Studying the Effects of Antihypertensives in Individuals at Risk for Alzheimer's Disease (SEAIRA) Pilot Study

NCT00980785

December 5, 2014

Protocol: "Studying the Effects of Antihypertensives in Individuals at Risk for Alzheimer's Disease (SEAIRA) Pilot Study"

Principal Investigator:
Cynthia Carlsson, MD, University of Wisconsin

Co - Investigators:
Whitney Wharton, Ph.D., Emory University (Original Principal Investigator at University of Wisconsin)
James H. Stein, MD, University of Wisconsin
Maritza Dowling, PhD, University of Wisconsin
Carey Gleason, PhD, University of Wisconsin

Collaborator
Claudia Korcarz, PhD, University of Wisconsin

Funding Sponsor
Alzheimer's Disease Initiative of the State of Wisconsin

Contact information:
Contact information Emory University:
Whitney Wharton, PhD
Assistant Professor
Emory University
Department of Neurology
WWHC 1841 Clifton Rd., NE
Atlanta, GA 30329
w.wharton@emory.edu

Contact Information University of Wisconsin:
Cindy Carlsson, MD, MS
Department of Medicine
2500 Overlook Terrace
Madison, WI 53507
cmc@medicine.wisc.edu
608.280.7000

Introduction

Please note, this study completed enrollment as of 3/1/2011. Per the 5 year renewal policy, this study remains open for tissue and data analysis only. Data and specimens continue to be stored at the UW-Madison. When specimens are analyzed, they will be analyzed at UW-Madison and the de-identified data resulting from the specimen analysis may be sent to Emory University for further analysis.

Project Summary

High blood pressure (BP) in midlife is predictive of Alzheimer's disease (AD) in later life. Similarly, reductions in BP are associated with protection against AD. Treatment with antihypertensive medications, specifically angiotensin converting enzyme inhibitors (ACE-I) such as ramipril, is associated with up to a 55% reduction in the prevalence of AD, suggesting a potentially promising role for ACE-I in the prevention of AD. It is unknown however 1) whether ACE-Is will have the same effect on CSF Aβ levels in humans as in animal models 2) whether ACE-Is induce changes associated with vascular function (i.e. levels of CSF angiotensin converting enzyme (ACE) and peripheral endothelial function) and 3) whether there are interactions between ACE-I-induced changes in CSF Aβ, CSF ACE and indices of vascular function.

One mechanism by which antihypertensives may protect against AD is via Aβ neuropathology. In order to better understand the mechanisms through which ACE-I may modify CSF Aβ and possibly AD risk, we propose a randomized, double-blind, placebo-controlled pilot clinical trial, enrolling 20 middle-aged (age range 40 – 70 years), mildly hypertensive (between 130 – 160 mmHg mean systolic and between 70 – 100 mmHg mean diastolic) participants, who are adult children of an individual with AD. The main objective of this trial is to examine the effects of the ACE-I, ramipril, on 1) CSF Aβ levels 2) CSF ACE levels and 3) peripheral endothelial function as measured by brachial artery flow-mediated vasodilation (FMD) and aortic augmentation index (AAIx), in middle-aged adults with mildly elevated BP, who are at increased risk of developing AD.

Hypothesis: mildly hypertensive, middle-aged, non-demented, adult children of persons with AD will exhibit lower CSF Aβ and ACE levels and improved endothelial function following 4 months of ramipril therapy. These vascular effects have been tied to cognition. Later work will examine whether ramipril may reduce the risk of AD in later life.

Specific Aim 1: To determine the effects of 4 months of ramipril therapy on CSF Aβ levels in mildly hypertensive, non-demented adult children of persons with AD when compared to subjects taking placebo.

Specific Aim 2: To evaluate the effects of 4 months of ramipril therapy vs. placebo on CSF ACE levels in mildly hypertensive, non-demented adult children of persons with AD when compared to subjects taking placebo.

Specific Aim 3: To determine the effects of 4 months of ramipril therapy on the cardiovascular outcome variables of brachial reactivity and aortic augmentation index in non-demented adult children of persons with AD when compared to subjects taking placebo.

RESEARCH PLAN

A. Hypotheses and Specific Aims

A.1. Rationale:

High blood pressure (BP) in midlife is predictive of Alzheimer's disease (AD) in later life. Similarly, reductions in BP are associated with protection against AD [1, 2] such that even small reductions (<5mmHg systolic and <3 mmHg diastolic) are associated with improvements in Mini Mental State Examination (MMSE) [1] scores. Specifically, converging evidence indicates that lowering BP alters the pathobiological factors that contribute to AD (see Birns et al. review [2]). Treatment with antihypertensive medications, specifically angiotensin converting enzyme inhibitors (ACE-I) such as ramipril, is associated with up to a 55% reduction in the prevalence of AD, suggesting a potentially promising role for ACE-I in the prevention of AD [3]. ACE-I improve endothelial function outside of the central nervous system and decrease Aβ (i.e. a substance thought to contribute to neuronal damage in AD) in animal models, suggesting that these are mechanisms by which ACE-Is may protect against AD [4]. It is unknown however 1) whether ACE-Is will have the same effect on cerebrospinal fluid (CSF) Aβ levels in humans as in animal models 2) whether ACE-Is induce changes associated with vascular function (i.e. levels of CSF angiotensin converting enzyme (ACE) and peripheral endothelial function) and 3) whether there are interactions between ACE-I-induced changes in CSF Aβ, CSF ACE and indices of vascular function.

In order to better understand the mechanisms through which ACE-I may modify CSF Aβ and possibly AD risk, we propose a randomized, double-blind, placebo-controlled pilot clinical trial, enrolling 20 middle-aged (age range 40 –70 years), mildly hypertensive (130 – 160 mmHg mean systolic and 70 – 100 mmHg mean diastolic) participants, who are adult children of an individual with AD. The main objective of this trial is to examine the effects of the ACE-I, ramipril, on 1) CSF Aβ levels 2) CSF ACE levels and 3) peripheral endothelial function as measured by brachial artery flow-mediated vasodilation (FMD) and aortic augmentation index (AAIx), in middle-aged adults with mildly elevated BP, who are at increased risk of developing AD based on familial history.

Our overarching hypothesis is that mildly hypertensive, middle-aged, non-demented, adult children of persons with AD will exhibit lower CSF Aβ and ACE levels and improved endothelial function following 4 months of ramipril therapy. These vascular effects have been tied to cognition. Later work will examine whether ramipril may reduce the risk of AD in later life.

The proposed trial has the potential to draw attention to an extremely promising avenue in AD prevention. Specifically, a therapeutic intervention that reduces Aβ levels may delay the onset of AD pathology, or possibly one day serve as a treatment strategy. Pilot data collected from the current investigation will be used to support a NIH Pathway to Independence grant application (K99/R00), which will investigate the effects of centrally acting ACE inhibitors on biomarkers for AD (CSF and MRI perfusion), in middle-aged persons at risk for AD. Of note, the K99/R00 proposal will not only have the power and the length of follow-up needed to address the variables included in the current proposal, but it will also be designed to examine cognitive task performance. Importantly, the NIA has stated that funding pharmacologic research in order to understand the pathobiology of AD is of the highest priority. The current project serves to provide preliminary data, as well as to address initial questions surrounding the relationship

between AD and vascular factors before a larger, more expensive and more targeted research project is warranted.

A.2. Hypothesis:

We hypothesize that mildly hypertensive, middle-aged, non-demented, adult children of persons with AD will exhibit lower CSF Aβ and ACE levels and improved endothelial function following 4 months of ramipril therapy. These vascular effects have been tied to cognition. Later work will examine whether ramipril may reduce the risk of AD in later life.

A.3. Specific Aims:

The present study is guided by the following specific aims:

Specific Aim 1: To determine the effects of 4 months of ramipril therapy on CSF Aβ levels in mildly hypertensive, non-demented adult children of persons with AD when compared to subjects taking placebo.

Specific Aim 2: To evaluate the effects of 4 months of ramipril therapy vs. placebo on CSF ACE levels in mildly hypertensive, non-demented adult children of persons with AD when compared to subjects taking placebo.

Specific Aim 3: To determine the effects of 4 months of ramipril therapy on the cardiovascular outcome variables of brachial reactivity and aortic augmentation index in non-demented adult children of persons with AD when compared to subjects taking placebo.

B. Background and Significance:

B. 1. Vascular Risk Factors and Alzheimer's disease Neuropathology: Alzheimer's disease (AD) is the most common dementing illness and is associated with significant morbidity and mortality among older adults. As many as 5.2 million people in the United States are currently living with AD, and this number is expected to exceed 14 million by the year 2050 [5]. In Wisconsin alone, 100,000 individuals age 65 and older were diagnosed with AD in 2000. By the year 2010, the Alzheimer's Association predicts that Wisconsin will see a 10 % increase in AD, bringing the number of affected persons to a startling 110,000 individuals over the age of 65. Although breakthroughs in AD treatments have been made, these therapies work mainly to delay further loss of function rather than restore cognitive abilities. Irreversible neuronal loss may limit efficacy of therapies once clinical signs of AD have developed, thus, emphasizing the importance of developing primary preventive strategies for use in high-risk individuals during the preclinical phase.

