January 2020

The Effects Of Antioxidant Status, Omega-6 To Omega-3 Ratio, And Dietary Diversity On Cognitive Performance

Jonathan Santiago

The Effects of Antioxidant Status, Omega-6 to Omega-3 Ratio, and Dietary Diversity on Cognitive Performance

---

A Thesis

Presented to

The Faculty of the Department of Psychology

University of North Dakota

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

By

Jonathan Santiago

Thomas Petros

August, 2020

This thesis, submitted by jonathan.santiago in partial fulfillment of the requirements for the Degree of Master of Arts in Psychology from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

DocuSigned by:
Thomas Petros
C4A022288E24476...
Thomas Petros

DocuSigned by:
Richard Ferraro
1F0DCCE89ECF416...
Richard Ferraro

DocuSigned by:
2C877E791A994CE...
Joseph Miller

____
Name of Committee Member 3

____
Name of Committee Member 4

____
Name of Committee Member 5

This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

DocuSigned by:
Chris Nelson
13D0157109424B1...
Chris Nelson
Dean of the School of Graduate Studies

5/5/2020
Date

PERMISSION

Title	The Effects of Antioxidant Status, Omega-6 to Omega-3 Ratio, and Dietary Diversity on Cognitive Performance
Department	Clinical Psychology
Degree	Master of Arts

Jonathan Santiago
8/2/2020

Abstract

Past research on the relationship between nutrition and cognitive performance has suggested that dietary diversity decreased the risk of cognitive decline among Japanese older adults, that a lower ratio of Omega 6 to Omega 3s is linked to a better MoCa score, and that Vitamin E was positively associated with higher scores on a variety of cognitive tests. The main purpose of this study was to compare younger adults (18-35) and older adults (65+) on measures of nutritional intake and examine how nutritional impact influences performance on a neuropsychological battery. Participants were recruited from North Dakota. There were two sets of participants separated into groups based on age: one group was 58 people 18 to 31 years of age, recruited from UND. The other group was 21 people 60 to 85 years of age. Participants completed a 72-hour dietary record and both the WAIS Vocabulary Subtest and RBANS battery. Dietary diversity was positively related to cognitive performance on the RBANS Full Scale SS, the IMI, and DMI. an increase in Omega-3 FAs was associated with a decrease in RBANS Full Scale SS and Story Recall, while an increase in the Omega-6: Omega-3 ratio was associated with a decrease in the DMI. Vitamin E and Beta-Carotene were associated with better scores on the Attention Index and List Learning respectively, while more Vitamin C was associated with decreased performance on LL. Future research should use people from diverse backgrounds, a longitudinal design to account for lifestyle factors, and control the food consumed.

Nutritional Intake and Cognitive Performance

In a world where we are starting to better understand the relationships between our behavior and health, researchers have put increasing amounts of effort into studying the relationship between nutritional intake and cognitive performance. For the purposes of this study, cognitive performance will be defined as psychological functioning in the following domains: executive functioning, language, attention, and memory. This research interest in nutrition is in part because the average life expectancy around the world has mostly increased; one study suggests that by 2060, the elderly population (people 65 years and older) will be expected to grow from 17.4% to nearly 30% worldwide (Vazour et al., 2017). Despite this cultural shift in age, there is a paucity of research concerning the short-term effects of nutrition on cognitive performance and currently there are no dietary recommendations that are universally accepted to mitigate against age-related cognitive decline. One challenge in nutritional research is that randomized placebo-controlled trials (RCTs) are very difficult to conduct because the effects of some nutrients are supposed to build up over a long period of time and may be confounded by individual differences in cognitive aging (Vazour et al., 2017). One way nutritional research is conducted in longitudinal paradigms where a variety of independent variables including nutritional intake, socioeconomic status, past medical history, and others are analyzed together and inferences about the effects of specific macro/micronutrients, types of food, and diversity of food intake on cognitive performance are made.

Oxidative Stress and Antioxidants

A number of authors have attributed health declines with age to the deleterious effects of oxidative stress. Marrocco, Altieri, & Peluso (2017) define oxidative stress as the imbalance in the redox characteristics in some cellular environment. Redox characteristics refer to an equilibrium or an electrical homeostasis, which is essential in ensuring important cellular functionality (Marrocco, Altieri, & Peluso, 2017). This imbalance can be the result of production of reactive species through biochemical processes (i.e. exercise and converting food into energy), exposure to damaging agents (i.e. radiations and environmental pollutants), or limited antioxidant system capabilities (National Center for Complementary and Integrative Health, 2016). Reactive oxygen and nitrogen species (ROS/RNS) produced under oxidative stress have been known to damage all cellular biomolecules (lipids, sugars, proteins, etc.). Oxidative stress has been studied in relation to diseases such as cancer, cardiovascular disease, Alzheimer's disease, Parkinson's disease, and diabetes (National Center for Complementary and Integrative Health, 2016). In order to mitigate uncontrolled ROS increase, human beings have developed natural defense systems. One of the primary defense system components produced are antioxidants, which are natural compounds that inhibit oxidation. Examples of antioxidants are nonenzymatic molecules like Vitamins A, C, and E, and several other molecules present in some foods such as selenium and carotenoids (beta-carotene, lycopene, etc.) (National Center for Complementary and Integrative Health, 2016). The role of antioxidant supplements in preventing chronic diseases has been extensively studied; however, most research was unable to link a reduction in chronic disease with antioxidant supplement status (National Center for Complementary and Integrative Health, 2016).

Methods of Conceptualizing and Measuring Nutritional Intake

Research on nutrition and health has yielded four main methods of measuring nutritional intake: diet history, diet recall (typically 24hrs, but can extend to a week or more), food record, and food frequency questionnaire (FFQ) (Shim, Oh, & Kim, 2014). While there is no standardized method for diet history, typically this method entails a highly trained interviewer (typically a nutritionist) conducting a 1-2 hour interview with the participant to discern food intake, typical meal patterns, and other salient information related to their diets. Usually diet history also involves two or more nutritional intake methods (24-hr diet recall, 3-day food record, or checklist of foods consumed) but the exact methodology can vary between studies (Shim, Oh, & Kim, 2014). Diet History is designed to be a measure of total dieting behavior (e.g. when someone eats and how often someone eats), but generally has been found to overestimate nutrient intakes compared with other nutritional intake methods (Peter et al. 2015). While the diet history method can provide detailed information on the type and frequency of foods eaten, the exact amounts of food intake can be compromised by poor recall ability or variations in the standard portion size for the individual. In terms of validity, the error in this method has not been calculated empirically due to difficulty in observing intakes and difficulty in validating the recall period (Péter et al. 2015). In terms of reliability, repeated dietary history has been demonstrated to be reproducible based on a 24-hr urinary nitrogen excretion (a test which can tell researchers how much protein the participant has been ingesting) compared to a 1-month diet history. Limitations to this method include high resource cost (a trained interviewer is required), high respondent burden, and lengthy administration time (1-2 hours) (Péter et al. 2015).

Diet recall is when an interviewer asks the respondent to recall every food and beverage item that they have consumed within a certain time period (usually 24-72 hours). Amounts are estimated with portion size measurement aids (for example, a picture of a 16oz serving of mashed potatoes) and food descriptions are also collected. While this method tends to underestimate energy intake (specifically, adult women and overweight adolescents tend to underreport intake) and is typically indicative only of a very select time frame (1-3 days on average) (Péter et al. 2015), it is an easy-to-administer, low cost, quick way to obtain information about a respondent's nutritional intake. In terms of validity, there is a standardized method of administering diet recalls that provides estimates of mean nutrient intakes for groups of people, but not individuals (Péter et al. 2015). There may also be difficulty in determining portion sizes due to faulty memory and if the diet recalls are self-report, participants may provide biased responses. In terms of reliability, the types of food and beverage that a person consumes generally changes on a fairly regular basis, so day-to-day variability must be considered (Péter et al. 2015).

