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Relationship between Nut Consumption and Memory Domains of Cognitive Function Among Elderly Seventh-day Adventists

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RELATIONSHIP BETWEEN NUT CONSUMPTION AND MEMORY DOMAINS OF COGNITIVE FUNCTION AMONG ELDERLY SEVENTH-DAY ADVENTISTS

By

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A Dissertation in Partial Fulfillment of the Requirements for the

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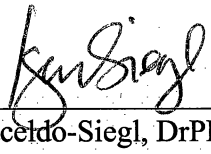

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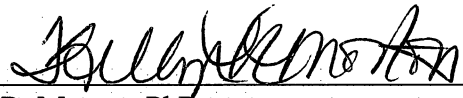

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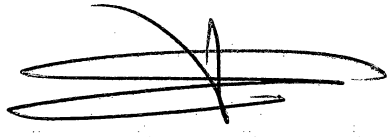

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ABSTRACT OF DISSERTATION

RELATIONSHIP BETWEEN NUT CONSUMPTION AND MEMORY DOMAINS OF COGNITIVE FUNCTION AMONG ELDERLY SEVENTH-DAY ADVENTISTS

by

Angeline B. David, MHS

Doctor of Public Health Candidate in Nutrition

Loma Linda University, Loma Linda, CA 2011

Karen Jaceldo-Siegl, Chair

Objective. To determine the relationship between consumption of all types of nuts, tree nuts, and peanut and peanut butter and specific memory domains of cognitive function in a community-dwelling elderly population.

Design. Cross-sectional analysis using clinic data from the Biopsychosocial Religion and Health Study and dietary intake data from the Adventist Health Study-2.

Subjects/Methods. Adults aged 50 and older were administered the California Verbal Learning Test-II (CVLT) Short Form and provided self-report frequency and portion sizes of nut consumption, anthropometric measures, and other demographic, lifestyle, and clinic data. CVLT scores were age- and gender-strata specific z-scores normed to a national sample.

Results. Although memory scores were significantly predicted by the combined effect of nut intake, caloric intake, ethnicity, education, gender, age at memory testing, history of

cardiovascular disease or diabetes, sedentary physical activity, consumption of animal products, and anthropometric measures, intake of all nuts, tree nuts and peanuts were not significantly associated with CVLT scores independent of other variables. Age, gender, education, ethnicity, and waist-to-hip ratio (WHR) were significantly associated with at least one CVLT domain, with ethnicity and WHR showing significance with all five domains under investigation, even after adjustment. WHR maintained significance even when including adjusting for body mass index (BMI). There is a general trend of lower CVLT score for higher BMI, except for those aged 70-79 years where the opposite trend occurs; however, these trends are not statistically significant.

Conclusions. Our findings show no statistically significant relationship between nut consumption and memory function among an elderly Seventh-day Adventist population after adjusting for demographic, clinical, and lifestyle factors. Waist-to-hip ratio is significantly associated with memory, suggesting the need to further investigate the role of adiposity in cognitive ability in a healthy population of elderly adults.

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ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

A. Statement of the Problem

The normal aging process is associated with changes in biological systems, including the neurological system. Changes in the structural, functional, and molecular aspects of the brain are associated with altered cognitive abilities. Even in "normal" aging marked declines in processing speed, problem solving, working (short-term) memory, and long-term memory are observed throughout the ages of 20 to 80 years (Glorioso & Sibille, 2011). Dementia is a progressive neurodegenerative disease that is characterized by an accelerated decline in cognitive abilities and has a prolonged preclinical phase that is similar to normal aging. Mild Cognitive Impairment (MCI), which has an increased risk of progressing to dementia, is characterized by cognitive impairments beyond those expected to occur in a specific age and education strata but without affecting daily activities.

Most dementias cannot be reversed or halted with currently available therapies although some can slow progression, improve quality of life, ease burden on caregivers, or delay admission to a nursing home. Novel methods of primary and secondary prevention are crucially needed in order to attenuate the downward trajectory of cognitive decline in otherwise healthy adults.

Nutritional factors and nutrient deficiencies have long been associated with cognitive function, and research in these lines are continuing to grow in number and

scope. Certain vitamins have been linked to cognitive function, as have macronutrients such as omega-3 fatty acids. Free radicals, which are produced as a result of high levels of metabolic activity in the brain, can increase vulnerability of neural tissues to oxidative damage when not under tight regulation. The antioxidant vitamins A, C, E, and carotenoids have been associated with improved cognitive function and less dementia (Del Parigi, Panza, Capurso, & Solfrizzi, 2006). Deficiencies of the B vitamins, which play prominent roles in structure of cerebral tissue, oxygen uptake, cholinergic activity, and homocysteine metabolism, have also been associated with cognitive declines. The long-chain omega-3 fatty acids eicosapentaeoic acid (EPA) and docosahexaenoic acid (DHA) are also important structural components of neuronal tissue, and have been associated with delaying cognitive decline. Although the mechanisms of action are yet to be elucidated, the numerous antioxidant and anti-inflammatory components of plant foods have proven to be protective against development of neurodegenerative diseases (Shukitt-Hale, Lau, & Joseph, 2008).

Nuts contain high levels of these beneficial molecules and have been the subject of investigation for numerous health outcomes, including cognitive function. Nuts were found to be protective against brain aging in animals (J. A. Joseph et al., 1999; J. A. Joseph et al., 1998; Shukitt-Hale et al., 2008) and may be similarly beneficial in humans (Shukitt-Hale et al., 2009). Two independent studies on rats indicate improved working memory after short-term walnut supplementation and improved memory retention after supplementation with almond paste (Kulkarni, Kasture, & Mengi, 2010; Willis, Shukitt-Hale, Cheng, & Joseph, 2009). In a recent epidemiological study within a middle-aged

cohort, Nooyens et al (2011) found significant positive associations between quintiles of total nut consumption and four baseline measures of cognitive function. These results suggest efficacy of nuts in attenuating memory deficits in the normal aging process of humans, thus reducing the likelihood of progression to certain dementias and other neurodegenerative conditions.

Although nuts are relatively high in fat content, they have been useful in weight management and in improving lipid profile (Griel & Kris-Etherton, 2006; Sabate & Ang, 2009; Wien, Sabate, Ikle, Cole, & Kandeel, 2003). In addition to other negative health consequences, obesity has been implicated in pathology of neurological conditions including dementia (Anstey, Cherbuin, Budge, & Young, 2011; Knecht, Ellger, & Levine, 2008). The growing trend of obesity among the elderly gives cause to be concerned about the resultant increase in morbidity (Jensen & Rogers, 1998; Mitchell, Catenacci, Wyatt, & Hill, 2011).

B. Purpose of the Study

The purpose of the present research is to investigate the association between consumption of nuts and cognitive ability in a community-dwelling elderly population. We hypothesized that dietary nut consumption is positively associated with specific domains of memory. In addition, we investigated the association between measures of obesity and memory within an elderly cohort.

C. Research Questions

1. Does increasing amounts of nuts, tree nuts, or peanuts, expressed as tertiles of intake, improve the ability of an elderly population to remember a supraspan list

of common household items?

2. Does increasing levels of adiposity, measured as body mass index or waist-to-hip ratio, decrease the ability of an elderly population to remember a supraspan list of common household items?

D. Significance to Nutrition

Dementia contributes substantially to disability among the elderly (Sousa et al., 2009). With a rapidly expanding elderly segment of the world's population, the burden of disease due to cognitive decline associated with normal aging can be expected to concomitantly increase. Foods rich in antioxidants have exhibited the ability to slow cognitive decline in animal and human studies. Other foods rich in omega-3 fatty acids have shown efficacy. Nuts are nutritionally dense foods that have been associated with numerous health benefits, including improving cardiovascular disease risk factors and augmenting weight loss, and recent findings indicate usefulness in preventing cognitive decline. Diet also affects adiposity levels, which can then affect cognitive ability. Memory loss is among the first symptoms of progressive degenerative disorders such as MCI and Alzheimer's Disease. If dietary modifications, such as eating nuts or weight maintenance, can alleviate memory impairments in aging adults, they may potentially improve quality of life or delay progression to more serious cognitive impairments.

CHAPTER 2

REVIEW OF LITERATURE

A. Prevalence of Dementia and Neurodegenerative Diseases

Dementia is a chronic, non-specific illness that affects higher cognitive processes including memory, reasoning, language, perception, judgment, and decision-making. Personality changes and behavioral problems can negatively affect social relationships which can lead to progressive declines in quality of life. Estimated prevalence rates of dementia and MCI are 13.9% and 22.2%, respectively, in the US (Plassman et al., 2008; Plassman et al., 2007). With the growing elderly population in the United States and throughout the world, the economic, social, and health burden of dementia can also be expected to rise. The World Alzheimer Report 2009 estimated that 35.6 million people worldwide would have had dementia in 2010, which will nearly double every 20 years to 65.7 million in 2030 and 115.4 million in 2050 (Prince & Jackson, 2009). The World Health Organization's Global Burden of Disease (WHO, 2008) reports that neuropsychiatric conditions, including Alzheimer's and other dementias, accounts for one-third of years lost due to disability among adults.

The term "dementia" is used to describe an array of symptoms that are often caused by changes to brain structure and function. Sub-types of dementia can be described according to common presenting features of disease, whether or not the disease is progressive, and which areas of the brain are affected. The major forms of dementias are described in Table 2.1. Diagnosis of dementia and its subtypes is based on

observations of the individual's cognitive deterioration and functional disability; however, there is increasing evidence for impact of behavioral and psychological symptoms on disease outcome and burden (McKeith & Cummings, 2005). The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) defines dementia as declines in memory and one other cognitive task compared to a previous functional level and to an extent that compromises activities of daily living.

Varying diagnostic criteria and definitions for MCI are currently in use. Criteria developed by researchers at the Mayo Clinic have been applied in numerous studies. Based on results from a number of neuropsychological tests and conference between clinicians, participants were diagnosed with MCI if they had the following: 1) memory complaints; 2) normal activities of daily living; 3) normal general cognitive function; 4) abnormal memory for age; and 5) no dementia (Petersen et al., 1999). Petersen (2004) later assigned these criteria specifically to the amnestic MCI clinical subtype and distinguished it from multiple domain-MCI plus amnesia, multiple domain-MCI without amnesia, and single nonmemory domain MCI, which appear to differ in incidence, conversion to demetia, and etiology (Roberts et al., 2012; Sachdev et al., 2012).

B. Relation Between Normal Aging, MCI and Dementia

As individuals age, they may experience minor cognitive changes in memory and speed of information processing. These age-related cognitive declines are normal and do not necessarily indicate presence of dementia. MCI is a condition where decline is more pronounced but not enough to warrant a dementia diagnosis; however, many individuals progress from MCI to dementia.

Cohorts of nondemented subjects indicate that the progression to symptomatic dementia is preceded by an asymptomatic preclinical phase and early memory impairment is associated with subsequent onset of demented illness (P. Chen et al., 2001). Although some older adults with cognitive complaints may perform normally on memory tests, they may exhibit similar patterns of changes in their brain structure as MCI patients (Saykin et al., 2006) thus increasing the likelihood of these individuals progressing to further cognitive impairments and conversion to AD. For instance, Chao et al (2010) determined that individuals who were neither demented nor clearly healthy and who had no other neuropyschological impairment (classified by researchers as "preMCI") differed from normal controls but not from MCI subjects in entorhinal cortex, fusiform, and frontal gray matter volume.

The normal aging process involves anticipated declines in the function of various cognitive domains. Beginning at about age 25, individuals may lose ability for numerical or arithmetic ability and declines in processing speed. In the late 30's or 40's, or even as late as the 50's or 60's, episodic or declarative memory begins to fail. Reasoning, verbal ability, and visuoperceptual skills begin to decline in the 50's and 60's and word knowledge, vocabulary, and word reading decline in the 70's. MCI is indicated at the earliest point of accelerated cognitive decline along the pathway to Alzheimer's Disease (AD) development (Petersen, 2003). Further impairment leads to a clinical diagnosis of probable AD and finally to definite AD diagnosis. It is noteworthy that amnestic MCI patients (i.e., those with memory disorders) are at increased risk of progressing to AD

diagnosis (Albert & Blacker, 2006) and that at least half, and up to 70%, of dementias are caused by AD (Graves, 2004).

As mentioned, significant memory impairments greater than that expected by age is one of the first signs of a progressive disease. One cohort of nondemented individuals over the age of 75 experienced a mean annual decline of 1.6 points per year (95% CI: -2.0 to -1.1) in total cognitive score and decline of 1.5 points per year (95% CI: -2.1 to -0.9) in the memory domain (Cullum et al., 2000). Petersen et al (1999) found that MCI study subjects had impaired memory scores compared to control subjects, though still within normal ranges, yet had greater functioning in all other cognitive measures compared to AD subjects. Determining effective interventions to slow memory decline may then be instrumental in retarding progression to MCI and finally to dementia.

C. Memory Formation

The exact mechanisms of memory formation are still under investigation, but in general, three sequential steps have been identified: 1) encoding; 2) consolidation and storage; and 3) retrieval. Information obtained through sensory systems are first registered or perceived and then encoded by undergoing further processing for identification and association. The encoded information (i.e., engram) is then converted in the hippocampus into a form that is suitable for storage. Retrieval is then the process of drawing the stored memory into one's consciousness. Short-term memory is limited in capacity and lasts only a few seconds to a minute. Long-term memory stores much larger quantities of information for an unlimited duration.

The brain has two major systems for long-term memories: declarative (explicit) and procedural (non-declarative or implicit). Declarative memories can be further classified as episodic (events) or semantic (consisting of facts about places, things, people) and involve the medial temporal lobe and hippocampus. These memories must be recalled into consciousness in order to be used. Conversely, procedural memories are recalled unconsciously and consist of perceptual and reflexive motor skills that involve the cerebellum, striatum, amygdala, and simple reflex pathways. These memories are further classified as associative or non-associative. Non-associative memories do not require unconscious linking of cause and effect, such as eating or driving, and are classified as habituation (a decrease in response to a repeated stimulus) and sensitization (elevated response to stimuli following one of greater intensity). Associative learning is classified into classical conditioning and operant conditioning. Classical conditioning was demonstrated by Pavlov's dog and involves associating an unconditioned stimulus (e.g., presentation of meat powder) that has an unconditioned response (e.g., salivation) with a conditioned stimulus (e.g., ringing bell) so that the response can be elicited solely by the conditioned stimulus even in the absence of the unconditioned stimulus. Operant conditioning is learning to associate a reward with a certain behavior and is also called trial-and-error learning. The remainder of this discussion will focus on explicit memory.

Synaptic plasticity, which involves the remodeling of neuronal networks, has long been considered to be the primary physiological mechanism of memory formation. One form of plasticity is long-term potentiation (LTP), which is the prolonged and coordinated activation of several neurons afferent to the hippocampus and which can last for days or

even weeks. The resulting increase in excitatory post-synaptic potential causes an influx of calcium into the post-synaptic cell due to activation of N-methyl-D-aspartate (NMDA) receptors glutamate, which is a neurotransmitter released by the pre-synaptic neuron. Calcium then binds to calmodulin activating calcium-calmodulin dependent protein kinase (CaMKII), which is necessary for LTP induction through three different pathways (Lisman, 2003). LTP induction can also activate synthesis of new proteins which promote growth and stabilization of synapses. It is likely that both CaMKII activation and protein synthesis are needed for new memory formation.

