formulas to two of Littmann's formulas and found that the formula derived by Bengtsson and Krakau underestimated by 0.91% when compared to Littmann's formula based on the anterior corneal diameter and the refractive power of the eye; Littmann's formula based on the axial length and refractive power of the eye underestimated by 3.6% (Monsour, 1990). Our Littmann adjustment was slightly modified to take into consideration the use of a non-telecentric camera in this study (Rudnicka, et al., 1997). The difference found here, more than 43% between values, could not therefore be explained solely by the calculation method used.

Clinical experience indicates that the true AVR should approximate 2/3. Our "Big6" values were closer to this figure than our Parr-Hubbard values. Of the compared studies, those of Wong (2006) and Ikram (2004) are closest to this value, while the value of 0.89 in the Beaver Dam Eye Study (Wong, et al., 2004) was farthest. We therefore felt that our data best fit

with clinical experience and the body of literature as a whole when we used the raw data from the Knudtson-modified formulas, rather than adjusting for camera or ocular magnification. If the IVAN software is calibrated for our camera magnification as we suspect, and if the change in camera magnification is negligible between eyes, then applying an adjustment formula would give the effect of a "double adjustment."

Telecentric cameras, such as the Zeiss camera used by Littmann and other investigators, allow for rays to emerge parallel to the optic axis after refraction by the camera system (Rudnicka, et al., 1998). The Canon camera used in this study was non-telecentric, and thus there was a linear relationship between camera magnification (p) and ocular refraction (Rudnicka, et al., 1998). The magnification effect of different cameras as given by Rudnicka and colleagues (Rudnicka, et al., 1998), if plotted against refractive error, would yield a relatively flat slope for non-telecentric cameras, like ours, compared to a much steeper slope for telecentric cameras like the Zeiss (Nancy Coletta, 2007, unpublished data). Indeed, our calculated camera magnification varied little between subjects, remaining close to the mean of 1.86 degree/mm for most subjects, as indicated by the small standard deviation of ±0.04.

While the literature as a whole seems to agree that adjusting for camera magnification in some way is important, the number of different methods proposed indicates there may not be one ideal way to do so. Any retinal measurements are strongly dependent on camera position, the optic nerve, and the quality of the photographs (Spencer, et al., 1995). All of the methods for adjustment were derived based on measurements of optic disc size, while our

measurements are based on the reflectivity profile of blood vessels. Perhaps these two methods -- and thus any formulas to adjust for magnification -- are not readily interchangeable. This may have been the case for Spencer and colleagues, who compared measurements of vertical diameter of the optic disc obtained with a scanning laser ophthalmoscope (HRT, Heidelberg Engineering, Germany) against planimetric methods and found a significant difference (Spencer, et. al., 1995). In addition, while strong evidence supports the presence of a camera magnification factor, most studies shown in **Table 7** used a different type of camera, and in some cases, the type of camera was not reported. In choosing a camera for our study, we used the digital Canon camera because it was readily available and we felt we had reliable information about the optics of the device, but even our Parr-Hubbard values were quite different from those of the Atherosclerosis Risk in Communities Study (Hubbard, et al., 1999), which used Parr-Hubbard values and photos taken with a Canon film camera.

The differences between the two CRAE values acquired from the same eye was relatively small for most eyes, with a mean of 1.13 μm, and the mean difference in CRVE between the two images was even smaller, 0.31 μm. Because the same grader read all images, these differences are consistent with a small effect of the pulse cycle and a larger effect of image variability, as reported by Knudtson, et al. (2004).

### *Conclusions*

Although we collected ocular biometric data and were prepared to adjust our measurements for ocular and camera magnification, the raw CRVE and CRVE calculated by the IVAN program was most comparable to the combination of clinical sense and literature values. This may be due to a combination of factors, including the limited range in the biometric parameters (refractive error, corneal curvature, axial length) found in our participants, the camera used in the study, and the conversion factors used by the IVAN program itself. Therefore, the raw data using the Knudtson-modified Parr-Hubbard formulas (Knudtson, et. al., 2003), and not the adjusted measurements, were used in subsequent analyses.

# CHAPTER 4: PHYSICAL EXAM, SEROLOGY, AND ASSOCIATIONS WITH RETINAL VASCULATURE

## *Background*

Retinal imaging has demonstrated that patients with early Alzheimer disease (AD) have narrower retinal venules than age-matched controls (Berisha, et al., 2007; Feke et al., 2006). Because of the homologies between the retinal vasculature and the cerebral vasculature (Patton, et al., 2005) such vascular alterations in the eye may reflect those occurring in the brain, supporting the theory that vascular changes do indeed occur early in AD (de la Torre, 2002). The ability of retinal imaging to detect these changes in early AD suggests that it may be a useful, noninvasive tool in the assessment of risk for AD.

The effects of systemic conditions on the retinal vasculature have been well documented. Therefore, in any study examining the retinal vasculature, the effect of any coexisting conditions must be considered. The two conditions whose effects on retinal vasculature have been most extensively studied are hypertension and diabetes. Hypertension is associated with generalized retinal arteriolar narrowing, which is more commonly seen in vessels without arteriosclerosis (Wong, Klein, Klein, Tielsch, Hubbard, and Nieto, 2001), while focal narrowing and dilatation are observed simultaneously in vessels with moderate to severe arteriosclerosis, and thus usually in older persons (Wong, et al., 2001). Chronic elevated blood pressure leads to the exudative phase, which is characterized by hemorrhages, microaneurysms, exudates, and cotton wool spots (Patton, et al., 2005; Wong, et al., 2001), although the pathophysiology leading to the exudation is somewhat controversial, since

exudation has been observed both with and without endothelial cell necrosis (Wong, et al., 2001), and changes such as retinal hemorrhages and cotton wool spots have been related to current rather than previous blood pressure levels (Wong and MacIntosh, 2005b). Alterations in retinal microvasculature may persist even after blood pressure control is improved (Sharrett, et al., 1999). Diabetes also leads to exudation, tissue edema, and microaneurysms, as well as ischemia and retinal neovascularization (Patton, et al., 2005).

An exhaustive discussion of all conditions that influence retinal blood vessel diameters is beyond the scope of this thesis, but a selection of the more common systemic and ocular conditions that affect the retinal vasculature is summarized in **Table 8**, next page. Because the retinal vasculature can be viewed *in vivo* using non-invasive techniques, and because some changes may occur in the eye before more generalized manifestations of these conditions can be detected, evaluation of the retina has been proposed as a potential screening tool for these conditions. In addition, because of the effects on ocular health, it is important that individuals with these conditions have their eyes checked regularly.

Mean arterial blood pressure (MABP) has been shown to have an inverse relationship with CRAE (Kaushik, Kifley, Mitchell, and Wang, 2007; Wong and McIntosh, 2005a; Leung, et al., 2003) and with AVR (Hubbard, et al., 1999), consistent with the belief that MABP is an indicator of end-organ perfusion pressure (Leritz, Salat, Milberg, Williams, Chapman, Grande, Rudolph, Schnyer, Barber, Lipsitz, Fischl, and McGlinchey, 2010). MABP of 106 mmHg or higher is considered indicative of mild hypertension (*American Heart Association*).

**Table 8: Summary of Selected Retinal Vascular Findings**

| *Retinal Arteriole Narrowing* | *Retinal Venule Narrowing* |
|---|---|
| **Glaucoma** (Leung, et al., 2003)<br>**Elevated homocysteine** (Wong, et al., 2006)<br>**Systemic and diastolic blood pressure** (Wong, et al., 2006)<br>**Mean arterial blood pressure** (Leung, et al., 2003)<br>**Hypertension** (Ikram, et al., 2006)<br>**Pre-hypertension** (Ikram, et al., 2006)<br>**Current alcohol** (Wong, et al., 2006) | **Dislipidemia** (Wong, et al., 2006)<br>**Alzheimer disease** (Berisha, et al., 2007) |
| *Retinal Arteriole Widening* | *Retinal Venule Widening* |
| **Diabetes** (Wong, et al., 2006)<br>**Current cigarette smoking** (Wong, et al., 2006)<br>**Higher levels of plasma fibrinogen** (Wong, et al., 2006) | **Diabetes** (Wong, et al., 2006)<br>**Current cigarette smoking** (Wong, et al., 2006)<br>**Inflammatory condition** (Ikram, et al., 2006; Wong, et al., 2002) **or systemic markers of inflammation** (Wong, et al., 2006)<br>**Obesity** (Wong, et al., 2006)<br>**Endothelial dysfunction** (sICAM-1, PAI-1), (Wong, et al., 2006)<br>**Progression of cerebral small vessel disease** (Ikram, et al., 2006) |
| *Other Blood Vessel Findings: Arteriolar Calibers* | *Other Blood Vessel Findings: Venular Calibers* |
| **Females> males** (Wong, et al., 2006)<br>**Hispanics and Blacks >> Whites** (Wong, et al., 2006) | **Hispanics, Blacks, and Chinese > Whites** (Wong, et al., 2006)<br>**Blood pressure has little effect on caliber** (Wong, et al., 2006; (Leung, et al., 2001; Sharrett, et al., 1999). |

***Purpose***

The purpose of this study was to identify associations between retinal vessel caliber and risk factors for AD in patients at increased risk for, but without clinically evident dementia. Our hypothesis was that healthy individuals with increased risk for AD, identified in the U-CARE and OAIC studies, would demonstrate retinal vessel caliber changes according to risk for AD. Specifically, we expected that retinal venule blood column diameter would be thinner in healthy individuals without dementia but who possess a genetic risk factor for AD, APOE4.

***Methods***

Participants enrolled in the U-CARE and OAIC studies at the VA Boston Healthcare System were invited to participate in this prospective, cross-sectional study, according to the protocols described in Chapters 1 and 2. As mentioned previously, the U-CARE and OAIC studies are cross-sectional studies of community dwellers, aged 50 to 70 years, and 55 to 84 years, respectively. Participants were screened for and excluded if they had clinical dementia. All participants underwent a physical exam, basic neurological examination, blood testing, and cognitive testing. Demographic data (such as gender, date of birth, place of birth, ethnicity, years of education, handedness), a detailed medical history including review of systems and medications, family history, physical exam (height, weight, body mass index or BMI, blood pressure, pulse, cardiac exam, lung exam, neurologic exam), blood labs (low density lipoprotein or LDL cholesterol, high density lipoprotein or HDL cholesterol, triglycerides, glycosylated hemoglobin or HbA1c, calculated mean glucose, fasting glucose, creatinine, albumin, and thyroid stimulating hormone or TSH, and genetic testing for the

presence of APOE4) were collected, and some participants also underwent an EKG (A list of neuropsychological and physical exam measures is given in **APPENDIX A**, page 93). Mean arterial blood pressure (MABP) was calculated using the formula MABP = 1/3 (systolic-diastolic) + diastolic (Leritz, et al., 2010).

A trained reader blind to APOE4 status determined central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) following the methods described in detail in Chapter 3.

As described earlier, two-sample t-tests were used to examine differences of continuous variables (shown in **Table 9**, next page) between the two groups (APOE4+/APOE4-). For each continuous variable of the entire sample, simple linear regression was used for analysis. For continuous variables that differed significantly between the two groups, one-way analysis of covariance (ANCOVA) for two independent samples (using quartiles of continuous values) was used, in order to determine whether the difference had a significant effect on blood vessel diameter, using an online software program (Vassarstats, Lowry, R., http://faculty.vassar.edu/lowry/VassarStats.html, Poughkeepsie, NY ). This was done with CRAE, CRVE, and AVR separately.

***Results***

The exam and serologic data for the two groups (APOE4-/APOE4+) are shown in **Table 9.** Neither HbA1c, fasting glucose, HDL cholesterol, LDL cholesterol, triglycerides, calculated

total cholesterol, creatinine, TSH, albumin, nor height differed significantly between the two groups. Weight differed significantly between the APOE4+ and APOE4- groups (P= 0.033), as did BMI, which was known for 25 of the 26 participants (P = 0.01).