A food record (also known as a food diary) is a record that the respondent keeps on their person that includes all foods, beverages, and their ingredients for a certain amount of time (typically 3, 7, or 14 days). These records can be added to non-consecutively over the course of a year to estimate a person's typical nutritional intake, but they tend to underestimate overall energy intake and when people eat out they tend to describe food less accurately than when they cook at home (Péter et al. 2015). In terms of validity, multiple days can be collected to get a more whole and accurate picture of a person's dietary intake, but the potential for respondent bias should be considered as well. Proper instruction and intermittent monitoring can increase validity; for example, having respondents weigh their portions can provide more accurate data.

A Food Frequency Questionnaire (FFQ) collects information about the frequency of food and beverage intake over a certain period of time (1 week-1 year) and is typically self-administered. More of a subjective instrument, the FFQ can be used to estimate nutrient content and can help to identify which foods are consumed often, not often, or not at all. This method tends to overestimate energy and some nutrients; estimates become less accurate when food is grouped together and generally preparation details and specific details about foods are unknown (Péter et al. 2015).

Macro and Micronutrients

Macronutrients are chemical compounds that provide humans with most of their energy and are absorbed primarily by consuming vegetables and animals. There are three main types of macronutrients: carbohydrates, proteins, and fats. Carbohydrates, also known as sugars, are categorized based on the number of molecules present: Monosaccharides (single sugars) form compounds like glucose (blood sugar) and fructose (fruit sugar), Disaccharides (double sugars) form compounds like sucrose (table sugar) and lactose (milk sugar), and Polysaccharides (or complex carbohydrates) form compounds like glycogen (what our muscles use for energy) and starch (grain sugar) (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 6, 2005). Carbohydrates function as the body's main short-term energy source and tends to be stored as fat when leftover energy remains.

If carbohydrates are the main energy source of the body, then proteins constitute the major structural components for all the cells in the body. One of the many functions of a protein is to act as enzymes, which catalyze biochemical processes and build up or break down other molecules. Proteins also allow different chemicals to be transported through our blood; they compromise our muscles and other physical features such as our hair and nails. Proteins are made up of amino acids, which can form a variety of three-dimensional shapes in order complete specific tasks for the body. Protein mainly comes from eating meat, nuts, legumes, and other sources like dairy and fungi (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 10, 2005).

Fats are another major source of energy for the body and aid in the absorption of fat-soluble vitamins and carotenoids. Because nutritional research has had inconclusive and conflicting results concerning the effects of consuming fats, there are no dietary guidelines for an Adequate Intake (AI) or Recommended Dietary Allowance (RDA) of fats (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 8, 2005). Fats are made when glycerol (a naturally occurring carbohydrate) is esterified (a chemical process in which an acid and alcohol molecule combine) with fatty acids (hydrocarbon chains that contain a methyl (CH_{3-}) and a carboxyl (-COOH) end) (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 8, 2005). Fatty acids can be categorized as: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and trans fatty acids. Saturated fats, which are saturated simply because all of their carbon atoms are attached to hydrogen atoms, mainly come from animal byproducts such as meat and dairy and are associated with energy and normal protein functioning. Monounsaturated fats, which have one double bond (basically, the chain has a different structure than a saturated fatty acid) typically occur in plant sources such as canola and olive oils and are essential in myelin production and in membrane structural lipids (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 8, 2005).

Polyunsaturated (meaning more than one double bond present) fats (PUFAs) can be further categorized into Omega-6 (n-6) and Omega-3 (n-3) polyunsaturated acids. Omega-6 acids are heavily prevalent in the Western (American) diet and can be found in foods such as poultry, corn, wheat, cereals, and many more foods (National Cancer Institute, 2018). Long-chain Omega-3 fatty acids come from the fatty tissue of cold-water fish such as salmon and come in the form of either EPA (eicosapentaenoic acid) or DHA (docosahexaenoic acid)(DRFM, 2005), which have been linked to a variety of health benefits that will be discussed later on. Short-chain

Omega-3 fatty acids come from plant sources such as flaxseed and walnuts and take the form of ALA (alpha-linolenic acid). PUFAs contain the only two essential fatty acids (the rest can be synthesized in the body through natural processes): linolenic and alpha-linolenic acids. A lack of the Omega-6 linolenic acid can lead to reduced growth and other adverse medical symptoms, such as scaly rashes (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 8, 2005). The Omega-3 ALA has been linked to preventative effects for coronary heart disease, arrhythmias, and thrombosis (*Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, Chapter 8, 2005).

Finally, trans fatty acids are unsaturated fatty acids that become more hardened than other types of fatty acids and typically come from foods containing hydrogenated oils or margarine. Unlike other fatty acids, trans fats are a relatively recent discovery caused by the popular use of processed foods and oils back in the 1950s and are linked to increased rates of heart attacks and strokes (American Heart Association, 2016).

Micronutrients can be divided into vitamins (organic nutrients) and minerals (inorganic nutrients) and are required by our bodies in much smaller doses than macronutrients. Vitamins are mainly attained through food consumption and each of the 13 known vitamins have specific roles in body functioning and are: Vitamin A, Provitamin A (Beta-Carotene), Vitamins B1, B2, B6, B12, Biotin, Vitamin C, Vitamin D, Vitamin E, Folic Acid, Vitamin K, Niacin, and Pantothenic Acid (DSM, 2016). Minerals are also mainly acquired through food sources and are divided into trace elements such as copper, iodine, manganese, selenium, and zinc along with macro elements such as calcium, magnesium, potassium, and sodium. Both vitamins and minerals are essential for normal cellular functioning and health; deficiencies of either compound can lead to various diseases and health problems such as scurvy, anemia, or osteoporosis.

Dietary Diversity

According to Ruel (2003) Dietary Diversity (DD) is a concept that refers to an individual consuming foods and nutrients from a variety of sources that capture the majority of human nutritional needs. Ruel (2003) attempted to summarize studies of the association between DD and individual nutrient adequacy, as well as establish an operational framework for how to define and validate measures of DD. Ruel begins her analysis with some helpful examples of common terms used in the literature to either describe or directly refer to the concepts of DD. According to Ruel (2003), Dietary Diversity (also sometimes known as Dietary Variety) is defined "as the number of different foods or food groups consumed over a given reference period". Dietary Quality, a more challenging concept to operationalize, was not found to have any type of "official" definition in the literature the author reviewed. Ruel (2003) did liken Dietary Quality to another term, Nutrient Adequacy, which refers to the "achievement of recommended intakes of energy and other essential nutrients" (Ruel, 2003, pg. 3912S). However, there is no standardized list of nutrients established, and it seems that researchers tend to use a somewhat exhaustive list of nutrients when assessing Nutrient Adequacy.

Ruel (2003) goes on to describe the methods as to which DD is generally calculated by researchers. It seems that in essence, DD is typically measured by summing the number of foods and food groups consumed over a specific reference period (usually 1-3 days, although some have used periods of 7-15 days). The literature then diverges in terms of how exactly counts of food or food groups are defined based on the country in which DD is being measured. For example, developing countries like Ethiopia may have their DD calculated differently than a country with more accessible food groups like the United States of America. Essentially, researchers have used a variety of DD measures based on different food and food group classification systems and different reference periods. This makes comparisons between studies somewhat difficult to interpret; Ruel (2003) then goes on to describe how DD has been validated in developing countries, concluding that DD has been extensively validated against Dietary Quality (e.g. Nutrient Adequacy) and that a consistent positive association between DD and nutrient adequacy seems to exist based on past findings. Ruel (2003) finishes her summary by listing the inner mechanisms of DD that should be better operationalized for future research. One issue is the idea of measuring individual foods (i.e. bread) as opposed to measuring food groups (i.e. grains/wheat as a whole); her research states that for measurement purposes that variance was just as well explained by 5 major groups (dairy, meat, grains, fruits, and vegetables) as it was by the variety within those specific food groups. Other considerations when measuring DD are the concept of portion size, the scoring system used to classify high/low DD groups, and the recall period for participants completing DD questionnaires.

