THE RELATIONSHIP OF INFLAMMATORY MARKERS TO COGNITIVE FUNCTION IN A POPULATION-BASED SAMPLE

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DEDICATION

To Rob, for seeing me through.

THE RELATIONSHIP OF INFLAMMATORY MARKERS TO COGNITIVE FUNCTION IN A POPULATION-BASED SAMPLE

By

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Looking back, I never imagined I would have completed a doctoral dissertation with the words "chemoattractant proteins" or "lipoprotein-associated phospholipase" coming even remotely near my typing fingers. But here we are, many "phospholipases" later, and I have many people to whom I owe much gratitude for helping to make this experience such a rich one.

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My parents have continually held my hand on this road of self-discovery, and I’ve had a rich and very full life because of their love and support. Their encouragement to try those things that made my heart sing, to love who I was meant to, and take risks that others thought were unwise have shaped who I am today. Thank you could never be enough. No one is a luckier son.

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THE RELATIONSHIP OF PRO-INFLAMMATORY MARKERS TO COGNITIVE FUNCTION IN A POPULATION-BASED SAMPLE

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BACKGROUND: C-reactive protein (CRP), Interleukin-18 (IL-18), Monocyte chemoattractant protein (MCP-1), and Lipoprotein-associated phospholipase (Lp-PLA_2) are pro-inflammatory blood markers that appear to play critical roles in atherosclerosis and vascular disease, and have been linked to Alzheimer's Disease, vascular dementia, and subclinical levels of cognitive decline. The present study investigated the relationship of these four inflammatory markers to cognitive function prospectively, and examined the potential impact of vascular risk factors (i.e., hypertension, hyperlipidemia, smoking status, alchol intake, diabetes mellitus, waist circumference, Cystatin C) and APOE 4 as mediators of cognitive function. METHOD AND RESULTS: Participants include 1904 individuals with CRP, IL-18, MCP-1, and Lp-PLA_2, vascular risk factor and APOE 4 data collected as part of the Dallas Heart Study I initiated in 1999, who returned to the Dallas Heart Study II (8 years later) who completed the Montreal Cognitive

Assessment (MoCA) as part of a larger clinical research protocol. A significant yet weak correlation was found for Lp-PLA_2 (r=.09, p<.01) and MoCA scores, with significant correlations only for men (r=.24, p<.01). None of the other inflammatory markers were associated with MoCA scores. An increased number of vascular risk factors was not related to lower MoCA total scores [F(5,1621)=1.56, p=.168)]. The presence of the APOE 4 allele did not impact the relationship between concentrations of blood markers and cognitive function as hypothesized. In logistic regression analysis, only the demographic variables of Caucasian race and education were significant, and decreased the odds of membership into lowest MoCA tertile group. CONCLUSIONS: Results did not support a relationship between mid-life inflammation and vascular risk factors and later cognitive function in this healthy, middle-aged sample. Demographic factors were the only consistent variables associated with cognitive performance. The minimum level of significant inflammation in this sample may have attenuated the results. Follow-up studies to examine progression of inflammation and vascular risk in relation to cognitive function will help to further examine these relationships.

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Glossary of Terms

Adipocyte: Also known as lipocytes. Fat cells.

Chemokine: Small cytokines used for chemotaxis (the attracting of specific cells to certain areas.)

Cytokine: Molecules (proteins) secreted by specific immune system cells that act in mediating communication between cells and modulating the behavior of cells.

Complement binding: The binding of an antibody to its specific antigen.

Complement cascade: Series of biochemical events that compromises the body's response to foreign cells/antigens. Classically, it is initiated by the binding of an antibody to its antigen and ends with a chemical complex that causes the rupture of the cell membrane. A complement cascade can also make a foreign cell body more susceptible to phagocytosis (the ingestion of foreign substance or cellular debris). In layman's terms, some foreign bodies get punctured which causes them to explode, while others get consumed.

Endothelial cells: Cells that line the inner surface of all vessels in the circulatory system.

HDL: High Density Lipoprotien. Allows lipids to be transported in water-based bloodstream, and thus, removed. Often called the "good cholesterol."

HIV-1 transactivator protein Tat: The HIV-1 transactivator protein Tat increases the release of MCP-1.

Interleukins: A group of cytokines (proteins/molecules used for signaling and communication in the immune system). Initially thought only to be produced by and used for signaling between white blood cells. Now known to be produced by or work on a variety of cell types involved in the immune system.

Intimal thickness: The thickness of inner layers of an arterial wall. In practice, however, it is always the intimal-medial thickness. The intima is only the endothelial cell lining. The media is the middle layer consisting of elastic and fibrous connective tissue. Thickening of the intimal-medial thickness is correlated with atherosclerosis.

LDL: Low density lipoprotein. A lipoprotein (fat + protein) molecule that transports cholesterol and triglycerides within the bloodstream, and predominantly transports cholesterol from the liver to peripheral tissues. LDL is often called the "bad cholesterol" due to its association with atherosclerosis.

Leukocytes: Most nonspecific term for all white blood cells. Leukocytes includes monocytes, macrophages, lymphocytes, neutrophils, eosinophils, and basophils.

Macrophage: The largest type of phagocyte. Other types are neturophils and monocytes.

MCP-1: A chemokine that attracts monocytes. The monocyte infiltration in the brain is thought to be a major factor in HIV related dementia.

Moiety: Part of a larger group or structure. In organic chemistry, a specific segment or region of a molecule.

Phagocytes: A cell that absorbs harmful microorganisms, waste material, or other foreign bodies. Can be a white blood cell.

Phagocytic activity: Specific types of white blood cells called phagocytes "eat" foreign cells and particles and cellular debris by engulfing them. They have multiple functions: 1. Dispose of the foreign cell or material. 2. Present parts of the foreign material (antigens) to the immune system. 3. Activate other cells in the immune/inflammatory response.

Plasminogen activator inhibitor-1: inhibits fibrinolysis (the degradation of blood clots) by inhibiting certain serine proteases (enzymes that cut the peptide bonds of proteins).

Pravastatin: A lipid-lowering drug. Pravastatin reduces the production of cholesterol. This is often marketed under name of Pravachol.

Porcine model (pigs): Experiments on pigs.

RANTES: Acronym for **R**egulated on **A**ctivation, **N**ormal **T** **E**xpressed and **S**ecreted. Also known as CCL5. A chemokine (i.e. chemotactic cytokine) that functions as a selective attractant for T lymphocytes and monocytes. It plays an important role in attracting leukocytes to areas of inflammation.

Restenosis: The reoccurrence of narrowing of a blood vessel lumen (channel or cavity in the vessel).

Single nucleotide polymorphism or SNP (pronounced "SNiP"): when a specific DNA sequence (i.e. gene) between members of a group or species differs only by one nucleotide (A,T,G,C). The gene is said to have two alleles. Example: AT**T**CGACT and AT**C**CGACT.

Transforming growth factor beta 1 (TGF-ß1): family of cytokines with many functions: cellular growth and proliferation, cellular differentiation and apoptosis (programmed cellular death). Generally (but not always) have suppressive/inhibitory functions in the immune system.

VLDL: Very Low Density Lipoprotein. Carries endogenously produced lipids (cholesterol and triglycerides) within the bloodstream. Often converted to LDL within the blood.

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
APOE 4	Apolipoprotein E 4
CXCL 1	Chemokin Ligand 1
CSCL 2	Chemokin Ligand 2
CRP	C-reactive protein
CT	Computed Tomography
CHD	Coronary Heart Disease
DM	Diabetes Mellitus
GFR	Glomerular Filtration Rate
HDL	High-Density Lipoprotein
HLD	Hyperlipidemia
HTN	Hypertension
IFNY	Interferon-Gamma Inducing Factor
IL	Interleukin
IL-6	Interleukin-6
IL-18	Interleukin-18
LDL	Low-Density Lipoprotein
Lpa	Lipoprotein a
Lp-PLA$_2$	Lipoprotein-Associated Phospholipase
LTBR	Lymphotoxin beta receptor
MMP-9	Matrix Metalloproteinase-9
MRI	Magnetic Resonance Imaging
MMSE	Mini Mental State Examination
MCP-1	Monocyte Chemoattractant Protein-1
MoCA	Montreal Cognitive Assessment
MI	Myocardial Infarction
MPO	Myeloperoxidase

PLGF	Placenta Growth Factor
PAPPA	Pregnancy-Associated Plasma Protein A
VaD	Vascular Dementia
WC	Waist Circumference

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Introduction

Cognitive impairment has become an increasingly important public health issue as the American population ages (Anderson, 2008). Severe cognitive impairment, as occurs in Alzheimer disease (AD) and vascular dementia (VaD), is the final occurrence in a chain of events initiated and aggravated by factors that operate long beforehand (Kidd, 2008). Thus, it is logical that the cognitive effects of these factors may be detectable many years before severe impairment develops, and a possible role for vascular risk factors in cognitive decline has been suggested. Inflammation has been associated with both AD and with atherosclerosis, the underlying cause of VaD and stroke (Lowe & Pepys, 2006; Ravaglia, et al., 2007). Among many others, some of the most often investigated factors include 1) C-reactive protein, 2) Interleukin-18 (IL-18), 3) Lipoprotein-associated phospholipase (LP-PLA_2, and 4) Monocyte Chemoattractant Protein (MCP-1).

Inflammatory Markers

C-Reactive Protein (CRP)

Of the known inflammatory markers, C-reactive protein (CRP) has shown the strongest relationship to atherosclerosis. CRP is a member of the class of acute-phase reactants whose levels rise sharply during inflammatory processes (Yeh & Willerson, 2003), and is also believed to have a role in early defense system against infections. The relationship between CRP and future cardiovascular events has been consistent throughout the literature, and appears to be independent of age, smoking, blood pressure, and diabetes (Ridker, 2003; Zacho, et al., 2008).

Although CRP is largely synthesized in the liver, it is also produced in brain and other organs (McGeer & McGeer, 2004). In fact, a major finding linking blood inflammatory markers to AD was the discovery that the complement cascade (i.e. series of biochemical events that compromises the body's response to foreign cells/antigens) including CRP, is fully activated in AD (McGeer, Rogers, & McGeer, 2006). Elevations in plasma CRP have also been associated with higher risk of AD and VaD ((K. Nilsson, Gustafson, & Hultberg, 2008; Ravaglia, et al., 2007; Yamamoto, et al., 2005).

Despite a relationship between CRP and cardiovascular risk, the relationship between CRP and cognitive decline is less clear. In 78 subjects with cardiovascular disease followed over one year, for example, high levels of CRP were associated with subtle declines in attention-executive-psychomotor performance ($p = .04$) after adjusting for the effects of age and baseline cognitive performance (Hoth, et al., 2008). Other work, however, that examined 4231 female participants aged 66 and over, has demonstrated that cognitive function scores did not vary in relation to CRP concentration that was measured 4-7 years earlier (Weuve, Ridker, Cook, Buring, & Grodstein, 2006). Thus, it is not well understood how CRP concentrations affect cognitive performance.

Interleukin-18 (IL-18)

Interleukins are secreted by leukocytes (both macrophages and T-cells) and endothelium to stimulate the body's immune response to trauma. This response is more pronounced after burns or other damage leading to inflammation. Elevated levels of IL-18 have been linked to risk for coronary heart disease (Blankenberg, et al., 2003; Blankenberg, et al., 2002), although this relationship has not always been consistent (Zirlik, et al., 2007), and in the majority of healthy people, IL-18 is not detectable

(Gracie, Robertson, & McInnes, 2003). Older, male, and obese individuals with elevated IL-18 were more likely to have a history of diabetes, hypertension, tobacco use, lower HDL, hypertriglyceridemia, previous myocardial infarction, and lower glomerular filtration rate (GFR) than those with subclinical IL-18 levels (Zirlik, et al., 2007).

Researchers have also examined the relationship between IL-18, AD and VaD, with higher levels of IL-18 in the dementia groups compared with healthy controls (Bossu, et al., 2007; Malaguarnera, Motta, Di Rosa, Anzaldi, & Malaguarnera, 2006). More recently, IL-18 production by peripheral blood cells was found to be increased in patients with AD, and was correlated with severity of cognitive impairment on the MMSE (Bossu, et al., 2008). Little if any research to date has investigated the relationship between IL-18 and cognitive decline.

Lipoprotein-associated phospholipase (Lp-PLA_2)

Lp-PLA_2, also known as platelet activating factor acetylhydrolase (PAF-AH), is a member of the phospholipase A_2 enzyme superfamily, and it is considered a pro-inflammatory mediator involved with vascular inflammatory processes. Lp-PLA_2 attaches to LDL cholesterol particles in the bloodstream and often adheres to arterial walls, which induces oxidation and triggers the atherogenic process. Lp-PLA_2 can promote the buildup of plaque in the arteries, and also produce molecules that attract immune cells to arterial walls. These molecules bind to monocytes, which are then converted to macrophages. This process can increase the amount of atherosclerotic buildup within arterial walls (Caslake & Packard, 2003).

There has been a consistent linking of Lp-PLA_2 levels to risk of atherosclerotic disease (Ballantyne, et al., 2004; Caslake & Packard, 2005; Packard, et al., 2000),

although this finding has not always been robust (Blake, Dada, Fox, Manson, & Ridker, 2001). Associations between elevations in Lp-PLA_2, AD and VaD have only been recently examined, and one investigation found that elevated levels of Lp-PLA_2 were associated with an increased risk of dementia (van Oijen, et al., 2006). Lp-PLA_2 has not been empirically studied in relationship to cognitive decline, however.

Monocyte Chemoattractant Protein-1 (MCP-1)

Monocyte Chemoattractant Protein-1 (MCP-1) is a key chemokine in the pathogenesis of autoimmune diseases, chronic inflammatory disorders, and neuroinflammatry disease (Izikson, Klein, Luster, & Weiner, 2002; Luster, 1998). It is secreted by endothelial and monocyte-like cells (Chen, et al., 2003) and appears to be inactive in the normal brain (Yamagami, et al., 1999). Increased MCP-1 concentrations facilitate inflammatory responses and initiate mononuclear cell recruitment during vascular injury (J. Nilsson, 1993).

Several studies found plasma MCP-1 levels highest in acute coronary syndromes, and lowest among healthy controls, despite overlap of levels between groups (Cipollone, et al., 2001; Economou, et al., 2001; Nishiyama, et al., 1998). Increases in MCP-1 levels have also been associated with the development and progrssion and AD (Fenoglio, et al., 2004; Sun, et al., 2003), yet little if any research has been conducted on the presence of MCP-1 and vascular dementia. The relationship between MCP-1 and cognitive decline following cardiac surgery has been investigated (Reis, et al., 2007), and it was found that MCP-1 levels did not change in relationship to changes in cognition.

Vascular Risk Factors

Hypertension

There is a potential association between vascular inflammation and HTN, and studies have been conducted that link the inflammatory processes associated with HTN to several inflammatory blood markers, including IL-18 (Zirlik, et al., 2007), CRP (Chae, Lee, Rifai, & Ridker, 2001), MCP-1(de Lemos, et al., 2003), and Lp-PLA_2 (Persson, Hedblad, Nelson, & Berglund, 2007). Whether inflammation causes structural and functional changes in arterial walls that lead to HTN, or is simply a consequence of HTN remains unclear (Sprague & Khalil, 2009). The inverse relationship between blood pressure elevations and cognitive dysfunction has not yet been fully established, though epidemiologic studies have found that untreated hypertension during mid-life has been associated with cognitive dysfunction in later life (Kilander, Nyman, Boberg, Hansson, Kithell, 1998; Launer, Masaki, Foley, Havlik, 1995).

Hyperlipidemia

Lower levels of HDL have been associated with higher CRP levels (Cushman, et al., 2005), and IL-18 (Zirlik, et al., 2007). MCP-1 levels have also been found to be elevated in hyperlipidemic patients (Kowalski, et al., 2003). Approximately 80% of LP-PLA_2 is bound to LDL while 15-20% is bound to HDL, suggesting that the former might be more attributable to atherosclerosis and vascular disease, while the later may be cardio-protective (Chapman, McQuillan, Beilby, Thompson, & Hung, 2006). Midlife elevations in serum cholesterol have been linked to cognitive impairment measured 21 years later through a large neuropsychological battery including the Wechsler Memory

Scale-Revised, the Boston Naming Test, and the Wechsler Adult Intelligence Scale (Kivipelto, et. al, 2001).

Diabetes Mellitus

In recent years, there has been an increased understanding of the relationship between inflammatory processes and tissue damage associated with diabetes mellitus (DM). Several studies demonstrate an association between DM and CRP (Festa, D'Agostino, Tracy, & Haffner, 2002; Goldberg, 2009), MCP-1 (Herder, et al., 2006), IL-18 (Herder, et al., 2009; Miyauchi, Takiyama, Honjyo, Tateno, & Haneda, 2009), and Lp-PLA_2 (Yang, et al., 2007), and accelerated cognitive decline in DM patients versus normal controls has been identified (Cukierman-Yaffe, et al., 2009; Fontbonne, Berr, Ducimetiere, & Alperovitch, 2001).

Cigarette Smoking

Cigarette smoking is a major risk factor for cardiovascular disease, and approximately 20% of deaths due to cardiovascular disease have been directly linked to smoking (Bazzano, He, Muntner, Vupputuri, & Whelton, 2003). Smoking has been associated with traditional risk factors for cardiovascular disease and atherosclerosis, and recent research has shown that cigarette smoking can cause an elevation in pro-inflammatory markers as well, including CRP (Bazzano, et al., 2003; de Lemos, et al., 2003), MCP-1 (Shimada, et al., 2009; Zou, Hong, & Dai, 2009), and IL-18 (McKay, et al., 2004). Smoking has also been associated with cognitive impairment in mid- life as measured by the MMSE and Acitivities of Daily Living Scale (Zhou, et. al., 2003).

Waist Circumference

Obesity as measured by increased waist circumference (i.e., greater than 102 cm for men, and 88 cm for women) has been linked to risk factors of cardiovascular disease including HTN, HDL, and type 2 diabetes mellitus, in addition to increased risk of cardiovascular morbidity and mortality, and systemic inflammation (Ghandehari, Le, Kamal-Bahl, Bassin, & Wong, 2009; Madsen, et al., 2009).

Associations have been found between waist measurements and levels of pro-inflammatory markers, including CRP (Bochud, et al., 2009; Eiriksdottir, et al., 2009), IL-18 (Botella-Carretero, et al., 2007; Madsen, et al., 2009) and MCP-1 (Chacon, et al., 2008; Kaur, et al., 2009). Little is known about the relationship of Lp-PLA_2 to waist circumference. The relationship between waist circumference and cognitive dysfunction is not clear, however, as some studies demonstrate an inverse relationship between increased waist circumference measurements and cognitive decline as measured by the Wechsler Adult Intelligence Scale, Halstead-Reitan Neuropsychological Battery, Wechsler Memory Scale Revised, and the Mini-Mental State Examination in a cross-sectional design (Dore, Elias, Robbins, Budge, and Elias, 2008; Jeong, et al., 2005), while others do not demonstrate this relationship.

Cystatin C

Cystatin C level reflects glomerular filtration rate (GFR), an important determinant of the blood concentration of many substances. GFR is the amount of plasma that the kidneys can clear of a particular substance in a particular unit of time (Laterza, Price, & Scott, 2002). Persons with low GFR (e.g., kidney failure) have persistent activation of the inflammatory response in the body, and thus, increased levels of pro-

inflammatory substances (Pecoits-Filho, et al., 2003). Low GFR has been associated with elevated levels in CRP (Bavbek, et al., 2008; Wasen, Isoaho, Vahlberg, Kivela, & Irjala, 2008), IL-18 (Nguyen & Devarajan, 2008), and MCP-1 (Ibrahim & Rashed, 2008; Tam, et al., 2009), with no extensive research being conducted on the association of GFR to Lp-PLA_2. Elevations in cystatin C concentration have also been associated with lower cognitive scores as measured by the Modified Mini Mental State Examination, and were more likely to experience decline over 7 years than those with intermediate or low levels (Yaffe, et al., 2008).

Apolipoprotein E 4 (APOE 4)

Apoliprotein E 4 (APOE 4) is one allele of a class of apolipoproteins that carry cholesterol, and are essential for normal catabolism of triglyceride-rich lipoproteins and amyloid and tau production (Gunzburg, Perugini, & Howlett, 2007). Due to its association with circulating levels of lipids, it is often viewed as a risk factor for atherosclerosis and coronary heart disease (Luc, et al., 1994; Novaro, Sachar, Pearce, Sprecher, & Griffin, 2003; Tiret, et al., 1994).

A link has been demonstrated between APOE 4 and the occurrence of reduced cognitive function associated with AD (Breitner, et al., 1999; Gunzburg, et al., 2007). There is also evidence of a relationship between APOE 4 genotype and cognitive decline across the lifespan independent of AD (Blair, et al., 2005; Kozauer, Mielke, Chan, Rebok, & Lyketsos, 2008), and studies have looked specifically at the association between two pro-inflammatory blood markers and APOE 4. One investigation found CRP levels to be within normal ranges in patients with AD (Licastro, Masliah, Pedrini, &

Thal, 2000), and another found that the presence of the APOE 4 allele did not affect the association between increased MCP-1 levels and AD (Pola, et al., 2004).

Current Study

The current study examined the relationship between inflammatory factors (MCP-1, CRP, IL-18 and Lp-PLA_2) and cognitive functioning 8 years later. It also investigated the impact of vascular risk factors (i.e., hypertension, hyperlipidemia, diabetes, waist circumference, smoking status, and cystatin c) on cognitive function. As established in the literature (Pearson, 2002; Rockwood, Middleton, Moorhouse, Skoog, & Black, 2009), vascular risk factors can have an additive effect on cognitive dysfunction. As such, number of vascular risk factors in relation to cognitive function were examined. Further, this study looked at the impact of APOE 4 on the relationships between inflammatory markers and cognitive function, a relationship that has been clearly established previous work (Kanaya, Abe, Sakai, Fujii, & Iwamoto, 2010).

The aim of this investigation was to study the relationship between cognitive function and four markers related to inflammation, using a large community-based sample of individuals living in Dallas County, Texas. It was hypothesized that: 1) There will be an inverse relationship between cognitive functioning and blood inflammatory markers, 2) Participants with a higher number of vascular risk factors will demonstrate lower performance on a global cognitive measure than those with fewer vascular risk factors, and that vascular risk factors and pro-inflammatory markers will predict performance on a global cognitive measure, and 3) Those participants with APOE 4 will demonstrate lower cognitive scores than those without this allele, and that the addition of

the APOE 4 allele will strengthen the relationship between pro-inflammatory factors and cognitive performance.

Methodology

Data were obtained from the Dallas Heart Study, a multi-ethnic, population-based, longitudinal study of biological and social factors in relation to cardiovascular health between different ethnicities and to support research on the underlying mechanisms that might contribute to these differences (Victor, et al., 2004). The Dallas Heart Study was initiated in 1999 and recruited participants via stratified random sampling from the greater Dallas metroplex, oversampling blacks to enrich the ethnic diversity of the sample. DHS I participants were recruited to participate in DHS II, which began in 2007 and ended in 2010.

Participants

Participants tested for the four blood markers of interest obtained from DHS I, who completed cognitive testing from DHS II were used in this investigation. As such, there was a mean time between blood marker collection and cognitive testing of approximately 8 years. All participants included met the following inclusion criteria:

1) Able to speak and read English
2) Had measurements of pro-inflammatory blood markers drawn from DHS I
3) Completed a valid Montreal Cognitive Assessment (MoCA)
4) Provided informed consent

Participants with a history of stroke were excluded from the study.

Data for the current analysis were drawn from the stratified random sample obtained in DHS I who returned to DHS II. Of those seen in DHS II (N=2,911), 796 participants were excluded because they were not present at DHS I, leaving 2,115 participants. One participant was excluded because they requested that their data not be used for research purposes, 46 duplicate data entries were deleted, and 40 additional participants were excluded due to missing data that prevented the calculation of a MoCA total score. Thirty-seven additional participants were excluded for history of stroke, and one was deleted due to unclear history of stroke. Although Spanish forms of the MoCA were used for Spanish speakers, clerical problems and potential lack of comparability of scores resulted in the elimination of an additional 86 participants, leaving a total of 1904 subjects. Complete data on all four pro-inflammatory markers drawn at DHS 1 were available for 997 of these participants and certain analyses were limited to this group of subjects. Sample size varied for individual analyses depending on missing data.