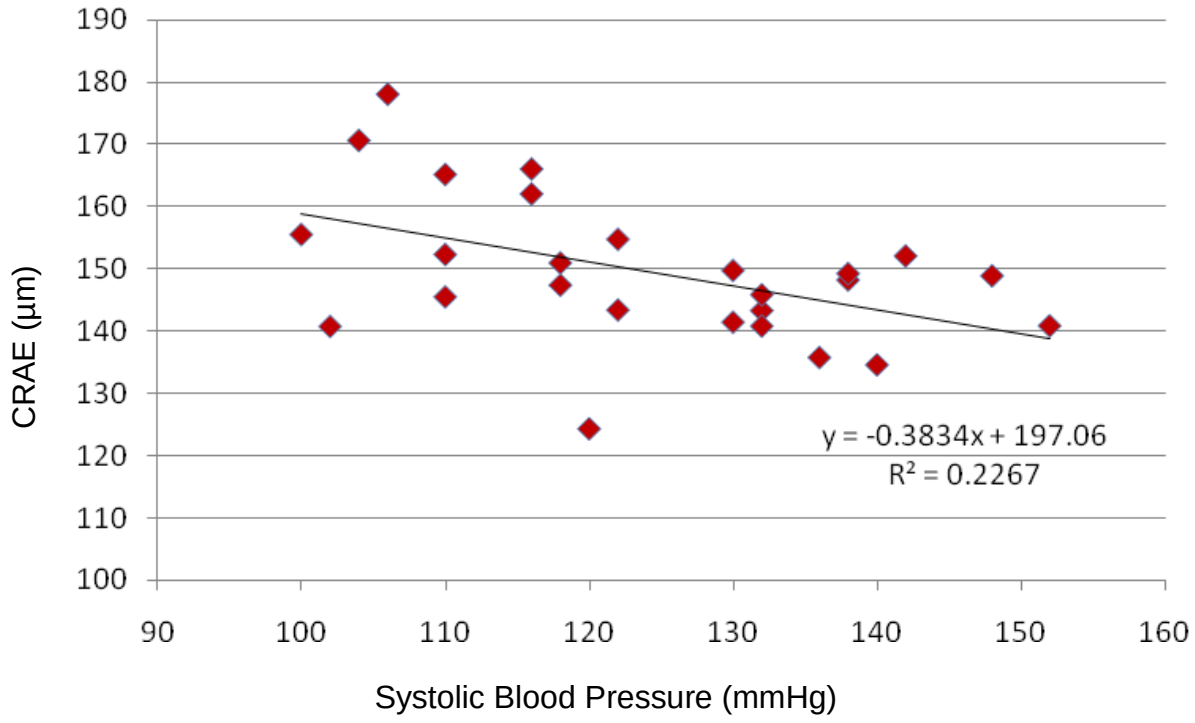
**Table 9: Serologic and Physical Data for Two Groups (APOE4-/+)**

| | ***Low Risk*** **APOE4(-), (n=18), Mean (range, SD)** | ***High Risk*** **APOE4(+), (n=8) , Mean (range, SD)** | **P** |
|---|---|---|---|
| HbA1c, % | **5.6 (**5.1-6.1, 0.3) | **5.5** (4.9-5.9, 0.4) | 0.815 |
| Glucose | **90.61** (68-108, 10.9) | **87.38** (65-107, 12.9) | 0.515 |
| HDL | **61.2** (41-135, 14.5) | **68.25** (25-94, 33.5) | 0.452 |
| LDL | **125.5** (56-194, 37.1) | **117** (76-171, 31.6) | 0.579 |
| Triglycerides | **95.9** (52-201, 38.3) | **93.1** (45-161, 32.6) | 0.858 |
| Calculated Total Cholesterol† | **205.86** (133.8-286, 43.2) | **203.88** (131.2-258.4, 52.0) | 0.920 |
| Creatinine | **0.97** (0.6-1.2, 0.2) | **0.98** (0.7-1.3, 0.2) | 0.94 |
| TSH | **1.87** (0.53-5.7, 1.2) | **2.6** (0.5-5.9, 2.1) | 0.271 |
| Albumin | **4.31** (3.7-5.4, 0.37) | **4.41** (4.1-4.9, 0.31) †† | 0.518 |
| Height, inches | **67.2** (62-73, 3.6)* | **66.4** (62-72, 2.9) | 0.633 |
| Weight, lbs | **173.6** (120-251, 33.9) | **143.3** (118-195, 25.3) | **0.033**** |
| BMI | **27.28** (21.9-36, 4.4)* | **22.71** (18.7-28, 3.2) | **0.01**** |
| Systolic BP, Standing (mmHg) | **125.8** (100-152, 14.7) | **120** (102-138, 15.6) | 0.363 |
| Diastolic BP, Standing (mmHg) | **74.3 (**52-90, 9.9) | **68.8 (**60-82, 7.0) | 0.163 |
| MABP (mmHg) | **91.5** (76.7-104, 9.4) | **85.8** (75.3-101.3, 8.5) | 0.161 |

*n=17
†Calculated total cholesterol = LDL + HDL + (triglycerides/5)
††n=7
**p <0.05

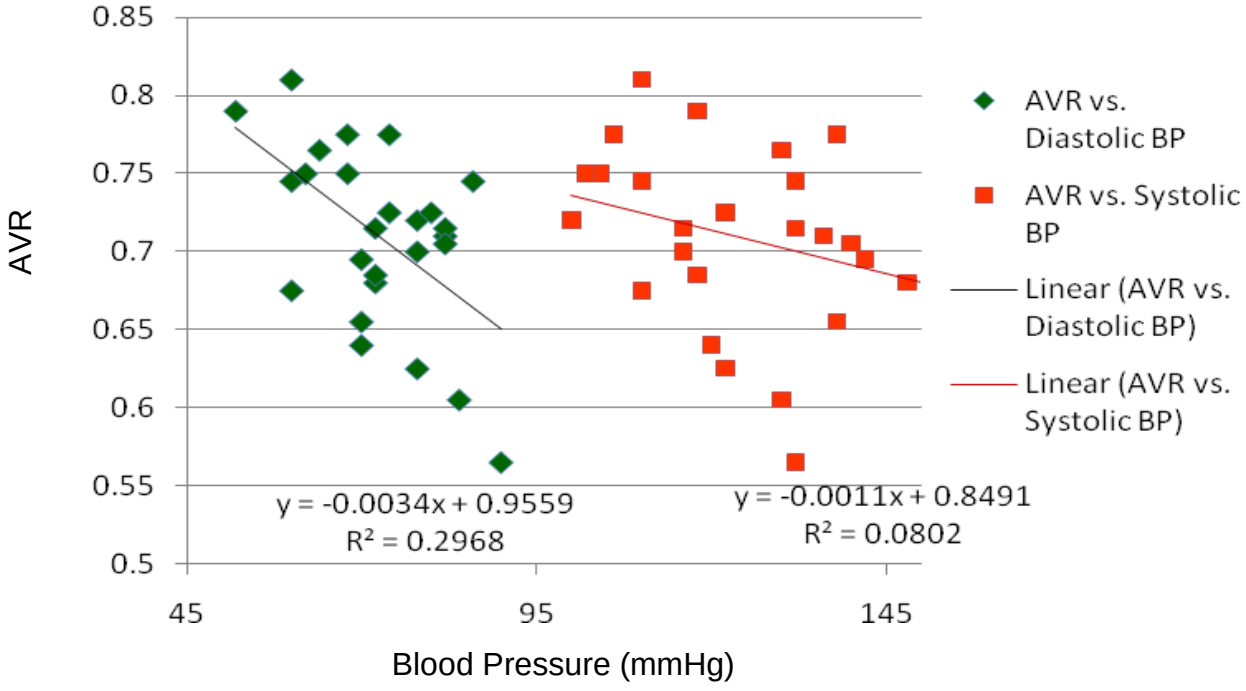
For the entire sample, none of the physical parameters, including age, gender, and education, correlated significantly with CRVE. Systolic blood pressure correlated significantly and negatively with CRAE ($y = -0.38335x + 197.063$, $R^2 = 0.23$, $P = 0.0140$), **(Figure 5**, below), but not with CRVE ($y = -0.20135x + 237.07$, $R^2 = 0.0162$, $P = 0.535$), and diastolic blood pressure did not correlate significantly with either CRAE ($y = -0.252106x + 168.119$, $R^2 = 0.0412$, $P = 0.340$) or CRVE ($y = 0.66776x + 162.90$, $R^2 = 0.0749$, $P = 0.176$). Systolic blood pressure did correlate with diastolic blood pressure ($y = 0.664166x + 75.7713$, $R^2 = 0.180$, $P = 0.0309$).

**Figure 5: CRAE vs. Systolic Blood Pressure For Entire Sample**



In all participants, MABP was below the cutoff of 106 considered to be indicative of mild hypertension (range 75.33-104.0). There was a trend toward a negative correlation between MABP and CRAE ($y = -0.4783x + 192.443$, $R^2 = 0.146$, $P = 0.055$), but MABP did not correlate significantly with CRVE ($y = 0.283997x + 186.62$, $R^2 = 0.0133$, $P = 0.575$). AVR did not correlate significantly with systolic blood pressure ($y = -0.00133x + 0.849086$, $R^2 = 0.0802$, $P = 0.161$), but it did correlate significantly and negatively with diastolic blood pressure ($y = -0.00339x + 0.955936$, $R^2 = 0.297$, $P = 0.0040$), **(Figure 6**, below), and with MABP ($y = -0.00315x + 0.991776$, $R^2 = 0.258$, $P = 0.0081$).

**Figure 6: AVR vs. Diastolic and Systolic Blood Pressure For Entire Sample**



Predictably, participants who were taking antihypertensive medications had significantly higher systolic blood pressure, mean diastolic blood pressure, and mean arterial blood pressure than those not taking antihypertensive medications (**Table 10**, below). AVR was significantly less in the medication group, but neither CRAE nor CRVE was significantly different between groups.

**Table 10: Comparisons Between Participants On and Off Antihypertensive Medications**

| | **No Meds (n=18)** | **On Meds (n=8)** | **P** |
|---|---|---|---|
| Mean Systolic BP (mmHg), (SD) | 118.7 (13.8) | 136 (8) | **0.003*** |
| Mean Diastolic BP (mmHg), (SD) | 69.9 (9) | 78.8 (7.3) | **0.022*** |
| MABP (mmHg), (SD) | 86.1 (8.8) | 97.1 (4) | **0.0016*** |
| CRAE (µm), (SD) | 151.56 (13.1) | 144.95 (6.6) | 0.192 |
| CRVE (µm), (SD) | 209.13 (22.24) | 218.78 (25.09) | 0.335 |
| AVR (SD) | 0.73(0.05) | 0.67 (0.06) | **0.011*** |

*** P<0.05**

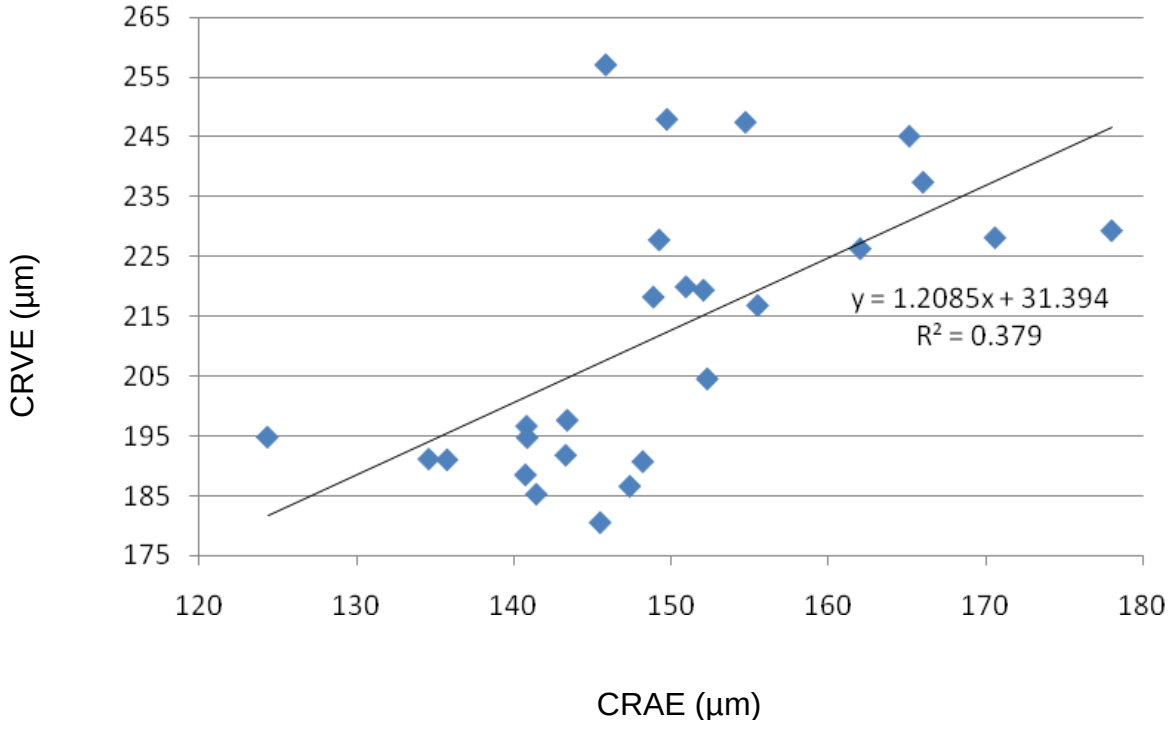
To analyze the differences in time since physical examination, Analysis of Variance (ANOVA) was performed on quartiles of time since initial exam. There were no significant differences between the groups in systolic blood pressure (P = 0.452), CRAE (P=0.276), CRVE (P=00.907), or AVR (P =0.673) (**Table 11**, below).