Recent findings indicate a role for reactive oxygen species (ROS) in LTP, learning and memory, and signal transduction pathways that support LTP and memory (Kishida & Klann, 2007). ROS that are generated due to neuron and glia signaling in the hippocampus during periods of increased neuronal activity lead to significant, long-lasting action potential firing and post-translational modifications in neighboring cells (Atkins & Sweatt, 1999) that enhance LTP (Infanger, Sharma, & Davisson, 2006).

A burgeoning area of investigation is in the neurogenesis that occurs in the adult brain and that appears to play a role in memory. It is specifically within the dentate gyrus of the hippocampus formation that these new neurons are born (Koehl & Abrous, 2011). Research in animals have shown a close interplay and linking of the existing neural network and newborn neurons in the hippocampus (Li & Pleasure, 2010). The dentate gyrus is responsible for receiving sensory inputs from the entorhinal cortex, septum, supramammillary region, posterior hypothalamus, and brain stem and relaying the information to the hippocampus. Coras et al (2010) demonstrated a highly significant

correlation between cell loss in the dentate gyrus and decreased ability to store and recall new memories ($r = 0.966$, $p = 0.001$).

D. Biology of Disease

Each form of dementia affects different regions of the brain to a greater or lesser extent, but there are some common features in the pathology of these diseases. These include oxygen flow to the brain, activity of neurons and neurotransmitters in the brain, and structural changes in the brain. The adult brain contains about 100 billion nerve cells that branch out to connect at nearly 100 trillion neural synaptic junctions. Dementia results from the failure of nerve cells to transmit messages through the neuron or across the synapses, which can eventually lead to cell death. Cell-to-cell signaling at the synapses can be blocked by plaques of β-amyloid proteins. In addition, tangles of *tau* protein formed within a cell disable transport of information and nutrients within the neuron. Individuals with AD, for example, experience spreading of plaques and tangles throughout the cortex in a predictable pattern. The earliest changes may occur 20 years prior to diagnosis as plaques and tangles begin to form in regions of the brain involved in learning and memory and thinking and planning. These then spread to areas involved in speaking and understanding and personal sense of space in mild to moderate stages. Finally, the tangles and plaques affect most of the cortex resulting in tissue loss throughout the brain and brain shrinkage. Shrinkage is especially pronounced in the hippocampus, the region of the cortex that is involved in forming new memories.

Inflammation (Gorelick, 2010) and oxidative stress (Mattson & Liu, 2002) have been implicated in the pathophysiology of cognitive decline and dementia. Normal

metabolic reactions in the brain utilize a large amount of oxygen rendering these cells especially prone to oxidative stress, which in turn contributes to the disease process of age-related neurodegenerative diseases. For example, the NADPH oxidase (NOX) family of enzymes are dedicated to the production of free radicals in neurons. They function to reduce molecular oxygen (O2) into superoxide (O2*) and other ROS. Although ROS is needed for LTP, as was discussed above, excessive amounts of oxidative stress leads to impaired processes and potentially neuronal death, indicating the need for a carefully modulated redox environment (Infanger et al., 2006).

E. Nutrition and Cognitive Function

The brain is a high-energy consumer, requiring 20% of energy provided by the diet and 20% of the inhaled oxygen (Roman, 2006). It then follows that nutritional factors and deficiencies, including vitamins, macronutrients, and even caloric intake, would be associated with cognitive function. Table 2.2 lists several recently published research studies on the relationship between diet and cognitive function among elderly humans and shows the divergent findings for various foods, nutrients, and diet patterns.

1. Diet patterns

Diet patterns, including caloric restriction and fasting, consumption of specific foods, and overall diet patterns, have also been investigated for the impact on cognition (J. Joseph, Cole, Head, & Ingram, 2009). Dietary restriction through intermittent fasting or caloric restriction appears to stimulate neurogenesis from neuronal stem cells and production of proteins that improve neuronal plasticity and resistance to oxidative damage (Mattson, Duan, & Guo, 2003). The Mediterranean diet pattern has

also been investigated for various health benefits but findings related to cognitive function are inconclusive. In one study, Feart et al (2009) demonstrated that a higher adherence to the Mediterranean pattern slowed cognitive decline with some cognitive testing, but was not associated with risk for dementia. Scarmeas et al (2009), however, found that subjects who had higher adherence to the Mediterranean diet had increasing protection against MCI and AD.

2. Foods and supplements

Specific foods and food groups, such as fish, nuts, berries, fruits, and vegetables, have been implicated in improving cognitive function or delaying cognitive decline; however, results are mixed. For example, Morris et al (2006) report a significant attenuation of cognitive decline with increasing vegetable consumption while Nooyens et al (2011) report no significant association of vegetable consumption and memory. Because fatty fish is a good source of n-3 fatty acids, fish and fish oil have also been investigated for effects on cognition. One study by van Gelder et al (2007) reported that older men who consumed fish had less cognitive decline after five years (p-value = 0.01) and a significant inverse dose-response effect between combined EPA and DHA intake and cognitive decline (p-value = 0.01).

Memory can specifically be improved with nutritional supplementation, according to a recent report by Chan et al (2010). This randomized clinical trial utilized a nutriceutical formulation containing folic acid, vitamin B12, vitamin E, S-adenosylmethionine, N-acetyl cysteine and Acetyl-L-carnitine. Participants consuming the pill performed significantly better on the California Verbal Learning Test's (CVLT-II)

short-delay recall ($p<0.001$) compared to placebo, although there was no significant difference in immediate recall and long-delay recall. Using a composite score composed with equal weights of immediate recall, short-delay and long-delay recalls, there was clinically significant improvement (effect size = 0.35) in participants administered the test formula. These results were similar when excluding participants over 74 years of age, indicating similar response among the very aged. Within the same study population, participants' scores on the Trail-making tests (Trails B - Trails A) returned to baseline levels ($p=0.39$) after a three-month wash-out period where treatment was withdrawn. They again showed significant improvement ($p=0.01$) compared to baseline when treatment was re-introduced.

3. Antioxidants

Antioxidants counteract the production of free radicals in neural tissue by inhibiting lipid peroxidation, programmed death of brain cells, damage to cell membranes, production of reactive oxygen species, and oxidative damage to proteins and DNA, by promoting an anti-inflammatory response, and by improving endothelial function (Lau, Shukitt-Hale, & Joseph, 2007). Morris et al (2002) showed that consuming a greater amount of the antioxidant vitamin E in foods or as supplements slows cognitive decline in an elderly population. Moreover, the antioxidant vitamins A, C, E, and carotenoids have been inversely associated with cognitive decline and dementia (Del Parigi et al., 2006).

4. Omega-3 fatty acids

The long-chain omega-3 fatty acids α-linolenic acid (ALA), eicosapentaeoic acid (EPA) and docosahexaenoic acid (DHA) are important structural components of neuronal tissue, and have been associated with delaying cognitive decline (van Gelder et al., 2007). The neuro-protective effects of the omega-3 family of fatty acids include opening of background K+ channels, preventing necrosis and apoptosis of motor neurons, and maintaining membrane fluidity (Bourre, 2006b). DHA is the primary fatty acid deposited in the brain, mainly occurring in the infant during the last trimester of pregnancy and the first two years of life.

Over 60% of the energy consumed by the adult brain is due to Na-K-ATPase enzyme activity, which accounts for more than 50% of dietary carbohydrates. Since DHA content of membranes affects activity of the Na-pump, deficiencies could lead to decreased energy metabolism and glucose transport (Bourre, 2006b). DHA levels in the hippocampus decrease with age and are found in lower levels among AD patients and are associated with memory loss (Su, 2010). Freemantle et al (2006) suggest that ALA and EPA contribute to the preservation of cognition in aging by producing ketones and promoting fatty acid oxidation rather than by the limited conversion to DHA in the brain.

5. Micronutrients

Deficiencies of the B vitamins thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), folate (B_9), and cobalamin (B_{12}) have been associated with cognitive declines. The B vitamins play prominent roles in structure of cerebral tissue, oxygen

uptake, cholinergic activity, and homocysteine metabolism. The cognitive importance of these vitamins, along with other micronutrients, has been reviewed extensively by Bourre (2006a). Those that have been implicated in affecting memory are B_6, B_9, and B_{12}. It is noteworthy that Bourre concludes that the primary quality of these nutrients in relation to brain function is their antioxidant activity.

F. Nuts and Cognitive Function

Nuts are nutritionally dense foods that are readily available in most regions of the world. The fatty acid and antioxidant profile of nuts warrant further investigation for their effect on cognitive function. In addition, adding almonds to the diets of community-dwelling adults have the effect of improving their overall nutrient intake pattern (Jaceldo-Siegl, Sabate, Rajaram, & Fraser, 2004), which may positively effect their overall health profile. Interestingly, this was true despite greater caloric consumption due to almond supplementation, indicating a special property of almonds, and perhaps other nuts, in providing health benefits.

Table 2.3 lists some of the primary nutrient and non-nutrients found in various nuts that have purported effects on cognitive function. Consuming tree nuts and peanuts that contain antioxidants can provide the antioxidant benefits in humans (Ros, 2009). Walnuts contain 13-21 mmol of total antioxidants per 100 g (Halvorsen et al., 2006; Halvorsen et al., 2002) and 3.5 ± 1.0 ng of melatonin per gram (Reiter, Manchester, & Tan, 2005). Reiter et al (2005) showed that rats fed a walnut diet experienced significantly increased blood levels of melatonin, which was positively correlated with total antioxidant capacity of serum. Melatonin has shown to have strong antioxidant

properties (Reiter et al., 2008; Reiter et al., 2003), with potency even greater than vitamins E, C, or β-carotene (Korkmaz et al., 2009). Melatonin's modes of action include the ability to directly scavenge free radicals, to stimulate antioxidant enzymes, to work synergistically with other antioxidant molecules, and to support mitochondrial activity and ATP generation (Reiter et al., 2003). In a placebo-controlled trial of hospitalized patients, Al-Aama et al (2010) found that low doses of supplemental melatonin reduced the risk of delirium by 15.6%.

Omega-3 long-chain polyunsaturated fatty acids, which are abundant in walnuts, are purported to inhibit age-related cognitive decline; however, a recent controlled trial of cognitively-healthy elderly showed no difference in cognitive function between fish oil supplements and placebo treatment groups (Dangour et al., 2010). It may be that antioxidants are necessary in tandem to provide stability to fatty acids, reduce free radical damage, and ultimately health benefits.

Results from animal studies indicate a protective affect on cognitive function from specific nuts (J. A. Joseph et al., 1998). Rats, for example, had improved working memory after short-term walnut supplementation and improved retention of memory after supplementation with almond paste (Kulkarni et al., 2010; Willis et al., 2009). In a study of rats with induced amnesia, administration of almond paste decreased cholinesterase activity in the brain and reduced cholesterol and triglyceride levels, and had the effect of reversing the amnesia (Kulkarni et al., 2010).

Few human studies, however, have been conducted. An early report by Fraser et al (1996) demonstrated an insignificant effect of nut consumption on overall severity of

cognitive impairment. But when assessing four specific cognitive domains including memory, Nooyens et al (2011) reported significant positive associations with quintiles of total nut consumption in a middle-age population of 2,613 adult men and women who were administered two rounds of cognitive testing from 1995 to 2007. At baseline, participants who consumed greater amounts of nuts showed better cognitive function in all cognitive domains (P for trend ≤ 0.01). The difference in cognitive function between the lowest and highest quintiles of nut intake in this population was similar to an age difference of 5-8 years.

Nut consumption may prove a win-win situation since they contain both omega-3 fatty acids and antioxidants. The present research will explore the utility of nut consumption in maintaining memory abilities in an older population. Looking at specific domains of memory may implicate specific steps in memory formation that may be affected by the nutritional profile of nuts, which to date has not been reported by other researchers.

G. Epidemiology and Risk Factors

Age is well known to be the strongest risk factor for dementia, with the odds of developing AD doubling every 5 years after age 65. Liddell et al (2007) demonstrated the rate of decline among individuals with AD, MCI, subjective memory complaint (SMC), and those with normal functioning. For every 10 years of advancing age, normals, SMC, MCI and AD patients showed a decline of 0.08, 0.08, 0.24, and 0.36 standard deviations, respectively, in delayed verbal memory recall and decline of 0.09, 0.1, 0.24, and 0.4 standard deviations, respectively, in memory recognition accuracy. The

trends were similar for two other measures of verbal memory. Several measures of working memory and sustained attention, executive function, and verbal processing also showed similar trends between normals and SMC patients and between MCI and AD patients. It is hypothesized that the age of onset of clinical dementia is related to the trajectory of brain reserve, or rate of neuronal loss, due to development during early life and other risk factors discussed below (Graves, 2004).

Several forms of dementia are linked to genetic mutations that can be passed from one generation to another. As such, family history increases the risk of developing these forms; however, the conditions can also develop in individuals with no history of disease in their family. The ε4 single-gene mutation of apolipoprotein E (APOE ε4) has been shown to be a risk factor for sporadic and familial Alzheimer's Disease and carriers have shown to have significantly greater cognitive decline compared to noncarriers (Jonker, Schmand, Lindeboom, Havekes, & Launer, 1998). Other genes of interest include *APP*, *preselin-1*, and *preselin-2*. Results have varied as to the gender differences in risk of dementia, which may differ by ethnicity and the type of dementia (J. H. Chen, Lin, & Chen, 2009).

Other health conditions such as atherosclerosis, high cholesterol levels, diabetes, and Down Syndrome can also increase the risk of dementia. Cholesterol buildup, which can result in atherosclerotic plaques, may lead to decreased oxygen delivery to the brain resulting in stroke or dementia. Diabetes is a risk factor for atherosclerosis and stroke and can also develop into dementia. Poor regulation of blood glucose levels has been

associated with poor performance on cognitive tests, particularly memorization (Bourre, 2006b).

Smoking is an independent risk factor for dementia and cognitive decline. In a meta-analysis of 19 prospective studies, researchers found a 27% increase risk for any dementia among current smokers compared to those who never smoked (Anstey, von Sanden, Salim, & O'Kearney, 2007). Certain environmental factors have also been implicated in risk of dementia, including metals such as aluminum, iron, copper, and zinc (J. H. Chen et al., 2009). Conversely, higher education has been shown to decrease risk for dementia and the impact of pathology prior to death (Brayne et al., 2010). Exercise has also been widely investigated as a protective factor. Meta-analysis of 30 randomized trials indicated that exercise training improved cognitive function, physical function, and behavior in affected elderly (Heyn, Abreu, & Ottenbacher, 2004).

Table 2.1. Forms of Dementia (NINDS, 2010).

