NUTRITION, VISION, AND COGNITION IN HEALTH: EGG (IONHEALTH-EGG) STUDY

A Dissertation

by

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ABSTRACT

PURPOSE: The purpose of this study is to evaluate the impact of the nutrients in eggs, specifically in the yolk, on the visual cognitive performance (VCP) in generally healthy older individuals. METHODS: One hundred six generally healthy men (40) and women (66) aged 50 to 75 years were randomly assigned to one of five dietary treatment groups: four egg whites (EW); two whole regular eggs (WE); two whole Omega-3 FA fortified eggs (O3E); four egg yolks (EY); and a no-egg control (NEC). Subjects maintained their usual dietary intake including the treatment modifications daily for 30 days. VCP was measured using the NeuroTracker™ CORE (NT) 3-Dimensional (3-D) software program (15 training sessions) during the final 2 weeks of the study. Participants recorded daily food intake during the first 2 weeks of treatment and each day they trained on the NT. Food logs were analyzed using Nutribase software. Statistical analyses were performed in SPSS using the paired sample t test, the one-way analysis of variance (ANOVA), repeated measures ANOVA, ANCOVA and multiple regression. RESULTS: The dietary intervention successfully created distinct dietary intake differences for choline, lutein, omega-3 fatty acids and cholesterol ($P<0.01$) between groups. Cognitive training improved VCP in all groups (+37%, $P<0.01$) but there were no differences between egg groups (P>0.10). There were significant increases in Lutein (($P=0.02$), Zeaxanthin ($P=0.004$) and L + Z ($P=0.006$) in the WE treatment group only.

CONCLUSION: Dietary and cognitive interventions were successful at altering dietary intake, significantly improving serum lutein and zeaxanthin in the whole egg treatment group and improving VCP among all treatment groups with limited impact on lipid levels. No egg consumption pattern was superior in improving cognitive responses.

DEDICATION

This PhD is dedicated to my parents, Tyrone and Alma Mynhier, who taught me the value of education, continual learning and daily progressive growth and consistently provided support, encouragement, and resources to excel. They also taught me about God and helped me develop my faith, which has been an essential resource in this journey and in my daily life.

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NOMENCLATURE

6-s	6 Seconds
3-D	3-Dimensional
3-D-MOT	3–Dimensional Multiple Object Tracking
AD	Alzheimer Disease
AH	Acetone:Hexane
AMD	Age-related Macular Degeneration
ANOVA	Analysis of Variance
AREDS2	Age-Related Eye Disease Study 2
AT	Activity Tracker
ABCA1	ATP binding cassette transporter A1
BBB	Blood Brain Barrier
BCO2	β-carotene Oxygenase 2
BIA	Bio-electrical Impedance Analysis
BMI	Body Mass Index
BCMO1	β-carotene monooxygenase 1
BP	Blood Pressure
BRB	Blood Retinal Barrier
CD	Cognitive Decline
CD36	Cluster of Differentiation 36
CE	Cholesterol Ester
cHFP	Custom Heterochromatic Flicker Photometry

CHOL	Cholesterol
CNS	Central Nervous System
CT	Cognitive Training
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DHA	Docosahexaenoic Acid
DM	Diabetes Mellitus
DR	Diabetic Retinopathy
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Eicosapentaenoic Acid
EW	Large Egg Whites
EY	Large Egg Yolks
fMRI	Functional Magnetic Resonance Imaging
GRAS	Generally Recognized As Safe
HDL-C	High Density Lipoprotein- Cholesterol
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
IONHealth-Egg	Nutrition, Vision, and Cognition in Health Study: Egg
IONSport	Nutrition, Vision, and Cognition in Sport
IRB	Institution Review Board
L	Lutein
LDL-C	Low Density Lipoprotein- Cholesterol

MAQ	Modifiable Activity Questionnaire
MCI	Mild Cognitive Impairment
MED	Mediterranean diet
MIND	Mediterranean- Dietary Approaches to Stop Hypertension Diet Intervention for Neurodegenerative Delay
MMSE	Mini Mental State Examination
MUFA	Monounsaturated Fatty Acids
MP	Macular Pigment
MPOD	Macular Pigment Optical Density
MRI	Magnetic Resonance Imaging
MZ	Meso-zeaxanthin
NB	NutriBase 19 Pro Edition Software
NEC	No Egg Control
NT	NeuroTracker™ CORE 3-Dimensional Software Program
O3E	Omega-3 Fatty Acid Fortified Eggs
Omega-3 FA	Omega-3 Fatty Acids
OS	Oxygen Saturation
PSQI	Pittsburgh Sleep Quality Index
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated Fatty Acids
RDA	Recommended Dietary Allowance
RDN	Registered Dietitian Nutritionist

ROS	Reactive Oxygen Species
RPE	Retinal Pigment Epithelium
SCD	Subjective Cognitive Decline
SD	Standard Deviation
SDLP	Standard Deviation of Lateral Position
SFA	Saturated Fatty Acid
SMI	Subjective Memory Impairment
SPMSQ	Short Portable Mental Status Questionnaire
SRB	Scavenger Receptor Class B Proteins
SRB-1	Scavenger Receptor Class B Type 1
SRB-2	Scavenger Receptor Class B Type 2
ST	Speed Threshold
T2DM	Type-2 Diabetes Mellitus
TAG	Triacylglycerols
TAOC	Total Antioxidant Capacity
tCSF	Temporal Cognitive Sensitivity Function
TG	Triglycerides
UV	Ultraviolet
VA	Visual Acuity
VCP	Visual Cognitive Performance
VLDL	Very Low-Density Lipoproteins
WE	Whole Large Eggs

WHAM	Wisconsin Hypo-alpha Mutant
Z	Zeaxanthin

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Global Concerns of Aging

Globally, approximately 50 million individuals experience dementia; it is expected that this number will triple by the year 2050. Without an identified treatment for this condition, there are tremendous social and financial implications.[1] It is projected that delaying the onset of dementia for just five years would generate a significant positive economic impact, both immediately and in the long-term.[2] Based on the model of progression of dementia associated with Alzheimer's Disease (AD), subjective cognitive decline (SCD) or subjective memory impairment (SMI), is the initial state where individuals self-recognize a gradual reduction in cognitive ability that is not preceded by an acute event causing it. Generally, these individuals are impacted by progressive memory decline that generates anxiety associated with it and is recognized by others; however, it is not reflected on objective tests or measures used for clinical diagnosis.[3] Some studies report that up to 60% of persons who have SCD advance to mild cognitive impairment (MCI) or AD over a 15-year period. Studies evaluating biomarkers of SCD have focused on neurodegeneration emphasizing the temporal region and applying various forms of imaging for analysis.[3,4]

Individual cognitive variability exists with the aging process and may include regional brain shrinkage, motor performance reduction, and cognitive decline in memory, processing rate, spatial awareness, judgement, and decision making. Situations requiring free recall and complex working memory tasks requiring attention, processing

ability and integration are commonly compromised in those with SCD.[3,4] Reduced spatial awareness, the inability to recognize the relationship between oneself and the environment and properly respond to changes also frequently occurs. The decline of processing speed and spatial ability can hinder or delay stimuli response resulting in accidents including, but not limited to, car crashes.[5] Studies evaluating driving responses of older persons identify concerns related to visual and cognitive distractions, difficulty of quick decision making and response, and lower standard deviation of lateral position (SDLP), a sensitive indicator of driving ability.[6-10]

Interventions

Cognitive Training

Intervention is required to slow the progression of cognitive decline (CD) and preserve existing abilities as well as reduce negative financial and societal impact associated with this condition. Pharmaceutical treatment is a potential intervention, however, new drugs require extensive, expensive clinical trials prior to approval and use. Alternative interventions that include cognitive training and addressing coinciding conditions of depression, anxiety, fitness, obesity, diabetes mellitus (DM) and personality alterations may be more efficient and effective.[4] A systematic review that evaluated non-pharmaceutical treatments in older persons with CD reported that cognitive training (CT) was more impactful in delaying decline in aging persons than other diverse interventions.[11] Studies that applied perceptual CT using three-dimensional

multiple object training (3-D MOT) reported this intervention successfully delivered cognitive benefits to elderly persons, including those with SCD.[4,12]

Nutrition

Nutrition has also been considered as an approach to mitigate CD. Nutrients such as lutein (L), zeaxanthin (Z), omega-3 fatty acids (Omega-3 FA) and vitamins A, B complex, E, and D have been evaluated for their ability to delay dementia onset.[3,4] A systematic review by Krause and Roupas reported that vitamins A, B complex components, E, and D, either individually or in combination, did not reduce or delay CD.[13] Several studies reported that L and Z supplementation improved cognitive performance in individuals of varying age groups, including older populations.[15- 20] Additionally, L was identified as a non-essential nutrient that contributed to reduction in age-related macular degeneration (AMD), however, this systematic review also reported that more evidence is required to identify any additional clinical benefits.[14] A systematic review that evaluated the relationship between Omega-3 FA and CD from 2010 to 2017 reported that 71% (n=14) of the randomly controlled trials that supplemented elderly persons with Omega-3 FA positively impacted one or more cognitive function domains.[21] Additionally, Omega-3 FA were reported to be effective in treating elderly persons with mild to moderate depression, a common complication of CD.[22]

Dietary Patterns

The association between dietary patterns, specific food groups and cognitive

status has also been studied. A systematic review evaluating the impact of fruit and vegetables on cognitive ability concluded that vegetables were linked to a reduced CD in aging persons; however, fruit did not provide the same benefit.[23] A study that evaluated the cognitive ability of institutionalized older people using food intake records and the Short Portable Mental Status Questionnaire (SPMSQ) reported fewer test errors by those who consumed larger amounts of cereals, eggs, oils and fats.[24] The Dietary Approach to Stop Hypertension (DASH) and Mediterranean (MED) diets have also been studied for their impact in improving cognitive performance. Both have shown some benefits when supplemented with other foods such as virgin olive oil or nuts and/or behaviors such as caloric control or exercise.[25-30] Based on these reported benefits, researchers united components of the DASH and MED diets, incorporated some modifications and created the MED-DASH Diet Intervention for Neurodegenerative Delay (MIND) diet, a dietary pattern that emphasizes berries, leafy green vegetables, a serving of fish weekly and limits consumption of animal products and saturated fat. When compared to the results of the DASH and MED diets, the MIND diet reported less incidence of CD on global cognition tests and cognitive domains. Additional research comparing the benefits of these diets is needed.[29,31]

Consideration of Eggs in the Intervention Approach

The egg has not been considered to have an important role in the proposed dietary patterns related to cognition; however, it provides several of the same nutrients found in the leafy green vegetables associated with improved cognition throughout the life span.[32-34] A large whole egg is a lower calorie (72 kcal), nutrient-dense, complete

protein that includes bioactive components that may reduce inflammation in certain populations. While several of the nutrients in the egg such as choline, L, and Z have been associated with enhanced cognitive performance, the egg yolk also contains phospholipids, which appear to be preferential in the formation of high-density lipoprotein cholesterol (HDL-C).[33,35,36] Eggs are known for their cholesterol (CHOL) content resulting in historical recommendations to limit eggs in the diet. However, the CHOL limitation was removed in the 2015-2020 Dietary Guidelines for Americans due to the increased number of research studies that failed to confirm the link between CHOL and cardiovascular disease (CVD). Instead, more recent studies are showing benefits of egg consumption related to increased HDL-C, lean muscle, and weight management.[33,36]

Nutrition, Vision, and Cognition in Sport (IONSport) Pilot Study

In our pilot study of healthy college students (IONSport), those who had higher intakes of nutrients rich in whole eggs had significantly higher training scores with the NeuroTracker™ CORE program (NT), a validated test that provides CT and measures visual cognitive performance (VCP). As the older adult population (age 60 and older) is growing with increased life expectancy and the incidence of dementia is expanding, the nutrients in eggs may contribute to a healthy diet pattern to delay CD.[1,2] To test this, men, and women aged 50-75, without cognitive dysfunction, followed one of five egg consumption patterns for 30 days. Subjects participated in NT cognitive testing during the last two weeks of the 30-day egg intervention. Egg consumption patterns included: no egg control (NEC), 4 large egg whites daily (EW, protein control), 2 large eggs daily

(WE), 2 large omega-3 fatty acid-enriched eggs daily (O3E), 4 large egg yolks daily (EY).

CHAPTER II

REVIEW OF LITERATURE

Theoretical Framework

Biological aging is a physiological change that organisms experience over time. Consequences associated with this process elevate the risks for functional decline and impairment including, but not limited to, cognitive performance. While several aging theories have been proposed, the free radical and mitochondrial theories were used in establishing a theoretical framework for this research. The free radical theory, proposed by Harman (1956), suggested that the aging process included the formation of free radicals resulting in biological damage.[37] The mitochondrial theory relates the aging process to damaged mitochondria that are exposed to oxidative stress resulting in increased formation of reactive oxygen species (ROS).[38,39] While humans have natural systems to reduce damage of free radicals, it has been suggested an imbalance in ROS and antioxidants results in an accumulation of free radical damage that initiates and advances the aging process.[39,40] This theory is supported by Serviddio, et al., who reported that aged rats had significantly lower mitochondrial membrane potential in the liver, kidney and heart when compared to younger rats.[41] Other researchers also used this theoretical framework for various conditions including AMD, atherosclerosis, ischemia, AD, frailty syndrome and renal dysfunction.[39,42-44]

Macular Pigment Carotenoids

The free radical and mitochondrial theories are linked to this research based on the antioxidant and light-filtering functions of the macular pigment (MP) carotenoids. Lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) comprise the macular pigment (MP) carotenoids. L and Z are non-provitamin A carotenoid isomers and MZ is the product of L catalyzation in the retinal pigment epithelium (RPE) by RPE65 isomerase enzyme.[45,46] L, Z, and MZ have a distinctive arrangement including two ionone rings with hydroxyl groups at each end of their structures, nine conjugated double bonds, and a compositional make-up of $C_{40}H_{56}O_2$. While each has varying stereoisomeric forms, the hydroxyl groups enhance their polarity beyond that of other vitamin A sources and enhances their antioxidant capacity and ability to protect photoreceptors in the macula .[45]

The retina, an organ prone to oxidation due to its high oxygen content and polyunsaturated fatty acid (PUFA) concentration, is uniquely favorable to the MP carotenoids. The MP carotenoids and their metabolites accumulate and are distributed to specific regions of the fovea and retinal layers where they appear to use their antioxidant and light-filtering properties to reduce the occurrence of ROS.[45] Studies of MP carotenoid reported their antioxidant properties positively influence those with AMD, diabetes mellitus (DM), atherosclerosis, severe traumatic brain injury, and healthy non-smokers.[45,47-53] Additionally, several studies have suggested an association between increased MP and cognitive performance.[54-56]

