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Mediation and Moderation Analysis of Nutrition, Inflammatory Biomarkers, and Cognition in Older Adults

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Mediation and Moderation Analysis of Nutrition, Inflammatory Biomarkers, and Cognition in Older Adults

by

Elizabeth Pauline Handing

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a Concentration in Aging Studies

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ABSTRACT

Nutrition can be viewed as a modifiable factor related to maintaining and preserving health in older adults. Previous studies have found that nutritional factors can influence cognitive abilities, however few studies have examined macronutrients and micronutrients as they relate to cognitive functioning. Research has yet to examine the mechanisms related to nutrition, cognition and aging in an older adult population from a holistic and interactive perspective.

This dissertation examined three research questions to better understand the relationship between age, nutrition, cognition, and inflammatory biomarkers. First, is nutrition related to cognition beyond demographic factors? Do individual nutrients serve as mediators? Second, are inflammatory biomarkers significant mediators to cognitive performance? Third, do nutrients and inflammatory markers interact as moderators to cognitive performance?

This study examined 1,317 adults 60 years and older from the National Health and Nutrition Examination Survey III (NHANES III). Macronutrients were collected from a retrospective 24-hour dietary recall, micronutrient values were obtained from blood serum/plasma for vitamin C, vitamin D, vitamin E, homocysteine, iron, folate, and inflammatory biomarkers values were obtained from blood serum/plasma for C-reactive protein, fibrinogen, and ferritin. Cognition was measured by 6 tasks: immediate and delayed word recall, immediate and delayed story recall, orientation, and digit subtraction. All tasks were then combined to form a global cognitive measure.

Results for question one found that after controlling for age, sex, education, and total calorie intake, higher intake of polyunsaturated fat was related to better global cognition and
delayed story recall score (std β= .08, p=.028, std β= .08, p=.04 respectively). Greater than 28% of calories from carbohydrate indicated worse global cognition and delayed story recall (std β= -.013, p=.028, and std β= -.158, p=.01). Higher intake of saturated fat and protein were related to worse digit subtraction scores (std β= -.160, p=.02, std β= -.064, p=.02). Higher serum vitamin C, D, and folate levels were related to better global cognition and digit subtraction. Additionally, higher serum vitamin C and D were associated with better orientation score, and folate was related to better immediate and delayed story recall. Building from these relationships, individual mediation models found that serum vitamin C, vitamin D, folate, and ferritin were significant mediators between age and the previously mentioned cognitive tasks.

Results for question two examined mediation between inflammatory markers and cognition and found that higher fibrinogen was related to worse global cognition and digit subtraction. Higher ferritin was associated with better delayed word recall.

Question three investigated the moderating relationship between age, nutrients, and biomarkers, and results found that folate and fibrinogen were significant moderators. Higher serum folate was related to better global cognition and immediate story recall. Ferritin values below 1.2 and above 3.2g/l indicated worse digit subtraction performance. Evidence for a dose-dependent relationship was confirmed.

Results from this project demonstrated that select nutrients (polyunsaturated fat, vitamin C, D, and folate), and inflammatory markers (ferritin and fibrinogen) were associated with cognitive performance across various cognitive domains. Consuming a diet rich in healthy fatty acids, and antioxidants may be beneficial for cognitive health. Future studies should continue to examine the underlying mechanisms connected to maintaining, preserving, and protecting cognitive abilities in older adults.
CHAPTER ONE:

INTRODUCTION

Significant contributions have been made in the field of cognitive aging investigating age-related cognitive changes. The general consensus is that some cognitive abilities decline with age such as memory, speed of processing, and working memory, while others remain stable or improve such as vocabulary (Craik & Bialystok, 2006; Park et al., 2002; Salthouse, 2009; Schroeder & Salthouse, 2004). Different cognitive tasks have different patterns in relation to age and there exist large variations in cognitive functioning among older adults. Many questions still remain as to what influences cognitive changes within individuals, between individuals, and across time. For the vast majority of older adults, maintaining cognitive function and preventing future decline is an important concern. One method to potentially improve cognitive aging is through examining modifiable risk factors such as diet and nutrition. A preventative approach, such as identifying important nutrients in healthy older adults, may provide beneficial evidence for promoting cognitive health and reducing the burden of disease in an aging population.

It is well established that aging is influenced by both environmental and biological factors. For example, education (Christensen et al., 1997), socioeconomic status (Cagney & Lauderdale, 2002), genetics (Caselli et al., 2009; Plassman, Williams, Burke, Holsinger, & Benjamin, 2010), physical activity (Kramer et al. 2006), and overall health status (Hultsch, Hammer, & Small, 1993) can have significant implications on cognitive health. Extensive research has been conducted examining lifestyle habits and health, however a new area of study is beginning to examine the connection between nutrition and brain health.
Nutrition is a complex, multifaceted issue and its association to brain functioning has been examined in terms of macronutrients (fat, carbohydrates, and protein), micronutrients (vitamins and minerals) and fatty acids. Numerous studies have identified single nutrients such as vitamin E, vitamin D, or omega-3 fatty acids as either protecting against or increasing risk of cognitive decline (Joseph et al., 1998; Masaki et al., 2000; Morris et al., 1998a), however a combined, holistic approach has seldom been examined.

Although an association between nutrition and cognition is growing, a new link to research involves the connection between inflammation, cognition, and nutrition. It is well known that inflammatory markers and oxidative stress may play key roles in aging and neurodegenerative diseases such as dementia and Alzheimer's disease (Dik et al., 2005; Schmidt et al., 2002; Teunissen et al., 2003). The release of C reactive protein (CRP) and other inflammatory markers may contribute to increased cognitive decline via the inflammatory pathway. Inflammatory markers are thought to be activated in response to stress and may be involved in the development of Alzheimer’s disease and cardiovascular disease (Tsigos & Chrousos, 2002).

Inflammation is known to increase with normal aging, however previous literature suggests that abnormally high CRP is associated with accelerated cognitive decline (Noble et al., 2010; Schram et al., 2007). Two other novel biomarkers, fibrinogen and ferritin will also be incorporated into this project to analyze inflammation and cognition. Few studies have focused on multiple dietary factors (macronutrients and micronutrients) as they pertain to cognitive function, and to our knowledge none have combined inflammation, nutrition, and cognition.

The current dissertation examined the relationship between nutritional factors and inflammatory biomarkers to differences in cognitive function among older adults from the
National Health and Nutrition Examination Survey from 1988-1994 (NHANES III). First, the dissertation examined how demographic characteristics and nutrition influence cognitive functioning performance. Second, macronutrients (fat, carbohydrates, and protein) and micronutrients (vitamin C, vitamin D, vitamin E, folate, iron, and homocysteine) were examined in a mediation framework which explained if they directly or indirectly served as a mediator between age and cognitive function. Next, inflammatory markers (C-reactive protein, fibrinogen, and ferritin) were examined as mediators to cognitive performance, and lastly, nutritional values and inflammatory markers were examined with age as moderators to cognitive function.

Previous epidemiological and animal studies have found significant effects for the role of diet on brain health, however there are several limitations in the current literature. First, dietary collection methods range from food frequency questionnaires, dietary recalls, to more demanding 24 hour dietary records, thus leading to differences in reliability and validity of nutritional intake over time (Thompson & Subar, 2001). Second, studies vary immensely in the use of cognitive performance measures. Often large epidemiological studies condense cognitive functioning into a global composite score or a brief battery of cognitive testing often lacking domain specific information, which is important for understanding normal vs. pathological cognitive changes. Third, research from animal studies has led to insights into the role of dietary factors and brain functioning as well as inflammatory factors and brain health, however complete studies in humans has not been examined thoroughly, thus providing a new area of innovative research.

The current project will address these limitations by examining nutritional influences (specifically macronutrients and select micronutrients) and inflammatory biomarkers on cognitive outcomes using data from NHANES III. Dietary values of macronutrients (fat,
carbohydrates, and protein), blood serum/plasma values of micronutrients (vitamin c, vitamin d, vitamin e, folate, iron, and homocysteine) and three inflammatory markers (CRP, fibrinogen, and ferritin) are examined in relation to five cognitive domains: immediate verbal memory (immediate object recall and immediate story recall) delayed verbal memory (delayed object recall and delayed story recall) working memory (serial subtraction task), orientation (questions about time and place) and global cognition (sum of correct responses from the six cognitive tasks). Specifically, nutrition and inflammation are examined as predictors of cognitive performance and the use of mediation models will allow us to describe the direct and indirect effects of diet and inflammation to cognitive functioning among older adults. The ability for nutrition to influence cognitive performance above and beyond factors related to sex, education, socio-economic status, and inflammation may provide new insights in understanding how nutrition can be beneficial for cognitive functioning and promote new directions for optimal aging.
CHAPTER TWO:
LITERATURE REVIEW

A great deal of literature has examined changes in cognition across the lifespan. In this chapter, literature is summarized on cognitive aging in older adults including a discussion on age-related variance in cognitive performance with reference to the cognitive reserve hypothesis. Next, a section summarizing research on modifiable factors is presented highlighting the role of nutritional factors (micronutrients and macronutrients) to cognitive function in older adults. Lastly, research on inflammatory markers (CRP, fibrinogen, and ferritin) is discussed as a potential pathway connected to alterations in cognitive performance in older adults.

Normative changes in cognition with age

Lifespan research indicates that fluid abilities such as information processing speed and working memory decline with advancing age, while crystalized abilities such as verbal memory remain stable (Baltes, Staudinger, & Lindenberger, 1999). Additional studies have refined and replicated this concept by examining changes in fluid and crystalized abilities and age-related differences. In a study by Park et al. (2002) memory performance across the lifespan was examined cross-sectionally in 324 adults ranging from 20-90 years old. Linear differences were evident in declines in speed of processing measures, working memory, long term memory, and short term memory, while knowledge based verbal abilities exhibited much smaller age-related variance. Evidence from longitudinal studies, suggests that normal age-related changes involve a linear decline in speed of processing, reasoning, and memory while vocabulary demonstrates an
upward trend peaking in the 50’s and later stability with advancing age (Salthouse, 2004; Schaie, 1994). These studies highlight how age related changes in cognition are task specific rather than general, however the timing and extent of such changes involve great variability between individuals.

**Mechanisms for age-related variance**

Given the heterogeneity of aging, identifying single mechanisms related to successful aging or pathological aging has proven challenging, yet several theories/hypotheses exist that serve as a foundation for future research. Potential mechanisms such as decreased inhibition (Zacks & Hasher, 1997), cognitive demand (Craik & Byrd, 1982) and dedifferentiation/compensation (Dennis & Cabeza, 2011) are important concepts in understanding patterns of cognitive aging, however the cognitive reserve hypothesis serves as an additional framework better connected to the scope of the current project.

**Cognitive reserve hypothesis**

The cognitive reserve hypothesis posits that life experience such as education and occupational attainment along with neurophysiological capacities may offer individuals cognitive advantages allowing some individuals to cope with brain pathology better than others (Scarmeas & Stern, 2003; Stern, 2002). Two types of cognitive reserve have been proposed: passive cognitive reserve and active cognitive reserve.

Passive cognitive reserve refers to innate biological and neurological correlates of brain structure and function. Passive reserve, also referred to as brain reserve, represents a specific threshold or cutoff for neuronal capacities in which all individuals will experience functional
limitations (Stern, 2009). It is proposed that genetics and early life factors play a large role in the determination of the threshold, which represents a quantitative, fixed capacity. This threshold capacity does not take into account individual differences in cognitive processing, coping strategies or compensatory mechanisms (Stern, 2002).

Active cognitive reserve is concerned with environmental factors such as level of intelligence, education, and occupational attainment as a predictor of more efficient processing and less functional deficits if damage to the brain occurs. Studies on healthy adult samples with high levels of intellectual functioning and an engaged lifestyle demonstrate maintenance of cognitive abilities over time (Hultsch, Hertzog, Small, & Dixon, 1999; Schooler & Mulatu, 2001). By compensating for expected declines in cognitive functioning, and building active cognitive reserve through lifestyle interventions, cognitive reserve can serve as a target for prevention and maintenance of functioning with age.

