ASSOCIATIONS OF ANTIOXIDANT CONSUMPTION FROM FOOD AND SUPPLEMENT SOURCES ON COGNITIVE FUNCTION IN OLDER ADULTS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Health, Nutrition, and Exercise Sciences

March 2024

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Oxidative stress, the imbalance of prooxidants and antioxidants, has been recognized as a possible risk factor for cognitive impairment. We postulate that increased dietary antioxidant consumption could help preserve cognitive function during aging. Dietary intake and cognitive function of 50 adults aged ≥65-years (66% women; aged 71.5±5.1 years) was assessed using Cronometer, an electronic nutrition analysis application, and the Saint Louis University Mental Status examination. The fisher's exact test ($p < 0.01$) identified a significantly lower percentage of cognitive dysfunction (31.6%; 6 of 19 participants) among participants that met the Recommended Daily Allowances (RDAs) for the antioxidants, vitamin C, vitamin E, selenium, and manganese, and a higher percentage of cognitive dysfunction among those that did not meet the antioxidant RDAs (74.2%; 23 of 31 participants). However, mean cognitive scores differed insignificantly between those meeting RDAs (26.2 ±4.2 points) and those that did not (24.9 ±2.9 points; $p = 0.07$).

ACKNOWLEDGMENTS

I owe many thanks to many people for their help in the completion of this thesis. First, I would like to express my gratitude to Dr. Rhee. She has been an integral part of my academic journey at NDSU, and I am so appreciative of her guidance and encouragement. Second, thank you to Dr. McGrath as this study could not have been executed without his strong mentorship. Third, thank you to Dr. Orr for her statistical insight and expertise. Fourth, thank you to Halli Heimbuch, the co-investigator of this study, for her hard work and dedication. Fifth, thank you to Joshua Batesole and Maren Berntson for their assistance with data collection. Lastly, thank you to Curt Doetkott for his help with the statistical analysis of data.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
BMI	Body Mass Index
CERAD	Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Battery
EBMT	East Boston Memory Test
FFQ	Food Frequency Questionnaire
MIS	Memory Impairment Screen
MMSE	Mini-Mental Status Examination
Mn	Manganese
MoCA	Montreal Cognitive Assessment
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
PHS	Physicians' Health Study
RAVLT	Rey Auditory Verbal Learning Test
RDA	Recommended Daily Allowance
ROS	Reactive Oxygen Species
SCWT	Stroop Color and Word Test
Se	Selenium
SLUMS	Saint Louis University Mental Status Examination
TICS	Telephone Interview for Cognitive Status
TMT	Trail Making Test
VFT	Verbal Fluency Test
WHS	Women's Health Study
3MS	Modified Mini-Mental Status Examination

CHAPTER 1. INTRODUCTION

Older adults (65+ years) account for about 17% of the current U.S. population (United States Census Bureau, 2021). This population is rapidly growing and projected to consist of 86 million people by 2050, accounting for 22% of the national population (United States Census Bureau, 2021). Parallelly, the prevalence of age-related conditions and diseases, such as dementia, are increasing as they are common in this population. It is projected that more than 9 million people in the U.S. could have dementia by 2030 (Population Reference Bureau, 2021). Severe deterioration of cognition can lead to a reduction of physical functioning, decreased independence, decline in the quality of life for the individual, and increased socioeconomical burden on society (Gray et al., 2021). There is currently no effective cure for dementia; therefore, studies investigating prevention strategies are of great importance. Literature suggests that diet is a modifiable factor that may reduce the age-related incidence of dementia (Eratne et al., 2018). Evaluating ways in which the next generation of older adults can preserve healthy cognitive status through the means of nutrition is the purpose of this study. Lifestyle interventions, such as those related to diet, are critical to maintain intact cognition and/or slow the rate of its decline.

There appears to be an agreement in the literature that oxidative stress plays a role in the pathogenesis of neurological decline (Cheignon et al., 2018; Eratne et al., 2018; Pisoschi et al., 2021; Wang et al., 2014). Evidence suggests oxidative stress may be a contributing factor to conditions of neurodegeneration, such as Alzheimer's disease (AD), due to an overabundance of free radicals and reactive oxygen species (ROS) in the body (Eratne et al., 2018). Antioxidants are compounds that provide protection against free radicals and ROS that may cause inflammation or damage in the body if not neutralized (Pisoschi et al., 2021). Seemingly, there is

a potential benefit from adding food sources of antioxidants to the diet to help mitigate further cognitive decline due to oxidative stress.

Available research regarding the effects of supplemental antioxidants on cognitive function are varied (Crosta et al., 2021; Devore et al., 2013; Grodstein et al., 2007; Kang et al., 2006; Kryscio et al., 2017; Naeini et al., 2014; Power et al., 2022). Available research examining the association of antioxidant consumption from food sources and cognitive status also varied, however, many studies cite finding a slower rate of decline in those with high and frequent intake of antioxidants from food over time. Still, researchers in this field call for additional studies due to a lack of sufficient evidence to assert a firm conclusion based on specific antioxidants (Devore et al., 2013; Engelhart et al., 2002; M. C. Morris et al., 2002; Naeini et al., 2014). A continued pursuit for additional evidence to conclude cognitive benefits from food- and supplement-based antioxidant consumption is warranted.

Statement of the Problem

Deterioration of cognitive function is common in the rapidly growing older adult population. There is not yet sufficient evidence to assert the relationship between the consumption of specific antioxidants from food or supplements and cognitive function. A need for more evidence exists as the potential of cognitive benefits from antioxidant consumption would further propel the field into building nutrition-based interventions.

Purpose of the Study

The purpose of this study was to examine the impact that consumption of antioxidants, from food and supplement sources, may have on cognitive function in the older adult population.

Research Questions

RQ1: Will older adults that meet the Recommended Daily Allowances (RDAs) of the antioxidants, vitamin C, vitamin E, selenium (Se), and manganese (Mn) (with or without dietary supplements), have higher cognitive scores than those who did not meet the RDAs (with or without dietary supplements)?

RQ2: Will different sources of antioxidants, food vs. supplement, exhibit similar relationships with cognitive function?

RQ3: Which antioxidant, carotenoids, vitamin C, vitamin E, Se, or Mn, will have a stronger positive association with cognitive function?

Hypotheses

H1: Older adults that meet the RDAs for the antioxidants, vitamin C, vitamin E, Se, and Mn from food alone or with supplements will have higher SLUMS scores than those that do not meet the RDAs for those antioxidants.

H2: Antioxidants from food sources will be more strongly associated with SLUMS scores than antioxidants from supplements.

H3: Carotenoids, vitamin C, and vitamin E will have a greater positive association with cognitive function than Se and Mn.

Limitations of the Study

Limitations of the study should be considered when interpreting the results. First, participants were asked to log the food, beverages, and supplements they consumed along with their respective portion sizes on Cronometer. Although participants were given a brief education on portion sizes, estimated portions sizes may have varied across the cohort due to personal bias and food and nutrition knowledge deficits. Second, it is challenging to pinpoint one nutrient as a

sole combative agent against cognitive decline due to the possibility of synergistic relations between various nutrients. Third, the small sample size (N=50) makes it challenging to produce results that are consistent with population-representative results, therefore, there is potential for a type 2 error occurring. Lastly, we did not control for cofounders that may have played a role in cognitive dysfunction such as genetics, diagnosis of an inflammatory disease, lack of physical activity, or history of inadequate nutrition.

CHAPTER 2. REVIEW OF LITERATURE

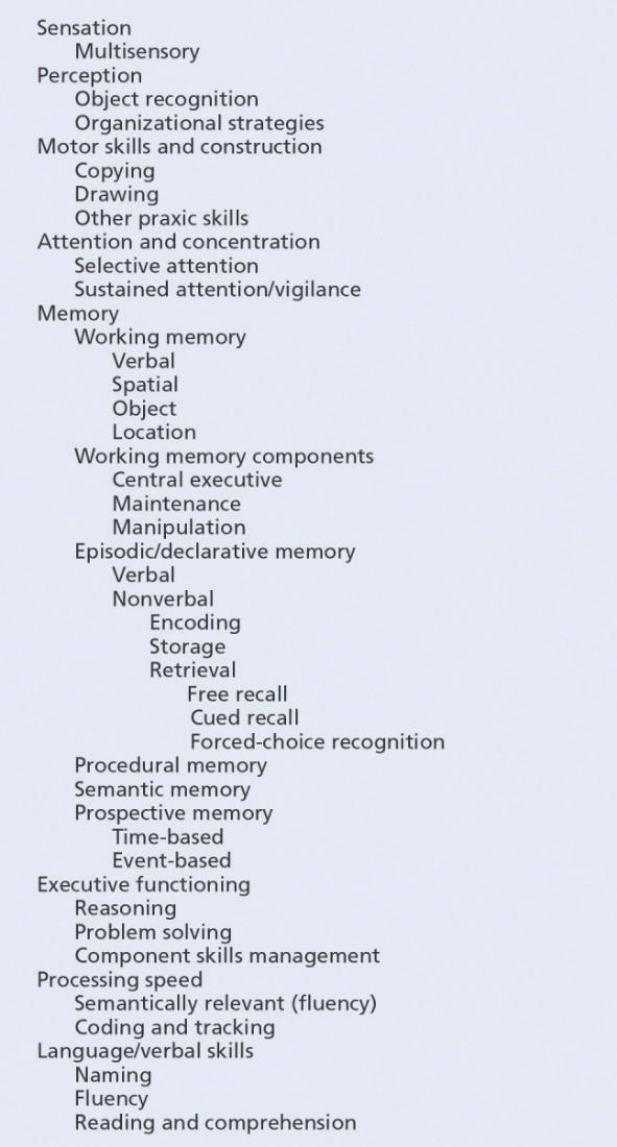
This literature review examined available research about the influence of antioxidant consumption, from food sources and/or supplements sources, on cognitive function in the older adult population. Online databases (PubMed, MEDLINE, and Web of Science) were used to find relevant research articles concerning oxidative stress-induced cognitive deterioration, its health impacts on the individual and society, and the potential of antioxidant consumption mitigating neurodegeneration in older adults. Search terms included the following used individually and in combination: antioxidant, carotenoids, beta-carotene, lycopene, vitamin C, vitamin E, selenium, manganese, oxidative stress, cognitive function, cognitive decline, cognitive impairment, older adult, dementia prevention, and mild cognitive impairment. From the databases' results, the investigator independently assessed which research studies were applicable and made the final decision to include or discard articles. All studies selected for review are included in the reference list.

Cognition

According to the American Psychological Association, cognitive functioning is defined as "the performance of the mental processes of perception, learning, memory, understanding, awareness, reasoning, judgment, intuition, and language" (American Psychological Association, n.d.). Cognition has several domains of which are classified and shown in Figure 1 (Harvey, 2019).

Figure 1

Domains of Cognitive Functioning: Presented as a Bottom-Up Conceptualization



Note. (Harvey, 2019).

Cognitive functioning is not static across the lifespan; all domains continuously change as an individual develops and ages. Some domains peak and decline at varying phases of life. For example, processing speed and working memory may peak as early as 20-30 years of age and

linearly decline throughout the remainder of life, while language/verbal skills may continue improve until late life (Juan & Adlard, 2019). It is generally accepted that a decline of cognition is a normal part of ageing (Onaolapo et al., 2019). Understanding how cognitive functioning changes across the lifespan is helpful to prevent and/or identify abnormalities. The eight domains (sensation, perception, motor skills and construction, attention and concentration, memory, executive functioning, processing speed, and language/verbal skills) can be measured individually using validated tools and tests.

Commonly Used Cognitive Status Examination Tools

Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Neuropsychological Battery

The CERAD neuropsychological battery was established by a grant from the National Institute on Aging to standardize procedures for evaluating and diagnosing patients with AD (J. C. Morris et al., 1989). This battery consists of several subtests; Verbal Fluency (naming as many animals as possible in one minute), Boston Naming Test (naming 15 pictured items; (Goodglass et al., 1983), Mini-Mental State Exam (detailed below; (Folstein et al., 1975), Word List Learning (10 nouns presented on three successive occurrences and later recalled), Constructional Praxis (copying 4 designs; (Rosen et al., 1984), Word List Recall (recall of the 10 nouns presented earlier), Word List Recognition (recognizing the 10 nouns from a list of 20 words), and Constructional Praxis recall (drawing the 4 designs from memory). The battery may take twenty to thirty minutes to administer.

East Boston Memory Test (EBMT)

The EBMT was created as a screening tool for cognitive impairment indicative of AD and other forms of neurodegenerative disease (Gfeller & Horn, 1996). It measures verbal

memory by orally presenting participants with a short, three-sentence story with each sentence containing two idea units. Subsequentially, participants are asked questions based on each idea unit and given one point for each correct answer. Scores may range from 0 to 6. Scherr et al. proposed normative data to establish what EBMT scores indicate: a score of 5 or 6 suggested no memory impairment, and a score of 4 or less is suggestive of memory impairment (Scherr et al., 1988). The EBMT can be administered in five to ten minutes.