Relationship Between Nutritional Intake and Health in Older Adults

The likelihood of developing neurodegenerative diseases increases with age; Polidori et al., (2009) examined whether dietary choices and antioxidant micronutrient status may mitigate these choices. The authors collected 193 participants aged between 45-102 years old (93 males, 100 females) and had them undergo a full physical/neurological examination to assess clinical conditions and identify any major organ diseases. The participants were also administered a neuropsychological examination; they completed the Mini-Mental Status Exam, clock drawing task, and DemTect Scale, which is a screening tool used for dementia. Participants were excluded if they showed any signs of cognitive impairment. The participants then completed a food-frequency questionnaire (FFQ) to assess amount of fruit and vegetables consumed in the last two weeks and had their blood tested for a micronutrient analysis. Based on answers to FFQ, 94 subjects were in high intake (HI) group and 99 subjects were in low intake (LI) group. A statistical analysis was then performed in which all nutrient and biomarker values (e.g. blood plasma content, micronutrient levels) normalized by log-transformation and then analyzed by the general linear model to compare the high-intake (HI) and low-intake (LI) groups. Age, gender, BMI, education, total cholesterol, LDL- and HDL- cholesterol, triglycerides, and albumin were introduced as covariates. The results revealed that healthy elderly participants who consumed a diet rich in fruits and vegetables have higher plasma levels of lipophilic antioxidant micronutrients (e.g. beta-carotene or vitamin E), lower levels of biomarkers related to oxidative stress, and better scores on neuropsychological evaluation (e.g. Mini-Mental Status Exam) compared to subjects with low intakes of fruits and vegetables. This result is independent of gender, education, BMI, lipid profile, and albumin levels but, most importantly, is independent of age. Micronutrient levels are a good indicator for fruit and vegetable intake and important in influencing the in vivo oxidant/antioxidant balance in healthy subjects. There has not been a study so far that has examined such a wide range of ages and the results of this study were consistent with prior research conducted on cognition and nutrition. In summary, the authors were able to show that healthy subjects consuming the recommended intakes of 5 daily portions of fruits and vegetables have higher circulating levels of antioxidant micronutrients and lower levels of oxidative stress biomarkers as well as better cognitive scores in comparison to healthy subjects consuming one daily portion or less of fruit and vegetables.

Another study done by Otsuka et al. (2017) found that dietary diversity decreased the risk of cognitive decline among Japanese older adults. The authors analyzed data from the National

Institute for Longevity Sciences- Longitudinal Study of Aging (NILS-LSA), which is a project in which data has been collected for several periods of years and assessed detailed health information such as medical checkups, physical fitness tests, and nutritional examinations. For their study, they assessed cognitive functioning through a Japanese version of the Mini-Mental Status Exam (MMSE), which is a widely used brief screening test for dementia and tested a total of 570 participants (298 men, 272 women) who were aged between 60-81 years old. The participants also completed a 3-day dietary record in which they recorded and weighed the food that they ate after being recruited for the study. After performing a generalized estimating equation (GEE), the authors found that there is evidence that higher dietary diversity reduces the risk for cognitive decline in community-dwelling older adults. In previous studies, similar Asian cultures reported that lower rice intake and higher soybean, vegetable, and dairy consumption was associated with less cognitive decline in aging adults (Otsuka et al., 2017). These results were very consistent with the present study, where lower dietary diversity (such as mainly consuming a cereal diet), had an adverse effect on cognitive function.

When trying to determine which specific facet of dietary diversity may be related to cognitive performance preservation, Beydoun and colleagues (2015) looked at dietary antioxidant intake and its association with cognitive function in an ethnically diverse sample of US adults. Prior research they cite suggests that oxidative stress plays a vital role in neurodegenerative processes that relate to cognitive impairment and dementia (especially AD). Specific dietary antioxidants, mostly β-carotene (and other carotenoids), Vitamin C and Vitamin E, were shown to inhibit biological processes related to oxidative stress, leading the authors to hypothesize that dietary antioxidants can potentially improve middle-aged adults cognitive performance and eventually delay onset of AD in aging adults. The authors gathered participants from an ongoing prospective cohort study called the HANDLS study (Healthy Aging in Neighborhoods of Diversity across the Life Span, N= 3,720), in which a representative sample of African Americans and white Americans (30-64 years old) living in Baltimore were recruited.

After restricting the total sample size to participants with complete and reliable cognitive tests as well as predictor/covariate variables, the authors were left with a sample of 1,274 participants that did not vary from the original sample on sex, age, race, or poverty/income ratio distribution. The authors used nine cognitive tests covering 7 domains of functioning (global, attention, learning/memory, executive functions, visuo-spatial/visuo-construction ability, psychomotor speed, language/verbal): the Mini-Mental State Examination (MMSE), the California Verbal Learning Test immediate and delayed free recall (CVLT-List A & CVLT-DFR), the Benton Visual Retention Test (BVRT) Animal Fluency test (AF), Brief Test of Attention (BTA), Trail Making test (A & B), Clock Drawing Test (CDT), Card Rotations (CR) and Identical Pictures (IP). The authors then used the US Department of Agriculture's (USDA) Automated Multiple Pass Method (AMPM), which is a computerized interview, to administer two 24-hr dietary recalls. An online database converted grams of USDA food codes into specific nutrients consumed per day and the average of the two days was used after the summation of nutrient intakes was calculated for both days. Specifically they looked at four areas of interest: Vitamins A, C, and E divided by energy intake and expressed as a retinol equivalent, and also the sum of five carotenoids (α-carotene, β-carotene, lutein + zeaxanthin, β-cryptoxanthin and lycopene) termed "total carotenoids". The authors also assessed a baseline measure of affective and depressive symptoms by using the Center for Epidemiologic Studies Depression Scale (CES-D). In terms of covariates and potential confounds, socio-demographic/lifestyle factors such as age, sex, race/ethnicity, marital status, completed years of education, poverty income ratio, measured body mass index (BMI), lifetime drugs use, smoking status, literacy ability, and total energy intake (specifically certain B-vitamins and n-3 unsaturated fatty acids) were examined and added to subsequent analyses.

The primary results of the study were that Vitamin E was positively associated with higher scores on a variety of cognitive tests (CVLT-List A, AF, & IP). These effects were only partially mediated by the CES-D scores of the participants (their depressive symptoms). Basically, dietary Vitamin E (as opposed to a supplement) was positively associated with performance in domains of verbal memory, verbal fluency, and psychomotor speed (but only among women). It is worth noting that in Beydoun et al.'s (2015) study, only 10.1% of their sample actually met the Estimated Average Requirement (EAR) of Vitamin E and that many isoforms of Vitamin E exist that should be further examined to parse out the true meaning of these results.

Sydenham, Dangour, & Lim (2012) conducted an intervention review in which they reviewed randomized controlled trials (RCTs) to determine the effects of omega-3 PUFA supplementation for the prevention of dementia and cognitive decline in cognitively healthy older people. They examined data from a total of 3536 cognitively healthy older adults ages 60-80 (first study=743, second study= 302, and third study= 2911) that were involved in three separate RCTs; in two studies, the participants consumed gel capsules which either contained omega-3 PUFA or sunflower or olive oil (placebo) for 6 or 24 months. In the other study, participants received tubs of margarine spread for 40 months (regular versus margarine fortified with omega-3 PUFA). All three RCTs intended to measure the effects of omega-3 PUFA supplementation on measures of cognitive functioning. In the first two studies, participants completed a battery of tests designed to measure cognitive functioning at both baseline and at final follow-up at 24 months later. The tests they used were designed to assess different domains such as memory, processing speed, and executive functioning and included: the Word Learning Test, the Wechsler Digit Span Test (forward and backward), Trail Making Test, the California Verbal Learning Test, and the Stroop Test. In the third study, the authors used the MMSE at baseline and final follow-up. In all three studies, there were no benefits to cognitive functioning among cognitively healthy older adults as a result of omega-3 PUFA supplementation. The authors recommend conducting studies of longer duration in order to determine if omega-3 PUFA supplementation can have preventative effects on cognitive decline in older adults.