A major indication of preclinical AD pathology is Aβ accumulation [6]. The accumulation of Aβ plaques has received much attention and could prove to be a modifiable target for AD prevention. In AD patients, Aβ plaques are particularly prominent in the CA1 region of the hippocampus, a brain region important for memory processes and one that is selectively impaired in the presence of elevated BP levels [7]. Specifically, scientists propose that Aβ plaques precede cognitive decline in AD patients. Thus, a therapeutic intervention that reduces Aβ levels may delay the onset of AD pathology, or possibly one day serve as a treatment strategy. CSF Aβ levels are reliable biomarkers of underlying AD neuropathology and have been proposed as a biomarker to monitor treatment efficacy [8].

B. 2. Vascular Risk Factors and Alzheimer's disease Neuropathology: An increased risk of AD has been associated with numerous vascular risk factors, including hypercholesterolemia,

[9] hyperhomocysteinemia, [10] diabetes mellitus, [11-13] obesity, [11, 14, 15] physical inactivity, [16] elevated inflammatory markers [17] and particularly hypertension [9]. Advancing age increases exposure time to age-dependent vascular risk factors, suggesting that the effects of "age" on cognitive decline may be mediated in part via vascular risk factors [18]. Cerebrovascular dysregulation and Aβ deposition are findings in preclinical AD pathology, are integrally related to vascular risk factors, [19-21] and are processes that work synergistically to accelerate neuronal degeneration [19]. In the Atherosclerosis Risk in Communities cohort of middle-aged persons (mean age 55), the effects of hypercholesterolemia, diabetes, and ApoE ε4 on declines in memory over a 6-year interval were additive [22], with greater declines occurring in persons with increased risk factors. This suggests that vascular risk factors and ApoE ε4 may act synergistically in causing cognitive decline. If aging contributes to AD risk, in part, through the accumulation of vascular risk factors, then modifying these risk factors in midlife may reduce the detrimental cognitive effects in later life. Thus, it is critical that future research investigates the interaction and relative influence of vascular risk factors, genotype, gender, and age on the development and progression of AD.

Hypertension has been closely linked with vascular dementia and cognitive decline [23]. Additionally, elevated BP in mid-life has been linked to the development of AD decades later, leading to speculation that BP regulation plays a key role in the progression of AD. Recent epidemiological [24] observational [7, 25], clinical [3, 23, 26] and basic science [24, 27] suggests that some antihypertensive medications may reduce the risk for AD, and even improve cognition in patients with AD [28].

B. 3. ACE-I Improve Cognition in Humans with AD by Decreasing Aβ Levels: One mechanism by which antihypertensives may protect against AD is via Aβ neuropathology [4, 24]. Some of the most promising clinical [3, 26] observational [29] and basic science [24, 27] data examining the effects of antihypertensives on AD comes from studies using ACE-I. Angiotensin converting enzyme (ACE), a component of the renin–angiotensin system (RAS), has been shown to facilitate in the accumulation of Aβ plaques [30, 31], while ACE-I are reported to reduce Aβ deposition [32]. The chief rationale for using ACE-I is based on findings that ACE receptors are over-expressed in the hippocampus and throughout blood brain barrier (BBB) cells in patients with AD [30 {Savaskan, 2005 #5900, 33]. Furthermore, many ACE-Is such as the one in the current proposal, have the ability to cross the BBB, a factor that may contribute to ACE-I selective ability to treat AD via prevention of Aβ deposition. In fact, studies have demonstrated that treatment with centrally acting ACE-I decreases Aβ in animal models of AD [32], decreases AD incidence [34] and improves cognitive performance in AD patients [35]. These preclinical studies suggest that centrally acting ACE-Is may have AD-modifying activity in participants with AD or in those at high risk of developing AD.

Targeting modifiable risk factors, such as elevated BP, that contribute to Aβ accumulation and subsequent AD pathology may be important in developing effective preventive strategies for AD. The mechanisms through which adjustable risk factors contribute to neuronal damage may potentially be modified. ACE is associated with elevated BP and the accumulation of Aβ plaques [16, 19-22]. If the presence of Aβ leads to neuronal damage in part through deregulation of ACE levels and elevated blood pressure, then modifying Aβ accumulation through the use of ACE-Is may potentially reduce the risk of developing AD in high-risk individuals.

B. 4. Significance: The proposed 4-month randomized, double-blind, placebo-controlled, pilot clinical trial will provide information concerning possible mechanisms underlying the potential efficacy of ACE-I to reduce the risk for AD. If ramipril improves CSF Aβ levels and these changes correlate significantly with changes in CSF ACE levels, peripheral measures of endothelial function and/or cognition in persons at risk for AD, it would strengthen the evidence

for the role of ACE-I in AD prevention. Preventing or delaying the onset of AD would have a profound population health and economic impact on the people of Wisconsin and worldwide.

B. 5. Importance of Prevention: Recent estimates of the projected rapid increase in the prevalence of AD emphasize the importance of early detection, intervention, and prevention of the disease.[36] There is increasing evidence that the clinical syndrome of AD is preceded by a prolonged and currently irreversible preclinical phase characterized by neuropathological, [37] functional, [38, 39] structural, [40] metabolic, [39, 41] and cognitive changes[42-45] typical of AD. There is growing evidence that a person's risk of developing AD may be influenced by lifestyle, [46-52] diet, [53-55] vascular risk factors, [9, 10, 12, 14, 17, 56, 57] use of vitamin supplements, [58-60] and use of nonsteroidal anti-inflammatory agents [61-63]. However, there are currently no known effective interventions targeting these potentially modifiable factors to either prevent the development of MCI, postpone the conversion of MCI to AD, [64, 65] or slow the progression of established disease [66]. The increasing prevalence of AD, lack of effective treatments, and increasing identification of potentially modifiable risk factors emphasize the critical need for systematic research targeting preclinical diagnosis and identification of early intervention and prevention strategies.

B. 6. Modifiable and Non-modifiable Risk Factors for AD.
Identification of modifiable risk factors for AD is critical to developing effective prevention strategies for the disease. Although some risk factors for AD cannot be changed, the mechanisms through which these risk factors contribute to the pathobiology of AD could be altered. Studies of preclinical AD have focused on persons with ApoE ε4, which has been linked to metabolic, structural and cognitive changes that are suggestive of AD in asymptomatic persons. Clinically this translates into an earlier age of AD onset [67] and increased conversion of Mild Cognitive Impairment (MCI) to AD in ApoE ε4 carriers [68-70]. ApoE ε4 is also associated with increased incidence of cardiovascular disease,[71] central nervous system (CNS) lipid dysregulation, [72, 73] and impaired resting CBF in cognitively healthy young adults [56]. The association of ApoE ε4 with vascular disease and the rapidly converging data supporting the association of vascular risk factors [9, 71, 74-76] with AD pathology supports the hypothesis that some of the detrimental effects of ApoE ε4 may be favorably altered by targeting modifiable vascular risk factors.

B. 7. Justification for Using Participants at Risk for AD: One approach to investigating the relative influences of modifiable vascular factors, such as hypertension, on the development of AD is to study neurobiological factors occurring in middle-aged individuals who do not have dementia, but may be at increased risk for developing the disease. One such group is the adult children of persons with AD who, unlike older siblings of persons with the disease, provide an opportunity to prospectively study the interplay between vascular factors that occur in the evolution of preclinical disease and the eventual development of symptomatic AD. Surprisingly, little is known about this cohort, and most data about family history as a risk factor for AD has

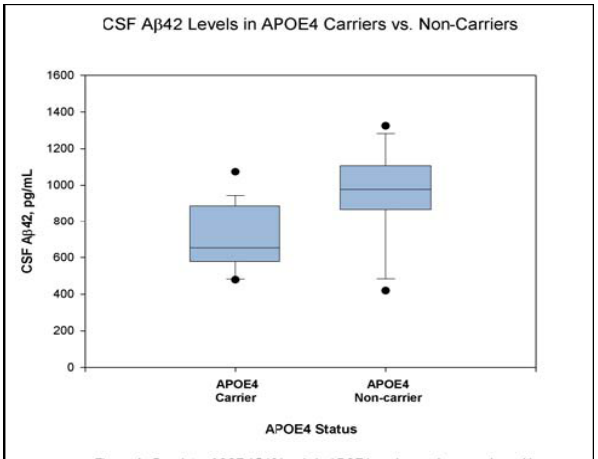


Figure 1. Boxplots of CSF Aβ42 levels in APOE4 carriers and non-carriers with median, 10th, 25th, 75th, and 90th percentiles.

been developed from mixed samples of first-degree relatives. In the REVEAL study, the calculated AD risk associated with a family history and ApoE ε4 was additive and is illustrated for Caucasian women in **Figure 1** [77]. As shown, the risk that a 55-year-old Caucasian woman will develop AD by age 80 is less than 10% for the general population, 31% for a female with a family history of AD but without ApoE ε4, and 50% if she has a positive family history and one ApoE ε4 allele. Another study[78] found that persons with a family history of dementia and ApoE ε4 had a 9-fold higher age-specific rate of developing dementia over a 4-year period compared with those without a family history and no ApoE ε4. In twin and family studies, presence of one or more ApoE ε4 in probands accounts for some, but not all, of the increased risk associated with a positive family history of AD.[79-82] Thus, the study of family members provides unique opportunities to characterize the precursors of AD, including modifiable vascular risk factors, and as of yet unidentified genetic markers.