Memory Impairment Screen (MIS)

The MIS is a brief screening tool for neurodegenerative diseases that evaluates the cognitive domain of memory (Buschke et al., 1999). Participants are shown four items on a piece of paper – checkers, saucer, telegram, and Red Cross – and asked to read them aloud. The administrator states that each item belongs to a different category (i.e., game, dish, message, and organization). Participants are asked to identify which item fits into each category; failure to complete this task may suggest cognitive impairment. Once the task is completed, the participants are asked to remember each item before engaging in a distractor activity for two to three minutes. Then, participants are asked to recall the four items; 2 points are awarded for every recalled item. If participants are unable to recall an item, the administrator will provide a category clue (i.e., What was the organization?). For each item correctly recalled with a category clue, 1 point is awarded. The maximum score of this screening tool is 8, with a score of 5-8 indicating no impairment and a score of ≤ 4 indicating possible cognitive impairment. Administration of this tool can be fulfilled in about five to ten minutes.

Mini-Mental Status Exam (MMSE)

Folstein et al. devised the MMSE in 1975 (Folstein et al., 1975). At that time, many of the available batteries were lengthy and burdensome to clients, therefore, there was a need for a

simplified assessment tool to evaluate mental status. The MMSE contains 11 questions divided into two sections. The first section covers the cognitive domains of orientation, memory, and attention through verbal responses from participants. The second section covers language and motor function by asking the participants to follow commands and draw a complex figure. The exam's maximum total score is 30 points; a score of ≤ 23 points is indictive of cognitive impairment. The exam is not timed, and typically takes about five to ten minutes to administer.

Modified Mini-Mental Status Test (3MS)

The purpose of altering the MMSE to create the 3MS was to cover a broader scope of cognitive domains and enhance the reliability and validity of scores while retaining the brevity, objective scoring, and ease of administration of the MMSE (Teng & Chui, 1987). The 15-question extension of the MMSE assesses attention and concentration, long-term and short-term memory, language, constructional praxis, abstract thinking, and verbal fluency. Scores can range from 0 to 100 points; ≤ 77 points suggests dementia with 87% sensitivity and 89% specificity determined by Bland & Newman (Bland & Newman, 2001). This test can be administered in about ten minutes.

Montreal Cognitive Assessment (MoCA)

The MoCA, a brief screening tool for mild cognitive impairment, evaluates short-term and working memory, attention and concentration, language, and executive function (Nasreddine et al., 2005). It involves sequencing numbers and letters, naming animals, drawing a 3-D cube and a clock, and recalling words. The maximum possible score is 30 points; a score of ≥ 26 points is considered normal, while ≤ 25 points may be suggestive of cognitive dysfunction. The MoCA requires about ten minutes to administer.

Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT is a verbal memory test originally developed in 1941 by Andre Rey for French-speaking adults; an English version was adapted in 1959 (Taylor, 1959). The multistage process proceeds as follows: the administrator verbally presents 15 words (List A) to the participant, who will be asked to repeat them based on memory. This will be repeated four more times for a total of five trials. Next, the administrator will verbally present 15 new words (List B) to the participant, who will be asked to repeat the words from List B based on memory in one attempt. The participant will be asked to remember as many words as possible from List A. Then, a 20-minute delay will be implemented. Following the delay, the participant will be asked to recall as many words as possible from List A. Finally, a list of words will be given to the participant to read from which they must indicate which words were from List A. One point is earned for each correct answer. There are three summary scores: Immediate (the sum of scores from the first five trials), Learning (the score of Trial 5 minus the score of Trial 1) and Forgetting (the score of Trial 5 minus the score of the recall immediately following the delay). Currently, there is no universal normative data for the RAVLT as scores in different contexts (e.g., ranging age groups) make them difficult to obtain.

Saint Louis University Mental Status Examination

The SLUMS is a 30-point, 11-question assessment tool for mild cognitive impairment and dementia that examines the domains of attention, memory, language, executive function through animal naming, clock drawing, digit span, size differentiation and figure recognition (Tariq et al., 2006). Two scoring scales are differentiated by the participant's level of completed education. Scoring for an individual with a high school education or greater is as follows: 27-30 points normal cognition; 21-26 points mild neurocognitive disorder; 1-20 points dementia.

Scoring for an individual with less than a high school education is as follows: 25-30 points normal cognition; 20-24 points mild neurocognitive disorder; 1-19 points dementia. Execution of the SLUMS requires about five to ten minutes.

Stroop Color-Word Test (SCWT)

The SCWT evaluates the cognitive domains of attention and concentration and processing speed by testing one's ability to concentrate on a certain task while being presented with distracting stimuli, simultaneously (Stroop, 1935). A participant will be shown three tables of color words (e.g., red, blue, purple), one at a time. The first table will present color words printed in black ink. The participant will be asked to read aloud as many words on the first table as possible within 45 seconds. The second table of words presents the same words, although each word is printed in the ink color its name indicates (i.e., the word "purple" is printed in purple ink). The participant is asked to read aloud as many words on the second table as possible within 45 seconds. The third table of words presents the same words, although the color of ink in which each word is presented contradicts the spelled word (i.e., the word "red" is printed in blue ink). The participant is asked to name aloud the color of ink each word is printed in as fast as possible for 45 seconds. The method of scoring commonly used was proposed by Golden (1978). Each correctly named item is calculated for each table (W, C, CW). A CW score is predicted (Pcw) using the following equation: $Pcw = (W \times C)/(W + C)$. Then, Pcw is subtracted from CW to obtain an inference score based on table 1 (W) and table 2 (C) scores; a positive inference score indicates performance better than expected, and a negative inference score indicates poorer performance than expected.

Symbol Digit Modalities Test (SMDT)

The SDMT is a paper-pencil tool that screens for cognitive dysfunction by examining attention, processing speed, and motor skills (Smith, 1982). The participant is asked to match as many numbers with corresponding geometric figures as possible in 90 seconds; a reference key is presented to the participant indicating which numbers match which figures (e.g., 1 = triangle). Scoring is based on the number of correct matches made within the time allotted (110 points possible). This test takes about five minutes to administer.

Trail Making Test (TMT)

The TMT was initially developed in 1944 as a part of the Army Individual Test Battery used to assess the presence of brain injuries in soldiers (Washington, DC War Department, Adjutant General's Office, 1944). Decades later, it was integrated into clinical use through the Halstead Reitan neuropsychological battery (Reitan & Wolfson, 1993). The test provides information on attention, processing speed, working memory and executive function. The test is divided into two parts, A and B. In TMT-A, participants are given a sheet of paper with encircled numbers 1 through 25 scattered around the page. They are asked to draw lines with a pencil to connect each number in numerical order at maximal speed. In TMT-B, participants are given a sheet of paper with encircled numbers 1 through 25 and encircled letters A through Y scattered around the page. They are asked to connect the circles in an alternating fashion between numbers and letters (e.g., 1, A, 2, B, 3, C, etc.) at maximal speed. Increased time to complete the tasks or the inability to complete either or both tasks may suggest cognitive impairment. The TMT requires five to ten minutes to complete.

Telephone Interview for Cognitive Status (TICS)

The TICS is a brief test administered over the telephone to evaluate overall cognitive function when in-person tests are impractical or inefficient, however, it can be administered in-person (Brandt et al., 1988). Furthermore, it is a useful tool to evaluate cognition in those who are visually impaired as TICS does not require vision to complete. The test is highly correlated with the MMSE and was standardized for adults aged 60-98 years. The TICS contains 11 test items that evaluate attention and concentration, memory, language, praxis skills, and takes less than 10 minutes to complete and score. The TICS Total score (a maximum of 41 points) can be interpreted by four qualitative impairment ranges: unimpaired (33 to 41 points), ambiguous (26 to 32 points), mildly impaired (21 to 25 points), and moderately to severely impaired (0 to 20 points).

Verbal Fluency Test (VFT)

Verbal fluency refers to the ability to retrieve memories and communicate thoughts through words. The VFT examines the cognitive domain of language and verbal functioning through semantic (categorical) fluency and/or letter fluency tasks. Semantic fluency is measured by the number of items the participant can name from a specified category (e.g., animals, fruits, colors.) in one minute. Phonemic (letter) fluency is measured by the number of words starting with a specified letter (often F, A, or S) the participant can list in one minute. Tombaugh et al. presented normative data for the VFT (specifically, the animal naming and FAS tasks) from a sample of 1,300 cognitively intact individuals (defined by scoring higher than 23 points on the MMSE) aged 16 to 95 years (Tombaugh et al., 1999). On the animal naming semantic task, adults aged 60-69, 70-79, 80-89, and 90-95 named 17.6 (±4.7), 16.1(±4.0), 14.3(±3.9), and 13.0(±3.8) animals, respectively. On the FAS phonemic task, adults aged 60-69, 70-79, 80-89,

and 90-95 listed an average of 38.5(±13.7), 34.8(±12.8), 28.9(±11.7), and 28.2(±11.0) words, respectively. This suggests that scores below what has been indicated in each respective age group may indicate decreased cognitive function.

The Effect of Cognitive Decline on Daily Living

Cognitive decline, whether gradual or progressive, may coincide with impairment of activities of daily living (ADL) and/or instrumental activities of daily living (IADL) (Gray et al., 2021) such that the ability to perform these activities are dependent on cognitive abilities, such as complex thinking (Mlinac & Feng, 2016). ADLs include fundamental skills required to perform basic needs such as toileting, bathing, dressing, eating, and rising from a chair or bed (Mlinac & Feng, 2016). IADLs are complex functions that require a higher level of thinking (e.g., reasoning, planning, multitasking) to perform such as managing finances or medications, shopping for groceries, or preparing meals (Mlinac & Feng, 2016). Due to the high level of mentation required to perform IADLs, impairments in these activities may serve as an early indicator of cognitive decline (Marshall et al., 2011). In 2018, it was estimated that 25.4% of older Americans were living with at least one IADL impairment (Knoll et al., 2023). Impairment of cognition and subsequent reduction of physical function capacity can lead to serious implications including reduced independence, decreased quality of life, and increased healthcare costs. Dementia is one of the costliest health conditions in the United States. In 2020, informal care for people with dementia (i.e., unpaid care provided by family members and friends) was estimated to amount to 15.3 billion hours valuing about $256.7 billion (Alzheimer's Association, 2021). Additionally, in the same year, it was estimated that an average total of lifetime care for someone with dementia was $373,527. The atrophy of daily living that coincides with the progression of dementia not only negatively impacts the diagnosed individual but may also pose

a heavy burden on the family which further implies the importance of interventions to mitigate cognitive decline.

Defining Neurodegenerative Diseases

Dementia is defined as a collection of cognitive deficits such as difficulty with memory, language, problem-solving, and other mental skills (Alzheimer's Association, 2023). AD, Cerebrovascular disease, Frontotemporal degeneration (FTD), Hippocampal sclerosis (HP), Lewy body disease, Parkinson's disease (PD), and other mixed pathologies can cause dementia. Ambiguity of nomenclature for neurodegenerative diseases has been an issue for many decades (Knopman et al., 2019). For example, the terms dementia and AD are commonly misused interchangeably. Inconsistent use of dementia terminology proliferates challenges in communication between researchers, clinicians, and laypersons. Recently, members of the Dementia Nomenclature Initiative suggested a new framework to describe neurogenerative disorders and diseases, which considers symptomatic presentation within the domains of cognition, behavior/psychiatric, motor, or other neurological symptoms, defined further by severity (i.e., none, minimal, mild, or moderate, severe), and physiological evidence (Petersen et al., 2023). Initiation of this framework may improve disease labeling and thereby enhance health literacy and understanding for affected persons and their families, refine pharmaceutical and psychosocial interventions, shorten the duration between diagnosis and initiation of intervention, and ameliorate future research endeavors.

Alzheimer's Disease

AD is the most common cause of dementia, affecting an estimated 6.7 million people (Alzheimer's Association, 2023). It is characterized by deficits in cognitive function secondary to accumulation of extracellular beta-amyloid, a toxic protein fragment, which leads to

hyperphosphorylation of tau protein, a microtubule-associated protein, and the creation of intracellular neurofibrillary tangles in the brain (Abeysinghe et al., 2020). Brain scans, such as positron emission tomography (PET) and cerebrospinal fluid (CSF) testing help clinicians accurately diagnose AD by quantifying accumulation of beta-amyloid and tau proteins. The greatest risk factors for AD are increased age, genetics (specifically the inheritance of the apolipoprotein E4), and family history of the disease (National Institute on Aging, 2023). Modifiable risk factors include physical activity, staying mentally and socially active, smoking, education, blood pressure, and diet (Alzheimer's Association, 2023). It is well-accepted that AD pathogenesis begins decades before the onset of measurable symptoms (Martins et al., 2018), therefore, preventative measures should be taken long before diagnosis.