Although not specifically stated in the cognitive reserve hypothesis, nutrition may directly or indirectly be related to cognitive maintenance in later life through disease reduction, prevention of health related declines, and better functioning in older age. Like education, nutrition is a cumulative process influenced by socio-economic resources, access, and personal preferences. By examining a nutritional component within the cognitive reserve framework, this may help to explain unaccounted variability above and beyond factors related to cognitive reserve. Like active cognitive reserve, nutrition can potentially modify or influence cognitive functioning by protecting the brain and body against pathologies.
Modifiable factors: Nutrition and cognitive performance

Extensive research has been conducted examining a healthy diet and risk for disease (i.e. cardiovascular disease, cancer, and diabetes) and a new area of study is beginning to examine the connection between nutrition, brain health, and inflammatory markers. The following sections will present literature on dietary patterns and cognition, specifically the Mediterranean Diet, followed by a section on micronutrients and cognitive function, macronutrients and cognition, and lastly the role of inflammatory markers on subsequent cognitive performance.

Dietary patterns influence on cognitive functioning

The Mediterranean Diet one of the most widely researched diets linked with reduced incidences of cardiovascular disease (Estruch et al., 2013), cancer (Trichopoulou, Lagiou, Kuper, & Trichopoulos, 2000), metabolic syndrome (Esposito et al., 2004), and Alzheimer’s disease (Scarmeas, Stern, Tang, Mayeux, & Luchsinger, 2006; Sofi, Macchi, Abbate, Gensini, & Casini, 2010). The broad characteristics of this diet include; high amounts of fruits, vegetables, breads, potatoes, beans, nuts, olive oil, dairy products (yogurt and cheese), fish, poultry, and eggs in small amounts, wine in low to moderate amounts (1-2 glasses), and red meat consumed a few times per month or in small amounts (Willett et al., 1995). Although widely researched, results remain inconsistent to cognitive outcomes in normal aging populations.

For example, Feart and colleagues (2009) found that adherence to the Mediterranean diet, measured prospectively, was associated with a slower decline on the Mini Mental Status Exam (MMSE) but not associated with the risk for dementia over a 5 year follow up. These results suggest a relationship between Mediterranean diet adherence and cognition, however this study
was conducted in a European sample, the study had a relatively short follow up, and a limitation of only the MMSE to measure cognitive functioning make the findings hard to generalize.

Another study from Gu et al. (2010) prospectively measured nutrition intake in 2,149 older adults part of the Washington Heights-Inwood Columbia Aging Project (WHICAP) using a 61 item semi-quantitative food frequency questionnaire administered at baseline and re-examined every 1.5 years for a mean follow up of 3.96 years. They found that a food combination of a diet rich in omega-3, vitamin E, folate, low saturated fat intake, and vitamin B12 was associated with lower risk for Alzheimer’s disease. This pattern has similarities to the Mediterranean Diet and suggests a healthy diet can reduce risk for Alzheimer’s disease. This study is unique for collecting multiple measures of dietary information including a variety of nutrients (macro and micronutrients), but lacks adequate sensitivity to identify individual nutrients. It also lacks evidence for explaining the underlying biological pathways involved in cognitive decline.

In conclusion, examining dietary factors from a holistic prospective may offer insights into the synergistic effects of diet and its impact on cognitive functioning. Preserving and maintaining cognitive function in older age is an important issue and lifestyle factors, especially nutrition coupled with biological markers deserves greater research.

**Micronutrients and cognitive functioning**

Micronutrients are composed of vitamins and minerals, which are critical for cellular function, membrane function, and modulating brain activity (Fernstrom, 2000; Ferry & Roussel, 2011; McDaniel, Maier, & Einstein, 2003). The prevalence of nutritional deficiency is not uncommon in older adults and may be due to impaired appetite, taste, reduced calorie intake,
gastrointestinal tract issues, polypharmacy, malabsorption, and/or a lack of nutritional education (Ferry & Roussel, 2011). In this section a review of micronutrients, specifically vitamin D, antioxidants, and omega-3 fatty acids will be discussed in relation to cognitive health in older adults.

**Association of vitamin D to cognitive function**

One of the most common nutrient deficiencies found in older adults is a lack of vitamin D (Kritchevsky & Houston, 2012). With advancing age, activation of 25-hydroxyvitamin D [25(OH)D] into the biological form of vitamin D may be reduced due to compromised liver and kidney function (Prentice, Goldberg, & Schoenmakers, 2008). In a nationally representative cross sectional study using data from the NHANES III, Llewellyn and colleagues (2010) examined the relationship of serum 25(OH)D levels and cognitive impairment in adults 65 and older. Results determined that deficient levels of 25(OH)D (i.e. <25mmol/L) were associated with increased risk for cognitive impairment (HR 3.94, p=.017). Although not clearly defined, it is recommended for serum concentrations of 25-(OH)D to be at or above 75 nmol/l (30 ng/ml) (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2007; Pearce & Cheetham, 2010).

On the other hand a study examining 25(OH)D and cognitive performance in older adults found a positive relationship to executive functioning performance (Buell et al., 2009). In this study older adults with >20ng/mL (above the deficiency threshold) showed significantly better scores on the Trail Making A and B tasks, matrix reasoning, and digit symbol tasks. Implications from this study are valuable and suggestive of the importance for maintaining adequate vitamin D levels. Mental fluid abilities such as executive function and processing speed are known to
decrease with age, and if older adults adhere to proper vitamin D recommendations, age–related cognitive changes may be modified or improved. Clearly, the correction of vitamin D deficiency may have immense potential for preventing cognitive impairment and preserving cognitive function in older adults.

**Association of antioxidants to cognitive functioning**

Dietary antioxidants have also been investigated widely in relation to cognitive health and functioning (Jama et al., 1996; Morris et al., 1998b; Morris, Evans, Bienias, Tangney, & Wilson, 2002; Morris, Evans, Tangney, Bienias, & Wilson, 2006). For example, vitamin E, C, and beta-carotene from dietary sources based upon a food frequency questionnaire was examined in a longitudinal population based study of older adults and found that individuals in the highest quintile of vitamin E intake (median intake= 5,682 mg/day) showed a 36% reduction in rate of cognitive decline over 3.2 years. Individuals in the upper quintiles of intake had higher predicted baseline scores than those in the lowest quintile (Morris et al., 1998b). Vitamin C and beta-carotene were not statistically related to cognitive decline however they did show trends for reduced rates of decline. Other studies exploring the relationship of cognitive performance and antioxidant intake have found similar protective effects of vitamin E (Perrig, Perrig, & Stahelin, 1997), while others have demonstrated cognitive improvements with beta-carotene and vitamin C (La Rue et al., 1997) or found no effects at all (Kalmijn, 2000; Perkins et al., 1999). Findings remain mixed over the role of vitamin E, C, and beta-carotene in cognitive function among older adults.
Omega-3 fatty acids and cognitive functioning

Another dietary factor related to cognition and brain health is omega-3 polyunsaturated fatty acids. Omega 3- fatty acids from fish may be associated with improved brain functioning by activating intracellular pathways, insulin signaling, reducing oxidative stress (Ferry & Roussel, 2011; Solfrizzi et al., 2005).

In a cross sectional population based study of 1,613 middle aged men and women from the Netherlands (Doetinchem Cohort Study), researchers were interested in examining fish intake, total fat, saturated fatty acids, monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) intake and cognitive function (Kalmijn et al., 2004). Diet was assessed using a 128-item food frequency questionnaire and an extensive cognitive battery was administered. The lowest 10% of participant scores on the cognitive tasks were categorized as having cognitive impairment. Dietary factors between the cognitively normal individuals and cognitively impaired individuals did not statistically differ except with regards to fatty fish consumption and calculated eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in which cognitively normal adults showed higher intake. Fatty fish, higher amounts of EPA and higher DHA were significantly associated with a decreased risk of overall cognitive impairment (Odds Ratio=.77, p < .05, OR=.81, p < .05, respectively) and better speed of processing performance (OR=.72, p < .05, OR=.71, p < .05) after controlling for age, sex, education, smoking, alcohol consumption, and energy intake.

To test these assumptions in a double-blind, randomized placebo-control trial among older adults, Van de Rest et al. (2008) examined if a fish oil supplements containing 1,800 mg/day of EPA-DHA, 400 mg/day of EPA-DHA, or placebo would show improvements in cognitive performance over a 26 week intervention. Results showed that higher plasma
concentrations of EPA and DHA increased to 238% compared to baseline while no significant differential effects were seen in cognitive performance among older adults. These results coupled with evidence from Kalmijn et al. (2004) demonstrate variability in reported findings for the role of omega-3 fatty acids and cognitive performance. More studies are needed to examine if cognitive benefits of omega-3 fatty acids from intake of fatty fish only or if supplements can provide a similar effect.

In conclusion, research on micronutrients including vitamin D, antioxidants, and omega-3 fatty acids play an important role in cognitive health, and more evidence is needed to examine the neurobiological effects of these nutrients on brain health. Future studies should specifically compare dietary micronutrient intake in conjunction with macronutrients to examine if nutrient interactions exist and how these patterns may alter brain functioning.

Macronutrients and cognitive functioning

The majority of literature has focused on examining micronutrients in relation to cognitive functioning, while literature on macronutrients is limited. Macronutrients are dietary components that make up the majority of one’s diet including fat, carbohydrates, and protein (Kritchevsky & Houston, 2012). Animal models examining these factors have been the most successful because of the ability to directly manipulate dietary factors and provide complete environmental control. Human trials may be limited due to ethical standards and adherence, however, both approaches are useful for understanding the role and mechanisms of diet to cognitive performance.

In human epidemiological studies, it has been shown that intake of high saturated fat diets may be related to increased risk for Alzheimer’s disease (AD) (Freeman, Haley-Zitlin,
A study conducted by Morris et al (2003) examined dietary fat intake and risk of AD in a longitudinal community-based population (Chicago Health and Aging Study). Dietary intake was collected using a food frequency questionnaire at baseline in 815 community dwelling dementia free older, average age of 73, of whom 131 participants developed dementia after a mean follow up of 3.9 years. Fat intake was aggregated into quintiles and individuals with the highest intake of saturated fat and trans-unsaturated fat (median= 25.1 grams/day and median= 4.8 grams/day) were associated with increased risk of AD (Relative Risk= 3.6, \( p = .21 \), RR=5.2, \( p = .09 \), respectfully) where as omega-6 polyunsaturated fatty acid (PUFA’s) and monounsaturated fatty acids (MUFA’s) were related to a decreased risk (RR= .30, \( p = .10 \), RR=.20, \( p = .10 \)) after controlling for age, sex, race, education, and apolipoprotein E genotype. Further examination of animal fat and vegetable fat were investigated and only vegetable fat was statistically significantly related to lower risk in the multivariable model (RR= .20, \( p = .002 \)). An underlying biological mechanism for the observed associations suggests that high fat diets are related to altered membrane functioning, insulin resistance, oxidative stress, inflammation, and altered vascularization (Freeman & Granholm, 2012; Kalmijn et al., 1997; Parrott & Greenwood, 2007; Uranga & Keller, 2010).

Work by Kaplan and colleagues (2001) examined the relationship of dietary protein, carbohydrates, and fat intake and cognitive performance in older adults using a formulated drink experiment. Eleven men and eleven women ages 61-79 years old consumed 300mL of a either a pure protein (whey), carbohydrate (glucose), fat (safflower oil), or a non-energy placebo drink on four separate mornings. Cognitive tests included the Rey Auditory-Verbal Learning Test (AVLT) list recall, Wechsler immediate and delayed paragraph recall, Trail Making Test A and
B, and a visual attention task which were assessed 15 minutes and 60 minutes after consumption of the drinks. Plasma glucose was collected 15 minutes, 60 minutes, and 90 minutes post drink consumption to examine the potential role of blood glucose and cognitive function.