Type	Common Behavioral and Cognitive Features
Alzheimer's Disease (AD)	Decline in nearly all cognitive abilities, including memory, movement, language, judgment, behavior, abstract thinking, visuospatial problems, and delusions.
Vascular Dementia	Sudden presence of symptoms after a stroke, which may or may not progress over time with sudden changes; symptoms similar to stroke patient, including depression and incontinence, and cognitive impairment similar to AD without personality or emotional changes.
Lewy Body Dementia	Memory impairment, poor judgment, confusion, visual hallucinations, parkinsonian symptoms, and fluctuations in severity of symptoms
Frontotemporal Dementia	Exhibit socially inappropriate behavior, loss of speech and language, compulsive or repetitive behavior, increased appetite, motor problems
HIV-associated Dementia	Impaired memory, apathy, social withdrawal, difficulty concentrating, problems with movement
Huntington's Disease	Irritability, anxiety, depression progressing to severe dementia and psychotic behavior
Dementia Pugilistica	Dementia and parkinsonism, poor coordination, slurred speech
Corticobasal Degeneration	Symptoms begin by affecting one side of body, poor coordination, memory loss, dementia, visual-spatial problems, apraxia, halting speech, dysphagia
Creutzfeldt-Jakob Disease	Muscular coordination, personality changes, impaired memory, judgment, and vision, insomnia, depression
Gerstmann-Straussler-Scheinker Disease	Ataxia and progressive dementia
Fatal Familial Insomnia	Insomnia, poor reflexes, dementia, hallucinations, coma
Familial British Dementia & Familial Danish Insomnia	Progressive dementia, paralysis, loss of balance

Table 2.2. Published research studies relating major nutrients, foods, and diet patterns with cognitive function among the elderly.

Author (Date)	Location	Study Design	N	Age (years)	Main Exposure of Interest	Primary Findings
Correa Leite et al (2001)	Italy	Cross-sectional	1651	65-69	Healthy diet according to WHO guidelines	Better healthy diet score associated with lower cognitive deficit
Dangour et al (2010)	UK	Clinical trial	744	70-79	EPA and DHA	No difference between intervention arms
Feart et al (2009)	France	Prospective cohort	1410	>65	Adherence to Mediterranean diet	Higher adherence to diet was associated with slower cognitive decline but not dementia risk
van Gelder et al (2007)	Netherlands	Prospective cohort	210	70-89	Fish consumption, EPA and DHA intake	Fish consumers had less cognitive decline in 5 years; inverse dose-response effect of combined EPA + DHA intake on cognitive decline
Huijbregts et al (1998)	Europe	Cross-sectional	1049	70-91	Healthy diet according to WHO guidelines	Mixed association between healthy diet and cognitive impairment across cohorts
Krikorian et al (2010)	USA	Clinical trial	12	78.2 (sd=5)	Concord grape juice	Improved cognitive performance among grape juice arm compared to placebo

Abbreviations: EPA = eicosapentanoic acid; DHA = docosahexanoic acid; WHO = World Health Organization

Table 2.2 (continued). Published research studies relating major nutrients, foods, and diet patterns with cognitive function among the elderly.

Author (Date)	Location	Study Design	N	Age (years)	Main Exposure of Interest	Primary Findings
Morris et al (2005)	USA	Prospective cohort	3718	>65	Fish consumption	Fish consumption associated with slower rate of cognitive decline
Morris et al (2002)	USA	Prospective cohort	2889	65-102	Vitamin E	Vitamin E intake associated with less cognitive decline
Nooyens et al (2011)	Netherlands	Prospective cohort	2613	43-70	Habitual fruit & vegetable intake	High intake of some fruits and vegetables, including nuts, associated with better cognitive function at baseline
van de Rest et al (2008)	Netherlands	Clinical trial	302	>65	EPA & DHA supplements	No difference between intervention arms

Abbreviations: EPA = eicosapentanoic acid; DHA = docosahexanoic acid; WHO = World Health Organization

Table 2.3. Nutrient and non-nutrient composition of select nuts, per standard serving sizes as defined by the Adventist Health Study-2 (U.S. Department of Agriculture, 2011).

	Almonds	Cashews	Peanuts	Black Walnuts	Mixed Nuts*
Serving Size (g)	18.9	9.5	18.0	16.2	19.6
Calories (kcal)	109.0	52.0	102.0	100.0	116.0
Protein (g)	4.0	1.7	4.6	3.9	3.4
Total fat (g)	9.3	4.1	8.9	9.6	10.1
Carbohydrate (g)	4.1	2.9	2.9	1.6	5.0
Magnesium (mg)	51.0	28.0	30.0	33.0	44.0
Thiamine (mg)	0.04	0.04	0.12	0.01	0.04
Riboflavin (mg)	0.19	0.01	0.02	0.02	0.04
Folate (mcg)	9.0	2.0	43.0	5.0	10.0
Vitamin E (alpha-tocopherol, mg)	5.0	0.1	1.5	0.3	--
ALA (mg)	0.001	--	0.001**	0.325	.037**
Tryptophan (g)	0.04	0.03	0.05	0.05	0.05
Phenylalanine (g)	0.2	0.1	0.2	0.2	0.2

* Mixed nuts, dry roasted with peanuts, no salt added
** Represents undifferentiated n-3 fatty acids
Abbreviations: ALA = alpha-linoleic acid

CHAPTER 3

METHODOLOGY

A. Study Subjects

Adventist Health Study-2 (AHS-2) participants were recruited from Seventh-day Adventist (SDA) churches and congregations in the United States and Canada from 2002-2007. During the period of recruitment into the AHS-2 study, there were over one million SDAs living in the United States and Canada, with an estimated 90,000 black and 260,000 non-black church attendees eligible for recruitment (Butler et al., 2008). Church attendees were included if they were at least 30 years of age and sufficiently fluent in English to complete data collection forms.

Over 96,000 adults enrolled, of which 25,000 are blacks and the remaining are mostly white. The AHS-2 cohort has widely varying diet patterns including 48% non-vegetarians, 16% semi- or pesco-vegetarian, 28% lacto-ovo vegetarian, and 8% total vegetarians (Jaceldo-Siegl et al., 2011). The population also consumes varying amounts of foods such as soy and nuts and the vast majority abstain from tobacco use and alcohol.

Twenty thousand AHS-2 respondents were then randomly selected to participate in the Biopsychosocial Religion and Health Study (BRHS). Out of the 10,988 respondents, a subset was invited to participate in clinic visits if they resided within driving distance of the clinic centers located in Loma Linda and Los Angeles, CA and were at least 50 years of age at time of recruitment with a goal of obtaining 500 total

participants. Figure 3.1 illustrates the sampling plan and overall timeline. Five hundred thirty-six adults participated in the in-person clinic visit.

Past studies conducted among the Adventist population have contributed greatly to the scientific understanding of the effect of diet on numerous health conditions (J. Chan, Knutsen, Blix, Lee, & Fraser, 2002; Hailu, Knutsen, & Fraser, 2006; Jacobsen, Knutsen, & Fraser, 1998), including dementia (Giem, Beeson, & Fraser, 1993). Seminal studies among Adventists living in California demonstrated the beneficial health effects of nut consumption on heart disease (Fraser, Sabate, Beeson, & Strahan, 1992; Sabate & Fraser, 1994). A calibration sub-study of the current AHS-2 cohort provides added validity to diet information (Jaceldo-Siegl et al., 2011), thus increasing confidence in research findings.

B. Study Design

Secondary analysis of AHS-2 and BRHS data was conducted to examine the relationship between intake of nuts and memory and measures of adiposity and memory. Details of the AHS-2 and BRHS study design have been published (Butler et al., 2008; Lee et al., 2009). Briefly, AHS-2 participants completed a baseline questionnaire, which included data on medical history, demographic factors, and dietary habits. The BRHS questionnaire obtained information on social and psychological factors and religious practices and beliefs. An average of four years after enrollment in AHS-2, 536 older adults participated in the BRHS clinic visit and provided anthropometric measurements and cognitive and physical performance assessment. In appreciation of their time, participants were given a health report at the conclusion of the clinic visit.

C. Data Collection

1. Nut consumption

A quantitative food frequency questionnaire (FFQ) was used to asses dietary intake of AHS-2 participants. The food list included items that are commonly consumed by US Adventists and that significantly contribute to the 26 nutrient, phytochemical, and mineral indices related to the major study hypotheses. Additionally, results from piloting the FFQ warranted the inclusion of foods specific to Black Adventist populations (Akbar, Jaceldo-Siegl, Fraser, Herring, & Yancey, 2007). The final questionnaire included a food list of over 200 fruits, vegetables, legumes, grains, oils, dairy, fish, eggs, beverages, meats and commercial products such as supplements, cereals, and vegetarian protein products. Participants were also asked to write in food items that were not present in the food list and to specify the brand names and product information (*e.g.*, regular, low-fat or "lite"). Categorical frequency of consumption options ranged from never or rarely to more than six servings per day while portion sizes included a stated standard, one-half the standard, or 1.5 times the standard. Pictures of common foods or beverages were provided to assist in estimating portion sizes. The baseline questionnaire, including the FFQ, has been validated for intake of several nutrients and foods (Jaceldo-Siegl et al., 2011; Jaceldo-Siegl, Fraser, Chan, Franke, & Sabate, 2008; Jaceldo-Siegl et al., 2010). Specifically for nuts, de-attenuated validity correlations ranged from 0.39 to 0.59 (Jaceldo-Siegl et al., 2011). Uncorrected and de-attenuated correlation coefficients for nuts are presented in Table 3.1.

Nut intake for peanuts, walnuts, almonds, cashews, mixed nuts, and peanut butter was queried on the FFQ. Frequency of intake included eight options: never or rarely; 1-3 per month; 1 per week; 2-4 per week; 5-6 per week; 1 per day; 2-3 per day; and 4+ per day. Standard portion size was stated as 1 tablespoon for peanut butter, 14 nuts for mixed nuts, 35 halves for peanuts, 8 halves for walnuts, 16 whole nuts for almonds, 6 whole nuts for cashews, and 12 nuts for write-in entries. The NDS-R 2008 (NDS-R, Nutrition Coordination Center, Minneapolis, MN, USA) database was used to calculate serving sizes with the following standard grams per serving: peanut butter = 16.13 g; mixed nuts = 19.6; peanuts = 18 g; walnuts = 16.2 g; almonds = 18.9 g; and cashews = 9.45 g. Grams of nut intake were then adjusted for total caloric intake using the residual method (W. C. Willett, Howe, & Kushi, 1997). The present analysis defined three nut variables: 1) peanuts, which included peanuts and peanut butter; 2) tree nuts, which included walnuts, almonds, cashews, and mixed nuts; and 3) all nuts, which combined all types of peanuts and tree nuts. Each nut variable was categorized by tertiles of intake.

2. Memory measures of cognitive function

The California Verbal Learning Test-Second Edition Short Form (CVLT) was administered to study participants during clinic visits. The CVLT Short Form is an abridged version of the longer CVLT, involving a shorter list of words (9 versus 16), only one list rather than two used with the CVLT-II, and fewer recall trials. The CVLT is used for individuals with more severe cognitive impairments or as a screening tool. More than 50 indicators of cognitive performance are measured by the CVLT, including short and

long-delay recall, semantic clustering, primacy and recency, learning slope, response bias, and intrusions.

A word list is used to assess both the amount of verbal material remembered and how verbal learning occurs. The nine items fall into one of three categories (clothing: hat, sweater, belt; fruit: cherries, lemon, peaches; and tools: wrench, pliers, drill), which are read to the subject in randomized order at a pace of about one second per word. The participant is given four trials to recite the words in any order. Next follows a short 30-second distraction activity (counting backwards from 100) before the subject is asked to recite the words using free recall without prompting. Then a 15-minute distraction involving non-verbal physical testing is performed before the subject is asked to recite the words using free recall (without prompting) and cued recall (with prompting for words that fall under the three categories). Finally, the long-delay yes/no recognition asks the subject to decide whether or not each item from a list of 27 words was on the original list of nine words.

Z-scores standardized to national norms from five CVLT measures were used in analysis (see Table 3.2). Z-scores were generated within each strata for gender and 10-year age category with participants ages 90 and above included in the 80-89 age bracket. Core verbal learning ability is reflected by performance on Immediate-Recall Trial 4 while the Trial 1 score provides reflects auditory attention. The Short-Delay Free Recall reflects the effect of memory performance attributable to both the short-delay interval and interference from the verbal distractor exercise. Likewise, the Long-Delay Free Recall assesses forgetting rates over a longer interval but without the interference

caused by a verbal distractor. The Long-Delay Cued Recall can identify individuals who make intrusion errors by naming words not on the test list. Intrusion errors can range from mild to severe and are more prevalent among those with more serious types of memory disorders.

3. Anthropometrics

Anthropometric measurements were obtained at clinic visits. Weight was measured using a Tanita scale with the participant standing without shoes, socks and heavy outer garments. Height was likewise measured. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist and hip circumferences were measured with participants standing in a relaxed position. Dividing waist circumference by hip circumference yielded the waist-to-hip ratio (WHR).

4. Covariates

Age at time of memory testing was calculated using the date of birth and date of clinic visit. Gender was also included in the models. Highest attained education was queried on the baseline AHS-2 questionnaire using the following nine categories: grade school; some high school; high school diploma; trade school diploma; some college; Associate degree; Bachelors degree; Masters degree; and Doctoral degree. Attained education was analyzed as ordinal data and categories with few cases were collapsed as indicated in Table 3.2. Because the study population is composed primarily of non-Hispanic Whites and Black African American, all other ethnic groups (4.9% of study sample) were categorized together with Whites.

The baseline AHS-2 questionnaire contained a multiple-question section on physical activity. Duration of moderate, vigorous, and extremely vigorous physical activity on a usual week day, usual Saturday, and usual Sunday was obtained with the following options: never do; less than 20 minutes; 20-39 minutes; 40-59 minutes; at least one but less than two hours; at least two but less than three hours; at least three but less than six hours; and more than six hours. Minutes of activity was calculated using, for the most part, the median number of minutes in each category, except that "never do" was given zero (0) minutes, "less than 20 minutes" as 20 minutes, and "more than six hours" as 360 minutes. Average minutes per day of each activity level was calculated separately by averaging the minutes for Sunday, minutes for Saturday, and five times the minutes for week day.

In order to account for dietary factors other than nuts, a three-category variable was defined to indicate relative amount of consumption of animal products including meats, fish, dairy, and eggs as determined by the AHS-2 baseline FFQ. Categories included low, moderate, and high intake of animal products. Daily caloric intake was also calculated from the FFQ using NDS-R 2008 database.

Previous diagnosis of health conditions that may affect cognition was obtained from the BRHS questionnaire. Separate variables were created for history of diagnosis of cardiovascular disease (stroke lasting 24 hours, TIA, angina pectoris, or high blood pressure), type 2 diabetes, and depression. A time variable was also created in order to isolate the effect of the varying period between completion of the FFQ and cognitive testing.

D. Data Analysis

BRHS participants were excluded if they had missing or invalid diet data (n=38), were aged less than 50 years of age at the time of memory testing (n=5), or had missing medical history information (n=23). Missing data for other important variables were imputed using mean substitution. Missing age at cognitive testing values were imputed with the mean age for each gender and ethnicity strata. Means for each age and gender strata were imputed for missing values for caloric intake, level of attained education, body mass index, physical activity variables, and time between AHS-2 questionnaire and memory testing. The final analytic sample consisted of 167 males and 278 females (total N = 445).