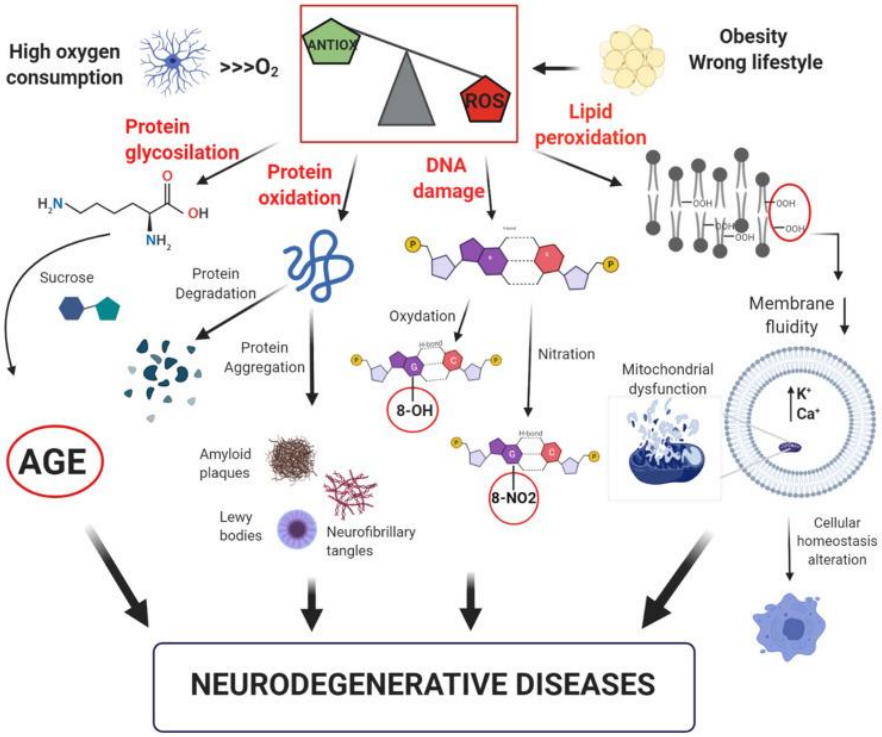
Oxidative Stress and Age-Related Cognitive Decline

Oxygen is a highly reactive non-metal with two unpaired electrons that can accept a pair of electrons from an electron donor (Gulcin, 2020). The mitochondria of living organisms use oxygen in the process of oxidative phosphorylation to produce energy in the form of adenosine triphosphate (ATP) (He et al., 2017). Through this process, free radicals, which are highly unstable atoms with one or more unpaired electron that have a high affinity for reacting with other molecules, are produced as byproducts (Gulcin, 2020). Reactive oxygen species (ROS), oxygen-centered free radicals, occur in living organisms during normal respiration and cellular metabolism, and are involved in the necessary processes of immune response, inflammation, synaptic plasticity, and memory and learning (Anraku et al., 2018; Grimm & Eckert, 2017). However, problems may arise if ROS, or other prooxidant species, overwhelm the antioxidants in the organism to create a state of oxidative stress which may lead to cellular damage, harming proteins and DNA and inducing lipid peroxidation, and mitochondrial dysfunction (Grimm &

Eckert, 2017). This process of oxidation-induced degeneration is depicted in Figure 2 (Franzoni et al., 2021).

Figure 2

Oxidative Stress: Imbalance of Antioxidants and Free Radicals.



Note. (Franzoni et al., 2021)

According to Gulcin, there are two possible mechanisms giving rise to oxidative stress: 1) the number of reactive species is increased due to chronic inflammation, or 2) antioxidant concentration in the organism is decreased due to mutated antioxidant enzymes, toxins, or inadequate intake of natural antioxidants (Gulcin, 2020). Given that mitochondria regulate oxidation-reduction (redox) homeostasis in living organisms, mitochondrial dysfunction creates an environment conducive to further degeneration (Grimm & Eckert, 2017). Dysfunction of mitochondria due to oxidative stress may be one of the first events in the pathogenesis of

neurodegenerative diseases. Additionally, neurons in the brain are non-dividing post-mitotic cells that cannot be replaced in the event of damage (Ionescu-Tucker & Cotman, 2021), therefore, it is not surprising that the brain is highly susceptible to degeneration with aging.

The free radical theory of aging, postulated by Harman, indicates aging and age-related degenerative diseases are caused by free radical reactions inside the body (Harman, 1992). Furthermore, this theory suggests slowing the rate of free radical reactions may increase an organism's lifespan. As the literature has evolved, current studies similarly suggest that oxidative stress caused by free radicals is associated with the pathophysiology of chronic diseases, such as chronic obstructive pulmonary disease, atherosclerosis, cancer, and neurodegenerative diseases (Forman & Zhang, 2021; He et al., 2017).

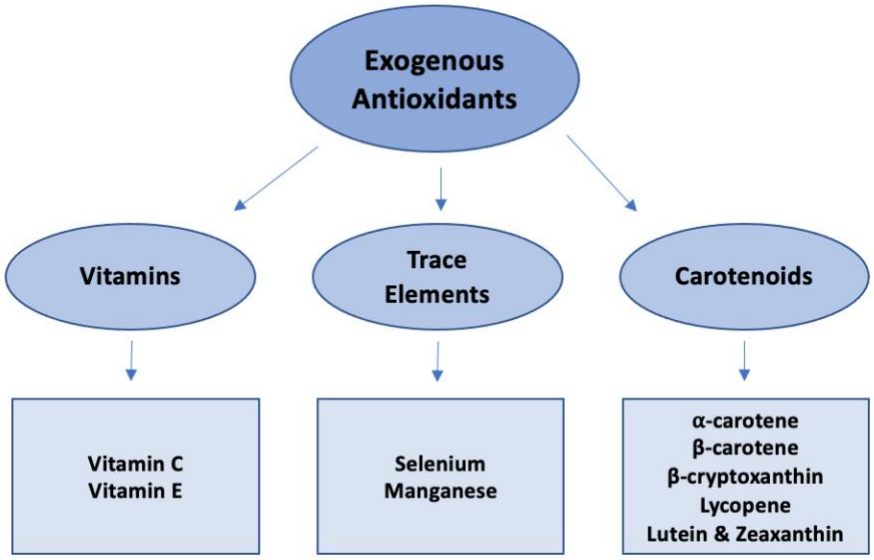
Antioxidants

Definition and Classification

Halliwell originally defined an antioxidant as "a substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell, 1990). For the sake of this literature review, exogenous sources of carotenoids, trace elements, and vitamins will be highlighted (Figure 3).

Figure 3

Antioxidant Classification



Carotenoids

Carotenoids are colorful, liposoluble pigments that contain a polyisoprenoid structure, a long, conjugated chain of double bonds and a near bilateral symmetry around the central double bond (Milani et al., 2017). They can be categorized as provitamin A or non-provitamin A carotenoids. Beta-carotene, alpha-carotene, and beta-cryptoxanthin are known as provitamin A carotenoids as they serve as precursors of vitamin A; after consumption, they convert into vitamin A in the intestines (Milani et al., 2017). Lycopene, lutein, and zeaxanthin are known as non-provitamin A carotenoids as they do not convert into vitamin A. Carotenoids are responsible for the deep green, yellow, orange, and red pigments found in fruits and vegetables such as bell peppers, carrots, cantaloupes, tomatoes, broccoli, spinach, and sweet potatoes. As antioxidants, carotenoids are known to stabilize unpaired electrons after radical quenching, deactivate peroxyl radicals, and quench oxygen in both in vitro and in vivo models (Mendonça et al., 2022). The

presence of the conjugated double bonds empowers them to accept electrons from reactive species and thereby neutralize free radicals (Milani et al., 2017).

Vitamins

Ascorbic acid, also known as vitamin C, is a six-carbon compound that contains two acid-ionizing groups (Ballaz & Rebec, 2019). This water-soluble vitamin, which is necessary to consume to avoid developing Scurvy, is abundantly found in citrus fruits, bell peppers, broccoli, and Brussel sprouts (National Institutes of Health, 2021c). According to the Food and Nutrition Board at the Institute of Medicine of the National Academies, USA, the recommended dietary allowance (RDA) for vitamin C is 90 mg per day for men and 75 mg per day for women aged 51 years and older (National Institutes of Health, 2000). The amount of vitamin C humans can absorb at one time is limited to the sodium-dependent vitamin C (SVCT1) transporters in the gut, therefore, consumption of over 400 mg/day saturates the plasma and the excess is excreted (Savini et al., 2008). The highest concentration of ascorbic acid in the human body is in the brain (Franzoni et al., 2021) which further denotes its importance for cognitive function. As an antioxidant, it buffers oxidizing species by protecting the membrane of phospholipids and efficiently scavenging free radicals (Mendonça et al., 2022).

Vitamin E includes eight lipophilic compounds; four tocopherols (α, β, δ, γ) and four tocotrienols (α, β, δ, γ) (Mendonça et al., 2022). All forms of vitamin E have a chromanol ring and a 16-carbon phytyl-like chain (Mendonça et al., 2022). Vitamin E is found most abundant in oils, such as wheat germ, sunflower, and safflower oil, green leafy vegetables, sunflower seeds, hazelnuts, and peanuts (National Institutes of Health, 2021). Plant tissues primarily contain the α-tocopherol form of vitamin E, while seeds and their derived products contain mainly γ-tocopherol. The RDA for vitamin E for men and women ages 51 years and older is 15 mg per

day (National Institutes of Health, 2000). Tocopherols and tocotrienols exhibit radical-scavenging properties through the donation of hydrogen from the phenolic group on its chromanol ring (Szewczyk et al., 2021).

Trace Elements

Selenium (Se) is a micronutrient that plays important roles in reproduction, thyroid hormone metabolism, DNA synthesis, and reactive oxygen species scavenging (National Institutes of Health, 2021b). The RDA for individuals 51 years and older is 55 µg per day (National Institutes of Health, 2000). The content of Se in plant products varies based on the Se content of the soil they were grown in; similarly, the content of Se in animal products varies based on the diet of the animal (Bjørklund et al., 2022). Selenium yeast, Brazilian nuts, garlic, onions, chicken, and beef are good sources of Se (Kieliszek & Błażejak, 2016). There are several antioxidant enzymes giving this trace element its antioxidant property; Glutathione peroxidase, superoxide dismutase (SODs), and thioredoxin reductase are enzymes that protect against oxidative stress (Steinbrenner & Sies, 2013). Glutathione peroxidase catalyzes the reduction of hydrogen peroxide, lipid hydroperoxides, and organic hydroperoxides to water (Forman & Zhang, 2021).

Manganese (Mn) is a trace element that plays an important role as a cofactor for many enzymes involved in amino acid, cholesterol, glucose, and carbohydrate metabolism, bone formation, reproduction, and immune response (National Institutes of Health, 2021a). Food sources of Mn include whole grains, clams, oysters, mussels, nuts, soybeans and other legumes, rice, and leafy vegetables. Adequate Intake (AI) of Mn is 2.3 mg/day and 1.8 mg/day for men and women aged 51 years and older, respectively (National Institutes of Health, 2001). Mn is a

component of the antioxidant enzyme SODs, which catalyzes the conversion of superoxide anion free radical to hydrogen peroxide thereby stabilizing it by becoming oxygen and water.

Measuring Antioxidants Activity

Measuring antioxidant activity is difficult because each individual antioxidant can have a different and complex mechanism of action. For example, some antioxidants prevent production of ROS, some scavenge prooxidant species, and some repair oxidative damage (Flieger et al., 2021). “Antioxidant activity” and “antioxidant capacity” are important terms to describe antioxidant measurement assays that are often incorrectly used interchangeably. Kinetic-based assays measure “antioxidant activity” by the rate constant of reaction between reactants or scavenging percentages per unit of time (Flieger et al., 2021). “Antioxidant capacity” is the efficiency of antioxidants to inhibit oxidative damage (Flieger et al., 2021).

There is no golden standard assay used to measure antioxidant activity (Pinchuk et al., 2012) and, therefore, many different methods are used in antioxidant research. However, there are a few more commonly used assays.

Oxygen Radical Absorbance Capacity (ORAC)

Developed in 1992, the ORAC measures the amount of protection an antioxidant provides to the target molecule (fluorescein probe) against ROS, specifically the peroxyl radical (Cao et al., 1993; Carvalho et al., 2023). The important concept of this assay is that when fluorescein is exposed to radical species, it will be decomposed by oxidation; if an antioxidant is added to the equation before oxidation begins, the ROS will be quenched and the fluorescein will be preserved (Cao et al., 1993). ORAC provides a quantitative value of the antioxidant’s radical scavenging ability based on free radical inhibition time and degree of inhibition compared to that

of Trolox, a water-soluble vitamin E analogue, that serves as a positive control that inhibits fluorescein decomposition in a dose dependent manner (Cao et al., 1993; Gupta, 2015).

Ferric Reducing Antioxidant Power (FRAP)

Established in 1996, the FRAP measures the antioxidant's ability to reduce the ferric ions to ferrous irons in an aqueous solution (Benzie & Strain, 1996). When the reduction occurs, a blue color is produced and subsequentially measured calorimetrically at 700 nanometers. Because this assay is completed in an aqueous solution, the antioxidants being tested must be water-soluble (e.g., vitamin C). Quantitative results are expressed in Fe2+ equivalents which are in μM.

Trolox Equivalent Antioxidant Capacity assay (TEAC)/ABTS assay

The TEAC assay was originally postulated in 1993 and improved and renamed to the ABTS assay in 1999. The ABTS assay is based on the antioxidant's ability to absorb a pre-generated 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) radical cation, which has a deep blue color (Miller et al., 1993). When an antioxidant is introduced, it reduces the cation by donating hydrogen atoms and visibly bleaching the deep blue color. Absorption of the color is measured at wavelengths 415 nanometers (nm), 645 nm, 734 nm and 815 nm and compared to that of Trolox. This assay is applicable for lipophilic and hydrophilic systems.