Beneficial effects were seen for each macronutrient as follows: carbohydrate improved Trail Making Test A and B performance after 60 minutes, with a trend for improvement after 15 minutes, fat ingestion impaired AVLT word list recall after 60 minutes and a trend for carbohydrate at 15 minutes and 60 minutes impairing AVLT word recall. All three macronutrients showed improved scores on immediate and delayed paragraph recall after 15 minutes while no effect of drink was found for paragraph recall 60 minutes later. Results indicate that only the carbohydrate drink caused plasma glucose levels to increase over time speculating that glucose levels did not influence the relationship of fat and protein intake to cognitive performance. Results demonstrate that energy intake from carbohydrates, fat and protein can independently influence specific cognitive tasks. However a limitation of this study should be noted because improved scores over a short time (15-60 min) and repeated exposure (4 days of testing) may have biased the results due to practice effects.

Long-term exposure of high fat intake is a common theme in the American or “Western Diet”. This diet emphasizes red meat, refined sugars, cheese, margarine, and a lack of fruits and vegetables (Freeman et al., 2013). Fat consumption from these foods are often in the form of saturated fatty acids (SFA) and trans fatty acids compared to healthier fats such as omega-3 polyunsaturated fatty acids.

Population based epidemiological studies have seldom examined the longitudinal effects of consistent both macro and micronutrients and cognitive function. More research is needed in
examining the potential mechanisms behind the relationship to cognitive performance, particularly as it relates to risk for impairment and disease in older adults.

**Inflammation and aging**

There is potential for diet to positively influence cognitive performance, but the underlying biological pathways are not fully understood. The hypothalamic-pituitary-adrenal axis (HPA) is the central mechanism governing the body’s neuroendocrine and immune response to physical and psychological stressors. Literature suggests that chronic hormonal events due to the functioning of the HPA axis may alter the inflammatory pathway (Luciano, Marioni, Gow, Starr, & Deary, 2009; Schmidt et al., 2002). Activation of microglia cells can trigger the release of proinflammatory cytokines which can contribute to cognitive decline, cognitive impairment and be a risk factor for Alzheimer’s disease (Akiyama et al., 2000).

Experimental studies suggest an association between the pathogenesis of cognitive decline and elevated inflammatory markers including CRP, fibrinogen, and ferritin, however research remains mixed over these findings (Marioni et al., 2009; McDade, Lindau, & Wroblewski, 2011). By examining this pathway in a national representative sample and in connection to dietary factors, inflammatory biomarkers may offer insights for future interventional studies to preserve and maintain healthy cognitive function.

**Summary: Limitations of current work and future considerations**

The literature reviewed in this section highlights research on nutrition, inflammation and cognitive functioning among older adults. Several limitations are evident in the existing literature, involving the scope and approach to dietary examination research. As presented, studies often
focus on nutrient status of single micronutrients and macronutrients while less work has examined a combined retrospective approach. By incorporating inflammatory markers with the association to diet and cognition, this project serves as an untapped area of research. NHANES is a nationally represented sample, including more than 1,000 older adults with valid cognitive, diet, and inflammatory marker data. The aims of this dissertation are to examine the following research questions:

**Research Questions**

**Research Question 1:**

Is there a relationship between nutritional factors (macronutrients: dietary fat, carbohydrates, protein, and micronutrients: serum vitamin C, serum vitamin D, serum vitamin E, serum folate, serum iron, and plasma homocysteine) and cognitive performance in immediate verbal memory, delayed verbal memory, working memory, orientation, and global cognitive functioning?

**Question 1: Part A**

Does nutrition influence cognitive performance above and beyond demographic factors such as sex, education, income, race, total calorie intake, and body mass index?

*Hypothesis.* Based upon previous studies, it is expected that higher serum vitamin C, D, and E will show positive associations to cognitive performance because they are strong antioxidants, while higher values of homocysteine will lead to worse cognitive performance. It is also expected to see a positive association with PUFA fat intake and cognitive performance.
**Question 1: Part B:**

Do nutritional factors mediate the relationship between age and cognitive functioning?

*Hypothesis.* A significant mediation is expected between age, nutrition, and cognitive functioning. As stated previously, vitamin C, D, E and PUFA dietary fat intake will positively influence cognition scores, while homocysteine and inflammatory markers will negatively. Vitamin D and iron are expected to decline with age and homocysteine and CRP to increase with age.

**Research Question 2**

Do inflammatory markers CRP, fibrinogen, and ferritin serve as mediators between age and cognitive performance?

*Hypothesis.* Previous studies indicate significant relationships with inflammatory markers and poorer cognitive performance, particularly with CRP. In this study similar results are anticipated with CRP, fibrinogen, and ferritin to mediate the relationship between age and cognitive performance.

**Research Question 3**

Do significant inflammatory biomarkers and nutritional factors interact with age as moderators to cognitive performance?

*Hypothesis.* By examining the interaction between biomarkers and nutritional factors in older adults, we expect to find a significant interaction for CRP and antioxidant vitamins (vitamin C, and E) serving as an antagonist to cognitive function. Elevated inflammation and higher intake of antioxidant vitamins will result in an interaction with age and antioxidants will improve cognitive abilities while inflammation with attenuate cognitive performance. High intake of carbohydrates and saturated fat will both lead to worse cognitive performance.
CHAPTER THREE:

METHOD

National Health and Nutrition Examination Survey Approach

The National Health and Nutrition Examination Survey (NHANES) is an ongoing research survey sponsored by the National Center for Health Statistics to assess the health and nutritional status of individuals in the United States. NHANES is a cross sectional, nationally representative study that uses a multi-stage probabilistic design. NHANES I-III were collected in four to six year cycles and beginning in 1999, the study gathered information on a yearly basis. NHANES has been used in epidemiological studies, health sciences, and policy-related research and is considered to be one of the most comprehensive ongoing nutritional studies in the United States for examining nutritional factors and health outcomes (Wright et al., 2002).

The NHANES dietary approach consists of the following procedures: After consent and agreement to participate is gathered, individuals complete an in-person interview followed by a comprehensive physical examination and blood draw in a mobile examination center (MEC). The in-person interview collected information regarding demographic, socioeconomic, health, cognitive function and dietary status. Dietary data was collected using a 24-hour dietary recall in-person at the household interview using a computer-assisted personal interview methodology with a multipass format and standardized probes. Responses from the 24-hour dietary recall were then imputed and calculated using the US Department of Agriculture food composition databases (USDA, 2004).
**Dietary assessment technique**

The 24-hour dietary recall is considered to be the gold standard for dietary analysis. In this technique a trained interviewer asks a participant to recall all foods and beverages consumed over the past 24 hours. The interviewer elicits questions regarding the amount and preparation of food and often uses a five stage multipass probing system (Guenther, DeMaio, Ingwersen, & Berlin, 1996; Moshfegb, Borrud, Perloff, & LaComb, 1999). For example, the first pass is the initial recall which highlights the basic general intake. The second pass, often termed the “forgotten list” probes the participant about any forgotten foods such as sweets, non-alcoholic drinks, or snacks. The third stage asks the participant to recall the time and occasion of each intake, and the fourth stage is a detailed report of preparation and portion of intake. Participants are either shown or described several portion sizes and asked to provide the best estimate. The last stage or “final review probe” is when the interviewer asks for the last time about all intakes of foods and beverages from the past 24-hours. Once the interview is completed, data is entered into a computer based nutritional software program for nutrient analysis.

Advantages of this method are that it is relatively brief, does not require writing or literacy, is less burdensome to the participant compared to other food records, provides estimates of absolute intake, and is an accurate and validated measure (Block, 1982; Conway, Ingwersen, & Moshfegh, 2004). Disadvantages of the 24-hour record are the inability of a single day to describe usual intake, self-reporter bias, possible under/over estimation of consumption, inaccuracies for individuals with memory impairments, and is resource intensive for coding and data entry (Kritchevsky & Houston, 2012; Thompson & Subar, 2001).
Macronutrient values: Dietary recall

Macronutrients were calculated from the NHANES 24-hour dietary recall values which were imputed from the United States Department of Agriculture food and nutrient composition database. Macronutrient variables include: total fat (gm), protein (gm), and carbohydrates (gm). Fat intake will be expressed as % of calories from dietary protein and will be examined by three types: grams of total saturated fatty acids, grams of total monounsaturated fatty acid (MUFA), and grams of total polyunsaturated fatty acid (PUFA). Protein will be examined as % of calories from protein, grams of animal protein, and grams of vegetable protein. Carbohydrates will only be expressed as % of calories from carbohydrates.

Micronutrient values: Blood serum/plasma

In order to examine micronutrient intake, blood assays were included for select nutrients and biomarkers. Approximately 6 tablespoons of blood was drawn via venipuncture on individuals participating in NHANES MEC visit. Laboratory specimen from the blood was processed, stored and shipped to various laboratories for analysis according to NHANES processing manual (NHANES, 1998).

Selection of blood micronutrients were based upon previous research (Bowman et al., 2012; Gu & Scarmeas, 2011). Serum vitamin C (mg/dL), serum vitamin D (ng/mL), serum vitamin E (μg/mL), serum folate (ng/mL), serum iron (μg/dL), and plasma homocysteine (μmol/L).

Participants were asked to fast for at least 12 hours prior to testing, however the reliability was not consistent throughout the sample. Participants who did fast for at least 12
hours will be compared to those who did not and if a statistical difference emerges, fasting will be controlled for as a covariate.

**Inflammatory biomarker samples**

Serum C-reactive protein (CRP) (mg/dL) is considered a strong measure of inflammation, tissue damage, and acute phase response to an infectious disease (Gabay & Kushner, 1999). It can also be used to measure the body’s response to inflammation from chronic conditions, such as arthritis, as well as environmental toxins such as smoking. Higher values represent presence of inflammation. Plasma fibrinogen (mg/dL) is an essential blood-clotting factor that increases with age and is involved in a range of other functions, including platelet aggregation and smooth muscle proliferation (Kamath & Lip, 2003). Higher plasma fibrinogen (generally above 400 mg/dL) is related to increased risk of thrombosis, stroke, and atrial fibrillation (Kamath & Lip, 2003). Serum ferritin (ng/mL) is an iron binding protein that can detect inflammation, and evaluate risk for anemia (Patel, 2008). Higher values may represent hemochromatosis, liver disease, hyperthyroidism, or rheumatoid arthritis, while low values represent iron deficiency.

**Cognitive measures**

Cognitive functioning was measured in adults 60 years and older during the MEC interview. NHANES III (1988-1994) included six cognitive tasks which were categorized into five cognitive domains.
Immediate verbal memory

Participants completed an immediate word recall task adapted from the Mini Mental Status Exam (MMSE) (Folstein, Folstein, & McHugh, 1975). Participants were told three object, “apple, table, penny”, and asked to repeat and remember the three words. Participants also completed an immediate story recall in which the interviewer read a story to the participants from the East Boston Memory task (Gfeller & Horn, 1996). “Three children were alone at home and the house caught on fire. A brave fireman managed to climb in a back window and carry them to safety. Aside from minor cuts and bruises, all were well.” Participants were then asked to repeat the story to the interviewer who recorded if six key details were presented correctly in their recall: three children, house on fire, fireman climbed in, children rescued, minor injuries, everyone well.

Delayed verbal memory

After a short delay and continuation of other cognitive tasks, participants were asked to recall the three objects “Apple, table, penny”. Correct recall of each item was recorded and scored. Participants were also asked to recall the story heard previously. Accurate recall of the six key details was recorded and scored.

Working memory

Participants completed a serial subtraction task from the Wechsler Adult Intelligence scale (Wechsler, 1997). Participants were told the following scenario: “If you have $20 and you take away $3, how many dollars do you have left? Keep subtracting $3 from the answer until I tell you to stop.” Responses were recorded for 5 trails.
Orientation

Six questions from the MMSE that evaluated time and place were included into the orientation domain. Questions included were: 1) what is today’s date? 2) what is the day of the week? what is your complete address: 3) street, 4) city/ town, 5) state, and 6) zip code. Correct responses to the questions were totaled.

Global cognition

A composite score reflecting correct responses from all six cognitive tasks were totaled for a global cognition score rating from 0-29.

Demographic variables

Information on age, gender, ethnicity, education, income, and body mass index was collected from a self-reported questionnaire. Age was coded as a continuous variable, gender was categorical, and ethnicity was categorized as White (Non-Hispanic White), or Black (Non-Hispanic Black). Education was categorized as less than college graduate or above, and income was categorized as earning ≤ $19,999 or earning ≥ $20,000. Body mass index (BMI) was calculated as weight (kg)/ height (m$^2$) and classified according to standard categories: Underweight (BMI<18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9), and obese (BMI >30). Physical activity was categorized as physically active vs no physical activity based upon answering yes to any of the following, “In the past month did you… run, jog, bicycle, swim, do aerobics, garden/yard work, lift weights, or other aerobic activity?”