Power analysis was performed using G*Power 3.1.2 (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). For a two-tailed post hoc power analysis with an alpha level of 0.05, effect size of 0.28 (Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2010), and total sample size of 445, there was 99% probability of detecting a difference if it truly exists. Using the same criteria, 331 total participants would be needed to achieve 95% power, which is easily met by this study sample.

Initial data analysis indicated that moderate, vigorous, and vigorous physical activity levels were not related to any of the CVLT measures under investigation ($p>0.05$), and were therefore excluded from further analyses. The same held true for previous diagnosis of depression. Multivariate regression techniques evaluated CVLT z-scores as dependent variables and all nuts, tree nuts, peanuts, BMI, and WHR as independent variables in separate models. Specifically, analysis of covariance was used

for tertiles of nut intake while multiple linear regression was used for anthropometric variables. All analyses were performed using Stata/IC version 11.1 (StataCorp, College Station, TX).

E. Strengths and Limitations

The primary strengths of this study are the comprehensive ascertainment of dietary and lifestyle factors, use of standardized memory scores, and large sample size. As has been already mentioned, the AHS-2 food frequency questionnaire has been validated for nut and seed intake and can be depended on to provide a moderately good estimation of actual intake. Additionally, the CVLT was able to provide information on specific learning and memory domains of cognitive function that have not yet been evaluated against nut consumption. The CVLT measures are also age- and gender-standardized to an external US population, thus allowing for greater generalization. Because the study population is composed entirely of Seventh-day Adventists, a religious group that abstains from alcohol and tobacco, these potential confounders are also naturally controlled for in analysis. This study was limited to an older population (mean = 69, s.d. = 11.6) who are at greater risk of cognitive decline.

One of the limitations of this study is the limited generalizability due to the study population being part of the same religious body, which may affect other dietary and lifestyle factors, and being drawn from southern California. There may also be concern regarding the time lapse between collection of baseline information, including diet, and cognitive testing. Although this is a notable concern, the prospective data collection may be useful in determining a time effect. It can also be shown that the dietary pattern in this

population is fairly consistent over time and that changes in diet are minimal over short periods. Nevertheless, a time variable was introduced into analysis to determine any differences in outcome related to the length of time between completion of the FFQ and cognitive testing. The most pressing limitation is associated with the cross-sectional design of the analysis. Causation cannot be evaluated in this study. In fact, reverse causation is of concern but cannot be investigated.

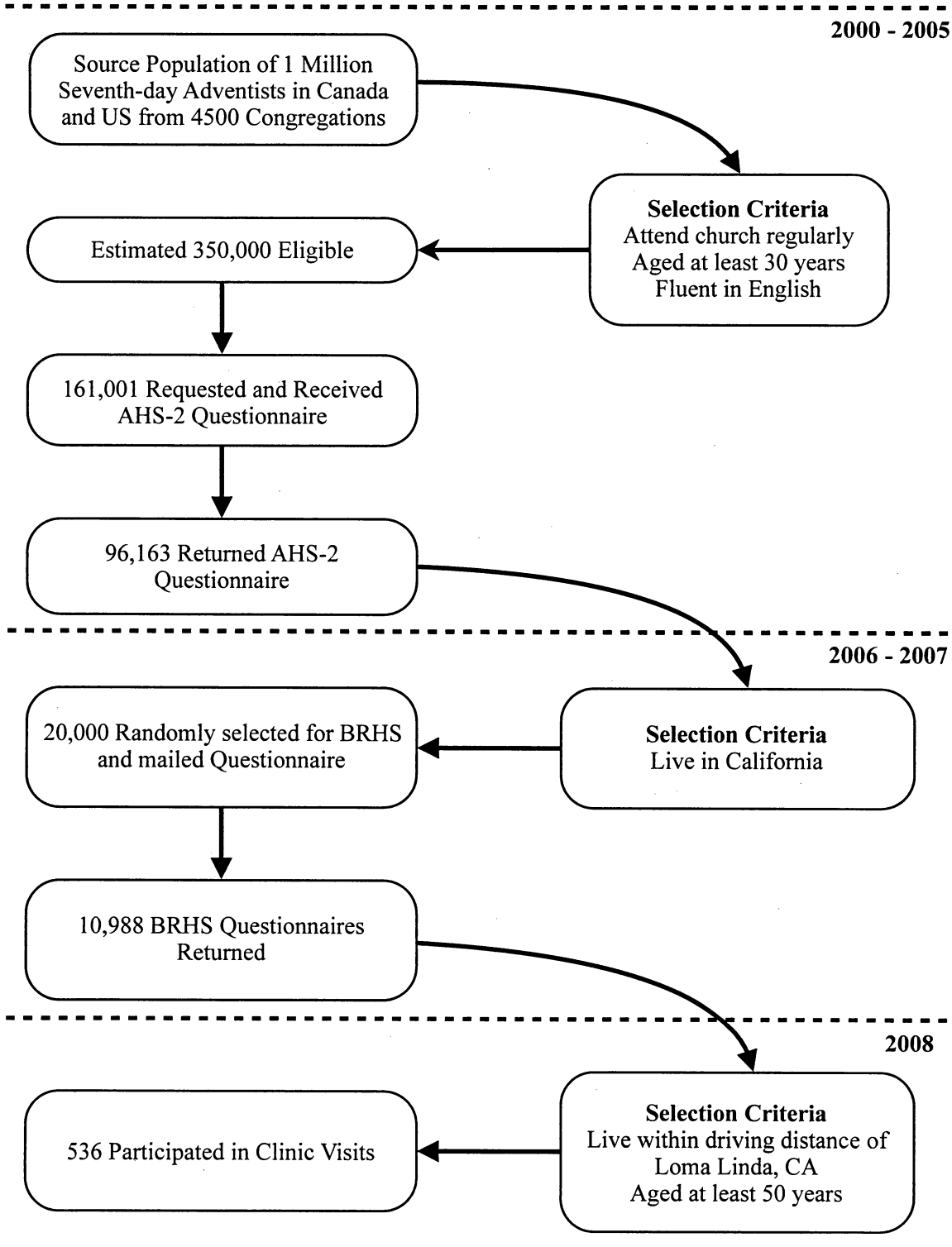

Figure 3.1. Sampling plan and timeline of the Adventist Health Study-2 (AHS-2) and Biopsychosocial Religion and Health Study (BRHS)

Table 3.1. Energy-adjusted validity correlations for nut and seed data from the Adventist Health Study-2 Calibration Study (Jaceldo-Siegl et al., 2011).

	Whites		Blacks	
	De-attenuated Correlation	95% CI	De-attenuated Correlation	95% CI
Peanuts	0.40	0.31 - 0.48	0.27	0.15 - 0.37
Peanut butter	0.59	0.52 - 0.65	0.41	0.30 - 0.50
Tree nuts	0.58	0.49 - 0.65	0.39	0.28 - 0.50
All nuts and seeds	0.58	0.47 - 0.66	0.47	0.38 - 0.55

Table 3.2. Variable definitions and scales of measurement used in analysis

Variable	Description	Coding	Scale of Measurement
Dependent Variables			
Immediate-recall, Trial 1	Level of correct recall performance for trial 1	Age- and gender-standardized z-score	Interval
Immediate-recall, Trial 4	Level of correct recall performance for trial 4	Age- and gender-standardized z-score	Interval
Short-delay Free Recall	Level of correct recall after 30-second verbal distractor	Age- and gender-standardized z-score	Interval
Long-delay Free Recall	Level of correct recall after 15-minute non-verbal distractor	Age- and gender-standardized z-score	Interval
Long-delay Cued Recall	Level of correct recall after 15-minute non-verbal distractor providing cues	Age- and gender-standardized z-score	Interval
Independent Variables			
Total nuts	Calorie-adjusted grams of intake of all nuts	Tertiles	Ordinal
Tree Nuts	Calorie-adjusted grams of intake of tree nuts	Tertiles	Ordinal
Peanuts	Calorie-adjusted grams of intake of peanuts and peanut butter	Tertiles	Ordinal
BMI	Body mass index	kg/m^2	Interval
WHR	Waist-to-hip ratio	Ratio	Interval

Table 3.2 (continued). Variable definitions and scales of measurement used in analysis

Variable	Description	Coding	Scale of Measurement
Other Variables			
Sex	Male or female	1 = Male 2 = Female	Binary
Age	Age at cognitive testing	Years	Interval
Ethnicity	Ethnicity	1 = White or Other 2 = Black	Binary
Education	Highest attained education	1 = Less than college 2 = Associate's/Bachelor's 3 = Master's/Doctoral	Ordinal
Animal product consumption	Relative amount of meats, fish, dairy, or egg consumed regularly	1 = Low 2 = Moderate 3 = High	Ordinal
Calories	Caloric intake	kcal/day	Interval
Napping	Daytime time spent napping	Minutes/day	Interval
Sedentary behavior	Daytime time spent sedentary	Minutes/day	Interval
Physical activity	Time spent in moderate, vigorous, or extremely vigorous activity	Minutes/day	Interval
Cardiovascular disease history	Previous diagnosis of stroke lasting 24 hours, TIA, angina pectoris, or high blood pressure	0 = No 1 = Yes	Binary

Table 3.2 (continued). Variable definitions and scales of measurement used in analysis

Variable	Description	Coding	Scale of Measurement
Diabetes history	Previous diagnosis of type 2 diabetes	0 = No 1 = Yes	Binary
Depression history	Previous diagnosis of depression	0 = No 1 = Yes	Binary
Time	Time between completion of food frequency questionnaire and clinic visit	Years	Interval

CHAPTER 4

FIRST PUBLISHABLE PAPER

Adiposity and Domain-Specific Memory Function in the Elderly:

Findings from the Adventist Health Study-2

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ABSTRACT

Objective. To describe the association between memory function and measures of adiposity in a community-dwelling elderly population.

Methods. Adults aged 50 years and older residing in southern California (n = 445) were administered the California Verbal Learning Test-II (CVLT) Short Form and provided self-report medical, clinical, and lifestyle data as part of the Biopsychosocial Religion and Health Study and Adventist Health Study-2. Measures of body mass index (BMI) and waist-to-hip ratio (WHR) were obtained at clinic visits.

Results. Standardized z-scores from five memory domains of the CVLT (Immediate Recall Trial 1, Immediate Recall Trial 4, Short-delay Free Recall, Long-delay Free Recall, and Long-delay Cued Recall) were significantly associated with age, gender, ethnicity, education, and WHR after adjusting for other variables. Although not statistically significant, we found better memory scores for those above 80 years of age with increased WHR. WHR was significantly associated with memory scores even after adjusting for BMI, though BMI did not independently show significant relationships with any memory score.

Discussion. The study population shares similar factors that affect memory as do other populations. WHR is a stronger predictor than BMI for memory function, possibly due to the action of metabolic factors associated with central obesity.

Background

The normal aging process is associated with declines in processing speed, problem solving, working (short-term) memory, and long-term memory throughout the ages of 20 to 80 years (Glorioso & Sibille, 2011). Even among those with "normal" cognitive declines due to aging, impairments can decrease quality of life for the individual as well as family and caregivers. A marked decline in memory function is often the first sign of cognitive impairment among the elderly, and may signal dementia pathology. The complex processes involved in memory formation suggest that a broad and comprehensive approach to intervention is necessary to support cognitive health , which is achievable even in the elderly (Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2010).

The most important risk factors for Alzheimer's disease, which is the most common form of dementia, have been classified as genetic, vascular, and psychosocial, while nutrition and other lifestyle factors as well as occupational exposures are still under investigation (Povova et al., 2012). Population-based epidemiological studies afford the ability to analyze the combined effect of these exposures in order to determine the magnitude of their individual effect within a community. Moreover, identifying the primary risk factors within a population may help screen for those at risk, signal the need for early intervention, and ultimately lead to attenuation of or delay in the downward trajectory of cognitive decline with targeted treatment.

Seventh-day Adventists (SDAs) are a religious group that have been widely studied for their health outcomes, including a number of cohorts since the 1950s. This

population is unique in that most SDAs adhere to a religiously-motivated abstinence of smoking and alcohol, thus controlling for these strong confounders, while having a wide range of health experiences and lifestyle habits (Fraser, Sumbureru, Pribis, Neil, & Frankson, 1997; Montgomery et al., 2007; Tonstad, Butler, Yan, & Fraser, 2009). Although the Adventist Health Study cohorts have shown that SDAs live longer than non-SDAs in the US (Fraser, 2005; W. Willett, 2003), they suffer from the same causes of morbidity and mortality but at a later age than non-SDAs (G. E. Fraser & D. Shavlik, 1997; G. E. Fraser & D. J. Shavlik, 1997). This phenomenon allows evaluation of health outcomes among an older population group that survive longer without clinical disease, but who eventually succumb to the same illness as the general populace.

The worsening obesity epidemic is of particular concern in the United States and is increasingly more so with the recognition that obesity has adverse effects on brain structure and cognition (Gunstad, Lhotsky, Wendell, Ferrucci, & Zonderman, 2010; Stanek et al., 2011). Moreover, the obesity paradox, which states that excess weight, which is typically a risk factor for morbidity and mortality, may actually improve survival among the elderly, confounds the impact of excess weight on morbidity among the elderly, even among SDAs (Kyulo, Knutsen, Fraser, & Singh, 2012). The parallel increases in the number of adults living longer, incidence of dementia, and prevalence of obesity warrants a better understanding of how these factors interplay and what measures can be done to limit the burden on individuals and societies.

The current SDA cohorts, including the Adventist Health Study-2 (AHS-2) and Biopsychosocial Religion and Health Study (BRHS), have obtained extensive dietary,

lifestyle, medical history, biometric, and psychosocial data on 511 adults over the age of 50 years. Data collection includes memory assessment using the California Verbal Learning Test-II Short Form (CVLT). The present study used data from this population of older community-dwelling adults to examine the relationship between measures of adiposity and specific memory domains of cognitive function.

Methods

Participants. Details of the design and recruitment of the AHS-2 cohort have been published (Butler et al., 2008; Lee et al., 2009). In brief, AHS-2 participants were adult (aged at least 30 years) members of SDA churches located in the United States and Canada. They completed a baseline questionnaire on numerous demographic and dietary factors between 2002-2007. Twenty-thousand randomly-selected AHS-2 respondents completed the BRHS questionnaire from 2006-2008, which included information on medical history. Among these, 511 attended clinic visits if they resided within driving distance of the clinic center located in Loma Linda, CA. Study protocol was reviewed and approved by the Loma Linda University's Institutional Review Board. The present research utilizes data obtained from self-reported questionnaires and in-person clinic visits, excluding those with missing or invalid diet data (n=38), those who were less than 50 years of age at the time of memory testing (n=5), or who had missing medical history information (n=23). The final analytic sample consisted of 167 males and 278 females (total N = 445).

Cognitive performance testing. Memory function scores were obtained with the California Verbal Learning Test-Second Edition Short Form (CVLT) during clinic visits.