Studies Investigating Antioxidants and Cognitive Function

Research investigating antioxidants' role in cognitive function has often been performed through intervention or observational study designs. The following studies are original human research published in 2000 or thereafter. To be included, participants had to be aged 55 years or older at baseline, and dietary intake and cognitive function data must have been collected. Additionally, to be included, the studies must have investigated at least one of the following

vitamins or minerals that possess antioxidant properties and how it may have impacted cognition when consumed: carotenoids (including alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene), vitamin C, vitamin E (including alpha-tocopherol, beta-tocopherol, delta-tocopherol, gamma-tocopherol), Se, and Mn.

Antioxidant Intervention Studies

Carotenoids

The Physician's Health Study (PHS) II, a randomized, placebo-controlled trial, was spearheaded in 1997 to examine the potential of preventing cardiovascular disease, cancer, age-related eye disease, and cognitive decline through vitamin supplementation (Christen et al., 2000). Participants from the original PHS, circa 1982, were invited to participate in the extending study PHS II, and 7,641 men accepted. An additional 7,000 men 55 years and older were newly recruited. Four vitamin supplements were tested: 1) alternate-day beta carotene (50 mg Lurotin) or placebo, 2) alternate-day vitamin E (400 IU) or placebo, 3) daily ascorbic acid (500 mg) or placebo, and 4) daily multivitamin (Centrum Silver) or placebo. Participants were randomly assigned in a 2x2x2x2 factorial trial to receive a combination of the supplements listed above. Grodstein et al. focused on examining the effects of beta carotene supplementation on cognitive function (Grodstein et al., 2007). Cognitive function was assessed through a cognitive battery consisting of the TICS (Brandt et al., 1988), immediate and delayed recall measures of the EBMT (Gfeller & Horn, 1996) delayed recall of a 10-word list, and category fluency – naming animals in 1 minute. Evidence showed that short-term beta-carotene supplementation (mean of one year) had no impact on cognition scores from baseline to follow-up. Long-term beta-carotene supplementation (mean of 18 years) had a significant positive impact on cognition scores as participants with long-term supplementation had higher global cognition scores and

performed significantly better on verbal memory than those with the placebo (Grodstein et al., 2007). These results suggest long-term beta-carotene may provide cognitive benefits for memory in older men.

Crosta et al. sought to evaluate the potential function of antioxidants as preventative agents for onset and progression of cognitive impairment in a double-blind, randomized, controlled study (Crosta et al., 2021). Eighty participants (68.8% female) aged 60 years or older with no indication of cognitive dysfunction were randomly assigned to either the active group that consumed a supplement tablet consisting of four bioactive compounds (bacopa, lycopene, astaxanthin, and vitamin B12) orally once a day for eight weeks or the control group that consumed a placebo with the same intake schedule. The supplement tablet was composed of 80,000 mg bacopa, 100,000 mg lycopene, 160,000 mg astaxanthin, and 6,000 mg vitamin B12 per dose. Currently, there is no RDA for lycopene. Porrini and Riso suggest the average daily intake of lycopene in the United States ranges from 6.6–10.5 mg/day for men and 5.7–10.4 mg/day for women (Porrini & Riso, 2005); Imran et al. cite the daily intake of lycopene in the Unites States being greater than 7 mg (Imran et al., 2020). A dietitian met with all participants to evaluate current dietary habits, correct nutritional deficiencies, and standardize diets relatively. Cognitive function was assessed at baseline and after eight weeks (±two days) of treatment by the TMT part A and B, VFT, MoCA, MMSE, and RAVLT. Results presented a statistically significant decrease ($p < 0.0001$) in time required to complete the TMT B in the active group (-17.63 seconds), whereas the time required to complete the TMT B in the control group exhibited a statistically significant increase (+3.46 seconds, $p < 0.0001$). The active group displayed a significantly higher decrease in required time to complete TMT A (−6.86 s) than in the control group (−0.37 s). Letter fluency of VFT in the active group was significantly higher in the active

group (+5.28 vs. +1.07 words; $p < 0.001$). No other statistically significant differences were found for the MoCA, MMSE, or RAVLT. The present study provides encouraging evidence that the daily consumption of an antioxidant mix including lycopene may positively influence cognitive performance in cognitively intact, generally healthy, older adults, but it cannot be directly associated with lycopene alone as the other elements of the supplement may have synergistically contributed to the intervention's outcome.

Vitamins C and E

In a double-blind, randomized, placebo-controlled trial, Naeini et al. examined the effect of vitamin E and C consumption on cognitive performance in older adults in Isfahan, Iran (Naeini et al., 2014). Two hundred and fifty-six older adults (53% female) aged 60-75 years with cognitive impairment were recruited and randomly assigned to consume 300 mg of vitamin E and 400 mg of vitamin C or placebo daily for one year. Cognitive function was assessed with the MMSE (Folstein et al., 1975) at baseline and 6- and 12-months after intervention initiation. No difference was observed between the group receiving the antioxidant supplements and the group receiving a placebo based on MMSE scores.

In 1976, the Nurses' Health Study (NHS) began when 121,700 female registered nurses aged 35-55 responded to mailed questionnaires about their health and lifestyle. The study's main motivation was to research the potential long-term effects of oral contraceptives. In 1980, a food frequency questionnaire (FFQ) was added and administered every 4 years, and from 1995 to 2001 the TICS was implemented (Brandt et al., 1988). In 2012, Devore et al. used data from 16,010 women aged 70 years or older to investigate the association between antioxidants and cognition (Devore et al., 2013). Results showed that higher, long-term intake of supplemental vitamin C was associated with worse cognitive decline, specifically in global cognition ($p =$

0.001). Similarly, higher, long-term intake of total vitamin C (both dietary and supplemental) was also associated with worse cognitive decline in global cognition ($p = 0.006$), the verbal score ($p = 0.04$), and TICS score ($p = 0.006$). However, a positive relationship was found between greater dietary vitamin C intake and verbal cognition. No association was found between dietary or supplemental vitamin E and cognition in multivariable-adjusted models.

In a sub-study of the Women's Health Study (WHS), supplementation of 600 IUs (402 mg) of vitamin E every other day over an 8-week intervention period was initiated in a cohort of 39,876 healthy women aged 65 years and older to exam its effect on cognitive function (Kang et al., 2006). Cognitive function was assessed through a cognitive battery consisting of the TICS (Brandt et al., 1988) and EBMT (Gfeller & Horn, 1996). The mean time between the first and the last cognitive assessment was 4.0 years (range of 2.6-5.7 years). Results provided no indication that vitamin E supplementation higher than the RDA provides any cognitive benefits in the cohort of women 65 years and older.

Vitamin E and Selenium

In the Prevention of Alzheimer's Disease with Vitamin E and Selenium (PREADVISE) primary prevention trial, 3,786 men were selected from the parent study Selenium and Vitamin E Cancer Prevention Trial (SELECT) to determine if antioxidant supplementation of vitamin E or selenium (used alone or in combination) could prevent dementia in older men (Kryscio et al., 2017). The participants were required to be at least 60 years old, enrolled in a SELECT participating site, and have no presence of dementia or neurological condition that affected cognition. A supplement of vitamin E (400 IU (268 mg)/day), Se (200 mcg/day), vitamin E and Se together, or placebo were given to participants once a day for a median of 5.4 ± 1.2 years. The MIS (Buschke et al., 1999) and a modified TICS (TICS-M; (de Jager et al., 2003) were

administered annually to assess cognitive function. Findings in this cohort study suggested no significant evidence of prevention for dementia through supplementation of vitamin E or Se or a combination of the two antioxidants.

Selenium

Rita Cardoso et al. executed a randomized controlled pilot trial to investigate the impact of Brazil nut consumption on cognitive performance on older adults with mild cognitive impairment (Cardoso et al., 2021). According to the National Institutes of Health, the Brazil nut is one of the most abundant food sources of Se (National Institutes of Health, 2021e). Twenty adults aged 60 years or older (mean age 77.7 ±5.3 years) were randomly assigned to the treatment group (3 males, 8 females) or the control group (3 males, 6 females). The treatment group was asked to consume one Brazil nut (mean weight of one nut was 5 grams providing approximately 289 µg of selenium) each day for six months, and the control group was asked to maintain their typical dietary patterns for six months. Cognitive function was assessed at baseline and again after 6 months using the CERAD neuropsychological battery (J. C. Morris et al., 1989). One subtest is the constructional praxis subtest where participants are presented with four drawings (diamond, circle, cube, and overlapping rectangles) one at a time that they must copy within a maximum of two minutes per drawing. Additionally, the WAIS-III Digit Span, WAIS-III Digit Symbol Substitution, and TMT-A were administered to assess attention; the Free and Cued Selective Reminding Test and Wechsler Logical Memory evaluated memory; the clock drawing test evaluated visuospatial functions; the TMT-B and SCWT assessed executive function; and phonemic and semantic verbal fluency evaluated language. Cognitive performance was similar between groups at baseline. Results showed a significant improvement in verbal fluency ($p = 0.007$) and the constructional praxis subtest ($p = 0.031$) in the treatment group after

six months of daily Brazil nut consumption. No other considerable indications of effect were found during analysis.

Dietary Observation Studies

Carotenoids

Using data from the NHS, Devore et al. examined the association of long-term antioxidant intake from food sources and cognitive decline among older women aged 70 years or older (Devore et al., 2013). Dietary and cognition data were collected through a semiquantitative food frequency questionnaire (Willett et al., 1985) and the TICS (Brandt et al., 1988) which were administered every four years. Antioxidant intake was averaged from the participant's first dietary assessment through their last to obtain a stable measure reflecting long-term diet. An average of five dietary assessments over a mean of 17 years were collected from 16,010 women. Evidence suggested a long-term higher intake of lycopene from food sources may be related to slower cognitive decline in global and verbal cognition scores. A greater intake of lutein, zeaxanthin, and lycopene was also related to a better cognitive status of global cognition score. Strong positive associations were found between carotenoid intake and overall cognition.

Christensen et al. investigated associations between dietary and supplemental lutein (L) and zeaxanthin (Z) with cognitive function among older adults. Researchers used a nationally representative sample of 2,796 participants (53.6% female) aged 60 years or older from the 2011-2014 National Health and Nutrition Examination Survey (NHANES) (Christensen et al., 2020). An estimation of L and Z intake (including dietary and supplemental sources) was taken from two non-consecutive 24-hour diet recalls. All participants' intake amounts were then organized into quartiles. Assessment of cognitive function was performed with three tests: the CERAD Word Learning subtest (CERD W-L), the Animal Fluency test, and the DSST. A

positive association between cognitive performance and L and Z from food sources was found from the results, but a negative association was found between cognitive performance and supplemental L and Z. Greater total L and Z intake was significantly associated with higher scores on all three cognitive tests. Results from this study suggest higher intakes of L and Z are correlated with better cognitive function, although the use of only two 24-hour dietary recalls to assess participants' typical intake is a limitation that should be considered when evaluating the findings.

Vitamins C and E

In the Cache County Study, 3,632 participants (56.9% women) aged 65 years and older were examined to evaluate the relationship between cognitive decline and antioxidant intake in the span of seven years (Wengreen et al., 2007). Participants completed a 141-food item FFQ, and their consumption of vitamins C and E were modeled in quartiles of intakes. The 3MS (Teng & Chui, 1987) was used to assess cognitive function at baseline and at least one follow-up. Results showed that participants in the highest quartile of vitamin C intake had higher average 3MS baseline scores and experienced slower rates of cognitive decline in the seven-year span than those in the lowest quartiles of vitamin C intake. Similarly, participants in the highest quartile of vitamin E intake had slower rate of cognitive decline compared to those of lower quartiles of vitamin E. Investigators also found that participants with vitamin C and E intakes in the top 25% of the distribution intake scored an average 0.92 points higher on the MMSE than those in the lowest 25% of the distribution intake of vitamins C and E ($p = 0.05$). The Cache County study results suggest consuming higher quantities vitamins C and E may maintain cognition status and/or slow the rate of cognitive decline for older adults.

Vitamin E

Morris et al. aimed to find associations between vitamin E intake and reduced age-related cognitive decline in a longitudinal population-based study (Morris et al., 2002). Investigators recruited 2,889 community residents (62% female) aged 65 to 102 years to complete a modified Harvard self-administered FFQ that measured intake for the past year (Rockett et al., 1995) and four cognitive tests (EBMT, SDMT, MMSE, and a measure of perceptual speed) at baseline and follow-up. The average length between baseline and follow-up was 3.2 years (range: 1.8-5.9 years). Morris et al. found less cognitive decline in participants in the highest quintiles of vitamin E from foods or supplements. A linear inverse association was found between vitamin E intake from food sources and cognitive decline in age-adjusted analyses. The rate of cognitive decline for participants in the highest vitamin E quintile was lower by 36% compared with that of participants in the lowest quintile, which was statistically significant ($p = 0.05$). Additionally, participants that consumed vitamin E supplements had significantly less cognitive decline than participants without vitamin E supplementation who had low food intake of vitamin E ($p = 0.04$). These findings suggest that vitamin E may play a key role in mitigating cognitive decline as persons age, and supplemental vitamin E may provide cognitive benefits for those with a low vitamin E intake from food sources.