A study conducted in 2017 by Andruchow et al. examined the effects of the ratio of omega-6 to omega-3 fatty acids on spatial memory and cognitive status. The authors used 52 healthy participants (33 women and 19 men aged 60 to 75) recruited from the greater Montreal area and were screened for mild cognitive impairment, dementia, and/or depression. Participants completed three tasks designed to measure spatial memory: a wayfinding task, The 4 on 8 Virtual Maze, and the probe trial. Only a subset of participants completed the wayfinding task (20 women, 14 men) which essentially consisted of participants navigating through a virtual town made up of streets, buildings, alleys, and eight landmarks. Participants were asked to take the shortest route to a predetermined target location after exploring the virtual town for 20 minutes; accuracy and amount of time to complete the task were assessed and participants were given a score based on how many trials they completed successfully. All participants completed the 4 on 8 Virtual Maze (4/8 VM) which is a virtual navigation task based on the rodent eight-arm radial maze. There are a minimum of five trials, with each trial having two parts; in each part of the task, participants are placed in the center of a radial maze in which each of the eight pathways has a pit at the bottom. In the first part of the task, only four of the pathways are available and participants are supposed to retrieve objects in those pits and then remember which pathways they have used because in the second part all of the potential pathways are accessible. In the second part of the task, participants were supposed to use new pathways to find the objects at the bottom of the pits; to do this, one of two strategies was utilized: a spatial strategy in which the location of objects in relation to landmarks is used to remember pathways and a response strategy in which numbering arms from the starting position is used to remember pathways. To move on to the probe trial, the criterion was that participants complete the second part of the 4/8 VM task three times without error. When participants reached criterion, they started the first part of the probe trial which was identical to the first part of the 4/8 VM task. However, during the second part of the probe task, which was similar to the second part of the 4/8 VM task, all external landmarks were removed so that participants who used a spatial strategy instead of a response strategy were prone to making more errors; in addition to the amount of errors, the researchers conducted a verbal interview to determine which strategy the participant was using. Participants also completed a FFQ designed to measure their dietary intake for the past year; these results were used to calculate approximate omega-6 to omega-3 fatty acid ratios. Finally, participants were given the Montreal Cognitive Assessment Test (MoCA; which is comparable to the MMSE) and also underwent a neuropsychological battery in order to assess different cognitive domains: the Rey Auditory Verbal Learning Task (RAVLT; used to assess verbal memory), the Rey-Osterrieth Complex Figure Test (ROCFT; used to assess visuospatial memory), and the Test of Non-Verbal Intelligence (TONI-III; used as a measure of IQ).

Results from Andruchow et al.'s study concluded that the ratio of omega-6:3 FA was strongly associated with participants' ability to form a spatial representation of a virtual town and their ability to learn a virtual navigation task. They also found that overall cognitive status was associated with the ratio of omega-6:3 FA; specifically, the lower the ratio of omega-6:3 FA, the more accurate participants were on the wayfinding task when they searched for target landmarks. Therefore, a lower ratio of omega-6:3 FA was associated with a better ability to form a spatial ability of the environment. Participants with the lower ratio also had a faster learning rate for the 4/8 VM task; these results were even more robust after education and age were introduced as covariates. This is the first study that has ever been able to predict how the ratio of omega-6:3 in healthy older adults could influence spatial memory and cognition. In addition to these results, a lower ratio of omega-6:3 FA also predicted better performance on the MoCA, although there were no associations between dietary status and performance on the RAVLT, ROCF, and NONI-III.

Study Purpose

A number of studies have explored the impact of nutrition on cognitive performance, however, none of them have directly compared people from different age groups (e.g. younger and older adults). The main purpose of this study will be to compare younger adults (18-35) and older adults (65+) on measures of nutritional intake and examine how nutritional impact influences performance on a neuropsychological battery. None of the studies mentioned above have explored the relationship between dietary diversity status, antioxidant status, and omega-3 to omega-6 PUFA ratio on cognitive functioning; the current study will examine if cognitive performance can be predicted by these nutritional indicators. One of the main tests used to assess cognitive decline/impairment in the studies examined is the MMSE; however the present study this study used a more sensitive measure of cognitive functioning, the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). The RBANS is a more sensitive measure of cognitive functioning because it has been validated in community dwelling “normal” elderly samples and has also demonstrated excellent estimates of Alzheimer's Disease diagnostic accuracy (Duff et al., 2008). The independent variables (or predictors) in the present study will be dietary diversity, antioxidant status, ratio of omega-3 to omega-6 PUFAs and verbal ability, while the dependent variable will be performance on the RBANS.

Methods

Participants

Participants were recruited from the Greater Grand Forks and Fargo areas in North Dakota. There were two sets of participants separated into groups based on age: one group was 58 people 18 to 31 years of age, recruited from UND (younger age group). The other group was 21 people 60 to 85 years of age (older age group). Participants were recruited using flyers placed in Grand Forks as well as through the SONA network, which is an online participant pool consisting of University of North Dakota undergraduate students. In addition, for the older adult's group, the principal investigator worked with North Dakota State University's (NDSU's) Cognitive Aging Lab to recruit some recurring participants in their lab. A total of 2 of the participants in the older group were recruited in this manner. Participants in the older group received $10 as compensation for their time to complete the study. Participants in the younger group received a total of 1.5 class credits through SONA over two appointments. All participants were required to be 18 years regardless of UND status. Exclusion criteria included: a history of concussions (3 or more), a history of severe mental illness or cognitive impairment, if they take medications with anticholinergic properties, if they have had a stroke, if they have had a head injury, if they are registered as a sex offender, and if they have significant dietary restrictions. A total of 3 participants (2 older and 1 younger) were excluded from this study due to having a history of concussions.

Materials

Demographic Questionnaire. A basic demographic questionnaire that assessed biological sex, age, ethnicity, years of education, perceived health status, and medication status was administered to all participants.

72-hr Diet Record. For the younger age group, participants were instructed to visit the lab and receive their 72-hour diet record with instructions on how to complete it. In the older age group, participants were mailed a 72-hour diet record (food journal) that they used at home to record all the food and beverages they consumed within a 72-hour period. Participants were required to bring in their 72-hr food journal when they attended the in-lab portion of the study.

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). The RBANS (RBANS; Randolph, 1998) is a test battery that is administered to an individual to assess attention, language, visuospatial/constructional abilities, and immediate/delayed memory. There are 12 subtests with alternative forms, taking about 45 minutes to complete. The 12 subtests comprise 5 indices: Immediate Memory Index (IMI), Delayed Memory Index (DMI), Visioconstructional Index (VCI), Language Index (LI), & Attention Index (AI). The IMI is made of the List Learning (LL) and Story Memory (SM); the DMI is made of the List Recall (LR), Story Recall (SR), Figure Recall (FR), & List Recognition (LRe) subtests; the VCI is made of the Line Orientation (LO) and Figure Copy (FC) subtests; the LI is made of the Picture Naming (PN) and Semantic Fluency (SF) subtests; and the AI is made of the Digit Span (DS) and Coding (CD) subtests. Using this measure was intended to help mitigate potential ceiling effects that could be obtained by young people completing another measure of cognitive functioning (e.g. the MMSE).

Weschler Adult Intelligence Scale IV Vocabulary Subtest- The Vocabulary subtest of the Wechsler Adult Intelligence Scale-IV (WAIS-IV; Wechsler, 2008) was used to estimate intellectual ability. In this test participants were read 30 vocabulary words of increasing difficulty and asked to provide a definition orally. The WAIS-IV vocabulary is widely used in studies of cognitive aging to determine potential differences in general intelligence.