**Table 11: Analysis of Variance: Quartiles of Time Since Physical Examination**

| | 1st QTR | 2nd QTR | 3rd QTR | 4th QTR | P |
|---|---|---|---|---|---|
| n | 7 | 6 | 6 | 7 | - |
| Mean Years (SD) | 83.71 (0.099) | 99.17 (0.24) | 104.67 (0.353) | 107.86 (0.23) | - |
| Range (yrs) | 0.836-1.112 | 1.121-1.677 | 1.723-2.649 | 2.666-3.299 | - |
| Systolic BP (mmHg) Mean (Range, SD) | 125.42 (102-148, 16.07) | 118.33 (104-152, 17.22) | 120.33 (110-130, 10.46) | 130.57 (100-140, 14.0) | 0.452 |
| CRAE (µm) Mean (Range, SD) | 145.91 (124.36-162.04, 11.55) | 156.52 (140.87-170.61, 12.33) | 151.80 (140.83-178.02, 14.10) | 145.21 (134.61-155.52, 7.71) | 0.276 |
| CRVE (µm) Mean (Range, SD) | 212.98 (186.51-257.19, 25.29) | 217.66 (180.42-245.22, 25.24) | 210.13 (185.18-247.56, 23.52) | 208.15 (190.66-248.04, 23.15) | 0.907 |
| AVR Mean (Range, SD) | 0.69 (0.57-0.79,0.07) | 0.72 (0.68-0.81,0.05) | 0.73 (0.63-0.78,0.05) | 0.70 (0.61-0.78,0.06) | 0.673 |

As expected, CRVE correlated significantly and positively with CRAE (y = 1.2085x + 31.94, $R^2$ = 0. 379, P = 0.000814), **(Figure 7,** below).

**Figure 7: CRVE vs. CRAE For Entire Sample**



AVR, but not CRAE or CRVE, was significantly lower in males (mean 0.68) than in females (mean 0.74, P = 0.0055). Because male participants had higher blood pressure than females, analysis of covariance (ANCOVA) was performed to determine whether the correlations between AVR and gender still existed after adjusting for blood pressure, and the results are shown in **Table 12**, below. The correlation between AVR and gender persisted after adjusting for systolic blood pressure (P =0.169) but not diastolic blood pressure (P = 0.101) or MABP (P = 0.95).

**Table 12: Blood Pressure and Blood Vessel Measurements by Gender***

| | **Males (n=13)** | **Females (n=13)** | **P** |
|---|---|---|---|
| Mean Systolic Blood Pressure (BP), (mmHg), (SD) | 129.7 (12.8) | 118.3 (14.5) | **0.0444**** |
| Mean Diastolic BP (mmHg), (SD) | 77.6 (7.0) | 67.7 (8.9) | **0.0046**** |
| MABP (SD) | 94.9 (6.9) | 84.5 (8.8) | **0.0028**** |
| CRAE (µm) | | | |
| Observed (SD) | 146.05 (10.7) | 153.01 (12.1) | 0.134 |
| Adjusted for Systolic BP | 148.76 | 150.98 | 0.643 |
| Adjusted for Diastolic BP | 147.07 | 152.66 | 0.331 |
| Adjusted for MABP | 148.81 | 150.93 | 0.702 |
| CRVE (µm) | | | |
| Observed (SD) | 216.40 (23.9) | 207.80 (22.3) | 0.352 |
| Adjusted for Systolic BP | 218.57 | 205.63 | 0.201 |
| Adjusted for Diastolic BP | 213.46 | 210.74 | 0.809 |
| Adjusted for MABP | 216.25 | 207.96 | 0.466 |
| AVR | | | |
| Observed (SD) | 0.680 (0.06) | 0.740 (0.04) | **0.0055**** |
| Adjusted for Systolic BP | 0.683 | 0.738 | **0.0169**** |
| Adjusted for Diastolic BP | 0.692 | 0.729 | 0.101 |
| Adjusted for MABP | 0.691 | 0.730 | 0.095 |

* Adjusted values were adjusted using Analysis of Covariance (ANCOVA), as described in text.

****P<0.05**

There were no significant differences in spherical equivalent refraction (P = 0.929) or corneal curvature (P = 0.076) between the APOE4- and APOE4+ groups. Mean axial length was significantly greater in the APOE4- group (25.28mm) than in the APOE4+ group (24.30mm, P = 0.012).

There were no significant differences in CRAE or AVR between the APOE4- and APOE4+ groups (see **Table 13**, below). CRVE was smaller in the APOE4+ group (206.07 µm) than in the APOE4- group (214.78 µm), but this difference was not significant (P = 0.385 for two tail, one-tail P = 0.193).

**Table 13: CRVE, CRAE, and AVR by APOE4 Group, Adjusted for Weight and Axial Length***

| | **APOE4(-), (n=18) mean** | **APOE4(+), (n=8) , mean** | **P** |
|---|---|---|---|
| CRVE (µm), (range, SD) | **214.78** (186.51 – 257.19, 23.8) | **206.07** (180.42- 229.37, 21.6) | 0.385 |
| Adjusted for Weight | 213.60 | 208.73 | 0.659 |
| Adjusted for Axial Length | 215.44 | 204.59 | 0.355 |
| CRAE (µm), (range, SD) | **149.17** (124.36 – 170.6, 11.1) | **150.33** (140.76-178.02, 13.9) | 0.823 |
| Adjusted for Weight | 149.17 | 148.63 | 0.825 |
| Adjusted for Axial Length | 149.27 | 150.11 | 0.889 |
| AVR (range, SD) | **0.695** (0.57-0.79, 0.06) | **0.73** (0.66 – 0.81, 0.05) | 0.182 |
| Adjusted for Weight | 0.706 | 0.712 | 0.643 |
| Adjusted for Axial Length | 0.679 | 0.734 | 0.174 |

* Values were adjusted using Analysis of Covariance (ANCOVA, see text)

Analyses of Covariance (ANCOVA) were used to adjust CRVE, CRAE, and AVR for weight and axial length separately (**Table 13**, above). With both CRVE and AVR, adjusting for weight made the differences between the two groups less significant, while doing so had relatively little impact on the difference in CRAE between the two groups. Adjusting for axial length made none of the differences between groups reach statistical significance. Before adjusting for weight or axial length and analyzing the entire sample, axial length did not correlate significantly with the observed values of CRAE ($y = -0.52403x + 162.62$, $R^2 = 0.00179$, $P = 0.838$), CRVE ($y = 0.441971x + 201.06$, $R^2 = 0.00033$, $P = 0.930$), or AVR ($y = -0.00311x + 0.787$, $R^2 = 0.00258$, $P = 0.805$). Weight was not significantly associated with observed values of CRAE ($y = -0.07317x + 161.55$, $R^2 = 0.0451$, $P = 0.298$) or CRVE ($y = 0.154432x + 186.73$, $R^2 = 0.0523$, $P = 0.262$). Weight, however, did have a significant and negative relationship with observed AVR in the entire sample ($y = -0.00077x + 0.837$, $R^2 = 0.207$, $P = 0.0195$), but height did not ($y = -0.00646x + 1.142$, $R^2 = 0.146$, $P = 0.597$). **Figure 8a,** next page, shows the observed CRVE distribution by APOE4+ and APOE4-, while **Figure 8b** shows the observed AVR distribution by APOE status.

For the 25 participants for whom BMI was available, using ANCOVA to adjust for BMI made CRVE, CRAE, and AVR less significant between the two groups **(Table 14**, page 60). Without adjusting for BMI and analyzing the sample as a whole, BMI did not significantly correlate with CRAE ($y = -0.2097x + 154.28$, $R^2 = 0.0062$, $P = 0.707$), CRVE ($y = 1.2786x + 178.08$, $R^2 = 0.0582$, $P = 0.245$), or AVR ($y = -0.0044x + 0.8239$, $R^2 = 0.109$, $P = 0.107$).