Selenium

Ferdous and colleagues conducted a secondary analysis of data from the NHANES 2011-2014 to evaluate the association between Se and cognition (Ferdous et al., 2023). Cognitive function was determined by the CERAD neuropsychological battery (Morris et al., 1989). To assess Se intake from food and supplement sources, an average intake of Se was calculated from two non-consecutive 24-hour diet recalls. Participants (n =1681; 53.4% female; aged 65 years or

older) were categorized into groups, adequate vs. inadequate Se intake, based on Se's Estimated Average Requirement (EAR) (45 mcg/day). Participants with adequate Se intake scored significantly higher on the CERAD (25.1 points, standard error [SE] =0.34) compared to those with inadequate intake (22.6 points, SE=0.78; $p<0.01$). However, those with inadequate Se intake were also found to have significantly lower average energy intake (851 calories, SE=25.3) than those with adequate Se intake (1,899 calories, $p<0.001$). Upon further investigation, CERAD scores were not significantly different when adjusted for energy intake which suggests energy intake was a better predictor for CERAD scores than Se. When interpreting these results, we must consider the possibility of underreporting/underestimating food intake or the presence of malnutrition as they may have influenced the results given the very low average calorie intake of the inadequate Se group (i.e., 851 calories).

Manganese

Zhao et al. extracted data from the Health and Retirement Study 2013/2014 to examine the relation of dietary trace minerals (iron, copper, zinc, and Mn) with cognitive function (Zhao et al., 2023). Data from 6,863 participants (59.3% women) with a mean age of 66.7 ± 10.5 years were used. Dietary intake of said trace minerals was calculated from a semi-quantitative FFQ, and a 27-modified TICS assessed cognitive function. Linear regression models calculated the mean differences in global cognitive function scores by quintiles of intake levels of iron, copper, zinc, and Mn. Results found that higher Mn intake was associated with better cognitive scores ($P = 0.001$) when adjusted for age, sex, race, but were not significant in the fully adjusted models ($P = 0.368$). Overall, insignificant associations were found between Mn and cognitive function in this study.

Summary

The rate of which the older population is increasing in the U.S. is parallel to that of the dementia prevalence, thereby creating a need for preventative strategies against neurodegeneration. It has been suggested that oxidative stress-induced cognitive decline could be mitigated by antioxidant consumption. Much of the evidence suggesting a potential for cognitive benefits associated with the consumption of antioxidants hails from observational studies. There is not sufficient evidence to deduce any individual antioxidant effective against cognitive deterioration overall or on a specific cognitive domain, and investigators in the field call for ongoing action to be taken on this topic.

CHAPTER 3. METHODOLOGY

Study Design

The purpose of this cross-sectional study was to investigate the association of antioxidant consumption, from food and supplement sources, and cognitive function in older adults. A protocol amendment request was approved by North Dakota State University Institutional Review Board (IRB) on June 19, 2023; see appendix A.

Participants

Participants were recruited via email invitation from the pool of individuals that completed participation in the GRip Assessment for Protocol Enhancements (GRAPE) in Older Adults study. Inclusion criteria consisted of the following.

- Aged at least 65-years
- Not undergoing current cancer treatment
- Have not been diagnosed with severe dementia by a healthcare provider
- Able to engage in physical activity
- Are ambulatory

Participants were excluded if they failed to complete a full three-day food log on Cronometer (or in an alternative way agreed upon by co-investigators) or did not complete a second visit.

According to the power analysis for ANOVA, a sample size of $n_1 = 17$ participants that met the RDAs for vitamin C, vitamin E, selenium, and manganese, and $n_2 = 17$ participants that did not will ensure that the test of hypothesis has 80% power to detect a 1-unit difference in mean cognitive function (SLUMS) scores (Sullivan, 2017).

Data Collection

All collected data were saved as a hard copy (see appendix B), on a OneDrive folder, and on an external flash drive. The Excel sheet collected an email address, date of birth, demographical data (age, sex, race), anthropometric data (height, body mass, body mass index [BMI]), cognition scores (SLUMS), and Cronometer's nutrition analysis of three-day food records. Cronometer's nutrition analysis data were exported through the administrative Cronometer account. Nutrition analysis included total carotenoids, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene, vitamins C and E, selenium, and manganese from food, beverage, and supplement sources. Each day of dietary data had its own sheet within the Excel document. Email address, demographic, and cognition data were manually extracted from the GRAPE in Older Adults study's hard copy collection; this use of secondary data was approved by that study's principal investigator on May 4th, 2023; see Letter of Support in appendix C.

Tools

Health Status Survey

See appendix D for the self-report questionnaire used to collect demographic characteristics. Demographic data were extracted from GRAPE in Older Adult study's data collection to be used in this study.

Seca 286 Measuring Station

Standing height and body mass were measured to the nearest 0.1 centimeter and the nearest 0.1 kilogram, respectively. BMI was also collected to the nearest 0.1 kg/m^2.

Saint Louis University Mental Status Examination

The Saint Louis University Mental Status (SLUMS) Examination was utilized to measure cognitive function from participants (Tariq et al., 2006). The SLUMS is a 30-point, 11-question assessment tool to identify mild cognitive impairment and dementia. Each question examines a specific cognitive domain(s):

Question 1-3: Attention, immediate recall, and orientation

Question 4 and 7: Delayed recall with interference

Question 5: Numeric calculation and registration

Question 6: Memory (immediate recall with inference in a time constraint)

Question 8: Registration and digit span

Question 9: Visual spatial

Question 10: Visual spatial and executive function

Question 11: Executive function and extrapolation.

Scoring for an individual with a high school education or greater is as follows: 27-30 normal cognition; 21-26 mild neurocognitive disorder; 1-20 dementia. Scoring for an individual with less than a high school education is as follows: 25-30 normal cognition; 20-24 mild neurocognitive disorder; 1-19 dementia. See appendices E and F.

Cronometer

Cronometer is a comprehensive nutrition tracking application that was used to collect dietary intake data from participants digitally and remotely (Cronometer, 2023). The Cronometer application can be downloaded on a mobile device or accessed through the website on a desktop computer. Cronometer has four different versions: Cronometer Basic, Cronometer Gold, Cronometer Pro, and Cronometer Pro Plus. Cronometer Basic is free to the public, and users can

track their exercise and the macronutrient content (carbohydrate, protein, fat) of the food they record on the app. The public can upgrade to Cronometer Gold, which requires a monthly/yearly fee, to gain access to additional features such as a recipe importer, nutrition score, fasting timer, the micronutrient content of their intake, and more. Cronometer Pro is a version exclusively used by healthcare professionals, nutritionists, dietitians, universities, and research teams, and requires a monthly/yearly fee. Through this version, professionals can access their clients'/participants' nutrition records from which the application will calculate with extensive detail the amount of total energy (kcal), grams of fat, fiber, carbohydrates, and protein, percentage of total calories from carbohydrates, fats, and proteins (macronutrient distribution), total carotenoids, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene, retinol, total carotenoids, vitamins A, C, D, E (beta-tocopherol, alpha-tocopherol, delta-tocopherol, gamma-tocopherol), selenium, and manganese. Cronometer Pro Plus is similar, and requires a monthly/yearly fee, however, it is used by larger institutions like hospitals or healthcare professionals that require HIPAA, staff training, and application programming interface (API) access. Cronometer Pro was the most appropriate version for this study. Approval for the use of Cronometer software was received on April 24, 2023; see appendix G.

Participants were asked to visit the Cronometer website on a computer or download the Cronometer application on a mobile device and sign-up for a free account with their primary email address. A professional Cronometer profile was created from which investigators invited participants via email to join Cronometer Pro under this study's paid subscription. This allowed investigators to gain access to participant nutrition records.

Additional Resources for Participants

Participants were given brief education on food portion sizes from a trained researcher to promote the likelihood of accurate dietary records across the cohort. The "Portion Sizes Guide" from University of Minnesota Extension was explained and given to the participant for reference (University of Minnesota Extension, 2019); see appendix H. Post-visit, participants were emailed a link to a pre-recorded YouTube tutorial video that was created by Cronometer to reinforce the information they received.

Procedures

Email invitations to participate in this study were sent to potential participants; see appendix I. Two visits were required to complete data collection.

During visit one, participants were given a consent form to inform them of the purpose and procedures of this study; see appendix J. Height, weight, and BMI were obtained using the Seca 286 Measuring Station. The Cronometer application was downloaded onto participant's mobile device (or accessed on a laptop computer) where they registered for an account on which they were asked to track three days of food intake, two weekdays and one weekend day of eating to reflect usual eating behavior. (If a participant wished not to use Cronometer, they were asked to track their intake for three days by writing it on paper. In this alternative case, researchers created an internal Cronometer profile for the participant and manually entered the participant's intake data for analysis.) Verbal and written instructions for utilizing Cronometer and a brief education on food portion sizes using the "Portion Size Guide" from University of Minnesota Extension (University of Minnesota Extension, 2019) were provided by researchers. A second visit was scheduled with each participant before the end of visit one. A post-visit email, that

included important reminders, contact information of researchers, and additional resources, was sent to each participant; see appendix K.

During the second visit, outlier or unusual data within food logs were discussed with participants, if applicable, to ensure integrity of data. Participants that chose to record their intake on paper were asked to bring their hardcopy to the second visit where it was reviewed and discussed with a researcher. Once nutrition data were verified, compensation was provided ($25 in cash).

Data Analysis

SAS 9.4 (SAS Institute, Cary, NC, USA) was used for statistical analysis. Two-sample t-tests were employed to assess differences in mean cognition scores for those with adequate or inadequate intake of the antioxidants, vitamin C, vitamin E, selenium, and manganese based on the Recommended Daily Allowances (RDA) (carotenoids were not included in this test as they do not have intake recommendations). The fisher's exact test evaluated proportional differences within cognition categories between those meeting or not meeting RDAs. Pearson correlation coefficients were calculated for all nutrition variables to identify potential linear associations with cognition scores. An alpha level of <0.05 was used to indicate statistical significance.

CHAPTER 4. MANUSCRIPT

Abstract

Background

Oxidative stress, the imbalance of prooxidants and antioxidants, has been recognized as a possible risk factor for cognitive impairment. We postulate that increased dietary antioxidant consumption could help preserve cognitive function during aging.

Methods

Dietary intake and cognitive function of 50 adults aged ≥65-years (66% women; aged: 71.5±5.1 years) were examined. Three-day food logs, which encompassed intake of all food, beverages, and supplements, were collected from each participant using Cronometer, an electronic nutrition tracking and analysis application. The Saint Louis University Mental Status examination assessed cognition using a 30-point scale to categorize participants with normal cognition, mild neurocognitive disorder, or dementia. Cognitive dysfunction was defined as having a score below the "normal" cognition threshold (<27 points with a high school education or <25 points with less than a high school education).

Results

The fisher's exact test ($p < 0.01$) identified a significantly lower percentage of cognitive dysfunction (31.6%; 6 of 19 participants) among participants that met the Recommended Daily Allowances (RDAs) for the antioxidants, vitamin C, vitamin E, selenium, and manganese, and a higher percentage of cognitive dysfunction among those that did not meet the antioxidant RDAs (74.2%; 23 of 31 participants). However, mean cognitive scores differed insignificantly between those meeting RDAs (26.2 ± 4.2 points) and those that did not (24.9 ± 2.9 points; $p = 0.07$).

Conclusion

We found little evidence to support the hypothesis that older adults meeting the RDAs for antioxidants, specifically vitamin C, vitamin E, selenium, and manganese, would have higher cognition scores than those that do not; however, a higher proportion of cognitive dysfunction was found among those that did not meet antioxidant RDAs compared to those that did.

Introduction

Older adults (65+ years) account for about 17% of the current U.S. population (United States Census Bureau, 2021). This population is rapidly growing and projected to consist of 86 million people by 2050, and account for 22% of the national population (United States Census Bureau, 2021). Parallelly, the prevalence of age-related conditions and diseases, such as Alzheimer's disease (AD), are increasing as they are common in this population. It is projected that more than 9 million people in the U.S. could have dementia by 2030 (Population Reference Bureau, 2021). Severe deterioration of cognition can lead to a reduction of physical functioning, decreased independence, decline in the quality of life for the individual, and increased socioeconomical burden on society (Gray et al., 2021). There is currently no effective cure for dementia; therefore, studies investigating prevention strategies are of great importance. Literature suggests that diet is a modifiable factor that may reduce the age-related incidence of dementia (Eratne et al., 2018). Ascertaining the relationship between dietary habits, such as antioxidant consumption, and cognitive function in older adults is the purpose of this study.

There appears to be an agreement in the literature that oxidative stress plays a role in the pathogenesis of neurological decline (Cheignon et al., 2018; Eratne et al., 2018; Pisoschi et al., 2021; Wang et al., 2014). Evidence suggests oxidative stress may be a contributing factor to conditions of neurodegeneration due to an overabundance of free radicals and reactive oxygen

species (ROS) in the body (Eratne et al., 2018). Antioxidants are compounds that provide protection against free radicals and ROS that, if not neutralized, may cause inflammation or damage in the body (Pisoschi et al., 2021). Seemingly, there is a potential benefit from adding food sources of antioxidants to the diet to help mitigate further cognitive decline due to oxidative stress.

Available research regarding the effects of supplemental antioxidants on cognitive function are varied (Crosta et al., 2021; Devore et al., 2013; Grodstein et al., 2007; Kang et al., 2006; Kryscio et al., 2017; Naeini et al., 2014; Power et al., 2022). Available research examining the association of antioxidant consumption from food sources and cognitive status also varied, however, many studies cite finding a slower rate of decline in those with high and frequent intake of antioxidants from food over time. Still, researchers in this field call for additional studies due to a lack of sufficient evidence to assert a firm conclusion based on specific antioxidants (Devore et al., 2013; Engelhart et al., 2002; M. C. Morris et al., 2002; Naeini et al., 2014). A continued pursuit for additional evidence to conclude cognitive benefits from food- and supplement-based antioxidant consumption is warranted.