The Food Processor Nutrition Analysis Software- The Food Processor ™ is a program designed to analyze menus, diets, foods, recipes, and even fitness needs of clients. It features an extensive food and nutrition database of more than 100,000 foods and food items that includes popular foods, ingredients, and recipes. Its analytic software is able to compute 172 nutritional components including macronutrients, micronutrients, and amino acids. It generates both spreadsheet and single nutrient reports for quick and efficient data viewing (https://esha.com/products/food-processor/).

Procedure

For the older adult group, individuals who called to express an interest in the study were mailed a 72-hour diet record and scheduled a time to participate in the study. They were asked to complete the dietary food record on one weekend and two weekdays immediately prior to their appointment. When the participant arrived at the laboratory the informed consent was obtained, then participants completed a demographics questionnaire. Participants then completed the WAIS-IV vocabulary subtest to measure for individual differences. Afterwards, participants completed the RBANS and then were debriefed on the study. For the younger group, most of the procedure was the same, except that they were scheduled for two separate sessions. During the first session, participants were given the informed consent form and the 72-hour food record with instructions on how to complete it. During the second session, participants filled out the demographics questionnaire, completed the WAIS Vocabulary subtest, and the RBANS. The responses from the 72-hour diet records were examined and were input into The Food Processor nutritional database to effectively analyze macronutrient/micronutrient content, dietary diversity and antioxidant status (https://esha.com/products/food-processor/).

Results

The means and standard deviations for the demographic data (Table 1a), Dietary Intake data (Table 1B), RBANS Index data (Table 1C) and RBANS subscale data (Table 1d) are presented below. A series of independent samples t-tests were conducted to compare age groups on all measures. An * in the title of the measure indicates that significant Age differences were observed on the indicated measure.

Demographics Data:

Table 1A:

Age Group	Age*	Years of Education*	Health Rating	WAIS Vocabulary* Raw Scores	WAIS Vocabulary* Scaled Scores
Young					
Mean	19.43	12.78	3.86	35.47	11.14
SD	2.01	1.04	0.74	8.25	2.45
Older					
Mean	72.24	16.24	3.90	42.14	11.81
SD	7.02	2.39	0.77	9.37	2.91

$^*P<.05$

Table 1B:

Age Group	Vitamin A RAE (mcg)	Vitamin E (mg)	Selenium (mcg)	Omega 3 Fatty Acids (g)	Omega 6 Fatty Acids (g)	Dietary** Diversity Score (1-12)	Omega 6:3** Ratio
Young							
Mean	369.23	4.15	49.63	0.68	5.93	9.76	10.68
SD	470.29	3.17	31.24	0.64	3.65	1.37	5.08
Older							
Mean	597.23	3.88	55.76	1.02	8.05	10.33	8.50
SD	651.51	2.59	26.90	0.95	6.74	1.02	2.84

****p<.08; assuming heterogeneity of variance p<.0**

Table 1C:

Age Group	RBANS Full Raw	RBANS Full SS	RBANS* Immediate Memory Index	RBANS Visuo-construction Index	RBANS Language Index	RBANS Attention Index	RBANS Delayed Memory Index
Young							
Mean	506.38	101.41	104.19	101.60	104.19	101.79	94.59
SD	43.97	12.31	12.68	14.73	10.96	14.96	9.39
Older							
Mean	493.67	97.71	96.90	95.81	103.71	102.71	94.52
SD	43.97	13.20	12.90	15.86	11.23	14.60	15.09

Table 1D:

Age Group	RBANS* List Learning SS	RBANS Story Memory SS	RBANS** Figure Copy SS	RBANS* Semantic Fluency SS	RBANS Digit Span SS	RBANS Coding SS	RBANS** Story Recall SS	RBANS* Figure Recall SS
Young								
Mean	10.95	10.63	10.14	12.45	9.21	11.26	10.97	11.40
SD	2.62	2.58	3.86	2.99	3.05	3.04	2.55	3.06
Older								
Mean	8.90	9.95	8.43	10.57	10.38	10.52	9.81	8.81
SD	2.58	1.94	3.51	3.26	3.38	2.34	2.11	2.69

***p<.05, **p<.08**

A series of simultaneous multiple regression analyses were conducted with predictor variables of: Vitamin A International Units (IU), Vitamin A Retinol Activity Equivalent (RAE), Beta-Carotene, Vitamin C, Vitamin E, Vitamin K, Zinc, Omega-3 Fatty Acids (FA) & Omega-6 Fatty Acids (FA). Additionally, predictors included age, dietary diversity scores, Omega-6 FA to Omega-3 Ratio, & WAIS Vocabulary Raw Scores. All bivariate correlations between predictor variables were less than .8 and all indices of muticolinnearity satisfied conventional standards of acceptance (VIF<10 and tolerance >.1). Dependent variables included each RBANS global index: RBANS Full Scaled Score, Immediate Memory Index (IMI), Delayed Memory Index (DMI), Visuospatial/Constructional Index (VCI), Language Index (LI), and Attention Index (AI). In addition, dependent variables also consisted of select RBANS subtests including: RBANS List Learning (LL), Story Memory (SM), Figure Copy (FC), Semantic Fluency (SF), Digit Span (DS), Coding (CD), Story Recall (SR), & Figure Recall (FR). Four subtests were not included because they only produced percentiles instead of scaled scores, these subtests were: Line Orientation (LO), Picture Naming (PN), List Recall (LR), and List Recognition (LRe).

The results of the regression analyses presented below includes the regression coefficient (b), a standardized regression coefficient and the part correlation squared for the predictor variable and the dependent variable. The regression coefficient indicates the amount of change in the dependent variable that occurs for each unit change in the predictor variable. The standardized regression coefficient indicates the amount of standard deviation unit changes in the dependent variable that occur for each standard deviation change in the independent variable. The part correlation squared is the percent of variance in the dependent variable uniquely accounted for by each predictor variable.

Results by RBANS Index:

Table 2A: RBANS Full Scale Standard Score

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-11.723	3.252	-0.414	-3.605*	0.118
WAIS_V_RAW	0.758	0.148	0.543	5.119*	0.237
VitaminAIU	0	0.001	0.044	0.305	0.001
BetaCarotene	0.001	0.001	0.138	0.927	0.008
VitaminC	-0.037	0.028	-0.158	-1.324	0.016
VitaminE	0.651	0.511	0.156	1.274	0.015
Zinc	0.41	0.477	0.098	0.86	0.007
Omega3FA	-7.949	3.821	-0.47	-2.08*	0.039
Omega6FA	0.871	0.555	0.327	1.569	0.022
DDS	2.022	1.014	0.209	1.993*	0.036
Omega6to3Ratio	-0.751	0.379	-0.279	-1.978	0.035
VitaminARAE	0.002	0.004	0.066	0.444	0.002
VitaminK	-0.018	0.02	-0.124	-0.894	0.007

A simultaneous multiple regression of RBANS Full SS indicated that as Age increased the RBANS Full SS decreased. Additionally, as dietary diversity scores and WAIS Vocabulary scores increased, RBANS Full SS increased. In contrast, increases in Omega-3 Fatty Acids were associated with a decrease in RBANS Full SS.

Table 2B: RBANS Immediate Memory Index

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-14.12	3.476	-0.481	-4.063*	0.158
WAIS_V_RAW	0.684	0.158	0.471	4.319*	0.179
VitaminAIU	8.63E-06	0.001	0.002	0.016	0.000
BetaCarotene	0.001	0.001	0.16	1.044	0.01
VitaminC	-0.017	0.03	-0.07	-0.566	0.003
VitaminE	0.116	0.546	0.027	0.212	0.000
Zinc	0.687	0.51	0.158	1.348	0.017
Omega3FA	-6.198	4.084	-0.353	-1.518	0.022
Omega6FA	0.678	0.594	0.245	1.141	0.013
DDS	2.258	1.084	0.225	2.083*	0.042
Omega6to3Ratio	-0.482	0.406	-0.173	-1.189	0.013
VitaminARAE	-0.002	0.004	-0.074	-0.481	0.002
VitaminK	-0.002	0.021	-0.012	-0.084	0.000

A simultaneous multiple regression of RBANS IMI indicated that as Age increased the RBANS IMI decreased. Additionally, as dietary diversity scores and WAIS Vocabulary scores increased, RBANS IMI increased.