Xanthophylls

Sources

Lutein and Z are fat-soluble xanthophylls that must be consumed over the lifespan to achieve adequacy due to absence of endogenous production.[57,62] While L and Z are generally found in the same foods, dietary L content is much higher than Z.[57] Dietary MZ consumption is extremely low or possibly absent due to its presence in very limited food sources including fish skin and turtle fat.[45] Leafy green vegetables (spinach, kale) are primary sources of L and Z. However, these xanthophylls are also found in a variety of other foods including egg yolks, avocado, specific grains (maize, wheat varieties of einkorn, Khorasan, and durum), bright colored orange and yellow fruits (cantaloupe, oranges, red grapefruit) and vegetables (corn, orange and yellow peppers, carrots).[45,57,62-65]

Although there is no recommended dietary allowance (RDA) for xanthophylls, the typical diet was reported to include a total of 1.3-3 mg L and Z, and research suggested that 2-12 mg L and Z daily resulted in positive eye health outcomes.[45] Generally recognized as safe (GRAS)-approved L and Z supplements can deliver the xanthophyll benefits that are diminished in a diet low in these nutrients. The maximum dose tested for L and Z combined and MZ alone is 400 mg/kg body weight daily and 300 mg/kg body weight daily, respectively.[45] Based the secondary analysis of the Age-Related Eye Disease Study 2 (AREDS2), a supplement containing 10 mg L and 2 mg Z was more protective and safer than β-carotene in reducing AMD and yielding positive benefits to adults of varying ages with or without AMD and those with AD.[66]

Absorption

The absorption of L and Z is variable, based on a variety of factors including the source structure (food, supplement, esterification), composition of foods consumed with xanthophyll sources, processing and/or preparation methods, and growing, harvesting, and post-harvest management.[45,46,67-75] Lutein and Z are present in foods or supplements in either a unesterified (free) or esterified form with di-esterified xanthophylls often attached to palmitate. While eggs and most fruits and vegetables are reportedly in the unesterified form, other foods and supplements may have unesterified or esterified xanthophylls.[67,71] Research studies comparing the bioavailability of unesterified and esterified L reported increased absorption from unesterified L.[71] However, other studies reported significantly greater absorption with supplements containing esterified L.[67] Additional research that evaluated the absorption of esterified L reported the fat content of the foods consumed simultaneously influenced its absorption. Participants that consumed a higher fat meal (~40 g) with esterified L had greater absorption than those consuming one lower in fat (<6.5 g) suggesting that the amount of fat required to enhance L absorption may be influenced by the L esterification status.[68]

The lipid content of the food itself may also influence xanthophyll bioaccessibility. For example, consumption of eggs and avocados, both lipid-rich sources, resulted in increased serum and retinal absorption when compared to lutein-rich greens and supplements.[65,69,76,77] Dietary fibers including alginate also influenced absorption of xanthophylls. When comparing consumption of a starch-base to an alginate-base product, the starch-base product resulted in a significant increase in L

absorption when compared to baseline whereas the alginate-base product did not. A small study of women also reported only 40-74% L absorption when also consuming dietary fiber.[72,78]

The fatty acid composition of triacylglycerols (TAG) consumed with xanthophylls also appeared to influence absorption; however, inconsistencies between studies exist. One study used varying models including two *in vitro*, an animal model and human study to evaluate bioaccessibility and efficiency of L and Z absorption. The *in vitro* models used prepared potatoes, minced beef with chopped spinach or corn in a digestion model and sweet corn and spinach in the Caco-cell model, respectively. TAG mixes (trilauren, tripalmitin, triolein, trillinolein trieicosopentanoin, and tridocosahexaenoin) were added to identified fats (butter, palm oil, olive oil, sunflower oil, fish oil) in the *in vitro* experiments. Vegetables and TAG emulsions with butter, olive oil or fish oil were used in the animal study. The impact of saturated fatty acids (SFA) on L and Z in fruit and vegetables were also evaluated in humans. The SFA in butter significantly increased bioaccessibility of L in the *in vitro* and animal studies when compared to polyunsaturated fatty acids (PUFA) or monounsaturated fatty acids (MUFA). However, SFA had a negative relationship with L and Z absorption in the human study.[70] Another study reported increased L absorption when it was consumed with the PUFA when compared to SFA consumption.[71]

Food preparation using high heat cooking methods such as boiling, frying, and microwaving were shown to reduce xanthophyll levels in eggs.[79] On the other hand, pasteurization with high temperatures increased Z bioavailability by incorporating it into

micelles. It was suggested this processing method disrupted the food structure and enhanced Z extraction.[80] Growing and harvesting during agriculture production were also shown to influence carotenoid content of plant sources.[75]

Metabolism and Transport

Due to their lipophilic properties, xanthophylls are metabolized like other lipid-rich foods. Enzymes in the gut digest esterified L, and the free, unesterified, L is transported via micelles to enterocytes where it is absorbed passively or actively with the potential involvement of the scavenger receptor class B type 1 (SRB-1). Chylomicrons ferry L to the liver where it is incorporated in lipoproteins for travel through the blood stream and distribution to tissues.[45,46,81] Xanthophylls have a specific affinity to HDL for transport. However, they are also found in low density lipoprotein-cholesterol (LDL-C) and very low density lipoproteins (VLDL).[45,81-85] One study most frequently cited supporting HDL as the primary lipoprotein transporter of L and Z compared the dietary intake of the Wisconsin hypo-alpha mutant (WHAM) and control chickens. The WHAM chickens were the select model because 1) they did not have the ATP binding cassette transporter A1 (ABCA1), 2) they had minimal levels of HDL-C and, consequently, 3) had very low serum L and Z levels. The WHAM chicken was associated with humans who also have a mutation resulting in very low HDL-C. The WHAM chickens fed a high L diet had a noticeable presence of L in plasma and other tissues that receive it via LDL-C and VLDL transport, but minimal L in the retinal area. This study supported the function of HDL-C as the primary transporter of xanthophylls to the retina.[83]

Specific Protein Transporters

While the macula is a prime storage area for L and Z, the specific transporters to that site and other tissues including adipocytes continue to be studied.[86,87] Scavenger receptor class B proteins, including cluster of differentiation 36 (CD36), SRB-1 and SRB-2, all appear to have a role in xanthophyll transport and distribution to tissues. CD36 contributes to uptake of long-chain fatty acids into adipocytes. Due to the adipose tissue being a prime storage location of lycopene and L, adipose cellular cultures were used to study the uptake of L in adipocytes by CD36. Results of a study that used overexpression of C36 in cells supported its role in L absorption in adipose tissue.[86] In addition to supporting the role of SRB proteins in the macula, a study that measured L, Z, and MZ uptake by cells overexpressing SRB proteins supported the role of SRB-2 in the macula and displayed its affinity to Z and MZ and preferential uptake with increased HDL. However, increased L uptake occurred with a higher LDL concentration. This study also reported a variance in SRB retinal concentration location; CD36 was isolated to the RPE, while SRB-1 and SRB-2 were distributed throughout the retina and in the RPE.[87]

Activity in the Retina and Brain

While xanthophyll research has advanced, there are still questions about its absorption and functions in the retina and brain. The primate is the non-human model often used for MP research in these regions because, like humans, it has a macula.[88-90] Additionally, tissues of decedents of varying ages and “macular pigment mice”, those without β-carotene oxygenase 2 (BCO2), have been useful in supporting this body of

research.[48,91-96] It has been reported that L and Z preferentially traverse the blood retinal barrier (BRB) and are stored in the central retina.[88] The retina, an extension of the central nervous system (CNS), contains the macula lutea and the RPE.[45] The concentration of these carotenoids in the human eye are in the macula and range between 0.1 and 1 mM.[62] The fovea, located in the center of macula, has the highest concentration of L from prenatal through young childhood.[57,60] The macula also contains StARD3 and GSTP1, carotenoid binding proteins, which are specific to their respective xanthophyll, L (StARD3) and Z (GSTP1). The binding proteins strengthen the antioxidant properties and influence the distribution of their respective xanthophyll in the retina.[45,47]

While L and Z are selectively stored in the macula, it has been reported that L and Z cross the blood brain barrier (BBB) in a similar manner that they enter the retina.[62,88,96] Originally, Vishwanathan, et al. identified a significant relation between retinal L and Z in various regions of the primate brain.[88] Additional research using the primate model identified trans-L throughout all brain regions and suggested it served as an antioxidant protecting DHA in the brain.[90] Studies also suggested that the L accumulation in the tissues of the infant brain served as an antioxidant and may be an indicator of the infants general nutritional health.[92,93] When examining xanthophyll content of the adult brain, one study showed L and Z were concentrated in the cerebellum region, another reported a significant concentration of L in the occipital cortex, and a different one reported the frontal region as a prime location of L.[94-96] A study examining StARD3, the L-binding protein, reported its presence in the glial

regions of the brain, implicating its accumulation in this area.[45] While L has been identified as the primary brain carotenoid, L and Z together comprise 66-77% of the carotenoids in brain tissue including the frontal, occipital, and temporal cortices, cerebellum, and pons.[97]

Assessment Measures

Subjective and objective evaluations can be used to evaluate xanthophyll status. Common subjective measures include using participant responses or food logs.[47] A measure commonly used to assess MP is custom heterochromatic flicker photometry (cHFP), a validated non-invasive technique. Participants see a flickering stimulus between wavelengths of approximately 460 nm (blue light) to 540 nm (green light) and attempt to eliminate it based on their perception by adjusting the blue light. Ratios of different areas are identified and used to calculate the MP optical density (MPOD). The reliability of the measurements obtained from this technique are reliant on the participant's ability to adjust the blue light and their compliance in following directions.[47] This measure is commonly used in research studies because it does not require pupil dilation and is cost effective compared to other tests.[47,98] Analysis of serum L and Z concentrations are often used to assess L and Z adequacy. Serum L and Z levels span from 0.1–1.44 μmol/L, and 0.07–0.17 μmol/L, respectively, and appear reflective of dietary xanthophyll consumption.[62,97,99] Other objective tools used to measure xanthophyll status are dual-wavelength autofluorescence imaging, single or dual-wavelength reflectometry, resonance raman spectroscopy, and fluorescence lifetime

imaging ophthalmoscopy. These tests are most often used in clinical settings, require pupil dilation, and are expensive.[47]

While serum analysis and cHFP are commonly used research measures, the results they provide differ. Serum analysis are reflective of acute L and Z dietary intake and do not include MZ unless intentional supplementation has been used. [62,97,99] However, MPOD reflects the retinal MP including L, Z and MZ. It appears to be more stable with a slow turnover and limited impact of acute changes in dietary intake.[55,97,99,100] Both serum and MPOD have been used as assessment measures evaluating the relation between xanthophylls and cognitive performance. While there were inconsistent associations between serum xanthophylls and cognition, there was a more consistent relation between MPOD and cognitive test performance.[100] This study and other related research have identified MPOD as a biomarker for its concentration in the brain.[88-96,100]

Factors Affecting Xanthophyll Serum and MPOD Levels

Lifestyle, Body Fat, and Obesity

Varying factors have been reported to influence serum L and Z and MPOD levels, which supports individual variability of xanthophyll absorption. Smoking is one lifestyle behavior that has been associated with reduced MPOD. It was suggested this may be due to increased inflammation.[46] Individual adiposity was another factor that influenced MPOD levels. Serum L and Z and MPOD were measured in healthy young adults with a body mass index (BMI) of 23.4 kg/m^2. While there was not a significant relation identified between body fat and serum xanthophylls, those with higher body fat

had significantly lower MPOD. When analysis was conducted based on gender, men displayed a significant inverse association of body fat to MPOD while women did not.[101] An additional study that compared BMI to MPOD in healthy adults also displayed an inverse connection between these measures. Male and female participants with a BMI >29 and body fat of >27% had the lowest MPOD levels when compared to those with a lower BMI and percent body fat. While dietary L and Z intake was significantly lower in the group with the higher BMI, their serum L and Z levels were only slightly lower. It has been proposed that there is competition between the retina and adipose tissue for this fat soluble nutrient and that dietary L is deposited in adipocytes.[102] The study that supported the role of CD36 in L absorption in adipose tissue potentially aligns with this hypothesis.[86] Obesity, oxidative stress, increased inflammation and lower HDL are often associated with low MPOD and frequently occur in conditions that also have been reported to have low MPOD such as AMD and metabolic syndrome.[103,104]

Ethnicity and Genetics

Ethnicity and genetic variants also appear to impact serum xanthophylls and MP. An observational study that evaluated MPOD in subjects who were 70 years or older reported no significant change in MPOD due to age. However, race was reported to have a significant impact on MPOD when comparing black and white participants. The study, which included 23% black participants, reported the MPOD of black subjects when compared to whites was 41% lower.[105] Other studies that evaluated the relation between MPOD and ethnicity reported whites had a greater risk for lower L and Z.[104,106] One of the studies that identified white, non-Hispanic as higher risk for lower xanthophyll levels

also suggested that the genetic variants in those of African descent may be protective from AMD.[106] Genotypes were reported to impact MP absorption and stability.[104] A study that sought to determine the impact of genetic variants on individual response to MPOD and serum xanthophyll levels reported genetic variants from CD36, β-carotene monooxygenase 1 (BCMO1) and ABCG8 influenced MPOD and plasma L by 38% and 25%, respectively. It was also noted that participants with the lowest MPOD at baseline displayed the greatest response to dietary L consumption. Additional findings included identification of significant associations between BCOM1, CD39, MPOD and plasma L suggesting these genetic variants have a role in L metabolism.[107]

Functions

Retinal Development and Protection

Lutein and Z have varying functions in processes ranging from vision enhancement, neural activity, and cognition.[57,59] Xanthophylls appear influential throughout the lifespan with a distinct role in physiological retinal and CNS development during gestation.[58,62,93] MP protects the retina and improves visual acuity (VA) by filtering damaging ultraviolet (UV) (~380 nm) and short-wave blue lights.[46,57,61,108-110] Persons with a higher MP experienced reduced irritation and oxidative stress from blue light with 400-500 nm wavelengths.[46] Adults supplemented with L alone or combination of L and Z had positive blue light filtering capacity, improved contrast response enhancing night driving, reduced visual complications due to extended computer time, less visual fatigue and greater visual glare response.[57,58,109] The filtering ability and antioxidant properties associated with high MP may also reduce the incidence

of visual aging issues such as cataracts and AMD.[57,109,110]