Methods

This study was approved by North Dakota State University Institutional Review Board. Participants *(n*= 50, 66% female; aged ≥65 years) were recruited from the pool of those that had partaken in the GRip Assessment for Protocol Enhancements (GRAPE) in Older Adults study. Data collection took place from July 2023 through October 2023.

Two visits were required to complete data collection. During visit 1, participants signed informed consent which permitted researchers to obtain cognitive scores via the St. Louis University Mental Status (SLUMS) examination and demographical data (age, birthday, race,

ethnicity, and education level) from GRAPE in Older Adults study's data collection. SLUMS scores ranged from 0-30 points, which is adjusted for completed education level. For an individual with a high school education or greater, 27-30 points represented normal cognition, 21-26 points mild neurocognitive disorder, and 1-20 points dementia. For an individual with less than a high school education, 25-30 points represented normal cognition, 20-24 points mild neurocognitive disorder, and 1-19 points dementia. Participants were then invited to download the nutrition-tracking application Cronometer (Cronometer Pro, Cronometer, Revelstoke, BC, Canada) onto their smartphone and sign up for a free account. A thorough tutorial for the application was administered by trained personnel. A detailed food log was kept for three days (two weekdays and one weekend day) to assess habitual eating habits. If a participant wished against using Cronometer due to technological skill level or perception of high burden, the participant was given the option to log intake with paper and pen. A food portion size education was administered by either a Registered Dietitian Nutritionist or a Nutrition Science student using a portion size guide created by Minnesota State University Extension (University of Minnesota Extension, 2019). This increased the quality of data as it attenuated personal bias and estimation errors across the cohort. Height (centimeters), weight (kilograms), and body mass index (kilograms/meters^2) were recorded using the Seca 286 Measuring Station (Seca, Chino, CA). Visit 2 was roughly 1-2 weeks later. Researchers reviewed each day of the participant's food diary and asked relevant questions regarding portion sizes, forgotten foods, brand names, etc. to ensure its accuracy. From the 3-day food logs, nutrient averages for vitamin C, vitamin E, selenium (Se), and manganese (Me) were computed and used for analysis.

Data Analysis

SAS 9.4 (SAS Institute, Cary, NC, USA) was used for statistical analysis. Two-sample t-tests were employed to assess differences in mean cognition scores for those with adequate or inadequate intake of the antioxidants, vitamin C, vitamin E, selenium, and manganese based on the Recommended Daily Allowances (RDA) (carotenoids were not included in this test as they do not have intake recommendations). The fisher's exact test evaluated proportional differences within cognition categories between those meeting vs not meeting RDAs. Pearson correlation coefficients were calculated for all nutrition variables to identify potential linear associations with cognition scores. An alpha level of <0.05 was used to indicate statistical significance.

Results

Characteristics of Participants

Characteristics of the sample (*n*=50) are presented in Table 1. Participants were aged 71.5 ± 5.1 years, with a majority being female (*n*=33, 66%), all identifying as white persons, and 49 reporting an ethnicity of non-Hispanic (missing ethnicity data for *n*=1). In this study, cognitive dysfunction is defined as a SLUMS classification of mild neurocognitive disorder or dementia. Cognition status via the SLUMS classified 42% (*n*=21) of the cohort with normal cognition, 50% (*n*=25) with mild neurocognitive disorder, and 8% (*n*=4) with possible dementia. Education level varied across the cohort; 8% (*n*=4) completed a high school diploma/GED or equivalent, 26% (*n*=13) completed some college or vocational training, 6% (*n*=3) completed an associate degree, 30% (*n*=15) completed a bachelor's degree, and 30% (*n*=15) completed a graduate degree.

Table 1

Characteristics of Participants

Characteristics	
Gender, *n* (%)	
Male	17 (34)
Female	33 (66)
Age (years), mean±SD	71.5±5.1
BMI (kg/m^2), mean±SD	30.4±6.5
Race, *n* (%)	
White	50 (100)
Ethnicity, *n* (%)	
Not Hispanic or Latino	49 (98)
Missing data	1 (2)
Education Level, *n* (%)	
High school diploma/GED or equivalent	4 (8)
Some college	13 (26)
Associate degree	3 (6)
Bachelor's degree	15 (30)
Graduate degree	15 (30)
Cognition Status, *n* (%)	
Normal	21 (42)
Mild neurocognitive disorder	25 (50)
Dementia	4 (8)

Note. n = number of participants; SD = standard deviation

Cognition Scores and Antioxidant Intake

The average consumption of each nutrient was computed for each participant using their 3-day food log. Table 2 demonstrates the difference in SLUMS scores among participants that met the RDA (yes) for the following antioxidants or not (no): vitamin C (75 mg/day for women and 90 mg/day for men), vitamin E (15 mg/day), Se (55 mcg/day), Mn (1.8 mg/day for women and 2.3 mg/day for men), or all four nutrients. Total intake and SLUMS scores are displayed as mean ± standard deviation. Mean SLUMS score for those that met and did not meet the RDA for

all four antioxidants were 26.2 ± 4.2 points and 24.9 ± 2.9 points, respectively. Two-sample t-tests revealed no significant difference in mean SLUMS scores based on meeting or not meeting the RDA for vitamin C (p=0.747), vitamin E (p=0.249), Se (p=0.465), Mn (p=0.625), or all four antioxidants (p=0.186).

Table 2

Cognition Scores for Participants Meeting vs Not Meeting Recommended Daily Allowances (RDA) for Vitamin C, Vitamin E, Selenium, and Manganese

Antioxidants	Participants *n* (%)	Total Intake[1] mean±SD	SLUMS Score (points) mean±SD†
Vitamin C, mg/day			
Yes	32 (64)	349±363	25.5±4.03
No	18 (36)	44.1±21.3	25.2±2.18
Vitamin E, mg/day			
Yes	21 (42)	60.4±78.7	26.0±4.02
No	29 (58)	6.51±3.41	24.9±2.97
Selenium, mcg/day			
Yes	37 (74)	112±56.1	25.6±3.72
No	13 (26)	31.7±12.4	24.8±2.62
Manganese, mg/day			
Yes	29 (58)	4.99±2.2	25.6±4.05
No	21 (42)	1.20±0.510	25.1±2.49
All Four			
Yes	19 (38)		26.2±4.17
No	31 (62)		24.9±2.90

Note. n = number of participants; SD = standard deviation; mg = milligrams; mcg = micrograms; SLUMS = Saint Louis University Mental Status Examination; Yes = met RDA; No = did not meet RDA

[1]Total intake based on food and supplement sources combined

†Two-sample t-tests were conducted to assess differences in mean SLUMS scores

*$p < 0.05$

Table 3 depicts further categorization of participants with normal cognition, mild neurocognitive disorder, or dementia based on their SLUMS score. The fisher's exact test identified significant proportional differences within cognition categories between those meeting the RDAs vs. those not meeting the RDAs. Higher proportions of cognitive dysfunction (defined as a SLUMS score below the "normal" cognitive threshold; <27 points with a high school education or <25 points with less than a high school education) are observed in participants that did not meet RDAs. Of participants that met the RDAs of all four antioxidants, 13 classified with normal cognition, 4 with mild neurocognitive disorder, and 2 with dementia. In other words, 31.6% of participants (*n*=6) had cognitive dysfunction. Of those that did not meet the RDA of all four antioxidants, 8 adults were classified with normal cognition, 21 with mild neurocognitive disorder, and 2 with dementia, meaning 74.2% of participants (*n*=23) had cognitive dysfunction. There is a similar trend for each antioxidant individually.

Table 3

Categorized Cognition Scores Stratified by Adherence to Recommended Daily Allowance (RDA) for Specific Antioxidants

Antioxidants	Normal *n* (%)	Mild Neurocognitive Disorder *n* (%)	Dementia *n* (%)	Fisher's Exact p-value
Vitamin C				
Yes	18 (56.2)	10 (31.3)	4 (12.5)	0.001*
No	3 (16.7)	15 (83.3)	0 (0)	
Vitamin E				
Yes	13 (61.9)	6 (28.6)	2 (9.5)	0.024*
No	8 (27.6)	19 (65.5)	2 (6.9)	
Selenium				
Yes	19 (51.4)	14 (37.8)	4 (10.8)	0.018*
No	2 (15.4)	11 (84.6)	0 (0)	
Manganese				
Yes	17 (58.6)	8 (27.6)	4 (13.8)	0.001*
No	4 (19.0)	17 (81.0)	0 (0)	
All Four				
Yes	13 (68.4)	4 (21.1)	2 (10.5)	0.002*
No	8 (25.8)	21 (67.7)	2 (6.5)	

Note. n = number of participants; Yes = met RDA; No= did not meet RDA

*$p < 0.05$

Table 4 presents relationships between SLUMS scores and various nutrition variables via Pearson's correlations. Furthermore, nutrition variables were stratified by food sources (dietary), supplement sources (supplemental), and food and supplement sources combined (total). Age and SLUMS scores exhibited a weak negative correlation ($r = -0.289$, $p = 0.041$), indicating a cognitive score decline of 1 point for every 5 years (or about 0.2 points decrease per year). Of nutrition variables, supplemental selenium and SLUMS scores were positively correlated ($r = 0.306$, $p = 0.031$), and supplemental vitamin C trended towards a significance relationship with SLUMS scores ($r = 0.263$, $p = 0.065$). No other nutrition variable was associated with cognition.

Table 4

Pearson's Correlations for Various Variables with Cognition Scores

Antioxidants	Intake mean±SD	Correlation Coefficient (r)	p-value
Vitamin C, mg/day			
Total	239±325	0.245	0.086
Dietary	79.0±61.5	-0.041	0.776
Supplemental	161±312	0.263	0.065
Vitamin E, mg/day			
Total	29.1±57.1	0.224	0.118
Dietary	6.40±4.20	-0.109	0.453
Supplemental	22.8±56.6	0.233	0.103
Selenium, mcg/day			
Total	90.8±60.0	0.176	0.223
Dietary	74.5±59.5	0.063	0.663
Supplemental	16.4±22.2	0.306	0.031*
Manganese, mg/day			
Total	3.40±2.50	0.124	0.3903
Dietary	2.30±1.90	-0.035	0.808
Supplemental	1.10±1.70	0.226	0.115
All Carotenoids[1], mg/day	6.68±5.10	0.014	0.923
Alpha-carotene	0.836±1.12	-0.055	0.704
Beta-carotene	3.14±3.06	-0.097	0.501
Beta-cryptoxanthin	0.144±0.309	-0.196	0.172
Lutein & Zeaxanthin	2.59±4.70	-0.027	0.854
Lycopene	3.46±4.60	0.144	0.318

Note. SD = standard deviation; mg = milligrams; mcg = micrograms

[1]Includes alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein & zeaxanthin, lycopene combined

*$p < 0.05$

Supplement Intake

Additionally, cognitive scores were also evaluated based on whether participants took an antioxidant supplement (n=39) or not (n=11). Supplements were included if it contained vitamin C, vitamin E, Se, Mn, alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene, or lutein & zeaxanthin. Of participants that ingested at least one antioxidant supplement, 21 classified with normal cognition, 15 classified with mild neurocognitive disorder, and 3 classified with possible dementia. Of participants that did not consume any supplemental antioxidants, 10 were classified with mild neurocognitive disorder and 1 with dementia. Again, conflicting results were observed depending on whether the data were analyzed as continuous or categorical. A paired t-test concluded no difference in means from the supplement group vs no supplement group ($p = 0.111$), while the fisher's exact test concluded that there was a significant difference in proportions of cognitive dysfunction between these two groups ($p = 0.002$).

Discussion

In this research, we found little evidence to support that meeting the Recommended Daily Allowance for the antioxidants of vitamin C, vitamin E, Se, and Mn may help preserve cognitive function in older adults. Adults meeting the RDAs did not have significantly higher cognition scores than those that did not meet the RDAs when data were interpreted as continuous via two-sample t-test. However, when interpreting these results, two considerations should be made. First, this statistical test assumes that the data follows a normal distribution, however, cognition scores for those that met the RDA for individual antioxidants and/or all four were left-skewed, violating this assumption. Second, t-tests are sensitive to outliers. Two participants that met the RDAs for all four antioxidants had two of the lowest cognition scores (18 and 15 points). These data points may have decreased the mean cognition score and inflated the variance of data of

those meeting the RDAs, impeding statistical significance. To demonstrate this sensitivity, we conducted a post hoc analysis where we excluded the lowest cognition score (15 points) and repeated the two-sample t-test. Results indicated a significant difference in mean cognition scores between those that met the RDAs for all four antioxidants (26.8±3.26 points) and those that did not (24.9±2.90; $p = 0.0422$). Given that this participant's SLUMS score (15 points) was less than two standard deviations of the mean cognition score, and the accuracy of their food log is questionable due to their classification in the dementia category, this outlier should be excluded from the dataset. When cognition scores were assessed as categorical data, the fisher's exact test suggested that cognitive function is associated with antioxidant consumption, and people that do not meet RDAs for one or more of the specified antioxidants may be more likely to have cognitive dysfunction. The proportion of cognitive dysfunction was lower among those that met all four antioxidants' RDA compared to those that did not.