The CVLT has been normed to a national sample of 1,087 individuals aged 16 to 89 years and is used as a screening tool or to assess severe impairments. Administration followed standard CVLT procedures (Delis, Kramer, Kaplan, & Ober, 2000), which involves reading a nine-item word list to the subject four times and instructing them to recite the words in any order after each reading. All words fall under one of three categories: clothing, tools, and fruit. After a short 30-second verbal distraction activity (subject counts backwards from 100), they are asked to recall the same list of words without providing prompts. Following this, after a longer 15-minute distraction involving non-verbal physical testing, the subject is asked to recite the words without prompting and again by verbally prompting for words that fall under each of the three categories.

Standardized *z*-scores were generated using the computerized CVLT Comprehensive Scoring System (Pearson, San Antonio, TX), normed to the standardization sample within each 10-year age and gender strata. Individuals 90 years and older were included in the 80-89 age group. Higher scores on the CVLT reflect better functioning. Scores evaluated in the present study include the standardized scores for the first and fourth recall trials (Immediate Free Recall Trials 1 and 4, IRT1 and IRT4), scores for the trial following the short delay (Short-Delay Free Recall, SDFR), and the two scores following the long delay (Long-Delay Free Recall, LDFR, and Long-Delay Cued Recall, LDCR).

Anthropometric measures. Anthropometric measurements were obtained at the in-person clinic visit. Weight was measured using a Tanita scale with the participant standing and with shoes, socks and heavy outer garments removed. Height was also

measured without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist and hip circumferences were measured with participants standing in a relaxed position. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Other variables. Demographic variables were obtained from the baseline AHS-2 questionnaire. Age at time of memory testing was determined by subtracting the date of birth from the date of clinic visit. Gender was also included in models. The study population was composed primarily of non-Hispanic Whites and Black African American, therefore persons of all other ethnic groups (4.9% of study sample) were categorized together with Whites. Highest attained education was analyzed as ordinal data with three categories (less than college degree; Associate's/Bachelor's degree; and Master's/Doctoral degree).

The BRHS questionnaire queries for previous diagnosis of or recent treatment for cardiovascular diseases (CVD) and type 2 diabetes. CVD included previous diagnosis of stroke lasting 24 hours, transient ischemic attack, angina pectoris, or hypertension.

A food frequency questionnaire including a list of over 200 fruits, vegetables, legumes, grains, oils, dairy, fish, eggs, beverages, meats and commercial products such as supplements, cereals, and vegetarian protein products was included in the AHS-2 baseline questionnaire. To account for overall dietary habits in the present analysis, participants were categorized into three groups based on the relative consumption (low, moderate, or high) of animal products including meats, fish, poultry, dairy, and eggs.

The AHS-2 questionnaire also obtained information on duration of napping during the day, time spent in sedentary behavior, and moderate, vigorous, and extremely vigorous physical activity. These were queried separately for a usual week day, usual Saturday, and usual Sunday using the following options: 1) never do; 2) less than 20 minutes; 3) 20-39 minutes; 4) 40-59 minutes; 5) at least one but less than two hours; 6) at least two but less than three hours; 7) at least three but less than six hours; and 8) more than six hours. Minutes of activity was calculated using, for the most part, the median number of minutes in each category, except that "never do" was assessed as zero (0) minutes, "less than 20 minutes" as 20 minutes, and "more than six hours" as 360 minutes. Average minutes per day for each activity category was calculated by summing the number of minutes spent in that activity on Sunday plus minutes for Saturday plus five times the minutes for a week day, then dividing by seven days per week.

Analysis. Missing data values were imputed using mean substitution within each age and gender strata for attained education and physical activity variables. Initial data analysis indicated that moderate, vigorous, and vigorous physical activity levels were not related to any of the CVLT measures under investigation ($p>0.05$), and were therefore excluded from further analyses; however time spent in sedentary behavior was retained. Multivariate regression analysis evaluated CVLT z-scores as outcome variables in separate models. All statistical analyses were performed using Stata version 11 (StataCorp, College Station, TX).

Results

Baseline characteristics of the study population are shown in Table 4.1. The population consisted of nearly two-thirds Whites and two-thirds females with a mean age of nearly 70 years. Over three-quarters of the participants have a college education, over half (53.0%) of whom have graduate degrees. Fourty-five percent reported having a previous CVD diagnosis, while few reported having been diagnosed with type 2 diabetes. Most study participants (55.5%) consumed animal products on a regular basis.

The average (standard deviation) scores for IRT1, IRT4, SDFR, LDFR, and LDCR were -0.05 (1.4), 0.15 (1.0), 0.59 (1.3), 0.44 (0.9), and 0.26 (0.9), respectively. In multivariate regression, medical history variables were not significant but were retained in models in order to account for disease status. Likewise, consumption of animal products was retained in order to account for variation in diet. Time spent in sedentary behavior was also not significantly associated with any of the CVLT scores after adjusting, but retained in models as a proxy for physical activity.

Results of multiple regression analysis indicated that the combined effect of the aforementioned variables significantly predicted scores for IRT1 (R^2=0.08, F(11,433) =3.64, p<0.001), IRT4 (R^2=0.07, F(11,433)=2.84, p<0.01), SDFR (R^2=0.11, F(11,433) =5.08, p<0.001), LDFR (R^2=0.15, F(11,433)=6.85, p<0.001), and LDCR (R^2=0.16, F (11,433)=7.5, p<0.001). Moreover, age, gender, education, ethnicity, and WHR were independently associated with at least one CVLT domain. WHR was significantly associated with four CVLT domain scores when adjusting for age, gender, education, ethnicity, CVD and diabetes history, diet, and sedentary time (Table 4.2) and also when

adjusting for BMI (data not shown). BMI, however, showed no significance when adjusting for other variables (Table 4.2) and WHR (data not shown).

We further investigated the presence of interaction between WHR and age. Although the interaction term was not significant for any CVLT score, we found distinct patterns. Figure 4.1 displays the mean CVLT z-scores by the lowest and highest tertiles of WHR and for each 10-year age group. The first, second, and third tertile groups have mean (sd) WHR values of 0.77 (0.05), 0.88 (0.03), and 0.99 (0.04), respectively. In general, IRT1 scores were lower for higher WHR. But among the oldest participants aged 80 and older, those with lower WHR also had lower IRT1 scores (mean=-0.52, sd=1.08) compared to those with higher WHR (mean=-0.22, sd=1.64). A similar pattern emerged for IRT4 and SDFR scores.

Body mass index was categorized as < 25 kg/m^2 (classified as "thin") or ≥ 25 kg/ m^2 (classified as "overweight") for the purpose of assessing interaction with age. The age*BMI interaction term was marginally significant for SDFR ($F(3, 428)=2.61$, p=0.0514) but not for other memory measures. Figure 4.2 displays CVLT scores by BMI category for each age group. There is a general trend of lower CVLT score for higher BMI, except for those aged 70-79 years where the opposite trend occurs. When comparing mean predicted scores after adjustment, thin 50-59 year olds had significantly higher SDFR scores compared to thin 70-79 year olds ($\beta = -1.2$, p=0.001). There was no difference between these age groups who were classified as overweight ($\beta = -0.10$, p=0.646). Comparing the same age groups' IRT4 scores reveal the same pattern for thin ($\beta = -0.86$, p=0.002) and overweight ($\beta = 0.00$, p=0.986) participants.

Discussion

We found in the present study that memory function among elderly Seventh-day Adventists is significantly associated with age, gender, ethnicity, and education. These factors are known to be associated with cognitive function in other study populations, suggesting similarities between SDAs and non-SDAs. A measure of adiposity, specifically waist-to-hip ratio, was also significant after adjusting for demographic, clinical, and lifestyle factors. Although the effect of waist-to-hip ratio did not vary significantly by age group, we do see interesting trends that warrant further investigation.

Advancing age has consistently been shown to be associated with cognitive decline (Park & Reuter-Lorenz, 2009; Soares et al., 2012), including working, short-term, and long-term memory (Park et al., 2002). These declines are also apparent in non-pathological, or "normal," cognitive aging and involves the interplay of numerous other factors, including early and mid-life cognition, genetics, medical conditions such as cardiovascular disease, changes in the structure of the brain, and diet and lifestyle (Deary et al., 2009). The current population represents a wide age range of individuals in mid-life and later allowing us to investigate the cognitive experiences across a large portion of the lifespan within the context of other determining factors. Future research objectives will enable us to assess the change in cognition over time in this same study population.

It is possible that the lower scores for older participants is due to poorer learning rather than retention (Matzel, Wass, Kolata, Light, & Colas, 2009). This may, in part, explain why age is significantly associated with the immediate recall and short-delay CVLT scores (IRT1, IRT4, and SDFR), but not the long-delay trials (LDFR and LDCR).

For instance, the IRT1 score is dependent on auditory attention, which, if adequately trained, may improve working memory (Adcock et al., 2009). LDFR and LDCR reflect retention, which do not differ between the younger and older participants in our study following the repetitive learning of the immediate recall and SDFR trials. These findings suggest that elderly individuals may benefit from targeted auditory attention training or simply repetition of data in order to have the same level of retention as their younger counterparts.

Our findings on the association between measures of obesity and cognition add to a growing body of literature on the same (Anstey et al., 2011; Cohen-Mansfield & Perach, 2011; Gunstad et al., 2010; Kerwin et al., 2011; Levine & Crimmins, 2012; Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005). Although it has been suggested that cardiorespiratory fitness and physical activity are more important than obesity for health outcomes (McAuley & Blair, 2011), we found no association between BMI, cardiovascular disease history, or measures of activity level after adjusting for other variables; however, WHR was strongly associated with several CVLT domain scores in this population. Although the interaction terms between age and tertiles of WHR were not statistically significant, we saw trends of decreasing memory scores for age groups 50-59, 60-69, and 70-79 years across tertiles of WHR but increases in the same memory domains for those above the age of 80. We also found increasing memory scores across tertiles of WHR for all but the 50-59 year olds, with the increase for 80+ year olds being the most remarkable.

Our findings may be explained in part by the obesity paradox. Arena and Lavie (2010) review the evidence for the obesity paradox and propose a number of physiological explanations, one of which relates to the greater amount of circulating lipoproteins associated with increased adipose tissue and their role in reducing inflammation. Cognitive function has been shown to be inversely related to inflammation (Rosano, Marsland, & Gianaros, 2012); therefore, it may be that the higher visceral fat in the older ages operates in a similar fashion as in the case of cardiovascular diseases to protect against cognitive decline. Other investigators have found that the relationship between obesity and cognition varied by age (Cohen-Mansfield & Perach, 2011), central adiposity (Kerwin et al., 2011), and presence of sarcopenia (Levine & Crimmins, 2012). Similarly, our findings suggest that central obesity, as reflected by WHR, may be a stronger factor than BMI in regards to cognitive ability among the elderly. Moreover, any benefit of being overweight may not be manifested until very late in life, *i.e.*, after 80 years of age.

Due to the cross-sectional aspect of our analysis we were not able to determine if reverse causation is at work, specifically that memory impairments preceded and affected changes in adiposity. Others, though, have linked weight loss later in life with incidence of dementia (Ogunniyi et al., 2011) and have suggested that weight loss may precede dementia by up to 20 years in women (Knopman, Edland, Cha, Petersen, & Rocca, 2007). The elderly often experience changes in their eating patterns, thus affecting numerous metabolic processes (Wilson & Morley, 2003), which should be investigated with respect to cognition. For instance, adiponectin, a regulator of glucose and fatty acid catabolism,

is inversely related to BMI and waist measures (Milewicz, Jedrzejuk, Dunajska, & Lwow, 2010; Poppitt et al., 2008; Stepien et al., 2012). The relation between adiponectin and cognition is mixed. Une et al (2011) report that adiponectin levels in plasma and cerebrospinal fluid are significantly higher in those with cognitive impairment compared to normal controls and data from the Framingham Heart Study showed that higher adiponectin levels significantly predicted all-cause dementia and Alzheimer's disease among women (van Himbergen et al., 2012). On the other hand, Kamogawa et al (2010) demonstrated a 54% decrease in odds of cognitive impairment with a 10 gm/L increase in plasma adiponectin among males. It is possible that, among our oldest study participants, those with greater central obesity had different levels of adiponectin or other metabolic factors compared to their thin counterparts, thus explaining their higher memory scores.

The limitations of cross-sectional study design are inherent in this study. Although associations between exposure and outcome can be assessed, causal inference should be avoided. Moreover, self-reported medical history of cardiovascular disease and diabetes, which are known risk factors of poorer cognitive function, were used introducing the potential for recall and reporting biases; however, these variables are currently being validated using medical records. Other self-reported data include physical activity and diet information.

Nevertheless, the present cohort of elderly Seventh-day Adventists has provided additional information and direction for future research in the area of healthy brain aging. We find that this study population has similar factors associated with cognition as other groups, allowing for confidence in the generalizability of research findings. The domain-

specific memory scores enable analysis of specific aspects of learning and retention. And finally, proposed areas for intervention, especially among the most advanced age groups, is of especial interest for public health and medical practitioners.

Table 4.1. Selected characteristics of the study population of 445 elderly Seventh-day Adventists.

	Mean	Range
Age at memory testing (years)	69.4	50.1 - 96.4
Male, n (%)	167 (37.5)	
White/Other ethnicity, n (%)	287 (64.5)	
Highest attained education, n (%)		
Less than college degree	107 (24.0)	
Associate's/Bachelor's degree	159 (35.7)	
Master's/Doctoral degree	179 (40.2)	
CVD diagnosis, n (%)	200 (44.9)	
T2D diagnosis, n (%)	53 (11.9)	
Animal product consumption, n (%)		
Low	198 (44.5)	
Moderate	74 (16.6)	
High	173 (38.9)	
Physical activity and leisure time (min/day)		
Nap during day	21.4	0 - 360
Sedentary time	40.1	0 - 360
Moderate physical activity	89.0	0 - 360
Vigorous physical activity	38.5	0 - 360
Extremely vigorous physical activity	13.3	0 - 360
Body mass index (kg/m^2)	27.4	17.3 - 49.1
Waist-hip ratio	0.9	0.6 - 1.2

CVD = Cardiovascular disease, including previous diagnosis of stroke lasting 24 hours, TIA, angina pectoris, or high blood pressure. T2D = type 2 diabetes.

Table 4.2. Multiple regression models* relating the standardized CVLT domain scores to anthropometric measures.

	Waist-to-Hip Ratio		Body Mass Index	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
IRT1	-2.128 (-3.888 to -0.367)	**0.018**	0.009 (-0.017 to 0.034)	0.503
IRT4	-1.427 (-2.747 to -0.107)	**0.034**	-0.004 (-0.024 to 0.015)	0.649
SDFR	-2.132 (-3.751 to -0.513)	**0.010**	0.002 (-0.021 to 0.026)	0.855
LDFR	-1.185 (-2.326 to -0.045)	**0.042**	-0.001 (-0.018 to 0.016)	0.914
LDCR	-0.859 (-1.989 to 0.271)	0.136	0.009 (-0.007 to 0.026)	0.268

*Models include age, gender, education, ethnicity, history of CVD and diabetes, diet pattern, and sedentary time.
Abbreviations: IRT1 = Immediate Recall Trial 1; IRT4 = Immediate Recall Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall.