Measures

The Montreal Cognitive Assessment

The MoCA was utilized to assess cognitive functioning because of its brevity and reported sensitivity. It was developed as a screening tool for detection of Mild Cognitive Impairment (MCI) in individuals with cognitive complaints, and has been found to have superior sensitivity and specificity to the Mini Mental State Examination in detection of MCI (Nasreddine, et al., 2005). The MoCA is a 30-point test that requires approximately 10 minutes to administer. It is comprised of items from the following cognitive domains: visuospatial ability, naming, memory, attention, language, abstraction, and orientation. A suggested cut off of ≤26 has been reported for detection of mild cognitive impairment

(Nasreddine, 2005). MoCA scores were analyzed as a continuous variable, and were further divided into tertiles for comparison of group differences in demographic, vascular risk factor, and biomarker data. A copy of the MoCA and additional details on the test can be in found in Appendix E.

Pro-Inflammatory Markers

The four inflammatory markers investigated, CRP, MCP-1, Lp-PLA_2, and IL-18, were chosen based on the availability of data from the DHS I in relation to their known or putative cardiovascular impact (de Lemos, et al., 2003; Victor, et al., 2004). High-sensitivity CRP measurements were performed. The minimal detectable range of the assay used in this analysis was 0.1 mg/L, and the upper limit is 20 mg/L. The minimal detectable concentration for the MCP-1 assay was 40 pg/ml, and the upper end of the reportable range is 2,000 pg/ml (Deo, et al., 2004a). Both Lp-PLA_2 and IL-18 procedures have been described elsewhere (Brilakis, et al., 2008; Zirlik, et al., 2007). For correlational analyses, biomarker concentration data were used as continuous variables. Clinically significant elevations for MCP-1, Lp-PLA_2, and IL-18 were based on scores that were greater than 1 standard deviation above the mean for the entire sample. The 220 participants (6.5%) with CRP values > 20 mg/L were treated as having CRP elevations.

Vascular Risk Factors

Hypertension was defined as an average systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg, or if the participant was using antihypertensive treatment. Hypercholesterolemia was defined as LDL ≥ 160 mg/dl, a total cholesterol of ≥ 240 mg/dl, or if the participant was currently using statin

medication. Subjects were considered to have a positive diabetes mellitus status is they obtained a fasting glucose level of ≥ 126 mg/dl, or if they were using hypoglycemic medications. Waist circumference measurements were in centimeters (cm), obtained 1 cm above the iliac crest, and were defined as significant if > 80 cm for women, and > 102 cm for men. Smoking status was obtained via questionnaire, and categorized participants as either current smokers or past smokers, or never smoked. All variables were used dichotomously.

Procedures

In DHS I, three separate sequential visits with participants were used to collect epidemiologic and biologic data. Demographic information, medical history, and vascular risk factor variables were collected at DHS Visit 1 via a 60-minute, computer-assisted health interview conducted in participants home by trained study personnel. Inflammatory markers were collected during Visit 2, which was also conducted at the home of each participant. No data was used that was collected at Visit 3. All participants signed informed consent and had no knowledge of the hypotheses of this study at the time of their initial participation.

Subjects returning for DHS II participated in a day long visit, where subjects provided social and medical history, underwent a physical examination and imaging studies, and provided both blood and urine samples. The Montreal Cognitive Assessment (MoCA) was administered to all consenting participants and efforts were made to administer this test at the beginning of the day to minimize the possible effects of fatigue. MoCA administration took approximately 10 minutes per subject. Trained personnel administered all MoCA tests, and efforts were made to ensure inter-rater reliability for

MoCA scoring and administration. Scoring followed standard MoCA procedures, and each test was double-checked for accuracy prior to entry into the data bank. All scored MoCA's were then entered into the DHS data bank, and data protected through university security procedures. Once entered, scores were spot-checked for final accuracy. Total MoCA scores were analyzed as a continuous variable, and were further divided into tertiles in order to allow for comparisons between the highest and lowest scoring subject groups. The suggested one-point education correction for those with <= 12 years of education was not applied since education was used as a covariate when appropriate.

Data Analysis

All data were analyzed using SPSS version 18 (SPSS, Inc., Chicago IL). Missing data were filtered out via list wise deletion. Analyses on missing data versus complete data were performed in order to compare participants with missing data to those without on similar measures using chi-square tests for categorical variables, and t-tests for continuous variables, with no significant differences noted. In order to account for potentially spurious findings in the large sample size being investigated, a more stringent significance level of .01 was used in all analyses (Cohen, 1988). CRP demonstrated a markedly skewed distribution, and these data were logarithmically transformed (log10 transformation) for the use in parametric testing, though the results for the study are reported without this transformation as is consistent with previous work (Cushman, 2005). Gender differences were examined using both chi square analysis and independent samples t-tests.

To investigate the relationship between each of the pro-inflammatory markers (i.e., CRP, IL-18, Lp-PLA_2, and MCP-1) and cognitive function as measured by the

MoCA, partial Spearman correlation analysis that controlled for education and age was utilized. Spearman's correlation analysis was used to account for the non-normality of data distribution that violated the assumptions of Pearson's correlation. To assist with clarification of clinical interpretation of these correlations, the strength of significant correlation coefficients was designated as small (.10-.29), medium (.30-.49), and large (>.50) as per standard convention [Cohen, 1988]. A new variable was created that identified participants with or without an elevation on each biomarker (as described above). These new variables were used in ANCOVAs in order to determine if there were mean differences in MoCA scores between participants with and without elevated values.

To investigate the relationship between number of vascular risk factors and cognitive function, a variable was created that quantified the number of risk factors, and then partial Spearman correlations, controlling for age and education, were conducted between this new variable and MoCA scores. Logistic regressions were then conducted to identify which inflammatory markers (i.e., CRP, MCP-1, IL-18, and Lp-PLA2), vascular risk factors (i.e, HTN, HLD, DM, waist circumference) and demographic variables best predict lowest MoCA scores. For these analyses, the lowest MoCA tertile group was used as the dependent variable. Vascular risk factors and pro-inflammatory markers were entered in Step 1 of the analyses via entry method in order to obtain an unadjusted full model, followed by backward stepwise method in order to obtain a reduced, unadjusted model. In Step 2, demographic variables were added to obtain an adjusted model, and this final model was entered via entry method in order to maximize the sample size. The Hosmer Lemeshow goodness-of-fit test was used in order to identify the model with best overall fit.

To determine if those participants with the APOE 4 allele obtained lower MoCA scores than those without this allele, a variable was created that identified presence or absence of APOE 4 in participants. ANCOVAs were used to compare mean MoCA scores between groups while controlling for age and education. To determine if the presence of the APOE 4 allele strengthened the relationship between pro-inflammatory blood markers and cognitive function, APOE 4 status was added to the regression models that were used in pervious analyses.

Results

Demographic Characteristics

Descriptive information can be seen in Table 1. The majority of participants in the study were Black (54%) and more than half were female (58%).

Insert Table 1 about here

Subjects were relatively well educated, with a mean of 13.6 years (SD = 2.7; range = 1 to 20 years. MoCA total scores had a mean of 23.4 (SD =4.0; range 7 to 30). Mean age of the sample was 42.94 years (SD =10.52; range 18-65).

Health and Biological Characteristics

Health and biological characteristics for the sample are presented in Table 2. The majority of the sample was non-diabetic (79.4%), normotensive (68.1%), and had normal

cholesterol values (76.8%). Most participants in the study were non-smokers (74%), and identified themselves as current drinkers (72%). Quantity of alcohol was not assessed in this analysis. The most frequently occurring APOE allele pattern in this population was E3/E3 (48%), and 28% of the group had at least one E4 allele.

Insert Table 2 about here

MoCA data

MoCA performance by gender and ethnicity can be viewed in Table 3. ANCOVAs were conducted to assess differences between group means with age and education controlled for in the analysis. Caucasian participants had significantly higher MoCA total scores than all other racial groups, while Black subjects demonstrated significantly lower MoCA total scores than Hispanic participants. Education was significantly correlated with MoCA total score [r=.43, p<.001)], as was age [(r= -.199, p=.000].

Insert Table 3 about here

Pro-Inflammatory Marker Data by Gender

Differences between concentrations of biomarkers by gender were investigated using ANCOVA, with education and age as covariates in the model. There were significant differences between males and females for CRP, IL-18, and Lp-$PLA_{2,}$ (See Table 4). Females had significantly greater CRP concentrations and significantly lower IL-18 and $LPPLA_2$ concentrations than men. There were no significant differences for gender for on MCP-1 elevations.

Insert Table 4 about here

Inflammatory Markers and Ethnicity

Black subjects had significantly greater concentrations of CRP than Caucasians [$F(3,1649)=10.64$, $p<.001$)]. Caucasians had significantly greater concentrations of Lp-PLA_2 than Blacks and Hispanics [($F($3, 1603)=31.26, $p<.001$] (see Table 5). There were no differences in MCP-1 [($F(3)$, 1645)=3.87, $p=.896$], or IL-18 [$F(3, 1665)=7.87$, $p=.996$].

Insert Table 5 about here

Hypothesis One

Hypothesis one stated that there would be an inverse relationship between cognitive function and inflammatory marker concentration. A partial Spearman's correlation analysis, controlling for age and education showed a weak but statistically significant correlation between MoCA total scores and levels of Lp-PLA_2 [(n=994; r=.09, p=.003)] (Table 6). When this analysis was conducted by gender, the significant correlation between MoCA total scores and Lp-PLA_2 concentration was only found in men (r=.24, p<.001) (see Table 7). No significant correlations between concentration of CRP, MCP-1, IL-18 and MoCA total scores were found. Additionally, ANCOVAs revealed no significant mean differences between MoCA total scores between those participants who demonstrated elevations on each marker versus those who did not, as seen in Figure 1.

Insert Tables 6 & 7, & Figure 1 about here

Hypothesis Two

To investigate the relationship between the number of vascular risk factors and global cognitive function, a new variable was created that quantified the number of vascular risk factors for each participant. These factors included HTN, HLD, DM, cigarette smoking, waist circumference, and cystatin C elevations. It should be noted that no subjects demonstrated elevations on all 6 variables. As can be seen in Table 8, no

significant differences were found between groups with increasing numbers of vascular risk factors.

Insert Table 8 about here

Logistic regression was used to predict assignment to lowest MoCA tertile group from demographic and health variables. The reduced, adjusted model of the logistic regression analysis showed no significant findings in terms of vascular risk factors. The final adjusted model of this regression identified education as decreasing the odds of lowest MoCA membership tertile assignment (See Table 9). When biomarker variables were added to this model, there were no significant predictors of lowest tertile group membership, while education and Caucasian served to decrease the odds of lowest MoCA tertile membership (Table 10).

Insert Tables 9 & 10 about here

Hypothesis Three

The third hypothesis of this study was to examine if the presence of the APOE4 allele strengthened the relationship between inflammatory markers and cognitive function. Using ANCOVA to control for education, there was no significant difference

between participants with and without an E4 allele on the MoCA [$F(1, 1643) = .244$, $p=.780$). Logistic regressions revealed no significant impact of the presence of the E4 allele on cognitive function, and the presence of this allele did not strengthen the relationship between blood marker elevations and cognitive function as hypothesized. These results can be viewed in Table 11. In this analysis, the final adjusted model indicated education and Caucasian race as being protective of membership assignment into the lowest MoCA tertile group. The E4 allele status was not present in the final model.

Insert Table 11 about here

Discussion

Pro-Inflammatory Factors and Demographic Variables

Women had significantly greater concentrations of CRP, and lower levels of IL-18, and Lp-PLA_2 than men. Consistent with the current findings, some investigators have has found higher CRP concentrations in women (Cushman, et al., 2005; de Lemos, et al., 2003), and elevated IL-18 levels in men (Blankenberg, et al., 2003; Zirlik, et al., 2007), but we did not find greater concentrations of MCP-1 in women, as has been reported by others (de Lemos, et al., 2003). The relationship of Lp-PLA_2 and gender is less clear, as some studies find no gender differences (Albert, Glynn, Wolfert, & Ridker, 2005; van

Oijen, et al., 2006), while previous research with The Dallas Heart Study found lower Lp-PLA_2 concentrations in women (Brilakis, et al., 2008), consistent with the current results. Other investigations, however, have found that the relationship between pro-inflammatory levels and gender was moderated by body composition and fat distribution with levels of subcutaneous fat contributing to inflammatory levels. (Cartier, et al., 2009; Detopoulou, et al., 2009). Thus, gender differences in pro-inflammatory markers remain unclear, and may be moderated by other physiological factors.

Black participants had significantly greater CRP levels than Caucasians, while Caucasian participants had significantly greater concentrations of elevations of IL-18 than Blacks and Hispanics, as well as increased levels of Lp-PLA_2 as compared to Hispanic participants. Some studies have found a similar pattern of CRP elevation in Blacks compared to Caucasians (Cushman, et al., 2005; Davis, Crow, Chambers, Meek, & Chambers, 2009), while others have shown Caucasians to have higher MCP-1 elevations than Blacks and Hispanics (de Lemos, et al., 2003; Deo, et al., 2004b). Consistent with the current findings, IL-18 has been shown lowest in Blacks, of intermediate levels of elevation in Hispanics, and was highest in Caucasians (Brilakis, et al., 2008). Thus, there have been largely mixed findings in this area, but these results are mostly consistent with other reports of ethnic differences in inflammatory markers.

CRP concentration differences in Black participants may be accounted for by differences in a clustering of metabolic risk factors, and hence, an inflammatory response, that have been shown to be more prominent in people of African descent (Kalra, et al., 2005). However, very little research has been conducted on the etiology of ethnic differences in inflammatory markers for comparison. It remains possible that

racial socioeconomic disparities may account for differences in health behaviors, and medical treatment of illnesses associated with inflammatory marker response.

Hypothesis One

The hypothesis of an relationship between cognitive function and biomarker levels was not significant, as the only correlation was an association between Lp-PLA_2 and MoCA scores, but in the positive direction. When this was analyzed by gender, the significant results were found only in men, but again in the positive direction. There were no significant relationships between levels of CRP, MCP-1, and IL-18 and MoCA scores. Given that Lp-PLA_2 has only been recently studied in terms of its potential impact on cognition, this finding warrants further investigation. It was surprising that measures of other inflammatory biomarkers, especially those that have been more robustly associated with cognitive function (i.e., CRP) in some previous studies, did not result in significant correlations.

However, other studies have also failed to find such associations, suggesting that inflammatory markers, at least in healthy samples, are not useful predictors of cognitive function (Weuve, et al., 2006). In fact, it has been reported that a *decrease* in MCP-1 occurs in patients with mild cognitive impairment who converted to AD over a one-year follow-up (Galimberti, 2006). Although information on biomarker concentrations are likely to inform pathologies that have inflammatory processes as a component, its utility as a predictive variable may only be useful when combined with other molecules or additional biochemical data (Galimberti, 2006).

The biochemical mechanism(s) through which inflammatory factors may influence cognition remains unclear. In the current study, only Lp-PLA_2 was associated

with cognitive function measured at a later time, and not in the expected direction. In fact, elevations in Lp-PLA_2 served as a protective factor, yet only weakly. Currently, it is not clearly understood whether biomarker elevations contribute to the atherosclerotic process, are a reaction related to it, or elevate as a parallel process that occurs simultaneously with atherosclerosis (Schunkert & Samani, 2008). However, of all the blood markers investigated in this analysis, Lp-PLA_2 may be both a risk factor for as well as a protective factor for cardiovascular illness, as this biomarker attaches to both LDL and HDL in the blood (Caslake & Packard, 2005). Despite the weak association found in this study, it is possible that the manner by which Lp-PLA_2 affects the atherosclerotic process has more direct impact on cognition than do the other markers in this investigation due to it's direct association with lipoproteins in the blood.

Hypothesis Two

The hypothesis that a greater number of vascular risk factors would result in lower cognitive function was not supported, with no significant differences in mean MoCA total scores and increased number of vascular risk factors. Although this finding is inconsistent with others studies that have examined the impact of traditional vascular risk factors on cognition (Rockwood, et al., 2009), the relative health of the sample may account for these findings. It may be that the severity of vascular risk burden and duration of risk factors are more important than the mere presence of each condition when understanding the relationship between vascular illness and cognitive dysfunction (Villeneuve, Belleville, Massoud, Bocti, and Gauthier, 2009).

Hypothesis Three

The hypothesis that the presence of the APOE4 allele would strengthen the relationship between elevation in pro-inflammatory blood markers and cognitive function was not supported. This finding is contrary to other reports that link this allele to cognitive dysfunction (Blair, et al., 2005; Bookheimer, & Burggren, 2009). While the APOE 4 allele has been well established as a risk factor for AD (Blair, et al., 2005; Strittmatter, 2000), the impact of this allele on cognition remains unclear (Heun, et al.; Hsiung, Sadovnick, & Feldman, 2004), particularly in a healthy, mostly middle-aged population.

Some studies provide inconclusive results with regard to APOE4 and the development of atherosclerosis (Davignon, Cohn, Mabile, & Bernier, 1999; Slooter, et al., 2001) and therefore APOE4 may not have a direct impact on cognitive dysfunction associated with the atherosclerotic processes as hypothesized. However, the lack of correlations between biomarkers, vascular risk factors, and cognitive function in this sample made it unlikely that APOE4 would have much impact on the associations. Additionally, some research has suggested an association between APOE4 and intimal thickening, not plaque (Altamura, 2007), while cognitive dysfunction has been shown to be related to plaque indexes but not intimal thickening associated with atherosclerosis (Silvestrini, et al., 2009).

Protective Factors for Cognitive Function

Those subjects with higher levels of education and who were of Caucasian ethnicity had a lower likelihood of obtaining MoCA scores in the lowest tertile. Level of education and its negative association with cognitive test performance has been well

established (Bennett, et al., 2003; Evans, et al., 1993). Ethnic differences were also associated with MoCA performance in this analysis. It is possible that disparities in quality of education between ethnicities and the influence of socioeconomic status on the opportunity for high quality education can account for these differences (Sirin, 2004).

Limitations

The current study had several limitations. Lack of significant correlations between MoCA and biomarker data may be due to the design of this study, which is neither cross-sectional nor longitudinal. During the 8 years between pro-inflammatory marker measurement and assessment of cognitive functioning, participants in the sample may have experienced significant changes in their health, their health behaviors and lifestyles. Along these lines, patients with medical conditions such as hypertension, diabetes, and hyperlipidemia may have experienced changes in their illness or treatment regimen during the 8 years between DHS I and DHS II (Koh, et al., 2004). Additionally, it is possible that those individuals suffering health conditions that were caused or exacerbated by elevations in pro-inflammatory markers during DHS I did not return for follow-up in DHS II, creating a healthy survivor effect. The current study, then, may have not assessed those individuals with biomarker elevations high enough to impact cognitive function (Baillargeon & Wilkinson, 1999).

As noted earlier, the biomarkers under consideration are acute phase reactants, and their elevations may be due to non-atherosclerotic inflammatory processes (i.e., burn, infection, injury). These elevations, then, could be misleading in terms of their association with atherosclerosis and cognitive dysfunction due to atherosclerotic disease. Having a better understanding of the etiology of the rise in blood markers at the time they

were measured, and then statistically controlling for those participants whose levels may not be directly associated with the atherogenic process, may have proven useful. As measurements of inflammatory markers were only examined at one point in time, examination of current levels with cognition may prove more clinically useful, as this would make possible direct associations between concentration of inflammatory factors and cognition. Duration of biomarker elevations in participants was unknown and examination of DHS II levels when available will be informative.

The MoCA itself has limitations. It was developed as a cognitive screening measure, and more subtle differences in cognitive domains may not be as evident with this tool, as might be seen with a more in depth neuropsychological battery. The MoCA is a relatively new assessment tool, and there is limited research using the measure in non-clinical populations, so few comparisons can be made between this study and others. As the DHS sample was relatively well educated, these findings may not generalize well to less educated populations. Several important participant factors were also not assessed, including presence of dementia, history of traumatic brain injury, or the presence of inflammatory processes that are not associated with atherosclerosis such as autoimmune disorders, arthritis, infection, burn, or tissue injury. Corticosteroid use should also be assessed in future work due to the relationship between these medications and inflammatory marker elevations (Antoniu, 2010; Habib, 2009). Additionally, these findings may not generalize to populations that are less educated, more unhealthy, and older.

Future research utilizing concomitant measures of inflammation and more detailed assessment would help to further explore this area. Future work should also consider following a group of participants longitudinally with both cognitive and

inflammatory marker assessments taken at baseline and final point of study. This would help examine how changing concentrations of inflammation impact cognitive functioning over time.

Conclusions

In a large, community-based sample, inflammatory biomarker data were compared with cognitive function measured 8 years later to examine their relationship. In this investigation, a significant but weak positive correlation was found between LP-PLA_2 and cognition, yet no relationships were found between elevated CRP, MCP-1, IL-18 and a brief index of global cognitive function. Participants with a greater number of vascular risk factors did not demonstrate overall lower cognitive performance. The presence of the APOE4 allele did not impact the relationship between biomarkers, vascular risk factors and global cognitive function as hypothesized. Education and Caucasian ethnicity served as protective factors for cognitive impairment. Thus, the current study suggests that demographic factors may have greater impact on mid-life cognitive performance than measures of inflammation or vascular risk factors *per se*. It is also possible that our findings with regard to inflammatory markers and cognitive function differ from others due to the 8-year time gap between blood marker measurements and cognitive function assessment, as well as the relative health and minimal pro-inflammatory blood marker elevations of our sample.