Antioxidant and Anti-inflammatory Activity

Lutein and Z are effective antioxidant and anti-inflammatory agents that protect both the retina and brain, locations that are high in PUFA and vulnerable to oxidation.[45,111-115] In a study where peroxide was used to promote retinal oxidation of omega-6 PUFA, xanthophylls exhibited antioxidant protection by reducing oxidation under non-enzymatic conditions.[112] Lutein and Z were also influential in reducing oxidation and inflammation under oxidative stress in neonates and conditions of traumatic brain injury in rats.[53,113] Additional studies recruited healthy participants to evaluate the impact of xanthophylls on inflammatory markers.[52,114] Persons ages 18 to 25 were given a placebo or 13 mg or 27 mg xanthophyll supplement for a six-month period. Both supplement groups displayed significantly reduced inflammatory markers and significant improvements in serum xanthophyll levels, total antioxidant capacity (TAOC), MPOD, and brain derived neurotrophic factor when compared to the controls. Participants also displayed improved performance on some cognitive tests.[114] In a separate study, participants ages 20 to 80 years were given either a placebo or 10 mg or 20 mg L supplements daily for 12 weeks. Both supplement groups had significant increases in serum xanthophyll levels and TAOC. However, only the 20 mg supplement group had a significant reduction in the inflammatory marker, C-reactive protein, when compared to the placebo.[52] MP was also reported to reduce the level of ROS associated with both AMD and DR.[45,47,115] Additionally, L and Z appeared to have a role in

regulating functional properties of synaptic membranes, increasing membrane stability, and enhancing communication at gap junctions.[58,111]

Visual Cognitive Performance (VCP)

MPOD concentration was reported to influence VCP response time and visual processing.[108,116-118] A randomized placebo-controlled study conducted on young people evaluated the relation between MPOD and temporal contrast sensitivity function (tCSF), which correlates with the rate of visual processing. Subjects who were supplemented with L and Z had a significant improvement in both MPOD and processing speed.[116] Similarly, a double-blind placebo-controlled study that used MPOD and temporal vision as criteria to evaluate the visual processing speed of young adults ages 18-32 years reported significant benefits of L and Z. Study participants who consumed either Z or combined L and Z supplementation had a significantly higher MPOD and temporal vison processing than the placebo-group supporting a correlation with L and Z and visual processing speed.[117] Additionally, a year of L and Z supplementation significantly increased MPOD in the brain (hippocampus, frontal and occipital cortex) over this duration and improved visual remembrance, attention ability, and reasoning skills. This study suggests that L and Z supplementation may result in enhanced neural efficiency.[118]

Functional Benefits in Aging

Although atrophy has been reported to occur in identified regions of the aging brain, nutrients, including L and Z, have been shown to have functional benefits in older persons.[55,97,100,119-122] In a study of those older than 50 years, MPOD levels were associated with performance on diverse cognitive tests; there was a significant

association between MPOD and those with lowest test scores.[120] A study of older adults who completed a range of cognitive tests identified a significant association between MPOD concentration and enhanced visual processing; however, the same benefit was not associated with serum L and Z.[100] Additionally, a study that evaluated L and Z serum levels, MPOD and cognitive ability reported a significant association between MPOD and cognitive test scores. However, this same association did not exist between L and Z serum levels. Instead, there was only a significant association with L and Z serum levels and a single cognitive test.[55]

Functional magnetic resonance imaging (fMRI), a technique that has been used to assess the brain transformations that occur with aging, was used to evaluate the impact of L and Z on neural efficiency during the completion of identified tasks in aging persons.[97,121] This study linked L and Z serum concentration and MPOD to more efficient neural processes including enhanced visual performance.[97] Retinal and serum L and Z were also evaluated when participants completed specific tasks during a fMRI scan. Similarly, those with greater levels of L and Z demonstrated neural efficiency in various regions of the brain associated with visual processing and ability to make decisions.[122]

Three-Dimensional Multiple Object Tracking (3-D MOT)

Three–dimensional multiple object tracking (3-D MOT) software has been used with varying groups including aging persons to improve cognitive ability.[4,123-129] The 3-D MOT training program was identified as a robust intervention to evaluate individual cognitive skills and to enhance VCP. The validated 3-D MOT program is designed to

train four main aspects of perceptual-cognitive function including 1) attention while tracking multiple digital objects 2) over a large visual field 3) with individual task performance measured using a maximal speed threshold (ST), and 4) visual cues that are three-dimensional (3-D).[123-125] The CT process involves tracking the spatial location of multiple target spheres, identified before initiating the trial, among other distractors identical in shape and color. The spheres move at a given speed within a 3-D virtual space, colliding with one another or the edges of the screen and changing direction, as well as passing in front of or behind one another. If the participant correctly identifies the spheres that were originally highlighted after six seconds (6s) of movement, the speed will increase for the next trial. However, if one or more spheres were missed, the speed of sphere movement will decrease for the next trial in an one-up, one-down staircase pattern.[130] When comparing the number of objects adults could track, older adults could track three objects while younger persons had the ability to track four or five.[131]

Benefits of 3-D MOT Training

The individualized speed and difficulty levels of the 3-D MOT training were shown to consistently challenge trainees and enhance their VCP.[124-129] Studies using the 3-D MOT program reported improved attention span, memory, reaction time and demonstrated quantitative changes in brain function in college age subjects who participated in 10 training sessions.[125] When NT training was used to compare the performance of younger and older healthy adults, the younger subjects had higher performance scores, but both groups had equivalent learning progression. While the

score progression of the younger participants leveled off at the fifth week, the scores of the older subjects were still improving when the study ended.[126] Studies using the 3-D MOT program to train elderly subjects reported a significant improvement in VCP including cognitive ability, processing speed and attention when compared to controls.[123,127] Further, older adults who had subjective cognitive decline (SCD) showed significant improvement in memory and VCP after 3-D MOT training when compared to controls.[4]

The 3D-MOT training is suggested to be equivalent to processing requirements in dynamic environments. Legault and Faubert tested the 3-D MOT program to see its impact on biological motion perception or the ability to recognize movement of humans and respond to it. The experimental group that trained weekly on the 3-D MOT for five weeks performed significantly better on biological motion tasks when compared to controls. This training may be useful to enhance the ability of aging persons in dynamic activities requiring anticipation of human movement. An example may include recognizing a cue for a potential collision while driving and avoiding it.[12]

Summary

In summary, solutions must be identified to enhance the quality of life for aging persons and their caregivers and reduce negative personal and societal financial implications due to an increased aging population and the associated prevalence of cognitive decline in this population. Enhanced nutritional status is one potential approach to resolving these issues. Observational research studies have shown a correlation between the L and Z dietary intake and increased MP in the retina and brain.

Additionally, the functional properties of xanthophylls appear to display benefits that have the potential to positively impact conditions such as progressive dementia, AMD, CVD and DM. Combining the benefits of xanthophyll and the 3-D MOT cognitive training program provides a promising solution to the societal challenges of aging and cognitive decline.

CHAPTER III IONSPORT PRELIMINARY STUDY

Materials and Methods

The purpose of the IONSport preliminary study was to examine the influence of free-living nutritional intake on visual cognitive performance (VCP) in young healthy adults. The NeuroTracker™ CORE (NT) 3-Dimensional (3D) software program was used to identify individual perceptual cognitive ability and provide training. Ten daily food logs were used to evaluate nutrition intake. We were unaware of any studies that evaluated the effect of nutrient intake to visual cognitive training (CT) using the NT software.

Subjects

Men and women aged 18 to 33 years were recruited for the IONSport Study using a convenience sampling strategy. Email, posting fliers and direct contact with student groups on the Texas A&M University campus were used to recruit participants. Individuals with a BMI less than 18, relying upon a pacemaker, experienced vertigo, or had difficulty with 3D viewing were excluded. Persons who were unable to distinguish between yellow and orange due to color blindness were also excluded since the training program required the ability to distinguish these colors. All participants signed an informed consent, and the study protocol was approved by the Texas A&M University Human Subject's Institution Review Board (IRB).

Screening and Baseline

Prior to the start of the study, baseline data including blood pressure (BP); heart rate (HR) (Omron Healthcare, Inc. Bannockburn, Il.); visual acuity (VA) using the

Snellen Chart; and body composition using the Biometric Beurer BF 520 BIA technology (Beurer, Germany) was collected.[132,133] Subjects completed the Pittsburg Sleep Quality Index (PSQI) and the Modifiable Activity Questionnaire (MAQ) to provide detailed sleep and physical activity patterns.[134,13]

Nutrition Education and Monitoring

Subjects were instructed to continue their normal eating behaviors and physical activity throughout the study. Participants received direct guidance and were also emailed five, one-to-two-minute online instructional videos on how to properly measure and document food and beverage consumption prepared by a registered dietitian nutritionist (RDN). Each food log was reviewed daily by an RDN and if necessary, additional details were collected to ensure high quality food logs that were complete and accurate. Subjects were asked to log all food and beverages consumed on each of the 10 cognitive training days. Food logs were entered into Nutribase 19 Pro (NB) for analyses.

Visual Cognitive Testing

Participants completed 15 cognitive training sessions using the NT 3-D software program over 10 training days that included alternating single and double training sessions with 4-5 interspersed days of no training (15 total days of the study). During the first training session, the CT procedures were explained to participants. Participants were seated in a chair aligned 4½’ from a 3-D television in a dark, quiet testing room with the seat adjusted so that their eyes were positioned at the center of the screen. They wore active 3-D glasses and noise-cancelling headphones during testing to avoid distractions.

Participants who relied on glasses for corrected vision wore them under the 3-D glasses when training.

Each training session included tracking the spatial location of four pre-identified target spheres that were initially a different color from the other four spheres. Once identified, these spheres became identical in color to the four other spheres. All eight identical spheres moved among each other at a given speed within a 3-D virtual space, passing in front of or behind each other, colliding with each other or the edges of the screen and changing directions. After six seconds (6-s) of movement, the spheres stopped, and the participant picked out the four pre-identified spheres. If the subject selected all four of the correct spheres, the speed of sphere movement increased for the next 6-s trial. If one or more spheres was missed, the speed of sphere movement decreased for the next trial in an one-up, one-down staircase pattern.[130] Subjects performed 20 trials within a single training session obtaining a "speed threshold," (ST) the level at which the participant correctly tracked and selected the correct objects 50% of the time. The final ST for each training session and the progression over 15 sessions were the primary determinants of cognitive performance.

Each of the 10 CT days included collection of recent physical activity, fluid intake, most recent urine color (validated urine color scale), BP, HR, readiness to perform, body composition, Stanford sleep questionnaire and hours of sleep the previous night.[136-138]

Statistical Analysis

IBM SPSS software (v 27) was used for all the analyses. Data are presented as estimated mean ± SD and $P<0.05$ was considered statistically significant. Ten-day nutrient averages of all food and beverage logs provided by NutriBase 19 Pro Edition, v. 19.2 software (NB) (CyberSoft, Inc, Houston, TX) were used for nutrition data analyses.

The mean and maximal performance levels were used to identify individual NT performance and associations between the NT ST and the specified nutrients CHOL, choline, L and Z. Since eggs are a rich source of many of the nutrients reported to influence perceptual cognitive performance, the nutrients in two large whole eggs (WE) daily were used to establish limits for data analysis.[32-34] Choline and CHOL were analyzed simultaneously since these nutrients are highly correlated in the diet preventing evaluation of their independent effects. Participants were divided into two groups with one group consuming more than two WE worth of CHOL (>400 mg/day) and choline (>237 mg/day) and the second group consuming less than the amount of CHOL and choline in two WE.

Lutein and Z were also analyzed simultaneously using <2,000 μg/day as the dividing marker between the two groups of participants since this amount is associated with the highest risk of AMD. Covariates analyzed included gender, protein (g/kg/day), carbohydrate intake (kcal/day), hydration, VA, and sleep.

Results

The IONSport Study enrolled 109 participants, and 99 (37 males, 62 females) had complete data sets for analyses (10 daily food records and 15 NT sessions in 15

days). The independent sample t test was used to determine significance among participant characteristics. Table 1 shows the baseline characteristics of the population with significant differences in the males in age (P<0.05), weight, body fat and BMI (P<0.001). There were no significant differences among the female participants.

Table 1. Participants Baseline Characteristics

	Males (N=37)	Females (N=62)
Age (years)	22.7 ± 3.0# (18-29)	20.9 ± 3.6 (18-33)
Weight (kg)	80.4 ± 10.4* (62-109)	60.5 ± 9.2 (46-85)
Body fat (%)	12.7 ± 3.5* (5-19)	23.7 ± 5.5 (15-40)
BMI ($kg \cdot m^{-2}$)	25.3 ± 2.7* (19-30)	22.5 ± 3.1 (17-31)

Data are presented as mean ± SD. Denotes significant difference
*P<0.001, # P<0.05.

Participants submitted dietary records of their normal intake each day they participated in CT resulting in 10 food records each. Intake was averaged and analyzed and used to identify nutrients that potentially influenced VCP. Table 2 shows the participant mean intake of calories, macronutrients, and nutrients of interest. The mean intake of carbohydrates (g/day), protein (g/kg/day), and riboflavin (vitamin B2; mg/day) were higher than the Dietary Reference Intakes (DRI) for this population. The mean fat intake for men and women was slightly higher than the recommended 30% of total calories, and cholesterol intake was higher than the recommended 300 mg/day in males.

The mean choline intake for males and females was 50% or less than the DRI recommendations. There is not a DRI for L and Z, but 2-12 mg L and Z daily is recommended for good eye health.[45] There were significant differences in caloric intake, macronutrients, CHOL, and choline for male participants but not for the nutrients L, Z, or riboflavin.

Table 2. Participants Average Dietary Intake For 10 Days

	Males (N=37)	Females (N=62)
Calories (kcal/day)	2240 ± 489*	1710 ± 394
Protein (g/day)	124.2 ± 0.56*	79.7 ± 26.7
Protein (g/kg/day)	1.54 ± 49.4#	1.34 ± 0.44
Carbs (g/day)	251.2 ± 77.3*	206.4 ± 56.8
Fat (g/day)	82.5 ± 49.4*	65.4 ± 23.0
Cholesterol (mg/day)	440.9 ± 318.9*	233.6 ± 163.5
Lutein/Zeaxanthin (μg/day)	2359 ± 6062	2178 ± 4105
Vitamin B2 (mg/day)	3.7 ± 10.3	1.3 ± 1.6
Choline (mg/day)	274.1 ± 264.1*	135.2 ± 119.7

Data are presented as mean ± SD. Denotes significant difference *P<0.001, # P<0.05.

Cholesterol (CHOL) and Choline

Controlling for gender, protein and carbohydrate intake, the high CHOL/choline group performed significantly better than the low group (P<0.028) as seen in Figure 1. In separate analyses, data indicated that men performed better than women (P<0.001)

and very high protein consumption (>1.4 g/kg/day) was associated with poor cognitive performance (*P*=.013).