**Figure 8a: Observed CRVE Distribution by APOE Status**

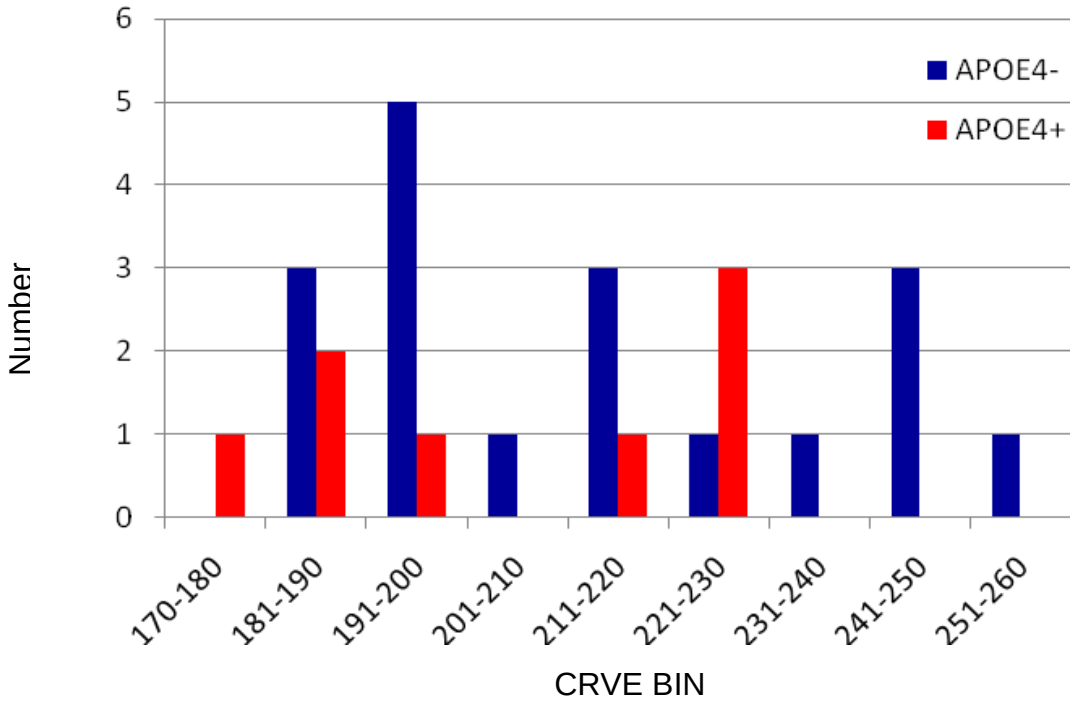



**Figure 8b: Observed AVR Distribution by APOE Status**

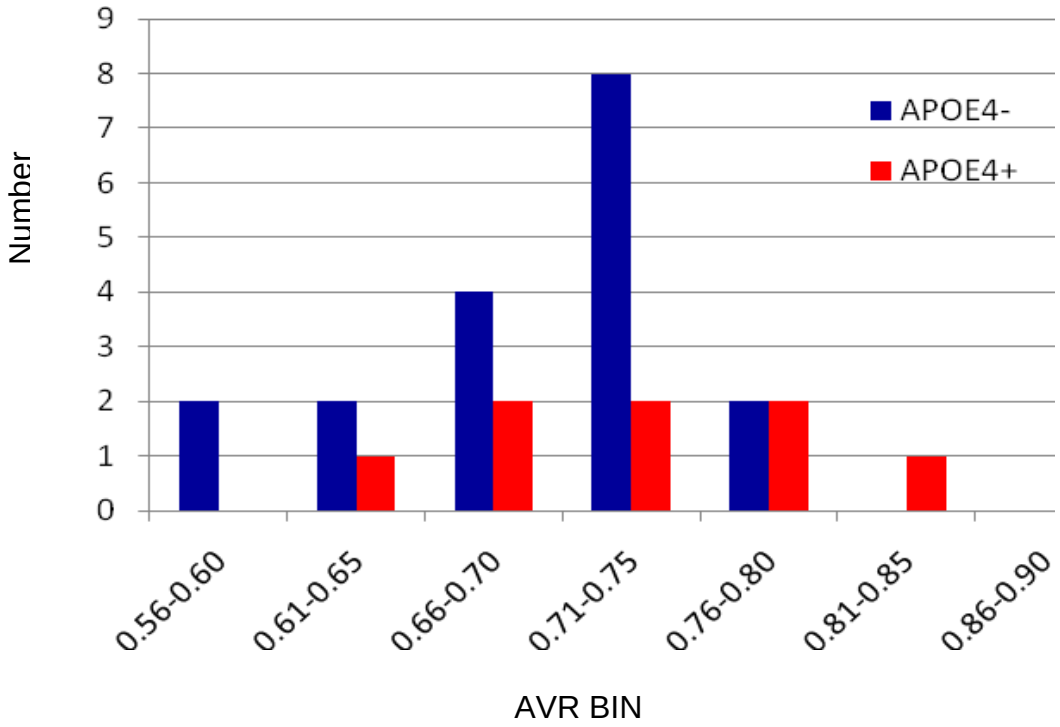


**Table 14: CRVE, CRAE, and AVR by APOE4 Status, Adjusted for BMI***

| | APOE4(-), n=17 | APOE4(+), n=8 | P |
|---|---|---|---|
| CRVE (µm)<br>Observed Mean (SD)<br>Adjusted for BMI | <br>213.45 (23.86)<br>211.74 | <br>206.07 (21.55)<br>209.69 | <br>0.466<br>0.864 |
| CRAE (µm)<br>Observed Mean (SD)<br>Adjusted for BMI | <br>148.18 (10.62)<br>148.36 | <br>150.32 (13.87)<br>149.95 | <br>0.673<br>0.794 |
| AVR<br>Observed Mean (SD)<br>Adjusted for BMI | <br>0.699 (0.06)<br>0.704 | <br>0.732 (0.05)<br>0.721 | <br>0.195<br>0.560 |

*Adjustments were made using Analysis of Covariance (ANCOVA), see text.

***Discussion***

APOE4 genotype has been associated with cardiovascular risk factors, but primarily fasting plasma glucose (Scuteri, Najjar, Muller, Andres, Morrell, Zonderman, and Lakatta, 2005). Our finding of higher weight, and thus BMI, in the APOE4+ group than in the APOE4- group is inconsistent with Scuteri, et al., and could be coincidental. However, because BMI negatively correlated with CRAE and positively correlated with CRVE in the Multi-Ethnic Study of Atherosclerosis (Wong, et al., 2006), it was anticipated that adjusting for the higher BMI in the APOE4+ group would adjust the mean CRVE toward the null hypothesis, as it did. Since BMI is an integer calculated directly from weight and has a smaller range than weight, the reason we did not detect a significant association between BMI and observed AVR in our sample as a whole may have been because our sample size was relatively small.

Wong and coworkers found a significant thinner retinal arteriolar caliber in males than females, and no significant difference in retinal venular caliber between genders (Wong, et al., 2006), so it is not surprising that we found a lower AVR in males in our study. We would expect CRAE to be thinner in males than in females in our study as well, and while we found the mean in males to be smaller than that in females, the difference was not significant in our small sample.

Blood pressure was expected to correlate negatively with CRAE, and systolic blood pressure did so in this study. As expected, MABP and diastolic blood pressure significantly and negatively correlated with AVR. The lack of a significant correlation found between MABP and CRAE is likely due to the narrow range of MABP found in our participants. To be included in this study, participants needed to have well-controlled blood pressure, and therefore a number of individuals with high blood pressure were excluded. In the Blue Mountains Eye Study, for example (Kaushik, et al., 2007), MABP was analyzed in groups of $<100$, 100-109, 110-119, and $\geq 120$. Of our participants, 23 would have fallen into the first group, and only 3 would fall into the second group. None of our participants had hypertension, as defined by American Heart Association by MABP of 106 or higher.

Lower AVR with higher MABP is consistent with the results of the Atherosclerosis Risk in Communities Study (ARIC) (Hubbard, et al., 1999). In the Multi-Ethnic Study of Atherosclerosis (MESA), Wong and coworkers found CRAE correlated strongly and inversely with systolic blood pressure ($P< 0.001$); and after controlling for age, gender, race-

ethnicity, and center, with diastolic blood pressure as well (Wong, et al., 2006). One might speculate whether the need for these adjustments indicate that the effects of systolic blood pressure on the retinal vasculature are more easily detected than those of diastolic blood pressure; this might explain why diastolic blood pressure did not correlate significantly with CRAE in our small sample, even when there was a significant correlation between AVR and diastolic blood pressure, and between systolic and diastolic blood pressure. Or perhaps our method, using fundus photographs taken at different points in time, is more sensitive to changes in CRAE with higher systolic blood pressure -- when the heart is beating -- than with diastolic blood pressure, or between heartbeats.

Similarly, these correlations may not be strong enough to translate into a significant association seen between AVR and systolic blood pressure, even when CRAE increases with increasing systolic blood pressure. There may be some, although less significant, association between systolic blood pressure and CRVE, and in this small sample, this may mask any correlations between AVR and systolic blood pressure. Wong and coworkers found CRVE tended to decrease with increasing systolic blood pressure but, just as with our study, this trend did not reach statistical significance (P= 0.20) (Wong, et al., 2006).

However, our participants taking antihypertensive medications had significantly higher systolic blood pressure, mean diastolic blood pressure, and mean arterial blood pressure than those not taking them. It is not surprising, then, to find AVR to be less in the medication group, even though neither CRAE nor CRVE was significantly different between groups.

Generalized retinal arteriolar narrowing has been independently related to past blood pressure levels in several studies, including the Atherosclerosis Risk in Communities (ARIC) Study (Sharrett, et al., 1999) and the Cardiovascular Health Study (Wong, et al., 2002) and may indicate previous effects on the arterioles from previous elevated blood pressure (Wong and MacIntosh, 2005b).

As discussed in Chapter 2, the mean time difference between physical examination and the acquisition of retinal images was 1.88 years in this study. It may be possible that blood pressure changed during that time. However, at the time of retinal photography, none of the participants who reported a change in medications during the physical exam indicated a change in hypertensive medication. Standard of care indicates adults with uncomplicated hypertension should have an in-office blood pressure goal of 140/90 mmHg or lower, and that goal is even lower for adults with complications; changes in medication regimen are indicated when the goal is not met (Institute for Clinical Systems Improvement, 2008). In this study, the stable medication regimen and history of well-controlled blood pressure would indicate that the blood pressure continued to be stable in the participants whose data were used for analysis.

The ARIC Study showed that normotensive adults with generalized retinal arteriolar narrowing at baseline were 60% more likely to be diagnosed at with hypertension over a 3-year period (Wong, et al., 2004), a finding essentially duplicated by the Beaver Dam Eye Study and the Blue Mountains Eye Study (Wong and McIntosh, 2005b). These studies

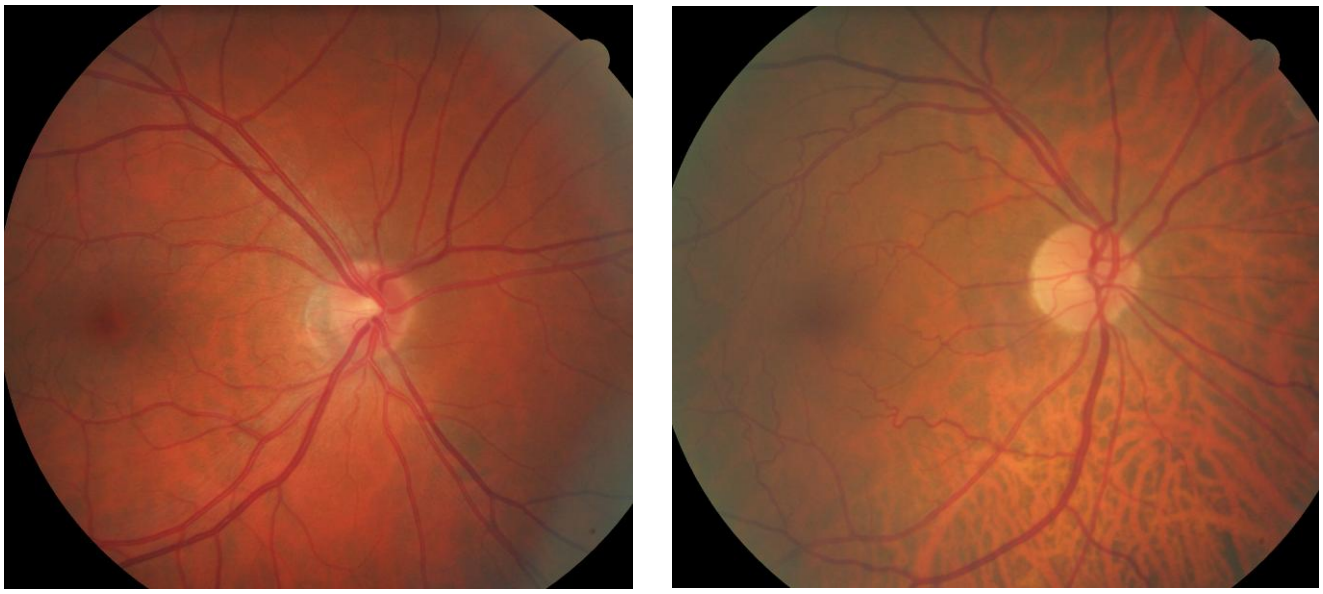
provide evidence that generalized retinal arteriolar narrowing may be an early marker for hypertension (Wong and McIntosh, 2005b). However, when also considering that, as stated above, retinal arteriolar narrowing was found to be related to past blood pressure levels measured 3 and 6 years previously in the ARIC Study, even after adjusting for current blood pressure (Wong, et al., 2001), the evidence as a whole suggests that generalized retinal arteriolar caliber may be a more robust indicator of blood pressure or vascular status over a period of several years than any single blood pressure measurement taken within that time.

While BMI did not show a significant correlation with CRVE, there were 4 participants in the APOE4- group who had a BMI over 30, which by definition is considered to fall into the obese category. None of the participants in the APOE4+ category had a BMI above 29. Because BMI has been positively correlated with CRVE (Wong, et al., 2006) we expected that adjusting for BMI should adjust our data in favor of the null hypothesis. If we excluded everyone with a BMI over 30, there is still a significant difference in BMI for the APOE4- group (mean = 25.43, n =13) compared to the APOE4+ group (mean = 22.71, n =8, P = 0.035), and there is no significant difference found in CRVE between the APOE4- group (mean = 211.16μm) compared to the APOE4+ group (mean = 206.07μm, P = 0.595). Finally, if we excluded everyone in the APOE4- group with a BMI over 26, the mean BMI for the APOE4- group becomes 23.8 and more closely matches that of the APOE4+ group (P = 0.375), and the mean CRVE for the remaining APOE4- group is 214.51μm (n = 8), but the difference in CRVE is still not statistically significant (P = 0.417).