Table 1

Demographic Characteristics

	N	Min	Max	M (*SD*)
Age	1904	18	65	42.94 (*10.52*)
Education	1904	1	20	13.59 (*2.69*)
Gender	N (%)			
Male	801 (*42.1*)			
Female	1103 (*57.9*)			
Ethnicity				
Black	1019 (*53.5*)			
White	637 (*33.5*)			
Hispanic	207 (*10.9*)			
Other	41 (*2.2*)			

Table 2

Health and Biological Characteristics

	N	Min	Max	M (SD)
Waist Circumference (cm)	1564	56.0	164.0	99.55 (17.04)
ApoE Status				
E2/E2	13 (.7)			
E2/E3	191 (10.0)			
E2/E4	52 (2.7)			
E3/E3	910 (47.8)			
E3/E4	417 (21.9)			
E4/E4	61 (3.2)			
Alcohol Intake				
Current Drinker	1372 (72.1)			
Recent Abstainer	321 (16.9)			
Lifetime Abstainer	208 (10.9)			
Missing	3 (.2)			
Smoking				
Current Smoker	491 (25.8)			
Past Smoker	320 (16.8)			
Never Smoked	1091 (57.3)			
Missing	2 (.1)			
Diabetes				
Diabetic	157 (8.2)			
Non-diabetic	1511 (79.4)			
Missing	236 (12.4)			
Hypertension				
Yes	578 (30.4)			
No	1296 (68.1)			
Missing	30 (1.6)			
Hypercholesterolemia				
High Cholesterol	195 (10.2)			
No Cholesterol	1463 (76.8)			
Unknown	10 (.5)			
Missing	236 (12.4)			

Table 3

MoCA Total Score by Gender and Ethnicity

	Male		Female		Overall	
	N	M *(SD)*	N	M *(SD)*	N	M *(SD)*
Black	389	21.62 (2.94)	630	22.13 (4.13)	1019	21.94 (4.06)
White	299	25.61 (2.78)	338	25.82 (2.88)	637	25.72 (2.83)
Hispanic	87	22.74 (3.52)	120	23.28 (3.82)	207	23.05 (3.70)
Other	26	23.46 (3.90)	15	24.07 (3.08)	41	23.68 (3.60)
Total	801	23.29 (3.95)	1103	23.41 (4.08)	1904	23.36 (4.03)

* Variables controlled for in model: Age and Education.
**White group significantly higher MoCA total score than all other groups; Black group also significantly lower MoCA total score than Hispanic group. $F(1, 1898) = 90.17$, $p \leq .001$

Table 4

Inflammatory Markers by Gender

	Gender	Mean	Std. Deviation
MCP-1	Male	203.32	187.64
	Female	194.57	178.50
	Total	197.75	181.83
CRP*	Male	3.76	4.63
	Female	6.01	5.87
	Total	5.20	5.56
IL-18* *	Male	729.84	752.47
	Female	620.71	651.63
	Total	660.34	691.57
Lp-PLA_2***	Male	199.73	58.26
	Female	178.06	52.74
	Total	185.93	55.76

*Significant Difference for CRP F (1, 994) = 35.92, p <.001
**Significant Difference for IL-18 F (1, 994) = 6.33, p =.012
***Significant Difference for LPPLA_2 F (1,994), p <.001

Table 5

Pro-Inflammatory Markers by Race

	Ethnicity	Mean	SD	N
MCP-1	Black	192.59	190.97	855
	White	208.10	156.56	574
	Hispanic	184.91	91.53	180
	Other	185.56	77.72	37
	Total	196.99	169.08	1646
CRP[1]	Black	5.91	6.02	859
	White	4.00	4.88	581
	Hispanic	4.63	5.40	179
	Other	3.58	4.83	36
	Total	5.05	5.62	1655
IL-18	Black	629.589	619.73	580
	White	736.65	809.720	343
	Hispanic	594.44	467.50	104
	Other	677.83	893.67	19
	Total	662.08	682.304	1046
Lp-PLA_2[2]	Black	176.93	60.05	840
	White	207.39	55.15	560
	Hispanic	189.35	50.59	176
	Other	195.94	49.21	33
	Total	189.28	58.82	1609

[1]Black group significantly higher than White group for CRP $F(3,1649) = 10.64$, $p < .001$
[2]White group significantly higher than Blacks and Hispanics for Lp-PLA_2 $F(3,1603) = 31.26$, $p < .001$

Table 6

Partial Spearman Correlation between MoCA scores and Inflammatory Markers

		MCP1 (n=1646)	CRP (n=1655)	IL-18 (n=1046)	Lp-PLA_2 (n=1609)
MoCA Total Score	Correlation	-0.03	-0.06	0.02	0.09
	Sig.	.345	.079	.567	.003
	df	994	994	994	994

Variable controlled for in model: Age and Education

Table 7

Partial Spearman's Correlation for MoCA Total Scores and Biomarkers by Gender

			MCP-1 (n=1646)	CRP (n=1655)	IL-18 (n=1046)	Lp-PLA$_2$ (n=1609)
Male	MoCA Total Score*	Correlation	0.02	-0.07	-0.02	0.24
		Significance	.782	.164	.677	.000
		df	359	359	359	359
Female	MoCA Total Score	Correlation	-0.02	-0.02	0.00	0.06
		Significance	.593	.669	.999	.119
		df	632	632	632	632

Variable controlled for in model: Age and Education

Table 8

MoCA Total Score by Number of Vascular Risk Factors

# Vascular Risk Factors	N(%)	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
0 Factors	384 (20%)	23.44	0.19	23.07	23.82
1 Factor	577 (30%)	23.64	0.15	23.34	23.93
2 Factors	407 (22%)	23.13	0.18	22.78	23.48
3 Factors	182 (10%)	23.11	0.27	22.58	23.63
4 Factors	65 (3%)	23.78	0.45	22.90	24.65
5 Factors	15 (1%)	22.34	.921	20.53	24.14

Variables controlled for in model: Age and Education

No significant difference between means $F(5, 1621) = 1.56$, $p=.168$

Table 9

Logistic Regression Predicting Lowest MoCA Tertile with Demographic and Health Variables

		B	S.E.	Wald	df	Sig.	OR	95% C.I.for OR Lower	Upper
Model 1	Education	.100	.024	17.40	1	.000	1.10	1.06	1.16
	High Cholesterol	.290	.779	.138	1	.710	1.34	.290	6.16
	Normal Cholest.	-.128	.200	.408	1	.523	.880	.594	1.30
	Hyptertensive	-.156	.498	.098					
	Hypertensive	-.133	.496	.072	1	.788	.875	.331	2.31
	Current Smoker	-21.66	284.05	.000	1	.633	.955	.719	1.27
	Past Smoker	-0.243	.194	1.566	1	.211	.784	.536	1.15
	Never Smoked	-0.085	.169	.253	1	.615	.919	.660	1.28
	Cystatin C	.202	.269	.566	1	.452	1.25	.723	2.07
	Diabetic	.127	.235	.291	1	.590	1.14	.716	1.80
	Wasit Circum.	-.008	.004	3.92	1	.048	.992	.984	1.00
	Current Drinker	.219	1.44	.023	1	.879	1.24	.074	20.89
	Recent Abstainer	.094	.165	.322	1	.570	1.09	.794	1.52
	Lifetime Abstainer	-.128	.243	.277	1	.599	.880	.546	1.42
	Black	.077	.448	.030	1	.054	.926	.385	2.26
	White	-.135	.450	.030	1	.764	1.15	.474	2.76
	Hispanic	.039	.480	.006	1	.936	.962	.375	2.46
	Age	.003	.007	.165	1	.684	1.00	.989	1.01
	Constant	.767	.475	2.61	1	.106	2.154		
Final Model	Education	-.105	.023	20.02	1	.000	1.11	1.06	1.16
	Constant	.742	.370	4.02	1	.045	2.100		

65.5% Correctly Predicted into Lowest MoCA tertile; N=985

Table 10

Logistic Regression Predicting Lowest MoCA tertile with Demographic, Health, and Biomarker Variables

							95% C.I.for OR		
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
Model 1	High Cholesterol	.338	.936	.131	1	.718	1.40	.224	8.78
	Normal Choles.	-.236	.268	.779	1	.377	.789	.467	1.33
	Hyptertensive	.320	.634	.254	1	.614	1.38	.397	4.77
	Normotensive	-.022	.201	.012	1	.912	.978	.659	1.45
	Current Smoker	-21.80	40.92	.000	1	.999	.000	.000	.012
	Past Smoker	-.379	.275	1.90	1	.169	.685	.399	1.17
	Never Smoked	-.154	.233	.437	1	.508	.857	.542	1.35
	Waist Circum.	-.010	.006	2.841	1	.092	.990	.979	1.00
	Cystatin C	.316	.387	.667	1	.414	1.37	.642	2.92
	Diabetic	.366	.307	1.42	1	.233	1.44	.790	2.63
	Non Diabetic	.876	.378	1.22	1	.465	1.07	.578	.542
	Current Drinker	.273	.226	1.46	1	.227	1.31	.844	2.05
	Past Drinker	.095	.316	.091	1	.763	1.10	.592	2.05
	Never Drank	.978	.465	.121	1	.744	1.20	.896	2.00
	MCP-1	.000	.000	.490	1	.484	1.00	.999	1.01
	CRP	-.005	.016	.078	1	.780	.995	.964	1.03
	IL-18	.000	.000	.018	1	.894	1.00	1.00	1.07
	Lp-PLA2	.000	.002	.087	1	.768	1.00	.997	1.01
	Black	.176	.596	.087	1	.768	1.19	.371	3.83
	White	.086	.600	.021	1	.886	1.09	.336	3.53
	Hispanic	-.021	.580	.001	1	.971	.979	.314	3.05
	Education	.098	.034	8.51	1	.004	1.10	1.10	1.18
	Constant	-.994	1.09	.834	1	.361	.370		
Final Model	Education	-.890	.030	8.848	1	.005	1.093	1.031	1.159
	White	-1.63	.043	15.28	1	.000	5.03	2.45	11.87
	Constant	4.97	1.02	23.91	1	.000	144.75		

55.2% Correctly Predicted into Lowest MoCA Tertile Group; N=696

Table 11

Logistic Regression Predicting Lowest MoCA Tertile with Demographic, Health, Biomarker, and APOE4 Variables

							95% C.I.for OR	
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
Model 1	High Cholesterol	.365	.93	.935	1	.576	1.42	.222	9.04
	Low Cholesterol	-.235	.262	.738	1	.364	.820	.489	1.37
	Hypertensive	.254	.635	.389	1	.689	1.38	.391	4.64
	Normotensive	.001	.189	0.00	1	.997	1.00	.690	1.45
	Current Smoker	-21.98	40.80	0.00	1	1.00	.000	.001	.067
	Past Smoker	-.467	.228	.176	1	.675	.908	.580	1.42
	Never Smoked	-.097	0.33	13.61	1	.080	0.30	0.16	0.57
	Diabetic	.344	.306	1.30	1	.265	1.43	.756	2.56
	Waist Circum.	-.057	.067	4.98	1	.033	.987	.974	.998
	Current Drinker	.276	.223	1.60	1	.251	1.35	.889	2.04
	Recent Abstainer	.065	.332	.045	1	.854	1.01	.576	1.95
	Cystatin C	.301	.392	.349	1	.553	1.21	.623	2.57
	MCP-1	.000	.000	.278	1	.592	1.00	.993	1.00
	CRP	.000	.018	.000	1	.991	1.00	.954	1.03
	IL-18	.000	.000	.159	1	.646	1.00	1.00	1.26
	Lp-PLA2	.000	.002	.186	1	.693	1.00	.967	1.03
	Male	-.376	.189	4.40	1	.034	.622	.449	.974
	Black	.213	.601	.127	1	.726	1.23	.324	4.02
	White	-.086	.601	.017	1	.001	1.08	.332	4.00
	Hispanic	.408	.651	.392	1	.531	1.50	.420	5.38

Table Continues

Table 11 (Continued)

Logistic Regression Predicting Lowest MoCA tertile with Demographic, Health, Biomarker, and APOE4 Variables

							95% C.I. for OR	
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
	Education	.087	.031	7.66	1	.006	1.09	1.02	1.16
	E4 Versus no E4	.152	.568	.071	1	.789	1.164	1.05	1.45
	Constant	1.03	.812	1.62	1	.203	2.81		
Final Model	Education	-0.08	.004	3.92	1	0.09	.991	.981	1.01
	White	-.025	.961	3.26	1	0.04	.892	.975	.992
	Constant	.926	.471	3.85	1	.044	2.51		

79.2% Correctly Predicted Into Lowst MoCA Tertile Group; N=58

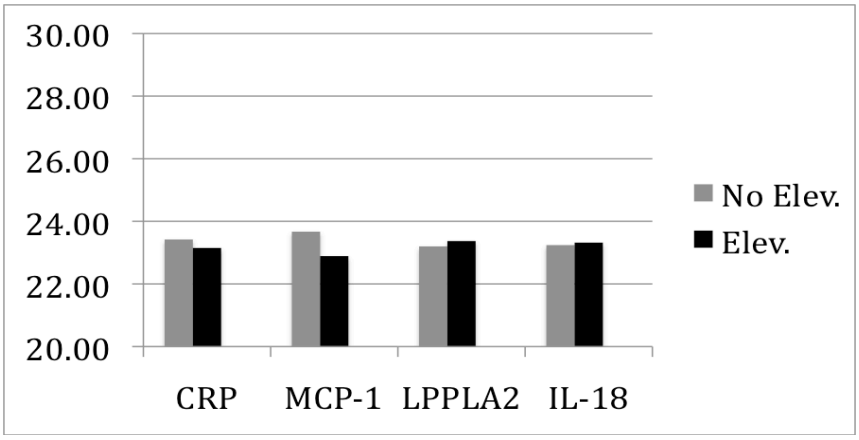


Figure 1

MoCA Total Score Means by Biomarker Elevation Versus No Elevation

No significant differences between MoCA means for CRP $F(1,908) = .134$, $p = .715$, MCP-1 $F(1,908) = 1.104$, $p = .294$, Lp-PLA_2 $F(1,908) = .055$, $p = .814$, or IL-18 $F(1,908) = .015$, $p = .904$

APPENDIX A
COMPLETE LITERATURE REVIEW

CHAPTER ONE
Review of the Literature

Inflammatory factors and Their Relationship to Atherosclerosis and Vascular Disease

Cognitive impairment has become an increasingly important public health issue as the American population ages (Anderson, 2008). The most common causes of severe cognitive dysfunction in the elderly are Alzheimer's disease (AD) and cerebrovascular disease in the form of vascular dementia (VaD). Severe cognitive impairment is the final occurrence in a chain of events initiated and aggravated by factors that operate long beforehand (Kidd, 2008). Thus, it is logical that the cognitive effects of these factors may be detectable many years before severe impairment occurs. The aim of this investigation is to study the relationship between cognitive function and four markers related to inflammation, using a large community-based sample of individuals living in Dallas County, Texas. Inflammatory factors were chosen for investigation because inflammation has been associated with both AD and with atherosclerosis, the underlying cause of VaD and stroke (Lowe & Pepys, 2006; Ravaglia, et al., 2007). The current study will attempt to determine if there is an association between several well-known inflammatory factors and a measure of cognitive function, the Montreal Cognitive Assessment (MoCA).

Four inflammatory factors will be explored in terms of their relationship to atherosclerosis and cardiovascular health. These include: 1) C- reactive protein, 2) Interleukin-18 (IL-18), 3) Lipoprotein-associated phospholipase (LP-PLA_2), and 4) Monocyte Chemoattractant Protein-1 (MCP-1). These factors will then be reviewed in context of both AD and VaD. Next, research investigating the relationship between these

four factors and cognitive decline will be reviewed. Finally, covariates of these blood inflammatory markers will be addressed.

C-Reactive Protein (CRP)

Of the known inflammatory markers, C-reactive protein (CRP) bears the strongest relationship to atherosclerosis. CRP is a member of the class of acute-phase reactants whose levels rise sharply during inflammatory processes (Yeh & Willerson, 2003). This dramatic rise is due to a rise in the plasma concentration of interleukin 6 (IL-6), which is produced by macrophages and adipocytes. CRP binds to the phosphocholine of bacterial cell walls and is thought to assist in complement binding to foreign substances and to damaged cells. It binds to a receptor on macrophages and enhances their phagocytic activity (Pepys & Hirschfield, 2003). It is also believed to have a role in early defense system against infections. CRP has no specificity in differentiating disease entities, yet research has shown it to be a powerful predictor for cardiovascular disease (Yeh & Willerson, 2003).

CRP also binds the phosphocholine of oxidized low-density lipoprotein (LDL), upregulates the expression of adhesion molecules in endothelial cells, and increases LDL uptake into macrophages. Additionally, CRP helps to stimulate Interleukin-8 (IL-8) release from monocytes, increases the release of Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-18 (IL-18), TNF-alpha, and increases plasminogen activator inhibitor-1 expression and activity. Normal reference ranges for blood tests are less than 5-6 mg/l, with the American Heart Association suggested cutoffs for CRP of <1, 1-3, and >3 mg/L suggesting lower, moderate, and higher relative risk for vascular disease (Ridker, 2009).

The relationship between CRP and future cardiovascular events has been consistent throughout the literature, and appears to be independent of age, smoking, blood pressure, and diabetes (Ridker, 2003). In fact, one study found CRP to be a strong predictor of risk of myocardial infarction (MI) 20 years after obtaining patient blood samples (Sakkinen, et al., 2002). The foregoing study, from the Honolulu Heart Program, used proportional hazards regression models with and without adjustment of confounding risk factors, and examined a male population. During the first five years of follow-up, the odds of an MI rose significantly with increasing levels of CRP when CRP was included in the model as a continuous risk factor. At 10 to 15 years of follow-up, a 2.1-fold excess in the odds of an MI was found in the top versus the bottom quartile of CRP (95% CI: 1.1-4.2). The overall finding was a 1.6-fold excess in the odds of an MI for men in the top versus bottom quartile of CRP (95% CI: 1.1-2.2) (Sakkinen, et al., 2002).

In a study that analyzed the baseline CRP levels and 10-year incidence of first MI or death related to coronary heart disease in the elderly, CRP levels were associated with increased 10-year risk of coronary heart disease, regardless of the presence of cardiac risk factors (Cushman, et al., 2005). Elderly subjects with elevated vascular risk displayed greater burden of inflammation (greater than 50% had CRP levels greater >3 mg/L at baseline, and <14% had CRP <1mg/L) when they were compared to middle-aged adults, thus indicating that CRP levels increase with age, after controlling for smoking, hypertension, disabetes, hyperlipidemia, and regular aspirin use (Cushman, et al., 2005).

In the Copenhagen City Heart Study, cross-sectional data indicated a strong association between CRP level and vascular risk (Zacho, et al., 2008). The researchers investigated the relationship between elevated CRP levels with risk of ischemic heart disease and cerebrovascular disease; CRP single-nucleotide polymorphisms with CRP

levels; and CRP polymorphisms with risk of ischemic heart and cerebrovascular disease. The researchers studied four independent cohorts of Caucasian people of Danish descent drawn from The Copenhagen City Heart Study, The Copenhagen General Population Study, The Copenhagen Ischemic Heart Disease Study, and The Copenhagen Carotid Stroke Study. The groups were defined such that no person appeared in more than one analysis group, and all participants were age and gender matched. The population consisted of 10,276 participants from the general population. Out of this sample, 1,786 subjects had ischemic heart disease and 741 had ischemic cerebrovascular disease.

The risk of ischemic heart disease and ischemic cerebrovascular disease was increased by a factor of 1.6 to 1.3, respectively, in those participants who had CRP levels above 3 mg/L, compared to those participants who had CRP levels below 1 mg/L. (Zacho, et al., 2008). Although CRP polymorphisms were associated with marked increased in CRP levels ($p<0.0001$), CRP polymorphisms were not associated with an increased risk of ischemic cerebrovascular or ischemic heart disease (p for trend, 0.28 to 0.94) (Zacho, et al., 2008)

Along these lines, another investigation sought to understand the relationship between CRP levels and cardiovascular disease while controlling for traditional risk factors associated cardiovascular illness (low-density lipoprotein cholesterol (LDL), high-density lipoprotein (HDL), blood pressure, smoking status, body mass index (BMI), history of diabetes, history of CHD, history of peripheral artery disease, and history of stroke or transient ischemic attack. (Sattar, et al., 2007). In this study, 5,680 participants aged 70-82 years of age (mean 75.8 years) were recruited. Of these, 865 participants had preexisting vascular disease (including coronary, cerebral, or peripheral) or increased risk due to hypertension, smoking, or diabetes.

Participants were randomized to receive either pravastatin (40mg/day) or a placebo, and were followed every 3 months, with a mean 3.2 years of follow up. CRP levels were higher in subjects who had a subsequent primary end-point event (defined as definite or suspected death from cardiovascular heart disease), compared with those who did not [mean 3.64 mg/L (SD, 3.08 mg/l) versus 3.01 mg/l (SD, 3.05 mg/L, $p<0.0001$]. However, baseline CRP added minimally to risk prediction beyond conventional predictors and did not relate to the extent of pravastatin benefit [hazard ratio 1.36 (95% CI, 1.15 to 1.61)] after adjusting for traditional predictors and body mass index (Sattar, et al., 2007).

Interleukin-18 (IL-18)

Interleukins are secreted by leukocytes (both macrophages and T-cells) and endothelium to stimulate the body's immune response to trauma. This response is more pronounced after burns or other damage leading to inflammation, but in the majority of healthy people, IL-18 is not detectable (Gracie, Robertson, & McInnes, 2003). Interleukins can act as both inflammatory (i.e, IL-1b, IL-6, IL-18, TNF-alpha) and anti-inflammatory (i.e., IL-4, IL-6) cytokines.

Il-18 is a relatively recently discovered cytokine, and its in vivo function is largely unexplored (Gracie, et al., 2003). It is pro-inflammatory, and is produced primarily by macrophages. After stimulation by IL-18, white blood cells release interferon gamma, another vital cytokine that plays an important role in the body's immune response following acute inflammation (Gracie, et al., 2003). In fact, IL-18 was originally named and identified as an interferon-gamma inducing factor (IFNy-inducing factor), and its role in the production of IFNy is particularly pertinent to atherosclerosis

due to the effect of IFNy on the progression of stable to unstable plaques (Gupta, et al., 1997). IL-18 is expressed in a wide range of cells, including macrophages, T cells, B cells, Kupfer cells, osteoblasts, dendritic cells, microglia and astrocytes (Gracie, et al., 2003).

Several lines of research link elevated levels of IL-18 to risk for coronary heart disease. A population-based study of coronary events, The Prospective Epidemiologic Study of Myocardial Infarction (PRIME) (Blankenberg, et al., 2003), investigated the relationship between baseline plasma levels of IL-18 and the subsequent incidence of coronary events over a 5-year follow-up. The sample included 10,600 healthy European men aged 50-59 years. Plasma IL-18 level was identified as an independent predictor of coronary events over other inflammatory markers such as CRP, IL-6, fibrinogen, and lipids.

In a prospective study of 1,229 patients with a documented history of coronary artery disease, plasma levels of IL-18 and other markers of inflammation (i.e., CRP, fibrinogen, IL-6, and lipid serum levels) were measured. In this sample, 95 persons died of cardiovascular related events during the follow-up period (median period, 3.9 years). Serum CRP levels were significantly higher in those who died (68.4 versus 58.7 pg/mL; P =0.0001). The investigators then conducted a hazard risk ratio for future cardiovascular death, and found that with increasing levels of IL-18 quartiles, hazard risk ratio for death also increased (68.4 versus 58.7 pg/mL; P = 0.0001 CI 1.21 to 1.76; P for trend = 0.0001). Even after adjustment for other factors, including CRP and fibrinogen, the relationship between IL-18 and the hazard risk ratio remained unchanged. These results suggest that patients within the highest quartile of IL-18 had a 3.3 fold risk of

hazard increase compared with those in the first quartile (95% CI, 1.3 to 8.4, P=0.01) (Blankenberg, et al., 2002).

In other studies, the relationship between plasma levels of IL-18 and atherosclerosis was less clear. In the Dallas Heart Study, elevated IL-18 plasma levels were associated with risk factors for atherosclerosis, but this effect was attenuated after accounting for traditional cardiovascular risk factors (Zirlik, et al., 2007). Elevations of IL-18 were found in older, male, and obese participants, and these participants were more likely to have a history of diabetes, hypertension, tobacco use, lower HDL, hypertriglyceridemia, previous MI, and lower renal glomerular filtration rate. Elevations of plasma IL-18 were not associated with alcohol intake, hypercholesterolemia, family history of MI, or stable angina. As noted, this study found that higher levels of IL-18 were associated with poor cardiovascular outcomes in patients with stable coronary artery disease, acute coronary syndromes, and with restenosis after angioplasty. After adjusting for traditional risk factors (i.e., body mass index, diabetes, hypertension, tobacco use, alcohol use, hypercholesterolemia, etc.) the relationship between IL-18 and subclincial atherosclerosis disappeared. These findings suggest that IL-18 does not associate with carotid atherosclerosis as assessed by intimal thickness, and this finding has been replicated (Chapman, McQuillan, Beilby, Thompson, & Hung, 2006).

Lipoprotein-associated phospholipase (Lp-PLA_2)

Lp-PLA_2, also known as platelet activating factor acetylhydrolase (PAF-AH), is a member of the phospholipase A_2 enzyme superfamily. Lp-PLA_2 is considered a pro-inflammatory mediator involved with vascular inflammatory processes. Lp-PLA_2 attaches to LDL cholesterol particles in the bloodstream and often adheres to arterial

walls, inducing oxidation and triggering the atherogenic process. Lp-PLA_2 can promote the buildup of plaque in the arteries, and also produce molecules that attract immune cells to arterial walls. These molecules bind to monocytes (a type of white blood cell that, in response to inflammation, moves quickly to elicit an immune response), which are then converted to macrophages. This process can increase the amount of atherosclerotic buildup within arterial walls (Caslake & Packard, 2003).