Chronic conditions including asthma, chronic obstructive pulmonary disease, cancer, diabetes, and rheumatoid arthritis will be examined. Reporting on health variables are confirmed
from a questionnaire asking “Has a doctor ever told you that you have:______”. Medications related to inflammation will be investigated. Medication use will include: Non-Steroidal Anti-Inflammatory Drugs (aspirin, ibuprofen, naproxen, indomethacin, diclofenac, ketoprofen), Cox-2 inhibitors (celecoxib and etoricoxib) and use of statins (lovastatin, pravastatin, and simvastatin).

**Statistical Analyses: Multiple Regression, Mediation, and Moderation**

All statistical analyses will be conducted using SAS 9.4 (SAS Institute, Cary, NC). Two-tailed p-values .05 will be used unless otherwise specified.

Before conducting the main analysis described below, distributional properties of study variables will be examined to determine if variance stabilizing or normalizing transformations should be applied. Outliers will be excluded from analyses if the score is three standard deviations or more away from the mean. Log transformations will then be applied to non-normally distributed data, typically required for inflammatory biomarkers (CRP, ferritin, and fibrinogen). To identify potential confounding variables, demographic variables including sex, education, ethnicity, income, body mass index, physical activity, and prescription/medication drug usage (anti-inflammatory medications, and supplement intake) will be examined as categorical variables. Should even marginal differences be found (p < .1), the identified variables will be entered as covariates in all subsequent analyses. Age, cognitive scores, nutrient values, and inflammatory markers will be examined as continuous variables. Correlation matrices will be examined for preliminary relationships and possible multicollinearity among all variables.
**Research Question 1:**

Is there a relationship between nutritional factors (macronutrients: dietary fat, carbohydrates, protein and micronutrients: serum vitamin C, serum vitamin D, serum vitamin E, serum folate, serum iron, and plasma homocysteine) and cognitive performance in immediate verbal memory, delayed verbal memory, working memory, orientation, and global cognitive functioning?

**Question 1: Part A:**

Does nutrition influence cognitive performance above and beyond demographic factors such as sex, education, income, race, total calorie intake, and body mass index?

The first analytic approach is to examine correlations between demographic factors, nutrient values and cognitive performance. Next after controlling for demographic factors, multiple regression will examine the linear and quadratic relationship between nutrients/inflammatory markers and cognitive performance for each task.

**Question 1: Part B:**

Do nutritional factors mediate the relationship between age and cognitive functioning?

The next analytic approach will involve examining the relationship of three variables (age, nutrition and cognition) using a statistical mediation approach. A mediator is considered to be a third variable that accounts for the relationship between a predictor and a criterion variable and represents a general mechanism through which the variables influence each other (Baron & Kenny, 1986). The basic approach follows a path model of causal outcomes. First, there must be a significant relationship between the predictor variable (X) and the outcome variable (Y), second, the predictor (X) must be related to the mediator variable (M), third, the mediator (M)
must be related to the outcome variable (Y). Lastly, when the mediator is added to the model (X- >M->Y), the relationship between X and Y after controlling for M should be significantly reduced to zero for full mediation to occur (Preacher & Hayes, 2004).

Mediation in this study includes three variables; age, cognition, and nutrition. Mediation will be calculated as model X ->Y (where X represents age and Y is cognitive domain score) and model X-> M -> Y where M represents a nutrient as a mediator between age and cognitive performance. The bootstrapping technique will be utilized which runs iterations (typically 10,000) of empirically represented sampling distributions of the indirect effects (Hayes, 2009).

**Research Question 2:**

Do inflammatory markers (CRP, fibrinogen, and ferritin) serve as mediators between age and cognitive performance?

The same basic analytic approach described previously will be applied here except mediation analyses will be calculated as model X-> M -> Y where M represents an inflammatory marker as a mediator between age and cognitive performance.

**Research Question 3:**

Do significant inflammatory biomarkers and nutritional factors interact as moderators to cognitive performance?

The last research question offers an alternative explanation for the relationship between nutrition and cognitive functioning by including the interaction of significant nutritional factors and age (a moderator) as they relate to cognitive performance. A moderating variable is defined as a variable that affects the direction and/or strength of the relationship between a predictor and
an outcome variable (Baron & Kenny, 1986). It is expected that inflammatory markers will be influenced by age and interact as they relate to cognitive performance.

Moderators, expressed as an interaction, can either a) enhance the relationship (both the predictor and moderator influence the outcome variable in the same direction and have an additive effect), b) buffer the relationship (the moderator weakens the effect of the predictor to the outcome), or c) be an antagonist (the moderator and predictor affect the outcome but the interaction is in opposite directions) (Frazier, Tix, & Barron, 2004).

To test moderation, the PROCESS analytic tool developed by Hayes (2013) was utilized. Age will be centered at the mean in order to interpret the regression coefficients and interaction with nutrients/inflammatory markers. To probe for an interaction and identify where a moderator is significantly related to the outcome, the Johnson-Neyman technique is applied. An advantage to using the Johnson-Neyman approach is the ability to generate regions of significance where the moderator significantly relates to the outcome (Hayes, 2013). It can allow for multiple regions to be identified and is not restricted to +/- 1 standard deviation above and below the mean. This can be beneficial particularly for variables that may have a dose-dependent relationship.

**Statistical power and sample size**

Power analyses were examined a priori for sufficient effect size in multiple regression analyses using G*Power 3.1.1. The sample size recommendation was 485 individuals tested with up to six predictors, 80% power, two tailed p-value = .05, and a large effect size (0.35) (Cohen, 1992). The current project will include more than 1,000 older adults providing evidence for sufficient power and interpretation abilities.
Given the scope of the project, which examines multiple hypotheses through use of advanced statistical modeling (multiple regression, mediation, and moderation), there is a need to control for potential Type 1 error inflation. When multiple independent variables/predictors are added to a model, the probability of rejecting the null hypothesis when it is true increases thus increasing Type 1 error (Shaffer, 1995). In order to provide satisfactory results, the Bonferroni correction will be used to correct Type 1 error (Holm, 1979).
CHAPTER FOUR:
RESULTS

In this section, an overview of sample demographic characteristics is presented first. Then results from each research question are described through the text and with corresponding tables/figures.

Sample Characteristics

A total of 1,317 adults 60 years and older were included in the study. A flowchart of participants is shown in Figure 1. Subjects had an average age of 70.76 years old, 53% were females, 63.55 % white, 74.72% had less than a college education, and 68.34% of the sample was overweight (defined as a BMI greater than 25). Average calorie intake was 1,754.83 kcal/day, a mean intake of 32.32% of calories from fat, 51.9% of calories from carbohydrates, and 15.85% of calories from protein.

Approximately half the sample (50.3 %, n= 663) fasted for 12 hours or more prior to the blood draw. Seventy-one percent (n=933) reported their health as good, very good, or excellent and 68.8% reported participating in physical activity in the past month. Seventy-one percent reported taking prescription medications in the past month with an average of 2.9 medications per day. About half the sample (52.3%, n= 689) reported taking vitamins or mineral supplements, with an average of 1.9 per day.
Chronic conditions within the sample included the following: 46% have high blood pressure, 43.5% with arthritis (22.0% rheumatoid arthritis and 26.0% with osteoarthritis), 33.0% with high blood cholesterol, 13.1% with diabetes, 7.7% have had cancer, 7.0% have congestive heart failure, 6.8% have a thyroid disease, 6.5% have had a stroke, and 5.7% have asthma. Regardless of a chronic condition diagnosis, 41.1% reported taking aspirin, and 15.1% ibuprofen. Cox-2 inhibitors were taken by 2.0% of the sample (n=26), and statins were used in 5.0% (n=66).

Average cognitive scores are stated below, higher score represent better performance:
- Immediate word recall (mean= 2.97, standard deviation (SD)= .18, range= 1.0-3.0),
- Delayed word recall (mean= 2.66, SD= .60, range= 1.0-3.0),
- Immediate story recall (mean= 3.81, SD= 1.04, range= 1.0-5.0),
- Delayed story recall (mean= 4.10, SD= 1.25, range= 1.0-6.0),
- Orientation (mean= 5.67, SD= .64, range= 1.0-6.0), and
- Digit subtraction (mean= 4.48, SD= 1.11, range= 1.0-5.0).

Initial correlations with demographic variables, results found that increasing age was significantly related to worse cognitive performance on all tasks (Table 1). Females performed significantly worse on the digit subtraction task. More years of education was related to better performance on all tasks. Increasing age was related to lower calorie intake, lower fat intake, higher carbohydrate intake, and lower protein intake (Table 2). Females consumed less total calories including fat and carbohydrates. Increasing age was related to higher vitamin C, E, folate, and homocysteine values (Table 3). Females had higher values of vitamin C, E, and folate, but lower values of vitamin D, iron, and homocysteine. Table 4 presents demographic factors and inflammatory markers showing that with increasing age, CRP and ferritin decreased.
Research Question 1: Part A:

Is there a relationship between nutritional values and cognitive performance? Does nutrition predict cognitive function beyond demographic factors?

The analytic approach used here was to first examine zero-order correlations among each cognitive task and individual nutritional factors (macronutrients and micronutrients), inflammatory markers, and demographic factors. Next, based upon significant correlations, variables were entered into a multiple regression model controlling for demographic variables (age, sex, education, and total calorie intake). Additionally in the multiple regression models, nutritional values were examined linearly and quadratically as predictors of cognitive function. Bonferroni adjustment was used to correct for Type 1 error which can occur when conducting multiple comparisons.

Zero-order correlations

Macronutrients. Results indicated significant relationships among macronutrients and cognitive functioning (Table 5). Higher fat intake was positively related to better global cognition and digit subtraction performance. When dietary fat was broken down into constituent types, more grams of saturated fat was related to better global cognition, immediate and delayed story recall, and digit subtraction. Higher intake of monounsaturated fatty acid was associated with better global cognition, delayed word recall, immediate and delayed story recall, and digit subtraction. Higher polyunsaturated was significantly related to all cognitive tasks except for immediate word recall. Higher percentage of calories from protein was related to worse digit subtraction performance. Meanwhile higher intake of animal protein and vegetable protein were related to better performance of global cognition, digit subtraction, and delayed story recall.
Higher percentage of calories from carbohydrates was related to worse global cognition and immediate story recall.

**Micronutrients.** Higher blood values of vitamin C and D were related to better global cognition and orientation scores. Additionally, higher vitamin D was also associated with better digit subtraction score. Higher values of vitamin E were related to better global cognition and delayed story recall performance. Serum homocysteine was negatively related to global cognition, immediate word recall, and delayed story recall. Higher levels of serum iron were associated with better digit subtraction and delayed story recall. Higher levels of homocysteine were related to worse global cognition, immediate word recall, and delayed story recall.

**Inflammatory Biomarkers.** CRP did not show a significant association to cognitive performance. Higher fibrinogen was related to worse global cognition, digit subtraction, delayed word recall, delayed story recall, orientation, and digit subtraction. A positive association was found between higher levels of ferritin and better delayed word recall performance.

**Multiple Regression Analyses**

To determine if significant relationships remained above and beyond demographic characteristics, multiple regression models were examined controlling for age, sex, education, and total calorie intake. Nutrient markers were examined as linear and quadratic effects.

**Macronutrients.** Presented in Table 6 are standardized regression coefficients between macronutrients as linear and quadratic terms.

**Linear Relationships:** Higher intake of polyunsaturated fat was significantly related to better global cognition and delayed story recall when examined linearly (Standardized β=.080,
Higher intake of protein was related to worse digit subtraction by .064 standard units, \(p\)-value .021.