No antioxidant had a strong association with cognition regardless of source (food vs supplement) via Pearson's correlations, although supplemental selenium presented a weak, positive correlation with cognition scores. There was no evidence that cognition scores differed among participants taking at least one type of antioxidant supplement compared to those that did not take any antioxidant supplements.

Much of the literature that suggests a relationship with dietary antioxidants and cognitive function hails from observational studies. Morris et al. conducted a longitudinal study with 2,889 older adults aged 65 to 102 years old and found that vitamin E intake from food and supplements was associated with less cognition decline, which suggests neuroprotection properties; however, within the highest quintile for vitamin E intake the median was 11.7 IU/day, which is below the RDA (15 mg/22.4 IU) (Morris et al., 2002). The Cache County Study found evidence from 5,092

participants to suggest high intakes of vitamin C, vitamin E, and carotene may delay cognition decline in older adults (Wengreen et al., 2007). The Rotterdam Study, a prospective cohort study with 5,395 participants, found that those with higher intake of vitamin E from food (> 15.5 mg/day) were 43% less likely to develop dementia compared to those with the lowest intake of vitamin E (<10.5 mg/day) (Engelhart et al., 2002).

Additionally, few studies have found a relationship between cognition and antioxidant supplements. Ferdous et al. detected a significant relationship with Se intake and higher cognition scores (Ferdous et al., 2023). However, the participants with low intake of Se also had very low average calorie intake which may influence the association that was presented, therefore, the possibility of malnutrition impacting cognition must also be considered. Cardoso and colleagues found daily selenium supplementation through a Brazil nut improved verbal fluency and construction praxis over a 5-year period in their randomized controlled pilot trial (Cardoso et al., 2021). Conversely, in a cohort study where 200 micrograms of supplemental selenium were given daily (alone or in combination with 400 IU of vitamin E) over a 7-year period, no effect on incidence of dementia was found in 3,786 men aged 65 ± 5.2 years old (Kryscio et al., 2017). Grodstein et al. found significantly better cognitive scores, specifically in verbal memory, in men with long-term (mean treatment duration of 18 years) beta-carotene supplementation (50 mg, alternating days) when compared to the placebo group (Grodstein et al., 2007). Short-term beta-carotene supplementation did not demonstrate an impact on cognition in that study. It is generally agreed upon that cognitive decline may begin years before the appearance of signs or symptoms (Karr et al., 2018), therefore, it is plausible that, long-term, consistent intake of antioxidants starting in midlife or earlier may be required to acquire cognitive benefits.

Two double-blind, randomized control trials (RCTs) failed to produce any significant evidence to elucidate a relationship between antioxidants and cognitive scores (Kang et al., 2006; Naeini et al., 2014). Naeini et al. conducted a study where participants received the intervention (300 mg vitamin E [DI-alpha-tocopherol] plus 400 mg of vitamin C) or placebo daily for 1 year. Kang et al. gave generally healthy older women 600 IU [alpha-tocopherol acetate] every other day for a mean duration of 5.6 years which did not impact cognition. Both RCTs used doses of vitamins C and E that were much higher than the RDAs, which suggests mega doses of these vitamins may not be beneficial short-term or long-term.

Limitations of the current study should be considered when interpreting the results. First, participants were asked to log the food, beverages, and supplements they consumed along with their respective portion sizes on Cronometer. Although participants were given a brief education on portion sizes, estimated portions sizes may have varied across the cohort due to personal bias and food and nutrition knowledge deficits. Second, it is challenging to pinpoint one nutrient as a sole combative agent against cognitive decline due to the possibility of synergistic relations between various nutrients. Third, the small sample size *(*N=50) makes it challenging to produce results that are consistent with population-representative results, therefore, there is potential for a type 2 error occurring. Lastly, we did not control for cofounders that may have played a role in cognitive dysfunction such as genetics, diagnosis of an inflammatory disease, lack of physical activity, or history of inadequate nutrition.

In this research, we did not find strong evidence to conclude that antioxidants, from food or supplemental sources, could be used as a preventative strategy against neurodegeneration. Given the cross-sectional study design, we were unable to evaluate participants' individual dietary patterns from earlier life to determine if it had impacted their current cognition status.

Additionally, it should be noted that an older individual noticing signs of cognitive decline may be inclined to engage in health-bettering behaviors, such as eating a more healthful diet or consuming supplements, which had the potential to impact our study's findings. Future studies should investigate long-term intake of antioxidants starting as early as childhood to investigate the possible link of antioxidant intake and cognitive function in later life. Observational studies have shown encouraging evidence, but longitudinal, randomized control trials would better elucidate the neuroprotective properties of antioxidants.

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APPENDIX A. IRB APPROVAL

NDSU NORTH DAKOTA STATE UNIVERSITY

06/19/2023

Dr. Yeong S Rhee
Health, Nutrition & Exercise

IRB Approval of Protocol #IRB0004768, "Interrelations between antioxidant and fiber consumption, cognition, and pancreatic and lung cancer risk in older adults"

Co-investigator(s) and research team:

- Yeong S Rhee
- Kelly Raye Knoll
- Halli Heimbuch
- Ryan McGrath

Approval Date: 06/19/2023
Expiration Date: 06/18/2026
Research site(s): This study will take place in the Healthy Aging North Dakota (HAND) lab in Research Building 2 on the NDSU main campus.
Funding Agency:
Review Type: Expedited category # 4,5,7

The above referenced protocol has been reviewed in accordance with federal regulations (Code of Federal Regulations, Title 45, Part 46, *Protection of Human Subjects*).

Additional approval from the IRB is required:

- Prior to implementation of any changes to the protocol.
- For continuation of the project beyond the approval period. A task will automatically generate for the PI and Co-PI 8 weeks prior to the expiration date. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved for continuation prior to the expiration date.

Other institutional approvals:

- Research projects may be subject to further review and approval processes.

A report is required for:

- Any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence.
- Protocol Deviations
- Any significant new findings that may affect risks to participants.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

NDSU has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

RESEARCH INTEGRITY AND COMPLIANCE
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | ndsu.research@ndsu.edu
Shipping Address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

APPENDIX B. DATA COLLECTION SHEETS

HAND-DIET-NUTRITION DATA COLLECTION PACKET

Participant ID: _____

Participant email: _____

Participant phone number: _____

Investigator: _____

Visit 1 date: _____ Season: Summer / Fall

Scheduled date for visit 2: _____ Season: Summer / Fall

Anthropometrics

Height	*Nearest 0.1 cm*	
Weight	*Nearest 0.1 kg*	
BMI	kg/m^2	

Your Disease Risk

Pancreatic cancer risk	
Lung cancer risk	

Participant ID: _____

HAND-MAIN Extracted Data

Demographics	
Birth date (MM/DD/YYYY)	
Age	
Sex	
Race	
Ethnicity	
Level of Education	
SLUMS	
Q1	/1 point
Q2	/1 point
Q3	/1 point
Q5	/3 points
Q6	/3 points
Q7	/5 points
Q8	/2 points
Q9	/4 points
Q10	/2 points
Q11	/8 points
Overall score	/30 points
Score based on level of education	

APPENDIX C. LETTER OF SUPPORT

Healthy Aging NORTH DAKOTA

HEALTH, NUTRITION, AND EXERCISE SCIENCES

NDSU COLLEGE OF HUMAN SCIENCES AND EDUCATION

May 4, 2023

Dear North Dakota State University Institutional Review Board,

My name is Dr. Ryan McGrath and I am the PI of protocol IRB0004266 titled, *Grip Assessment for Protocol Enhancements (GRAPE) in Older Adults*. This note is to provide support for use of demographic and cognitive functioning data from IRB0004266 to IRB0004768 titled, *Interrelations between antioxidant and fiber consumption, cognition, and pancreatic and lung cancer risk in older adults*. Please contact me directly if you have questions.

Thanks,

Ryan McGrath, PhD
Assistant Professor
Department of Health, Nutrition, and Exercise Sciences
North Dakota State University

APPENDIX D. HEALTH STATUS SURVEY

PAR ID: _____

Completion Date: _____

Investigator Name: _____

Health Status Survey

Please complete the following questions as accurately as possible.

Descriptive Information

What is your age? _____

What gender do you identify with?

A. Male
B. Female

Please circle the ethnicity that you most identify with:

A. Hispanic or Latino
B. Not Hispanic or Latino

Please circle the race that you most identify with:

A. American Indian/Alaska Native
B. Asian
C. Native Hawaiian or Other Pacific Islander
D. Black or African American
E. White
F. More than One Race
G. Other, please detail: _____

Please circle marital status:

A. Single
B. Married
C. Widowed
D. Living with non-married partner
E. Other, please detail: _____

Please circle the highest level of education you have completed:

A. Some High School
B. High School Graduate/GED or Equivalent
C. Some College or Vocational Training
D. Completed Associate Degree
E. Completed Bachelor Degree
F. Completed Graduate Degree

What is your status of employment (select all that apply)?

A. Full-time
B. Part-time
C. Unemployed and Looking for Work
D. Retired
E. Volunteer
F. Unpaid At-Home Work

How many people are currently living in your household? _____

Which is your dominant hand? _____

Which is your dominant leg? _____

Please list medications you are taking and for what purpose.

APPENDIX E. SLUMS EXAMINATION

Healthy Aging NORTH DAKOTA HEALTH, NUTRITION, AND EXERCISE SCIENCES NDSU COLLEGE OF HUMAN SCIENCES AND EDUCATION

PAR ID: _____

Completion Date: _____

Investigator Name: _____

SLUMS Exam

1. **What day of the week is it?**

2. **What is the year?**

3. **What state are we in?**

4. **Please remember these five objectives. I will ask you what they are later.**
   Apple Pen Tie House Car

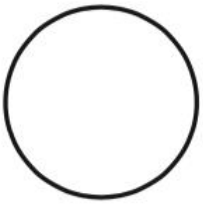
5. **You have $100 and you go to the store and buy a dozen apples for $3 and a tricycle for $20.**
   a. **How much did you spend?**
   b. **How much do you have left?**

6. **Please name as many animals as you can in one minute.**

7. **What were the five objects I asked you to remember?**

8. **I am going to give you a series of numbers and I would like you to give them to me backwards. For example, if I say 42, you will say 24.**
   a. 87 b. 648 c.8537

9. **This is a clock face. Please put in the hour markers and the time at ten minutes to eleven o'clock.**



10. **Please place an X in the triangle.**

a. **Which of the above figures is largest?**

11. **I am going to tell you a story. Please listen carefully because afterwards, I am going to ask you some questions about it.**

> Jill was a very successful stockbroker. She made a lot of money on the stock market. She then met jack, a devastatingly handsome man. She married him and had three children. They lived in Chicago. She then stopped work and stayed at home to bring up her children. When they were teenagers, she went back to work. She and Jack lived happily ever after.

a. **What was the female's name?**

b. **When did she go back to work?**

c. **What work did she do?**

d. **What state did she live in?**

APPENDIX F. SLUMS EXAMINATION SCORE SHEET

Healthy Aging NORTH DAKOTA HEALTH, NUTRITION, AND EXERCISE SCIENCES NDSU COLLEGE OF HUMAN SCIENCES AND EDUCATION

SCORE SHEET

__/1 **1. What day of the week is it? 1pt**

__/1 **2. What is the year? 1pt**

__/1 **3. What state are we in? 1pt**

4. Please remember these five objectives. I will ask you what they are later.

a. Apple Pen Tie House Car

__/3 **5. You have $100 and you go to the store and buy a dozen apples for $3 and a tricycle for $20.**

a. How much did you spend**? 1pt**

b. How much do you have left? **2pts**

__/3 **6. Please name as many animals as you can in one minute.**

a. 0-4 animals. **0pts** **b**. 5-9 animals. **1pt** **c**. 10-14 animals. **2pts**

d. 15+ animals. **3pts**

__/5 **7. What were the five objects I asked you to remember? 1pt for each one correct.**

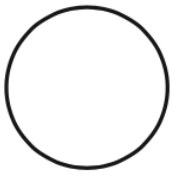
8. I am going to give you a series of numbers and I would like you to give them to me backwards. For example, if I say 42, you will say 24.

__/2 a. 87. **0pts** b. 648. **1pt** c.8537. **1pt**

9. This is a clock face. Please put in the hour markers and the time at ten minutes to eleven o'clock.

__/4 Hour markers okay. **2pts**

Time correct. **2pts**



__/2 **10. Please place an X in the triangle. 1pt**

a. Which of the above figures is largest? **1pt**

11. I am going to tell you a story. Please listen carefully because afterwards, I/m going to ask you some questions about it.

APPENDIX G. CRONOMETER APPROVAL

Cronometer Vendor Evaluation

Requested By: Kelly Knoll
Requested Date: 02-06-2023
Send to Purchasing: No
Reviewed By: Jeff Gimbel
Review Date:04-24-2023
Cost: $540
Summary:

- To collect nutrition data (dietary intake record) for a Master's thesis pilot project within the Healthy Aging North Dakota study in the HNES department of NDSU.