Lp-PLA_2 interacts with the part of the molecule in which the sn2 fatty acid has been altered (Caslake & Packard, 2005). The enzymatic activity of this protein can produce lysophosphatidylcholine and oxidized non-esterified fatty acids. These oxidized fatty acids can, in turn, trigger the atherogenic process through the promotion of pro-inflammatory markers. Approximately 80% of circulating Lp-PLA_2 is bound to LDL, approximately 15-20% is bound to HDL, and the remainder of this marker is bound to very low density lipoproteins (VLDL) particles in the blood. Thus, it has been suggested that Lp-PLA_2 that has bound to HDL might have cardioprotective properties, while Lp-PLA_2 that has bound to LDL might be more directly attributable to atherosclerosis at all stages in the process, from lipoprotein oxidation to endothelial dysfunction and plaque growth (Caslake & Packard, 2005).

There are two predominant streams of literature regarding the role of Lp-PLA_2 in cardiovascular disease. One stream investigates the deficiency of the factor, primarily within a Japanese population (Hiramoto, Yoshida, Imaizumi, Yoshimizu, & Satoh, 1997), and the second explores Lp-PLA_2 as a risk factor in epidemiology surveys in predominantly Western populations (Blankenberg, et al., 2003; Packard, et al., 2000).

Miwa et al. (1988) first identified a deficiency of the Lp-PLA_2 in the Japanese population (Miwa, et al., 1988). The deficiency resulted from a single point mutation

present in approximately 27% of the Japanese population (Stafforini, et al., 1996). Research on this mutation revealed an association with stroke (Yoshida, et al., 1998), peripheral artery occlusive disease (Unno, et al., 2000) and aortic aneurysms independent of other risk factors (Unno, et al., 2002).

The second stream of literature examines Lp-PLA_2 in Western populations, which has shown a consistent linking of Lp-PLA_2 levels to risk of atherosclerotic disease. In the West Scotland Coronary Prevention Study, a nested case-control design in 580 cases and 1,160 age and smoking-matched controls, was employed to investigate the relationship between Lp-PLA_2 and cardiovascular disease. In this study, Lp-PLA_2 level was found to be an independent risk factor for cardiovascular disease, with a twofold increased risk when Lp-PLA_2 levels were in the highest quintile compared with the lowest quintile (Caslake & Packard, 2005). Furthermore, this relationship was not influenced by other markers of inflammation including CRP, white cell count, or more traditional risk factors such as fibrinogen and lipid parameters (Packard, et al., 2000).

In an experimental, porcine model of diabetes and hypercholesterolemia, Lp-PLA_2 was found to augment inflammatory responses of leukocytes associated with proatherogenic conditions (Shi, et al., 2007). Additionally, this study found that a product of Lp-PLA_2, lysoPC, augmented the expression of several inflammatory mediators, while an inhibitor of Lp-PLA_2 stopped inflammatory responses that were induced by oxidized LDL in vitro (Shi, et al., 2007).

A preliminary report from the Atherosclerosis Risk in Communities Study showed that both Lp-PLA_2 and CRP were associated with coronary heart disease risk, even after adjusting for age, race, and gender (Ballantyne, et al., 2004). Specifically, Lp-PLA_2 level was weakly correlated with LDL-C (r=.036) and total cholesterol (r=0.23) in

both men and women. There was also a weak positive correlation with triglyceride Lp-PLA_2 levels (r=.013). Lp-PLA_2 levels in the highest tertile (≥422 ug/L) were associated with increased coronary heart disease (CHD) risk (1.78 HR, 95% CI 1.33 to 2.38) even when adjusted for age, gender, and race (Ballantyne, et al., 2004). There was an increased risk ratio for the highest versus lowest quintile of Lp-PLA2, as well as for the highest versus lowest tertile of CRP. Even at low levels of LDL, Lp-PLA2 was significantly associated with CHD in models that had been fully adjusted (Ballantyne, et al., 2004).

This finding has not always been robust. In the Women's Health Study, for example, Lp-PLA_2 was found to be a predictor of cardiovascular risk in a sample defined as having myocardial infarction, stroke, or death due to coronary heart disease (matched for age and smoking with equal number of controls), but the association was attenuated in multivariate analysis when other factors were included, especially LDL cholesterol (Blake, Dada, Fox, Manson, & Ridker, 2001).

Monocyte Chemoattractant Protein-1 (MCP-1)

Monocyte Chemoattractant Protein-1 (MCP-1) is a key chemokine in the pathogenesis of autoimmune diseases, chronic inflammatory disorders, and neuroinflammatory disease (Luster, 1998; Izikson, Klein, Luster, & Weiner, 2002). It is secreted by endothelial and monocyte-like cells (Chen, et al., 2003), and appears to be inactive in the normal brain (Yamagami, et al., 1999). Increased MCP-1 concentrations facilitate inflammatory responses and initiate mononuclear cell recruitment during vascular injury (Nilsson, 1993).

MCP-1 is a member of the largest family of chemokines, the CC chemokines (Coll, Alonso-Villaverde, & Joven, 2007). MCP recruits monocytes, memory T-cells, and dendritic cells to any areas of tissue injury, infection, and inflammation. It is widely recognized that insults to endothelium or smooth muscle cells initiate the production of chemoattractants. As macrophage death occurs, formation of a destabilizing lipid-rich core forms within the plaque, ending in extensive infiltration of leukocytes. Most of the leukocytes found in these lesions are monocytes or macrophages (approximately 80%), and T lymphocytes (approximately 10-20%) (Coll, et al., 2007). MCP-1, therefore, might play an important role in the initiation and pathogenesis of cardiovascular diseases.

Several studies found plasma MCP-1 levels highest in acute coronary syndromes, and lowest among healthy controls, despite overlap of levels between groups (Cipollone, et al., 2001; Economou, et al., 2001; Nishiyama, et al., 1998). First, in the Nishiyama et al study (1998), levels of circulating MCP-1 were measured in 46 patients with acute coronary syndrome and 30 patients with stable exertional angina. Plasma MCP-1 antigen levels were significantly higher in patients with acute coronary syndrome than in patients with angina ($p < 0.001$).

Cipollone, et al. (2001), tested plasma levels of MCP-1 before and 1, 5, 15, and 180 days after patients underwent percutaneous transluminal coronary angioplasty (PTCA). At baseline, MCP-1 levels did not differ, but following the procedure, restenotic patients had significantly higher ($p<0.0001$) elevated levels of MCP-1 than non-restenotic patients. Additionally, higher levels of MCP-1 were associated with restenosis, and were significantly correlated with increased monoctye activity. These findings also demonstrated that plasma level measured 15 days following the procedure was the only significant independent predictor of restenosis (beta=0.688, $p<0.0001$),

suggesting that MCP-1 production and macrophage accumulation may play an important role in restenosis after coronary angioplasty. The authors concluded that increased understanding of the mechanisms by which MCP-1 is produced and behaves after arterial injury may increase understandings of new therapies that limit the development of atherosclerosis (Cipollone, et al., 2001).

Economou et al. (2001) compared 28 patients undergoing PTCA with 28 healthy controls. Before the procedure, MCP-1 plasma in PTCA patients (441± 64 pg/ml) was similar to patients with coronary artery disease (430 ± 24 pg/ml) and significantly higher when compared with healthy controls (145± 17 pg/ml, $p<0.01$). At 3 months and 6 months following PTCA, MCP-1 levels rose significantly, ($p<0.01$). These results suggests that MCP-1 levels are stimulated and elevated in patients with coronary artery disease, and remain elevated for at least six months inpatients following PTCA. This elevation in MCP-1 levels may be due to the stimulation of the inflammation process that follows PTCA (Economou, et al., 2001).

In another study, MCP-1 levels were measured in 279 healthy volunteers, and 2,270 patients with acute coronary syndromes (de Lemos, et al., 2003). MCP -1 levels were significantly lower for healthy volunteers, 157 pg/ml (25^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles were 124, 196, 240, and 274 respectively) than for patients with coronary syndromes, 178 pg/ml (25^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles were 128, 238, 325, and 392 respectively) ($p<0.0001$) (de Lemos, et al., 2003). Additionally, MCP-1 levels were positively associated with female gender, older age, diabetes, hypertension, prior coronary artery disease, history or physical examination evidence of congestive heart failure, elevated levels of B-type natriuretic peptide, and renal insufficiency (de Lemos, et al., 2003).

Inflammatory Markers and their Association with Dementia

CRP

Alzheimer's disease (AD), the most common form of dementia, is neuropathologically characterized by the presence of neurofibrillary tangles and neuritic plaques, widespread neuronal loss, and the presence of amyloid-B peptide deposits. (Zaciragic, et al., 2007). Although CRP is largely synthesized in the liver, it is also produced in brain and other tissue (McGeer et al., 2001). In fact, a major finding linking blood inflammatory markers to AD was the discovery that the complement cascade, including CRP, is fully activated in AD (McGeer, Rogers, & McGeer, 2006). Once activated, this complement cascade appears to be a major cause of cell death in patients with AD (McGeer, et al., 2006).

Elevations in plasma CRP are associated with higher risk of AD (Engelhart, et al., 2004). CRP is present in the neuritic plaques and cortical neurons of AD patients but is undetectable in normal brains (Finch & Morgan, 2007). In one study, 37 subjects with probable AD (McKhann et al., 1984) were selected from a community-dwelling, geriatric population. This group was compared to 33 age-matched healthy controls. The results indicated significantly higher serum CRP concentration in patients with AD (Dimopoulos, et al., 2006). Mean CRP levels for those with AD (mean ± S.D 1.53 ± 0.97 mg/l) were significantly higher than those of healthy controls (mean ± S.D 0.72 ± 0.61 mg/l, $p<0.01$).

Zaciragic and colleagues (Zaciragic, et al., 2007) investigated serum CRP levels in 15 hospitalized and overweight patients aged 65 and over (mean age 73.46 years) with

AD, and 15 age-matched controls (mean age 69.93 years). All control participants had an MMSE score >28. Body mass index (BMI) and waist/hip ratio (WHR) was calculated for all participants. Age, systolic and diastolic blood pressure, BMI, and WHR did not differ significantly between groups. CRP levels in the group with probable AD were significantly higher than among the healthy controls ($p<0.0001$).

CRP levels have also been shown to increase in patients diagnosed with vascular dementia (VaD) (Nilsson, Gustafson, & Hultberg, 2008; Ravaglia, et al., 2007; Yamamoto, et al., 2005). One study consisted of 428 patients (182 males and 246 females) who underwent diagnostic procedures for psychiatric, nuerological, somatic, and laboratory investigations. Patients with AD were diagnosed in accordance with the NINCDS-ADRDA criteria (McKhann, et al., 1984), and those with VaD fulfilled the NINDS-AIREN criteria (Roman, et al., 1993). A comprehensive psychometric battery was used that measured several cognitive domains including verbal, visuospatial construction ability, attention, and episodic memory. Dementia was diagnosed in 239 patients, AD was diagnosed in 86 patients, VaD in 107 patients, and mixed VaD and AD and unclassified dementia in the remaining 46 patients. Participants with VaD demonstrated significantly higher CRP levels than those with AD ($p<0.0001$, CRP >3mg/l, 23 % in AD, vs. 55% in VaD). The authors did, however, find these results surprising as many previous studies demonstrated elevated CRP levels in patients with AD. Although levels of CRP were elevated in patients with VaD, the elevations did not reach significance when compared to controls.

In the Conselice Study of Brain Ageing (CSBA), the relationship between baseline levels of CRP, serum interleukin 6 (IL-6), plasma alpha-1-antichymotrypsin, and hyperhomocysteninemia and risk of incident of AD and VaD were investigated in 804

participants (427 women, 377 men, mean age 73.6 years; Ravaglia, et al., 2007). Education, APOE 4, history of cardiovascular disease, history of stroke, moderate physical activity, body mass index, plasma total homocysteine, serum creatinine, serum folate, serum vitamin B12, serum CRP, serum Interleukin 6, and plasma alpha-1 antichymotrypsin were determined. Covariates in the study included socio-demographic variables (i.e., age at baseline, gender, and educational status), physical activity (e.g., sedentary versus active lifestyle), diagnoses of stroke and cardiovascular disease, and body mass index. An analysis of the data indicated that risk of all-cause dementia was significantly associated with high levels of all markers, either alone or in combination, except for IL-6. Hazard ratios were calculated in order to determine an estimate of relative risk. This analysis resulted in 1.63 [1.1-2.39] for CRP, 1.62 [1.10-2.38] for ACT, 1.81 [1.22-2.68] for CRP/IL6, and 1.97 [1.25-3.10] for CRP/IL-6/ACT. Risk of AD was significantly associated only when markers were in combination [CRP/IL6, hazard ration=1.73 (1.04-2.86), and CRP/IL6/ACT hazard ratio 1.98 (1.11-3.51)].

Risk of VaD demonstrated a significant relationship with only CRP (hazard ratio=2.67 [1.31-5.46]), ACT (hazard ratio=2.64 [1.23-5.67]), and CRP/IL-6 (hazard ratio=2.11 [1.05-4.22]). After adjusting for confounders, CRP/IL-6 was the only marker significantly associated with both all-cause dementia (hazard ratio=1.57 [1.03-2.41]) and VaD (hazard ratio=2.56 [1.21-5.50]). Additionally, elevated CRP was significantly associated with VaD (hazard ratio=2.35 [1.00-5.52]). Interestingly, no inflammatory marker was associated with AD (Ravaglia, et al., 2007).

IL-18

Several studies have examined the relationship between IL-18, AD and VaD. Levels of IL-18 and transforming growth factor beta 1 (TGF-ß1), have been found elevated in patients with AD and VaD as compared with non-demented, age-matched subjects (Malaguarnera, Motta, Di Rosa, Anzaldi, & Malaguarnera, 2006). In this investigation, 40 patients with AD (16 men, 24 women, aged 55-90 years old, with a mean age of 73.2, ± 7.08 years) and 35 patients with VaD (19 men, 16 women, aged 61-88 years, with a mean age of 75.9, ± 6.91 years) were incorporated into the study over a three-year period. Patients were diagnosed as having AD who satisfied the following three criteria: 1) criteria on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition-revised (DSM-IV-R), 2) diagnostic criteria of the National Institute of Neurological and Communicative Disorders Association, and 3) those scoring ≤ 4 points on Hachinski's ischemic score were diagnosed as having AD. Controls included 30 healthy elderly participants (15 men and 15 women, aged 60-83 years, with a mean age of 73.5 ± 3.24 years of age).

Results revealed that IL-18 levels were significantly higher in AD and VaD subjects than in the healthy controls (43.62 ± 28.26 pg/mL, 26.38 ± 14.54, and 8.67 ± 3.03, respectively, $p < 0.001$). Further, IL-18 levels were significantly higher in AD patients compared with VaD patients. This suggests that the inflammatory cytokine system is more widely affected in AD than VaD (Malaguarnera, et al., 2006). The author's interpretation was that higher plasma levels of IL-18 in AD might result from the systemic expression of activated microglia which causes the expression of IFN-γ in neurons and, in turn, activates the microglia in a positive feedback loop that amplifies the immune response. The differences between AD and vascular dementia groups may be

due to size of the ischemic infarction (Malaguarnera, et al., 2006).

More recently, IL-18 production by peripheral blood cells was found to be increased in patients with AD, and was correlated with severity of cognitive impairment in 30 AD subjects and 25 healthy controls (Bossu, et al., 2008). Although no differences in IL-18 levels between AD patients and controls were found (mean pg/mL 306.6 ± 22.6 for patients with AD compared to mean pg/mL 312.4± 23.5 for healthy controls), there was a significant increase in the production of IL-18 by peripheral blood cells when compared to healthy controls indicating that IL-18 production is elevated during the disease process associated with AD (repeated measures ANOVA; $F=44.398$, $p<0.0001$). Additionally, a significant correlation was found between IL-18 production and cognitive decline in patients with AD, implicating inflammatory processes in the etiology of cognitive decline ($r=-0.407$, $p<0.05$) (Bossu, et al., 2008).

Lp-PLA_2

Associations between elevations in Lp-PLA_2, AD and VaD have only been recently researched, and only one investigation evidenced an association between Lp-PLA_2 and dementia. Van Oijen et al. (2006) conducted research with a population-based cohort as part of the Rotterdam Study. A random subcohort of 1,742 subjects was drawn from the at-risk group, and 77 had developed dementia during the follow-up period. An additional 225 participants with dementia from outside the subcohort were added. Out of the 302 incident dementia patients, AD was diagnosed in 222, including 22 with AD accompanied by cerebrovascular disease, 44 with VaD, 14 with dementia due to Parkinson's disease, and 22 with dementia due to other causes.

Results revealed that increasing levels of Lp-PLA_2 were associated with an increased risk of dementia. Specifically, hazard ratios were calculated and those participants in the upper quartile had a 56% higher risk of developing dementia than those in the lower quartile (van Oijen, et al., 2006). Controlling for BMI, HDL cholesterol, total cholesterol, carotid plaques, current smoking, systolic blood pressure, diabetes mellitus, C-reactive protein, white cell count, or the presence of the APOE e4 allele did not affect the estimates (van Oijen, et al., 2006). Thus, little research has been conducted on Lp-PLA_2 and this factor's relationship to cognitive functioning. However, the investigation that has been conducted did indicate an association between this factor and risk of dementia, indicating that elevations in LP-PLA_2 may have a potential role in the inflammatory processes associated with dementia risk.

MCP-1

Research has demonstrated a link between MCP-1 levels and AD, yet little if any research has been conducted on the presence of MCP-1 and vascular dementia. In research related to MCP-1 levels and the pathogenesis of AD, Sun and colleagues (2003) measured the levels of pha(1)-antichymotrypsin (ACT), alpha(1)-antitrypsin (AAT), interleukin-6 (IL-6), MCP-1 and oxidized low-density lipoprotein (oxLDL) in matched CSF and plasma of 141 patients with probable AD. A significant relationship between CSF and plasma levels of ACT ($r=.04$, $p<0.001$), IL-6 ($r=0.74$, $p<0.0001$), MCP-1 ($r=0.71$, $p<0.001$), and a significant yet weak relationship between CSF and plasma oxLDL ($r=0.22$, $p<0.05$) (Sun, et al., 2003). The fact that MCP-1 was found to be upregulated in the brains of patients with AD supports the potential role of MCP-1 in the pathogenesis of AD.

A study that investigated the relationship between the HIV-1 transactivator protein Tat MCP-1 and expression of dementia in patients with HIV (Conant, et al., 1998) found MCP-1 in the brains of patients with HIV-1 associated dementia. MCP levels in the cerebrospinal fluid of AIDS patients with and without dementia were examined. Specifically, the researchers collected Tat protein and serum MCP levels from four condition groups, all n=10: HIV-1 patients with associated dementia (HIVD), HIV-1 patients without dementia (HIVN), patients with multiple sclerosis (MS), and patients with non-inflammatory neurological conditions (NIN).

Both the HIVD and HIVN groups had significantly elevated levels of MCP-1 ($p<0.01$) when compared with those participants in both the MS and NIN groups (Conant, et al., 1998). Additionally, patients in the HIVD group had significantly higher levels of MCP-1 than those patients in the HIVN group ($p<0.002$). Further, MCP-1 levels were significantly higher in the CSF when compared to the serum in HIVD patients, suggesting that MCP-1 was intrathecally synthesized (Conant, et al., 1998).

There is also evidence that the risk of AD might be influenced by a particular polymorphism of the MCP-1 gene, the -2518 A/G polymorphism (Fenoglio, et al., 2004; Pola, et al., 2004). This single nucleotide polymorphism, found in the MCP-1 gene regulatory region at position -2518, influences the level of MCP-1 expression during the body's response to inflammation, or in its response to an inflammatory stimulus (Fenoglio, et al., 2004). Additionally, it has been reported that European-American men who have the A-2518G allele have higher levels of MCP-1 than men who do not, and may help explain differences in severity of pathologies among individuals who suffer from the same inflammatory diseases (Gonzalez, et al., 2002).

To investigate the potential importance of MCP-1 in the pathogenesis of AD and the effect of the presence of the A-2518G allele, researchers studied the distribution of the allele in 269 patients with AD and 203 healthy controls (Gonzalez, et al., 2002). Patients in the research group consisted of 191 women and 78 men, with mean age at disease onset of 75 years. All patients in this group underwent a series of examinations, including medical, neurological and screening laboratory tests, neurocognitive evaluation, and MRI, CT and PET, if indicated by a physician.

Dementia severity was assessed using the Clinical Dementia Rating (CDR) (Morris, 1993) and the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975). Disease duration was defined as the duration of time between patient's first reported symptoms and the formal clinical diagnosis. The 269 patients were divided into those with early disease onset (early disease onset group; <65 years, n=30), and those with late disease onset (late disease onset group; ≥ 65 years, n=239). The control group consisted of 203 subjects, matched for age and ethnic background (110 women, 93 men, mean age 72 years). None of the participants in the control group had developed dementia at a 6-month follow-up examination (Gonzalez, et al., 2002).

Results from the study were mixed. No differences in the frequency of the A-2518G single nucleotide polymorphism were found between AD patients and controls ($p > 0.05$), nor were there significant differences in MCP-1 levels found between AD and controls (930.1 ± 24.9 pg/ml versus 900.4 ± 33.6 pg/ml, $p>0.05$). However, when the researchers stratified for the presence of the mutated A-2518G allele, a significant difference was found in the increase of MCP-1 mean levels in the serum of AD patients (957.3 ± 28 pg/ml versus 871.4 ± 39.3 pg/ml, $p=0.03$), but in the control group, no significant finding emerged (932.4 ± 513.6 pg/ml versus 899.7 ± 58.8 pg/ml, $p>0.05$).

Thus, the researchers found that the effects exerted by the polymorphism occurred mostly in patients with AD, though there was a slight yet not significant finding in the control group (Gonzalez, et al., 2002). It was concluded that a possible explanation for this stronger effect in those patients with AD might be due to the interaction of the polymorphism with other activating factors specifically expressed during the development of AD (Gonzalez, et al., 2002).

In an examination of dementia patients in an ethnically homogenous Italian population (Pola, et al., 2004), 141 patients (mean age 77.6 ± 5.4 years and male to female ratio of 58:83) were studied. All participants in the study underwent brain CT scan, structured interview, formal neuropsychological testing, and MMSE. The Hachinski ischemic score was also used to help distinguish between AD, and multi-infarct dementia (MID). Controls for the study (mean age 76 ± 6.8 years, male to female ratio 99:107) were 206 age and gender-matched individuals, and MMSE and CT assessed cognitive function in these participants scan of the brain.

There was a significant difference in genotype distribution between AD patients and controls ($p<0.0001$), the frequency of the GG genotype was almost four times higher in those with AD than in the control group (21.3% versus 5.4%), and the G allele was significantly more frequent in AD (39.7% versus 25.0%, $p<0.0001$) (Pola, et al., 2004). Additional logistic regression analysis was used to adjust for the possible confounding factors of G/G genotype, hypertension, hypercholesterolemia, diabetes, and cardiovascular diseases. The researchers found that the GG genotype of the MCP-1 gene polymorphism was an independent risk factor for AD, and that patients who carried the GG genotype had risk of developing AD more than 5 times greater than AA homozygous individuals [OR 5.5 (95% CI, 2.3-12.9), $p<0.0001$] (Pola, et al., 2004).

The researchers also found that the GG genotype of the MCP-1 gene to be associated with increased risk of AD both in the absence [OR 2.0 (95% CI 1.1-3.5), p=0.009] and the presence [OR 8.5 (95% CI 3.6-20.3), p<0.0001] of the APOE ε4 allele. The above results are consistent with the hypothesis that inflammation is important in the pathogenesis of AD. However, these results, and those of other studies investigating the relationship between the polymorphism and MCP-1 levels, may support the hypothesis that differences in ethnic backgrounds and other clinical demographic variables may be influencing factors.

Relation of Inflammatory Factors to Cognitive Decline

Lp-PLA_2 and its relationship to cognitive decline has not been examined empirically. However, several studies have been conducted investigating the relationship between CRP and MCP-1 and cognitive decline, and one has looked at the relationship between IL-18 and cognitive function in octogenarians. These will be reviewed below.