C. Preliminary Studies

Prior research conducted by the PI and Co-Is of the proposed study has led to the development of the current project [83-85]. **Dr. Wharton (PI)** has successfully conducted research in cardiovascular risk factors, and BP in particular. She independently designed and conducted an observational study (N = 105) which reported a positive relation between blood pressure and cognition, confirming an inverted U-shaped relationship between BP and aging. Results were presented at scientific meetings and published in a high profile journal [84]. Also, Dr. Wharton currently has 2 manuscripts under review that focus on cardiovascular risk factors in the elderly. In addition, Dr. Wharton is involved in many aspects of the Kronos Early Estrogen Prevention Study (KEEPS), a randomized, double-blind, placebo controlled clinical trial examining the effects of estrogen therapy on cognition and cardiovascular endpoints. This pilot study will provide Dr. Wharton with the opportunity to combine her experience as a research investigator in cardiovascular function and her experience with randomized controlled trial (RCT), by conducting her first RCT as the principal investigator in conjunction with experienced clinical investigators from the WCMP and cardiology. **Dr. Carlsson (Co-I)** has conducted research closely related to the current proposal, including studies involving CSF collection in middle-aged adults at risk for AD, which is population of interest in the present application. Data from a recently completed study at UW showed that CSF Aβ is a factor directly related to the pathologic changes in AD brains and that lower levels have been shown to predict AD progression in older adults with memory loss [86]. These findings support the rationale for the *current proposal and stress the need to address the critical questions posed in this project.*

2014 Update: Results of this trial were published at the annual Alzheimer's Association International Conference and in the Journal of Alzheimer's Disease in 2012. Results were also used as preliminary data in a NIH K01 Career Development application which was awarded to the PI of this trial in 2014, which builds on these findings. As of December 2014 there are 4 pending grant applications that build on this research. Since the completion of this study, the PI and others have conducted similar research showing the protective effects of RAS acting antihypertensives on multiple outcomes including hippocampal volume, cerebral blood flow, brain inflammatory markers, cognitive function and perhaps most importantly, reduced disease progression from MCI to AD over a 4 year period.

C. 1. Vascular Risk Factors Are Associated with CSF Biomarkers of AD Progression in At-Risk Adults: In participants at risk for developing AD, higher BMI and total cholesterol levels in men were associated with lower CSF $A\beta_{42}$ levels, suggesting possible gender differences in the effects of vascular risk factors on the pathobiology of AD (**Figure 2**) [85]. In preliminary baseline data from the ongoing ESPRIT trial (PI: Carlsson), at risk, ApoE ε4 carriers with increased systolic blood pressureand BMI had CSF $A\beta_{40}$ and $A\beta_{42}$ levels and $A\beta_{40/42}$ ratios suggestive of preclinical disease progression. This relationship was not noted in ApoE ε4 non-carriers (data not shown). Thus, gender and ApoE ε4 may interact with vascular risk factors to influence risk for AD.

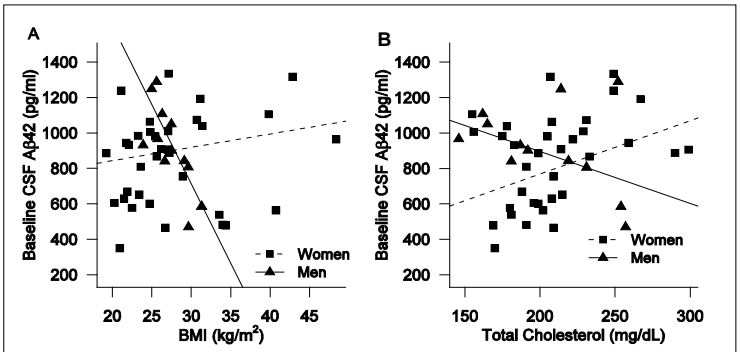


Figure 2. PAST Study. Baseline CSF Aβ42 vs. BMI (A) & total cholesterol (B) by gender. Figures display raw values (points) and predicted values for women and men (lines) using Robust-Estimation Wilcoxon-Score General Linear Modeling.

C. 2. Successful Recruitment Efforts in Studies Including Lumbar Punctures

Fifty-seven subjects enrolled in the UW IRB approved PAST Pilot and PAST studies, with 50 of those participants undergoing CSF collection. Fifty-one individuals have completed the studies (46 with CSF data). Subjects tolerated the lumbar punctures well without any unexpected adverse effects. Nine of 97 (9.3%) lumbar punctures have resulted in post-lumbar puncture (LP) headaches. We have had no other complications to date. All of these participants willingly chose to continue participating in the study and undergo a follow-up LP, which did not result in headache. Based on our experience, we are confident that the inclusion of lumbar punctures in the proposed study will have no significant effect on recruitment for the proposed trial.

C. 3. Preliminary Safety Data in Studies Including Lumbar Punctures in Healthy Adults
Both the PAST Pilot and PAST studies were approved by the UW Human Subjects Committee. All subjects provided informed consent and had intact capacity for medical decision-making (see Appendix for example consent form). As noted above, 9.3% of LPs have resulted in post-LP headaches. We have had no other complications to date. In our studies, we used atraumatic Sprotte 20-gauge spinal needles initially then changed to an even smaller needle, Sprotte 22-gauge. These specific atraumatic spinal needles have been shown in clinical trials to reduce the incidence of post-LP headache. For the proposed trial we will be using either a 22-gauge Sprotte or a 24-gauge Sprotte, depending on the body habitus of the participant. Incidence rates range from 4-6.3% for headaches after procedures using this needle type, depending on the gauge of the needle. Similarly to the current study, the protocols for the 2 studies mentioned above included 4 months of treatment with a medication (cholesterol lowering) vs. placebo in participants at risk for developing AD.

D. Research Design and Methods
This section contains 1) a summary of the experimental protocol and recruitment and 2) a summary of the experimental procedures.

D.1. Summary of Experimental Protocol and Recruitment

D. 1. 1. Participants: Twenty middle-aged, asymptomatic adult children of persons with AD will be randomized for this 4 month RCT. Inclusion/exclusion criteria are listed in **Table 1.** The majority of participants will be recruited from the Wisconsin Comprehensive Memory Program (WCMP) registry. Also, adult children of persons with AD seen in UW Assessment Clinics will be asked by the clinician about participation in this study. These methods have been successfully utilized by all co-investigators in previous clinical trials [85]. Participants may also be recruited via newsletters and information provided at educational seminars.

Given the extensive resources of the WCMP, including trained study personnel and a full-time subject recruiter, we anticipate that the study can start very promptly. We should be able to enroll 2-3 subjects per month. In an unrelated study, Dr. Carlsson (Co-I) was able to recruit 1-2 subjects per week. Enrolling subjects at our proposed rate of 2 to 3 per month will allow us to finish recruitment in 1 year and complete data collection by the middle of year 2. This will leave 6 months for final data analyses, preparation, and publication of manuscripts.

TABLE 1. INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA:
Age 40 - 70 years
Mean Resting Blood Pressure between 130 - 160 systolic and 70 - 100 diastolic
Parent with Alzheimer's disease
EXCLUSION CRITERIA:
Current invlovement in another investigational drug trial
Current invlovement in WRAP
Potassium > 5.0
Dementia based on DSMIV criteria
MMSE < 27
Current blood pressure medication (< 4 months from screening)
Weight loss medication
Contraindications for LP
Known diagnosis or history of hospitalization for congestive heart failure
Elevated creatinine (females > 1.3 mg/dL or males > 1.4 mg/dL at baseline)
Diabetes Type I and II
Known adverse reaction to an ACE-I or an angiotensin receptor blocker
Pregnant or nursing women

D. 1. 2. Inclusion/Exclusion Criteria

These are summarized in **Table 1.** Designated creatinine levels are based on the assumption that almost all participants will be Caucasian and will be between the ages of 40 and 70.

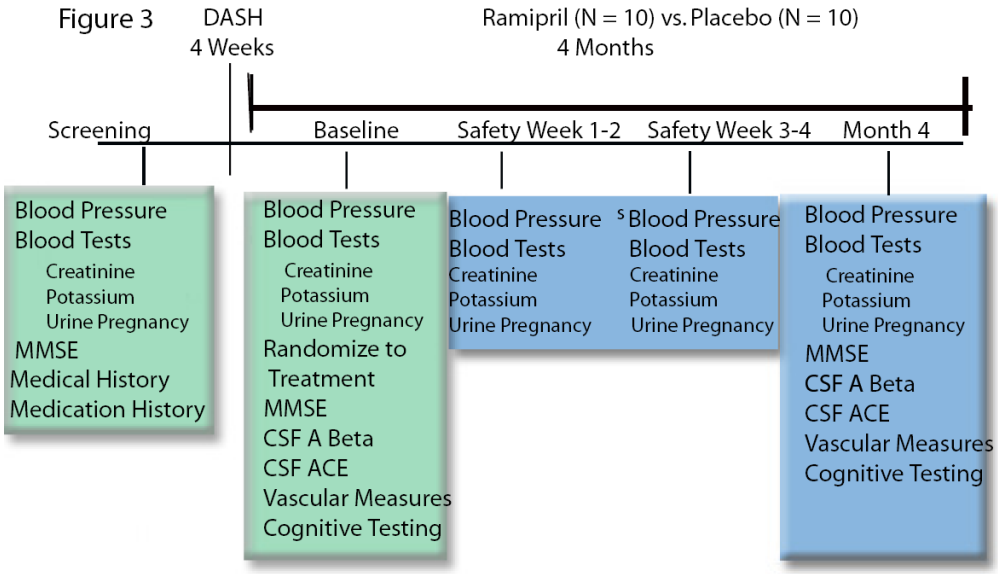
D. 1. 3. Study Procedures

Individuals will attend a total of 5 visits over the course of the 5 month study (**Figure 3**). Twenty (20) individuals will be randomized to treatment for 4 months. At the initial screening visit, participants will complete a short questionnaire about their medical history, medication history, have safety labs assessed and will have their blood pressure measured. Also, individuals will be given information on a self-paced, standardized wellness program including exercise material and a nutrition guide, called the Dietary Approaches to Stop Hypertension (DASH) [87] to be completed over the next 4 weeks +/- 5 days to adjust for the participant's schedule. The DASH is recommended in national hypertension guidelines [87] and information regarding exercise and fitness is based on guidelines from the National Institute on Aging. The purpose of the wellness program is to educate participants on ways to improve their health, specifically BP, in ways that do not involve medication. After the 4 week (+/- 5 days) wellness program, BP will be taken to assess the success of the wellness program. Participants should be seated 5 minutes

with their legs uncrossed before BP measures are taken. A total of 3 readings will be taken. The average of these 3 readings will designate the mean, which will be used to assess study inclusion.