Table 2C: RBANS Delayed Memory Index

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-7.075	3.149	-0.284	-2.247*	0.055
WAIS_V_RAW	0.417	0.143	0.339	2.906*	0.092
VitaminAIU	0	0	0.086	0.547	0.003
BetaCarotene	0.002	0.001	0.228	1.39	0.021
VitaminC	-0.04	0.027	-0.192	-1.462	0.023
VitaminE	0.211	0.495	0.057	0.426	0.002
Zinc	0.259	0.462	0.07	0.56	0.003
Omega3FA	-6.872	3.701	-0.462	-1.857	0.038

Omega6FA	0.963	0.538	0.41	1.79	0.035
DDS	2.105	0.982	0.248	2.143*	0.05
Omega6to3Ratio	-1.077	0.367	-0.454	-2.93*	0.094
VitaminARAE	0.001	0.003	0.039	0.24	0.001
VitaminK	-0.02	0.019	-0.157	-1.028	0.012

A simultaneous multiple regression of RBANS DMI indicated that as Age increased the RBANS DMI decreased. Additionally, as dietary diversity scores and WAIS Vocabulary scores increased, RBANS DMI increased. Also increases in omega6 to omega3 ratio led to decreases in the DMI.

Table 2D: RBANS Visuospatial/Constructional Index

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-10.062	4.515	-0.295	-2.229*	0.06
WAIS_V_RAW	0.405	0.206	0.24	1.969	0.047
VitaminAIU	-8.74E-05	0.001	-0.02	-0.122	0.0001
BetaCarotene	0	0.002	-0.049	-0.288	0.001
VitaminC	-0.03	0.039	-0.105	-0.767	0.007
VitaminE	0.442	0.709	0.088	0.623	0.005
Zinc	1.178	0.662	0.233	1.78	0.038
Omega3FA	-7.992	5.305	-0.393	-1.506	0.027
Omega6FA	0.117	0.771	0.036	0.152	0.0002
DDS	0.972	1.408	0.084	0.69	0.006
Omega6to3Ratio	-0.747	0.527	-0.23	-1.418	0.024
VitaminARAE	0.003	0.005	0.089	0.518	0.003
VitaminK	0.006	0.027	0.033	0.207	0.001

A simultaneous multiple regression of RBANS VMI indicated that as Age increased the RBANS VMI decreased.

Table 2E: RBANS Language Index

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-5.593	3.084	-0.227	-1.813	0.035
WAIS_V_RAW	0.648	0.14	0.532	4.616*	0.228
VitaminAIU	0	0	-0.072	-0.46	0.002
BetaCarotene	0.002	0.001	0.242	1.497	0.024
VitaminC	0.004	0.027	0.021	0.165	0.0002
VitaminE	-0.214	0.485	-0.059	-0.441	0.002

Zinc	-0.323	0.452	-0.089	-0.715	0.005
Omega3FA	0.231	3.624	0.016	0.064	0.000
Omega6FA	0.271	0.527	0.117	0.514	0.003
DDS	1.786	0.962	0.212	1.857	0.037
Omega6to3Ratio	0.358	0.36	0.152	0.994	0.011
VitaminARAE	0.001	0.003	0.061	0.377	0.002
VitaminK	-0.019	0.019	-0.153	-1.012	0.011

A simultaneous multiple regression of RBANS LI indicated that WAIS Vocabulary scores increased, RBANS LI increased.

Table 2F: RBANS Attention Index

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-4.852	4.339	-0.146	-1.118	0.015
WAIS_V_RAW	0.626	0.198	0.381	3.168*	0.117
VitaminAIU	0.001	0.001	0.166	1.015	0.012
BetaCarotene	0	0.002	-0.017	-0.1	0.0001
VitaminC	-0.052	0.037	-0.189	-1.397	0.023
VitaminE	1.41	0.682	0.288	2.068*	0.05
Zinc	-0.385	0.636	-0.078	-0.605	0.004
Omega3FA	-5.721	5.099	-0.288	-1.122	0.015
Omega6FA	0.853	0.741	0.273	1.151	0.015
DDS	0.469	1.354	0.041	0.347	0.001
Omega6to3Ratio	-0.561	0.506	-0.177	-1.107	0.014
VitaminARAE	0.001	0.005	0.047	0.28	0.001
VitaminK	-0.02	0.026	-0.121	-0.766	0.007

A simultaneous multiple regression of RBANS AI indicated that as Vitamin E totals and WAIS Vocabulary scores increased, RBANS AI increased.

Results by Individual Subtest:

Table 3A: RBANS List Learning

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-3.839	0.764	-0.568	-5.024*	0.222
WAIS_V_RAW	0.149	0.035	0.448	4.293*	0.162
VitaminAIU	0	0	-0.131	-0.928	0.008
BetaCarotene	0.001	0	0.295	2.011*	0.035

VitaminC	-0.014	0.007	-0.249	-2.123*	0.04
VitaminE	0.084	0.12	0.084	0.696	0.004
Zinc	0.075	0.112	0.075	0.67	0.004
Omega3FA	-1.469	0.898	-0.364	-1.636	0.023
Omega6FA	0.181	0.131	0.285	1.387	0.017
DDS	0.523	0.238	0.227	2.194*	0.042
Omega6to3Ratio	-0.15	0.089	-0.233	-1.681	0.025
VitaminARAE	0.001	0.001	0.098	0.669	0.004
VitaminK	-0.002	0.005	-0.073	-0.533	0.003

A simultaneous multiple regression of RBANS LL indicated that as Age increased RBANS LL decreased and as WAIS Vocabulary scores increased performance improved. Increased consumption of Beta-Carotene was associated with increases in performance while increased consumption of Vitamin C was associated with decreased performance. Additionally, an increase in Dietary Diversity (DDS) was associated with increased performance on RBANS LL.

Table 3B: RBANS Story Memory

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-1.235	0.714	-0.226	-1.728	0.035
WAIS_V_RAW	0.086	0.033	0.318	2.643*	0.082
VitaminAIU	0	0	0.196	1.203	0.017
BetaCarotene	0	0	-0.091	-0.538	0.003
VitaminC	0.008	0.006	0.165	1.219	0.017
VitaminE	-0.059	0.112	-0.074	-0.529	0.003
Zinc	0.159	0.105	0.196	1.514	0.027
Omega3FA	-0.641	0.84	-0.196	-0.764	0.007
Omega6FA	0.049	0.122	0.096	0.403	0.002
DDS	0.241	0.223	0.129	1.08	0.014
Omega6to3Ratio	-0.011	0.083	-0.021	-0.128	0.0001
VitaminARAE	-0.001	0.001	-0.271	-1.6	0.03
VitaminK	0.003	0.004	0.094	0.599	0.004

A simultaneous multiple regression of RBANS SM indicated that as WAIS Vocabulary scores increased, performance increased while no other predictors reached conventional levels of significance.

Table 3C: RBANS Figure Copy

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-2.038	1.203	-0.237	-1.694	0.038
WAIS_V_RAW	0.05	0.055	0.118	0.914	0.011
VitaminAIU	-6.93E-05	0	-0.064	-0.364	0.002
BetaCarotene	0	0	0.067	0.371	0.002
VitaminC	-0.004	0.01	-0.06	-0.415	0.002
VitaminE	0.128	0.189	0.101	0.676	0.006
Zinc	0.263	0.176	0.207	1.491	0.03
Omega3FA	-0.414	1.414	-0.081	-0.293	0.001
Omega6FA	-0.112	0.205	-0.139	-0.546	0.004
DDS	0.082	0.375	0.028	0.218	0.001
Omega6to3Ratio	-0.078	0.14	-0.095	-0.553	0.004
VitaminARAE	0	0.001	-0.037	-0.205	0.001
VitaminK	-0.003	0.007	-0.071	-0.422	0.002

A simultaneous multiple regression of RBANS FC indicated that no predictors reached conventional levels of significance.