Axial length was smaller in the APOE4+ group than in the APOE4- group, which was unexpected, since this is a previously unpublished finding. However, as discussed in Chapter 3, a number of methods to adjust for camera magnification use axial length as a parameter. Given that the retinal details were farther from the camera in the longer eyes, we expected that adjusting for axial length would make the means of both CRAE and CRVE smaller in the APOE4+ group, as it did. Even with this adjustment, though, the differences between groups did not reach significance.

In this study, the mean CRVE in the APOE4+ group was smaller than that for the APOE4- group, but the difference did not reach significance. One must wonder whether the failure to detect a significant difference was because the study was not sufficiently powered. The recruitment goals were not met, and only 8 of the desired 20 APOE4+ participants were recruited. If the study were sufficiently powered, such that the mean CRVE of 214.78 μm for APOE4- and 206.07 μm for APOE4+ were found to be statistically significant, a reasonable question would be whether this difference of approximately 8 μm would be clinically detectable during ophthalmoscopy. **Figure 9,** next page, shows a side-by-side comparison of two right eyes with a difference of CRVE of approximately 8 μm. The difference in retinal venule width is difficult to appreciate by gross observation. Clearly, technological assistance is needed in order to quantify this difference during clinical exam.

**Figure 9: Side-By-Side Comparison of Two Right Eyes With CRVE Difference of Approx. 8 µm**



CRVE = 228.18 µm

CRVE = 219.97 µm

***Conclusions***

As expected, systolic blood pressure correlated significantly and negatively with CRVE, and MABP and diastolic blood pressure significantly and negatively correlated with AVR. MABP, however, did not show any significant association with CRAE, probably because of the narrow range of MABP in our cohort. Although the lack of change in blood pressure medications in our participants makes the possibility that their blood pressure changed since their physical exam unlikely, the ARIC and other studies showed an association between generalized arteriolar narrowing in normotensive adults and diagnosis of hypertension at 3-

years follow up (Wong, et al., 2004). But generalized retinal arteriolar narrowing was also found to be related to past blood pressure levels measured 3 and 6 years previously, even after adjusting for current blood pressure (Wong, et al., 2001). The combination of these simultaneous findings suggests that generalized retinal arteriolar caliber may be a more robust indication of blood pressure or general systemic vascular status over a period of years than any single blood pressure measurement taken within that time.

While BMI did not show a significant correlation with CRVE, there were 3 participants in the APOE4- group who had a BMI over 30, which by definition is considered to fall into the obesity category. None of the participants in the APOE4+ category had a BMI above 29. Because higher BMI was associated with a larger CRVE, adjusting for BMI predictably adjusted our data in favor of the null hypothesis.

Axial length was smaller in the APOE4+ group than in the APOE4- group, a previously unpublished finding. As expected, adjusting for axial length made the means of both CRAE and CRVE smaller in the APOE4+ group, but the differences between groups did not reach significance.

In this study, neither CRVE nor CRAE showed any significant difference when compared between the APOE4- and APOE4+ groups. Arguably, a larger subject group may have revealed a significant difference in venule caliber between the two groups. In addition, further advances in technology may be needed in order to detect small changes in retinal

vessel diameters, and to correlate them with the retinal vessel changes well-documented in large epidemiologic studies, in order to detect them clinically and before the onset of AD in healthy individuals.

# CHAPTER 5: COMPARISON OF COGNITIVE TESTING TO RETINAL BLOOD VESSEL DIAMETERS

This chapter is based on a poster abstract presented at the Annual Meeting for the Association for Research in Vision and Ophthalmology, 5 May 2010.

Typically, patients with early Alzheimer disease show loss of episodic memory, mediated by the medial temporal lobe (Levy and Chelune, 2007). Retinal imaging has revealed relatively narrow retinal venules in patients with early Alzheimer Disease (AD) (Feke, et al., 2006). No study has yet examined whether retinal venule diameter correlates with medial temporal lobe function in healthy individuals at risk for AD.

The purpose of this study was to determine if this narrowing is detectable in individuals without dementia but who possess a genetic risk factor, APOE4, for AD.

## *Methods*

For this prospective, cross-sectional study, a pool of healthy participants was recruited from participants enrolled in the U-CARE and OAIC studies at the VA Boston Healthcare System, according to the protocols described in Chapters 1 and 2. As mentioned previously, the U-CARE and OAIC studies are cross-sectional studies of community dwellers, aged 50 to 70 years, and 55 to 84 years, respectively. All had well-controlled blood pressure and HbA1c of 6.1 or less; none smoked or had dementia, diabetes, glaucoma, retinal defects, ocular

inflammation, diplopia, or history of intraocular surgery other than uncomplicated cataract surgery. Cognitive and physical measures are listed in **Appendix A**.

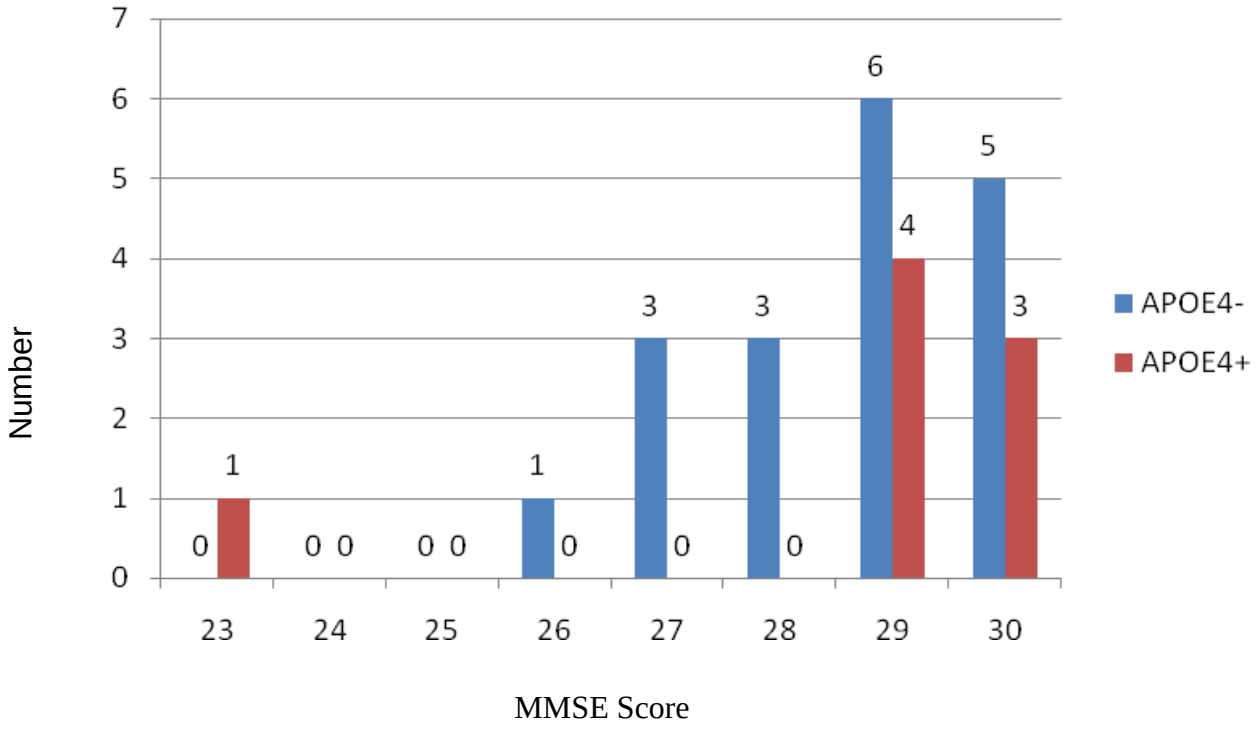
An examiner blind to the APOE status of the participants conducted a clinical exam including dilation with 0.5% tropicamide, A-scan, and acquisition of two 45-degree digital fundus photographs from each eye. A reader blind to APOE status determined central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) from fundus photographs using a semi-automated, published protocol (IVAN, University of Wisconsin, Madison). Relatively high AD risk was defined by the presence of the APOE4 allele.

Post-hoc analysis was completed to analyze the relationships between blood vessel data (CRVE, CRAE, and AVR) and cognitive testing. Simple linear regression was used to examine the correlation between each of the cognitive tests and the blood vessel data. CRVE, CRAE, and AVR were each analyzed separately. Bonferroni correction was used to correct for multiple testing in each domain. For those cognitive tests found to correlate significantly, simple linear regression was used to examine the effects of other parameters (age, education, blood pressure, etc.) and look for confounding variables. When confounders were found, variables were placed into bins, and analysis of covariance (ANCOVA) was used to determine the effects of these variables on the outcome, and compared with multiple regression, using an online software program (Vassarstats, Lowry, R., http://faculty.vassar.edu/lowry/VassarStats.html, Poughkeepsie, NY).

***Results***

Eight of 26 participants were positive for APOE4. Neither CRVE nor CRAE was significantly associated with APOE4, as shown previously in Chapter 4, **Tables 12 and 13**. **Figure 10**, below, shows the distribution of Mini-Mental State Exam (MMSE) scores for the entire sample (n=26). While one APOE4+ individual scored 23 on the MMSE, the rest of the APOE4+ participants scored 29-30.

**Figure 10: Mini-Mental State Exam (MMSE) Score Distribution for the Entire Sample**



None of the cognitive testing correlated significantly with CRVE. **Table 15**, below, shows tests of executive function and their correlation to CRAE. Three of the tests were negatively associated with CRAE, but with one of the tests, significance was lost after adjusting for multiple testing. CRAE was positively associated with performance on the Stroop test ($y = 0.9482x - 43.167$, $R^2 = 0.3292$, $P = 0.0022$), **(Figure 11a,** next page) and Category Fluency (Animals) ($y = 0.2464x – 17.465$, $R^2 = 0.3221$, $P = 0.0025$), **(Figure 12a**, page 74). When excluding the one individual with a MMSE score of 23 and analyzing the data for only those without cognitive impairment, the association between CRAE and Stroop scores became more significant ($y = 0.946617x - 42.155$, $R^2 = 0.342$, $P = 0.0021$), (**Figure 11b**, next page), as did the association between CRAE and Category Fluency (Animals) ($y = 0.245676x -16.978$, $R^2 = 0.372$, $P = 0.0012$), (**Figure 12b**, page 74).