Participants whose BP average remains in the targeted range (mean systolic 130 – 160 mmHg, and mean diastolic 70 - 100 mmHg) will be randomized to one of the two treatment arms (ramipril vs. placebo). Individuals whose BP is lowered as a result of the wellness program will not be included in the study (i.e. randomized). For subjects who are randomized, this visit will serve as their Baseline visit. The 20 subjects will be randomized in a 1:1 ratio by the UW Pharmaceutical Research Center (PRC) to receive an initial ramipril dose of 5mg to be taken once daily (N=10) vs. matching placebo (N=10).

Figure 3



At baseline and the 4 month follow-up visit, participants will have fasting CSF collected, complete tests of vascular reactivity **(See Vascular Endpoints below)**, provide an update on medical problems and medications, review any potential side effects and complete a 1 hour cognitive battery. A fasting blood draw will occur at the baseline visit to assess lipid profile, a potential factor in CSF a-beta levels. While cognitive task performance is not a primary outcome variable, we include this measure because cognition has been linked to BP and antihypertensive medication.

There will be 2 safety visits between the Baseline and Month 4 visits. The safety visits will be at 1) week 1-2 and 2) week 3-4 after the Baseline visit. During the 2 safety visits, participants will have safety labs (creatinine, potassium and urine pregnancy), have blood pressure taken, report any changes in medications or health as well as review and report potential side effects.

The primary endpoint of the proposed study is to determine the effect of ramipril therapy on CSF Aβ levels in a group at risk for developing AD. The secondary endpoints are the effect of centrally acting ramipril therapy on CSF ACE levels, biomarkers of peripheral vascular reactivity as measured by brachial artery flow-mediated vasodilation (FMD) and aortic augmentation index (AAIx) and cognitive task performance. Randomization will be stratified based on sex and APOE genotype, a risk factor for AD. Subjects and investigators will be blinded to the identity of the medication and BP readings obtained throughout the study. Dr. Carlsson will call in ApoE results to the PRC for randomization in order to maintain blinding. Compliance will be assessed by pill count at each of the follow-up visits. The study coordinator will review the possible side effects related to ramipril at each visit, including mild cough, hyperkalemia and changes in kidney function.

All outcomes outlined in the initial protocol, including ApoE testing, have been completed. However, the PI and her collaborators will continue to store all samples and tissue at the UW Madison for future testing.

D. 2. Summary of the Experimental Procedures

D. 2. 1. CSF Collection

The current study will use state-of-the-art collection techniques that are consistent with the "*NIA Biospecimens Best Practice Guidelines for the Alzheimer's Disease Centers*" [88]. Dr. Carlsson will perform lumbar punctures on all subjects that do not have contraindications to lumbar punctures (LPs). A neurologist and anesthesiologist will serve as back up for lumbar puncture procedures and LPs will be performed in the fasting state [89]. Subjects will be placed in the sitting position and the skin over L4-L5 will be prepped and draped in a sterile manner. A 25-gauge needle will be used to inject 1% lidocaine as a local anesthetic. A Sprotte 22-gauge spinal needle with introducer will be inserted into the L4-L5 interspace using sterile technique. Between 15-20 ml of CSF will be collected, and divided into four aliquots using four sterile, polypropylene tubes [90] from the UW Laboratory for both collection and storage. CSF samples will be transported to the CTRC processing area immediately after collection and distributed into 0.5 ml aliquots and the fourth CSF sample will be frozen for batched analysis in the Wisconsin Comprehensive Memory Program lab of Drs. Craig Atwood. Cynthia Carlsson, MD, MS, oversees the CSF Bank and has extensive experience in performing LPs. Over the past 5 years, Dr. Carlsson has performed over 250 LPs for research purposes for her own studies as well as the ADNI and ADCS DHA studies. For those participants consenting to storage of future samples, the third tube of CSF will be divided into 0.5 ml aliquots and stored in a -80º C freezer in the Madison VA GRECC for up to 10 years for future testing. Freezers are equipped with backup power and a telephone alarm system.

D. 2. 2. Vascular Endpoints

The primary vascular outcomes are FMD and AAIx. Dr. Stein (Co-Investigator and Director of the UW Atherosclerotic Imaging Research Program) will oversee collection and analyses of vascular endpoints. Endpoints consist of AAIx and brachial artery FMD. FMD is calculated as the ratio of brachial artery diameter after reactive hyperemia to baseline diameter, expressed as percentage change. Intraobserver reliability for measurement of the BA diameter is 0.987, reflecting an interclass correlation coefficient across all readings and conditions [91].

D. 2. 3. Laboratory Evaluation

Blood, urine and CSF samples will be collected after a 12-hour overnight fast at baseline and month 4 visits. Lipid profile from a fasting blood draw will be done at the baseline visit only. Potassium and creatinine levels will be measured from non-fasting blood draws done at screening and safety visits using enzymatic precipitation techniques on a Hitachi 747 analyzer with standard reagents. These studies will be performed in the UW Hospital Laboratory. Samples will be stored indefinably and may be used for ongoing Alzheimer's Disease related research. IRB approval will be obtained for any future use of data or samples not explicitly described in this protocol.

D. 2. 4. Cognitive Testing

Following blood and CSF collection, all subjects will be served a light breakfast. At the Baseline and Month 4 visits, cognitive tests will be administered by a trained technician according to protocols established for each test. The cognitive battery is expected to take 1½ -2 hours to

complete. To reduce practice effects, alternate forms of the above tests have been identified and validated to assure comparability. To control for variations in instrumentation, technicians will be trained to use standardized instructions for each test administered and the same technician will test a particular subject at each visit. In addition, change-scores will be determined for cognitive data obtained at baseline and month 4 visits. The change-scores of the ramipril group will be compared to the change scores of the placebo group. In this way, we will control for practice effects, and maximize internal validity (95). Details of each cognitive test are included in this application.

D. 3. Descriptions of Rationales for Each Specific Aim

D. 3. 1. Specific Aim 1: Effects of Ramipril on CSF Aβ40 and Aβ42

D. 3. 1. 1. Rationale for Evaluation of CSF Aβ42 Levels: Human genetic data have associated ACE with AD, and purified ACE has been reported to cleave synthetic Aβ in vitro. Also, some studies have found a salutary relationship between CSF Aβ42 levels and antihypertensive medications, though the exact mechanism is unclear. One study published recently found that low serum potassium levels caused my BP medications, were associated with low levels of Aβ42 in cerebrospinal fluid 24 years later in women aged 46 to 60 years of age. The study was published in the March 13 issue of *Archives of Neurology.* With AD progression, CSF Aβ levels drop as amyloid is deposited into the brain, emphasizing the importance of targeting individuals at risk for AD before clinical symptoms develop. The proposed randomized, placebo-controlled, pilot clinical trial will evaluate the effects of 4 months of ramipril therapy on CSF Aβ42 levels in cognitively healthy, middle-aged adult children of persons with AD.

D. 3. 1. 2. Using Cerebrospinal Fluid to Predict the Risk of AD: CSF biomarkers such as tau, Aβ40, and Aβ42 are emerging as promising research tools to identify those at high risk of developing AD [8, 92, 93]. CSF Aβ40 and Aβ42 levels have been shown to be reliable predictors of AD and risk of AD [8, 93]. CSF Aβ42 levels detect cognitive decline earlier than Aβ40 levels or tau protein, and, therefore, may be the most important CSF biomarker currently available in AD prevention research [94]. Aβ40, however, has more potent cerebrovascular effects and is the predominant form of Aβ in the cerebral blood vessels [19]. Thus, monitoring Aβ40 levels may provide important information on the mechanism through which CBF is altered in those at risk for AD. Some studies have shown that both CSF Aβ42 and tau levels, when used together, provide the greatest sensitivity in predicting persons at risk for cognitive decline [86]. Thus, although the primary outcome of the trial will be to monitor the effects of ramipril on CSF Aβ42 levels, Aβ40 and tau levels also will be evaluated.

Unfortunately, the *plasma* levels of Aβ40 and Aβ42 are unreliable due to low Aβ plasma concentrations, binding of Aβ to carrier proteins, and cross-reactivity between Aβ with several plasma proteins [95-101]. Some investigators have shown that plasma levels of Aβ42 are similar between AD patients and controls [101]. Others have noted an increase in plasma Aβ42 concentrations in 10 to 20% of sporadic AD patients [102, 103]. Because of the inaccuracy of plasma measures at this time, the proposed protocol will measure CSF Aβ levels. Since these CSF biomarkers have been shown to be stable when stored at -70°C for up to 15 years, extra tubes of CSF will be collected in those participants who agree to future testing of samples [8].