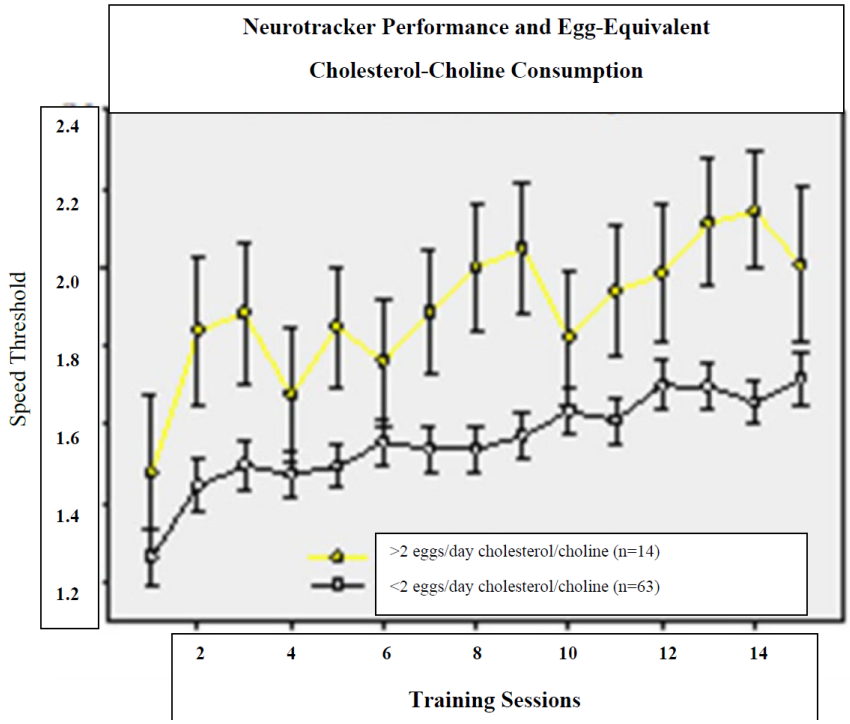

Figure 1 CHOL/ Choline Intake with Neurotracker Performance

Lutein (L) and Zeaxanthin (Z)

The mean ST from the first three sessions was compared to the ST mean of the last three session for the >2000 ug/day and the <2000 ug L and Z groups. After controlling for gender, there was not a significant difference between the L and Z groups (0.29±0.03 vs. 0.35±0.06, *P*>0.05) as seen in Figure 2. However, mean (1.57± 0.04 vs. 1.79±0.07, *P*=<0.001) and maximal (2.14±0.05 vs 2.36±0.09, *P*=0.031) cognitive performance as indicated by the ST was significantly higher in the high L and Z intake group.

Five participants in this study reported a family history of AMD. We compared the VCP of these participants to those without a history of AMD to determine predisposition effects. Figure 3 shows that participants with a family history of AMD had a lower mean speed threshold across 15 training sessions but this was not significant (P>0.05).

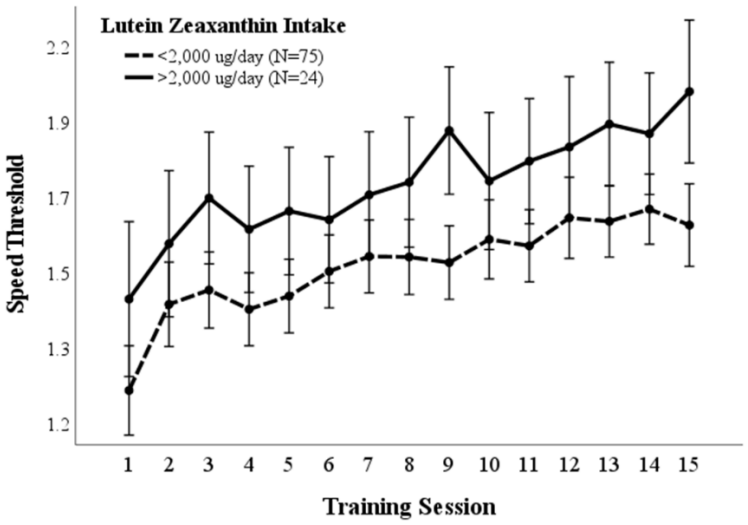

Figure 2 Lutein/Zeaxanthin Intake with Neurotracker Performance

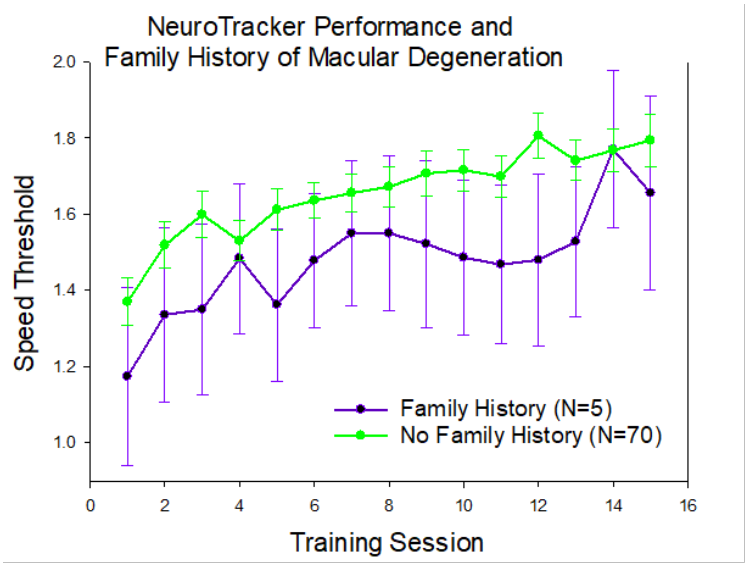

Figure 3 IONSport: Cognitive Performance With and Without Family History of AMD

Regression

The mean and the maximal ST of the first three NT sessions was compared to the final three sessions in the linear regression analysis. Independent variables evaluated included gender, L and Z, protein, riboflavin, and carbohydrate intake. Tables 3 and 4 display significant positive linear relation between L and Z and carbohydrate intake and the mean and maximal NT ST, respectively. Conversely, both show a significantly negative linear relation between protein intake and the mean and maximal NT ST. This analysis displays male subjects performed significantly better than females on the mean and maximal NT ST.

Table 3 Linear Regression Analysis with Neurotracker (NT) Mean Speed Threshold (ST)

Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
(Constant)	1.534	.215		7.130	.000
Gender 1=M, 2=F	-.208	.068	-.289	-3.047	.003
Lutein Zeaxanthin 2mg	.226	.073	.281	3.085	.003
Protein (g/kg/day)	-.210	.067	-.302	-3.140	.002
Riboflavin 1.8 mg	.155	.080	.186	1.924	.058
Carbohydrate (g/day)	.001	.000	.218	2.316	.023

Table 4 Linear Regression Analysis with Neurotracker (NT) Maximum Speed Threshold (ST)

Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
(Constant)	2.204	.283		7.797	.000
Gender 1=M, 2=F	-.296	.089	-.312	-3.332	.001
Lutein Zeaxanthin 2mg	.226	.097	.210	2.337	.022
Protein (g/kg/day)	-.266	.089	-.285	-2.997	.003
Riboflavin 1.8 mg	.176	.104	.162	1.697	.093
Carbohydrate (g/day)	.002	.001	.234	2.521	.013

Discussion

The linear regression models in Tables 3 and 4 support a positive relation between participant consumption of >2,000 µg/day L and Z and carbohydrates, the independent variables, and VCP. The results of this study align with other research

studies that show a positive relation between L and Z and enhanced cognitive performance. Eggs were used in this model due to their nutrient-rich profile that includes L, Z, and choline, nutrients that must be obtained in the diet. [16,21,57 34] Additionally, they provide several of the same nutrients found in the leafy green vegetables associated with improved cognition throughout the life span. However, eggs enhance the bioavailability of L and Z, fat soluble carotenoids, due to the high lipid level of the yolk.[16,34,63,69] Additional nutritional benefits of eggs are that they are a lower calorie (72 kcal), complete protein that provides phospholipids, amphipathic molecules that have been shown to enhance high-density lipoprotein cholesterol (HDL-C).[33,34,36] There were previous recommendations to reduce egg consumption due to CHOL content; however, more recent studies have shown benefits of egg consumption related to increased HDL-C, lean muscle, and weight management.[33,36]

Enhanced visual cognitive performance (VCP) by study participants aligns with the reported functions of L and Z including retinal protection and enhanced cognitive performance. L and Z, along with meso-zeaxanthin (MZ), comprise the macular pigment (MP). MP, measured by macular pigment optical density (MPOD), has been shown to reduce irritation and oxidative stress from short wave blue lights[46] Low MPOD is associated with the occurrence of AMD, a condition resulting in impaired VA and gradual loss of vision. Risks for low MPOD and AMD appear to be similar and include low intake of L and Z and genetic factors.[104,110,111] Those with reported family history in our study did not have clinical symptoms of AMD yet had a lower VCP and intake of L and Z. Supplementation with Studies have shown that supplementation of L resulted in

positive blue light filtering capacity, improved contrast response, and reduced visual complications.[57,58,109] Evaluating the relation of L and Z intake and VCP in younger adults with a family history of AMD may enhance our understanding of how AMD progression could be reduced.

While the highest concentration of the MP is in the macula, MPOD was also located in the brains of infants, healthy young adults and older male and female decedent donors.[92-96] MPOD has been identified as a biomarker for the MP concentration in the brain and was reported to influence cognitive performance, response time and visual processing.[34,58,88,96,118] A randomized placebo-controlled study evaluated the association of MPOD and temporal contrast sensitivity function (tCSF) which correlates with the rate of visual processing. Subjects who received L and Z supplementation significantly improved in MPOD and processing speed.[116]

Supplementing young fit subjects for four months with L and Z in a double-blind placebo-controlled study also significantly improved their CFF thresholds, response rate and their speed of processing.[117] A study that examined the relation of MPOD and pre-adolescent academic ability also reported a significant association with academic performance and MPOD[18] MPOD was also related to enhanced cognitive performance in older persons.[100] These studies may suggest that lutein and zeaxanthin supplementation may result in enhanced neural efficiency and contribute to VCP throughout the lifespan.[32,58]

Individuals should modify their dietary patterns to include L and Z to receive the benefits of enhanced VCP that these nutrients offer. The average adult's intake of L and

Z was reported at an average of 1–2 mg/day, which is low when compared to the 6 mg/day that is recommended to reduce the risk of macular degeneration.[57] Lutein and Z levels can be improved through increased consumption of nutrient-dense foods including avocado, cantaloupe, orange, kiwi, spinach, green leafy vegetables, orange and yellow pepper and carrots.[57,59,61-64,71] Whole eggs are a versatile, economic food that delivers an exceptional amount of nutrients in consideration of their caloric contribution to dietary intake.[34,] In addition to providing high biological value protein, eggs provide B vitamins, choline, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), including the Omega-3 FA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and L and Z, carotenoids that are not endogenously produced.[34] Based on the health benefit and nutrient density that eggs provide, it is an exceptional food to incorporate in the diet patterns of all persons. [33,34,36] For persons who are unable to achieve adequate levels of L and Z in the diet, supplementation is an option for consideration.[71]

Limitations

Limitations of this study include the subject population and the length of the study. The young, intelligent population limits the generalizability of the results to other age groups. A longer study would provide more reliable data. While we used 10 days of food logs in analyzing dietary intake, reliance on dietary recall to assess nutrient status may result in potential errors due to underreporting food and beverage consumption, inaccurate recall, inability to accurately assess portion sizes, and bias associated with education, economic status, age, and gender. Even if subjects accurately reported dietary intake, other biochemical assessment indicators are necessary to assess participant's MP

and absorption of nutrients consumed. While serum L and Z and MPOD have been used to measure L, Z and MP, serum L and Z levels represent the acute intake of these nutrients while MPOD provides a more reliable measure of MP status. biochemical indicators can be used for evaluation, MPOD would provide an objective measure of lutein and zeaxanthin and validate the reported dietary intake.[97,99]

Next Steps

This study supported the benefits of CHOL, choline, L and Z in enhancing VCP in young healthy adults. The lower VCP of participants with a family history of AMD when compared to those without stimulated our interest in conducting a future study with L as the dietary treatment and MPOD as the assessment parameter to enhance our understanding of the potential factors influencing AMD progression.

We are also interested in evaluating the impact of Omega-3 FA, particularly DHA, in relation to L and Z and its role in VCP due to its high concentration in the retina, influence on vision, and risk for oxidation. We were unable to evaluate the impact of Omega-3 FA on VCP in this study because the majority of our study population had a very poor intake of this nutrient.[61] We would like to understand the relation of Omega-3 FA to visual health and its potential role in slowing cognitive decline in aging populations.[18,22,26,61]

Conclusion

This study provided strong evidence that L, Z, and choline contribute to enhanced VCP. Cognitive testing scores and L consumption patterns should also be studied to evaluate any significant potential association with AMD. These studies

should incorporate baseline and post serum and MPOD analysis to confirm dietary records and identify changes in assessment measures that occur from baseline to the end of the study. Additionally, eggs should be studied further since they are a quality source of many nutrients including those that influenced enhanced cognitive ability in this study.

Studies also have shown cognitive decline (CD) with aging and a critical need for intervention due to the reduction of quality of life, increased challenges associated with this condition and the exorbitant personal and societal financial costs.[1,2] At the same time, studies have reported that cognitive training (CT) is an intervention that has benefitted those with subjective cognitive decline (SCD) [4,12,126] Based on the results of this and other studies, combining CT using the NT with a nutritional intervention such as eggs in the diet of older persons should be studied since this age group is expanding rapidly and is at high risk for CD. Eggs are an economical source of high biological value protein and nutrients, such as L, Z and choline which may enhance VCP in older persons.[32-34]

CHAPTER IV NUTRITION, VISION, AND COGNITION IN HEALTH: EGG (IONHEALTH-EGG) STUDY

Materials and Methods

The preliminary IONSport study performed on healthy men and women ages 18-35 years identified a positive relation between the nutrients found in eggs and enhanced visual cognitive performance (VCP). Based on these observations, the reported positive benefits of xanthophyll intake, and 3-D MOT cognitive training, a randomized controlled Nutrition, Vision, and Cognition in Health: Egg (IONHealth-Egg) study was implemented to evaluate the impact of the nutrients in eggs, specifically L and Z, on VCP in aging individuals.

Subjects

One hundred six generally healthy men (40) and women (66) aged 50 to 75 years were recruited for the IONHealth-Egg study using a convenience sampling strategy. Email, morning television announcements, posted fliers, and direct contact with seniors at local senior centers and community events including the Senior Expo and Mature Well Lifestyle Center cooking classes were the prime forms of recruitment.

Based on advertisements and recruitment, interested individuals contacted researchers via email, phone, or website to indicate interest and request more information. A standardized email explaining the details of the study and an informed consent form were emailed to all interested persons. Once the investigators received the signed consent form, prospective participants were asked to complete a questionnaire to

determine if they met preliminary inclusion criteria; the questionnaire addressed general health status, prescription and supplement use, food intake behaviors and visual health. Once preliminary inclusion criteria were confirmed, potential participants were scheduled to take the Mini Mental State Exam (MMSE), a validated screening instrument used to confirm the participant's cognitive capability to keep food logs throughout the study.[139]

Inclusion criteria required that all participants had a corrected visual acuity (VA) of at least 20/40 and women were two years post-menopausal. Persons who were vegan, had an egg allergy, took supplements with >6 mg L and/or >2 mg Z two months prior to the study or self-reported diagnoses of vertigo, AMD, diabetic retinopathy (DR), glaucoma, retinitis pigmentosa, optic neuropathy, retinal vascular occlusions, strabismus, autoimmune disorders related to visual health, MCI, dementia and/or AD were excluded. Persons who were unable to distinguish between yellow and orange due to color blindness were also excluded because the training program required the ability to distinguish these colors. In our pilot study, those with pacemakers were excluded because of the negative consequence of assessing body composition using BIA. However, we permitted those with pacemakers in this study if they met all other inclusion criteria, but we weighed them on a traditional hospital scale instead of using the BIA to gather body composition data.