CRP

Although the relationship between CRP and cardiovascular risk has been robust, the relationship between CRP and cognitive decline is less clear. A prospective study of 78 adults with established cardiovascular disease examined the association between CRP and five cognitive domains (i.e., global cognition, language, memory, visuospatial abilities, and attention-executive-psychomotor) (Hoth, et al., 2008). In patients followed over 1 year, high levels of CRP were associated with subtle declines in attention-executive-psychomotor performance (p = .04) after adjusting for the effects of age and

baseline cognitive performance, but not with change in language, memory, or visuospatial performance (Hoth, et al., 2008).

Plasma levels of CRP were also measured in 4231 older participants of the Women's Health Study and cognitive assessment was performed using 5 component tests (Weuve, Ridker, Cook, Buring, & Grodstein, 2006). A measure of general cognition was obtained using the Telephone Interview for Cognitive Status (Cook, Marsiske, & McCoy, 2009), a brief and reliable mental status examination that assessed orientation, immediate verbal recall, registration opposites, current events, serial subtraction, and counting among others. Verbal memory was assessed using the East Boston Memory test, and the Wechsler Memory Scale-Revised Logical Memory subtest (Wechsler, 1987). Additional delayed verbal memory tests, and category fluency were also included in the battery. In this study, cognitive function scores did not vary by quintile of CRP (Weuve, et al., 2006). Serum levels of CRP and cognitive function were also examined in a population-based sample of 97 women in Finland (Komulainen, et al., 2007). Specifically, 299 women (mean age 63.8 years) participated in a 1982 study to assess the association between CRP and cognitive function over a 12-year period. Of these women, 97 were available to participant again in the 2003 follow-up study.

Serum CRP levels and global cognitive function (MMSE) were assessed both at baseline and at 12-year follow-up. In the follow-up examination, cognitive assessment was augmented by the Word Recall Test for memory assessment (Heun, Burkart, Wolf, & Benkert, 1998), the Stroop Test (Golden, 1976), and the Letter-digit Substitution Test for cognitive speed (van der Elst, van Boxtel, van Breukelen, & Jolles, 2006). Disease as diagnosed by a physician, medication use, smoking, alcohol consumption (drinks/week), and physical activity (session per week) were also assessed at baseline and follow-up.

Depressive symptomatology was assessed using the Zung self-report, 20-item measure (Zung, 1965). Additionally, measures of body weight, height, waist circumference, and blood pressure were also measured at baseline and follow-up. Age, education, depression, hormone replacement therapy, smoking, LDL cholesterol and BMI were controlled for.

The researchers found that higher baseline CRP levels were associated with poorer memory performance at 12-year follow-up as indicated by the significant linear trend ($p=0.048$) for women with low (<1.0 mg/l), average (1.0-3.0 mg.l), and high (>3.0 mg.l) serum CRP levels. Higher serum CRP levels were also associated with poorer memory after adjustment for age, education, and depression (standardized regression coefficient $\beta = -0.757$, 95% CI -1.491 to -0.023, $p=0.043$), and additional adjustment for the use of hormone replacement therapy, smoking, serum LDL cholesterol, and BMI ($\beta = -0.709$, 95% CI -1.461 to 0.043, $p=0.064$). Adjustment for fasting blood glucose and systolic blood pressure slightly weakened the association ($\beta= -0.688$, CI 95% -1.466 to 0.070, $p=0.075$). Serum CRP level was not associated with cognitive speed (sum of z-scores from the Letter-digit Substitution Test and the Stroop Test; $p=0.886$) or MMSE ($p=0.803$) at 12-year follow-up.

MCP-1

One piece of research has looked at the relationship between MCP-1 and cognitive decline following cardiac surgery (Reis, et al., 2007). Researchers were interested in post-surgical concentrations of inflammatory makers in the CSF, and their potential relationship with neuropsychiatric complications. 12 patients who had been diagnosed with coronary heart disease participated in the "cardiac cohort" group (those patients who would be undergoing OP-CABG surgery), and 6 patients who were

scheduled for non-cardiac operations (4 for hemicolectomy and 2 for esophagectomy) made up the "non-cardiac" group. All participants scored 26 or higher on the MMSE to rule out gross dementia. The BDI, Hamilton Scale, and Geriatric Depression Scale were used to assess for depression. No participants were diagnosed with depressive symptomatology. CSF levels of IL-8, Rantes (a cytokine that is a member of interleukin-8 superfamily), IP-10, and MCP-1 were measured in both groups before surgery, and at one-week follow-up. At baseline, inter-group statistical analysis revealed no significant differences in blood markers ($p>.05$). Changes were observed in IL-8, with significant changes following surgeries for both groups (34.59 ± 7.15 vs. 99.45± 6.35 for cardiac group, $p=6.58 \times 10^{-9}$, and 27.44± 7.17 vs. 66.63 ± 15.18, $p=9.72 \times 10^{-5}$ for controls; Reis, et al., 2007).

Heart surgery also resulted in decreases in Rantes (19.87± 15.71 vs. 9.37± 3.65, $p=0.07$). The levels of both IP-10, and MCP-1 remained unchanged, however, following surgery for both groups (IP-10 254.41 ± 160.01 vs. 224.55 ± 214.39, $p=.39$, and MCP-1 140.37 ± 40.98 vs. 147.16 ± 37.98, $p=0.38$). The researchers found this result surprising, given that MCP-1 has demonstrated elevations following MI, and in patients with restenosis following percutaneous transluminal coronary angioplasty (Cipollone, et al., 2001; Matsumori, et al., 1997).

IL-18

Little research has been conducted on the effects of IL-18 on cognitive decline, but one investigation did look at a single nucleotide polymorhpism (SNP) of the IL-18 inflammatory marker in octogenarians (Krabbe, et al., 2009). This polymorphism is proposed to affect the binding transcription factors and transcriptional rate of cytokines,

and thus, act as a risk factor for cognitive decline through their effect on the production rate of cytokines. The researchers also examined SNPs specific to TNF-∝, IL-6, and IL-10 in a population of healthy 85-year-old participants from Denmark.

The group of participants was drawn from a larger research study that was initiated in 1964, and was made up of 976 individuals born in 1914. Follow-ups were conducted at the age of 60, 70, 75, 80, and 85, and the current investigation evaluated the inflammatory markers and their relation to cognitive decline in the 80-85 year old sample. The sample was comprised of 119 individuals who had valid WAIS IQ assessments, and valid blood samples. Exclusion criteria were diagnoses of rheumatoid arthritis (n=2), and self-reported acute illness in the two-week period preceding the collection of the blood samples, leaving a total of 112 participants for study.

Cognition was assessed using the Danish translation of the WAIS (Wechsler, 1958), and number of subtests completed by each participant varied as a result of the participants' level of exhaustion and stamina. Prorated Verbal and Performance scores were computed for all participants who completed at least two subtests within each of the domains, and Full Scale IQs were computed for those participants with Verbal and Performance IQs. To derive a change score for cognitive function, the scores from the assessments taken when participants were 85-years old were subtracted from assessments taken when participants were 80-years old. Additionally, the MMSE was administered in the 85-year old group to further assess cognitive functioning and as a dementia-screening tool.

As mentioned above, single nucleotide polymorphisms in the genes encoding IL-18, TNF-∝, IL-6, and IL-10 were collected via blood draw, and information about smoking (yes vs. no), alcohol consumption and physical activity [(1) "mainly sitting", 2)

"light physical activity", 3) "physical activity more than 4 hours per week, or sport or competitive sports")] were obtained from self-report measures. Alcohol consumption above 168g per week was used as a covariate, in addition to non-fasting serum cholesterol, BMI, mean arterial pressure (MAP), educational levels (scored on a 1-3 point scale) and vocational training (scored on a 1-5 point scale), and participants activities of daily living (which was measured using a scale of activities).

Results indicated that at age 85, participants homozygous for the IL-18-607G-137C halotype demonstrated lower Performance scores than the other participants when adjusted for sex and education (R^2= 24%). When the researchers controlled for BMI, smoking, MAP, and cholesterol levels, the association was stronger (R^2= 43%). Participants who carried the TNF-308GA allele had a tendency to have lower Verbal IQ at age 85 than GG carriers, but this result did not reach statistical significance when adjusted for smoking, BMI, MAP and cholesterol. This research did not demonstrate an association between any of these factors for those in the 80-year-old group. Neither IL-6-174, IL-1-1082 SNPs demonstrated association to IQ at for either the 80 year-old-group or the 85 year-old group. There were associations, however, between increases in TNF-∝, IL-6 and sTNFR-II and lower Full Scale IQ, and with both lower Verbal and Performance IQ scores, yet there no association between circulating levels of IL-18 and IQ scores at age 85.

A primary finding from this investigation is that the IL-18 halotype was a risk factor for lower Performance IQ in those participants over 85 (Krabbe, et al., 2009). IL-18 SNPs are associated with TNF-∝ and activation of the TNF system, which can reflect current levels of brain functioning (Dik, et al., 2005). The authors posited that these

factors are biomarkers of brain function individuals above the age of 80 (Dik, et al., 2005).

Thus, the relationship between MCP-1, CRP, and IL-18 and cognitive decline has not been a robust nor consistent finding throughout the literature, and little if any research on LP-PLA_2 has been conducted, so it is unclear how elevations of this factor could effect cognitive functioning. Further, no research has looked at the possibility that subclinical levels of cognitive decline could be related to prior elevations in those blood markers associated with atherosclerosis and vascular disease.

Potential Covariates associated with Blood Inflammatory Markers and Cognition

In order to be determine the potentially isolated affects of CRP, IL-18, Lp-PLA2, and MCP-1 on cognitive function, several covariates have been identified that will be utilized in the analysis of the data. As mentioned throughout the review of literature, various streams of research have examined variables that potentially contribute to atherosclerosis and levels of the pro-inflammatory markers under investigation, which can in turn potentially contribute to cognitive dysfunction. They include the following: hypertension (HTN), hyperlipidemia (HLD), diabetes status, smoking status, waist circumference, estimated glomerular filtration rate (eGFR), presence of apolipoprotein E 4 allele (APOE 4), as well as the demographic variables of age, gender, and ethnicity.

Hypertension

Hypertension (HTN) is a chronic medical condition in which there is an elevation in the pressure that aterial blood exerts against arterial walls. Hypertension is defined as a resting systolic blood pressure over 140 mm Hg and or a resting diastolic blood

pressure over 90 mm Hg (Beers, Porter, Jones, Kaplan, & Berkwits, 2006). Blood pressure increases with age, and approximately 2/3 of people over the age of 65 have HTN (Beers, et al., 2006).

There is a potential association between vascular inflammation and HTN, and studies have been conducted that link the inflammatory processes associated with HTN to several inflammatory blood markers, including IL-18 (Zirlik, et al., 2007), CRP (Chae, et al., 2001), and MCP-1(de Lemos, et al., 2003), and LP-PLA_2 (Persson, et al., 2007). Whether inflammation causes structural and functional changes in arterial walls that lead to HTN, or is simply a consequence of HTN remains unclear (Sprague & Khalil, 2009).

Hyperlipidemia

Hyperlipidemia (HLD) is an elevation of lipids in the blood stream that can accelerate the process of atherosclerosis, which in turn can increase the risk of heart disease, stroke and other vascular illness (Beers, et al., 2006). Factors contributing to HLD include obesity, sedentary lifestyle, smoking, or medical conditions including diabetes, kidney disease, hypothyroidism and pregnancy (Beers, et al., 2006). Men over the age of 45, women over the age of 55, and those with family history of HLD are at greater risk for developing the condition (Beers, et al., 2006).

There are five major families of blood lipoproteins; chylomicrons, very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Although the function of lipoproteins is to carry water-insoluble lipids and cholesterol in the bloodstream, slight functional differences exist. For diagnosis of HLD, LDL, otherwise known as “bad cholesterol”, and HDL, or “good cholesterol” are most relevant (Beers, et al., 2006).

Studies have found lower levels of HDL associated with higher CRP levels (Cushman, et al., 2005), IL-18 (Zirlik, et al., 2007). MCP-1 levels have also been found to be elevated in hyperlipidemic patients (Kowalski, et al., 2003). Approximately 80% of Lp-PlA_2 is bound to LDL while 15-20% is bound to HDL, suggesting that the former might be more attributable to atherosclerosis and vascular disease, while the later may be cardio-protective (Chapman, et al., 2006).

Diabetes Mellitus Status

Diabetes Mellitus (DM) is a disease in which the body does not produce adequate amounts of the pancreatic hormone, insulin, or when body cells become insensitive to the action of insulin in transporting glucose across cell membranes. This results in elevated glucose levels in blood which can lead to vascular disease, peripheral neuropathy and predisposition to infection (Beers, et al., 2006). There are two main types of DM; Type 1 (insulin dependent) or Type 2. In type 1 diabetes the pancreas secrets little to no insulin, thus those with type 1 DM are required to inject insulin. In type 2 DM, the pancreas still produces insulin, but not a sufficient amount. Type 2 DM often occurs in people over 35, and in those who are overweight. DM has also been shown to be a potent independent risk factor for atherosclerotic disease (Yang, et al., 2007).

In recent years, there has been an increased understanding between inflammatory processes and tissue damage associated with DM. Several studies demonstrate an association between DM and CRP (Festa, et al., 2002; Goldberg, 2009), MCP-1 (Herder, et al., 2006), IL-18 (Herder, et al., 2009; Miyauchi, et al., 2009), and LP-PLA_2 (Yang, et al., 2007). In fact, research demonstrates that biomarkers of inflammation can help identify individuals at risk for developing DM (Goldberg, 2009).

Cigarette Smoking

Cigarette smoking is a major risk factor for cardiovascular disease, and approximately 20% of deaths due to cardiovascular disease have been linked to smoking (Bazzano, et al., 2003). Smoking has been associated with traditional risk factors for cardiovascular disease and atherosclerosis, and recent research has shown that cigarette smoking can cause an elevation in pro-inflammatory substances. Those that have been researched include CRP (Bazzano, et al., 2003; de Lemos, et al., 2003), MCP-1 (Shimada, et al., 2009; Zou, et al., 2009), and IL-18 (McKay, et al., 2004). Little research has been conducted to determine an association between Lp-PLA_2 and cigarette use, so it is unclear what association cigarette use has on Lp-PLA_2 concentration within the blood.

Waist Circumference

Body fat is divided into two main storage compartments in the body, subcutaneous and intra-abdominal. It is composed of cells that contain trigylceride stores, and is influenced by age, gender, race, amount of physical activity, and total body adiposity (Ness-Abramof & Apovian, 2008). Being overweight or obese increases one's risk for cardiovascular disease, but the pattern of fat distribution is important in risk stratification. Specifically, more central and visceral fat accumulation (i.e., around the waistline) is associated with increased risk of DM and cardiovascular disease (Ness-Abramof & Apovian, 2008). The cutoffs for normal waist circumference, chosen by the National Heart, Blood, and Lungs institute, are > 40 inches (102 cm) in men, and >35

inches (88 cm) in women, independent of body mass index (BMI) or race (Ghandehari, et al., 2009).

Visceral body fat is most accurately measured by use of computed tomography (CT) or magnetic resonance imaging (MRI) (Ness-Abramof & Apovian, 2008). Waist circumference is thought to be an excellent surrogate measure of visceral fat, and has been strongly correlated with type 2 DM and cardiovascular disease (de Koning, Merchant, Pogue, & Anand, 2007; Ghandehari, et al., 2009; Ness-Abramof & Apovian, 2008). In fact, obesity as measured by increased waist circumference (i.e., greater than 102 cm for men, and 88 cm for women) has been linked to risk factors of cardiovascular disease including HTN, HDL, type 2 diabetes mellitus, increased risk of cardiovascular morbidity and mortality, and systemic inflammation (Ghandehari, et al., 2009; Madsen, et al., 2009).

Associations have been found between increased waist measurements and elevated levels of pro-inflammatory markers, including CRP (Bochud, et al., 2009; Eiriksdottir, et al., 2009), IL-18 (Botella-Carretero, et al., 2007; Madsen, et al., 2009), and MCP-1 (Chacon, et al., 2008; Kaur, et al., 2009), and little is known about the relationship of Lp-PLA_2 to waist circumference.

Glomerular Filtration Rate

Glomerular Filtration Rate (GFR) is the amount of plasma that the kidneys can clear of a particular substance in a particular unit of time (Laterza, et al., 2002). The best method of determining GFR rate is to measure how the kidney clears particular exogenous substances, including inulin, iohexol, DPTA, among others (Laterza, et al., 2002). However, these measures are both time and labor intensive, and costly in clinical

application. Measurements of endogenous blood substances are used in more routine monitoring (i.e. lab tests during physicals), and these include Cystatin C among others (Laterza, et al., 2002). Normal ranges for GFR are 70 ± 14 mL/min/m2 for men, and 60 ± 10 mL/min/m2 for women (Laterza, et al., 2002).

Persons with chronic renal failure demonstrate persistent activation of the inflammatory response in the body, and thus, increased levels of pro-inflammatory substances (Pecoits-Filho, et al., 2003). Low GFR has been associated with elevated levels in CRP (Bavbek, et al., 2008; Wasen, et al., 2008), IL-18 (Nguyen & Devarajan, 2008), MCP-1 (Ibrahim & Rashed, 2008; Tam, et al., 2009), with no extensive research being conducted on the association of GFR to LP-PLA_2.

Apolipoprotein E 4 (APOE 4)

Apoliprotein E 4 (APOE 4) is class of apolipoprotein that carries cholesterol, and is essential for normal catabolism of triglyceride-rich lipoproteins and amyloid and tau production (Gunzburg, et al., 2007). Due to its association with circulating levels of lipids, it is often viewed as a major risk factor for atherosclerosis and coronary heart disease (Luc, et al., 1994; Novaro, et al., 2003; Tiret, et al., 1994). APOE 4 is mapped to chromosome 19, and is polymorphic, with three major alleles, APOE 2, APOE 3, and APOE 4.

APOE 4 is a component of systemic amyloid deposits, and a link has been demonstrated between APOE 4 and the occurrence of Alzheimer's disease (AD) and associated cognitive dysfunction (Breitner, et al., 1999; Gunzburg, et al., 2007). Inheritance of the APOE 4 allele can convey a higher risk and earlier onset of AD, and although family history itself is a risk factor, the presence of the APOE 4 allele accounts

for approximately 50% of this genetic variance (Bookheimer & Burggren, 2009). There is also evidence of a relationship between APOE 4 genotype and cognitive decline across the lifespan of individuals without AD (Blair, et al., 2005; Kozauer, et al., 2008). There are studies, however, that have looked specifically at the association between two pro-inflammatory blood markers and APOE 4.

Demographic Variables

Several demographic variables have been shown to covary with inflammatory factors. Aging has been associated with complex changes in the immune system and inflammatory occurrences that are involved in age-related disease such as atherosclerosis and AD (Bruunsgaard, Pedersen, & Pedersen, 2001). Positive relationships between age and CRP (Cushman, et al., 2005; de Lemos, et al., 2003), MCP-1 (de Lemos, et al., 2003; Deo, et al., 2004b), IL-18 (Hung, McQuillan, Chapman, Thompson, & Beilby, 2005; Zirlik, et al., 2007), and LP-PLA_2 have been reported (Ballantyne, et al., 2004; Caslake & Packard, 2005).

In terms of gender, some research has indicated that higher elevations of CRP and MCP-1 are found in women (Cushman, et al., 2005; de Lemos, et al., 2003), while IL-18 levels were higher in men (Blankenberg, et al., 2003; Zirlik, et al., 2007). The relationship of Lp-PLA_2 and gender is less clear, as some studies find no gender differences in elevations of Lp-PLA_2. (Albert, et al., 2005; van Oijen, et al., 2006). However, the Dallas Heart Study found lower Lp-PLA_2 levels in women (Brilakis, et al., 2008), while another investigation found that the relationship between Lp-PLA_2 levels and gender was moderated by body composition and fat distribution (Detopoulou, et al., 2009).

There are also racial differences in elevations of certain pro-inflammatory markers. Some studies have indicated higher elevations of CRP in Blacks than in Caucasian or Hispanics (Cushman, et al., 2005; Davis, et al., 2009), while Caucasians have shown higher MCP-1 elevations than Blacks or Hispanics (de Lemos, et al., 2003; Deo, et al., 2004b). IL-18 was shown to be lowest in Blacks, demonstrated intermediate levels of elevation in Hispanics, and was found to be highest in Caucasians (Brilakis, et al., 2008).

Summary

The research cited above has demonstrated that inflammatory factors are associated with the development of atherosclerosis, vascular dementia, and AD. It has also been proposed that these inflammatory factors might have a direct effect on cognitive function, although this association has not been as thoroughly investigated. Thus, the proposed investigation will examine the potential relationship between inflammatory factors such as C- reactive protein, Lipoprotein-associated phospholipase ($Lp\text{-}PLA_2$), Interleukin-18 (IL-18), and Monocyte Chemoattractant Protein-1 (MCP-1) and levels of cognitive function, and will explore how HTN, hyperlipidemia, diabetes status, smoking status, waist circumference, glomerular filtration rate, APOE 4 allele, and demographic variables (i.e, age, gender, ethnicity) contribute to these potential relationships.

APPENDIX B

AIMS AND HYPOTHESES OF COMPLETE STUDY

Primary Aim I: To investigate the relationship between cognitive function and levels of blood inflammatory markers.

Hypothesis 1: There will be an inverse relationship between MoCA scores and CRP levels.

Hypothesis 2: There will be an inverse relationship between MoCA scores and IL-18 levels.

Hypothesis 3: There will be an inverse relationship between MoCA scores and MCP-1 levels in the blood.

Hypothesis 4: There will be an inverse relationship between MoCA scores and L_p-PLA_2 levels in the blood.

Primary Aim II: To investigate the relationship between vascular risk factors and cognitive function.

Hypothesis 6: Participants with multiple risk factors will obtain lower MoCA scores that those with a single risk factor.

Primary Aim III: To determine if the presence of the APOE 4 allele strengthens the relationship between inflammatory markers and cognitive function.

Hypothesis 7: Those participants with the presence of the APOE 4 allele will demonstrate a stronger inverse relationship between CRP and MoCA scores than those participants who do not have the presence of the APOE 4 allele.

Hypothesis 8: Those participants with the presence of the APOE 4 allele will demonstrate a stronger inverse relationship between IL-18 and MoCA scores than those participants who do not have the presence of the APOE 4 allele.

Hypothesis 9: Those participants with the presence of the APOE 4 allele will demonstrate a stronger inverse relationship between Lp-PLA_2 and MoCA scores than those participants who do not have the presence of the APOE 4 allele.

Hypothesis 10: Those participants with the presence of the APOE 4 allele will demonstrate a stronger inverse relationship between MCP-1 and MoCA scores than those participants who do not have the presence of the APOE 4 allele.

APPENDIX C

COMPLETE METHODOLOGY OF STUDY

Data was examined from the Dallas Heart Study (DHS) I and II. DHS I, initiated in 1999, was a population-based study designed to produce unbiased population estimates of biologic and social variables that could indicate differences in cardiovascular health between different ethnicities at the community level and to support hypothesis-driven research on the underlying mechanisms that might contribute to these differences (Victor, et al., 2004). The sample drawn from the DHS is a large and diverse sample, and allows for the examination of cognitive changes that may be associated with elevations in pro-inflammatory markers. The Dallas Heart Study began in 1999 and concluded in January of 2010.

Participants

DHS researchers selected a probability sample from the estimated population of 1.43 million civilian, non-institutionalized, English or Spanish-speaking adults, aged 18 to 65 years who had established primary residences in Dallas County, Texas, from July 2000 to January 2002. The United States Postal Service sequence file was used as the primary sampling frame, which included 841,943 housing unit addresses in Dallas County. This process helped to increase the efficiency of the sampling design by dispersing the sample throughout the country. 15,088 addresses were selected from 10 geographic strata of different ethnic compositions. In order to achieve the desired 50:50 split in ethnicity in the final sample, selection probabilities were greater in areas with larger concentrations of African Americans. Participants with data from the four blood

markers of interest obtained from DHS I, and cognitive testing from DHS II will be used in this investigation. All participants included did meet the following inclusion criteria:

1) Able to speak and read English
2) Had measurements of pro-inflammatory blood markers drawn from DHS
3) Completed a valid Montreal Cognitive Assessment (MoCA)
4) Provided informed consent.