D. 3. 2. Specific Aim 2: Rationale for Evaluation of Ramipril on CSF Markers of Angiotensin Converting Enzyme (ACE)
Overall, there is evidence that certain components of the renin-angiotensin system (RAS), the system that regulates ACE levels, may have a crucial role in learning and memory processes. Research has shown that there is a relationship between CSF ACE levels and AD. For instance, ACE gene variants are linked to AD risk in several populations. Specifically, studies have reported elevated levels of angiotensin-converting enzyme (ACE) in the hippocampus, parahippocampal gyrus, frontal cortex, and caudate nucleus of AD patients. The increased ACE activity may be directly responsible for cognitive impairment in AD because the enhanced formation of angiotensin II would result in an increased inhibitory effect of angiotensin II on acetylcholine release. Furthermore, Levels of CSF ACE reduced in Alzheimer's disease, Parkinson's disease, and supranuclear palsy [104].

D. 3. 3. Specific Aim 3: Rationale for Evaluation of Ramipril on Vascular Function
One objective of this trial is to examine the effects of the ACE-I, ramipril peripheral endothelial function as measured by brachial artery flow-mediated vasodilation (FMD) and aortic augmentation index (AAIx). The effect of ACE inhibitors on large and small artery endothelial function has been assessed in clinical trials in patients with essential hypertension. It is well established that ACE-I reduce blood pressure and improve FMD. For instance, one study reported that FMD corrected for resting diameter (FMDcorr) was lower in patients taking ACE-I (3.0±0.2%) compared with participants treated with beta-blockers (4.2±0.3%, $p<0.01$) [105].

D. 3. 4. Rationale for Cognitive Testing A three-year study of 3,217 elderly residents of Cache County, Utah, initially found that the use of some antihypertensive medications at baseline was associated with lower incidence of AD. More recently, ACE-I have been shown to influence cognitive task performance in clinical trials specifically targeting AD related memory problems. Of note, the current proposed study will incorporate the same cognitive outcome measure (the MMSE) that has been shown to be sensitive to ACE-I treatment. Two clinical trials, Syst-Eur and PROGRESS both reported improved cognitive test scores in participants with mild to moderate AD (controlled for vascular dementia) as a result of treatment with ACE-I.

Furthermore, Sink et al. examined the influence of ACE-I that do and no cross the blood brain barrier (BBB). The authors concluded that BBB crossing ACE-I participants in the BBB crossing group had a significantly lower incidence of developing AD as well as significantly reduced MMSE decline in AD patients compared to CCb and ACE inhibitors. This factor is of particular relevance because the ACE-I in the current proposal, ramipril, is an ACE-I that crosses the BBB. These results were presented at the AGS 2007 Annual Scientific Meeting: Abstract P36. Presented May 5, 2007.

D. 4. Data Analysis and Statistical Considerations The study objective is to evaluate the effects of 4 months of ramipril therapy vs. placebo on mean changes in CSF Aβ, ACE and mean changes in measures of peripheral endothelial function. While we are not aware of any research examining the effect of ACE-Is on CSF Aβ, cognitive improvements due to ramipril have been reported in as few as 5 days [29]. In reference to reductions in plasma ACE, ramipril is one of the most potent ACE-Is available and has a trough-to-peak ratio around 50% [106, 107]. When ACE-Is are injected intracerebroventricularly, Aβ reduction can occur in as few as 2 hours. A sample size of 10 subjects per arm should have greater than 80% power to detect an 8% change in CSF Aβ, the primary outcome, and greater than 90% power to detect significant

change in endothelial variables. Based on the sample size estimates, 20 subjects will be recruited for the 4-month clinical trial.

Clinical outcome parameters will be described by means and standard deviations at each measurement time point. Regression models will be used to evaluate predictors of change in CSF variables and endothelial outcomes. Changes in clinical outcome parameters (baseline, month 4) will be analyzed using repeated-measures analysis of variance (ANOVA) to assess the effects of ramipril vs. placebo, using covariates as applicable (ANCOVA). All tests will be two-tailed tests using a significance level of 0.05.

All data for the SEAIRA trial has now been collected at the UW Madison. Data collected as a part of this study will be shared with Dr. Whitney Wharton, the prior PI of the SEAIRA study, now Assistant Professor at Emory University. While at Emory University, Dr. Wharton plans to continue analyses on data collected during her time at the University of Wisconsin. We propose that this study now become a two-site study with Dr. Wharton's mentor and Co-I, Dr. Cindy Carlsson as the site PI for the University of Wisconsin. All physical data, data specimens including stored CSF and blood will remain at the UW. The study should however remain open so Dr. Wharton may continue data analyses for this study. Dr. Wharton will conduct the analyses at Emory with coded data. The UW ADRC research team will be the only institution with access to this code.

While SEAIRA is now complete, additional analyses on data from this trial need to be conducted. To accomplish this goal, data must be transferred to Emory University from the UW. Data will be sent electronically via email from the ADRC research team at UW Madison to Dr. Whitney Wharton at Emory University. The transfer will be overseen by new UW PI of the project – Dr. Cynthia Carlsson. All transmitted data will be coded. The code used to label data will be separate from the previously used SEAIRA trial ID number, so that Dr. Wharton will not know or have access to the key linking identities with the data. Data to be transferred consists of neuropsychological testing data, ApoE4 genotype, abeta and ACE levels and activity in CSF, family history status, race, gender, age, self-reported income category, handedness, education level, drug randomization assignment, symptom reports during the trial, lab data including HDL, LDL, cholesterol levels, potassium, creatine, CSF cell count, smoking history, exercise history, medication and medical history information and data confirming study medication compliance.

Data will be transferred immediately upon IRB approval. A member of the UW ADRC research team will transfer the coded data via email to Dr. Whitney Wharton at Emory. A data transfer will occur once IRB approval has been granted at both the UW and Emory University. This transfer will include an Excel and SPSS spreadsheet that was created by Dr. Wharton and contains all participant data collected thus far and is detailed above. All data to be transferred to Emory University will be coded and UW Madison will retain the key linking subject identities with the code numbers. Dr. Wharton will not have access to this key.

At Emory University, Dr. Wharton will store the data on her personal work computer. This computer is password protected, and the data saved on it is saved to a secure server. Dr. Wharton's computer is locked in her office when she is not there.

The University of Wisconsin will serve as the lead site/coordinating center for the study as all physical data, data specimens including stored CSF and blood will remain at the University of Wisconsin; however further data analysis will be conducted at both the UW and Emory University. Dr. Wharton will be responsible for receiving and analyzing data and developing and updating the study protocol as needed. The University of Wisconsin will communicate with and

disseminate information to Dr. Wharton at Emory as needed to discuss anything related to the physical data, W-ADRC or IRB requirements; however Emory will be responsible for communicating to the University of Wisconsin regarding data analysis progress, protocol updates or other changes to the study. Any unanticipated events or new information about the study will be disseminated to the University of Wisconsin and Emory University immediately.

The UW HS IRB will remain the IRB of record; however Emory University will provide approval of their involvement with the study (data analysis by Dr. Wharton only).

E. Human Subjects Research The University of Wisconsin has a humans subjects research Multiple Projects Assurance on file with the Office for Protection from Research Risks (M-1285). Furthermore, all key personnel have completed an educational program entitled "Human Subjects Protection Tutorial at the University of Wisconsin-Madison," adapted from the NIH Computer Based Training on Protection of Human Subjects. (A copy of the UW Human Subjects Training Certification Letter is included in the application.) In addition, all key personnel have completed appropriate training for the Health Information Portability and Accountability Act (HIPAA). Dr. Wharton, now at Emory University, has completed both Human Subjects and HIPAA training required by Emory University. Emory IRB reviewed the current project and determined that no IRB Review will be required at Emory.

E. 1. Involvement of Human Subjects. Twenty middle-aged, asymptomatic adult children of persons with AD will be randomized for this 4 month RCT. Subjects will be between the ages of 40 to 70 years. Special classes of subjects such as children, pregnant women, prisoners, and institutionalized individuals will not be enrolled. Children are specifically excluded because the goal of the study is to evaluate the effects of antihyperteisives in adult children of patients with AD. In order for young children to participate, one of their parents must have AD, meaning the parent would most likely be fairly young and have early-onset AD, which is clinically different from late-onset AD. **Young children** are not included in our study. **Pregnant women** are excluded because of regulations barring them from this type of research (*i.e.* the effects of ramipril are designated as pregnancy category 'D' by the Food and Drug Administration (FDA). This means that there have been studies in pregnant women that show the drug was associated with some risk for the fetus. Specifically, the use of ACE inhibitors during the second and third trimesters of pregnancy has been associated with fetal and neonatal injury including hypotension, neonatal skull hypoplasia, anuria, reversible or irreversible renal failure, and death. Current Wisconsin Registry for Alzheimer's Prevention (WRAP) participants are also excluded because the neuropyschological test battery may interfere with the primary endpoints of the WRAP study. Other exclusion criteria for study participants are listed in Section D.1. and Table 1. Subjects will complete health-related questionnaires, have fasting blood tests, lumbar punctures, and cognitive testing according to the study protocol summarized in Figure 3. All data for this trial has been collected and no further data collection will take place. In addition to data being coded with unique identifiers not known to Dr. Wharton, data is also now coded so as to not show identifiers such as DOB and visit date. These dates are now coded to reflect birth and visit month and year, but not the specific date of either.