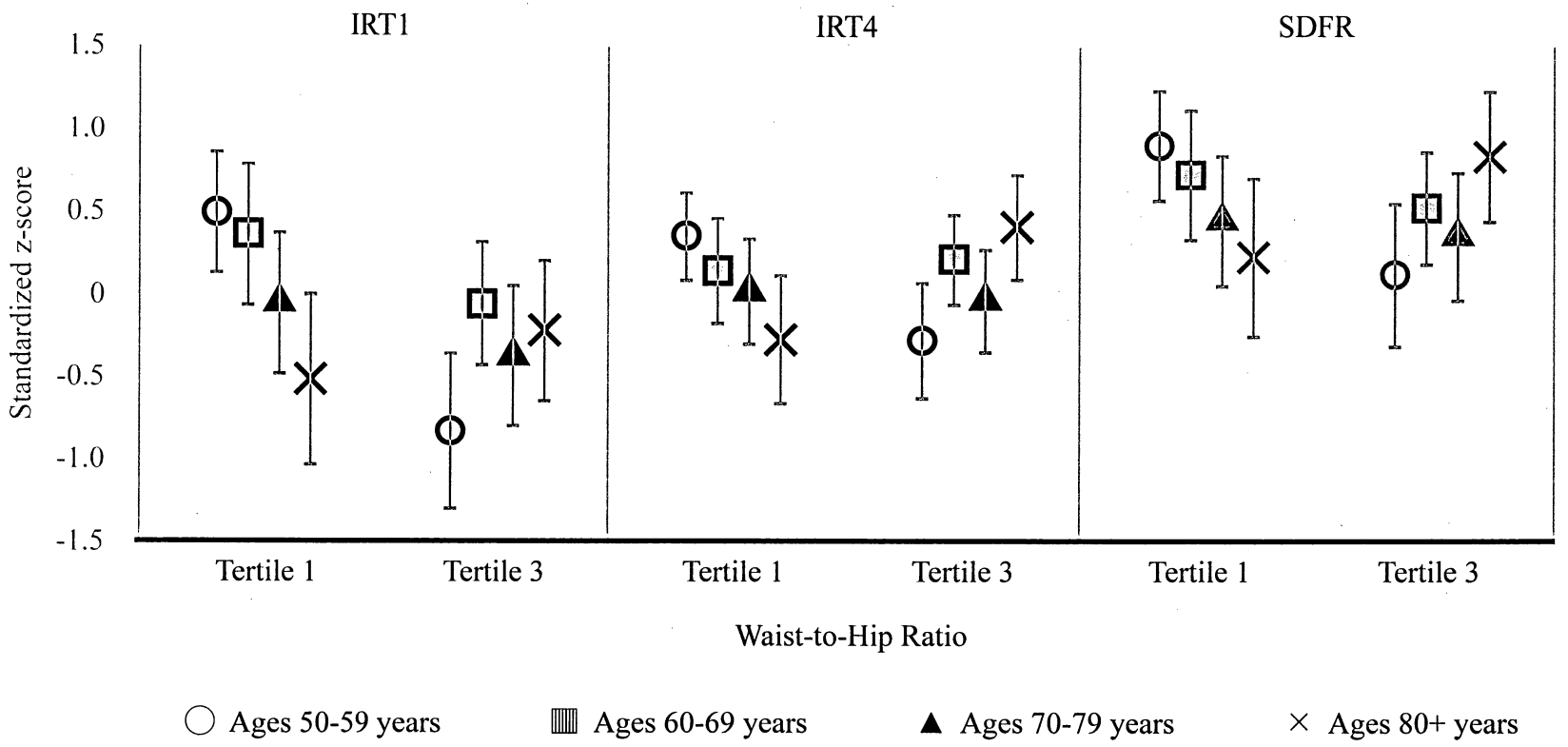

Figure 4.1. California Verbal Learning Test (CVLT) standardized scores for 445 Seventh-day Adventists by waist-to-hip ratio and age group. Abbreviations: IRT1 = Immediate Free Recall Trial 1; IRT4 = Immediate Free Recall Trial 4; SDFR = Short-delay Free Recall.

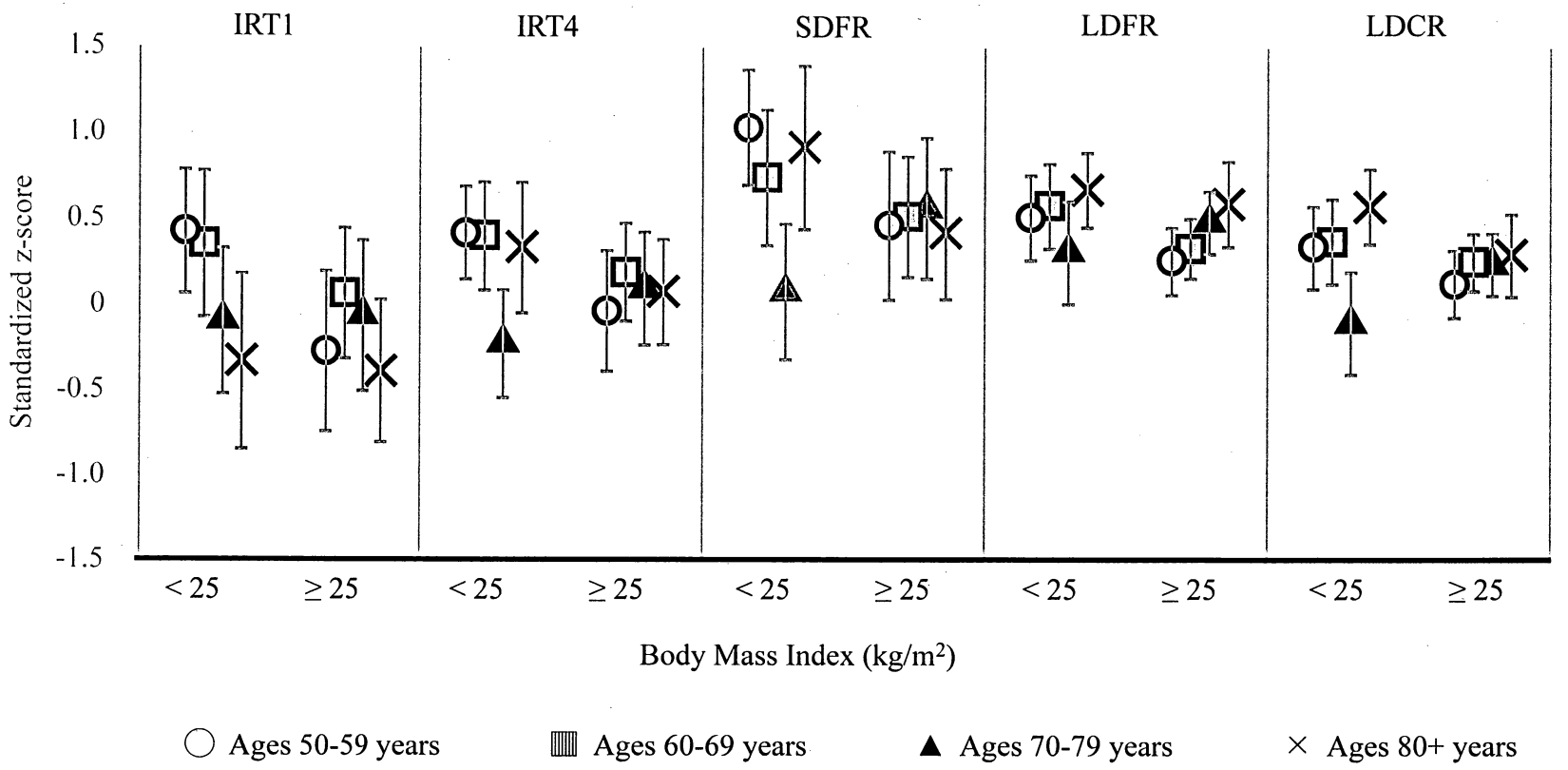

Figure 4.2. California Verbal Learning Test (CVLT) standardized scores for 445 Seventh-day Adventists by body mass index and age group. Abbreviations: IRT1 = Immediate Free Recall Trial 1; IRT4 = Immediate Free Recall Trial 4; SDFR = Short-delay Free Recall.

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CHAPTER 5

SECOND PUBLISHABLE PAPER

Nut Consumption and Memory Domains of Cognitive Function

Among Elderly Seventh-day Adventists

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ABSTRACT

Background. Eating nuts as part of the usual diet has shown to have positive effects on cardiovascular disease, but little has been done to examine the effect on cognitive function.

Methods. The study sample of 445 adults aged at least 50 years and living in southern California was drawn from the Adventist Health Study-2 and Biopsychosocial Religion and Health Study cohort. The California Verbal Learning Test-II (CVLT) Short Form was administered at clinic visits to measure memory function and a self-report quantitative food frequency questionnaire was used to ascertain amount and frequency of nut consumption as part of the usual diet.

Results. Tertiles of intake of all nuts, tree nuts, and peanuts were not significantly associated with standardized memory scores when adjusting for demographic, health, and lifestyle variables. We found an apparent trend of increasing scores with increasing amount of intake among younger participants, while older participants show a negative relationship; however, trends were not significant at $\alpha=0.05$.

Conclusion. These findings are among the first to investigate the association between consuming nuts and specific domains of memory and are useful in proposing future course of investigation.

Background

Over two decades ago, researchers at Loma Linda University reported that consuming nuts more than four times a week as a part of the usual diet decreased the risk of fatal cardiovascular disease and nonfatal myocardial infarctions among the first Adventist Health Study (AHS-1) cohort (Fraser et al., 1992). We now know that eating nuts provides benefits beyond those afforded for cardiovascular health (Sabate & Ang, 2009). Very little, however, has been reported in regards to the association between consuming nuts and cognitive ability, particularly in humans.

Advancing age is the strongest risk factor for cognitive decline, and even normal aging is associated with changes in the neurological system, including marked declines in processing speed, problem solving, working (short-term) memory, and long-term memory. Memory loss is often the first noticeable symptom of progressive degenerative disorders such as Alzheimer's disease. Dietary factors that are found in plant foods, including nuts, may protect against or attenuate the rate of cognitive decline, as has been demonstrated in studies of diet and cognition (Gu, Nieves, Stern, Luchsinger, & Scarmeas, 2010; Nurk et al., 2010), presumably by decreasing the damage to neuronal cells due to reactive oxygen species and inflammation (Ferrari, 2004).

Numerous vitamins act in concert to provide healthy brain activity; therefore, deficiencies in any important nutrient may alter cognitive function. The essential fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA), as well as antioxidants, are particularly important for healthy brain aging (Bourre, 2006a, 2006b). Nuts are a rich source of these vitamins, containing nearly the most antioxidants compared to other food

items (Halvorsen et al., 2006; U.S. Department of Agriculture, 2011). For instance, 100 g of almonds contain 12.0 g LA, 0.006 g ALA, and 0.3 g total antioxidants and 100 grams of walnuts contain 38.1 g LA, 9.1 g ALA, and 21.0 g total antioxidants (Halvorsen et al., 2002). In addition, adding almonds to the diets of community-dwelling adults can improve overall nutrient intake pattern (Jaceldo-Siegl et al., 2004), which may positively affect health and support retention of brain function, as has been demonstrated in animal studies (J. A. Joseph et al., 1998). Rats, for example, had improved working memory after short-term walnut supplementation and improved retention of memory after almond paste supplementation (Kulkarni et al., 2010; Willis et al., 2009). In rats with induced amnesia, almond paste decreased cholinesterase activity in the brain and reduced cholesterol and triglyceride levels as well as reversing the amnesia (Kulkarni et al., 2010). Evidence for an effect of nut consumption on cognition among humans has not yet been thoroughly explored.

The present study used data from older adults enrolled in the Biopsychosocial Religion and Health Study, BRHS (Lee et al., 2009), to investigate the relationship between consumption of nuts and specific memory domains of cognitive function. Examining specific domains of memory may implicate certain aspects of learning and memory that may be affected by the nutritional profile of nuts, which to date has not been reported by other researchers.

Methods

Participants. Adventist Health Study-2 (AHS-2) participants were recruited from Seventh-day Adventist (SDA) churches and congregations in the United States and Canada from 2002-2007 to examine lifestyle and cancer rates in Seventh-day Adventists (SDA) (Butler et al., 2008). A random subsample was selected to participate in the BRHS, 511 of whom participated in a clinic visit. These individuals resided near the study center located in Loma Linda, CA and were at least 50 years of age in 2007. The present research utilizes data obtained from self-reported AHS-2 questionnaires and BRHS clinic visits. Participants were excluded if they had missing or invalid diet data (n=38), were less than 50 years of age at the time of memory testing (n=5), or had missing medical history information (n=23). The final analytic sample consisted of 167 males and 278 females (total N = 445).

Nut exposure. The baseline AHS-2 food frequency questionnaire (FFQ) included over 200 items that are commonly consumed by SDAs living in the US and that significantly contribute to the 26 nutrient, phytochemical, and mineral indices related to the major study hypotheses. The FFQ, has been validated for intake of several nutrients and foods (Jaceldo-Siegl et al., 2011; Jaceldo-Siegl et al., 2008; Jaceldo-Siegl et al., 2010). Validity correlations for various nuts ranged from 0.3 to 0.6 (Jaceldo-Siegl et al., 2011).

Nut intake was estimated for five nuts (peanuts, walnuts, almonds, cashews, and mixed nuts), and peanut butter. Frequency of intake included eight options ranging from never or rarely to four or more times per day. Standard portion sizes were presented for

each type of nut and participants indicated if they consumed the standard amount, one-half or less the standard, or one-and-a-half times or more the standard at each serving. Missing responses for serving size was assumed as standard, while missing values for frequency of intake were dropped from analysis. Grams of nut intake was calculated using the NDS-R 2008 (NDS-R, Nutrition Coordinating Center, Minneapolis, MN, USA) database then adjusted for total caloric intake using the residual method (W. C. Willett et al., 1997). The present analysis defined three nut variables: 1) peanuts, which included peanuts and peanut butter; 2) tree nuts, which included walnuts, almonds, cashews, and mixed nuts; and 3) all nuts, which combined all types of peanuts and tree nuts. Each nut variable was categorized by tertiles of intake.

Memory testing. The California Verbal Learning Test-Second Edition Short Form (CVLT) was administered to study participants during in-person visits to the study clinic site. As per standard CVLT administration, a nine-item word list is read to the subject four times, and they asked to recite the words in any order after each reading (Immediate Recall Trials 1 through 4, IRT1 to 4). All words fall under one of three categories: clothing, tools, and fruit. Next follows a short 30-second distraction activity (counting backwards from 100) before the subject is asked to recite the words using free recall without providing prompts (Short-delay Free Recall, SDFR). A 15-minute distraction follows involving non-verbal physical testing, then the subject is asked to recite the words without prompting (Long-delay Free Recall, LDFR) and with prompting for words that fall under the three categories (Long-delay Cued Recall, LDCR). Age and gender standardized *z*-scores were generated from the CVLT standardization sample using the

CVLT Comprehensive Scoring System (Pearson, San Antonio, TX). Higher scores reflect better functioning. Individuals 90 years and older were included in the 80-89 age group.