**Table 15: Post-Hoc Analyses of Executive Function Tests vs. CRAE**

| Test of Executive Function | $R^2$ | P value |
|---|---|---|
| **Verbal Fluency (COWAT, total score)** | **0.0371** | **0.345** |
| **Category Fluency (Animals)** | **0.322** | **0.0025**** |
| Trails B (n=25 ) | 0.062 | 0.219 |
| Wisconsin Card Sorting Test (total correct) | 0.0076 | 0.671 |
| **Stroop** | **0.329** | **0.0022**** |
| Ruff Figural Fluency Test (speed + accuracy), n= 25 | 0.068 | 0.207 |
| **Clock-in-the-Box, executive subscore, n =25** | **0.238** | **0.0135*** |

N=26 unless noted otherwise.
*P < 0.05 (before Bonferroni correction)
**P < 0.007 (significant after Bonferroni correction)

**Figure 11a: Stroop Score vs. CRAE**

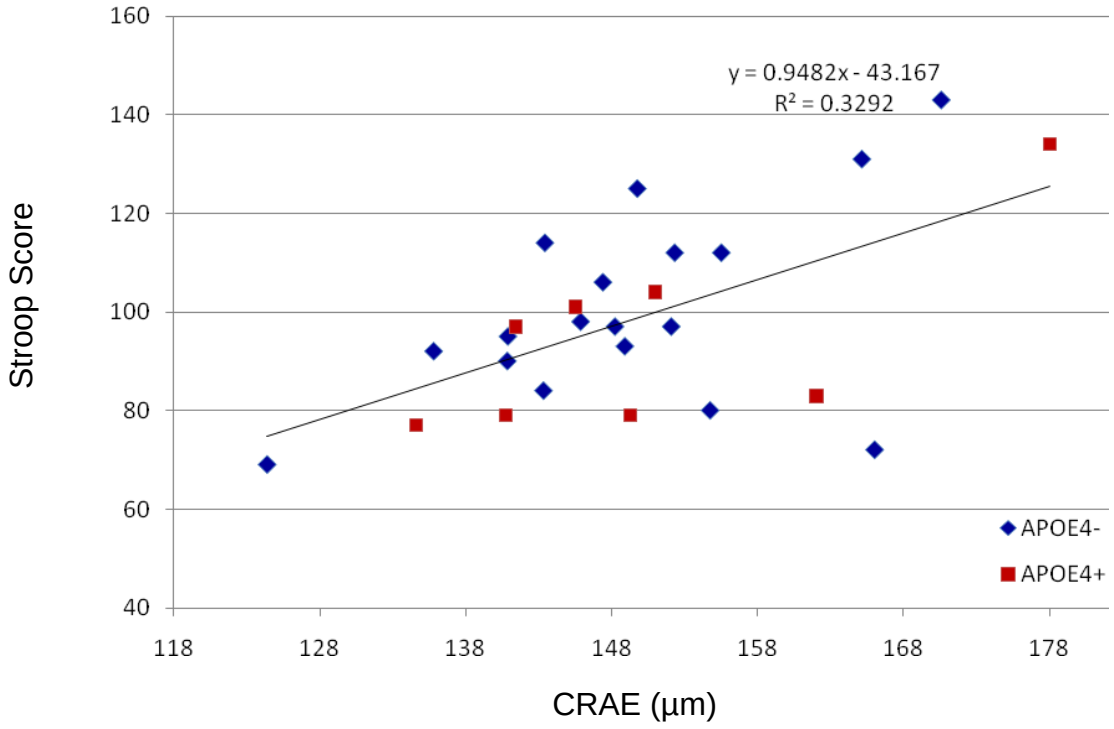



**Figure 11b: Stroop Score vs. CRAE, after Excluding One Participant with MMSE of 23**

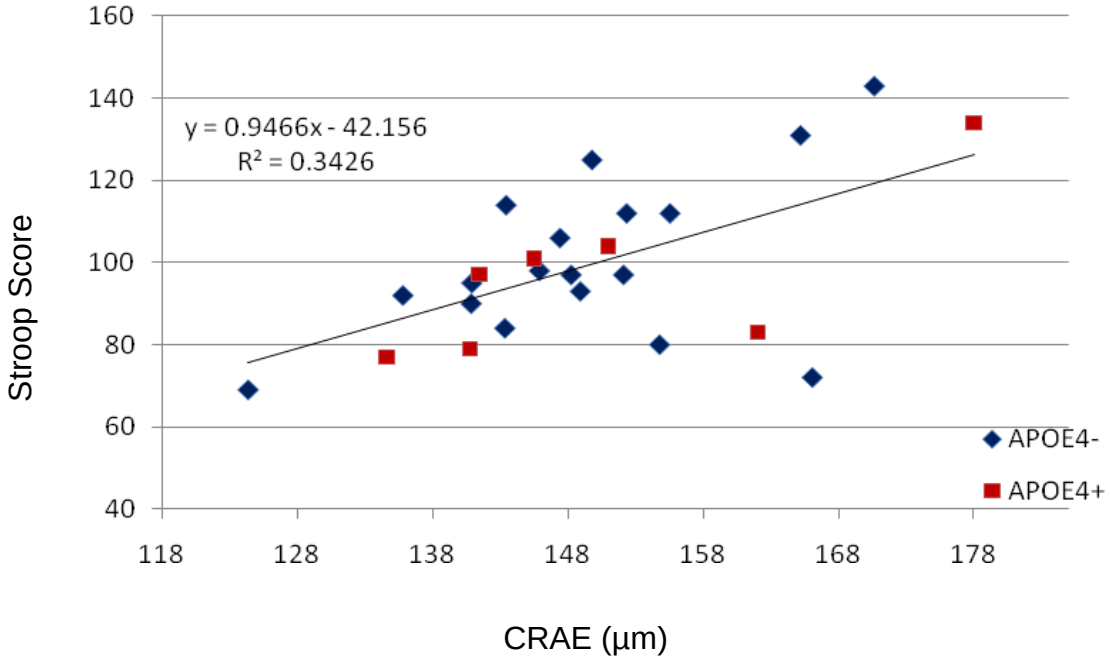


**Figure 12a: Category Fluency (Animals) vs. CRAE**

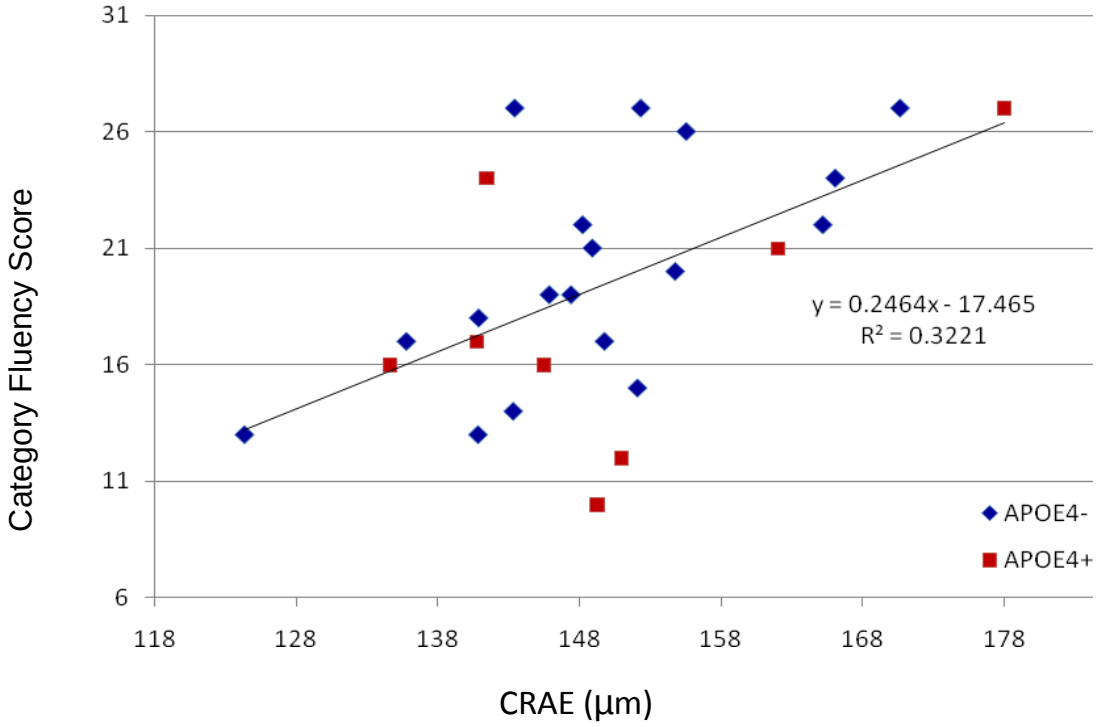
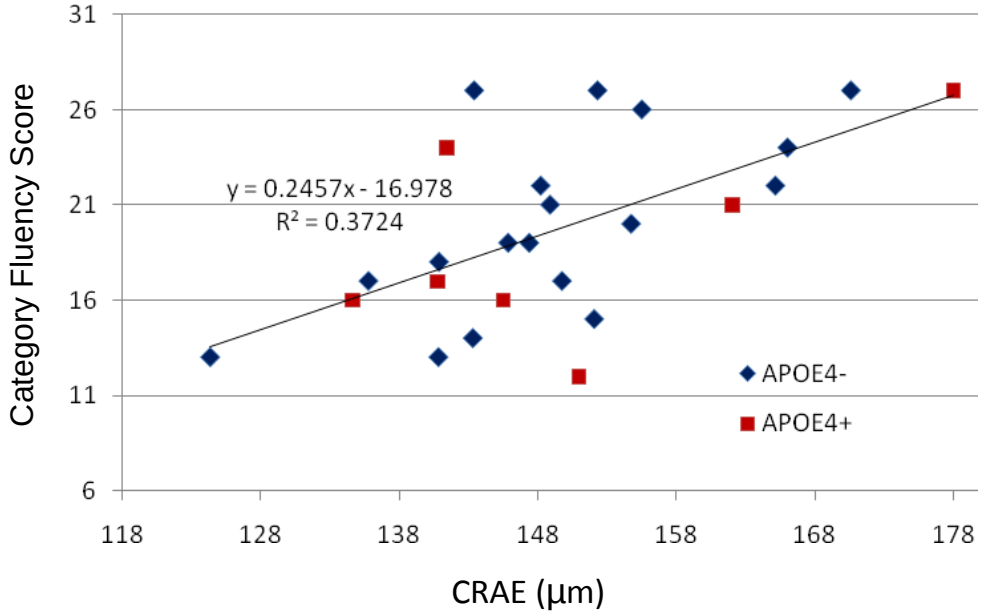



**Figure 12b: Category Fluency (Animals) vs. CRAE, after Excluding One Participant with MMSE of 23**



Neither the Stroop test nor the Category Fluency (Animals) test correlated significantly with APOE status, although in each of these tests, the means were higher for the APOE4- group. For the Stroop test, the mean score for the APOE4+ group was 94.25, and for the APOE4- group it was 100.56 (P= 0.457). For Category Fluency (Animals), the mean score for the APOE4+ group was 17.9, while it was 20.1 for the APOE4- group (P = 0.325).

Although CRAE did have a significant correlation with CRVE, CRVE did not by itself correlate significantly with the Stroop test ($y = 0.230076x + 49.816$, $R^2 = 0.0747$, $P = 0.177$) or with Category Fluency (Animals), ($y = 0.037009x + 11.535$, $R^2 = 0.0280$, $P = 0.414$). Systolic blood pressure, which had been found to be negatively and significantly associated with CRAE, showed a trend toward an inverse association with the Stroop test ($y = -0.51199x + 162.103$, $R^2 = 0.148$, $P= 0.0523$) and was negatively associated with Category Fluency (Animals), ($y = -0.1548+ 38.580$, $R^2 = 0.0235$, $P = 0.0235$). However, this was not significant after adjusting for multiple executive tests.

Among all 26 participants, in addition to CRAE, the Stroop test scores also correlated negatively with age ($y = -1.14405x + 177.9507$, $R^2 = 0.27759$, $P = 0.00569$), and this correlation was significant after adjusting for multiple executive tests. Together, age and CRAE explained more variability in the Stroop scores than either alone ($y = 44.7194 + 0.7436x_1 -0.8262x_2$, where $x_1$ = CRAE and $X_2$ = Stroop, Multiple $R^2 = 0.4586$, Adjusted Multiple $R^2 = 0.4115$). Analysis of Covariance (ANCOVA) between quartiles of CRAE adjusted for age showed the differences in means were still significant before Bonferroni

correction, but this finding should be interpreted with caution, since there were few and uneven numbers of data points in each quartile (**Table 16,** below). After repeating the analysis without the one participant whose MMSE score was 30, the association between the observed Stroop scores and age was still significant, but slightly less so (y= -1.11457 + 176.528, $R^2$ = 0.273, P = 0.0073).

**Table 16: Analysis of Covariance: CRAE Quartiles Adjusted for Age (n=26)**

| | 1st QTR | 2nd QTR | 3rd QTR | 4th QTR |
|---|---|---|---|---|
| n | 7 | 6 | 6 | 7 |
| Observed Means (µm) | 83.71 | 99.17 | 104.67 | 107.86 |
| Adjusted Means (µm) | 87.74 | 92.71 | 111.09 | 98.62 |
| Adjusted P | 0.0439 | | | |

Neither the Stroop nor Category Fluency (Animals) was significantly associated with AVR, BMI, weight, or diastolic blood pressure. Category Fluency (Animals) was positively correlated with education (y = 0.907848x + 5.347884, $R^2$ = 0.220, P = 0.0155), but this correlation was not significant after correcting for multiple testing.