E. 2. Sources of Research Material. CSF will be collected from subjects in this study for testing related to CSF Aβ and ACE levels. Blood samples will also be collected to monitor medication side effects and laboratory safety values. These specimens will be obtained specifically for research purposes and to ensure the safety of participants.

E. 3. Recruitment and consent procedures. Detailed recruitment procedures are described in Section D.1. If registrants are interested in participating, a research staff will meet with them to review the inclusion and exclusion criteria, risk, benefits, and consent form with them. Subjects will be given opportunity to review the risks and benefits of participating in the study and have their questions answered. A copy of both of the consent forms will be given to each subject.

All data collection for this study is now complete and no further participants will be recruited or enrolled. The data is being used in a manner to which the participants previously provided written consent and the original PI of the study is the individual requesting the data for purposes of data analysis and publication.

E. 4. Potential risks.

E. 4. 1. Cognitive testing: This is well tolerated by patients. Participants could become tired or frustrated by the difficulty of the cognitive tasks. However, the cognitive testing will last only approximately 1.5 hours, which is a relatively short duration in comparison to similar IRB approved protocols.

E. 4. 2. Neuroimaging:

E. 4. 3. Lumbar punctures: The most common complication of lumbar puncture is post-dural puncture headache. The rate of headache occurrence can be reduced to as low as 1.7% with use of an atraumatic 21-gauge Sprotte spinal needle [108] with a very small gauge (25 gauge with introducer), and using a gentle extraction technique now widely used in AD research. Bacterial meningitis is a very rare complication of lumbar puncture, occurring in fewer than 0.2% in one series [109]. Uncal or tonsillar herniation is a very uncommon complication, occurring in <1% of very high-risk subjects with known primary or metastatic neoplasms who underwent a lumbar puncture [110]. Using a smaller (24-gauge) Sprotte needle in this trial, we anticipate a reduction in post-LP headache rates to 5% (from our current rate of 9.3%). Only one of our previous subjects required a blood patch for the headache. Serious post-LP complications such as adhesive arachnoiditis are extremely rare, with estimates close to 0.4-0.6 per 10,000 procedures [111]. Therefore, we do not anticipate such significant complications during the course of this study. Dr. Carlsson will perform a neurological and fundoscopic exam on each subject prior to lumbar puncture and those participants thought to have papilledema or gross neurologic deficits consistent with a central nervous system mass will not have a lumbar puncture performed. Each subject will be called the day following his or her lumbar puncture to ask about any side effects. All subjects will be given a telephone number to reach a physician 24-hours per day.

E. 4. 4. Social: The social risk is a breach of confidentiality regarding risk for development of AD. Subject names and research data will be handled with utmost confidentiality and discretion. Subjects will be assigned a unique identification number that can be traced only by the research coordinator and Principal Investigator. All subject information will be kept in locked drawers, file cabinets, or secure computer files, with access only allowed to research personnel.

E. 4. 5. Ethical, Psychological, Legal, Other. Although participants will be given information on their plasma lipoprotein subclassification at the conclusion of their participation, they will not be given information about their APOE4 status, CSF or plasma biomarkers. This will be clearly

stated in the informed consent process. Although APOE4 allele is associated with a higher risk of developing AD, not all people with APOE4 develop AD and not all AD patients are APOE4 allele carriers. Also, there are no clear standards in cognitively healthy adults for identifying higher risk individuals using CSF or plasma biomarkers. Furthermore, although these markers *may* predict who is at higher risk of developing AD, they are still in the research stages and do not have proven roles in clinical practice. In addition, since there are no established effective preventive therapies for AD, providing individuals with individual risk data may only add to their psychological burden and not provide them any benefit. If, during the course of the study, a new medication is approved by the FDA for AD prevention in high-risk individuals, then current policies will be revised to ensure good clinical care for study participants. Insurability of participants may be affected if there is any breach of confidentiality, which we do not anticipate.

E. 5. Protection of Subjects. As stated above, the physical, social, and psychological risks associated with this study are small. Subjects will not be allowed to participate if their physician does not feel that it would be safe or prudent for them to do so. Subjects will be monitored for 30 minutes after lumbar puncture and will be called the next day after the procedure to inquire about adverse effects. Confidentiality will be assured by assigning a unique subject number to each subject. All subject information will be kept in locked drawers, file cabinets, or secure computer files, with access only allowed to research personnel. Data from this study may be shared with researchers of an associated protocol involving exercise and Alzheimer's disease risk in the event that the subject consents to participate in the associated exercise protocol. All data and samples will be housed at UW Madison.

E. 6. Risks and expected benefits for subjects and society. As above, the risks associated with this study are small. Although individual participants may not derive clinically apparent cognitive benefits from ramipril during the course of the study, they may benefit from future research that builds on these data should ACE-I eventually be proven to have a role in AD prevention. Subjects will receive free fasting lipid profiles as part of this study. Subjects will receive honoraria to reimburse them for their time and effort.

The benefits to society may be considerable. Society, specifically individuals at risk for AD, will benefit by learning if the markers indicating risk for cognitive decline can be modified by ACE-I therapy. This knowledge could affect decisions regarding initiation of ACE-I therapy for prevention of AD. Since the completion of this study, the PI and others have conducted similar research showing the protective effects of RAS acting antihypertensives on multiple outcomes including hippocampal volume, cerebral blood flow, brain inflammatory markers, cognitive function and perhaps most importantly, reduced disease progression from MCI to AD over a 4 year period.

E. 7. Inclusion Report

E. 7. 1. Inclusion of Women. All participants will be invited through clinic visits, recruitment registry or individual flyers to participate in this study. Since approximately 70% of AD cases are women, we expect that percentage to be reflected in our study population and we will emphasize the importance of AD prevention research in the context of women's health to those invited to participate.

E. 7. 2. Pregnancy and Contraception: For women of child bearing potential, it is important to note that ACE inhibitors are labeled with a pregnancy 'category D' by the Food and Drug Administration (FDA). This means that there have been studies in pregnant women that show the drug was associated with some risk for the fetus during the 2nd and 3rd trimesters. Because of this, premenopausal females who have not had a hysterectomy must agree to use at least one form of contraception during the study, in addition to a male partner who correctly uses a condom or is sterile. The medically accepted methods of birth control to use while enrolled in the study are:

- Oral contraceptives (either combined or progesterone only)
- Intrauterine device (IUD)
- Transdermal contraceptive patch
- Injectable progesterone
- Implants of levonorgestrel
- Double barrier method: condom + cervical cap or diaphragm with spermicidal agent

<http://www.fda.gov/CDER/drug/advisory/ACEI.htm>

E. 7. 3. <u>Inclusion of Minorities</u>: Since enrollment in this study requires subjects to come to UW CTRC for testing, the registrant population is primarily from Dane County. According to the 2000 Census, Dane County's total minority population between the ages of 45 to 70 years was 3% of the population (see Table 2). Based on this statistic, we will attempt to enroll 1-2 minorities.

Table 2. Dane County, Wisconsin, 2000 Census Data

	Number	**%**
Race/Ethnicity	**Ages 45-64**	**Ages 45-64**
White	85,244	93.7%
Black	2,187	2.4%
Hispanic	1,291	1.4%
Asian/Pacific Islander	1,522	1.7%
American Indian, Eskimo, or Aleut	217	0.2%
Other race	560	0.6%

E. 8. Data Safety and Monitoring

All participants will provide informed consent and the study will be overseen by a Data Safety and Monitoring Board (DSMB) consisting of a physician and statistician who are not directly involved in the study. Dr. Amy Kind is a physician who has served on DSMBs and has worked with the PI and Co-Is on previous projects. Similarly, Jodi Barnet MS, is a biostatistician who has also worked with the PI and Co-Is and has also been directly involved in clinical trials investigating the effects of statins on CSF Aβ and cognitive outcome variables. Additionally, Dr. Carlsson (Co-I) will serve on the DSMB and will be primary physician involved in this study. All side effects will be closely monitored. The most common side effects of ACE-I are mild cough, hyperkalemia or changes in kidney function. The most common side effect of lumbar puncture (LP) is headache, which is usually self-limited. At UW, the rate of headaches following LP is less than 5%. Dr. Carlsson will perform lumbar punctures for the proposed trial and has significant experience with this procedure, having performed over 260 lumbar punctures in the last five years with no complications. Participants will receive a phone call 24 hours after the LP to inquire about any post-procedure adverse effects. According to the Joint National Committee on Prevention (JNCP) VII guidelines, a dietary intervention for mild BP elevation for 4 months is

within the standard of clinical care [112]. All participants will receive wellness program information, which includes the DASH lifestyle intervention. Compliance will be measured via self report. Participants with contraindications to ACE-I therapy or LP will be excluded from participation. All subjects will be monitored for high blood pressure (systolic > 160 or diastolic > 100) and other possible side effects at each visit. At each visit, participants will be screened with blood pressure readings, blood tests, possible pregnancy tests and questionnaires to inquire about potential side effects. Participants with elevated potassium or creatinine will be advised to stop treatment and will have follow up labs until the abnormalities resolve. Should a participant's BP rise to unsafe levels (systolic > 160 or diastolic > 100) the participant will be advised to visit his/her primary care physician and the DSMB will be notified immediately. CSF collection will be completed according to guidelines put forth in the "*NIA Biospecimens Best Practice Guidelines for the Alzheimer's Disease Centers*." Once enrollment has ended, the DSMB will no longer meet; however will be available on an as-needed basis.