Covariates. Age at time of memory testing at the clinic visit was dichotomized as less than 70 years or 70 years and older. Because the study population is composed primarily of non-Hispanic Whites and Black African American, all other ethnic groups were categorized together with Whites. Highest attained education was queried on the baseline AHS-2 questionnaire using nine levels and further categorized into three ordinal levels (less than college degree, Associate's or Bachelor's degree, and Master's or Doctoral degree). Three variables were created to indicate self-reported history of diagnosis of cardiovascular disease (including stroke, transient ischemic attack, angina pectoris, and high blood pressure), type 2 diabetes, and depression.

The baseline AHS-2 questionnaire contained a multiple-question section on physical activity. Duration of moderate, vigorous, and extremely vigorous physical activity on a usual week day, usual Saturday, and usual Sunday was obtained. Average minutes per day of each activity level was calculated separately by averaging the minutes for Sunday, minutes for Saturday, and five times the minutes for week day.

Height and weight of participants were measured at the clinic visit with their shoes and heavy outer garments removed. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and dichotomized as "normal weight" (BMI < 25 kg/m^2) or "overweight" (BMI ≥ 25 kg/m^2). In order to account for overall dietary habits, participants were classified by their relative amount of

consumption (low, moderate, or high) of animal products including meats, fish, dairy, and eggs.. To control for the differing amount of time between completion of the FFQ and memory testing, a time variable measured the number of years between receipt of the FFQ by study staff and the date of the clinic visit.

Analysis. Missing data values were imputed using mean substitution. Missing age at cognitive testing values were imputed with the mean age for each gender and ethnicity strata. Means for each age and gender strata were imputed for missing values for caloric intake, level of attained education, physical activity variables, height, and weight. Initial data analysis indicated that time spent napping, and moderate, vigorous, and vigorous physical activity levels were not related to the CVLT measures under investigation ($p>0.05$), and were therefore excluded from further analyses. CVLT scores were compared across tertiles of nut intake using analysis of covariance. All statistical analyses were performed using Stata v.11 (StataCorp, College Station, Texas, USA).

Results

Individuals who reported higher intake of all nuts were typically male, non-Black, consumed low levels of animal products, had lower BMI, and had less sedentary time per day (Table 1). Participants consuming more nuts also consumed fewer total calories per day, with those in the second tertile of nut intake consuming the fewest ($p<0.01$). Energy-adjusted intake of nuts ranged from 0 to 186.3 grams per day of all nuts, 0 to 188.3 grams per day of tree nuts, and 0 to 129.5 grams per day of peanuts. Standardized z-scores on the CVLT ranged from -3 to 5 (IRT1), -3 to 3.5 (IRT4), -2.5 to 4.5 (SDFR), -2.5 to 3 (LDFR), and -3.5 to 2.5 (LDCR).

In univariate analysis (data not shown), a significant association was found between each of the standardized CVLT scores and ethnicity (Blacks score lower, $p<0.01$) and attained education (positive association, $p<0.01$); all but IRT1 were negatively associated with sedentary time ($p<0.05$); IRT1 was negatively associated with age ($p<0.05$); LDFR and LDCR were associated with gender (females score lower, $p<0.001$); IRT1 and IRT4 were negatively associated with napping during the day ($p<0.05$); SDFR was negatively associated with BMI ($p<0.05$); LDCR was associated with CVD diagnosis (those with CVD score lower, $p<0.05$) and vegetarian status (increasing meat consumption have lower scores, $p<0.01$); and SDFR, LDFR, and LDCR were associated with diagnosis of diabetes (those with diabetes score lower, $p<0.05$).

In multivariable regression analysis we found that intake of all nuts, tree nuts and peanuts were not significantly associated with any of the CVLT scores after adjusting for caloric intake, age, gender, education, ethnicity, history of CVD or diabetes, sedentary time, BMI, or vegetarian status (Table 2). Additional modeling was done by adding an interaction term between age and tertiles of nut intake. Although the interaction terms were not significant for any of the CVLT scores, an interesting pattern emerged. For example, looking at mean standardized z-scores for IRT1 across tertiles of all nut intake for participants aged less than 70 years (Figure 1A), we see a positive linear trend, while there is a negative linear trend among participants aged 70 years or older. The positive trend among those less than 70 years is also seen for IRT4 and SDFR across tertiles of all nut intake, for IRT1, SDFR, LDFR, and LDCR across tertiles of tree nut intake, and for

IRT1 and SDFR across tertiles of peanut intake (Figure 1). The trend among those older than age 70 is less consistent.

Discussion

This study is, as far as we know, the first published research to investigate the effects of consuming nuts on specific domains of memory function among a human population. An earlier study conducted in the first Adventist Health Study cohort (Fraser et al., 1996) found a null relationship between nut consumption and overall severity of cognitive impairment. In that study, cognitive testing using the Mini-Mental State Examination (MMSE) was completed 15 years following collection of dietary information among a population of 90 Seventh-day Adventists who were 75 to 93 years . Regression analysis in this older cohort yielded an insignificant negative slope (β= -0.0012; 95% CI: -0.0065 to 0.0041) between frequency of nut consumption and MMSE score, which is a similar finding to the negative slope for IRT1 found in the older age group of the present population. Although not statistically meaningful, these results indicate that future analyses should investigate age groups separately. By using the MMSE to measure cognition, the previous study was limited to assessing global function while we were able to assess specific areas of memory ability using the CVLT, which may provide a more accurate classification of participants' cognitive state (Rabin et al., 2009).

Nooyens et al (2011) reported significant positive associations between quintiles of total nut consumption and four specific cognitive domains among 2,613 middle-aged men and women. At baseline, participants who consumed greater amounts of nuts

showed better baseline cognitive function in memory ($p < 0.05$), information processing speed ($p < 0.05$), cognitive flexibility ($p < 0.01$), and a global cognitive score ($p < 0.01$). Nuts were not significantly associated with any outcome when assessing five-year change in function in the full model; however, nut consumption was significantly associated with less decline in memory ($p<0.05$) and global cognitive function ($p<0.05$) when not adjusting for cardiovascular risk factors. Participants were more likely to have greater consumption of fruits and vegetables if they were older, female, highly educated, smoke less, heavy alcohol consumers, inactive, and with higher HDL-cholesterol. In the present study, higher nut consumers also tended to be older and more highly educated, but were also more likely to be female and spend less time in sedentary behavior (Table 1). Less than 12% of the participants report ever smoking and less than 6% report current consumption of alcohol consumption. These important factors may at least partially explain the differences in the findings between the two studies. The European cohort study differed from the present analysis also in having a larger sample size, analysis of baseline and follow-up measures, use of four cognitive measures, and adjustment for health indicators such as blood pressure, HDL levels, waist circumference, etc. Future research objectives will enable us to assess the rate or amount of cognitive decline over time in this same study population, along with incorporating more clinical measures.

Cross-sectional analysis of a subsample of the PREDIMED clinical trial (Valls-Pedret et al., 2012) showed a significant positive association between walnut consumption and working memory ($p<0.05$). There are a number of differences between the PREDIMED and the current studies. PREDIMED study participants had a high

prevalence of cardiovascular risk factors (e.g., 72% with hyperlipidemia), as opposed to only 45% in the present study who reported a previous diagnosis of cardiovascular disease. This suggests that nuts may be of cognitive benefit among those at high risk vascular disease rather than those at low risk due to the contribution that vascular health has on cognitive impairment (Gorelick et al., 2011). The intake of total nuts and walnuts in the PREDIMED study ranged from 0-60 grams/day and 0-30 grams/day, respectively, while participants in the present study consumed as much as 203 grams/day of all nuts and 73 grams/day of walnuts (figures not adjusted for energy intake). In a separate analysis performed for the sake of comparison, we found that tertiles of walnut intake was not significantly associated with memory scores in our study. The high degree of variation among our population, combined with the smaller sample size, may have diffused any effect of nuts in this low risk population.

The relationship between nuts and memory may not have been strong enough to outweigh the effect of other dietary components. Participants who consumed greater amounts of nuts also consumed fewer animal products compared to low nut consumers ($p<0.001$). Overall diet pattern has been shown to affect cognition. It is evident that including a greater proportion of fish and plant foods, including nuts, rather than meats and high fat dairy decreases the odds of cognitive impairment (Gu & Scarmeas, 2011). A similar, though mixed, effect is found with increased adherence to the Mediterranean diet (Feart et al., 2009; Tangney et al., 2011; Vercambre, Grodstein, Berr, & Kang, 2012). A variable for overall diet pattern was included in the present study; however, future

analysis would benefit by accounting for specific food groups that may have overshadowed the effect of nuts.

The Adventist Health Study population is unique in terms of certain characteristics and behaviors that may affect their health outcomes. In this study, however, we were able to assess memory function using scores that are normed to a standardization population that is representative of the general US population (Delis et al., 2000), and to investigate specific domains of verbal learning and memory, though composite scores yield better clinical usability (Donders, 2008). Our population represented a wide age range (50 to 96 years at time of memory testing), thus representing both middle and older age groups that have varying degrees of risk for cognitive dysfunction. Self-reported nut consumption was the primary exposure variable, which has limitations due to recall bias; however, the intake questionnaire does have good validity, as has been mentioned.

In summary, we found no statistically significant association between amount of nuts consumed and memory function in this study population. Due to the cross-sectional design of the analysis, it is not possible to draw conclusions regarding causality, or the lack thereof. Diet data was collected an average of five years prior to memory testing and only baseline CVLT scores were used, thus we were unable to investigate the potential of reverse causality. Further analysis should consider other food groups that may be highly correlated with nut intake in this population and that also provide the same nutrients as nuts that are purported to support cognitive health. We were able to identify

intriguing trends comparing the older and younger participants, which also warrant further investigation.

Table 5.1. Characteristics of study population of elderly Seventh-day Adventists by tertiles of intake of all nuts (N = 445).

	Tertile 1 (N = 141)	Tertile 2 (N = 150)	Tertile 3 (N = 154)	p-value
Age, n (%)				
< 70 years	87 (36.4)	77 (32.2)	75 (31.4)	0.063
≥ 70 years	54 (26.2)	73 (35.4)	79 (38.4)	
Gender, n (%)				
Male	56 (33.5)	38 (22.8)	73 (43.7)	**< 0.001**
Female	85 (30.6)	112 (40.3)	81 (29.1)	
Ethnicity, n (%)				
White/Other	68 (23.7)	100 (34.8)	119 (41.5)	**< 0.001**
Black	73 (46.2)	50 (31.6)	35 (22.2)	
Attained Education, n (%)				
Less than college degree	39 (36.4)	36 (33.6)	32 (29.9)	0.096
Associate's or Bachelor's Degree	51 (32.1)	61 (38.4)	47 (29.6)	
Master's or Doctoral Degree	51 (28.5)	53 (29.6)	75 (41.9)	

Table 5.1 (continued). Characteristics of study population of elderly Seventh-day Adventists by tertiles of intake of all nuts (N = 445).

	Tertile 1 (N = 141)	Tertile 2 (N = 150)	Tertile 3 (N = 154)	p-value
Physical Activity, median min/day (range)				
Napping during day	20 (0 - 360)	18.6 (0 - 150)	20 (0 - 270)	0.854
Sedentary	28.5 (0 - 270)	22.1 (0 - 360)	20 (0 - 270)	**0.039**
Moderate	60 (0 - 334)	53.0 (0 - 347)	57.3 (0 - 360)	0.727
Vigorous	24.3 (0 - 347)	18.6 (0 - 360)	20 (0 - 330)	0.097
Extremely vigorous	0 (0 - 296)	0 (0 - 360)	0 (0 - 303)	0.108
Animal product consumers, n (%)				
Low	42 (21.2)	69 (34.9)	87 (43.9)	**< 0.001**
Moderate	28 (37.8)	26 (35.1)	20 (27.0)	
High	71 (41.0)	55 (31.8)	47 (27.2)	
BMI, n (%)				
< 25 kg/m^2	36 (20.9)	60 (34.9)	76 (44.2)	**< 0.001**
≥ 25 kg/m^2	105 (38.5)	90 (33.0)	78 (28.6)	
Caloric intake, median kcal/d (range)	1948 (1027 - 2120)	1630 (589 - 3969)	1842 (543 - 4357)	**< 0.001**
Time from FFQ to CVLT, median years (range)	5.1 (-0.3 - 5.7)	5.0 (-0.2 - 5.7)	4.9 (-0.1 - 5.6)	0.271

Table 5.2. Mean standardized cognitive test z-scores by tertiles of nut intake for all study participants.

	N	IRT 1				IRT 4				SDFR			
		Mean	95% CI		P-value	Mean	95% CI		P-value	Mean	95% CI		P-value
All Nuts													
Tertile 1	141	0.05	-0.19	0.29	0.399	0.17	-0.01	0.35	0.909	0.65	0.43	0.87	0.706
Tertile 2	150	-0.03	-0.25	0.20		0.12	-0.05	0.29		0.52	0.31	0.73	
Tertile 3	154	-0.18	-0.40	0.05		0.17	-0.00	0.33		0.61	0.40	0.81	
Tree Nuts													
Tertile 1	142	-0.02	-0.26	0.23	0.372	0.11	-0.07	0.29	0.874	0.59	0.37	0.81	0.976
Tertile 2	150	0.04	-0.19	0.27		0.16	-0.01	0.33		0.57	0.36	0.78	
Tertile 3	153	-0.19	-0.41	0.04		0.18	0.01	0.35		0.61	0.40	0.81	
Peanuts													
Tertile 1	146	-0.05	-0.28	0.18	0.882	0.14	-0.03	0.31	0.731	0.51	0.30	0.72	0.431
Tertile 2	147	-0.10	-0.33	0.13		0.11	-0.06	0.28		0.55	0.34	0.77	
Tertile 3	152	-0.02	-0.24	0.21		0.21	0.04	0.37		0.70	0.49	0.91	

Adjusted for caloric intake, age, gender, education, ethnicity, diagnosis of CVD, diabetes, and depression, sedentary time, BMI and vegetarian status. Abbreviations: IRT1 = Immediate Recall Trial 1; IRT4 = Immediate Recall Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall

Table 5.2 (continued). Mean standardized cognitive test z-scores by tertiles of nut intake for all study participants.