Data Requested:
- None

Data Generated:

- Daily Log of Nutrition / Food Intake

Data Stored:
- Name of Participants
- Daily Log of Nutrition / Food Intake

Data Classification:
- Private

Number of Records Stored:
- 100

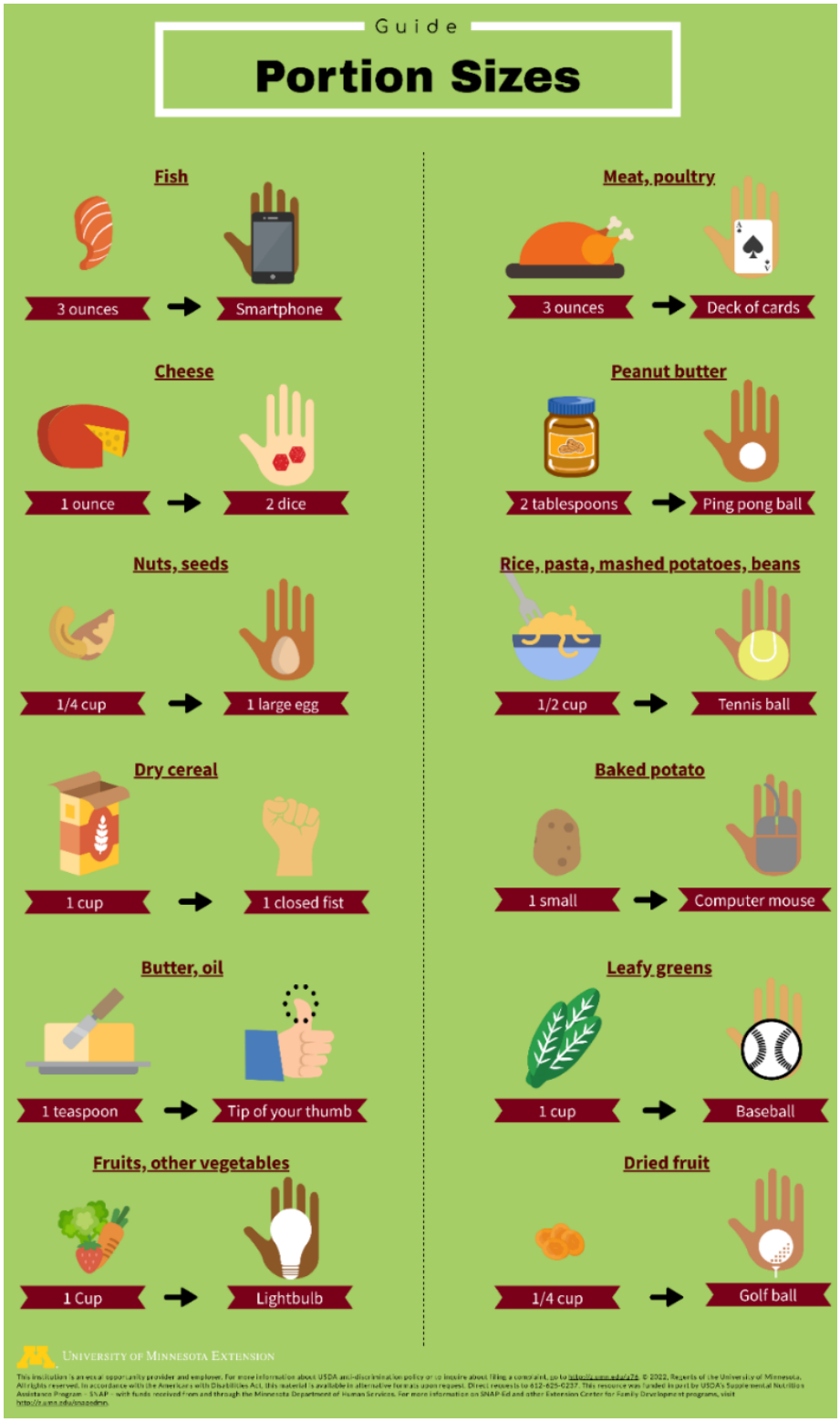
Notes:

-

Approved:

- Approved - JLG - 04-24-2023

APPENDIX H. PORTION SIZE GUIDE



Note: (University of Minnesota Extension, 2019)

APPENDIX I. EMAIL INVITATION TEMPLATE

Subject: HAND Nutrition Study Invitation

Message:

Hello [NAME],

Because you participated in the GRip Assessment for Protocol Enhancements (GRAPE) in Older Adults study, you are eligible and invited to participate in a sub-study focused on nutrition.

For this study, you will be asked to visit our lab at the NDSU main campus for two visits. The first visit will take about 45-60 minutes. You will be asked to:

- Complete two brief online surveys about cancer risk
- Access and/or download Cronometer, a nutrition tracking application on a mobile device or computer
- Register for a Cronometer account at no cost
- Complete a three-day food log using Cronometer, remotely

During the second visit, your completed food log will be reviewed, and compensation will be provided. This visit will take about 15 minutes. You will be given $25 for completing the study.

If you are interested, please suggest two days and times that will work for you, and we will be in touch. Please let us know if you have any questions.

Thank you,

Kelly Knoll and Halli Heimbuch

APPENDIX J. PARTICIPANT CONSENT FORM

NDSU NORTH DAKOTA STATE UNIVERSITY
Department of Health, Nutrition, and Exercise Sciences
NDSU Dept 2620
1301 Centennial Blvd
Fargo, ND 58102
(701) 231-7474

HAND Nutrition Study

This study is being conducted by:
Yeong Rhee, PhD, RD, advisor/principal investigator, yeong.rhee@ndsu.edu
Ryan McGrath, PhD, principal investigator, ryan.mcgrath@ndsu.edu
Kelly Knoll, co-investigator, kelly.knoll@ndsu.edu
Halli Heimbuch, co-investigator, halli.heimbuch@ndsu.edu

Key Information About This Study

This consent form is designed to inform you about the study you are being asked to participate in. Here you will find a brief summary about the study; however, you can find more detailed information later on in the form.

You will be asked to

- Allow the collection of your height, weight, and BMI measurements.
- Complete two brief online cancer risk surveys on pancreatic cancer and lung cancer from Your Disease Risk
- Register for and download Cronometer, a free nutrition tracking app
- Complete a three-day food log using Cronometer

You may be eligible if you are

- At least 65 years of age and generally in good health
- Not living with severe dementia
- Not receiving treatment for cancer (except minor skin cancer)
- Not living with a health condition such as stroke, multiple sclerosis or Parkinson's disease
- Healthy to take part in physical activity
- Able to walk
- Able to extend or curl your legs at the knee
- Able to squeeze a device on both hands without pain or limitations
- Completed the GRip Assessment for Protocol Enhancements (GRAPE) in Older Adults study (birth date, age, sex, race and ethnicity, level of education, Health Status Survey, and SLUMS scores must have been recorded)

Possible risks and/or discomforts:

- Increased awareness of your height, weight, and BMI
- Increased awareness of the food, drinks, and supplements you are consuming and their nutritional content
- Time spent completing a detailed three-day food log remotely

Benefits:

- Access to CronometerPro, a nutrition-tracking app
- For society, this study may give insight for future studies and interventions focusing on nutrition, cognition, and cancer.

Time commitment:

- Total: 90 to 105 minutes
- Two in-person visits
    - Visit 1: 45-60 minutes
    - Visit 2: 15 minutes
- Completing three-day food log using Cronometer, remotely (10 minutes each day)

Compensation:

- $25 cash after completing the two cancer risk surveys and the three-day food log

Privacy concerns:

- Your birth date, age, sex, race and ethnicity, level of education, and SLUMS scores will be collected from the GRip Assessment for Protocol Enhancements (GRAPE) in Older Adults study. By signing this consent form, you are granting permission to researchers of the HAND Nutrition study to collect these data points.
    - SLUMS scores indicate brain health and function.
- Your birth date, email address, and phone number will be stored and used for setting up your Cronometer account and communication purposes.
-

Why am I being asked to take part in this study?
We are looking into the relationships between antioxidant and fiber intake, cognition, and pancreatic and lung cancer risk in older adults.

What will I be asked to do?
First, you will be asked to come to the HAND lab. During this visit, you will be asked to stand on an electronic scale to measure your height, weight, and BMI. You will then be asked to complete two online surveys to estimate your risk for pancreatic cancer and for lung cancer based on your lifestyle history.

Second, you will be asked to download a nutrition-tracking app called Cronometer on your computer or mobile device.

You will be asked to record three days of food, drink, supplement, and medication intake using Cronometer within two weeks following your first in-person visit. Acute illness and chronic health diagnoses should also be reported each food log day.

Third, investigators will briefly discuss general portion sizes and provide you with a portion sizes guide handout. This will help you accurately record the amount of food, drinks, and supplements you consume.

Finally, you will be asked to return to the HAND lab for a second visit within the next two weeks to discuss completed food logs and receive compensation.

Where is the study going to take place, and how long will it take?
The total estimated time for participating in this study is 90-105 minutes. This study will include two in-person visits to our HAND lab in the Research 2 building on the NDSU main campus and a remote portion. The first in-person visit will take approximately 45-60 minutes. For the remote portion, plan for about 10 minutes for each day (three days) to record what you consumed that day. The second in-person visit will take approximately 15 minutes.


What are the risks and discomforts?
Some discomforts may include an increased awareness of your height, weight, and BMI and increased awareness of what you consume, and its nutritional value and the time spent recording all food, drinks, and supplements you consume for three days. It is not possible to know all potential risks in research; however, reasonable safeguards have been taken to lessen known risks. If new risks are found during the study, which may change your willingness to participate, we will tell you about these findings.


What are the expected benefits of this research?
Individual Benefits: You will get access to your estimated risks for pancreatic cancer and lung cancer. You will also get access to CronometerPro, a nutrition-tracking app, that lets you record and analyze your nutrient intake.
Societal Benefits: This study may guide future studies on nutrition, brain health, and cancer. Additionally, it may give useful insight for approaches to prevent and treat cognitive impairment and various cancers.

Do I have to take part in this study?
Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

Will it cost me anything to participate?
There will be no financial costs to participate.

What are the alternatives to being in this study?
Instead of being in this study, you may choose not to participate.

Who will have access to my information?
Some personal information will be collected during this study; this includes your birth date and email address and phone number. Your birth date will be stored and used for setting up your Cronometer account. Your email address and phone number will be stored and used for accessing your Cronometer account and for correspondence.
Your information will only be accessible to Dr. Yeong Rhee (advisor/principal investigator of this study), Dr. Ryan McGrath (co-investigator of this study and principal investigator of the main HAND study), Kelly Knoll (co-investigator), and Halli Heimbuch (co-investigator). All data will be digitally stored in a secure OneDrive folder, only accessible to the individuals listed above. Another digital form of data will be transferred and saved on a flash drive, which will be stored in a locked compartment in the HAND lab in the Research 2 building. An additional hard copy of data will be stored in the same secured compartment. Your personal information will not be shared at any time during the study with anyone outside of the researchers listed above. Identifiable information will be removed from research results. Research results may be shared to the public after the completion of the study through papers, posters, and oral presentations.

How will my information be used?
Your information will be used to set up your Cronometer profile; to process data; and for correspondence.
If you fail to complete a three-day food log within 2 weeks, your data will be removed from the study and be excluded from our data analysis. We will share information with you that may be helpful for your health in the future, including your estimated risk of pancreatic and lung cancer and information about your nutrition intake. You can decide whether you want to receive your study information.

Can my participation in the study end early?
You can stop your participation in this study at any time.

Will I receive any compensation for participating in the study?
Upon completion of all study activities, you will receive $25 in the form of cash on the second visit.

What happens if I am injured because of the study?
If you are injured during the course of this study, you should contact Dr. Yeong Rhee (principal investigator of this study) at (701) 231-7476 or Dr. Ryan McGrath (co-investigator of this study and principal investigator of the main HAND study) at (701) 231-6043. Treatment for the injury will be available including first aid, emergency treatment, and follow-up care as needed. Payment for this treatment must be provided by you and your third-party payer (such as health insurance or Medicaid). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

What if I have questions?
Before you decide whether you'd like to take part in this study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact Dr. Yeong Rhee at (701) 231-7476 or yeong.rhee@ndsu.edu; Kelly Knoll at kelly.knoll@ndsu.edu; or Halli Heimbuch at halli.heimbuch@ndsu.edu.

What are my rights as a research participant?
You have rights as a research participant. All research with human participants is reviewed by a committee called the *Institutional Review Board (IRB)* which works to protect your rights and welfare. If you have questions about your rights, an unresolved question, a concern or complaint about this research you may contact the IRB office at (701) 231-8995, toll-free at (855) 800-6717 or via email (ndsu.irb@ndsu.edu).

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form,
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

Your signature Date

Your printed name

Signature of researcher explaining study Date

Printed name of researcher explaining study

APPENDIX K. EMAIL POST-VISIT TEMPLATE

Subject: HAND Nutrition Study

Message:

Hello [NAME],

Thank you for participating in the nutrition sub-study of Healthy Aging North Dakota!

On Cronometer, please log 3 complete days of eating by [**date**]. Please include all food, beverages, and supplements that you consume and their respective portion sizes. Click here for a tutorial video on how to log food on the Cronometer app. Also, attached to this email is the Portion Sizes handout.

Make sure to "**mark your day as complete**" once you are finished logging food each day.

You have been scheduled to return to the HAND lab on [**date**] at [**time**]. Please mark your calendar.

If you have any questions or concerns about Cronometer, please contact me, [researcher's name] at [email] or [phone number].

Sincerely,

Kelly Knoll and Halli Heimbuch