References

1. McKhann, G., et al., *Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease.* Neurology, 1984. **34**(7): p. 939-44.
2. Birns, J., et al., *The effects of blood pressure reduction on cognitive function: a review of effects based on pooled data from clinical trials.* J Hypertens, 2006. **24**(10): p. 1907-14.
3. Staessen, J.A., et al., *Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators.* Lancet, 1997. **350**(9080): p. 757-64.
4. Eckman, E.A., et al., *Regulation of steady-state beta-amyloid levels in the brain by neprilysin and endothelin-converting enzyme but not angiotensin-converting enzyme.* J Biol Chem, 2006. **281**(41): p. 30471-8.
5. Hebert, L.E., et al., *Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050.* Alzheimer Dis Assoc Disord, 2001. **15**(4): p. 169-73.
6. Iadecola, C., et al., *SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein.* Nat Neurosci, 1999. **2**(2): p. 157-61.
7. Korf, E.S., et al., *Midlife blood pressure and the risk of hippocampal atrophy: the Honolulu Asia Aging Study.* Hypertension, 2004. **44**(1): p. 29-34.
8. Sunderland, T., et al., *Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease.* Jama, 2003. **289**(16): p. 2094-103.
9. Kivipelto, M., et al., *Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease.* Ann Intern Med, 2002. **137**(3): p. 149-55.
10. Seshadri, S., et al., *Plasma homocysteine as a risk factor for dementia and Alzheimer's disease.* N Engl J Med, 2002. **346**(7): p. 476-83.
11. Luchsinger, J.A., *Adiposity, hyperinsulinemia, diabetes and Alzheimer's disease An epidemiological perspective.* Eur J Pharmacol, 2008.
12. Luchsinger, J.A., et al., *Hyperinsulinemia and risk of Alzheimer disease.* Neurology, 2004. **63**(7): p. 1187-92.
13. Luchsinger, J.A., et al., *Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort.* Am J Epidemiol, 2001. **154**(7): p. 635-41.
14. Gustafson, D., et al., *An 18-year follow-up of overweight and risk of Alzheimer disease.* Arch Intern Med, 2003. **163**(13): p. 1524-8.
15. Whitmer, R.A., et al., *Central obesity and increased risk of dementia more than three decades later.* Neurology, 2008.

16. Yaffe, K., et al., *A prospective study of physical activity and cognitive decline in elderly women: women who walk.* Arch Intern Med, 2001. **161**(14): p. 1703-8.
17. Schmidt, R., et al., *Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study.* Ann Neurol, 2002. **52**(2): p. 168-74.
18. Lakatta, E.G. and D. Levy, *Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease.* Circulation, 2003. **107**(1): p. 139-46.
19. Iadecola, C., *Neurovascular regulation in the normal brain and in Alzheimer's disease.* Nat Rev Neurosci, 2004. **5**(5): p. 347-60.
20. Refolo, L.M., et al., *Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model.* Neurobiol Dis, 2000. **7**(4): p. 321-31.
21. Sparks, D.L., et al., *Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol.* Exp Neurol, 1994. **126**(1): p. 88-94.
22. Blair, C.K., et al., *APOE genotype and cognitive decline in a middle-aged cohort.* Neurology, 2005. **64**(2): p. 268-76.
23. Peila, R., et al., *Reducing the risk of dementia: efficacy of long-term treatment of hypertension.* Stroke, 2006. **37**(5): p. 1165-70.
24. Wang, J., et al., *Valsartan lowers brain beta-amyloid protein levels and improves spatial learning in a mouse model of Alzheimer disease.* J Clin Invest, 2007. **117**(11): p. 3393-402.
25. Fratiglioni, L., et al., *Occurrence of dementia in advanced age: the study design of the Kungsholmen Project.* Neuroepidemiology, 1992. **11 Suppl 1**: p. 29-36.
26. Tzourio, C., et al., *Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and cognitive decline in patients with cerebrovascular disease.* Arch Intern Med, 2003. **163**(9): p. 1069-75.
27. Hirawa, N., et al., *Long-term inhibition of renin-angiotensin system sustains memory function in aged Dahl rats.* Hypertension, 1999. **34**(3): p. 496-502.
28. Khachaturian, A.S., et al., *Antihypertensive medication use and incident Alzheimer disease: the Cache County Study.* Arch Neurol, 2006. **63**(5): p. 686-92.
29. Zuccala, G., et al., *Use of angiotensin-converting enzyme inhibitors and variations in cognitive performance among patients with heart failure.* Eur Heart J, 2005. **26**(3): p. 226-33.
30. Savaskan, E., et al., *Hippocampal estrogen beta-receptor immunoreactivity is increased in Alzheimer's disease.* Brain Res, 2001. **908**(2): p. 113-9.
31. Uehara, Y., et al., *Angiotensin II subtype-1 receptor antagonists improve hemodynamic and renal changes without affecting glucose metabolisms in genetic rat model of non-insulin-dependent diabetes mellitus.* Am J Hypertens, 1999. **12**(1 Pt 1): p. 21-7.
32. Barnes, J.M., et al., *Angiotensin II inhibits the release of [3H]acetylcholine from rat entorhinal cortex in vitro.* Brain Res, 1989. **491**(1): p. 136-43.
33. Savaskan, E., *The role of the brain renin-angiotensin system in neurodegenerative disorders.* Curr Alzheimer Res, 2005. **2**(1): p. 29-35.
34. Ohrui, T., et al., *Angiotensin-converting enzyme inhibitors and incidence of Alzheimer's disease in Japan.* J Am Geriatr Soc, 2004. **52**(4): p. 649-50.
35. Ohrui, T., et al., *Effects of brain-penetrating ACE inhibitors on Alzheimer disease progression.* Neurology, 2004. **63**(7): p. 1324-5.
36. Hebert, L.E., et al., *Alzheimer disease in the US population: prevalence estimates using the 2000 census.* Arch Neurol, 2003. **60**(8): p. 1119-22.
37. Braak, H. and E. Braak, *Alzheimer's disease: striatal amyloid deposits and neurofibrillary changes.* J Neuropathol Exp Neurol, 1990. **49**(3): p. 215-24.
38. Monsch, A.U., et al., *Comparisons of verbal fluency tasks in the detection of dementia of the Alzheimer type.* Arch Neurol, 1992. **49**(12): p. 1253-8.

39. Bookheimer, S.Y., et al., *Patterns of brain activation in people at risk for Alzheimer's disease.* N Engl J Med, 2000. **343**(7): p. 450-6.
40. Reiman, E.M., et al., *Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease.* Annals of Neurology, 1998. **44**(2): p. 288-91.
41. Reiman, E.M., et al., *Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E.* N Engl J Med, 1996. **334**(12): p. 752-8.
42. Baxter, L.C., et al., *Apolipoprotein E epsilon 4 affects new learning in cognitively normal individuals at risk for Alzheimer's disease.* Neurobiol Aging, 2003. **24**(7): p. 947-52.
43. Hsiung, G.Y., A.D. Sadovnick, and H. Feldman, *Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging.* Cmaj, 2004. **171**(8): p. 863-7.
44. Small, G.W., et al., *Predictors of cognitive change in middle-aged and older adults with memory loss.* Am J Psychiatry, 1995. **152**(12): p. 1757-64.
45. Cervilla, J., et al., *Premorbid cognitive testing predicts the onset of dementia and Alzheimer's disease better than and independently of APOE genotype.* J Neurol Neurosurg Psychiatry, 2004. **75**(8): p. 1100-6.
46. Scarmeas, N., et al., *Influence of leisure activity on the incidence of Alzheimer's disease.* Neurology, 2001. **57**(12): p. 2236-42.
47. Weuve, J., et al., *Physical activity, including walking, and cognitive function in older women.* Jama, 2004. **292**(12): p. 1454-61.
48. Wilson, R.S., et al., *Cognitive activity and incident AD in a population-based sample of older persons.* Neurology, 2002. **59**(12): p. 1910-4.
49. Luchsinger, J.A., et al., *Alcohol intake and risk of dementia.* J Am Geriatr Soc, 2004. **52**(4): p. 540-6.
50. Barnes, L.L., et al., *Social resources and cognitive decline in a population of older African Americans and whites.* Neurology, 2004. **63**(12): p. 2322-6.
51. van Gelder, B.M., et al., *Physical activity in relation to cognitive decline in elderly men: the FINE Study.* Neurology, 2004. **63**(12): p. 2316-21.
52. Wilson, R.S., et al., *Participation in cognitively stimulating activities and risk of incident Alzheimer disease.* Jama, 2002. **287**(6): p. 742-8.
53. Morris, M.C., et al., *Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease.* Arch Neurol, 2003. **60**(7): p. 940-6.
54. Kalmijn, S., et al., *Metabolic cardiovascular syndrome and risk of dementia in Japanese-American elderly men. The Honolulu-Asia aging study.* Arterioscler Thromb Vasc Biol, 2000. **20**(10): p. 2255-60.
55. Larrieu, S., et al., *Nutritional factors and risk of incident dementia in the PAQUID longitudinal cohort.* J Nutr Health Aging, 2004. **8**(3): p. 150-4.
56. Scarmeas, N., et al., *APOE genotype and cerebral blood flow in healthy young individuals.* Jama, 2003. **290**(12): p. 1581-2.
57. Kivipelto, M., et al., *Hypertension and hypercholesterolaemia as risk factors for Alzheimer's disease: potential for pharmacological intervention.* CNS Drugs, 2002. **16**(7): p. 435-44.
58. Khachaturian, A.S., et al., *Apolipoprotein E epsilon4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: The Cache County Study.* Arch Gen Psychiatry, 2004. **61**(5): p. 518-24.
59. Gray, S.L., et al., *Is antioxidant use protective of cognitive function in the community-dwelling elderly?* Am J Geriatr Pharmacother, 2003. **1**(1): p. 3-10.
60. Morris, M.C., et al., *Vitamin E and cognitive decline in older persons.* Arch Neurol, 2002. **59**(7): p. 1125-32.
61. Anthony, J.C., et al., *Reduced prevalence of AD in users of NSAIDs and H2 receptor antagonists: the Cache County study.* Neurology, 2000. **54**(11): p. 2066-71.