	N	LDFR				LDCR			
		Mean	95% CI		P-value	Mean	95% CI		P-value
All Nuts									
Tertile 1	141	0.50	0.35	0.66	0.431	0.32	0.17	0.47	0.611
Tertile 2	150	0.37	0.22	0.51		0.21	0.07	0.36	
Tertile 3	154	0.47	0.32	0.61		0.25	0.11	0.39	
Tree Nuts									
Tertile 1	142	0.44	0.28	0.60	0.961	0.26	0.10	0.41	0.657
Tertile 2	150	0.43	0.28	0.58		0.31	0.16	0.46	
Tertile 3	153	0.46	0.32	0.61		0.21	0.07	0.36	
Peanuts									
Tertile 1	146	0.45	0.30	0.60	0.452	0.24	0.09	0.38	0.311
Tertile 2	147	0.37	0.22	0.52		0.19	**0.04**	0.34	
Tertile 3	152	0.51	0.36	0.65		0.34	0.20	0.49	

Adjusted for caloric intake, age, gender, education, ethnicity, diagnosis of CVD, diabetes, and depression, sedentary time, BMI and vegetarian status. Abbreviations: IRT1 = Immediate Recall Trial 1; IRT4 = Immediate Recall Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall

Figure 5.1a. All Nuts

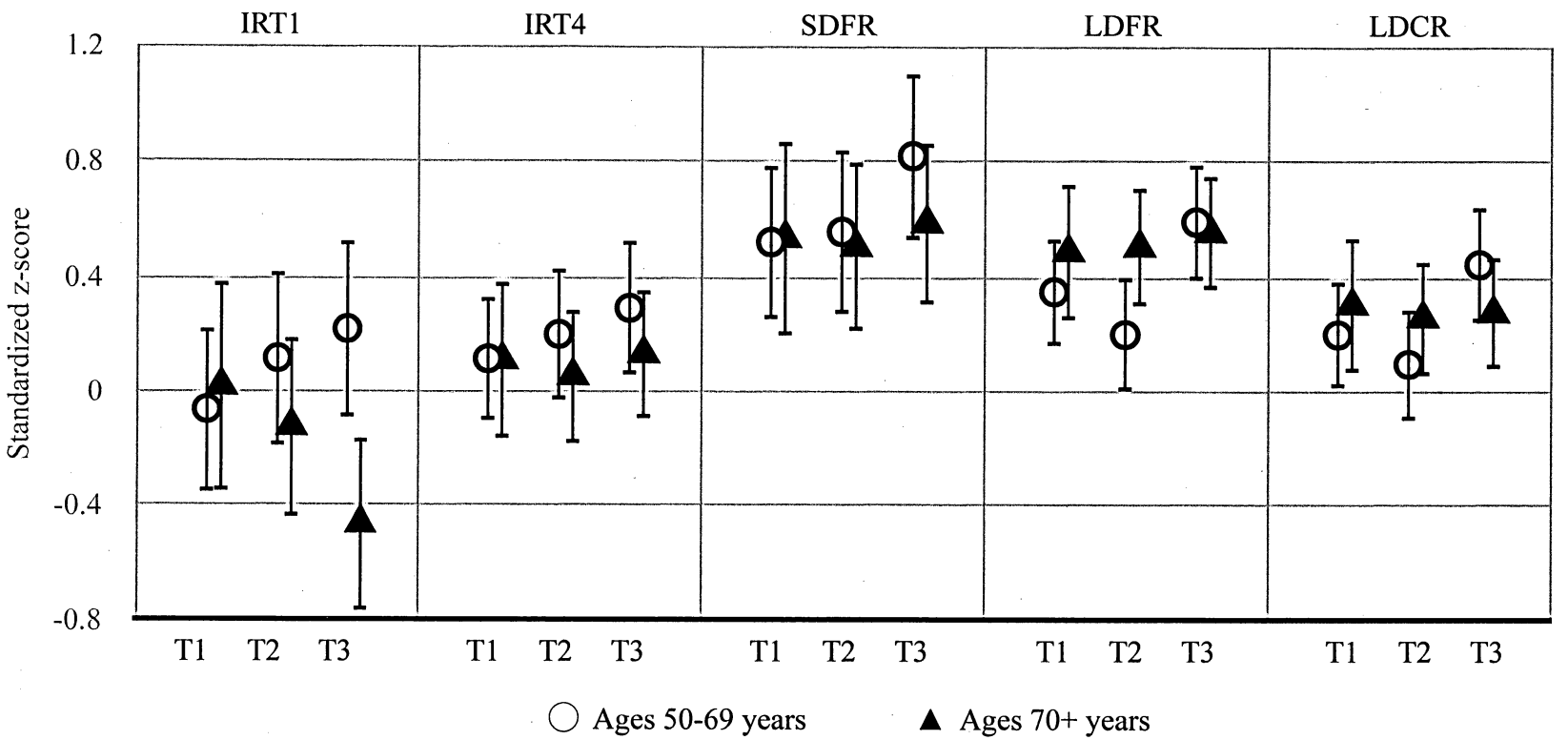

Figure 5.1. Age-specific standardized z-scores across tertiles of all nut intake (panel A), tree nut intake (panel B), and peanut intake (panel C). Abbreviations: IRT1 = Immediate Free Recall Trial 1; IRT4 = Immediate Free Recal Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

Figure 5.1b. Tree Nuts

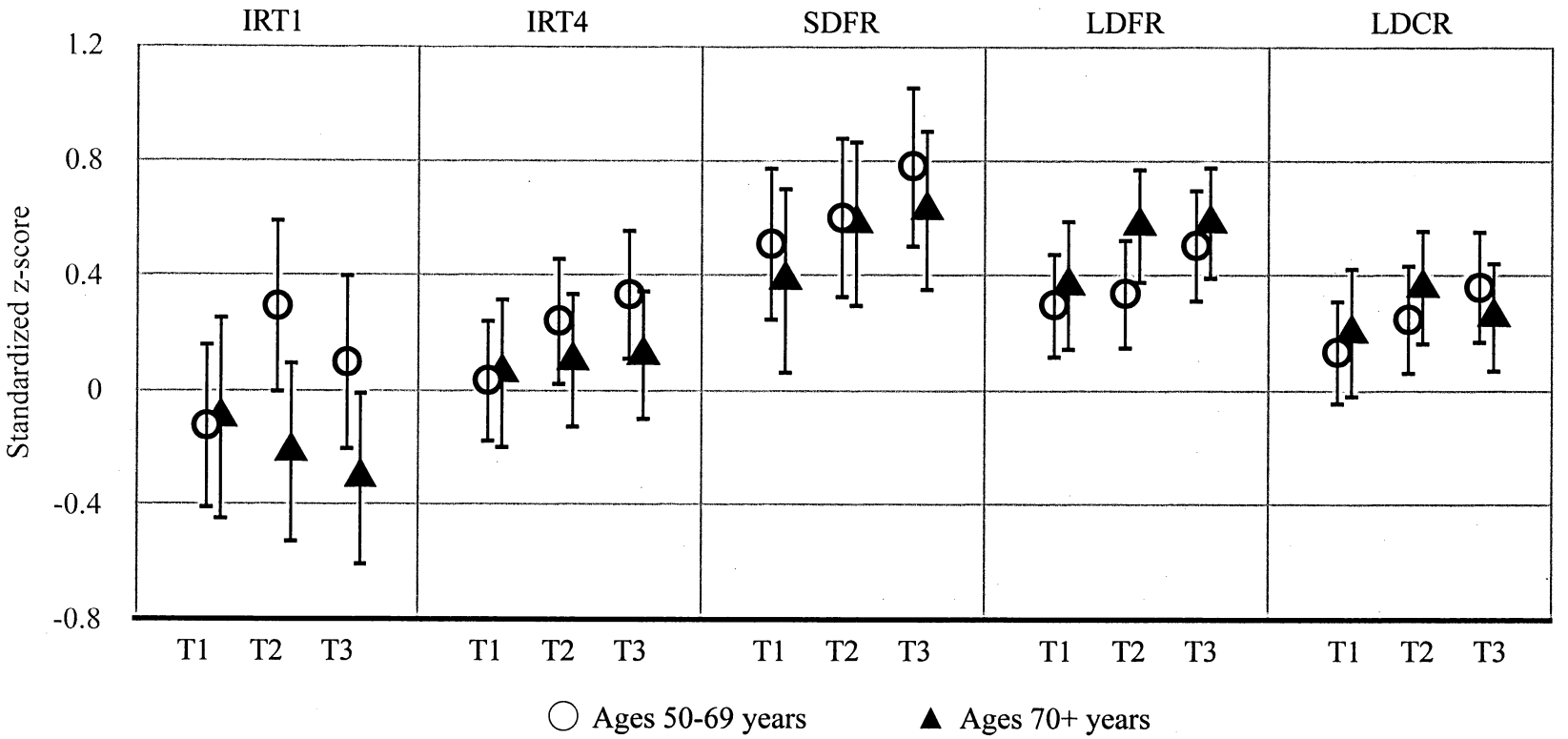

Figure 5.1 (continued). Age-specific standardized z-scores across tertiles of all nut intake (panel A), tree nut intake (panel B), and peanut intake (panel C). Abbreviations: IRT1 = Immediate Free Recall Trial 1; IRT4 = Immediate Free Recal Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

Figure 5.1c. Peanuts

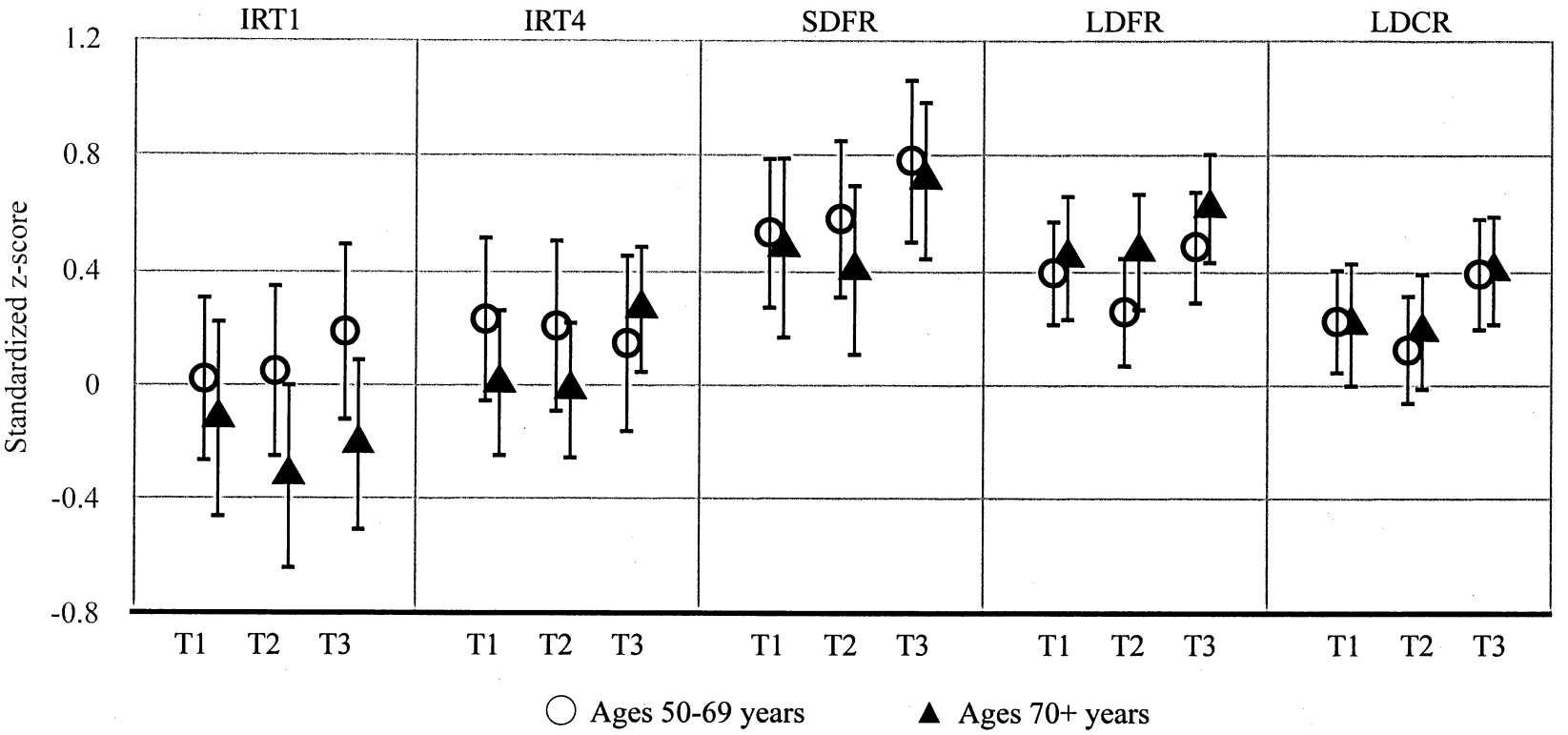

Figure 5.1 (continued). Age-specific standardized z-scores across tertiles of all nut intake (panel A), tree nut intake (panel B), and peanut intake (panel C). Abbreviations: IRT1 = Immediate Free Recall Trial 1; IRT4 = Immediate Free Recal Trial 4; SDFR = Short-delay Free Recall; LDFR = Long-delay Free Recall; LDCR = Long-delay Cued Recall; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

This research is among the first to investigate the association between nut consumption and cognitive function, and the first to examine specific domains of memory. We found no statistically significant association between tertiles of all nuts, tree nuts, and peanuts and memory domains of the California Verbal Learning Test-II; however, an interesting, though insignificant, trend was observed when stratified by age group. Among those aged 50-69 years of age, a positive association was apparent for the Immediate Free Recall Trial 1, which is a measure of auditory attention. Conversely, there was a negative trend among participants aged 70 years and above on the same memory measure.

We also find that this study population has the same risk factors for cognitive impairment as other groups, specifically, age, gender, educational attainment, and ethnicity. Moreover, we found that among younger participants those with normal BMI scored higher on several memory domains than those classified as overweight/obese, while the opposite was observed among participants above 70 years of age. Though also statistically insignificant, these findings suggest the presence of interaction between age and BMI. Waist-to-hip ratio was significantly associated with memory scores after adjusting for demographic, clinical, and lifestyle variables, including BMI.

We are limited in the ability to generalize these findings to the broader US population because study participants were Seventh-day Adventists living in southern

California. Dietary intake was also assessed by a self-reported food frequency questionnaire, which may have affects due to recall bias. The analysis was also cross-sectional, which precludes the ability to draw conclusions regarding causality, and also to determine the impact of reverse-causality. The relatively small sample size limited the number of covariates that could be assessed simultaneously in multivariate models and to investigate more specific type of nuts such as almonds and walnuts.

There are also a number of strengths to this study. Firstly, the study population represents a wide range of ages. Middle-aged participants are included, which allows the determination of factors that may affect cognition prior to the time when function begins to decline rapidly. Older ages are also part of the study population, thus allowing assessment of those who are at greater risk of cognitive decline. Secondly, only very few study participants reported having ever smoked or currently consume alcohol, thus controlling for these confounding factors. Memory scores are also normed to an external standardization sample, allowing for application of the findings to the greater US population. Nut intake in this population varies greatly, more so than other groups, thus providing sufficient variability in the exposure to detect a difference.

Findings of this analysis were instrumental in proposing new areas of research within this study population as relates to diet and cognitive function. Future analysis should consider additional confounding factors, such as apolipoprotein E genetic variants, estrogen status or use of hormonal therapy among women, religious behaviors and attitudes, and specific cardiovascular risk factors such as hyperlipidemia. More specific investigation of the differences between the ethnic groups should also be done.

Cognition is a complex process that may not show significant effects when assessing only one time point. A second round of memory testing has been completed with the current population, therefore allowing assessment of change in memory over time, as has been reported in other cohorts. Finally, consideration of other dietary factors is important, particularly those which provide similar nutrients as nuts. Other researchers have investigated diet patterns and specific food groups and their association with cognition and cognitive decline. The present study population affords the ability to determine if specific domains of memory are related to these broader levels of dietary exposure.

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