**Quadratic Relationships:**

Results found a significant quadratic relationship between carbohydrate intake and global cognition (Std. \(\beta = -.134, p\)-value .028) and delayed story recall (Std. \(\beta = -.158, p\)-value .012). To identify regions of significance, the Johnson-Neyman technique was used. An advantage to using this technique is its ability to identify the exact region of significance where a variable and outcome are statistically significant. In analyses for carbohydrates, those with intake greater than 25.6% of calories from carbohydrates resulted in significantly lower scores in global cognitive performance and intake of greater than 28.4% of carbohydrates lead to worse delayed story recall (shown in Figures 2 and 3). To note, the number of subjects with intake below these cutpoints (< 28%) was 20 people meaning the majority had consumption in the significant range. No upper limit was identified.

Saturated fat intake was quadratically related to worse digit subtraction score (Std. \(\beta = -.160, p\)-value .019). The Johnson-Neyman method identified a region of significance between 20.7-36.2 gm of saturated fat indicating worse digit subtraction performance (Figure 4). The number of subjects with values below 20.7 was \(n=742\), within the significant region \(n=398\), and above \(n=177\).

**Micronutrients.** Table 7 presents standardized regression coefficients between micronutrients linearly and quadratically for each cognitive task.

**Linear Relationships:** Greater values of serum vitamin C was significantly related to better global cognition (Std. \(\beta = .069, p\)-value .012), orientation (Std. \(\beta = .069, p\)-value .016) and digit subtraction scores (Std. \(\beta = .071, p\)-value .011). Serum vitamin D was also related to better
global cognition, orientation, and digit subtraction (Std. β= .060, p-value= .023, std. β= .058, p-value=.039, std. β= .066, p-value= .015, respectively). Serum folate was related to better global cognition (Std. β=.085, p-value=.023), immediate story recall (Std. β= .074, p-value=.008) delayed story recall (Std. β= .055, p-value=.046), and digit subtraction (Std. β = .059, p-value= .033), and serum iron was linearly related to better delayed story recall performance (Std. β= .061, p-value= .024).

**Quadratic Relationships:** Iron was quadratically related to worse global functioning (Std. β= -.306, p-value= .002), delayed word recall (Std. β= -.286, p-value=.005), and delayed story recall (Std. β= -.300, p-value=.003). To further examine the quadratic relationship low, normal, and high categories were created based upon the recommended reference range of 11-30μmol/L. Those with low serum iron levels performed significantly worse (Std. β= -.091, p-value <.001, n= 321), the normal range performed better (Std. β= .109, p-value <.001, n= 971), and above normal iron was related to worse performance (Std. β= -.094, p-value=.014, n= 25). For delayed word recall, above normal serum iron was related to worse performance (Std. β= -.064, p-value=.017), other categories were not significant. For delayed story recall, low serum iron indicated poorer performance (Std. β= -.112, p-value <.001) and within the normal range performed better (Std. β=.121, p-value <.001).

Vitamin E also showed a significant quadratic relationship with delayed word recall. A reference range of 11.6-42 μmol/L was used to create low, normal, and high groups. At low amounts of vitamin E (< 11.6 μmol/L), delayed word recall performance was significant worse (Std. β= -.057, p-value= .035, n=4).

**Inflammatory Biomarkers.** Table 8 presents standardized regression coefficients for CRP, fibrinogen, and ferritin. All inflammatory markers were log transformed for analyses.
**Linear Relationships:** Higher values of fibrinogen were related to a decrease of -.054 standard units on global cognition, \(p\)-value= .040, and -.061 units on digit subtraction, \(p\)-value= .023. Higher ferritin was associated with better delayed word recall (Std. \(\beta\)=.056, \(p\)-value= .040). No quadratic effects were significant.

**Research Question 1: Part B:**

Do nutritional factors mediate the relationship between age and cognitive functioning?

Based upon the previous findings, significant nutrients were entered as mediators in the following model \(X \rightarrow M \rightarrow Y\) where \(X\) represents age, \(M\) is the nutrient, and \(Y\) is the cognitive task. All models included sex, education, and total calorie intake as covariates. The a path represents the relationship between \(X\)-\(M\), the b path represents \(M\)-\(Y\), and c path represents the total effect of \(X\) through variable \(M\) to the outcome \(Y\). \(C'\) path represents the direct effect of \(X\)-\(Y\). For full mediation, path a and b must be significant and are multiplied \((ab)\) to determine the indirect effect.

\[ ab \] represents the amount of mediation and can be described using this equation:

\[ c = c' + ab \]

\((\text{total effect} = \text{direct effect} + \text{indirect effect})\)

To calculate the indirect effect, the bootstrapping method with bias corrected confidence estimates (based upon 5,000 iterations) was used to test the mediation hypothesis (Preacher & Hayes, 2004).

**Mediation analyses for nutrients**

**Macronutrients.** First, macronutrient models were examined with all three variables (fat, protein, and carbohydrates) as mediators. None of the multiple mediation models were
statistically significant, except for digit subtraction performance where protein intake was an indirect mediator, $a_{protein} = -0.054$, $p$-value < 0.001, $b_{protein} = -0.017$, $p$-value = 0.037, indirect effect = 0.009, confidence interval (CI) [0.0002 - 0.002] (shown in Figure 5). Next, each macronutrient was examined individually using significant results from previous multiple regression models. As expected, protein was a significant mediator with digit subtraction (Figure 6). No statistically significant mediation was found for saturated fat, polyunsaturated fat, or carbohydrates.

**Micronutrients.** All micronutrients were entered into one model. Results were not statistically significant except for folate and immediate story recall performance (Figure 7). When micronutrients were examined individually, vitamin C was a significant mediator for global cognition, orientation score, and digit subtraction (Figures 8-10). Vitamin D was also a significant mediator for global cognition and orientation (Figures 11-13). The quadratic term for vitamin E was not statistically significant. Folate was a significant mediator for global cognition, immediate story recall, delayed story recall, but not significant for digit subtraction (Figures 14-17). Examining serum iron linearly and quadratically resulted in no significant mediation.

**Research Question 2:**

Do inflammatory biomarkers mediate the relationship between age and cognitive functioning? From previous results, higher fibrinogen was related to worse global cognition and digit subtraction while higher ferritin was associated with better delayed word recall.

**Mediation analyses for inflammatory markers**

When examined in the mediation model, fibrinogen was not a significant mediator with global cognition: $a_{fibrinogen} = .002$, $p$-value = 0.790, $b_{fibrinogen} = -0.713$, $p$-value = 0.040, indirect effect = -0.002, CI = -0.002-.0009. Fibrinogen was not a significant mediator to digit subtraction
performance: $a_{\text{fibrinogen}} = .002, p\text{-value} = .790, b_{\text{fibrinogen}} = -.294, p\text{-value} = .023$, indirect effect = CI = -.0007-.0004. Ferritin was a significant mediator for delayed word recall (Figure 18).

**Research Question 3:**

Do significant inflammatory biomarkers and nutritional factors interact as moderators to cognitive performance?

The methodological approach for moderation in this section used an interaction term of age with each significant nutrient. The Process method (Hayes, 2013) was used with the Johnson-Neyman technique to identify regions of significance.

**Moderation analyses for nutrients and inflammatory markers**

*Macronutrients.* None of the macronutrients were significant moderators between age and cognitive functioning.

*Micronutrients.* Folate was a significant moderator between age and global cognition, and immediate story recall. The Johnson-Neyman region of significance could not be determined because the entire region was significant, so three levels of folate, low (1 SD below mean), average (mean), and high (1SD above mean) are depicted in Figures 19 and 20.

*Inflammatory Biomarkers.* Fibrinogen was a significant mediator between age and digit subtraction score. Figure 21 illustrates the Johnson-Neyman region of significance. Values below 1.5 and above 3.2 indicate significance. When examining the number of subjects in each region, 5 subjects had values less than 1.5, 690 were within 1.5-3.2, and 622 had values greater than 3.2. The recommended reference range for fibrinogen is 1.5-4.5 g/L. The interpretation of the Johnson-Neyman graph maps well onto the clinical threshold values demonstrating that fibrinogen has a critical value which may influence cognitive function in older adults.
CHAPTER FIVE:
DISCUSSION

Based upon the results and analyses, many of the findings are supported by the literature and theoretical perspectives. This section will first discuss the results of each research question in context to other scientific studies. The key areas that will be addressed are the role of macronutrients, micronutrients, and inflammation to cognition. Next, strengths and limitations of the current project will be discussed followed by recommendations for future research.

Research Question 1: Part A

Evidence for environmental factors to cognitive function

In research question one, the main objective was to determine if nutrition influenced cognitive function above and beyond demographic factors. Age is known to influence cognitive functioning and results indicated a negative association with all cognitive variables. Education on the other hand was shown to be positively associated with all cognitive tasks.

In this study, higher age was related to higher vitamin C, E, and folate intake, which was somewhat surprising. However these vitamins are very common in multivitamins and supplements. Supplement intake was examined and 52% of participants reported taking supplements in the past month. Within supplement and multivitamin files, each type of supplement could contain a different amount of each vitamin making it difficult to standardized and analyze intake. Additionally, because vitamin values in this study were from blood serum
values it is not possible to determine vitamin values from dietary sources vs supplements. Nonetheless, results indicated higher vitamin levels with advancing age.

**Cognitive reserve hypothesis**

Results from question one can be related to the cognitive reserve hypothesis framework indicating that educational attainment, an accumulated ability, corresponds to maintaining cognitive preservation in old age. Stern (2002) described cognitive reserve from the stance of active reserve and passive reserve. Active reserve refers to modifiable factors that can include level of education, intelligence, and occupation as they relate to preservation of cognitive functioning. In the present study, dietary factors (saturated fat, PUFA, protein, carbohydrates, vitamin C, D, E, folate, and iron) were related to cognitive abilities after controlling for sex, education, and age meaning that a relationship exists above and beyond these demographic factors. Nutritional components may add to a persons’ active cognitive reserve and be used as a modifiable factor influencing cognitive functioning in older age. Specific nutritional factors may impact brain functioning in different ways and thus was examined in more detail.

**Relationship between nutrition and cognitive function**

**Macronutrients**

Results from the current study found a relationship between higher intake of saturated fat and protein with worse digit subtraction scores, higher carbohydrates and worse global cognition and delayed story recall, and higher intake of PUFA demonstrated better performance in global cognition and delayed story recall.
A mechanism that might explain why our study found a negative association between higher intake of saturated fat, protein, and carbohydrates with worse performance may be due to the general “western” dietary pattern of consumption. The “western” diet followed by many Americans is high in saturated fat (meat, dairy, butter, margarine), high in simple carbohydrates (refined sugar, enriched flour), and low in healthy fatty acids such as omega-3 PUFA’s (Cordain et al., 2005; Fung et al., 2001).

**Type of fat and cognitive functioning**

Evidence is mounting that saturated fat intake can impair brain functioning and may cause atrophy in the hippocampus and prefrontal cortex in animals (Francis & Stevenson, 2013; Kanoski & Davidson, 2010). Moreover, higher saturated fat intake has also been associated with increased risk for mild cognitive impairment and Alzheimer’s disease (Eskelinen et al., 2008; Kalmijn et al., 1997; Morris, Evans, Bienias, Tangney, & Wilson, 2004).

A study by Okereke et al. (2012) examined dietary fat intake and cognitive function in community dwelling older adults and found that higher saturated fat was related to worse global cognition and verbal memory. Morris et al. (2004) also found significant results showing that high saturated fat intake was associated with greater decline in global cognition over 6 years.

Solfrizzi and colleagues (2005) examined the relationship between types of unsaturated fatty acid intake (MUFA and PUFA) and age related cognitive decline in older adults across eight years. High MUFA and PUFA were related to better cognitive performance as assessed by the Mini Mental Status Exam. The Mediterranean diet, which emphasizes fat intake from olive oil and fish high in omega-3 PUFA α-linolenic acid and has been related in numerous studies to
slower rates of cognitive decline in older adults (Kalmijn et al., 2004; Karr, Alexander, & Winningham, 2011; Panza et al., 2004; Tangney et al., 2011).