Table 3D: RBANS Semantic Fluency

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-2.895	0.893	-0.408	-3.241*	0.114
WAIS_V_RAW	0.13	0.041	0.371	3.195*	0.111
VitaminAIU	-7.81E-05	0	-0.087	-0.552	0.003
BetaCarotene	0	0	0.246	1.51	0.025
VitaminC	0.004	0.008	0.062	0.473	0.002
VitaminE	-0.135	0.14	-0.129	-0.962	0.01
Zinc	-0.033	0.131	-0.031	-0.25	0.001
Omega3FA	0.191	1.05	0.045	0.182	0.0003
Omega6FA	0.03	0.153	0.046	0.2	0.0004
DDS	0.596	0.279	0.246	2.14*	0.05
Omega6to3Ratio	0.168	0.104	0.249	1.613	0.028

VitaminARAE	0	0.001	0.083	0.507	0.003
VitaminK	-0.006	0.005	-0.17	-1.115	0.013

A simultaneous multiple regression of RBANS SF indicated that increased Age led to decreased SF performance. Also as WAIS Vocabulary scores and dietary diversity scores increased, performance increased while no other predictors reached conventional levels of significance.

Table 3E: RBANS Digit Span

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	0.517	0.964	0.073	0.536	0.004
WAIS_V_RAW	0.101	0.044	0.287	2.293	0.066
VitaminAIU	5.01E-05	0	0.056	0.328	0.001
BetaCarotene	0	0	-0.081	-0.461	0.003
VitaminC	-0.006	0.008	-0.104	-0.74	0.007
VitaminE	0.244	0.151	0.232	1.609	0.033
Zinc	0.052	0.141	0.05	0.369	0.002
Omega3FA	-0.032	1.133	-0.007	-0.028	0.000
Omega6FA	0.016	0.165	0.024	0.097	0.0001
DDS	-0.122	0.301	-0.05	-0.406	0.002
Omega6to3Ratio	-0.052	0.112	-0.076	-0.459	0.003
VitaminARAE	0	0.001	-0.066	-0.374	0.002
VitaminK	-0.004	0.006	-0.116	-0.708	0.006

A simultaneous multiple regression of RBANS DS indicated that no predictors reached conventional levels of significance.

Table 3F: RBANS Coding

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-1.888	0.853	-0.292	-2.214*	0.059
WAIS_V_RAW	0.094	0.039	0.294	2.414*	0.07
VitaminAIU	0	0	0.226	1.373	0.022
BetaCarotene	0	0	0.055	0.322	0.001
VitaminC	-0.01	0.007	-0.191	-1.4	0.023
VitaminE	0.151	0.134	0.158	1.124	0.015
Zinc	-0.19	0.125	-0.198	-1.516	0.028
Omega3FA	-1.631	1.002	-0.422	-1.628	0.032
Omega6FA	0.239	0.146	0.392	1.64	0.032
DDS	0.277	0.266	0.125	1.041	0.013
Omega6to3Ratio	-0.122	0.1	-0.198	-1.223	0.018
VitaminARAE	0.001	0.001	0.135	0.793	0.008
VitaminK	-0.002	0.005	-0.046	-0.292	0.001

A simultaneous multiple regression of RBANS CD indicated that as WAIS Vocabulary scores increased, performance on CD increased. Additionally, as age increased, performance on CD decreased.

Table 3G: RBANS Story Recall

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-1.744	0.768	-0.312	-2.27*	0.067
WAIS_V_RAW	0.05	0.035	0.18	1.418	0.026
VitaminAIU	-1.26E-05	0	-0.018	-0.104	0.0001
BetaCarotene	0	0	0.081	0.454	0.003
VitaminC	0.004	0.007	0.076	0.534	0.004
VitaminE	-0.077	0.121	-0.094	-0.64	0.005
Zinc	0.09	0.113	0.109	0.798	0.008
Omega3FA	-1.816	0.903	-0.544	-2.012*	0.052
Omega6FA	0.221	0.131	0.42	1.683	0.037
DDS	0.092	0.24	0.048	0.382	0.002
Omega6to3Ratio	-0.09	0.09	-0.169	-1.003	0.013
VitaminARAE	0	0.001	0.075	0.422	0.002
VitaminK	-0.001	0.005	-0.045	-0.269	0.001

The Story Recall subtest is a delayed passage recall which is meant to test long-term memory, as opposed to the "Story Memory" subtest, which measures immediate recall. A simultaneous multiple regression of RBANS SR indicated that as age and totals of Omega-3 Fatty Acids increased, performance on SR decreased.

Table 3H: RBANS Figure Recall

	Unstandardized Coefficients		Standardized Coefficients	t	Correlations
	B	Std. Error	Beta		Part R^2
AgeGroup	-3.445	0.905	-0.484	-3.807*	0.161
WAIS_V_RAW	0.031	0.041	0.088	0.749	0.006
VitaminAIU	-3.86E-05	0	-0.043	-0.269	0.001
BetaCarotene	0	0	0.055	0.331	0.001
VitaminC	0.004	0.008	0.062	0.472	0.003
VitaminE	0.18	0.142	0.171	1.265	0.018
Zinc	0.064	0.133	0.061	0.482	0.003
Omega3FA	-2.014	1.064	-0.473	-1.894	0.04
Omega6FA	0.18	0.155	0.269	1.167	0.015
DDS	0.277	0.282	0.114	0.98	0.011
Omega6to3Ratio	-0.277	0.106	-0.408	-2.62*	0.076
VitaminARAE	0.001	0.001	0.18	1.091	0.013
VitaminK	-0.002	0.005	-0.056	-0.365	0.001

A simultaneous multiple regression of RBANS FR indicated that as age and the Omega-6 FA to Omega-3 FA ratio increased, performance on FR decreased.

Discussion

In terms of findings related to nutritional intake, there was a mix of expected and unexpected results. Overall, however, results were mostly consistent with prior research done. Specifically, the results indicated age-related decline on most aspects measured on the RBANS, including the RBANS Full SS, with the exception of the Language and the Attention Index. The LI includes the subtests of Picture Naming and Semantic Fluency, while the AI includes the subtests of Digit Span and Coding. The only subtest among those indices not showing age differences was the SF subtest. These results are consistent with expected age related declines in various cognitive domains such as memory (Polidori et al., 2009). These age differences observed were not accounted for by vocabulary differences because age was tested after all other predictors in the equation.

Dietary diversity was positively related to cognitive performance on the RBANS Full Scale SS, the IMI, and DMI. In contrast, dietary diversity had no impact on the VCI, the LI, or the AI. In general, the results seem to suggest that dietary diversity is positively correlated to several measures of cognitive performance including overall performance, immediate memory, and delayed memory. Research by Otsuka et al (2017) has shown that dietary diversity is related to lower risk of cognitive decline in aging adults, this study suggested that dietary diversity was positively related to better overall RBANS performance and better memory which may be indicative of less cognitive decline in the future. Their research (2017) points out that there is a relative paucity of research on dietary diversity's relationship to cognitive performance. Future research could employ a longitudinal design to better understand the relationship between dietary diversity and potential cognitive decline as we age. Additionally, researchers could control the food that the participants ate to assess specific degrees of dietary diversity.

In terms of just Omega-3 FA levels, an increase in Omega-3 FAs was associated with a decrease in RBANS Full Scale SS and Story Recall, while an increase in the Omega-6: Omega-3 ratio was associated with a decrease in the DMI (specifically the Figure Recall and Story Recall subtests). Prior research from Sydenham, Dangour, & Lim (2012) failed to find any link between Omega-3 FAs and cognitive performance among healthy older adults, while this study actually was associated with various declines in cognitive performance due to Omega-3 FA intake. An important distinction to make is that they (2012) studied Omega-3 FA supplementation, while this study recorded both natural and supplemental intake of Omega-3 FAs. While there could have been errors in the amount of Omega-3 FAs participants recorded, future research should follow Sydenham, Dangour, & Lim (2012)'s recommendations of a longitudinal focus on the effects of Omega-3 FAs on cognitive decline and performance.