The benefits of participating in the study included individual body composition information, nutrition education with a full dietary analysis by a registered dietitian nutritionist (RDN), the opportunity to train on software being used by professional

athletes, and provision of eggs throughout the study. Participants were also provided a LETSCOM Fitness Tracker HR, Activity Tracker Watch with Heart Rate Monitor, IP67 Waterproof Smart Bracelet with Step Counter (Athleteks, LLC, Plantation, FL) that they were able to keep after study completion.

The study exposed participants to minimal risks including the potential for dizziness from the 3-D effects, bruising, soreness and/or infection due to blood draws, and/or emotional stress due to revealing medication usage and/or logging food consumption. Subjects with pacemakers were at risk for exposure to bio-electrical impedance analysis (BIA) technology and the Mighty Sat Fingertip Pulse Oximeter and those with physical hand limitations were at risk due to the measurement of hand grip strength. Risks were minimized by using color-coded charts to identify subjects with pacemakers and modifying the standard protocol for them.

All participants signed an informed consent and were identified by an assigned subject number. The study protocol was reviewed by the Texas A&M University Human Subject's Protection Program and/or the Institution Review Board (IRB).

Study Timeline

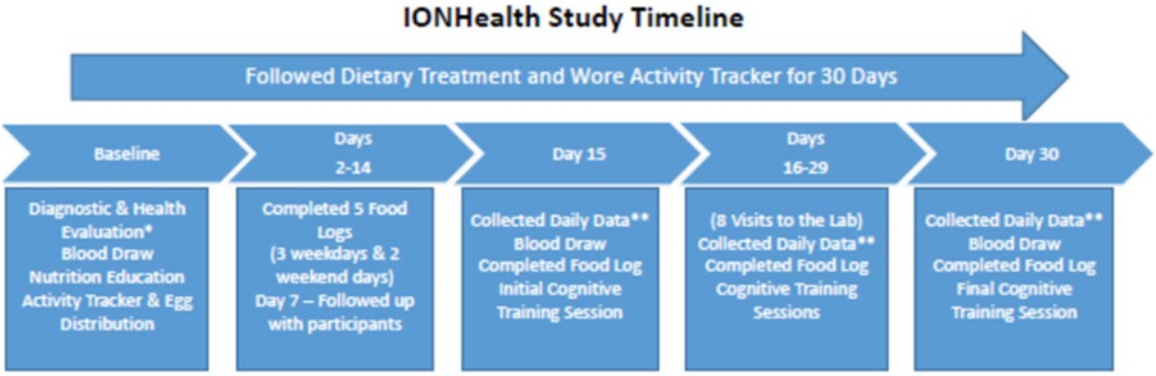

Figure 4 IONHealth-Egg Timeline
*Includes blood pressure (BP), heart rate (HR), vision acuity (VA), body composition (BIA), arterial oxygen saturation (SpO2), hand grip, standard questionnaire, Pittsburgh Sleep Quality Index (PSQI), and Modifiable Activity Questionnaire (MAQ)

The study timeline (Figure 4) required a 30-day commitment to eliminating or consuming eggs based on the dietary treatment, a minimum of 11 in-person visits to the lab on the Texas A&M University campus and participation in visual cognitive training (CT) during the last two weeks. Diagnostic and health evaluations were conducted at baseline. Fifteen food logs were completed during the study; five were completed during the first two weeks of the study and the remaining 10 were completed on each cognitive training day. Standard diagnostic measures, physical activity, and sleep patterns were recorded at each CT appointment. Serum collection was performed at baseline, days 15 and 30.

Dietary Treatment Groups

Each participant was randomly assigned to either a no-egg control (NEC) or one of the following dietary treatment groups: 4 large egg whites (EW) daily; 2 large whole

eggs (WE) daily; 2 large whole omega-3 fortified eggs (O3E) daily; or 4 large egg yolks (EY) daily. Participants were asked to follow their routine food and beverage intake other than the assigned dietary treatment guidelines. Participants in the egg treatment groups were permitted to prepare and consume them daily based on their preferred method.

Subjects were instructed to record all food and beverage consumption on provided food log forms or digitally for a minimum of 15 days, including three week and two weekend days during the first two weeks of the study and the remaining 10 on each training day. Resources provided to assist with accurate food documentation included written guidelines for the participant's treatment group, sample food logs displaying accurate food and beverage records and five RDN-prepared online instructional videos that demonstrated proper measurement and documentation of carbohydrates, proteins, fats and condiments, fruit and vegetables and dairy. The videos were emailed to each participant and were approximately one to two minutes in length.

Subjects who digitally recorded their intake were also emailed five NutriBase 19 Pro Edition -prepared 1-2 minute instructional videos describing how to set up a user profile, keep a food log using the software and export and email food logs to the researchers. Participants were asked to email the digitally entered food logs to the researchers on day 15 and each day they did cognitive testing thereafter.

All eggs used in the study, other than the large omega-3 FA eggs were from Keike Egg Farm (Burton, TX). Kroger Private Selection Christopher All-Natural Eggs Large Grade A-660 mg Omega-3 FA produced by Rose Acres (Seymour, IN) were

provided to the participants in the O3E treatment group. The nutritional content of the eggs varied based on the feed patterns.

Researchers purchased the eggs as needed throughout the study and maintained them at refrigerated temperatures in the lab until distributed. Participants were given a 15-day supply of eggs based on their treatment group on day 1 and again on day 15. They were blinded to the brand of eggs used in the study. All eggs were distributed in cartons that had a study label glued over any identifying information including nutrition content; Keike eggs were distributed in yellow styrofoam containers, and the Omega-3 FA eggs were in gray cardboard containers. The distinctive packaging helped researchers provide the correct eggs to the participants based on their treatment group.

Nutrition Assessment

All food and beverage logs were analyzed using NutriBase 19 Pro Edition, v. 19.2 software (NB) (CyberSoft, Inc, Houston, TX). Researchers entered the food intake records into NB using the subject's number for participants who logged their food. Researchers reviewed the digitally entered food logs for accuracy and uploaded them to the IONHealth study records. Nutrient averages were used in the final analysis.

Diagnostic and Health Assessment

Diagnostic baseline data included: BP; HR (Omron Healthcare, Inc. Bannockburn, Il.); VA using the Snellen Chart; body composition using the Biometric Beurer BF 520 BIA technology (Beurer, Germany); SpO_2 using the Mighty Sat Fingertip Pulse Oximeter (Masimo U.S.); and measurement of hand grip strength using the Camry

Electronic Hand Dynamometer (Camry Scale – USA, South El Monte, CA.).[132,133] When recording BIA, the scale was set with the participant's gender, their actual height and at a standard age of 60 years and non-athlete activity level. Diagnostic accommodations were made for participants with pacemakers and physical hand limitations; participants with pacemakers were weighed on a traditional hospital scale. Subjects with physical hand limitations did not have their hand grip strength measured.

Questionnaires were used to gather additional participant information. A standard questionnaire was used to identify usual dietary practices, nicotine use, concussion history, and video gaming activities. The Pittsburg Sleep Quality Index (PSQI) identified sleep patterns and issues that impacted sleep, and the Modifiable Activity Questionnaire (MAQ) provided extensive historic and current physical activity patterns.[134,135]

Data collected each training day included standard diagnostic tests, physical activity, and participant information using a standardized daily information form. Standard diagnostics included BP, HR, BIA, SpO_2, and hand grip strength. Physical activity and sleep patterns were recorded from the LETSCOM Fitness Tracker HR, Activity Tracker Watch that all participants wore throughout the study, except when showering or swimming. The daily information form provided the participant's readiness to perform, sleep status based on the Stanford sleep questionnaire, body composition, any medications taken over the last 12 hours, most recent exercise, beverages not recorded on the food log and hydration status using a clinically validated urine color scale.[136-138]

Visual Cognitive Training

The NT 3-D software program used in the IONSport pilot study was also used to evaluate individual VCP and provide CT to the older participants. Participants completed 15 NT training sessions over 10 days that included alternating single and double training sessions with 4-5 interspersed days of no training.

On the first training day, researchers explained the cognitive testing procedures to participants and set up the NT software using their assigned number. Participants were seated in a chair aligned 4½' from a 3-D television in a dark testing room with their eyes positioned at the center of the screen. Participants wore active 3-D glasses and noise-cancelling headphones during testing to avoid distractions. Participants who relied on glasses for corrected vision wore them under the 3-D glasses when training.

Each training session included tracking the spatial location of four pre-identified target spheres that were initially a different color from the other four spheres. Once identified, these spheres became identical in color to the four other spheres. All eight identical spheres moved among each other at a given speed within a 3-D virtual space, passing in front of or behind each other, colliding with each other or the edges of the screen and changing directions. After 6-s of movement, the spheres stopped, and the participant picked out the four pre-identified spheres. If the subject selected all four of the correct spheres, the speed of sphere movement increased for the next 6-s trial. If even one sphere was missed, the speed of sphere movement decreased for the next trial in a one-up, one-down staircase pattern.[130] Subjects performed 20 trials within a single training session obtaining a "speed threshold," (ST) the level at which the participant

correctly tracked and selected the correct objects 50% of the time. Speed threshold of each training session over the two-week period were used to identify significant training adaptations.

Although the task was virtual, the speed of the object movement was equivalent to real world speed to the viewer calculated as 68 cm/second at a speed of 1.0. The NT responses directly represented participant attention and previous work also demonstrated responses are associated with executive function, memory, and processing speed.[6,12,124]

Serum Analysis

Participant serum samples were stored in a -80°C freezer until processing. A maximum of 18 samples were thawed at room temperature for approximately 15 minutes prior to analysis of L and Z. When extracting L and Z, 200 µL serum was mixed with 200 µL distilled water and spiked with 200 µL beta-apo-8'-carotenal, the internal standard. Next, 200 µL non-acidified methanol was added, and the mixture was vortexed. A total of 2 mL acetone: hexane (AH, 1:3) was added incrementally over three extractions with each sample being vortexed and centrifuged after each addition. The upper AH supernatant was removed and added to the same test tube after each extraction. The cumulative sample was dried under nitrogen, and the remaining carotenoids were re-solubilized with 500 µL non-acidified methanol (1 ppm). The samples were filtered into amber colored storage vials using 0.22 µM polytetrafluoroethylene (PTFE) syringe filter, labeled and frozen in preparation for high performance liquid chromatography (HPLC). Blank and neat samples were used as quality standards throughout analysis. HPLC was run using the TSQ Altis LC triple

quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) that included 2.1 x 50 mm Thermo Scientific Accuore Vanquist C18 column. The mobile phase used was 50:50 Methanol: Acetonitrile with 0.05M ammonium acetate at a 0.5 mL/min, isocratic flow rate with positive ion spray voltage of 4200, 60 units of sheath gas, 2 units auxiliary gas and no sweep gas. The temperature of the ion transfer tube was 375°C and the vaporizer temperature was 50°C.

The quantitative and qualitative serum lipoprotein profile was evaluated with Lipoprotein Density Profiling and Density Gradient Ultracentrifugation and Fluorescence Spectrometry. The detailed description of the procedure for the measurement of the lipoprotein profiles for clinical research is described elsewhere by Larner, et al.[140] Enzyme-linked immunosorbent assay (ELISA) and chemical kits were purchased commercially and used to measure lipids. Apo B was analyzed using the Human Apo B/ Apolipoprotein ELISA Kit. CHOL and cholesterol esters (CE) were measured using the CHOL/CE Quantitation Kit, and triglycerides (TG) were measured using the TG Quantification Colorimetric /Fluorometric Kit. Lipoproteins were measured using the HDL and LDL/VLDL Quantitation Kit. Baseline and 30-day serum samples were used for analysis.

Statistical Analysis

IBM SPSS software (v 27) was used for all the analyses. Data are presented as estimated mean ± SD and $P<0.05$ was considered statistically significant. Statistical analyses were performed in SPSS using independent sample t test, the one-way analysis of variance (ANOVA), repeated measures ANOVA, ANCOVA and multiple regression.

ANOVA was used to analyze the differences between the control and treatment groups. The paired-sample t test was used to evaluate the differences in serum samples from baseline to the end of the study. Repeated measures ANOVA was used to evaluate cognitive training progression over time. Linear regression was used to identify significant independent predictors of VCP. Blood analysis was conducted at baseline, 15 days, and the study endpoint of 30 days.

Hypothesis

It was hypothesized that subjects who consumed the highest levels of L, Z, choline, CHOL and Omega-3 FA via daily EY would have a significantly greater response and overall VCP than NEC and that eating EY daily for 30 days would not have a significant negative impact on serum biochemical markers including CHOL, CE, Apo B, TG, and lipoprotein levels.

Results

Enrollment and Completion Data

One hundred twenty participants signed an informed consent and were identified by an assigned subject number. The study protocol was reviewed and approved by the Texas A&M University Human Subject's Protection Program, Institution Review Board (IRB).

Table 5 shows the numbers of those consented to participate, those who withdrew or completed the study by treatment group. The no-egg control (NEC) and two whole eggs/day (WE) groups both had five withdrawals compared to no withdrawals

from the four-egg yolk/day (EY) group. Three of those who withdrew from the NEC group reported the inability to exclude eggs from their diet for 30 days, while those that withdrew from the (WE) group were for various personal reasons. The national COVID pandemic resulted in immediately halting the study due to university-rules prohibiting in-person activities on campus throughout the spring and fall 2020 semesters. COVID and national quarantine requirements also resulted in one active study participant being dismissed from the study prior to completion. Of the initial participants who started the study, there were 106 persons who completed it.

Table 5 Study Enrollment and Completion

Treatment Group	Subjects Started	Withdrawals	Completions
No Egg – Control (NEC)	24	5	19
4 Egg Whites/Day (EW)	25	3	22
2 Whole Eggs/Day (WE)	24	5	19
2 Whole Omega-3 Eggs/Day (O3E)	23	1	22
4 Egg Yolks/Day (EY)	24	0	24
Totals	120	14	106

Control and Treatment Groups

Table 6 Control and Treatment Group Characteristics

Treatment Groups	Age (years)	Gender		BMI* (kg m^{-2})	RHR** (Beats/Min.)	Systolic BP*** (mmHg)	Diastolic BP*** (mmHg)
	Mean ± SD	Female	Male	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NEC (n=19)	61±9	14	5	26±7	70±10	117±10	76±7
EW (4/day) (n=22)	60±7	17	5	30±6	73±9	118±15	76±9
WE (2/day) (n=19)	63±7	12	7	27±6	72±11	125±18	80±13
O3E (2/day) (n=22)	61±7	8	14	28±5	74±8	124±13	78±6
EY (4 /day) (n=24)	63±6	15	9	27±4	68±10	127±12	80±8
Total	62±1	66	40	27±2	71±3	122±5	78±2

*Body Mass Index (BMI); **Resting Heart Rate (RHR); ***Blood Pressure (BP)

The final number of study participants was 106 including 40 men (38%) and 66 post-menopausal women (62%). Table 6 displays the similarities of the age, body fat, BMI, and BP of the NEC and egg treatment groups. The gender variance in the O3E group was most noticeable with most of the participants being male compared to the NEC and other treatment groups. Otherwise, general characteristics of the study groups were similar.