**Carbohydrates and cognitive performance**

A retrospective epidemiological study examining macronutrients intake in older adults, (Roberts et al., 2012) found that high carbohydrate diets may be related to increased risk of mild cognitive impairment (MCI) while total fat intake was protective. A sample of 937 older adults were measured at baseline (cognitively normal) and followed for cognitive testing every 15 months with an average follow up of 3.7 years. Two hundred subjects developed MCI over the study timeline and when macronutrient intake and comparing high carbohydrate intake (> 54% of total calories from carbohydrates) to low carbohydrate intake (< 47% of calories) risk for MCI increased by 89%. High total fat intake (>35% of calories from fat) showed a decreased risk (Hazard Ratio= .56, p=.03), however after further examination neither PUFA’s nor MUFA’s were statistically significant. The connection between higher carbohydrate and higher risk of MCI or dementia can be connected to the literature suggesting that insulin regulation and resistance is related to poorer cognitive function (Parrott & Greenwood, 2007).

Years of poor dietary habits can lead to earlier signs of vascular aging and be a risk factor for cognitive decline and neurodegenerative diseases. Overall, research studies suggest that high saturated fat and refined carbohydrates may be risk factor for cognitive decline while high PUFA intake has shown to be more protective. Because the current study was cross-sectional, speculations such as years of dietary intake cannot be proven, but with the knowledge that nutrition largely influences health, more research could examine habitual dietary pattern and cognitive function in older age.
The role of micronutrients to cognitive function

Results from the current study found significant relationships for better cognitive functioning with higher intake of vitamin C, D, and folate in the domains of global cognition, digit subtraction, orientation (vitamin C and D only), immediate and delayed story recall (folate only). Vitamin E was quadratically related to worse delayed word recall and iron was quadratically associated with worse global cognition, delayed word recall and delayed story recall.

Although micronutrients are not a main energy source for the body, vitamins and minerals are critical for cellular function, membrane function, and modulating brain activity (Fernstrom, 2000; Ferry & Roussel, 2011; McDaniel et al., 2003). Many vitamins, particularly fat soluble vitamins such as vitamin D and E may be dose dependent and hazardous when values are above the tolerable upper intake (Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud, 2007).

Research Question 1: Part B

Mediation of nutritional factors, age, and cognitive function

Based upon the previous significant relationships between nutrients and various cognitive tasks, results from mediation analyses identified several significant mediators. Nutrients included: higher protein and worse digit subtraction, higher vitamin C and D with better global cognition, orientation, and digit subtraction, and higher folate with better immediate story recall, delayed story recall, and digit subtraction performance. These findings are connected to relevant research studies and described in the section below.

Vitamin C. Vitamin C was a significant mediator between age, global cognition, orientation, and digit subtraction. These results indicated that serum vitamin C increased with
age, and higher serum vitamin C was related to better cognitive performance. Our results are consistent with several past studies and contribute new evidence that vitamin C can have beneficial qualities for cognitive functioning.

The mechanism related to the health benefits from vitamin C can be due to its antioxidant properties. Antioxidants are elements that donate an electron to an unstable molecule known as a free radical. Free radicals can create a buildup of reactive oxygen species and cause formation of oxidative stress leading to mitochondrial damage, membrane disruption, enzyme inactivity, and cell death (Benzie, 2003). Antioxidants help to reduce the amount of oxidative damage and can be beneficial for inhibiting lipid peroxidation (Sies & Stahl, 1995).

Support from the literature has found an association between vitamin C and cognitive performance. For example, in a study of 117 older adults vitamin C was examined in relation to global cognitive functioning (MMSE), immediate and delayed verbal memory, verbal fluency, and categorical verbal memory (Paleologos, Cumming, & Lazarus, 1998). The highest quartile of vitamin C intake (>284 mg) showed higher MMSE scores but was not significantly related to other cognitive tasks. Perrig et al. (1997) found evidence that high plasma vitamin C (>59μmol/L) was related to better performance in free recall, recognition and vocabulary. Other studies that examined serum vitamin C levels found no association to cognitive decline (Kalmijn et al., 1997; Perkins et al., 1999).

Evidence connecting vitamin C and cognitive abilities may be related to improved cognitive health by protecting the brain against oxidative damage, and new findings from our study demonstrate a relationship to orientation and digit subtraction performance. The mechanism and association to specific cognitive domains should be examined with additional studies.
Vitamin D. Vitamin D was also a significant mediator between age, global cognition, orientation, and digit subtraction. These results indicate that serum vitamin D increased with age, and higher serum vitamin D was related to better cognitive performance. Our results are consistent with several past recent studies examining vitamin D and cognition in older adults.

Vitamin D is one of the most common nutrient deficiencies found in older adults (Kritchevsky & Houston, 2012). In a study using data from NHANES III, Llewellyn and colleagues (2010) examined the relationship of serum 25(OH)D levels and cognitive impairment in adults 65 and older. Cognitive impairment was classified as scores in the lowest 10% on measures of immediate and delay verbal memory, orientation, and attention assessed by the MMSE, East Boston Memory task, and digit subtraction task. Results determined that low levels of 25(OH)D (i.e. <25mmol/L) were associated with increased risk for cognitive impairment (HR 3.94, \(p=0.017\)).

Annweiler and colleagues (2012) examined domain specific cognitive performance and found comparable results suggesting that low serum 25(OH)D deficiency may be particularly related to impaired mental shifting and information updating processes. In Annweiler’s study, older adults with >52mmol/L showed significantly higher scores on the Stroop task, verbal fluency, Trail Making Task B, and reaction time task. In another study examining 25(OH)D and cognitive performance in older adults, Brouwer-Brolsma et al., (2013) found a positive relationship between serum vitamin D and executive functioning and processing speed.

Our results are similar to current research indicating that serum vitamin D plays a role in cognitive functioning among older adults. In our study, vitamin D was a significant positive mediator meaning that serum vitamin D increased with age, and higher serum vitamin D was
related to better cognitive performance. This represents a new way of looking at serum vitamin D as it is related to maintaining cognitive abilities.

One limitation of our study could have been vitamin D supplementation, which may have led to higher serum values in older adults. With the variety and differences in supplements/multi-vitamin use, the comparison is not clear as to what amount of vitamin D was consumed from supplements alone. Sources of vitamin D could range from supplements to dietary sources to the amount of sunshine a person receives. The source could not be determined in this study.

Implications from this study are valuable and are suggestive of the importance for maintaining adequate vitamin D levels. Mental fluid abilities such as executive function and processing speed are known to decrease with age, and if older adults adhere to proper vitamin D recommendations, age-related cognitive changes may be modified or improved.

Folate (vitamin B9). Results from the current study found higher values of folate to mediate the pathway between age and global cognition, immediate story recall, delayed story recall, and digit subtraction. B-vitamins are important nutrients for brain health because of their role in neurotransmitter regulation of acetylcholine, serotonin, dopamine, and GABA, DNA integrity, and their participation in the methylation reaction of homocysteine/methionine metabolic cycle (Ferry & Roussel, 2011). Folate (vitamin B9) supplies the methyl group for conversion of methionine to S-adenosylmethionine (SAMe) and is closely associated the metabolism of homocysteine, vitamin B6, and B12 (Mattson & Shea, 2003). B-vitamin deficiencies can impair the conversion process and lead to elevated levels of homocysteine which may contribute to oxidative damage, cardiovascular disease, and cognitive impairment in older adults (Refsum, Ueland, Nygard, & Vollset, 1998; Reynolds, 2006; Seshadri et al., 2002).
More specifically, folate deficiency (<13 nmol/L) has been found to be related to lower MMSE scores and risk for dementia (Ramos et al., 2005; Riggs, Spiro, Tucker, & Rush, 1996).

Results from the current study, which found a positive relationship between folate and various cognitive domains, could be an indicator that higher values were leading to more efficient methionine synthesis, but it should be noted that higher intake may also be dependent upon vitamin B12 status (Barnes, Tian, Edens, & Morris, 2014). The study sample may have been using folic acid supplementation along with other B-vitamins, thus leading to a positive trend. Increasing folate levels in non-deficient populations should be cautioned and may have adverse side effects compromising DNA integrity and cell metabolism (Paul et al., 2015). In summary, folate values can have implications on verbal memory and executive function and should be considered as an important vitamin for maintaining cognitive function in older adults.

**Research Question 2:**

**Inflammatory markers and cognition**

The evidence supporting the role of inflammation in cognitive functioning and dementia pathology is growing. Inflammatory biomarkers vary across studies making the conclusions hard to generalize; nonetheless the mechanism has been postulated as a risk factor for cognitive decline. Results from the current project found a negative relationship for fibrinogen and worse global cognition and digit subtraction. Elevated ferritin was significantly related to better delayed word recall and served as a significant mediator in this relationship.

*Ferritin.* Our results were inconsistent with previous research studies suggesting that higher ferritin would hinder cognitive performance. This current study found that higher ferritin values were related to better delayed word recall.
Ferritin is an important iron storage protein in the body needed for cellular redox activity, myelination, extracellular transport, and antioxidant enzyme activity (Schipper, 2012). With advancing age, iron can accumulate in the central nervous system and can be redox-active leading to oxidative damage and age related neurodegeneration (Bartzokis et al., 2007; Stankiewicz & Brass, 2009). In a study by Ayton and others (2015) ferritin was measured from cerebrospinal fluid, and higher values were associated with declining cognitive performance over 7 years in 91 cognitively normal older adults. There is wide support that iron misregulation and hemochromatosis can play a role in the onset of Alzheimer’s disease (Bester, Buys, Lipinski, Kell, & Pretorius, 2013; Connor et al., 2001; Ke & Ming Qian, 2003).

Ferritin may be affected by age-related changes in iron binding capacities, ferritin storage, and cellular functioning a greater understanding of the mechanisms related and cognitive functioning are needed.

Fibrinogen. Our study provides support for fibrinogen status and poorer cognitive performance as shown in worse performance in global cognition and digit subtraction. Plasma fibrinogen serves as a blood coagulant protein and an indirect modulator of inflammation (Andreotti, Burzotta, & Maseri, 1999). Fibrinogen may increase blood viscosity, atherogenesis and thrombogenesis which serve as risk factors for stroke, and vascular dementia, and cerebrovascular disease (Luciano et al., 2009). Research has begun to include fibrinogen as an inflammatory marker with special attention to cognitive functioning in older adults.
Research Question 3:

Moderation between age and nutrient/biomarkers

Moderation analyses (interaction with age) were examined for all significant relationships found in question one. Results found no significant moderators among nutrients, but did find a significant moderator for fibrinogen and digit subtraction performance highlighting a dose-dependent response. The Johnson-Neyman region of significance was found below 1.3 g/l and above 3.2 g/l indicating poorer digit subtraction performance.

Literature has also found a connection between levels of fibrinogen as an inflammatory biomarkers and cognitive performance in older adults. For example, Rafnsson and others (2007) measured plasma fibrinogen, interleukin-6 (Il-6) and intercellular adhesion molecule (ICAM-1) showing poorer performance in immediate word recall, delayed story recall, Raven’s pattern matrices task, verbal fluency, and digit symbol substitution. More specifically, in multivariate analyses fibrinogen independently predicted 4 year decline in verbal memory and after statistical adjustment for cardiovascular risk factors was significantly related to a 4 year decline in the pattern matrices task of non-verbal reasoning (std β = -1.14, p <.05).

Additionally, from our results which found a threshold of 3.0 g/l, hyperfibrigenaemia can be an important contributor to poorer cognitive performance. Xu et al. (2008) investigated hyperfibrigenaemia (classified as fibrinogen > 3.0 g/l) with cognitive decline and found elevated fibrinogen to be a risk factor for dementia and mild cognitive impairment.

In conclusion, results from this study agree with research findings that fibrinogen as an indicator of inflammation and may have adverse outcomes on cognitive function.
Strengths

As presented, studies often focus on macronutrient intake or micronutrient intake and whole dietary analyses are scarce. Dietary intake and nutrition occur in combination and should be examined from a holistic perspective. This study has numerous strengths including the detail of dietary information and blood values from a nationally representative sample of over 1,000 older adults. NHANES III was also selected based upon the variety of cognitive tasks (6 measures) and richness of diet information including dietary intake and blood serum values.

Using blood serum values to measure micronutrients is a strength of this study because it can be a better indicator of long term nutrient status. Blood values particularly for vitamin D, folate, and iron are best examined as serum markers which show more stability and validity compared to self-reported dietary values (Prentice, 2008). Combining blood values for micronutrients and dietary recall for macronutrients allowed for the examination of nutrient status from multiple viewpoints.