62. Zandi, P.P., et al., *Reduced incidence of AD with NSAID but not H2 receptor antagonists: the Cache County Study.* Neurology, 2002. **59**(6): p. 880-6.
63. Hee Kang, J. and F. Grodstein, *Regular use of nonsteroidal anti-inflammatory drugs and cognitive function in aging women.* Neurology, 2003. **60**(10): p. 1591-7.
64. Petersen, R.C., et al., *Mild cognitive impairment: clinical characterization and outcome.* Arch Neurol, 1999. **56**(3): p. 303-8.
65. Petersen, R.C., et al., *Vitamin E and donepezil for the treatment of mild cognitive impairment.* N Engl J Med, 2005. **352**(23): p. 2379-88.
66. Courtney, C., et al., *Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial.* Lancet, 2004. **363**(9427): p. 2105-15.
67. Chapman, J., et al., *The effects of APOE genotype on age at onset and progression of neurodegenerative diseases.* Neurology, 2001. **57**(8): p. 1482-5.
68. de Leon, M.J., et al., *Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment.* Neurobiol Aging, 2006. **27**(3): p. 394-401.
69. Farlow, M.R., et al., *Impact of APOE in mild cognitive impairment.* Neurology, 2004. **63**(10): p. 1898-901.
70. Tilvis, R.S., T.E. Strandberg, and K. Juva, *Apolipoprotein E phenotypes, dementia and mortality in a prospective population sample.* J Am Geriatr Soc, 1998. **46**(6): p. 712-5.
71. Hofman, A., et al., *Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study.* Lancet, 1997. **349**(9046): p. 151-4.
72. Han, X., *The role of apolipoprotein E in lipid metabolism in the central nervous system.* Cell Mol Life Sci, 2004. **61**(15): p. 1896-906.
73. Puglielli, L., R.E. Tanzi, and D.M. Kovacs, *Alzheimer's disease: the cholesterol connection.* Nat Neurosci, 2003. **6**(4): p. 345-51.
74. Liu, H.C., et al., *ApoE genotype in relation to AD and cholesterol: a study of 2,326 Chinese adults.* Neurology, 1999. **53**(5): p. 962-6.
75. Hajjar, I., et al., *The impact of the use of statins on the prevalence of dementia and the progression of cognitive impairment.* J Gerontol A Biol Sci Med Sci, 2002. **57**(7): p. M414-8.
76. Launer, L.J., et al., *Midlife blood pressure and dementia: the Honolulu-Asia aging study.* Neurobiol Aging, 2000. **21**(1): p. 49-55.
77. Cupples, L.A., et al., *Estimating risk curves for first-degree relatives of patients with Alzheimer's disease: the REVEAL study.* Genet Med, 2004. **6**(4): p. 192-6.
78. Payami, H., et al., *A prospective study of cognitive health in the elderly (Oregon Brain Aging Study): effects of family history and apolipoprotein E genotype.* Am J Hum Genet, 1997. **60**(4): p. 948-56.
79. Green, R.C., et al., *Risk of dementia among white and African American relatives of patients with Alzheimer disease.* Jama, 2002. **287**(3): p. 329-36.
80. Loewenstein, D.A., et al., *The use of amnestic and nonamnestic composite measures at different thresholds in the neuropsychological diagnosis of MCI.* J Clin Exp Neuropsychol, 2007. **29**(3): p. 300-7.
81. Steffens, D.C., et al., *APOE and AD concordance in twin pairs as predictors of AD in first-degree relatives.* Neurology, 2000. **54**(3): p. 593-8.
82. Jayadev, S., et al., *Conjugal Alzheimer disease: risk in children when both parents have Alzheimer disease.* Arch Neurol, 2008. **65**(3): p. 373-8.
83. Stein, J.H., et al., *The effects of lipid-lowering and antioxidant vitamin therapies on flow-mediated vasodilation of the brachial artery in older adults with hypercholesterolemia.* J Am Coll Cardiol, 2001. **38**(7): p. 1806-13.
84. Wharton, W., et al., *Lower blood pressure correlates with poorer performance on visuospatial attention tasks in younger individuals.* Biol Psychol, 2006. **73**(3): p. 227-34.

85. Carlsson, C.M., et al., *Effects of simvastatin on cerebrospinal fluid biomarkers and cognition in middle-aged adults at risk for Alzheimer's disease.* J Alzheimers Dis, 2008. **13**(2): p. 187-97.
86. Andreasen, N., et al., *Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment.* Neurosci Lett, 1999. **273**(1): p. 5-8.
87. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.
88. *NIA Biospecimens Best Practice Guidelines for the Alzheimer's Disease Centers*. 2007 [cited 2008 April 22, 2008]; Available from: https://www.alz.washington.edu/BiospecimenTaskForce.html.
89. Watson, G.S., et al., *Insulin increases CSF Abeta42 levels in normal older adults.* Neurology, 2003. **60**(12): p. 1899-903.
90. Lewczuk, P., et al., *Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides.* Clin Chem, 2006. **52**(2): p. 332-4.
91. Carlsson, C.M., et al., *Managing dyslipidemia in older adults.* J Am Geriatr Soc, 1999. **47**(12): p. 1458-65.
92. Kanai, M., et al., *Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: a study in Japan.* Ann Neurol, 1998. **44**(1): p. 17-26.
93. Andreasen, N., et al., *Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment.* Acta Neurol Scand Suppl, 2003. **179**: p. 47-51.
94. Tapiola, T., et al., *Three-year follow-up of cerebrospinal fluid tau, beta-amyloid 42 and 40 concentrations in Alzheimer's disease.* Neurosci Lett, 2000. **280**(2): p. 119-22.
95. Pardridge, W.M., et al., *High molecular weight Alzheimer's disease amyloid peptide immunoreactivity in human serum and CSF is an immunoglobulin G.* Biochem Biophys Res Commun, 1987. **145**(1): p. 241-8.
96. Stern, R.A., J.Q. Trojanowski, and V.M. Lee, *Antibodies to the beta-amyloid peptide cross-react with conformational epitopes in human fibrinogen subunits from peripheral blood.* FEBS Lett,1990. **264**(1): p. 43-7.
97. Matsubara, E., B. Frangione, and J. Ghiso, *Characterization of apolipoprotein J-Alzheimer's A beta interaction.* J Biol Chem, 1995. **270**(13): p. 7563-7.
98. Zlokovic, B.V., *Cerebrovascular transport of Alzheimer's amyloid beta and apolipoproteins J and E: possible anti-amyloidogenic role of the blood-brain barrier.* Life Sci, 1996. **59**(18): p. 1483-97.
99. Jensen, M., et al., *Cerebrospinal fluid A beta42 is increased early in sporadic Alzheimer's disease and declines with disease progression.* Ann Neurol, 1999. **45**(4): p. 504-11.
100. Mehta, P.D., et al., *Amyloid beta protein 1-40 and 1-42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease.* Neurosci Lett, 2001. **304**(1-2): p. 102-6.
101. Mehta, P.D., et al., *Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease.* Arch Neurol, 2000. **57**(1): p. 100-5.
102. Scheuner, D., et al., *Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease.* Nat Med,1996. **2**(8): p. 864-70.
103. Tamaoka, A., et al., *Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease.* J Neurol Sci, 1996. **141**(1-2): p. 65-8.
104. Zubenko, G.S., et al., *Cerebrospinal fluid levels of angiotensin-converting enzyme in Alzheimer's disease, Parkinson's disease and progressive supranuclear palsy.* Brain Res, 1985. **328**(2): p. 215-21.

105. Buus, N.H., et al., *Large and small artery endothelial function in patients with essential hypertension--effect of ACE inhibition and beta-blockade.* Blood Press, 2007. **16**(2): p. 106-13.
106. Gosse, P., et al., *The effect of telmisartan and ramipril on early morning blood pressure surge: a pooled analysis of two randomized clinical trials.* Blood Press Monit, 2007. **12**(3): p. 141-7.
107. Vuong, A.D. and L.G. Annis, *Ramipril for the prevention and treatment of cardiovascular disease.* Ann Pharmacother, 2003. **37**(3): p. 412-9.
108. Engelhardt, A., S. Oheim, and B. Neundorfer, *Post-lumbar puncture headache: experiences with Sprotte's atraumatic needle.* Cephalalgia, 1992. **12**(4): p. 259.
109. Domingo, P., et al., *Iatrogenic streptococcal meningitis.* Clin Infect Dis, 1994. **19**(2): p. 356-7.
110. Lubic, L.G. and J.T. Marotta, *Brain tumor and lumbar puncture.* Arch Neurol Psychiatry, 1954. **72**: p. 568-572.
111. Chiapparini, L., et al., *Imaging and outcome in severe complications of lumbar epidural anaesthesia: report of 16 cases.* Neuroradiology, 2000. **42**(8): p. 564-71.
112. Chobanian, A.V., et al., *The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report.* Jama, 2003. **289**(19): p. 2560-72.