In contrast to the other Omega-3 related findings, in this study the Omega-6: Omega-3 FA Ratio was related to delayed memory as a whole; basically meaning that the more Omega-6 FAs people consumed in relationship to the amount of Omega-3 FAs they consumed, the worse they performed on delayed memory overall. Prior research from Andruchow et al. (2017) suggested that a lower Omega-6: Omega-3 FA ratio was related to better visuospatial ability and scores on the MOCA; the results of this study unexpectedly found that the ratio was related to better scores on measures of verbal memory (Story Recall). This study's finding of the negative relationship between the Omega-6: Omega-3 FA ratio and the Figure Recall subtest score was consistent with Andruchow et al. (2017)'s conclusions of better visuospatial memory and a lower ratio of Omega-6: Omega-3 FAs. Future research should examine the effects of the Omega-6: Omega-3 FA ratio with a more sophisticated story recall measure, such as the Wechsler Memory Scale-IV Logical Memory I + II subtests to confirm the story memory related results of this study.

Vitamin E and Beta-Carotene were associated with better scores on the Attention Index and List Learning respectively, while more Vitamin C was associated with decreased performance on LL. Prior research from Beydoun et al. (2015) has indicated that Vitamin E was related to improved scores on verbal memory, verbal fluency, and psychomotor speed, but this study supported that Vitamin E was related to better scores on tests measuring attention (although Coding, which measures psychomotor speed was positively related to Vitamin E in this study). The results of this study are somewhat consistent with Polidori et al.'s (2009) findings that antioxidants like Beta-Carotene and Vitamin E intake were positively associated with scores on the MOCA; future research should take into account the many different isoforms of Vitamin E and more analysis between these different types of Vitamin E and how they interact with cognitive decline should be conducted. The current study's findings regarding Vitamin C were not consistent with research from either Polidori et al. (2009) or Beydoun et al. (2015) and could be the result of erroneous food record analysis. More research should be done on the specific nature of Vitamin C and its relationship to verbal memory.

There were a variety of limitations to this study that could have affected results. For example, despite a dedicated effort to recruit older adults from the Greater Grand Forks and Fargo communities, we were not able to recruit the expected number of participants. This may have influenced what their average food consumption totals and RBANS scores turned out to be. There may have been discrepancies between what people reported eating on their food journals and what they actually ate. Despite being given clear instructions on how to record food content, totals, and preparation methods, there could have been some confusion, overreporting, or underreporting by the participants that could have influenced their overall results. Additionally, although the program used to create the reports for food journals contained a comprehensive nutritional database, The Food Processor, there may have been discrepancies between what participants recorded in their journals and what the database contained information on. This was especially true for local dishes and unorthodox/rarer dishes. The population used also was mainly Caucasian and from the Midwest United States, leading to a lack of diversity and therefore generalizability to the population at large.

General future directions for the study would entail including more older adults from a more diverse population and from more diverse backgrounds. Additionally, being able to control the food that the participants consumed over a 72-hour-period would lead to more consistency and accuracy in analyzing their nutritional data. Other more sophisticated neuropsychological measures that more accurately measure specific cognitive domains could be used to more efficiently parse out differences in neuropsychological functioning as well. However perhaps most importantly, using a longitudinal design that accounts for the myriad of other life factors experienced as we age could be crucial to better understanding the relationship between dietary intake and cognitive decline as we age.

Reference List

American Heart Association (2016, Nov 14.). Fats 101. Retrieved from http://atgprod.heart.org/HEARTORG/HealthyLiving/FatsAndOils/Fats-101_UCM_304494_Article.jsp#.W51-FOhKhPY

Andruchow, N. D., Konishi, K., Shatenstein, B., & Bohbot, V. D. (2017). A lower ratio of omega-6 to omega-3 fatty acids predicts better hippocampus-dependent spatial memory and cognitive status in older adults. *Neuropsychology*, *31*(7), 724-734. doi:10.1037/neu0000373

Beydoun, M. A., Fanelli-Kuczmarski, M. T., Kitner-Triolo, M. H., Beydoun, H. A., Kaufman, J. S., Mason, M. A., . . . Zonderman, A. B. (2015). Dietary Antioxidant Intake and Its Association With Cognitive Function in an Ethnically Diverse Sample of US Adults. *Psychosomatic Medicine, 77*(1), 68-82. doi:10.1097/psy.0000000000000129

DSM. What are Micronutrients? (2016). Retrieved September 15, 2018, from https://www.dsm.com/content/dam/dsm/cworld/en_US/documents/what-are-micronutrients.pdf

Duff, K., Humphreysclark, J., Obryant, S., Mold, J., Schiffer, R., & Sutker, P. (2008). Utility of the RBANS in detecting cognitive impairment associated with Alzheimers disease: Sensitivity, specificity, and positive and negative predictive powers. Archives of Clinical Neuropsychology, 23(5), 603-612. doi:10.1016/j.acn.2008.06.004

Food Processor Program: Nutrient Analysis Software. (2020). Retrieved from https://esha.com/products/food-processor/

Institute of Medicine. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: The National Academies Press. https://doi.org/10.17226/10490.

Marrocco, I., Altieri, F., & Peluso, I. (2017). Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxidative Medicine and Cellular Longevity, 2017*, 1-32. doi:10.1155/2017/6501046

National Cancer Institute. (2018, April 20). Table 2. Food sources of total omega 6 fatty acids (18:2 20:4), listed in descending order by percentages of their contribution to intake, based on data from the National Health and Nutrition Examination Survey 2005-2006. Retrieved from https://epi.grants.cancer.gov/diet/foodsources/fatty_acids/table2.html

National Center for Complementary and Integrative Health. (2016, May 04). Antioxidants: In Depth. Retrieved from https://nccih.nih.gov/health/antioxidants/introduction.htm

Otsuka, R. (2017). Dietary diversity decreases the risk of cognitive decline among elderly Japanese. *Geriatrics & Gerontology International, 17*(6), 1038-1039. doi:10.1111/ggi.13005

Péter, S., Saris, W., Mathers, J., Feskens, E., Schols, A., Navis, G., . . . Eggersdorfer, M. (2015). Nutrient Status Assessment in Individuals and Populations for Healthy Aging—Statement from an Expert Workshop. *Nutrients, 7*(12), 10491-10500. doi:10.3390/nu7125547

Polidori, M. C., Praticó, D., Mangialasche, F., Mariani, E., Aust, O., Anlasik, T., . . . Nelles, G. (2009). High Fruit and Vegetable Intake is Positively Correlated with Antioxidant Status and Cognitive Performance in Healthy Subjects. *Journal of Alzheimers Disease, 17*(4), 921-927. doi:10.3233/jad-2009-1114

Randolph, C. (1998). RBANS manual: Repeatable battery for the assessment of neuropsychological status. *San Antonio, TX: The Psychological Corporation*.

Ruel, M. T. (2003). Operationalizing Dietary Diversity: A Review of Measurement Issues and Research Priorities. *The Journal of Nutrition, 133*(11). doi:10.1093/jn/133.11.3911s

Shim, J., Oh, K., & Kim, H. C. (2014). Dietary assessment methods in epidemiologic studies. *Epidemiology and Health*. doi:10.4178/epih/e2014009

Sydenham, E., Dangour, A. D., & Lim, W. (2012). Omega 3 fatty acid for the prevention of cognitive decline and dementia. *Cochrane Database of Systematic Reviews*. doi:10.1002/14651858.cd005379.pub3

Vauzour, D., Camprubi-Robles, M., Miquel-Kergoat, S., Andres-Lacueva, C., Bánáti, D., Barberger-Gateau, P, &. Ramirez, M. (2017). Nutrition for the ageing brain: Towards evidence for an optimal diet. *Ageing Research Reviews, 35*, 222-240. doi:10.1016/j.arr.2016.09.010