This study combined macronutrients, micronutrients, and inflammatory biomarkers to examine cognitive performance in older adults using three different statistical methodologies. Mediation and moderation techniques are a strength of this study which identified significant pathways and interactions with multiple variables to better understand the relationship between age, cognition, nutrition, and inflammation. Findings from this study are supported by research and make new contributions including the mediating effect of protein, vitamin C, vitamin D, and folate as well as the significant moderating effects of folate and fibrinogen. This study is the first, to my knowledge, to use NHANES III to examine macronutrients, micronutrients, and markers of inflammation in relation to cognitive performance.
Limitations

A limitation of the current study is that it was cross-sectional with one dietary assessment and one baseline cognitive assessment. NHANES is not longitudinal by design and does not follow the same participants over time. Cross-sectional designs cannot address issues of reverse causation, meaning does poor diet cause changes in cognition or does poor cognition relate to poor dietary choices. Longitudinal designs are better suited for examining trends and dietary intake patterns over time.

Although this study contained over 1,000 participants, the variability in vitamin levels and inflammatory values were rather small. Particularly when examining cutpoints for quadratic effects, the number of subjects that were outside of a normal/reference range was typically less than 50 people. This would lead to low power and warn against making inferences from small subsets of the sample. Additionally, the study sample performed very well on all cognitive measures making the amount of variance small for individual cognitive tasks indicating a ceiling effect. Our strategy was to create a composite global cognition score to increase variability and range of scores. However a limitation of the composite score is that it cannot provide information about whether dietary factors more strongly impact specific cognitive abilities.

Another limitation of the study is the restriction of inflammatory markers to CRP, fibrinogen and ferritin. These markers may not be representative of all inflammatory pathways. Other markers such as interlukin-1 (IL-1), interlukin-6 (IL-6), tumor-necrosis factor –alpha (TNF-α) are more specific to cytokine production and the inflammatory response cascade with more evidence linked to mental abilities in older adults. The current dataset did not include these variables.
Although this study found significant relationships between diet, inflammation, and cognitive performance, other lifestyle and environmental factors can also play a role in cognitive functioning. Items such as amount of physical activity, access to food, quality of diet, mental health, and stress levels could have also influenced cognitive performance. Trying to tease a part lifestyle factors can be challenging and diet may be just one piece of the puzzle to a very complex problem.

**Future research directions**

Future studies should continue to examine the predictive role of macronutrients, micronutrients, and inflammatory markers with longitudinal designs and additional cohort studies. One example of a follow up study could include examines multiple waves of NHANES to find consistencies or differences over time. NHANES will release a new wave of data from 2011-2012 containing three cognitive measures (verbal fluency, digit substitution task, and CERAD word list learning), and would be the most recent dataset containing dietary information, inflammation, and cognitive function in community dwelling older adult sample. A study could combine NHANES III with wave 2001-2002 and 2011-2012 to examine trends over time could help identify long term nutritional intake values with various health and cognitive measures.

Future studies should be conducted longitudinally to examine pathways of causation and change over time between nutritional intake, status of inflammation, and cognitive functioning. Inferences can then be made to look at the direction of relationships and better understanding habitual dietary intake as it relates of age related changes. A randomized control dietary trial could also be conducted to study effects of changes in diet to changes in cognition. From this study, an emphasis could be placed on folate to study the effects of folate values and cognition in
older adults. Another dietary intervention study could be designed to examine macronutrient intake (i.e. alter ratios of carbohydrates, protein, and fat) and determine optimal intake values for cognitive performance. The ethical limitations of such as study would need to be addressed, but demonstrating experimental design using dietary modification could hold promising directions in understanding the relationship to cognitive health.

Another approach to studying diet and cognition is to look at food groupings and whether specific foods are more common in higher performing individuals compared to others. For example, do servings of fruits vs vegetables vs proteins have a significant relationship to long term cognitive performance? Can food groups be categorized and examined from a dietary pattern perspective?

All in all, future studies are needed to identify dietary factors that can mediate or moderate age related cognitive decline. Understanding the mechanism and biological pathway that occur over time can have significant implications for cognitive health particularly for an aging population.
CHAPTER SIX:
CONCLUSION

Findings from this national, retrospective study add to the literature on nutrition and inflammation as correlates of cognitive function in older adults. Diet should be considered as a primary modifiable strategy to preserving and maintain cognitive health. Identifying specific nutrients such as limiting saturated fat, carbohydrates, protein intake, and increasing polyunsaturated fatty acids, vitamin C, D, and folate may provide new knowledge for researchers to develop recommendations for preserving cognitive abilities with age. Chronic health conditions such as diabetes, stroke, cardiovascular disease, hypertension, cancer, and dementia may potentially benefit from identifying nutritional factors related to improving health and optimizing healthy aging.

The workings of diet and nutrition are an important area of research that involves an interdisciplinary approach to understanding the complex relationship to aging and cognitive functioning. Research remains promising for the benefits of a healthy diet as it relates to improved cognitive functioning. More research is needed to identify the neurobiological mechanisms involved in nutrition, inflammatory markers, and specific cognitive measures in order to understand the dynamic relationship and long-term impact on cognitive functioning.
TABLES AND FIGURES
Table 1. Pearson correlation coefficients among demographic and cognition variables

<table>
<thead>
<tr>
<th></th>
<th>Global Cognition</th>
<th>Immediate Word Recall</th>
<th>Delayed Word Recall</th>
<th>Immediate Story Recall</th>
<th>Delayed Story Recall</th>
<th>Orientation</th>
<th>Digit Subtraction</th>
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<tbody>
<tr>
<td>Age</td>
<td>-0.207*</td>
<td>-0.107*</td>
<td>-0.175*</td>
<td>-0.122*</td>
<td>-0.156*</td>
<td>-0.071*</td>
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<td>-0.107*</td>
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<tr>
<td>Income</td>
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<td>0.211*</td>
<td>0.082*</td>
<td>0.185*</td>
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<tr>
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<td>0.113*</td>
<td>0.124*</td>
<td>0.227*</td>
<td>0.257*</td>
<td>0.182*</td>
<td>0.257*</td>
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<tr>
<td>Body Mass Index</td>
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<td>0.048</td>
<td>-0.001</td>
<td>-0.041</td>
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Note: * = p < .001
Table 2. Pearson correlation coefficients among demographic variables and macronutrients

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<tr>
<th></th>
<th>Total Calories (kcal)</th>
<th>% of calories from fat</th>
<th>Total saturated FA (gm)</th>
<th>Total MUFA (gm)</th>
<th>Total PUFA (gm)</th>
<th>% of calories from protein</th>
<th>Animal Protein (gm)</th>
<th>Vegetable Protein (gm)</th>
<th>% of calories from carbs</th>
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<td>-0.100*</td>
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<td>-0.139*</td>
<td>-0.056*</td>
<td>0.118*</td>
</tr>
<tr>
<td>Female</td>
<td>-0.359*</td>
<td>-0.077*</td>
<td>-0.278*</td>
<td>-0.291*</td>
<td>-0.215*</td>
<td>0.047</td>
<td>-0.250*</td>
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<td>Income</td>
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<td>0.119*</td>
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<td>0.104*</td>
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<td>Body Mass Index</td>
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<td>-0.005</td>
<td>-0.026</td>
<td>0.060*</td>
<td>0.006</td>
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<td>-0.038</td>
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</table>

Note: FA= fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid, *= $p < .001$
Table 3. Pearson correlation coefficients among demographic variables and micronutrients

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C (mmol/L)</th>
<th>Vitamin D (nmol/L)</th>
<th>Vitamin E (umol/L)</th>
<th>Folate (nmol/L)</th>
<th>Iron (umol/L)</th>
<th>Homocysteine (umol/L)</th>
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<tbody>
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<td>0.045</td>
<td>0.062*</td>
<td>0.212*</td>
<td>-0.028</td>
<td>0.169*</td>
</tr>
<tr>
<td>Female</td>
<td>0.249*</td>
<td>-0.166*</td>
<td>0.203*</td>
<td>0.098*</td>
<td>-0.106*</td>
<td>-0.129*</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>-0.232*</td>
<td>-0.283*</td>
<td>-0.144*</td>
<td>-0.233*</td>
<td>-0.081*</td>
<td>-0.008</td>
</tr>
<tr>
<td>Income</td>
<td>0.112*</td>
<td>0.102*</td>
<td>0.093*</td>
<td>0.057*</td>
<td>0.138*</td>
<td>-0.103*</td>
</tr>
<tr>
<td>Years of Education</td>
<td>0.166*</td>
<td>0.106*</td>
<td>0.137*</td>
<td>0.140*</td>
<td>0.058*</td>
<td>-0.106*</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>-0.104*</td>
<td>-0.128*</td>
<td>-0.006</td>
<td>-0.141*</td>
<td>-0.028</td>
<td>-0.045</td>
</tr>
</tbody>
</table>

Note: * = p < .001
Table 4. Pearson correlation coefficients among demographic and inflammatory biomarkers

<table>
<thead>
<tr>
<th></th>
<th>ln_CRP</th>
<th>ln_Fibrinogen</th>
<th>ln_Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.068*</td>
<td>0.010</td>
<td>-0.105*</td>
</tr>
<tr>
<td>Female</td>
<td>0.032</td>
<td>-0.029</td>
<td>-0.178*</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.092*</td>
<td>0.098*</td>
<td>0.064*</td>
</tr>
<tr>
<td>Income</td>
<td>-0.111*</td>
<td>-0.090*</td>
<td>0.035</td>
</tr>
<tr>
<td>Years of Education</td>
<td>-0.092*</td>
<td>-0.085*</td>
<td>0.021</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.150*</td>
<td>0.082*</td>
<td>0.101*</td>
</tr>
</tbody>
</table>

Note: All inflammatory biomarkers were log transformed, * = \( p < 0.001 \)
Table 5. Zero-order correlation coefficients among nutrient values, inflammatory markers, and cognitive tasks