Data for the current analysis were drawn from the stratified random sample obtained in DHS I who returned to DHS II. Of those seen in DHS II (N=2,911), 796 participants were excluded because they were not present at DHS I, leaving 2,115 participants. One participant was excluded because they requested that their data not be used for research purposes, 46 duplicate data entries were deleted, and 40 additional participants were excluded due to missing data that prevented the calculation of a MoCA total score. Thirty-seven additional participants were excluded for history of stroke, and one was deleted due to unclear history of stroke. Although Spanish forms of the MoCA were used for Spanish speakers, clerical problems and potential lack of comparability of scores resulted in the elimination of an additional 86 participants, leaving a total of 1904 subjects. Complete data on all four pro-inflammatory markers drawn at DHS 1 were available for 997 of these participants and certain analyses were limited to this group of subjects. Sample size varied for individual analyses depending on missing data.

Procedures

In DHS I, three separate and sequential visits with participants were used to collect epidemiologic and biologic data. Visit 1 was to participants' households, and

consisted of 60-minute, computer-assisted health interviews of 6,101 participants (54% African American) with measurements of blood pressure, heart rate, and weight in adults 18 to 65 years of age. Trained field interviewers conducted these interviews. Participants self-assigned ethnic category from the same structured list of categories used in the Third National Health and Nutrition Examination Survey. Five blood pressure and heart rate measurements were then taken in the seated position using an automatic oscillometric device (Series #52,00, Welch Allyn, Inc., Arden North Carolina) that has been validated against direct catheter-based measurements of intra-arterial pressure. Field interviewers were trained to use the device and to select an appropriately sized cuff.

At visit 2, blood and urine were collected at the home of each participant. The blood was maintained at 4°C for ≤ 4 hours before being processed in the laboratory. Forty mL of blood collected in tubes containing citrate EDTA were centrifuged (1,000 *g* for 15 minutes) at 4°C. Plasma was removed and stored in 1-ml (n=1), 500-µl (n=2), and 100-µl (n≥ 60) aliquots at -80°C. In addition, plasma samples were sent to various laboratories for the quantification, sizing, and analysis of lipoproteins. Leukocytes were isolated from 40 ml of blood, and 1/3 of the cells were used to extract genomic DNA. The remaining 2/3 of the leukocytes isolated from blood were isolated and frozen so that they could later be used. Phlebotomy tubes were labeled with accession numbers (which included the phlebotomy date), participants' names, and bar code identifiers. Visit 3 consisted of extensive clinic examinations, and imaging studies with these participants were conducted using several modalities.

THE DHS II study was performed in a day at St. Paul Hospital, and included 2911 subjects. During this one-day long visit, patient's provided social and medical history, underwent a physical examination, and provided both blood and urine samples.

The Montreal Cognitive Assessment (MoCA) was administered to all consenting participants and efforts were made to administer this test at the beginning of the day to minimize the possible effects of fatigue. MoCA administration took approximately 10 minutes per subjectss. Trained personneladministered all MoCA tests, and efforts were made to ensure inter-rater reliability for MoCA scoring and administration. Scoring followed standard MoCA procedures, and each test was double-checked for accuracy prior to entry into the data bank. All scored MoCA's were then entered into the DHS data bank, and data protected through university security procedures. Once entered, scores were spot-checked for final accuracy. Total MoCA scores were analyzed as a continuous variable, and were further divided into tertiles in order to allow for comparisons between the highest and lowest scoring subject groups. The suggested one-point education correction for those with <= 12 years of education was not applied since education was used as a covariate when appropriate.

Data Analysis

All data was analyzed using SPSS version 18 (SPSS, Inc., Chicago IL). All missing data was filtered out via-list wise deletion. Analyses on missing data versus complete data was performed in order to compare participants with missing data to those without on similar measures using chi-square tests for categorical variables, and t-tests for continuous variables. Due to the large size of the sample being investigated, a significance level of .01 will be used in all analyses.

The first aim of the study involved investigating the relationship between each of the pro-inflammatory markers (i.e., CRP, IL-18, LP-PLA_2, and MCP-1) and cognitive function as measured by the MoCA. Correlation analysis was used to determine if there

was a relationship between blood markers and cognitive function. In order to assist with clarification of clinical interpretation of the correlations, the strength of significant correlation coefficients was designated as small (.10-.29), medium (.30-.49), and large (>.50) [Cohen, 1988]. In this preliminary analysis, no covariates were used. If analyses resulted in significant correlation between blood inflammatory markers and inflammation, logistic regressions were conducted to determine the model that best predicted cognitive dysfunction. For this analysis demographic variables were entered simultaneously in Block 1, and pro-inflammatory markers were added in Block 2.

The second aim of the study sought to investigate the relationship between vascular risk factors and cognitive function. Specially, it was hypothesized that there would be an inverse relationship between the number of vascular risk factors present in a participant and cognitive function as measured by the MoCA. For this hypothesis, a new variable was created that quantified the number of risk factors, and then correlations were conducted between this new variable and MoCA scores to determine this relationship. In more exploratory analysis, logistic regressions were conducted to investigate which combination of risk factors (i.e, HTN, HLP, DM, eGFR, waist circumference, and demographic variables) would best predict lowest scores on the MoCA. For this analysis, demographic variables were entered simultaneously in Block 1, and risk factor variables will be entered stepwise in Block 2.

The third aim of the study sought to determine if the presence of the APOE 4 allele strengthened the relationship between pro-inflammatory blood markers and cognitive function. More specifically, it was expected that those participants with the presence of the APOE 4 allele would demonstrate a stronger inverse relationship between blood markers and cognitive function. Additionally, it is expected that presence of the

APOE 2 allele would prove to ameliorate the relationship between inflammatory markers and cognitive function. APOE 4 was added to the logistic regressions described above. For these analyses, demographic variables were entered simultaneously into Block 1, inflammatory markers were entered into Block 2, and the APOE allele was entered into Block 3.

APPENDIX D

ADDITIONAL STUDY RESULTS

CHAPTER FOUR

Additional Study Results

Additional demographic data stratified by ethnicity and gender can be found in Tables 12 and 13. MoCA Total Scores for DHS participants as analyzed by ethnicity and gender can be viewed in Tables 12 and 13. ACNOVAs were conducted to determine differences in group means while controlling for age and education. In order to account for large unequal group sizes indicated by a significant Levene's test, the Brown-Forsythe F-ratio was utilized.

Caucasian participants had significantly higher MoCA total scores than all other racial groups, and Black subjects demonstrated significantly lower MoCA total scores than Hispanic participants. Education was significantly correlated with MoCA total score [r=.43, p<.001)], as was age [(r= -.199, p=.000]. There were no significant gender differences in MoCA total score for gender. Gender differences were examined using both chi square analysis and independent samples t-tests. Men were significantly more educated [t(1901) = 4.34), p <.001)], had significantly greater incomes above $40,000 per year [$x^2$(9) = 50.12), p <.001], had significantly greater waist circumference measurements [t(1562)=4.14, p<.001] and significantly lower BMI [t(1888) = -6.325, p<.001) than females. No significant differences were found in hypertension, diabetes, cholesterol or APOE status.

Insert Tables 12 and 13 about here

MoCA total scores were divided into tertile groups in order to investigate possible differences in demographic and biological variables between lowest scoring, middle scoring, and highest scoring individuals. These results can be viewed in Table 14. Significant differences were found between MoCA tertile groups for age [$F(3)=35.29$, $p<.001$], education [$F(3)=91.63$, $p<.001$] , waist circumference [$F(3)=9.27$, $p<.001$], smoking status [$\chi^2(4) = 23.80$, $p<.001$] , BMI [$\chi^2(6) = 25.12$] , hypertension [$\chi^2(2) = 65.96$, $p<.001$] , and diabetes [$\chi^2(2) = 12.16$, $p=.002$] .

Insert Table 14 about here

Aim One

Partial Spearman's correlations were conducted between MoCA total scores and CRP, IL-18, $LPPLA_2$, and MCP-1, with controlling for age and education and can be found in Table 6 and 7 above. Additional partial Spearman's correlations were conducted to determine if there were ethnic differences in associations between blood markers under consideration and global cognitive function, and those results can be viewed in Table 15. A significant correlation between CRP and MoCA total scores was found for Black participants ($r=-.100$, $p=.003$) There were no other significant correlations between MoCA total scores and other blood markers when analyzed by ethnicity.

Insert Table 15 about here

Aim Two

Several logistic regression analyses were conducted. When examining only demographic variables, education was the only significant variable predicting inclusion in the highest MoCA tertile group. However, only 55.1% were correctly predicted in this model (See Table 16). The other logistic regression analyses can be found in tables 9-11 above.

Insert Table 16 about here

Aim Three

The third aim of this study was to determine if the presence of the APOE 4 allele strengthened the relationship between inflammatory markers and cognitive function. A logistic regression was used to predict membership to lowest MoCA tertile group from demographic, biological, and health variables, with the addition of all APOE allele data added to this model. This analysis revealed no significant impact of the presence of APOE on cognitive function, and the presence of this allele did not strengthen the

relationship between blood marker elevations and cognitive function as hypothesized. In this analysis, 79.2% were correctly predicted into the lowest MoCA tertile, and the final adjusted model indicated education and being Caucasian decreased the odds of lowest MoCA tertile membership. These results can be viewed in Table 17.

Insert Table 17 about here

Correlations Between Biomarkers and MoCA domains

To better understand the relationship between biomarkers and domains of function assessed on the MoCA, variables were created for attention, executive function, language, and memory domains, and correlational analyses were then conducted with these variables and CRP, MCP-1, IL-18, and Lp-PLA_2. The attention domain was composed of Digit Span, Serial 7's and Vigilance. Executive function was composed of Trails, Cube drawing, Clock drawing, and Abstraction. Naming, Sentence Repetition, and Fluency composed the language domain, while Memory included Delayed Free Recall, Category recall, and Multiple Choice recall. Education was controlled for in this model. There were no significant correlations between CRP, MCP-1, Lp-PLA_2, and IL-18 and these domains. The results of this analysis can be viewed in Table 18.

Insert Table 18 about here

Pro-inflammatory Blood Markers by MoCA Tertile Group

To investigate if there were differences in mean elevations of inflammatory markers across MoCA tertile groups, MANCOVAs were conducted with education and age controlled for in the analysis. These results can be viewed in Table 19. Mean elevations for Lp-PLA_2 were significantly different by MoCA tertile, such that the group with the highest Lp-PLA_2 elevation was significantly different from those in the middle and lowest elevation groups [$F(2,992) = 7.30$, p=.001] , which were largely similar. No significant differences between mean elevations on all other biomarkers by MoCA tertile group were significant.

Insert Table 19 about here

Correlations Between MoCA Total Scores and Additional Biomarkers

Given that the current study did not demonstrate strong correlations between MoCA scores and CRP, MCP-1, IL-18, and Lp-PLA_2, exploratory analysis with additional biomarkers of inflammation drawn from the DHS I study was conducted. These markers included RAGE, MIP3, CCL11, Lipoprotein a (Lpa), ICAM1, lymphotoxin beta receptor (LTBR), VCAM 1, osteoprotegerin (OPG), caspase 3, adiponectin, CD40 ligand, interleukin-6 (IL-6), chemokin ligand 1 and 2 (CXCL 1, CSCL 2), arsin CAC agatston, leptin, placenta growth factor (PLGF), matrix

metalloproteinase-9 (MMP-9), pregnancy-associated plasma protein A (PAPPA), myeloperoxidase (MPO), and ana elisa units.

Correlation between these factors and MoCA total score resulted in a weak yet significant correlations for caspase 3 (r = -.012, p=.02). No other significant correlations were found. These results can be viewed in Table 20.

Insert Table 20 about here

APPENDIX E

ADDITIONAL TABLES OF RESULTS

Table 12

Descriptive Information by Ethnicity

	Caucasian M *(SD)* (N = 636)	Black M *(SD)* (N = 1019)	Hispanic M *(SD)* (N = 207)	Other M *(SD)* (N = 41)	Statistic	*p*
Gender - N (%)						
Male (%)	299 (47)	389 (38)	87 (42)	26 (63)	$\chi^2(3) = 20.21$	<.001*
Female (%)	338 (53)	630 (62)	120 (58)	15 (37)		
Education[1]	14.78 *(2.74)*	13.07 *(2.11)*	12.14 *(3.45)*	15.30 *(2.53)*	*F(3)* = 91.63	<.001*
Age[2]	44.48 *(10.46)*	42.85 *(10.46)*	39.27 *(10.02)*	40.85 *(10.23)*	*F*(3) = 13.81	<.001*
MoCA [3]	25.72 *(2.83)*	21.94 *(4.06)*	23.05 *(3.70)*	23.68 *(3.60)*	*F*(3) = 141.57	<.001*
Income N (%)						
<16K	32 (6)	208 (26)	24 (13)	4 (12)	$\chi^2(27) = 318.81$	<.001*
16-19,999K	11 (2)	50 (6)	15 (8)	2 (6)		
20-24,999K	24 (4)	90 (11)	25 (14)	1 (3)		
25-29,999K	31 (5)	89 (11)	14 (8)	1 (3)		
30-34,999K	45 (8)	86 (11)	15 (8)	3 (9)		
35-39,999K	31 (5)	59 (7)	19 (10)	3 (9)		
40-49,999K	72 (12)	79 (10)	18 (10)	4 (12)		
50-74,999K	143 (50)	102 (13)	32 (17)	8 (24)		
75-99,999K	93 (16)	36 (4)	12 (7)	6 (18)		
>=100K	96 (17)	17 (2)	11 (6)	2 (6)		
Waist Circum.[4]	96.66 *(16.29)*	102.22 *(17.50)*	98.22 *(15.89)*	92.58 *(13.85)*	*F*(3) = 14.59	<.001*

Table Continues

Table 12 (Continued)

Descriptive Information by Ethnicity

	Caucasian M *(SD)* (N = 636)	Black M *(SD)* (N = 1019)	Hispanic M *(SD)* (N = 207)	Other M *(SD)* (N = 41)	Statistic	*p*
APOE - N (%)						
E2/E2	5 (1)	8 (1)	0 (0)	0 (0)	$\chi^2(15) = 69.21$	<.001*
E2/E3	66 (11)	111 (13)	8 (4)	6 (16)		
E2/E4	11 (2)	39 (5)	1 (1)	1 (3)		
E3/E3	355 (61)	401 (48)	129 (71)	25 (68)		
E3/E4	134 (23)	238 (28)	41 (23)	4 (11)		
E4/E4	12 (2)	46 (6)	2 (1)	1 (3)		
Alcohol - N (%)						
Current Drinker	526 (83)	660 (65)	162 (78)	24 (59)	$\chi^2(6) = 93.00$	<.001*
Recent Abstainer	91 (14)	201 (20)	23 (11)	6 (15)		
Lifetime Abstainer	20 (3)	155 (15)	22 (11)	11 (27)		
Smoking - N (%)					$\chi^2(6) = 38.43$	<.001*
Current	146 (23)	294 (29)	43 (21)	8 (19)		
Past	148 (23)	127 (13)	37 (18)	8 (19)		
Never	341 (54)	598 (59)	127 (61)	25 (61)		

[1]Caucasian group significantly more educated than Black and Hispanic [1]Caucasina group significantly more educated than Black and Hispanic groups; Hispanic group significantly less educated than all other groups.

[2] Whites significantly older than Blacks and Hispanics

[3] Caucasian group significantly higher MoCA total score than all other groups; Black group also significantly lower MoCA total score than Hispanic group.

[4] Black group significantly higher waist circumference all other groups; Other group significantly smaller waist circumference than all groups.

Table 13

Demographic Information by Gender

	Male (N = 801) M *(SD)*	Female (N = 1103) M *(SD)*	Statistic	*p*
Ethnicity - N (%)				
African-American	389 *(49)*	630 *(57)*	$\chi^2(3) = 20.21$	<.001*
Caucasian	299 *(37)*	338 *(31)*		
Hispanic	87 *(11)*	120 *(11)*		
Other	26 *(3)*	15 *(1)*		
Education	13.90 *(2.78)*	13.36 *(2.60)*	$t(1901) = 4.34$	<.001*
Age	43.08 (*10.27*)	42.88(*10.70*)	*t(1902) =.163*	.687
MoCA Total Score	23.29 *(3.95)*	23.41 *(4.08)*	$t(1902) = -.659$	<.510
Median Income				
<16K	13%	20%	$\chi^2(9) = 50.12$	<.001*
16-19,999K	4%	6%		
20-24,999K	7%	10%		
25-29,999K	7%	9%		
30-34,999K	8%	10%		
35-39,999K	6%	7%		
40-49,999K	12%	10%		
50-74,999K	21%	15%		
75-99,999K	11%	8%		
>=100K	11%	5%		
Waist Circumference	101.62 *(14.84)*	98.03 *(18.35)*	$t(1562) = 4.14$	<.001*
APOE - N (%)				
E2/E2	3 *(.4)*	10 *(1)*	$\chi^2(5) = 5.40$	.370
E2/E3	84 *(12)*	107 *(11)*		
E2/E4	21 *(3)*	31 *(3)*		
E3/E3	396 *(57)*	514 *(54)*		
E3/E4	166 *(24)*	251 *(26)*		
E4/E4	21 *(3)*	40 *(4)*		

Table Continues

Table 13 (continued)

Demographic Information by Gender

	Male (N = 801) M *(SD)*	Female (N = 1103) M *(SD)*	Statistic	*p*
Alcohol - N (%)				
Current Drinker	626 *(78)*	746 *(68)*	$\chi^2(2) = 25.74$	<.001*
Recent Abstainer	112 *(14)*	209 *(19)*		
Lifetime Abstainer	63 *(8)*	145 *(13)*		
Smoking - N (%)				
Current	227 *(28)*	264 *(24)*	$\chi^2(2) = 15.67$	<.001*
Past	156 *(20)*	164 *(15)*		
Never	418 *(52)*	673 *(61)*		
BMI - N (%)				
<20	42 *(5)*	85 *(8)*	$\chi^2(3) = 57.07$	<.001*
20-25	184 *(23)*	236 *(22)*		
26-30	321 *(40)*	278 *(26)*		
>30	253 *(32)*	491 *(45)*		
Hypertension - N (%)				
Hypertensive	230 *(29)*	348 *(32)*	$\chi^2(1) = 1.67$	.197
Normotensive	557 *(71)*	739 *(68)*		
Diabetes - N (%)				
Diabetic	69 *(10)*	88 *(9)*	$\chi^2(1) = .280$	.597
Non-diabetic	631 *(90)*	880 *(91)*		
Hypercholesterolemia - N (%)				
Yes	87 *(13)*	108 *(11)*	$\chi^2(1) = .691$	.406
No	607 *(88)*	856 *(89)*		

Table 14

Demographic and Health Variables by MoCA Tertile

	Lowest M *(SD)* (N = 687)	Middle M *(SD)* (N = 558)	Highest M *(SD)* (N = 659)	Overall M *(SD)* (N = 1904)	Statistic	*p*
MoCA Total Score	18.97 *(2.85)* Min 7 Max 22	24.06 *(.80)* Min 23 Max 25	27.35 *(1.19)* Min 26 Max 30	23.36 (4.03) Min 7 Max 30	$F(2) = 65.88$	<.001*
Gender						
Male (%)	296 *(43)*	239 *(43)*	266 (40)	801 (42)	$\chi^2(2) = 1.13$	.569
Female (%)	391 *(57)*	320 *(57)*	392 (60)	1103 (58)		
Education	12.14 (*3.45*)	13.07 *(2.11)*	14.78 *(2.74)*	15.30 *(2.53)*	$F(3) = 91.63$	<.001*
Age	45.47 (*9.80*)	42.42 (*10.30*)	40.81 (*10.89)*	42.96 (*10.51*)	$F(3)=35.29$	<.001*
Ethnicity (%)						
Black	507 *(74)*	315 *(56)*	197 *(30)*	1019 *(54)*	$\chi^2(6) = 350.78$	<.001*
Caucasian	88 *(13)*	159 *(28)*	390 *(59)*	637 *(33)*		
Hispanic	80 *(11)*	70 *(13)*	57 *(9)*	207 *(11)*		
Other	12 *(2)*	15 *(3)*	14 *(2)*	41 *(2)*		
Waist Circum	101.74 *(17.31)*	99.63 *(16.18)*	97.32 *(17.23)*	99.55 *(17.04)*	$F(2) = 9.27$	<.001*
Cystatin C	.863 (*.24)*	.992 *(.29)*	.932 *(.31)*	.882(.32*)*	$F(2) = 1.099$	=.244
APOE						
E2/E2	6 *(1)*	4 *(1)*	3 *(1)*	13 *(1)*	$\chi^2(10)= 5.01$	.884
E2/E3	68 *(12)*	62 *(13)*	61 *(11)*	191 *(12)*		
E2/E4	20 *(3)*	16 *(3)*	16 *(3)*	52 *(3)*		
E3/E3	327 *(56)*	260 *(53)*	323 *(57)*	910 *(55)*		
E3/E4	141 *(24)*	132 *(27)*	144 *(25)*	417 *(25)*		
E4/E4	26 *(4)*	15 *(3)*	20 *(3)*	61 *(4)*		

Table Continues

Table 14 (Continued)

Demographic and Health Variables by MoCA Tertile

	Lowest M *(SD)* (N = 687)	Middle M *(SD)* (N = 558)	Highest M *(SD)* (N = 659)	Overall M *(SD)* (N = 1904)	Statistic	*p*
Smoking						
Current	209 *(30)*	154 *(28)*	128 *(19)*	491 *(26)*	$\chi^2(4) = 23.80$	<.001*
Past	102 *(15)*	101 *(18)*	117 *(18)*	320 *(17)*		
Never	376 *(55)*	304 *(54)*	411 *(63)*	1091 *(57)*		
BMI						
<20	36 *(5)*	28 *(5)*	63 *(10)*	127 *(7)*	$\chi^2(6) = 25.12$	<.001*
20-25	136 *(20)*	116 *(21)*	168 *(26)*	420 *(22)*		
26-30	217 *(32)*	181 *(33)*	201 *(31)*	599 *(32)*		
>30	288 *(42)*	232 *(42)*	224 *(34)*	744 *(39)*		
Hypertension						
Hypertensive	278 *(41)*	166 *(30)*	134 *(21)*	578 *(31)*	$\chi^2(2) = 65.96$	<.001*
Normotensive	396 *(59)*	385 *(70)*	515 *(79)*	1296 *(69)*		
Diabetes						
Diabetic	74 *(13)*	45 *(9)*	38 *(7)*	157 (*9)*	$\chi^2(2) = 12.16$	.002*
Non-diabetic	418 *(87)*	453 *(91)*	540 *(93)*	151 *(91)*		
High Cholesterol	77 *(13)*	51 *(10)*	67 *(12)*	195 *(12)*	$\chi^2(2) = 2.03$	.363
Yes	511 *(87)*	444 *(90)*	598 *(88)*	1463 *(88)*		
No						

Table 15

Partial Spearman's Correlations for Biomarker and MoCA Total Scores by Ethicity

			MCP-1	CRP	IL-18	$LPPLA_2$
Black	MoCA	Correlation	-0.07	-.100*	-0.05	-0.01
		Sig.	.052	.003	.270	.759
		N	855	859	580	840
White	MoCA	Correlation	0.01	-0.02	-0.05	0.00
		Sig.	.749	.594	.327	.986
		N	575	582	344	561
Hispanic	MoCA	Correlation	-0.11	0.06	-0.18	-0.01
		Sig.	.127	.430	.063	.943
		N	180	179	104	176
Other	MoCA	Correlation	0.22	-0.01	-0.24	-0.21
		Sig.	.189	.952	.332	.237
		N	37	36	19	33