***Discussion***

At the time of this writing, only five studies in PubMed have examined cognition and its relationship to retinal vessels, and two of these were part of the ARIC study (Wong, et al., 2002; Lesage, Mosley, Wong, Szklo, Knopman, Catellier, Cole, Klein, Coresh, Coker, and Sharrett, 2009). These studies are summarized in **Table 17**, next page. Executive memory tests are highlighted in yellow: Delayed Word Recall (DWR), Word Fluency (WF), and Verbal Fluency (COWAT).

In the ARIC study, any retinopathy was associated with a decrease in Delayed Word Recall (Wong, et al., 2002), and on 14-year follow-up, those participants with any retinopathy showed a greater decline in Word Fluency (Lesage, et al., 2009). In the Lothian Birth Cohort 1921 Study, suboptimal branching coefficients were associated with lower scores in Verbal Fluency (COWAT) (Patton, Pattie, MacGillivray, Aslam, Dhillon, Gow, Starr, Whalley, and Deary, 2007). In each of these three studies, there were no significant associations between cognitive testing and CRAE, CRVE, or AVR. However, the cognitive tests in these studies were limited to only a few, while in our study, dozens of tests were performed.

**Table 17: Retinal Vessels and Cognition: A Review of the Literature**

Executive memory tests highlighted: Delayed Word Recall (DWR), Word Fluency (WF), and Verbal Fluency (COWAT).

| | Study | N | Age | Exclusions | Retinal Assessment | Cognitive Testing | Summary of Findings |
|---|---|---|---|---|---|---|---|
| Liew, et al. , 2009 | Blue Mountains Eye Study | 1988 | 49-97 | Inability to complete MMSE or photography | 1. Any retinopathy<br>2. CRAE<br>3. CRVE | Folstein MMSE | 1. MMSE score ≤23 was associated with retinal venule dilation.<br>2. Retinopathy was associated with MMSE ≤ 23 in persons with hypertension but not in those without. |
| Wong, et al., 2002 | Atherosclerosis Risk in Communities Study (ARIC) | 8734 | 51-70 | Stroke, CNS-related drugs | 1. Generalized arteriolar narrowing (low AVR),<br>2. AV nicking,<br>3. Retinopathy:<br>a. Microaneurysms,<br>b. Hemorrhages,<br>c. soft exudates,<br>d. focal arteriolar narrowing | 1. Delayed Word Recall (DWR), a memory test used in assessing frontal lobe damage and early neurologic decline in elderly<br>2. Digit-Symbol Substitution Test (DSS), a measure of psychomotor speed<br>3. Word Fluency (WF), an executive function test | Any retinopathy (3a-d) was associated with decreased performance on all three cognitive tests, but the association was most significant with the DWR |
| Lesage, et al., 2009 | ARIC 14-year follow-up | 803 | Mean 58.4 | | Same as above except general arteriolar narrowing now defined as lowest quartile of CRAE, and focal arteriolar narrowing classified separately from general Retinopathy | | Those with any retinal abnormalities showed greater decline in WF (but not DSS or DWR) on follow- up. Those without had no decline in scores on follow-up. Retinopathy and focal narrowing were still significant in non-diabetics and non-hypertensives. |
| Baker, et al.,2007 | Cardiovascular Health Study (CHS) | 2211 | 69-97 | Stroke, anti-psychotic meds, anti-depressants | 1. Any retinopathy<br>2. Focal arteriolar narrowing<br>3. AV nicking<br>4. CRAE<br>5. CRVE | 1. Modified Mini-Mental State Exam (3MSE)<br>2. DSS | 1. Any retinopathy was related to lower DSS scores<br>2. NO significant associations in CRAE or CRVE with cognitive function. |
| Patton, et al., 2007 | Lothian Birth Cohort 1921 Study | 321 | ~83 | | 1. Branching coefficient (BC)<br>2. Median angle at arteriolar bifurcations<br>3. CRAE<br>4. CRVE<br>5. AVR | 1. Wechsler Logical Memory (WMS-R)<br>2. Verbal Fluency (COWAT)<br>3. Raven's Standard Progressive Matrices<br>4. General summary of 1-3 (G) | 1. Suboptimal BC associated with lower scores in G and COWAT.<br>2. Arteriolar deviation was associated with lower scores on WMS-R.<br>3. NO significant associations in CRAE, CRVE, or AVR with cognitive function. |

The Blue Mountains Eye Study was the first and only study to demonstrate an association between generalized venular caliber (venular dilation) and cognitive impairment (which was diagnosed as an MMSE score of 23 or less), but other microvascular signs were not associated with cognitive impairment (Liew, Mitchell, Wong, Lindley, Cheung, Kaushik, & Wang, 2009). This finding is inconsistent with earlier findings from the Cardiovascular Health Study, in which there were no associations found between CRAE, CRVE, and the Modified MMSE (Baker, Larsen, Kuller, Klein, Siscovick, Bernick, Manolio, and Wong, 2007). In our study, excluding the one participant with the lowest MMSE score and analyzing the findings for only those without any signs of cognitive impairment made the associations between CRAE and Stroop, as well as CRAE and Category Fluency, even more significant.

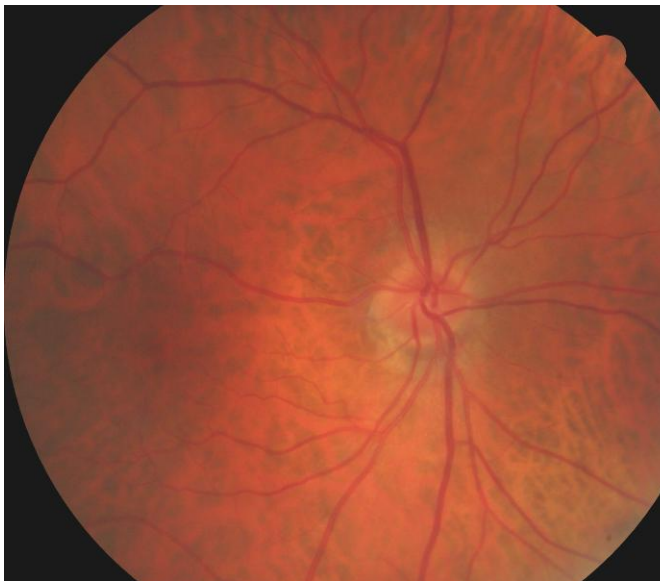
It is possible that the Stroop and Category Fluency tests are most indicative of the function of the parts of the brain whose circulation is most closely associated with that of the eye. Indeed, the frontal lobe, which mediates executive function, is supplied by the anterior cerebral artery and the middle cerebral artery; while the major blood supply of the eye, the ophthalmic artery, branches from the internal carotid artery just before the internal carotid joins the Circle of Willis and branches into the middle cerebral artery. Therefore, the origin of the middle and anterior cerebral arteries are anatomically very close to the origin of the ophthalmic artery.

In hypertensives, the negative association between blood pressure and executive function is widely established. Vascular cognitive impairment typically affects executive function before other domains (Leritz, et al., 2010; Levy and Chelune, 2007). However, in this study, all participants had well-controlled blood pressure and normal mean arterial blood pressure (MABP). None of our participants had dementia. As discussed in Chapter 4, systolic blood pressure was negatively associated with CRAE in this study. It may be possible, then, that early vascular changes affecting certain aspects of executive function are detectable in the retinal vasculature, even before they affect other areas of cognitive function, even in individuals whose blood pressure is thought to be well-controlled. **Figure 13**, next page, shows a side-by-side comparison of two right eye images from individuals with identical MMSE scores but very different observed CRAE values. The individual with the lower CRAE also achieved lower scores on the Stroop and Category Fluency (Animals) tests, despite achieving a high score on MMSE.

**Figure 13: Side-By-Side Comparison of Two Right Eyes with Equal MMSE Scores, Different CRAE Values**



CRAE =170.61 µm, Animals 27,Stroop 143, MMSE = 30



CRAE =140.76 µm, Animals 17, Stroop 79, MMSE= 30

***Conclusions***

In this study, retinal venular caliber was not associated with AD risk or cognition in healthy individuals. Retinal arteriolar caliber was associated with two measures of executive function, which is more typically affected early in vascular cognitive impairment. To our knowledge, this is the first study showing an association between generalized retinal vessel narrowing and cognitive function in healthy individuals without cognitive impairment.

In healthy individuals with normal mean arterial blood pressure, central retinal artery equivalent may serve as a non-invasive indicator of early microvascular changes subtly affecting executive function. Further research is needed to determine whether generalized retinal arteriolar narrowing may be an early marker for risk of vascular dementia.

# CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

Although we collected ocular biometric data and were prepared to adjust our measurements for ocular and camera magnification, the raw CRVE and CRVE calculated by the IVAN program were comparable to values in the literature. This may be due to a combination of factors, including the limited range in the biometric parameters (refractive error, corneal curvature, axial length) found in our participants, the camera used in the study, and the conversion factors used by the IVAN program itself. Therefore, the raw data, and not the adjusted measurements, were used in subsequent analyses.

As expected, systolic blood pressure correlated significantly and negatively with CRVE, and MABP and diastolic blood pressure significantly and negatively correlated with AVR. MABP, however, did not show any significant association with CRAE, probably because of the narrow range of MABP in our cohort.

This study was weakened by poorer recruitment than anticipated, despite all efforts, including the relaxing of systolic blood pressure criteria. The mean difference in time between the physical/cognitive testing in the U-CARE/OAIC studies and acquisition of retinal photographs in this study was 1.876 years. Although this time difference was larger than desired, it was smaller than that in the ARIC study (Patton, et al., 2005; Wong and McIntosh, 2005), and mounting evidence from that study as well as others supports the notion of general retinal arteriolar caliber as a more robust marker of general systemic

vascular health and blood pressure control over a period of a number of years than any one blood pressure measurement taken during that time.

While BMI did not show a significant correlation with CRVE, there were 3 participants in the APOE4- group who had a BMI over 30, which by definition is considered to fall into the obesity category. None of the participants in the APOE4+ category had a BMI above 29. Because obesity is associated with retinal venule widening, adjusting for BMI was expected to adjust our data in favor of the null hypothesis, and this was indeed the case. Excluding the individuals in the obesity category would not have changed the overall study outcome.

Neither CRVE nor CRAE showed any significant difference when compared between the APOE4- and APOE4+ groups. We attempted to minimize variability due to the pulse cycle and to multiple readers by taking two images of each eye and averaging their values, and by having the same grader read all images, but this was time-consuming. Further advances in technology are needed in order to detect small changes in retinal vessel diameters clinically, and to correlate them with the retinal vessel changes well-documented in large epidemiologic studies, in order to detect them before the onset of AD in healthy individuals.

In this study, retinal venular caliber was not associated with AD risk or cognition in healthy individuals. Retinal arteriolar caliber was associated with two measures of executive function, which is more typically affected early in vascular cognitive impairment. To our knowledge, this is the first study showing an association between generalized retinal vessel

narrowing and cognitive function in healthy individuals without cognitive impairment. Despite the ease with which circulation can be assessed non-invasively and *in vivo* within the eye, there is a paucity of literature examining retinal vasculature and cognitive function. Further research is needed to determine whether generalized retinal arteriolar narrowing may be an early marker for risk of vascular dementia.