<table>
<thead>
<tr>
<th></th>
<th>Global Cognition</th>
<th>Immediate Word Recall</th>
<th>Delayed Word Recall</th>
<th>Immediate Story Recall</th>
<th>Delayed Story Recall</th>
<th>Orientation</th>
<th>Digit Subtraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Calories (kcal)</td>
<td>0.147*</td>
<td>0.018</td>
<td>0.084*</td>
<td>0.063*</td>
<td>0.118*</td>
<td>0.063*</td>
<td>0.141*</td>
</tr>
<tr>
<td>% of calories from fat</td>
<td>0.060*</td>
<td>0.002</td>
<td>0.025</td>
<td>0.049</td>
<td>0.043</td>
<td>0.012</td>
<td>0.067*</td>
</tr>
<tr>
<td>Total saturated FA (gm)</td>
<td>0.118*</td>
<td>0.021</td>
<td>0.049</td>
<td>0.070*</td>
<td>0.092*</td>
<td>0.047</td>
<td>0.111*</td>
</tr>
<tr>
<td>Total MUFA (gm)</td>
<td>0.126*</td>
<td>0.011</td>
<td>0.068*</td>
<td>0.065*</td>
<td>0.109*</td>
<td>0.042</td>
<td>0.119*</td>
</tr>
<tr>
<td>Total PUFA (gm)</td>
<td>0.142*</td>
<td>-0.001</td>
<td>0.096*</td>
<td>0.058*</td>
<td>0.120*</td>
<td>0.071*</td>
<td>0.123*</td>
</tr>
<tr>
<td>% of calories from protein</td>
<td>-0.049</td>
<td>0.035</td>
<td>-0.038</td>
<td>-0.030</td>
<td>-0.020</td>
<td>-0.021</td>
<td>-0.083*</td>
</tr>
<tr>
<td>Vegetable Protein (gm)</td>
<td>0.101*</td>
<td>0.012</td>
<td>0.070*</td>
<td>0.076*</td>
<td>0.047</td>
<td>0.094*</td>
<td>0.070*</td>
</tr>
<tr>
<td>% of calories from carbs</td>
<td>-0.068*</td>
<td>-0.017</td>
<td>-0.028</td>
<td>-0.066*</td>
<td>-0.051</td>
<td>-0.012</td>
<td>-0.051</td>
</tr>
<tr>
<td>Serum Vitamin C</td>
<td>0.062*</td>
<td>0.009</td>
<td>0.022</td>
<td>0.037</td>
<td>0.025</td>
<td>0.065*</td>
<td>0.049</td>
</tr>
<tr>
<td>Serum Vitamin D</td>
<td>0.066*</td>
<td>0.020</td>
<td>-0.031</td>
<td>0.041</td>
<td>0.041</td>
<td>0.058*</td>
<td>0.095*</td>
</tr>
<tr>
<td>Serum Vitamin E</td>
<td>0.055*</td>
<td>0.025</td>
<td>0.015</td>
<td>0.046</td>
<td>0.058*</td>
<td>0.022</td>
<td>0.017</td>
</tr>
<tr>
<td>Serum Folate</td>
<td>0.051</td>
<td>0.022</td>
<td>-0.014</td>
<td>0.051</td>
<td>0.031</td>
<td>0.039</td>
<td>0.044</td>
</tr>
<tr>
<td>Serum Iron</td>
<td>0.046</td>
<td>-0.046</td>
<td>-0.007</td>
<td>0.052</td>
<td>0.073*</td>
<td>0.020</td>
<td>0.057*</td>
</tr>
<tr>
<td>Serum Homocysteine</td>
<td>-0.078*</td>
<td>-0.055*</td>
<td>-0.048</td>
<td>-0.019</td>
<td>-0.086*</td>
<td>-0.007</td>
<td>-0.046</td>
</tr>
<tr>
<td>Ln_ CRP</td>
<td>-0.034</td>
<td>0.017</td>
<td>-0.020</td>
<td>-0.022</td>
<td>-0.045</td>
<td>-0.013</td>
<td>-0.029</td>
</tr>
<tr>
<td>Ln_ Fibrinogen</td>
<td>-0.079*</td>
<td>0.016</td>
<td>-0.067*</td>
<td>-0.026</td>
<td>-0.054*</td>
<td>-0.054*</td>
<td>-0.075*</td>
</tr>
<tr>
<td>Ln_Ferritin</td>
<td>0.051</td>
<td>0.012</td>
<td>0.058*</td>
<td>-0.020</td>
<td>0.045</td>
<td>0.037</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Note: FA= fatty acid, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acid
All inflammatory biomarkers were log transformed
*= p <.001
Table 6: Regression models for cognition and macronutrients controlling for age, sex, education, and total calorie intake

<table>
<thead>
<tr>
<th></th>
<th>Global Cognition</th>
<th>Imm. Word Recall</th>
<th>Delayed Word Recall</th>
<th>Imm. Story Recall</th>
<th>Delayed Story Recall</th>
<th>Orient. Subtract</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of calories from Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear model</td>
<td>Std. B</td>
<td>R²</td>
<td>Std. B</td>
<td>Std. B</td>
<td>Std. B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.036</td>
<td>0.103</td>
<td>0.001</td>
<td>0.010</td>
<td>0.043</td>
<td>0.023</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.027</td>
<td>0.104</td>
<td>-0.007</td>
<td>-0.013</td>
<td>-0.004</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturated Fat</td>
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<tr>
<td></td>
<td>0.015</td>
<td>0.102</td>
<td>0.025</td>
<td>-0.044</td>
<td>0.066</td>
<td>0.006</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.101</td>
<td>0.104</td>
<td>0.016</td>
<td>-0.019</td>
<td>-0.052</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Monounsaturated Fat</td>
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<td>0.034</td>
<td>0.103</td>
<td>-0.008</td>
<td>0.006</td>
<td>0.055</td>
<td>0.051</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.041</td>
<td>0.103</td>
<td>0.001</td>
<td>0.008</td>
<td>-0.036</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyunsaturated Fat</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.080*</td>
<td>0.106*</td>
<td>-0.027</td>
<td>0.068</td>
<td>0.031</td>
<td>0.077*</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.040</td>
<td>0.106</td>
<td>-0.049</td>
<td>-0.038</td>
<td>-0.021</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>-0.038</td>
<td>0.103</td>
<td>0.031</td>
<td>-0.037</td>
<td>-0.029</td>
<td>-0.010</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>0.020</td>
<td>0.104</td>
<td>-0.039</td>
<td>-0.021</td>
<td>0.047</td>
</tr>
<tr>
<td>% of calories from</td>
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<tr>
<td>Animal Protein</td>
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<td></td>
<td>-0.021</td>
<td>0.103</td>
<td>0.031</td>
<td>-0.053</td>
<td>-0.009</td>
<td>0.011</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.050</td>
<td>0.103</td>
<td>-0.082</td>
<td>-0.023</td>
<td>0.004</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vegetable Protein</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.013</td>
<td>0.102</td>
<td>0.006</td>
<td>0.035</td>
<td>-0.012</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.134</td>
<td>0.104</td>
<td>-0.040</td>
<td>-0.039</td>
<td>-0.034</td>
</tr>
<tr>
<td>% of calories from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
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<td>-0.027</td>
<td>0.103</td>
<td>-0.007</td>
<td>-0.003</td>
<td>-0.047</td>
<td>-0.019</td>
</tr>
<tr>
<td>Quadratic model</td>
<td></td>
<td>-0.013*</td>
<td>0.106*</td>
<td>-0.045</td>
<td>-0.024</td>
<td>-0.077</td>
</tr>
</tbody>
</table>

Note: Imm.= immediate, orient= orientation, subtract= subtraction, *= p < .05
Table 7: Regression models for cognition and micronutrients controlling for age, sex, education, and total calorie intake

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Global Cognition</th>
<th>Imm. Word Recall</th>
<th>Delayed Word Recall</th>
<th>Imm. Story Recall</th>
<th>Delayed Story Recall</th>
<th>Orient. Subtract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.069*</td>
<td>0.107*</td>
<td>0.008</td>
<td>0.016</td>
<td>0.040</td>
<td>0.028</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.061</td>
<td>0.107</td>
<td>0.079</td>
<td>0.002</td>
<td>0.113</td>
<td>0.117</td>
</tr>
<tr>
<td>Vitamin D (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.060*</td>
<td>0.106*</td>
<td>0.031</td>
<td>-0.023</td>
<td>0.039</td>
<td>0.035</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.050</td>
<td>0.109</td>
<td>0.104</td>
<td>-0.022</td>
<td>-0.057</td>
<td>-0.012</td>
</tr>
<tr>
<td>Vitamin E (umol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.046</td>
<td>0.104</td>
<td>0.017</td>
<td>-0.002</td>
<td>0.041</td>
<td>0.052</td>
</tr>
<tr>
<td>Quadratic</td>
<td>-0.016</td>
<td>0.106</td>
<td>0.088</td>
<td>-0.204*</td>
<td>-0.103</td>
<td>-0.015</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.085*</td>
<td>0.109*</td>
<td>0.040</td>
<td>0.009</td>
<td>0.074*</td>
<td>0.055*</td>
</tr>
<tr>
<td>Quadratic</td>
<td>-0.057</td>
<td>0.110</td>
<td>0.011</td>
<td>0.019</td>
<td>-0.050</td>
<td>-0.041</td>
</tr>
<tr>
<td>Iron (umol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.031</td>
<td>0.103</td>
<td>-0.047</td>
<td>-0.011</td>
<td>0.043</td>
<td>0.061*</td>
</tr>
<tr>
<td>Quadratic</td>
<td>-0.306*</td>
<td>0.110</td>
<td>-0.018</td>
<td>-0.286*</td>
<td>-0.186</td>
<td>-0.030*</td>
</tr>
<tr>
<td>Homocysteine (umol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>-0.011</td>
<td>0.102</td>
<td>-0.025</td>
<td>0.014</td>
<td>0.021</td>
<td>-0.036</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.080</td>
<td>0.103</td>
<td>0.044</td>
<td>0.058</td>
<td>0.083</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Note: Imm.= immediate, orient= orientation, subtract= subtraction, *= p < .05
Table 8: Regression models for cognition and inflammation controlling for age, sex, education, and total calorie intake

<table>
<thead>
<tr>
<th></th>
<th>Global Cognition</th>
<th>Imm. Word Recall</th>
<th>Delayed Word Recall</th>
<th>Imm. Story Recall</th>
<th>Delayed Story Recall</th>
<th>Orient.</th>
<th>Digit Subtract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>-0.028 0.103</td>
<td>0.125</td>
<td>-0.022</td>
<td>-0.020</td>
<td>-0.039</td>
<td>-0.010</td>
<td>-0.013</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.007 0.103</td>
<td>-0.037</td>
<td>0.033</td>
<td>0.025</td>
<td>&lt;.001</td>
<td>-0.022</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Fibrinogen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td><em><em>-0.054</em> 0.105</em>**</td>
<td>0.024</td>
<td>-0.050</td>
<td>-0.012</td>
<td>-0.034</td>
<td>-0.044</td>
<td><strong>-0.061</strong>*</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.103 0.105</td>
<td>0.206</td>
<td>-0.002</td>
<td>0.444</td>
<td>0.198</td>
<td>-1.045</td>
<td>0.499</td>
</tr>
<tr>
<td><strong>Ferritin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.035 0.103</td>
<td>0.011</td>
<td><strong>0.056</strong>*</td>
<td>0.031</td>
<td>0.032</td>
<td>0.034</td>
<td>0.014</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.032 0.104</td>
<td>0.248</td>
<td>-0.178</td>
<td>-0.076</td>
<td>-0.073</td>
<td>0.127</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Note: All inflammatory biomarkers were log transformed, imm. = immediate, orient= orientation, subtract= subtraction *= p < .05
Figure 1. Flowchart of participants for the analytic sample
Figure 2. The Johnson-Neyman region of significance for percentage of calories from carbohydrates and global cognition score.

Note: The region to the right of 25.57 represents the region of significance ($p < .05$).
Figure 3. The Johnson-Neyman region of significance for percentage of calories from carbohydrates and delayed story recall score.

Note: The region to the right of 28.38 represents the region of significance ($p < .05$)
Figure 4. The Johnson-Neyman region of significance for grams of saturated fat and digit subtraction performance.

Note: The region between 20.7 and 36.2g represent the region of significance ($p < .05$).
Figure 5. Multiple mediation model with macronutrient intake as mediators to digit subtraction score. In the multiple mediation model, macronutrients were percentage of calories, covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 6. Mediation model with protein intake as a mediator between age and digit subtraction.

Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 7. Multiple mediation with micronutrient values as mediators between age and immediate story recall. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 8. Mediation model with serum vitamin C as a mediator between age and global cognition. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 9. Mediation model with serum vitamin C as a mediator between age and orientation score. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 10. Mediation model with serum vitamin C as a mediator between age and digit subtraction score. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 11. Mediation model with serum vitamin D as a mediator between age and global cognition. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 12. Mediation model with serum vitamin D as a mediator between age and orientation score. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 13. Mediation model with serum vitamin D as a mediator between age and digit subtraction. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 14. Mediation model with serum folate as a mediator between age and global cognition.

Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
**Figure 15.** Mediation model with serum folate as a mediator between age and immediate story recall. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
**Figure 16.** Mediation model with serum folate as a mediator between age and delayed story recall. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 17. Multiple mediation model with serum folate as a mediator between age and digit subtraction. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 18. Mediation model with log transformed term of ferritin as a mediator between age and delayed word recall. Covariates were sex, education, and total calorie intake.

Note: An asterisk indicates significance at the .05 alpha level.
Figure 19. Moderation of folate with age to global cognition.

All values were significant $p$-value <.05 and were covaried for sex, education, and total calorie intake.
Figure 20. Moderation of folate and age to immediate story recall.

All values were significant $p$-value <.05 and were covaried for sex, education, and total calorie intake.
Figure 21. Moderation of fibrinogen and age to digit subtraction score.

Values below 1.2 and above 3.2 represent the regions of significance.

Covariates included sex, education, and total calorie intake.
REFERENCES


Ayton, S., Faux, N. G., Bush, A. I., & the ADNI Initiative (2015). Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. Nature Communications, 6, 6760. doi: 10.1038/ncomms7760