Variables controlled for in the analysis: Age and Education

Table 16

Logistic Regression Predicting Lowest MoCA tertile for Demographic Variables

								95% C.I. for OR	
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
Model 1	Age	0.04	0.24	.443	1	.556	1.00	0.99	1.01
	Education	0.97	0.13	19.38	1	.000	1.13	1.39	1.59
	Male	-0.24	0.12	4.21	1	.039	.791	0.24	0.96
	Black	.111	.394	.080	1	.777	1.12	0.36	1.62
	White	.329	.395	.695	1	.405	1.39	2.31	13.46
	Hispanic	.093	.424	.049	1	.826	1.10	1.098	4.06
	Constant	-5.40	0.74	52.56	1	.000	0.00		
Final Model	Education	-0.98	0.13	18.746	1	.000	1.10	1.36	1.54
	Constant	-5.38	0.65	69.09	1	.000	0.00		

56.1% Predicted into Lowest MoCA Tertile Group

Table 17

Logistic Regression Predicting Lowest MoCA Tertile with Demographic, Health, Biomarker, and APOE4 Variables

								95% C.I.for OR	
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
Model 1	High Cholesterol	.365	.93	.935	1	.576	1.42	.222	9.04
	Low Cholesterol	-.235	.262	.738	1	.364	.820	.489	1.37
	Hypertensive	.254	.635	.389	1	.689	1.38	.391	4.64
	Normotensive	.001	.189	0.00	1	.997	1.00	.690	1.45
	Current Smoker	-21.98	40.80	0.00	1	1.00	.000	.001	.067
	Past Smoker	-.467	.228	.176	1	.675	.908	.580	1.42
	Never Smoked	-.097	0.33	13.61	1	.080	0.30	0.16	0.57
	Diabetic	.344	.306	1.30	1	.265	1.43	.756	2.56
	Waist Circum.	-.057	.067	4.98	1	.033	.987	.974	.998
	Current Drinker	.276	.223	1.60	1	.251	1.35	.889	2.04
	Recent Abstainer	.065	.332	.045	1	.854	1.01	.576	1.95
	Cystatin C	.301	.392	.349	1	.553	1.21	.623	2.57
	MCP-1	.000	.000	.278	1	.592	1.00	.993	1.00
	CRP	.000	.018	.000	1	.991	1.00	.954	1.03
	IL-18	.000	.000	.159	1	.646	1.00	1.00	1.26
	LP-PLA2	.000	.002	.186	1	.693	1.00	.967	1.03
	Male	-.376	.189	4.40	1	.034	.622	.449	.974
	Black	.213	.601	.127	1	.726	1.23	.324	4.02
	White	-.086	.601	.017	1	.001	1.08	.332	4.00
	Hispanic	.408	.651	.392	1	.531	1.50	.420	5.38

Table Continues

Table 17(Continued)

Logistic Regression Predicting Lowest MoCA tertile with Demographic, Health, Biomarker, and APOE4 Variables

							95% C.I. for OR	
		B	S.E.	Wald	df	Sig.	OR	Lower	Upper
	Education	.087	.031	7.66	1	.006	1.09	1.02	1.16
	E4 Versus no E4	.152	.568	.071	1	.789	1.164	1.05	1.45
	Constant	1.03	.812	1.62	1	.203	2.81		
Final Model	Education	-0.08	.004	3.92	1	0.09	.991	.981	1.01
	White	-.025	.961	3.26	1	0.04	.892	.975	.992
	Constant	.926	.471	3.86	1	.044	2.51		

79.2% Correctly predicted into lowest MoCA tertile group; N=584

Table 18

Partial Spearman's Correlations Between Biomarkers and MoCA Domains

		MCP-1	CRP	IL-18	$LPPLA_2$
Attention*	Correlation	-0.02	-0.08	-.006	0.07
	Significance	.485	.382	.841	.027
Executive Function*	Correlation	0.25	-0.07	.000	0.09
	Significance	.435	.154	.995	.022
Language*	Correlation	.006	-0.32	.038	.077
	Significance	.840	.102	.228	.025
Memory*	Correlation	-0.01	-0.02	.003	.004
	Significance	.741	.321	.992	.888

*Attention Domain composed of: Digit Span, Serial 7's, Vigilance
*Executive Fnx Domain composed of: Trails, Cube, Clock, Abstraction
*Language Domain composed of: Naming, Sentence Repetition, Fluency
*Memory Domain composed of: Delyed Free Recall, Category, Multiple Choice

Variables controlled for: Age and Education

Table 19

Proinflammatory Blood Markers by MoCA Tertile Group

	MoCA Tertile Groups			95% Confidence Interval	
		Mean	Std. Error	Lower	Upper
MCP 1	Lowest Tertile	205.67	10.05	185.94	225.40
	Middle Tertile	190.85	10.48	170.29	211.41
	Highest Tertile	195.38	10.47	174.83	215.93
IL-18	Lowest Tertile	692.43	38.26	617.36	767.51
	Middle Tertile	655.86	39.87	577.61	734.10
	Highest Tertile	629.59	39.85	551.38	707.79
Lp-PLA_2*	Lowest Tertile	181.30	3.06	175.29	187.31
	Middle Tertile	180.31	3.19	174.04	186.57
	Highest Tertile	196.02	3.19	189.75	202.28
CRP	Lowest Tertile	5.55	0.31	4.96	6.15
	Middle Tertile	4.72	0.32	4.09	5.34
	Highest Tertile	5.24	0.32	4.61	5.86

Variables Controlled for in model: Age and Education

* Groups Significantly Different on Lp-PLA_2 $F(2,992) = 7.30$, $p=.001$
Highest Elevation significantly different from Lowest and Middle Elevation

Table 20

Partial Spearman's Correlations Between MoCA Total Scores and Additional Biomarkers

	r	*p*
RAGE	.000	.940
MIP 3	.001	.743
CCL 11	-.08	.193
Lpa	-0.06	.192
ICAM 1	0.00	.833
LTBR	-0.04	.387
VCAM 1	.01	.897
OPG	-0.06	.671
Caspase 3	-.012	.020
Adiponectin	.05	.239
CD40 Ligand	--0.07	.230
Interleukin-6	0.05	.187
CXCL 1	-0.04	.455
CXCL 2	0.08	.075
Arsin CAC Agaston	0.00	.978
Leptin	0.07	.156
PLGF	.156	.020
MMP 9	-0.02	.834
PAPPA	0.03	.429
MPO	0.04	.429
Ana Elisa Units	0.00	.899

Variables Controlled for in analyses: Age and Education

APPENDIX F

THE MONTREAL COGNITIVE ASSESSMENT (MoCA)

Montreal Cognitive Assessment (MoCA)

The MoCA was utilized as the psychometric tool because of its brevity and sensitivity. It was developed as a screening tool for patients who present with mild cognitive complaints, and who usually perform in the normal range on the MMSE (Nasreddine, et al., 2005). Although there are several instruments currently in use by clinicians to assess cognitive impairment and dementia, the MMSE remains the most widely used, but is less useful in detecting mild cognitive changes (Ihl, Frolich, Dierks, Martin, & Maurer, 1992; Tombaugh & McIntyre, 1992; Wind, et al., 1997). In fact, approximately 80% of patients diagnosed with MCI score above 26 points on the MMSE, which has been clinically set as the diagnostic threshold for identification of dementia (Ihl, et al., 1992; Nasreddine, et al., 2005; Tombaugh & McIntyre, 1992; Wind, et al., 1997).

The MoCA (included as Appendix 1) is a 30-point test that requires approximately 10 minutes to administer. It is comprised of the following cognitive domains: visuospatial ability, naming, memory function, attention, language, abstraction, delayed recall, and orientation. Visuospatial abilities are assessed using a clock-drawing task, scoring 3 points for complete execution, and a three-dimensional cube drawing task, which receives 1 point for compete execution. Language is assessed using a three-item confrontation naming task (lion, rhinoceros, camel), repetition of two syntactically complex sentences (“ I only know that John is the one to help today”, and “The cat always hid under the couch when dogs were in the room”), and a phonemic fluency task (“tell me as many words you can think of that begin with the letter “f”).

Memory function is assessed via a 5-item word list that is repeated twice, followed by a 5-minute delayed recall trial that includes category cueing and multiple

choice. Executive function is assessed using an adaptation of the Trail Making Test Part B, the phonemic fluency task described above, and a two-item verbal abstraction task. Attention, concentration, and working memory are evaluated through a sustained attention task (target detection using tapping when the participant hears the letter "A"), a serial 7 subtraction task, and a digits forward and backward task. Finally, asking the patient for the date, month, year, day, and place of examination and city assesses orientation.

The MoCA was developed by Ziad Nasreddine (Nasreddine, et al., 2005) based on years of practice and his subsequent clinical intuition regarding domains of cognition often impaired in MCI. Initially, an easy to administer screening tool that examined 10 cognitive domains was developed. This tool was then modified over a 5-year period of clinical use. The first version was administered to 46 patients, mostly diagnosed with MCI or AD, and with an MMSE score of 24 or higher and impaired neuropsychological functioning. This subject pool was compared to 4 healthy controls. Five items were replaced due to poor discriminability, and scoring was adjusted to give increased weight to the most discriminant items (Nasreddine, et al., 2005)

The current form of the MoCA was then administered to three participant groups to help determine its sensitivity in detecting patients with AD and MCI: patients with mild AD, patients meeting criteria for MCI, and normal elderly controls. The MoCA was administered to all three groups, and sensitivity and specificity were compared with the MMSE using clinical diagnosis and neuropsychological evaluation as the criterion standard (Nasreddine, et al., 2005)

Tasks that discriminated between AD patients and normal controls were digit span, sustained attention, and serial 7 calculation. The authors note that these

components of the test require attentional processes that are largely preserved in patients with MCI (Nasreddine, et al., 2005). Patients with AD and MCI performed equally poorly on sentence repetition, and patients with MCI had greatest impairment on delayed recall. Summarily, then, all items were successful in discriminating between at least two of the groups being studied, and the majority of items on the MoCA discriminated between all three groups in a step-wise fashion (Nasreddine, et al., 2005).

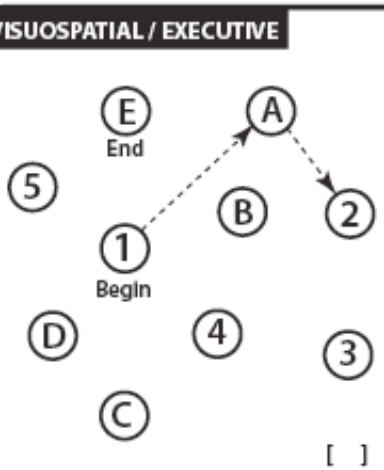
Sensitivity and specificity were then determined using clinical diagnosis as the standard for both patients and controls. A cut-off score of 26 was established as the best balance between sensitivity and specificity for the MCI and AD groups. Sensitivity was calculated separately for both the MCI and AD groups, and the MoCA demonstrated excellent sensitivity in detecting MCI (90%) and AD (100%). Specificity was defined as the percentage of normal controls that scored at or above the cutoff score of 26, and the MoCA demonstrated very good to excellent specificity (87%). Positive and negative predictive values for the MoCA were excellent for the MCI group (89% positive, 91% negative), as well as for AD (89% positive, and 100% negative) (Nasreddine, et al., 2005).

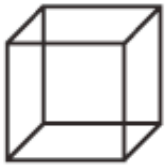
Test-retest reliability of the MoCA was measured on 26 participants. The measurements were taken on average 35.0 ± 17.6 days apart. The mean change score for the two administrations was 0.9 ± 2.5 points, and the correlation between the two evaluations was 0.92, $p<.001$). Internal consistency yielded a Cronbach alpha of 0.83, and item analysis revealed that trail making, cube drawing, clock drawing, naming, delayed recall, phonemic fluency, abstraction, and orientation discriminated reliably between all three groups, with AD participants performing most poorly, followed by those patients with MCI (Nasreddine, et al., 2005).

MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE





Copy cube

[ ] [ ]

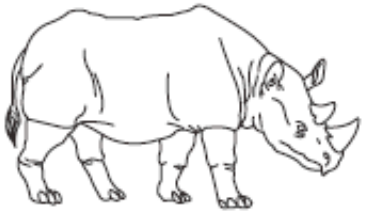
Draw CLOCK (Ten past eleven) (3 points)

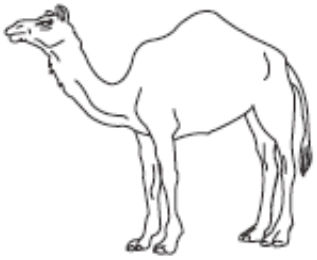
[ ] Contour [ ] Numbers [ ] Hands

POINTS

__/5

NAMING





[ ] [ ] [ ]

__/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

__/3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

__/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

__/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

__/2

DELAYED RECALL

Optional

	FACE	VELVET	CHURCH	DAISY	RED	
Has to recall words WITH NO CUE	[ ]	[ ]	[ ]	[ ]	[ ]	Points for UNCUED recall only
Category cue						
Multiple choice cue						

__/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

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Normal ≥ 26 / 30

TOTAL __/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

References

Albert, M. A., Glynn, R. J., Wolfert, R. L., & Ridker, P. M. (2005). The effect of statin therapy on lipoprotein associated phospholipase A2 levels. *Atherosclerosis, 182*(1), 193-198.

Anderson, L. (2008). *Assessing the impact of cognitive impairment in your state?* Paper presented at the National Center of Chronic Disease Prevention and Health Promotion, CDC.

Antoniu, S. A. (2010). Effects of inhaled therapy on biomarkers of systemic inflammation in stable chronic obstructive pulmonary disease. *Biomarkers, 15*(2), 97-103.

Baillargeon, J., & Wilkinson, G. S. (1999). Characteristics of the healthy survivor effect among male and female Hanford workers. *American Journal of Industrial Medicine, 35*(4), 343-347.

Ballantyne, C. M., Hoogeveen, R. C., Bang, H., Coresh, J., Folsom, A. R., Heiss, G., et al. (2004). Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation, 109*(7), 837-842.

Bavbek, N., Isik, B., Kargili, A., Uz, E., Uz, B., Kanbay, M., et al. (2008). Association of obesity with inflammation in occult chronic kidney disease. *Journal of Nephrology, 21*(5), 761-767.

Bazzano, L. A., He, J., Muntner, P., Vupputuri, S., & Whelton, P. K. (2003). Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States Ann Intern Med. 2003 Jun 3;138(11):I45; PMID: 12779312]. *Annals of Internal Medicine, 138*(11), 891-897.

Beers, M. H., Porter, R. S., Jones, T. V., Kaplan, J. L., & Berkwits, M. (Eds.). (2006). *The Merk Manual* (Eighteenth ed.). Whitehouse Station, NJ: Merck Researh Laboratories.

Bennett, D.A., Wilson, R.S., Schneider, J.A., Evans, D.A., Mendes de Leon, C.F., Arnold, S.E., Barnes, L.L., Blenias, J.L. (2003). Education modifies the relation of AD pathology to level of cognitive function in older persons. *Neurology, 60*, 1909-1915.

Blair, C. K., Folsom, A. R., Knopman, D. S., Bray, M. S., Mosley, T. H., Boerwinkle, E., et al. (2005). APOE genotype and cognitive decline in a middle-aged cohort. *Neurology, 64*(2), 268-276.

Blake, G. J., Dada, N., Fox, J. C., Manson, J. E., & Ridker, P. M. (2001). A prospective evaluation of lipoprotein-associated phospholipase A(2) levels and the risk of

future cardiovascular events in women. *Journal of the American College of Cardiology, 38*(5), 1302-1306.

Blankenberg, S., Luc, G., Ducimetiere, P., Arveiler, D., Ferrieres, J., Amouyel, P., et al. (2003). Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation, 108*(20), 2453-2459.

Blankenberg, S., Luc, G., Ducimetiere, P., Arveiler, D., Ferrieres, J., Amouyel, P., et al. (2003). Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction. *Circulation, 108*(20), 2453-2459.

Blankenberg, S., Tiret, L., Bickel, C., Peetz, D., Cambien, F., Meyer, J., et al. (2002). Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation, 106*(1), 24-30.

Bochud, M., Marquant, F., Marques-Vidal, P. M., Vollenweider, P., Beckmann, J. S., Mooser, V., et al. (2009). Association between C-reactive protein and adiposity in women. *Journal of Clinical Endocrinology & Metabolism, 94*(10), 3969-3977.

Bookheimer, S., & Burggren, A. (2009). APOE-4 genotype and neurophysiological vulnerability to Alzheimer's and cognitive aging. *Annual Review of Clinical Psychology, 5*, 343-362.

Botella-Carretero, J. I., Alvarez-Blasco, F., Martinez-Garcia, M. A., Luque-Ramirez, M., San Millan, J. L., & Escobar-Morreale, H. F. (2007). The decrease in serum IL-18 levels after bariatric surgery in morbidly obese women is a time-dependent event. *Obesity Surgery, 17*(9), 1199-1208.

Bossu, P., Ciaramella, A., Salani, F., Bizzoni, F., Varsi, E., Di Iulio, F., et al. (2008). Interleukin-18 produced by peripheral blood cells is increased in Alzheimer's disease and correlates with cognitive impairment. *Brain, Behavior, & Immunity, 22*(4), 487-492.

Breitner, J. C., Wyse, B. W., Anthony, J. C., Welsh-Bohmer, K. A., Steffens, D. C., Norton, M. C., et al. (1999). APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: the Cache County Study.[erratum appears in Neurology 2000 Jul 12;55(1):161-2]. *Neurology, 53*(2), 321-331.

Brilakis, E. S., Khera, A., McGuire, D. K., See, R., Banerjee, S., Murphy, S. A., et al. (2008). Influence of race and sex on lipoprotein-associated phospholipase A2 levels: observations from the Dallas Heart Study. *Atherosclerosis, 199*(1), 110-115.

Bruunsgaard, H., Pedersen, M., & Pedersen, B. K. (2001). Aging and proinflammatory cytokines. *Current Opinion in Hematology, 8*(3), 131-136.

Caslake, M. J., & Packard, C. J. (2003). Lipoprotein-associated phospholipase A2 (platelet-activating factor acetylhydrolase) and cardiovascular disease. *Current Opinion in Lipidology, 14*(4), 347-352.

Caslake, M. J., & Packard, C. J. (2005). Lipoprotein-associated phospholipase A2 as a biomarker for coronary disease and stroke. *Nature Clinical Practice Cardiovascular Medicine, 2*(10), 529-535.

Chae, C. U., Lee, R. T., Rifai, N., & Ridker, P. M. (2001). Blood pressure and inflammation in apparently healthy men. *Hypertension, 38*(3), 399-403.

Chapman, C. M., McQuillan, B. M., Beilby, J. P., Thompson, P. L., & Hung, J. (2006). Interleukin-18 levels are not associated with subclinical carotid atherosclerosis in a community population. The Perth Carotid Ultrasound Disease Assessment Study (CUDAS). *Atherosclerosis, 189*(2), 414-419.

Chen, Y., Hallenbeck, J. M., Ruetzler, C., Bol, D., Thomas, K., Berman, N. E., et al. (2003). Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. *Journal of Cerebral Blood Flow & Metabolism, 23*(6), 748-755.

Cipollone, F., Marini, M., Fazia, M., Pini, B., Iezzi, A., Reale, M., et al. (2001). Elevated circulating levels of monocyte chemoattractant protein-1 in patients with restenosis after coronary angioplasty. *Arteriosclerosis, Thrombosis & Vascular Biology, 21*(3), 327-334.

Cohen, J. (1998). A Power Primer. *Psychological Bulletin 112, 155-159.*

Coll, B., Alonso-Villaverde, C., & Joven, J. (2007). Monocyte chemoattractant protein-1 and atherosclerosis: is there room for an additional biomarker? *Clinica Chimica Acta, 383*(1-2), 21-29.

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., et al. (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proceedings of the National Academy of Sciences of the United States of America, 95*(6), 3117-3121.

Cook, S. E., Marsiske, M., & McCoy, K. J. (2009). The use of the Modified Telephone Interview for Cognitive Status (TICS-M) in the detection of amnestic mild cognitive impairment. *Journal of Geriatric Psychiatry & Neurology, 22*(2), 103-109.

Cukierman-Yaffe, T., Gerstein, H. C., Anderson, C., Zhao, F., Sleight, P., Hilbrich, L., et al. (2009). Glucose intolerance and diabetes as risk factors for cognitive impairment in people at high cardiovascular risk: results from the

ONTARGET/TRANSCEND research programme. *Diabetes Research & Clinical Practice, 83*(3), 387-393.

Cushman, M., Arnold, A. M., Psaty, B. M., Manolio, T. A., Kuller, L. H., Burke, G. L., et al. (2005). C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. *Circulation, 112*(1), 25-31.

Davignon, J., Cohn, J. S., Mabile, L., & Bernier, L. (1999). Apolipoprotein E and atherosclerosis: insight from animal and human studies. *Clinica Chimica Acta, 286*(1-2), 115-143.

Davis, K. A., Crow, J. A., Chambers, H. W., Meek, E. C., & Chambers, J. E. (2009). Racial differences in paraoxonase-1 (PON1): a factor in the health of southerners? *Environmental Health Perspectives, 117*(8), 1226-1231.

de Koning, L., Merchant, A. T., Pogue, J., & Anand, S. S. (2007). Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *European Heart Journal, 28*(7), 850-856.

de Lemos, J. A., Morrow, D. A., Sabatine, M. S., Murphy, S. A., Gibson, C. M., Antman, E. M., et al. (2003). Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation, 107*(5), 690-695.

Deo, R., Khera, A., McGuire, D. K., Murphy, S. A., Meo Neto Jde, P., Morrow, D. A., et al. (2004). Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *Journal of the American College of Cardiology, 44*(9), 1812-1818.

Desmond, D.W., Tatemichi, T.K., Paik. M., & Stern, Y. (1993). Risk Factors for cerebrovascular disease as correlates of cognitive function in a stroke-free cohort. *Archives of Neurology, 50*, 162.166.

Detopoulou, P., Nomikos, T., Fragopoulou, E., Panagiotakos, D. B., Pitsavos, C., Stefanadis, C., et al. (2009). Lipoprotein-associated phospholipase A2 (Lp-PLA2) activity, platelet-activating factor acetylhydrolase (PAF-AH) in leukocytes and body composition in healthy adults. *Lipids in Health & Disease, 8*, 19.

Dik, M. G., Jonker, C., Hack, C. E., Smit, J. H., Comijs, H. C., & Eikelenboom, P. (2005). Serum inflammatory proteins and cognitive decline in older persons. *Neurology, 64*(8), 1371-1377.

Dimopoulos, N., Piperi, C., Salonicioti, A., Mitropoulos, P., Kallai, E., Liappas, I., et al. (2006). Indices of low-grade chronic inflammation correlate with early cognitive deterioration in an elderly Greek population. *Neuroscience Letters, 398*(1-2), 118-123.

Dore, G.A., Elias, M.R., Robbins, M.A., Budge, M.M., and Elias, P.K. (2008). Relation between central adiposity and cognitive function in the Maine-Syracuse study: attenuation by physical activity. *Annals of Behavioral Medicine 35*(3), 341-350.

Economou, E., Tousoulis, D., Katinioti, A., Stefanadis, C., Trikas, A., Pitsavos, C., et al. (2001). Chemokines in patients with ischaemic heart disease and the effect of coronary angioplasty. *International Journal of Cardiology, 80*(1), 55-60.

Engelhart, M. J., Geerlings, M. I., Meijer, J., Kiliaan, A., Ruitenberg, A., van Swieten, J. C., et al. (2004). Inflammatory Proteins in Plasma and the Risk of Dementia. *Archives of Neurology, 61*(5), 668-672.

Eiriksdottir, G., Smith, A. V., Aspelund, T., Hafsteinsdottir, S. H., Olafsdottir, E., Launer, L. J., et al. (2009). The interaction of adiposity with the CRP gene affects CRP levels: age, gene/environment susceptibilty-Reykjavik study. *International Journal of Obesity, 33*(2), 267-272.