Table 7 Participant Medications and Vitamin Supplements

Treatment Groups	Medications			Vitamins
	Lipid Lowering	Antihypertensive	Hypoglycemic	
NEC (n=19)	0	3	1	8
EW (4/day) (n=22)	0	1	0	7
WE (2/day) (n=19)	3	4	1	8
O3E (2/day) (n=22)	2	6	2	5
EY (4/day) (n=24)	4	5	1	10

Individual participant information was also collected on survey forms. Out of the 106 participants who completed the study, four self-identified as having type 2 diabetes mellitus (T2DM) and 33 reported they were taking lipid lowering, antihypertensive, and/or hypoglycemic medications. Of those taking medication, the majority were taking antihypertensive medications while nine were taking those that lowered lipids. Both the O3E and EY groups had 10 subjects taking medications with the majority in both groups taking antihypertensive drugs. The EW group had the fewest participants taking medications with only one taking antihypertensive medications. Thirty-eight participants spread across all treatment groups reported taking various types of vitamins including, but not limited to, multivitamins, fish oil, vitamin D, B complex, zinc, and calcium. Participant medications and vitamins are reported on Table 7. All supplements that participants were taking were evaluated to confirm that participants were not and had not

taken supplements with >6 mg L and/or >2 mg Z two months prior to the study. Participants were permitted to continue taking medication and vitamins throughout the study as long as they met L and Z-related criteria.

Individuals who self-reported a diagnosis of AMD, DR, glaucoma, or other eye conditions that may negatively impact VCP were excluded from participation in this study. However, sixteen participants who completed the study reported a family history of AMD. Thirteen participants disclosed they had experienced a concussion in the past, with one having a severe concussion and the others being mild. Of the participants who reported their gaming experience, 63% (n=47) identified themselves as video-game players, while 25% (n=27) reported they did not play video games. Only two participants reported using nicotine, which was in the form of cigarettes.

Table 8 displays the average body composition among the treatment groups. Based on the one-way ANOVA, there was not a significant difference between groups in the categories of weight (P=0.21), body fat (P=0.24), muscle (P=0.06), bone (P=0.19) or water (P=0.21).

Table 8 Control and Treatment Group Body Composition

Treatment Groups	Weight (lbs.)	Fat %	Muscle (%)	Bone (lb.)	Water (%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NEC (n=19)	159±39	32±12	34±4	5±1	47±7
EW (4/day) (n=22)	186±50	38±8	32±4	6±1	44±5
WE (2/day) (n=19)	177±36	32±10	34±6	6±1	47±6
O3E (2/day) (n=22)	177±34	30±31	36±5	6±1	48±5
EY (4 /d ay) (n=24)	174±36	32±8	35±7	6±1	47±5

Dietary Intake

Based on completed and submitted food log records, participants appeared to be compliant with their diet treatment group by consuming the required number and type of eggs daily. They were asked to complete and submit a minimum 15 food logs including five days within the two-week period prior to cognitive testing and a food log every day they did cognitive testing (10 days). Ninety-seven participants submitted 11 or more food logs with 63% submitting 15 or more of them. All logs were used in dietary analysis. Table 9 shows the treatment group average of participant caloric and macronutrient consumption based on submitted food logs throughout the study. Based on the mean, the EY treatment group consumed more calories, protein, and fat than all other treatment and NEC groups; however, there were only significant

Table 9 Control and Treatment Group Caloric and Macronutrient Intake

Treatment Groups	Average Number of Food Logs Submitted	Calories (Kcal/day)	Carbohydrates (g/day)	Protein (g/day)	Fat (g/day)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NEC (n=19)	18	1783+416	186+56	77+22	76+27
EW (4/day) (n=22)	18	1582+367*	161+49	83+26	66+20**
WE (2/day) (n=19)	18	1735+380	169+60	79+16	81+19
O3E (2/day) (n=22)	16	1761+544	162+72	82+30	79+24
EY (4 /day) (n=24)	17	1947+582*	185+76	90+41	91+25**

differences in the caloric content ($P<0.01$), and grams of fat ($P<0.001$) between the EW and EY groups Perhaps the random group assignment influenced the dietary caloric and fat consumption patterns since EW are naturally lower in calories and fat than EY, and both treatment groups were assigned to consume either four EW or four EY, respectively, per day.

Choline, CHOL, L, Z, and Omega-3 FA were nutrients of interest due to their positive influence on cognitive performance in our pilot study participants. The average daily intake of these nutrients is displayed in Table 10 below. It is important to note that Nutribase, the software used for nutrient analysis combined L and Z together in the nutrition analysis. As expected, CHOL and choline intake were similar for the WE and O3E groups and was highest in the EY group. The amount of L and Z consumed was highest in the O3E group and was variable among the other groups with least amount consumed being the EW group. As expected, the O3E group was the highest in Omega-3

FA due the fortification of the O3E participants consumed. All the other groups consumed similar amounts of Omega-3 FA.

Table 10 Average Key Nutrient Intake Per Day

Control and Treatment Groups	Cholesterol* (mg/day)	Choline* (mg/day)	L + Z* (µg/day)	Omega-3 FA* (mg/day)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NEC (n=19)	176±48	96±80	1010±923	351±54
EW (4/day) (n=22)	147±38	76±68	597±670	315±33
WE (2/day) (n=19)	533±42	353±47	1041±637	308±32
O3E (2/day) (n=22)	536±63	386±65	2089±1824	1708±52
EY (4 /day) (n=24)	906±56	649±66	1498±927	390±31

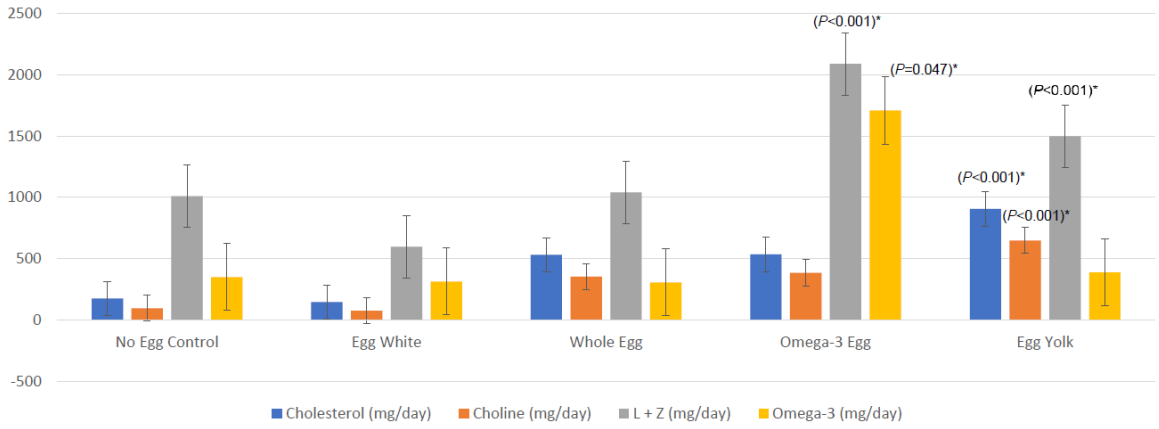

Figure 5 Average Key Nutrient Intake Per Group

The variance of intake among nutrients of interest, CHOL, choline, L, Z, and Omega-3 FA, is displayed in Figure 5. The average intake of CHOL (P<0.001), choline (P<0.001), L and Z (P<0.001), and Omega-3 FA (P=0.047) were significantly different between groups based on the one-way ANOVA single test. Therefore, the dietary

intervention successfully created distinct dietary intake differences for the diet treatment groups ($P<0.01$).

Sleep and Activity Patterns

Participants tracked their activity during the first two weeks of the study using the activity tracker they were provided at their initial study appointment. The research team recorded the subject's activity based on their activity tracker on the day of their first cognitive test and every day they came into the lab thereafter. Table 11 displays the mean and standard deviation of each treatment group's activity (steps), kcal burned, and sleep patterns. There were no significant differences between groups for activity ($P=0.51$), kcal ($P=0.73$) or sleep ($P=0.28$).

Table 11 Average Activity and Sleep Patterns Based on Control and Treatment Groups

Control and Treatment Groups	Activity (Steps/Day)	Kcals/Day (Walking)	Sleep (Hours/Day)
	Mean ± SD	Mean ± SD	Mean ± SD
NEC	15,637±7,777 (n=17)	660±300 (n=17)	6.5±0.4 (n=18)
EW (4/day)	17,487±9,675 (n=21)	691±416 (n=20)	6.6±0.6 n=21
WE (2/day)	18,641±10,499 (n=18)	891±580 (n=18)	6.5±0.6 (n=19)
O3E (2/day) (n=22)	19,399±8,135 (n=20)	918±438 (n=20)	6.8±0.96 (n=21)
EY (4 /day) (n=24)	18,761±10,174 (n=21)	830±467 (n=21)	6.3±0.85 (n=22)

Cognitive Testing

The NT 3-D software program was used to measure VCP. Figure 6 displays the mean ST for sessions 1-15 for each respective treatment group and the NEC. All groups

displayed improvement in VCP (+37%, $P<0.01$) from baseline to the end of the study. However, no treatment group performed significantly over the others.

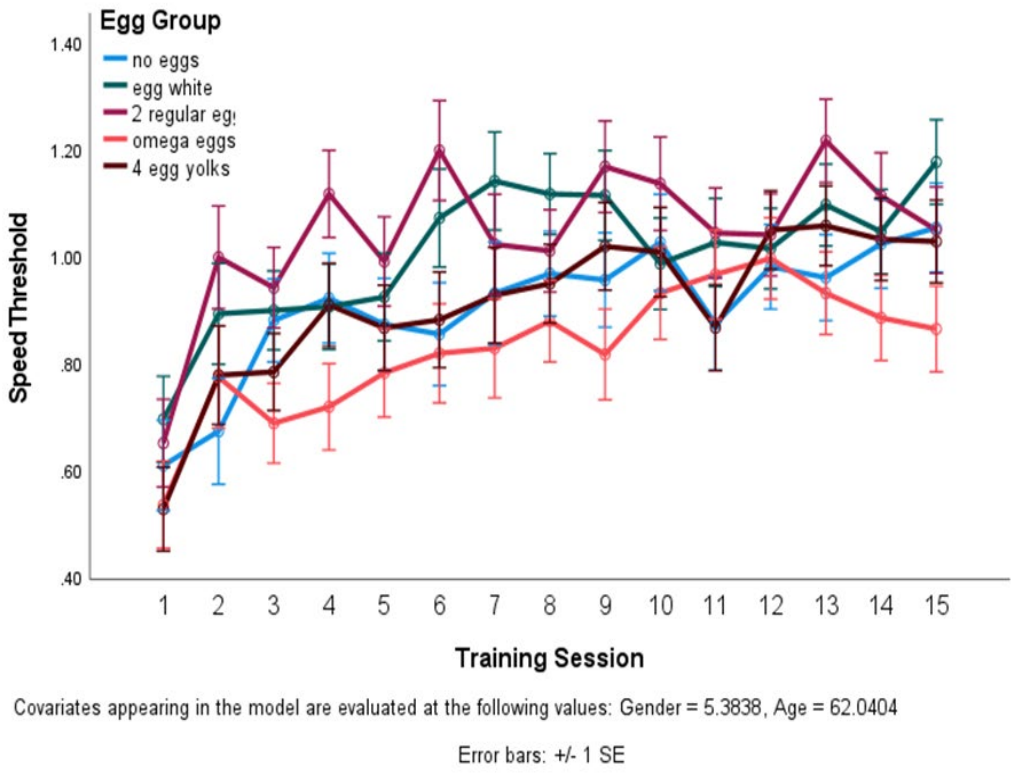

Figure 6 Cognitive Performance Based on Egg Treatment Group and NEC

Due to the initial variability of VCP and variance of participant gaming experience, the mean ST from sessions 1 through 5 were compared to those in sessions 6 through 15 for all treatment groups and NEC and are reported in Figure 7. The mean highest and lowest NT score for all treatment groups and NEC are also displayed. No significance among the groups was identified.

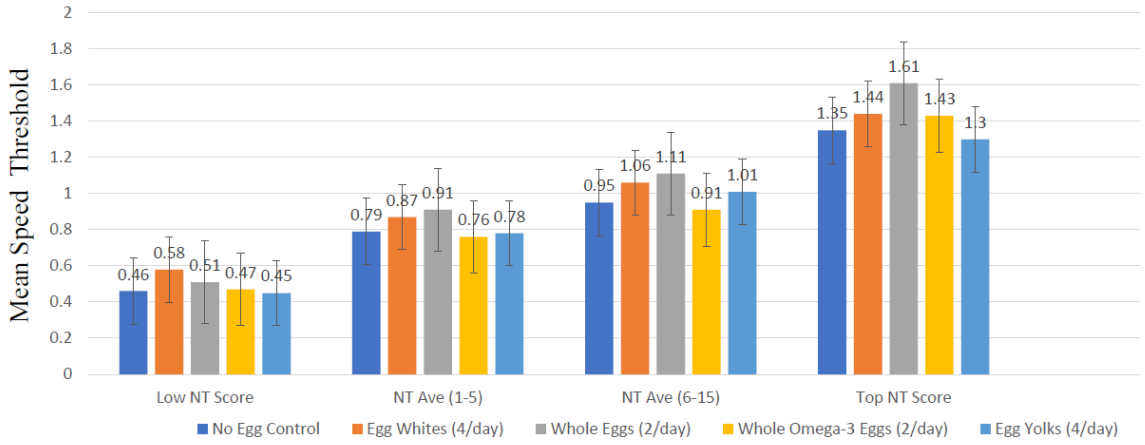

Figure 7 Neurotracker Scores Based on Treatment Groups and NEC

Figures 8 and 9 show the association of cognitive performance and age based on mean ST (figure 8) and maximal ST (figure 9). These figures display that cognitive performance in the aging population was significantly lower than those 18-30 year old pilot study participants, and that that the VCP was significantly higher in men than women. It also shows a negative association with a decline in NT scores across the 50-75 age range.