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## APPENDIX A: U-CARE AND OAIC NEUROPSYCHOLOGICAL AND PHYSICAL EXAMINATION STUDY MEASURES

| DOMAIN | TEST | VARIABLES | Possible Values |
|---|---|---|---|
| **Attention** | Digit Span | Digit Span Forward | 0-20 |
| | | Digit Span Backward | 0-20 |
| | | Digit Span Total | 0-40 |
| | | Digit Span Total Scaled Score | 0-20 |
| | | | |
| | Trail Making | Trails A | unlimited |
| | Mini Mental | Total score | 0-30 |
| | | | |
| **Memory** | | | |
| **Verbal** | CVLT | Learning Trial 1 | 0-16 |
| | | Learning Trial 2 | 0-16 |
| | | Learning Trial 3 | 0-16 |
| | | Learning Trial 4 | 0-16 |
| | | Learning Trial 5 | 0-16 |
| | | Total 1-5 | 0-80 (CVLT1 + CVLT2 + CVLT3 + CVLT4 + CVLT5) |
| | | Total Learning Standard Score (t-score) | 0-99 |
| | | List B | 0-16 |
| | | Short Delay Free | 0-16 |
| | | Short Delay Free Standard Score (z-score) | 0-99 |
| | | Short Delay Cued | 0-16 |
| | | Short Delay Cued Standard Score (z-score) | 0-99 |

| | | | |
|---|---|---|---|
| **Verbal (cont'd)** | | Long Delay Free | 0-16 |
| | | Long Delay Free Standard Score (z-score) | 0-99 |
| | | Long Delay Cued | 0-16 |
| | | Long Delay Cued Standard Score (z-score) | 0-99 |
| | | Free Recall Intrusions | unlimited |
| | | Cued Recall Intrusions | unlimited |
| | | Total Intrusions | Unlimited (FRINTRU + CRINTRU) |
| | | Total Intrusions Standard Score (z score) | 0-99 |
| | | Total Repetitions | unlimited |
| | | Recognition Hits | 0-16 |
| | | Recognition Hits Standard Score (z-score) | 0-99 |
| | | Recognition False Positives | 0-32 |
| | Logical Memory | Story A Recall | 0-25 |
| | | Story B 1st Recall | 0-25 |
| | | Story B 2nd Recall | 0-25 |
| | | Immediate Recall Total | 0-75 (LMIA+LMIB1=LMIB2) |
| | | Immediate Recall Total Scaled Score | 0-20 |
| | | Story A thematic | 0-7 |
| | | Story B 1st thematic | 0-8 |
| | | Story B 2nd thematic | 0-8 |
| | | Immediate thematic total | 0-23 (LMIATHE+ LMIB1THE+ LMIB2THE) |
| | | Immediate Thematic Total Scaled Score | 0-20 |
| | | Story A Delayed Recall | 0-25 |
| | | Story B Delayed Recall | 0-25 |
| | | Delayed Recall Total | 0-50 (LMIIA+LMIIB) |
| | | Delayed Recall Total Scaled Score | 0-20 |
| | | Story A Delayed Thematic | 0-7 |

| | | | |
|---|---|---|---|
| **Verbal (cont'd)** | | Story B Delayed Thematic | 0-8 |
| | | Delayed thematic total | 0, 15 (LMIIATHE+ LMIITHET) |
| | | Delayed thematic total scaled score | 0-20 |
| | | Recognition | 0-30 |
| | | Percent retention | 0-100 [LMIITOT/( LMIA+ LMIB2)]*100 |
| | | Percent retention scaled score | 0-20 |
| | | | |
| **Non-Verbal** | ROCF | Immediate Recall | 0-36 |
| | | Immediate Recall Standard Score (t-score) | 0-99 |
| | | Delayed Recall | 0-36 |
| | | Delayed Recall Standard Score (t-score) | 0-99 |
| | | Recognition | 0-24 |
| | | Recognition Standard Score (t-score) | 0-99 |
| | | | |
| | Visual Reproduction | Figure A immediate | 0-50 |
| | | Figure B immediate | 0-50 |
| | | Figure C immediate | 0-50 |
| | | Figure D immediate | 0-50 |
| | | Figure E immediate | 0-50 |
| | | Immediate total | 0-50 (AVRI+ BVRI+ CVRI+ DVRI+ EVRI) |
| | | Immediate total scaled score | 0-20 |
| | | Figure A delay | 0-50 |
| | | Figure B delay | 0-50 |
| | | Figure C delay | 0-50 |
| | | Figure D delay | 0-50 |
| | | Figure E delay | 0-50 |

| | | | |
|---|---|---|---|
| **Non-Verbal (cont'd)** | | Delay Total | 0-50 (AVRII+ BVRII+ CVRII+ DVRII+ EVRII) |
| | | Delay Total Scaled Score | 0-20 |
| | | Recognition total | 0-50 |
| | | Recognition total scaled score | 0-20 |
| | | Discrimination | 0-50 |
| | | Retention | 0-50 |
| | | Retention total scaled score | 0-20 |
| | | | |
| **Visuospatial** | ROCF | Copy | 0-36 |
| | | Rey Copy Standard Score (t-score) | 0-99 |
| | Clock Drawing | Total Score | 0-7 |
| | Clock-in-the-Box | Location | 0-1 |
| | | Object | 0-1 |
| | | Numbers | 0-1 |
| | | Time | 0-1 |
| | | Memory Subscore | 0-4 |
| | | Size | 0-1 |
| | | Number Order | 0-1 |
| | | Number Spacing | 0-1 |
| | | Hand length and origin | 0-1 |
| | | Executive Function Subscore | 0-4 |
| | | Total Score | 0-8 |
| | | | |
| | Visual Reproduction | Figure A copy | 0-50 |
| | | Figure B copy | 0-50 |
| | | Figure C copy | 0-50 |
| | | Figure D copy | 0-50 |
| | | Figure E copy | 0-50 |

| | | | |
|---|---|---|---|
| **Visuospatial (cont'd)** | | Copy total | 0-150 (AVRC+ BVRC+ CVRC+ DVRC+ EVRC) |
| | | Copy total Scaled Score | 0-20 |
| | | | |
| **Executive Functions** | Verbal Fluency | F words | Unlimited |
| | | A words | Unlimited |
| | | S-words | Unlimited |
| | | FAS total | (COWAF+ COWAA+ COWAS) |
| | | Perseverations F trial (raw) | 0-50 |
| | | Perseverations A trial (raw) | 0-50 |
| | | Perseverations S trial (raw) | 0-50 |
| | | Perseverations Total (raw) | 0-150 (FPERS+APERS+SPERS) |
| | | FAS total Standard Score (t-score) | 0-99 |
| | | | |
| | Category Fluency | Animals | Unlimited |
| | | Perseverations Animals (raw) | 0-50 |
| | | Animals Standard Score (t-score) | 0-99 |
| | | | |
| | Trails Making | Trails B | Unlimited |
| | | Trails B standard score (t score) | 0-99 |
| | | | |
| | WCST | Trials Administered | 0-128 |
| | | Total Correct | 0-128 |
| | | Total Errors | 0-128 |
| | | Errors standard score (t score) | 0-99 |
| | | Perseverative Responses | 0-128 |

| | | | |
|---|---|---|---|
| **Executive Functions (cont'd)** | | Perseverative Resp standard score (t score) | 0-99 |
| | | Perseverative Errors | 0-128 |
| | | Perseverative Errors standard score (t score) | 0-99 |
| | | Categories Completed | 0-6 |
| | Stroop | Color-word reading | 1-200 |
| | | Color-word reading Standard Score (t-score) | 0-99 |
| | Ruff 2&7 | Automatic Target detection speed | 0-300 |
| | | Automatic Speed Standard Score (t-score) | 0-99 |
| | | Automatic Target detection errors | 0-500 |
| | | Automatic Target detection accuracy | 0-100 [R27ASPD/( R27ASPD+ R27AERR)]*100 |
| | | Automatic Accuracy Standard Score (t-score) | 0-99 |
| | | Controlled Target detection speed | 0-300 |
| | | Controlled Speed Standard Score (t-score) | 0-99 |
| | | Controlled Target detection errors | 0-500 |
| | | Controlled Target detection accuracy | 0-100 [R27CSPD/( R27CSPD + R27CERR0]*100 |
| | | Controlled Accuracy Standard Score (t-score) | 0-99 |
| | | Total Speed | 0-300 |
| | | Total Speed Standard Score (t-score) | 0-99 |
| | | Total Accuracy | 0-300 |
| | | Total Accuracy Standard Score (t-score) | 0-99 |
| | Concept Generation | # Trials Administered Part A | 0-50 |

| | | | |
|---|---|---|---|
| **Executive Functions (cont'd)** | | Correct grouping A | 0-6 |
| | | Correct naming A | 0-6 |
| | | Repetitions A | 0-50 |
| | | # Trials Administered Part B | 0-50 |
| | | Correct grouping B | 0-6 |
| | | Correct naming B | 0-6 |
| | | Repetitions B | 0-50 |
| | | # Trials Administered Part C | 0-50 |
| | | Correct grouping C | 0-6 |
| | | Correct naming C | 0-6 |
| | | Repetitions C | 0-50 |
| | | | |
| **Language** | Boston Naming Test | Spontaneous | 0-60 |
| | | Semantic Cue | 0-60 |
| | | Phonemic Cue | 0-60 |
| | | Multiple Choice | 0-60 |
| | | Total score | 0-60 (BNTCOR+ BNTSEM) |
| | | Total score standard score (z-score) | 0-10 |
| | | | |
| **Intellectual Functions** | | | |
| | WTAR | Total correct | 0-50 |
| | | Estimated IQ | 0-134 |
| | LOFT (Spot-a-Word) | Total correct | 0-50 |
| | | | |
| **MOOD & ADLs** | GDS | Total score | 0-30 |

| | | | |
|---|---|---|---|
| **MOOD & ADLs (cont'd)** | BDI | Total score | 0-63 |
| | IADLS | Total Score | 0-27 |
| | Dysexecutive Questionnaire | Total Score | 0-80 |
| | | Total score percentile | 0-100 |

**MEDICAL EXAMINATION**

| | **VARIABLES** |
|---|---|
| Past medical history | |
| | Coronary artery disease |
| | Heart attack |
| | Bypass |
| | # of vessels, date of operation |
| | Angioplasty |
| | # of vessels |
| | Stent |
| | Post myocardial infarction |
| | When |
| | Congestive heart failure |
| | Hypertension |
| | Diabetes Mellitus |
| | Diabetic renal disease |
| | End organ disease |
| | Hypercholesterolemia |
| | How long |
| | Cramping in legs when walking |
| | Chronic obstructive pulmonary disease |

| | |
|---|---|
| Past medical history (cont'd) | History of cancer |
| | History of liver disease |
| | History of renal disease (not diabetes related) |
| | History of rheumatologic disease |
| | |
| Review of systems | Vision |
| | Hearing |
| | Neuropathy, other than DM |
| | Falls |
| | # in last month |
| | # in last year |
| **Blood pressure meds** | |
| | Diuretic |
| | Number of years |
| | Beta Blocker |
| | Number of years |
| | |
| | Ace Inhibitor |
| | Number of years |
| | Calcium channel blocker |
| | Number of years |
| | |
| | Aspirin (ASA) |
| | Nitrate use |
| | Lipid lowering agents |
| | Diabetes medications |
| | Hormone replacement therapy |
| | |
| Family History | Coronary artery disease |
| | Hypertension |
| | Diabetes mellitus |
| | Cognitive decline |

| | |
|---|---|
| Physical exam | Height |
| | Weight |
| | Body mass index |
| | Blood pressure seated trial #1 |
| | Blood pressure seated trial #2 |
| | Blood pressure standing trial #1 |
| | Blood pressure standing trial #1 |
| | Pulse |
| Cardiac exam | Carotid bruits |
| | Lateral PMI |
| | Irregular rhythm |
| | Murmur – homodyne signify |
| Lung exam | Abnormal sounds |
| Neuro exam | Muscle tone |
| | Tremor |
| | Fine coordination |
| | Finger nose missing target |
| | Abnormal gait |
| | DTR symmetric |
| | Cranial nerve symmetric |
| | Pathologic reflexes |
| | Neuropathology of foot |
| EKG | Rate |
| | QRS axis |
| | P axis |
| | Atrial-fibrillation |
| | Other rhythm disturbance |
| | Left ventricular hypertrophy |
| | Old q-wave infarction |
| | Bundle branch block - RBBB |
| | Bundle branch block - LBBB |
| | Bundle branch block - LAHB |
| | Bundle branch block - LPHB |

| | |
|---|---|
| | Active ischemia |
| Incontinence | Urinary tract infections |
| | Accidentally leaked urine past month |
| | If leakage how often |
| | How much does leakage bother you |
| | How many times up at night to pass urine |
| | Lost control of bowels |
| | How often |
| | Constipation |
| **BLOOD LABS** | |
| | LDL Cholesterol |
| | Glycohemoglobin Hemoglobin A1c |
| | Calculated mean glucose |
| **Chemistry** | |
| | Creatinine |
| | Glucose |
| | Triglycerides |
| | Albumin |
| | HDL-cholesterol |
| | Thyroid stimulating hormone |