Evans, D. A., Beckett, L. A., Albert, M. S., Hebert, L. E., Scherr, P. A., Funkenstein, H. H., et al. (1993). Level of education and change in cognitive function in a community population of older persons. *Annals of Epidemiology, 3*(1), 71-77.

Fenoglio, C., Galimberti, D., Lovati, C., Guidi, I., Gatti, A., Fogliarino, S., et al. (2004). MCP-1 in Alzheimer's disease patients: A-2518G polymorphism and serum levels. *Neurobiology of Aging, 25*(9), 1169-1173.

Festa, A., D'Agostino, R., Jr., Tracy, R. P., Haffner, S. M., & Insulin Resistance Atherosclerosis, S. (2002). Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes, 51*(4), 1131-1137.

Finch, C. E., & Morgan, T. E. (2007). Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. *Current Alzheimer Research, 4*(2), 185-189.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12*(3), 189-198.

Galimberti, D., Fenoglio, C., Lovati, C., Venturelli, E., Ilaria, G., Corra, B., Scalabrini, D., Cierici, F., Mariani, C., Bresolin, N., Scarpini, E. (2006). Serum MCP-1 levels are increased in mild cognitive impairment and mild alzheimer's disease. *Neurobiology of Aging, 27*(12), 1763-1768.

Ghandehari, H., Le, V., Kamal-Bahl, S., Bassin, S. L., & Wong, N. D. (2009). Abdominal obesity and the spectrum of global cardiometabolic risks in US adults. *International Journal of Obesity, 33*(2), 239-248.

Goldberg, R. B. (2009). Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. *Journal of Clinical Endocrinology & Metabolism, 94*(9), 3171-3182.

Golden, C. J. (1976). Identification of brain disorders by the Stroop Color and Word Test. *Journal of Clinical Psychology, 32*(3), 654-658.

Gonzalez, E., Rovin, B. H., Sen, L., Cooke, G., Dhanda, R., Mummidi, S., et al. (2002). HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proceedings of the National Academy of Sciences of the United States of America, 99*(21), 13795-13800.

Gracie, J. A., Robertson, S. E., & McInnes, I. B. (2003). Interleukin-18. *Journal of Leukocyte Biology, 73*(2), 213-224.

Gunzburg, M. J., Perugini, M. A., & Howlett, G. J. (2007). Structural basis for the recognition and cross-linking of amyloid fibrils by human apolipoprotein E. *Journal of Biological Chemistry, 282*(49), 35831-35841.

Gupta, S., Pablo, A. M., Jiang, X., Wang, N., Tall, A. R., & Schindler, C. (1997). IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. *Journal of Clinical Investigation, 99*(11), 2752-2761.

Habib, G. S. (2009). Systemic effects of intra-articular corticosteroids. *Clinical Rheumatology, 28*(7), 749-756.

Herder, C., Baumert, J., Thorand, B., Koenig, W., de Jager, W., Meisinger, C., et al. (2006). Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. *Diabetologia, 49*(5), 921-929.

Heun, R., Burkart, M., Wolf, C., & Benkert, O. (1998). Effect of presentation rate on word list learning in patients with dementia of the Alzheimer type. *Dementia & Geriatric Cognitive Disorders, 9*(4), 214-218.

Hiramoto, M., Yoshida, H., Imaizumi, T., Yoshimizu, N., & Satoh, K. (1997). A mutation in plasma platelet-activating factor acetylhydrolase (Val279-->Phe) is a genetic risk factor for stroke. *Stroke, 28*(12), 2417-2420.

Hoth, K. F., Haley, A. P., Gunstad, J., Paul, R. H., Poppas, A., Jefferson, A. L., et al. (2008). Elevated C-reactive protein is related to cognitive decline in older adults with cardiovascular disease. *Journal of the American Geriatrics Society, 56*(10), 1898-1903.

Hsiung, G. Y., Sadovnick, A. D., & Feldman, H. (2004). Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the

Canadian Study of Health and Aging. *CMAJ Canadian Medical Association Journal, 171*(8), 863-867.

Hung, J., McQuillan, B. M., Chapman, C. M., Thompson, P. L., & Beilby, J. P. (2005). Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. *Arteriosclerosis, Thrombosis & Vascular Biology, 25*(6), 1268-1273.

Ibrahim, S., & Rashed, L. (2008). Correlation of urinary monocyte chemo-attractant protein-1 with other parameters of renal injury in type-II diabetes mellitus. *Saudi Journal of Kidney Diseases & Transplantation, 19*(6), 911-917.

Ihl, R., Frolich, L., Dierks, T., Martin, E. M., & Maurer, K. (1992). Differential validity of psychometric tests in dementia of the Alzheimer type. *Psychiatry Research, 44*(2), 93-106.

Jeong, S.K., Nam, H.S., Son, M.H., Son, E.J., Cho, K.H. (2005). Interactive Effect of Obesity Indexes and Cognition. *Dementia and Geriatric Cognitive Disorders 19,* 91-96.

Izikson, L., Klein, R. S., Luster, A. D., & Weiner, H. L. (2002). Targeting monocyte recruitment in CNS autoimmune disease. *Clinical Immunology, 103*(2), 125-131.

Kalra, L., Rambaran, C., Chowienczyk, P., Goss, D., Hambleton, I., Ritter, J., et al. (2005). Ethnic differences in arterial responses and inflammatory markers in Afro-Caribbean and Caucasian subjects. *Arteriosclerosis, Thrombosis & Vascular Biology, 25*(11), 2362-2367.

Kanaya, K., Abe, S., Sakai, M., Fujii, H., & Iwamoto, T. Changes in cognitive functions of patients with dementia of the Alzheimer type following long-term administration of donepezil hydrochloride: relating to changes attributable to differences in apolipoprotein E phenotype. *Geriatrics & gerontology international, 10*(1), 25-31.

Kaur, S., Panicker, S. R., James, T., Sarma, P. S., Thankappan, K. R., & Kartha, C. C. (2009). Association of monocyte chemoattractant protein-1 -2518 polymorphism with metabolic syndrome in a South Indian cohort. *Metabolic Syndrome & Related Disorders, 7*(3), 193-198.

Kidd, P. M. (2008). Alzheimer's disease, amnestic mild cognitive impairment, and age-associated memory impairment: current understanding and progress toward integrative prevention. *Alternative Medicine Review, 13*(2), 85-115.

Kilander, L., Nymabn, H., Boberg, M., Hansson, L., Lithell, H. (1998). Hypertension is related to cognitive impairment: A 20-year follow up of 999 men. *Hypertension, 31*(2); 780-786.

Kivipelto, M., Helkala, E.L., Hanninen, T., Laakson, M.P., Hallikainen, M., Alhainen, K., Soininen, H., Tummilehto, J., Nissinen, A. (2001). Midlfie vascular risk factors and late-life cognitive impairment: A population-based study.

Koh, K. K., Quon, M. J., Han, S. H., Chung, W. J., Ahn, J. Y., Seo, Y. H., et al. (2004). Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation, 110*(24), 3687-3692

Komulainen, P., Lakka, T. A., Kivipelto, M., Hassinen, M., Penttila, I. M., Helkala, E. L., et al. (2007). Serum high sensitivity C-reactive protein and cognitive function in elderly women. *Age & Ageing, 36*(4), 443-448.

Kowalski, J., Okopien, B., Madej, A., Zielinski, M., Belowski, D., Kalina, Z., et al. (2003). Effects of atorvastatin, simvastatin, and fenofibrate therapy on monocyte chemoattractant protein-1 secretion in patients with hyperlipidemia. [Clinical Trial Controlled Clinical Trial]. *European Journal of Clinical Pharmacology, 59*(3), 189-193.

Krabbe, K. S., Mortensen, E. L., Avlund, K., Pilegaard, H., Christiansen, L., Pedersen, A. N., et al. (2009). Genetic priming of a proinflammatory profile predicts low IQ in octogenarians. *Neurobiology of Aging, 30*(5), 769-781.

Laterza, O. F., Price, C. P., & Scott, M. G. (2002). Cystatin C: an improved estimator of glomerular filtration rate? *Clinical Chemistry, 48*(5), 699-707.

Launer, L.J., Masaki, K., Petrovitch, H., Foley, D., Havlik, R.J. (1995). The asscociation between midlife blood pressure levels and late life cognitive function: The Honolulu Asia Aging Study. *The Journal of the American Medical Association, 274(*23), 1845-1851.

Licastro, F., Masliah, E., Pedrini, S., & Thal, L. J. (2000). Blood levels of alpha-1-antichymotrypsin and risk factors for Alzheimer's disease: effects of gender and apolipoprotein E genotype. *Dementia & Geriatric Cognitive Disorders, 11*(1), 25-28.

Luc, G., Bard, J. M., Arveiler, D., Evans, A., Cambou, J. P., Bingham, A., et al. (1994). Impact of apolipoprotein E polymorphism on lipoproteins and risk of myocardial infarction. The ECTIM Study. *Arteriosclerosis & Thrombosis, 14*(9), 1412-1419.

Luster, A. D. (1998). Chemokines--chemotactic cytokines that mediate inflammation. *New England Journal of Medicine, 338*(7), 436-445.

Madsen, E. L., Bruun, J. M., Skogstrand, K., Hougaard, D. M., Christiansen, T., & Richelsen, B. (2009). Long-term weight loss decreases the nontraditional cardiovascular risk factors interleukin-18 and matrix metalloproteinase-9 in obese subjects.

Malaguarnera, L., Motta, M., Di Rosa, M., Anzaldi, M., & Malaguarnera, M. (2006). Interleukin-18 and transforming growth factor-beta 1 plasma levels in Alzheimer's disease and vascular dementia. *Neuropathology, 26*(4), 307-312.

Matsumori, A., Furukawa, Y., Hashimoto, T., Yoshida, A., Ono, K., Shioi, T., et al. (1997). Plasma levels of the monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 are elevated in patients with acute myocardial infarction. *Journal of Molecular & Cellular Cardiology, 29*(1), 419-423.

McGeer, P. L., Rogers, J., & McGeer, E. G. (2006). Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. *Journal of Alzheimer's Disease, 9*(3 Suppl), 271-276.

McKay, A., Komai-Koma, M., MacLeod, K. J., Campbell, C. C., Kitson, S. M., Chaudhuri, R., et al. (2004). Interleukin-18 levels in induced sputum are reduced in asthmatic and normal smokers. *Clinical & Experimental Allergy, 34*(6), 904-910.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). 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, 34*(7), 939-944.

Miwa, M., Miyake, T., Yamanaka, T., Sugatani, J., Suzuki, Y., Sakata, S., et al. (1988). Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *Journal of Clinical Investigation, 82*(6), 1983-1991.

Miyauchi, K., Takiyama, Y., Honjyo, J., Tateno, M., & Haneda, M. (2009). Upregulated IL-18 expression in type 2 diabetic subjects with nephropathy: TGF-beta1 enhanced IL-18 expression in human renal proximal tubular epithelial cells. *Diabetes Research & Clinical Practice, 83*(2), 190-199.

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules.[see comment]. *Neurology, 43*(11), 2412-2414.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society, 53*(4), 695-699.

Ness-Abramof, R., & Apovian, C. M. (2008). Waist circumference measurement in clinical practice. [Review]. *Nutrition in Clinical Practice, 23*(4), 397-404.

Nguyen, M. T., & Devarajan, P. (2008). Biomarkers for the early detection of acute kidney injury. [Review]. *Pediatric Nephrology, 23*(12), 2151-2157.

Nilsson, J. (1993). Cytokines and smooth muscle cells in atherosclerosis. *Cardiovascular Research, 27*(7), 1184-1190.

Nilsson, K., Gustafson, L., & Hultberg, B. (2008). C-reactive protein: vascular risk marker in elderly patients with mental illness. *Dementia & Geriatric Cognitive Disorders, 26*(3), 251-256.

Nishiyama, K., Ogawa, H., Yasue, H., Soejima, H., Misumi, K., Takazoe, K., et al. (1998). Simultaneous elevation of the levels of circulating monocyte chemoattractant protein-1 and tissue factor in acute coronary syndromes. *Japanese Circulation Journal, 62*(9), 710-712.

Novaro, G. M., Sachar, R., Pearce, G. L., Sprecher, D. L., & Griffin, B. P. (2003). Association between apolipoprotein E alleles and calcific valvular heart disease. *Circulation, 108*(15), 1804-1808.

Packard, C. J., O'Reilly, D. S., Caslake, M. J., McMahon, A. D., Ford, I., Cooney, J., et al. (2000). Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. *New England Journal of Medicine, 343*(16), 1148-1155.

Pearson, T. A. (2002). New tools for coronary risk assessment: what are their advantages and limitations? [Review]. *Circulation, 105*(7), 886-892.

Pecoits-Filho, R., Heimburger, O., Barany, P., Suliman, M., Fehrman-Ekholm, I., Lindholm, B., et al. (2003). Associations between circulating inflammatory markers and residual renal function in CRF patients. [Research Support, Non-U.S. Gov't]. *American Journal of Kidney Diseases, 41*(6), 1212-1218.

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update.[erratum appears in J Clin Invest. 2003 Jul;112(2):299]. *Journal of Clinical Investigation, 111*(12), 1805-1812.

Persson, M., Hedblad, B., Nelson, J. J., & Berglund, G. (2007). Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. *Arteriosclerosis, Thrombosis & Vascular Biology, 27*(6), 1411-1416.

Pola, R., Flex, A., Gaetani, E., Proia, A. S., Papaleo, P., Di Giorgio, A., et al. (2004). Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism and risk of Alzheimer's disease in Italians. *Experimental Gerontology, 39*(8), 1249-1252.

Ravaglia, G., Forti, P., Maioli, F., Chiappelli, M., Montesi, F., Tumini, E., et al. (2007). Blood inflammatory markers and risk of dementia: The Conselice Study of Brain Aging. *Neurobiology of Aging, 28*(12), 1810-1820.

Reis, H. J., Teixeira, A. L., Kalman, J., Bogats, G., Babik, B., Janka, Z., et al. (2007). Different inflammatory biomarker patterns in the cerebro-spinal fluid following heart surgery and major non-cardiac operations. *Current Drug Metabolism, 8*(6), 639-642.

Ridker, P. M. (2003). Clinical application of C-reactive protein for cardiovascular disease detection and prevention.[see comment]. *Circulation, 107*(3), 363-369.

Ridker, P. M. (2009). C-reactive protein: eighty years from discovery to emergence as a major risk marker for cardiovascular disease. *Clinical Chemistry, 55*(2), 209-215.

Rockwood, K., Middleton, L. E., Moorhouse, P. K., Skoog, I., & Black, S. E. (2009). The inclusion of cognition in vascular risk factor clinical practice guidelines. *Clinical Interventions In Aging, 4*, 425-433.

Roman, G. C., Tatemichi, T. K., Erkinjuntti, T., Cummings, J. L., Masdeu, J. C., Garcia, J. H., et al. (1993). Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology, 43*(2), 250-260.

Sakkinen, P., Abbott, R. D., Curb, J. D., Rodriguez, B. L., Yano, K., & Tracy, R. P. (2002). C-reactive protein and myocardial infarction. *Journal of Clinical Epidemiology, 55*(5), 445-451.

Sattar, N., Murray, H. M., McConnachie, A., Blauw, G. J., Bollen, E. L., Buckley, B. M., et al. (2007). C-reactive protein and prediction of coronary heart disease and global vascular events in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). *Circulation, 115*(8), 981-989.

Schunkert, H., & Samani, N. J. (2008). Elevated C-reactive protein in atherosclerosis--chicken or egg? *New England Journal of Medicine, 359*(18), 1953-1955.

Shi, Y., Zhang, P., Zhang, L., Osman, H., Mohler, E. R., 3rd, Macphee, C., et al. (2007). Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. *Atherosclerosis, 191*(1), 54-62.

Shimada, T., Hiramatsu, N., Hayakawa, K., Takahashi, S., Kasai, A., Tagawa, Y., et al. (2009). Dual suppression of adipogenesis by cigarette smoke through activation of the aryl hydrocarbon receptor and induction of endoplasmic reticulum stress. *American Journal of Physiology - Endocrinology & Metabolism, 296*(4), E721-730.

Silvestrini, M., Gobbi, B., Pasqualetti, P., Bartolini, M., Baruffaldi, R., Lanciotti, C., et al. (2009). Carotid atherosclerosis and cognitive decline in patients with Alzheimer's disease. *Neurobiology of Aging, 30*(8), 1177-1183.

Sirin, S. R. (2004). *The relationship between socioeconomic status and school outcomes: Meta analytic review of research, 1990--2000*. Dissertation Abstracts International: Section B: The Sciences and Engineering.

Slooter, A. J., Bots, M. L., Havekes, L. M., del Sol, A. I., Cruts, M., Grobbee, D. E., et al. (2001). Apolipoprotein E and carotid artery atherosclerosis: the Rotterdam study. *Stroke, 32*(9), 1947-1952.

Sprague, A. H., & Khalil, R. A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical Pharmacology, 78*(6), 539-552.

Stafforini, D. M., Satoh, K., Atkinson, D. L., Tjoelker, L. W., Eberhardt, C., Yoshida, H., et al. (1996). Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. *Journal of Clinical Investigation, 97*(12), 2784-2791.

Strittmatter, W. J. (2000). Apolipoprotein E and Alzheimer's disease. *Annals of the New York Academy of Sciences, 924*, 91-92.

Sun, Y. X., Minthon, L., Wallmark, A., Warkentin, S., Blennow, K., & Janciauskiene, S. (2003). Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. *Dementia & Geriatric Cognitive Disorders, 16*(3), 136-144.

Tam, F. W., Riser, B. L., Meeran, K., Rambow, J., Pusey, C. D., & Frankel, A. H. (2009). Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CCN2) as prognostic markers for progression of diabetic nephropathy. *Cytokine, 47*(1), 37-42.

Tiret, L., de Knijff, P., Menzel, H. J., Ehnholm, C., Nicaud, V., & Havekes, L. M. (1994). ApoE polymorphism and predisposition to coronary heart disease in youths of different European populations. The EARS Study. European Atherosclerosis Research Study. *Arteriosclerosis & Thrombosis, 14*(10), 1617-1624.

Tombaugh, T. N., & McIntyre, N. J. (1992). The mini-mental state examination: a comprehensive review. *Journal of the American Geriatrics Society, 40*(9), 922-935.

Unno, N., Nakamura, T., Kaneko, H., Uchiyama, T., Yamamoto, N., Sugatani, J., et al. (2000). Plasma platelet-activating factor acetylhydrolase deficiency is associated with atherosclerotic occlusive disease in japan. *Journal of Vascular Surgery, 32*(2), 263-267.

Unno, N., Nakamura, T., Mitsuoka, H., Uchiyama, T., Yamamoto, N., Saito, T., et al. (2002). Association of a G994 -->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with risk of abdominal aortic aneurysm in Japanese. *Annals of Surgery, 235*(2), 297-302.

van der Elst, W., van Boxtel, M. P., van Breukelen, G. J., & Jolles, J. (2006). The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. *Journal of Clinical & Experimental Neuropsychology: Official Journal of the International Neuropsychological Society, 28*(6), 998-1009.

van Oijen, M., van der Meer, I. M., Hofman, A., Witteman, J. C., Koudstaal, P. J., & Breteler, M. M. (2006). Lipoprotein-associated phospholipase A2 is associated with risk of dementia. *Annals of Neurology, 59*(1), 139-144.

Victor, R. G., Haley, R. W., Willett, D. L., Peshock, R. M., Vaeth, P. C., Leonard, D., et al. (2004). The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. *American Journal of Cardiology, 93*(12), 1473-1480.

Villeneuve, S., Belleville, S., Massoud, F., Bocti, C., Gauthier, S., (1998). Impact of vascular risk factors and diseases on cognition in persons with cognitive impairment. *Dementia and Geriatric Cognitive Disorder, 27*, 375-381.

Wasen, E., Isoaho, R., Vahlberg, T., Kivela, S. L., & Irjala, K. (2008). Association between markers of renal function and C-reactive protein level in the elderly: confounding by functional status. *Scandinavian Journal of Clinical & Laboratory Investigation, 68*(6), 484-491.

Wechsler, D. 1958. The Measurement and Appraisal of Adult Intelligence. Williams & Wilkins.

Wechsler, D. (1987). *Wechsler Memory Scale-Revised.* San Antonio: The Psychological Corporation.

Weuve, J., Ridker, P. M., Cook, N. R., Buring, J. E., & Grodstein, F. (2006). High-sensitivity C-reactive protein and cognitive function in older women. *Epidemiology, 17*(2), 183-189.

Wind, A. W., Schellevis, F. G., Van Staveren, G., Scholten, R. P., Jonker, C., & Van Eijk, J. T. (1997). Limitations of the Mini-Mental State Examination in diagnosing dementia in general practice. *International Journal of Geriatric Psychiatry, 12*(1), 101-108.

Yaffe, K., Lindquist, K., Shlipak, M. G., Simonsick, E., Fried, L., Rosano, C., et al. (2008). Cystatin C as a marker of cognitive function in elders: findings from the health ABC study. *Annals of Neurology, 63*(6), 798-802.

Yamagami, S., Tamura, M., Hayashi, M., Endo, N., Tanabe, H., Katsuura, Y., et al. (1999). Differential production of MCP-1 and cytokine-induced neutrophil chemoattractant in the ischemic brain after transient focal ischemia in rats. *Journal of Leukocyte Biology, 65*(6), 744-749.

Yamamoto, H., Watanabe, T., Miyazaki, A., Katagiri, T., Idei, T., Iguchi, T., et al. (2005). High prevalence of Chlamydia pneumoniae antibodies and increased high-sensitive C-reactive protein in patients with vascular dementia. *Journal of the American Geriatrics Society, 53*(4), 583-589.

Yang, C. Y., Chen, H. H., Huang, M. T., Raya, J. L., Yang, J. H., Chen, C. H., et al. (2007). Pro-apoptotic low-density lipoprotein subfractions in type II diabetes. *Atherosclerosis, 193*(2), 283-291.

Yeh, E. T., & Willerson, J. T. (2003). Coming of age of C-reactive protein: using inflammation markers in cardiology. *Circulation, 107*(3), 370-371.

Yoshida, H., Imaizumi, T., Fujimoto, K., Itaya, H., Hiramoto, M., Yoshimizu, N., et al. (1998). A mutation in plasma platelet-activating factor acetylhydrolase (Val279Phe) is a genetic risk factor for cerebral hemorrhage but not for hypertension. *Thrombosis & Haemostasis, 80*(3), 372-375.

Zacho, J., Tybjaerg-Hansen, A., Jensen, J. S., Grande, P., Sillesen, H., & Nordestgaard, B. G. (2008). Genetically elevated C-reactive protein and ischemic vascular disease. *New England Journal of Medicine, 359*(18), 1897-1908.

Zaciragic, A., Lepara, O., Valjevac, A., Arslanagic, S., Fajkic, A., Hadzovic-Dzuvo, A., et al. (2007). Elevated serum C-reactive protein concentration in Bosnian patients

with probable Alzheimer's disease. *Journal of Alzheimer's Disease, 12*(2), 151-156.

Zirlik, A., Abdullah, S. M., Gerdes, N., MacFarlane, L., Schonbeck, U., Khera, A., et al. (2007). Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. *Arteriosclerosis, Thrombosis & Vascular Biology, 27*(9), 2043-2049.

Zou, N., Hong, J., & Dai, Q. Y. (2009). Passive cigarette smoking induces inflammatory injury in human arterial walls. *Chinese Medical Journal, 122*(4), 444-448.

Zou, H., Deng, J., Li, J., Wang, Y., Zhang, M., He H. (2003). Study of the relationship between cigarette smoking, alcohol drinking, and cognitive impariment in China. *Age & Ageing, 32*(2): 205-210.

Zung, W. W. (1965). A SELF-RATING DEPRESSION SCALE. *Archives of General Psychiatry, 12*, 63-70.