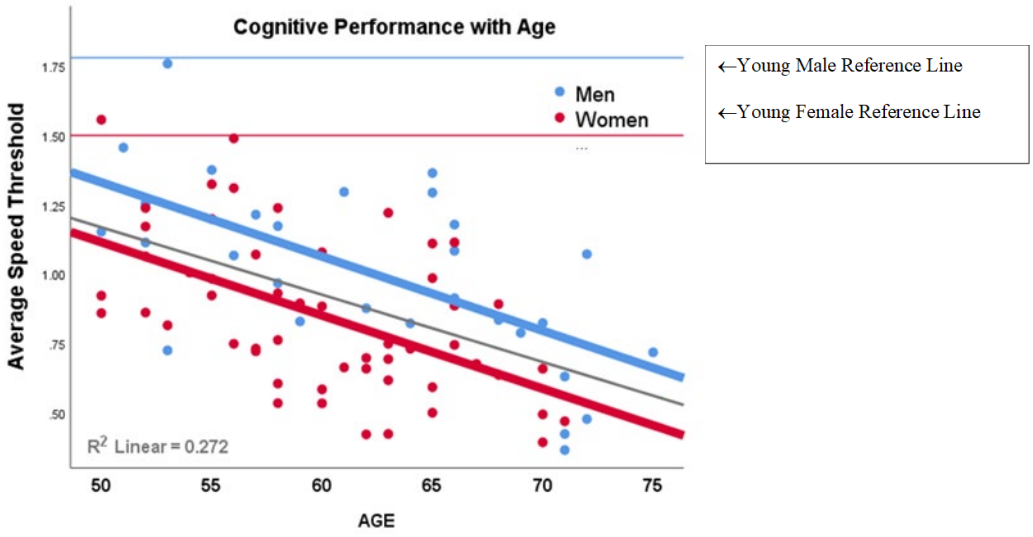

Figure 8 Cognitive Performance Based on Average ST

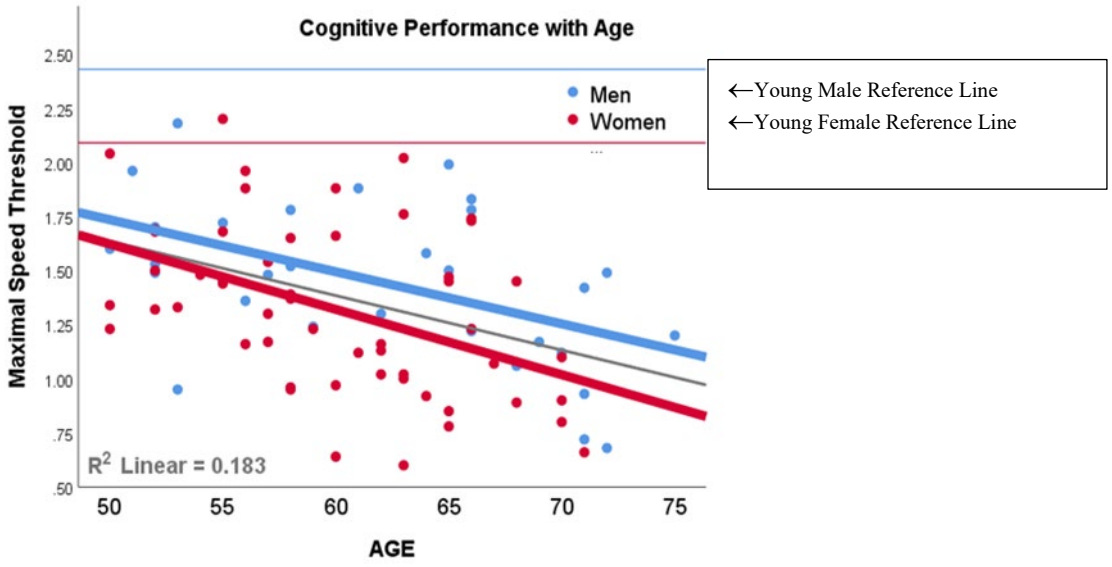

Figure 9 Cognitive Performance Based on Maximal ST

Due to the impact of AMD on visual performance, we evaluated the VCP of participants with a family history of AMD to examine predisposing factors of performance. Figure 10 displays the mean ST for sessions 1-15 for participants with and without a family history of AMD. While both groups displayed improvement, there were no significant differences between groups (*P*=0.25).

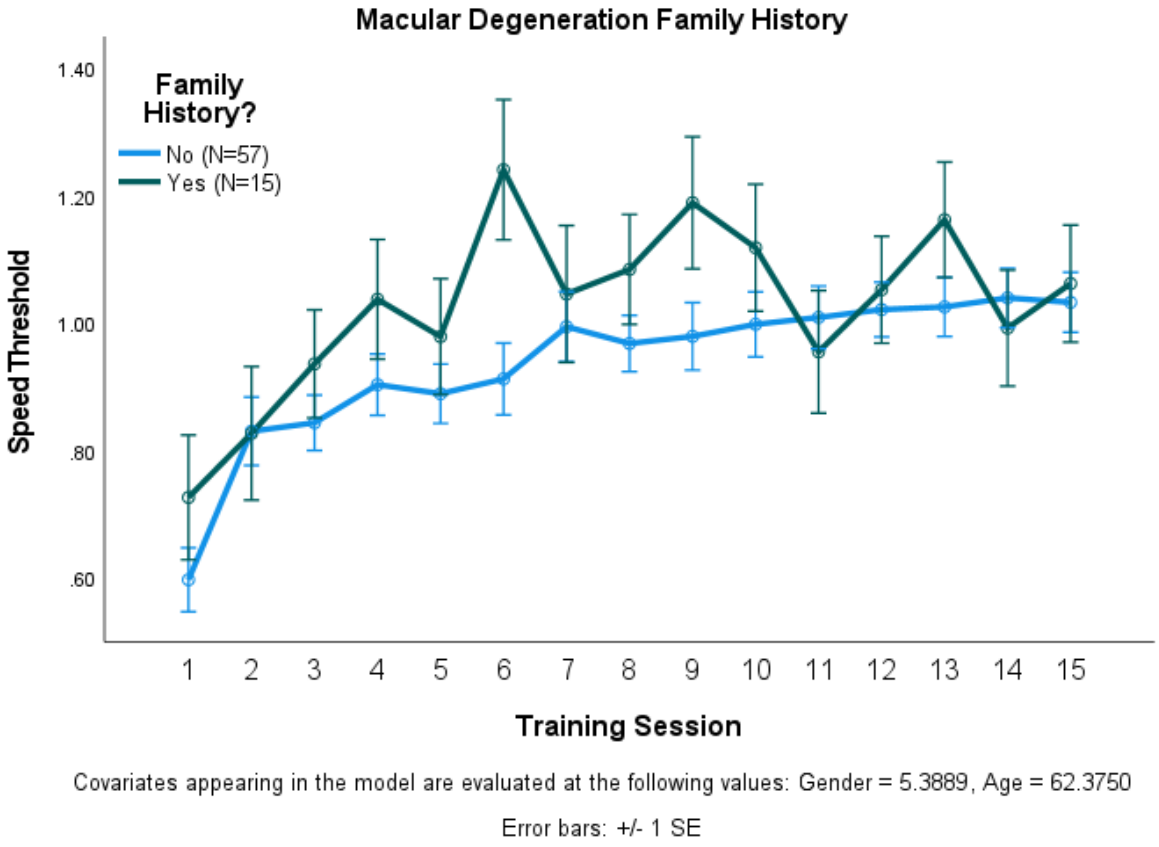

Figure 10 Cognitive Performance With and Without Family History of AMD

Figure 11 compares the mean VCP of participants with and without a family history of AMD among the treatment groups or NEC. Participants without a family history of AMD who were in the NEC, WE, and EY groups had a higher mean VCP than those with a family history of AMD. However, those with a family history of AMD in the EW and O3E group had a higher mean VCP than those without a family history but this was not significant.

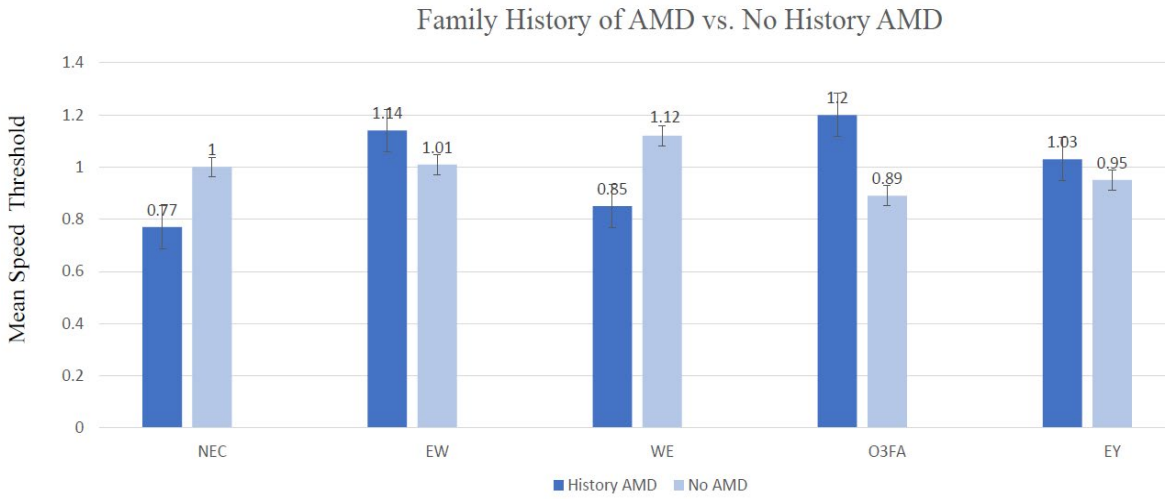

Figure 11 Comparison of Cognitive Performance With and Without Family History of AMD Based on Treatment Groups and NEC

The relation between L and Z intake in those who reported a family history of Macular Degeneration and VCP is described in Figure 12. The mean ST for each participant is labeled with their treatment group or NE. There was not a significant relation between family history of AMD and VCP.

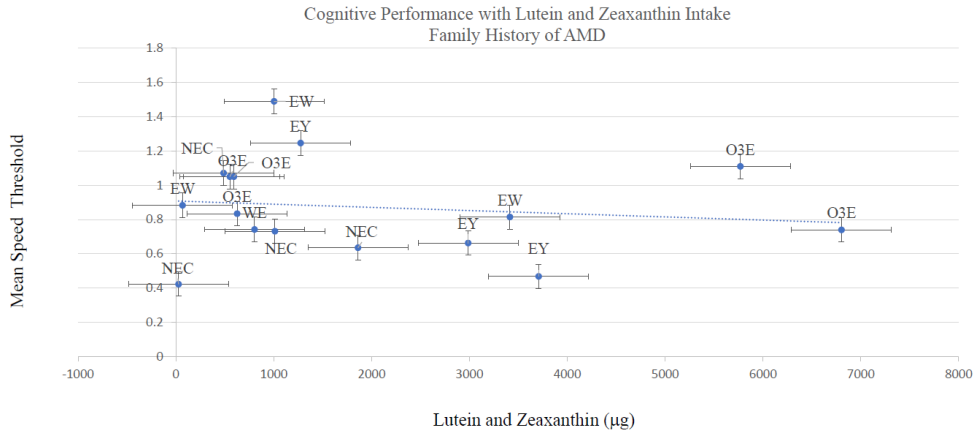

Figure 12 Impact of L and Z Consumption on VCP in Participants with a Family History of AMD

Individual adiposity was reported to have a potential impact on VCP.[101,102] A study of 682 participants (294 males, 388 females) identified a significant association of low MPOD, >27% body fat, and cognitive performance.[102] Figure 13 displays the gender association with higher percent body fat and VCP.

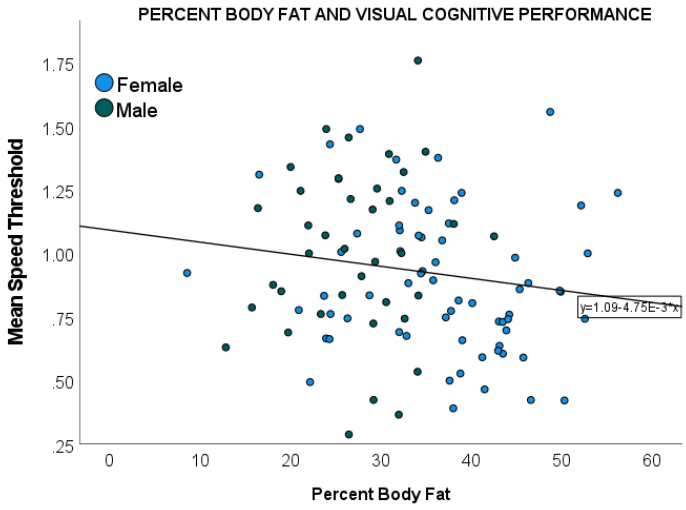

Figure 13 Association of Body Fat on VCP Based on Gender

Figure 14 compares the mean ST for sessions 1-15 for those above and below the gender specific median (27% men, 37% women). Adjusting for age (P<0.001) and gender (P=0.012), median body weight was significantly (P=0.015) associated to cognitive training.

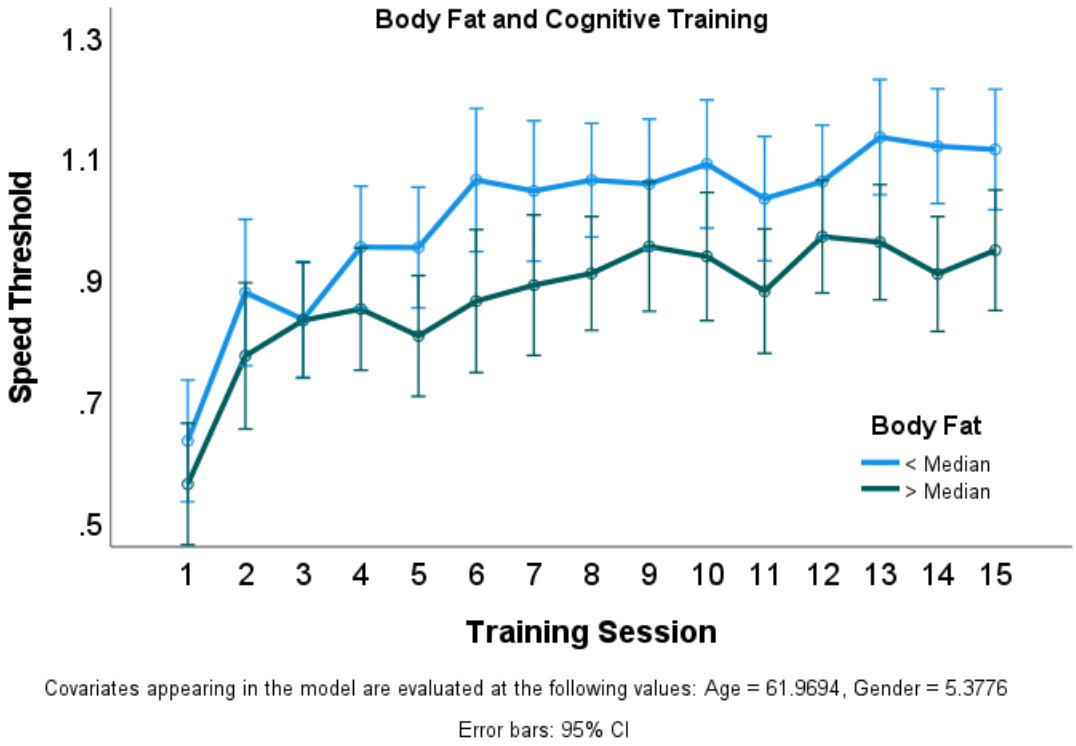

Figure 14 Cognitive Performance Based on Adiposity

Serum Analysis

Lutein and Zeaxanthin

The baseline and 30-day fasting blood levels for L and Z are reported in Table 12. Participants in the NEC and EW treatment groups experienced a small increase in

serum L, but both had declines in serum Z and L+Z levels. The EY group also displayed small changes in serum L and Z levels, which was unexpected since the yolk is rich in L and Z. The most noticeable changes in serum xanthophylls were in the WE and O3E groups with the WE treatment group having the most noticeable change with a 52% improvement in serum Z. ANOVA was used to evaluate the influence of the dietary treatment on serum L and Z between groups; however, there was not a significant difference between the L (P=0.35), Z (P=0.35) and L+Z (P=0.63) treatment groups. Figure 15 provides a graphic display of the L, Z, and L+Z serum changes based on the respective dietary treatment groups.

Next, pre- and post-serum levels for L, Z, and L + Z were analyzed using the paired sample t test. The WE treatment group had significant increases in L, Z and L + Z and the O3E treatment group had a significant increase in L + Z. When comparing serum L and Z within the WE group, these participants had a greater increase in Z than L. This also increased the significance of the L + Z value. No other egg treatment group had significant differences from baseline to the end of the study.

Table 12 Lutein and Zeaxanthin Serum Analysis

Treatment Group		Lutein (µmole/L)			Zeaxanthin (µmole/L)			Lutein + Zeaxanthin (µmole/L)		
		Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
NEC (n=15)	Mean	0.32	0.29	-9.4%	0.32	0.23	-29%	0.72	0.56	-23%
	SD	0.18	0.18		0.30	0.15		0.52	0.36	
EW (4/day) (n=17)	Mean	0.37	0.35	-4%	0.28	0.22	-17%	0.58	0.54	-7%
	SD	0.21	0.18		0.15	0.12		0.30	0.23	
WE (2/day) (n=15)	Mean	0.3	0.49	29% (*P*=0.02)	0.25	0.34	52% (*P*=0.004)	0.62	0.82	31% (*P*=0.006)
	SD	0.20	0.14		0.1	0.112		0.29	0.23	
O3E (2/day) (n=19)	Mean	0.41	0.5	22%	0.27	0.39	41%	0.67	0.90	34% (*P*<.01)
	SD	0.23	0.26		0.15	0.34		0.37	0.56	
EY (4/day) (n=18)	Mean	0.38	0.38	1%	0.27	0.27	<1%	0.66	0.68	4 %
	SD	.26	.23		.15	.15		.39	.4	

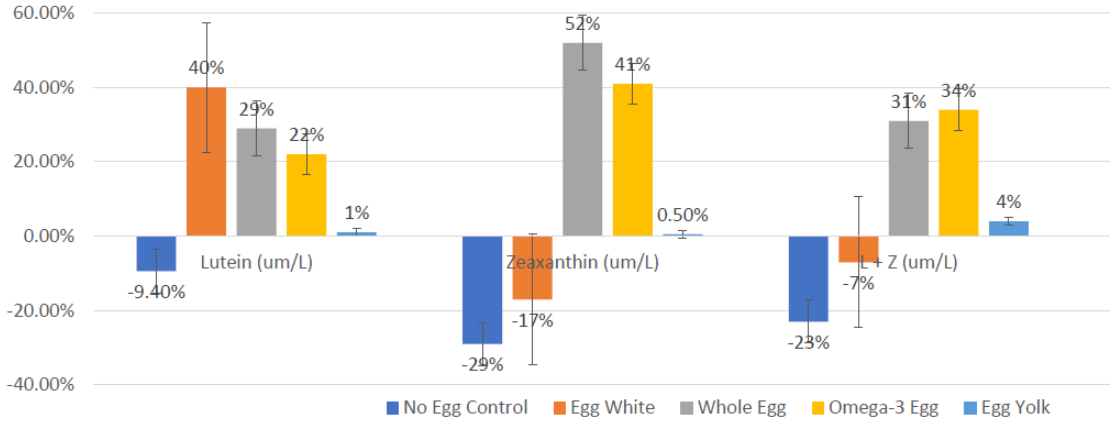

Figure 15 Percent Change in Serum L, Z, and L+Z Levels

Lipids

Serum CHOL and lipid biomarkers were also analyzed due to the increased intake of CHOL in the WE, O3E, and EY treatment groups. Pre (baseline) and post (30 day) serum levels were used to evaluate the percent change for CHOL and lipids. These values are displayed in Table 13. In the initial assessment, CE (P=0.006) and TG

(P=0.002) were significantly different between groups based on the one-way ANOVA. The two-sample t test was used to identify which treatment groups had significant difference in pre- and post-serum levels. There was a significant increase in CE in the EY group (P<0.001) and in TG for the O3E (P=0.02) and EY(P=0.003) groups only. There were no other levels of significance identified among lipid levels.

Table 13 Percent Change Based on Pre- and Post-Serum Lipid Levels

No Control and Treatment Groups	Total CHOL (mg/dl)	CE (mg/dl)	TG (NG/ul)	Apo B (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	HDL-C: LDL:C Ratio
	% Change (pre/post)	% Change (pre/post)	% Change (pre/post)	% Change (pre/post)	% Change (pre/post)	% Change (pre/post)	% Change (pre/post)
NEC	-20% (n=13)	13% (n=16)	4% (n=13)	-23% (n=16)	-22% (n=13)	1.6% (n=11)	-11% (n=9)
EW (4/day)	-1% (n=10)	-7% (n=19)	11% (n=20)	-12% (n=20)	-6% (n=12)	-22% (n=10)	-7% (n=8)
WE (2/day)	-4% (n=12)	24% (n=16)	47% (n=11)	-10% (n=15)	8% (n=10)	35% (n=10)	9% (n=7)
O3E (2/day)	12% (n=14)	-7% (n=19)	18% (n=17)	-18% (n=21)	2% (n=11)	-3% (n=10)	-2% (n=8)
EY (4/day)	-6% (n=13)	26% (n=17)	42% (n=15)	-11% (n=20)	22% (n=14)	35% (n=14)	7% (n=9)

Lipoprotein subfractions were also evaluated as they are reliable indicators for CVD risks. Table 14 displays the percent change of serum LDL and HDL subfractions from baseline to the end of the study. The change in LDL serum subfractions

Table14 Pre and Post Serum LDL-C and HDL-C Subfraction Changes (%)

Subfraction	NEC			EW			WE			O3E			EY		
	Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
TLR Mean	1094	1043	15% n=16	1175	1043	4% n=17	1302	1266	33% n=14	735	855	30% n=18	1092	1328	31% n=12
SD	631	747	92%	791	615	54%	1137	832	83%	301	486	91%	660	657	62%
LDL-1 Mean	384	280	-22 n=14	363	326	4% n=17	427	402	17% n=14	326	281	7% n=18	303	412	33% n=15
SD	120	97	32%	198	117	44%	166	176	62%	176	314	81%	97	223	52%
LDL-2 Mean	545	474	-7% n=16	523	265	13% n=18	509	543	25% n=14	484	440	-5% n=17	552	555	6% n=16
SD	134	136	38%	195	182	33%	243	192	79%	124	266	25%	180	184	46%
LDL-3 Mean	2024	2050	16% n=16	1856	1979	11% n=16	1558	1936	33% n=14	1940	2018	19% n=17	2065	1903	-7% n=16
SD	734	759	62%	785	721	35%	642	796	46%	1043	1065	68%	476	633	26%
LDL-4 Mean	3384	3186	-6% n=15	3534	3372	-2% n=18	3395	3127	-6% n=12	2850	3323	22% n=19	3173	3672	18% n=15
SD	984	1118	18%	1031	954	23%	1120	1329	28%	997	1306	54%	897	1049	28%
LDL-5 Mean	1547	1638	8% n=16	1411	1261	-12% n=15	1545	1567	5% n=14	1365	1384	5% n=18	1503	1762	25% n=17
SD	751	777	21%	372	493	15%	453	514	33%	413	444	25%	555	793	60%
HDL 2b Mean	3227	3294	6% n=15	2880	2590	-10% n=16	3081	2937	-2% n=14	3000	3224	8% N=18	3123	2767	-1% n=16
SD	1012	1156	32%	1223	1154	13%	1207	1506	36%	1109	1356	20%	1544	1180	40%
HDL 2a Mean	2674	2684	3% n=15	2697	2533	-5% n=18	2528	2354	-4% n=14	2692	2762	3% n=19	2649	2691	6% n=16
SD	570	402	19%	403	525	21%	527	600	28%	682	835	20%	692	533	26%
HDL 3a Mean	2425	2170	-9% n=16	2340	2374	4% n=18	2337	2109	-9% n=13	2398	2312	-1% n=18	2281	2443	8% n=16
SD	406	414	16%	377	422	23%	646	512	14%	500	427	22%	311	503	22%
HDL 3b Mean	1092	943	-8% n=16	989	908	-1% n=18	1107	941	-15% n=14	1011	1050	9% n=18	990	1063	10% n=16
SD	335	230	31%	262	208	36%	300	276	14%	275	277	31%	249	283	32%
HDL 3c Mean	699	695	8% n=16	626	648	34% n=18	702	624	-7% n=14	623	769	37% n=17	691	634	-2% n=15
SD	180	236	58%	236	189	94%	171	286	45%	175	141	66%	170	196	39%

among the diet treatment groups were variable across and within treatment groups. For example, the EY group was the only treatment group that displayed an increase in LDL-1. This group also had an increase in all other serum LDL subfractions over the study period, except LDL-3. The O3E group had a decrease in LDL-1 and LDL-5 but an increase in all the other LDL subfractions, while the WE group also had a decrease in

LDL-1 and an increase in all LDL subfractions. Based on ANOVA, there were no significant changes in among dietary treatment groups.

There were smaller overall changes in the serum HDL subfractions among the dietary treatment groups. Neither the EW nor WE groups displayed an increase in any serum HDL subfractions. However, NEC, 3OE and EY groups did display increases in some HDL subfractions. Both the NEC and 3OE groups had small increases in HDL 2-b, HDL 2-a, and HDL 3-c while the EY group experienced small increases in HDL-3a and HDL-3-b. Based on ANOVA, there were no significant changes in among dietary treatment groups.

Regression

Regression Tables 15 – 18 provide information about study participants and their responses to VCP. Age, gender, family history of AMD, fat median, and Omega-3 FA, and DHA were all considered in the linear regression. Table 16 reported the impact of age, gender, and family history on AMD on VCP. While age (P=0.002) and gender (P=0.02) both significantly impacted VCP, but there was not a significant association between a family history of AMD.

Table 15

Tests of Between-Subjects Effects

Transformed Variable: Speed Threshold Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	9.910	1	9.910	9.287	.003
Gender	10.376	1	10.376	9.724	.002
Age	24.035	1	24.035	22.525	.000
Egg group	8.654	4	2.164	2.028	.097
Error	98.166	92	1.067		

Table 16 Impact of Age, Gender and Family History of AMD

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	6.696	1	6.696	6.167	.015
Gender	6.112	1	6.112	5.630	.020
Age	10.733	1	10.733	9.886	.002
Mac Deg Hx	1.458	1	1.458	1.343	.251
Error	73.827	68	1.086		

Table 17 Association of Body Fat and Gender

Transformed Variable: ST Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	13.861	1	13.861	13.193	.000
Age	23.523	1	23.523	22.390	.000
Gender	6.944	1	6.944	6.610	.012
Fat Median	6.416	1	6.416	6.107	.015
Error	98.757	94	1.051		

a. Computed using alpha = .05

Regression Table 18 is the final regression analysis that evaluates the independent variables of age, gender, fat median, Omega-3 FA and DHA in relation to the dependent variable, VCP. Of these, age was the most significant variable that impacted VCP followed by gender. Fat median (P=0.005) and Omega-3 FA (P=0.032) both had significant impact on VCP, DHA was analyzed in this model but did not have a significant impact on VCP.

Table 18 Overall Regression of Factors Impacting VCP

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	T	Sig.
1	(Constant)	1.449	.358		4.044	.000
	Age	-.020	.004	-.441	-5.071	.000
	Gender	.185	.055	.295	3.349	.001
	Fat Median	-.149	.052	-.246	-2.880	.005
	Omega 3 grams	-.082	.038	-.193	-2.176	.032
	DHA grams	-.111	.059	-.160	-1.869	.065

a. Dependent Variable: Speed Threshold Average

Discussion

Factors Influencing VCP

This randomized dietary intervention study including four egg treatment groups and a NEC successfully created distinct dietary intake differences for choline, L, Omega-3 FA and CHOL (P<0.01) between groups. However, our hypothesis that the treatment group that consumed the highest levels of L, Z, choline, CHOL and Omega-3 FA group would have the highest VCP was not supported by the evidence. Instead, all groups improved VCP (+37%, P<0.01) with no significant differences between groups. Varying factors such as participant physical characteristics, nutrient consumption and absorption and bioavailability of the nutrients in eggs could have influenced some results.

Linear regression models identified increasing age as being one of the most significant negative factors influencing VCP. There was also a significant gender effect with men performing significantly better than women. When comparing these categories between the IONSport pilot study, there was a significant difference in performance and decline in the ST with progressing age and gender.

The final linear regression model (Table 18) identified significant effects of age, gender, percent fat median and Omega-3 FA on the dependent variable, VCP. Age and gender were consistently represented in all linear regression models. The significant effect of percent body fat with VCP aligns with other research studies that have also reported a negative relation with obesity, high percent body fat and decline in cognitive ability. [101,102] The negative relation of Omega-FA and VCP was somewhat surprising. There are several studies that report a positive association between Omega-3 FA and cognitive ability. One study evaluated the effect of Omega-3 FA including DHA and EPA on cognitive ability in elderly persons.[21,141,142] Low levels of Omega-3 FA influenced task performance and DHA and EPA were absent in those who had cognitive impairment. Another study that evaluated the impact of Omega-3 FA in an older population found a significant association between serum Omega-3 concentration and cognitive test performance. This study also reported positive benefits of DHA and cognitive ability.[141] A systematic review conducted from 2010 to 2017 and published in 2019 focused on the relation between Omega-3 FA and cognitive decline. Of the randomized control studies reviewed, 71% reported at least one domain of cognitive

function was met and concluded that Omega-3 FA may have a positive impact on coginition.[21]

Conversely, some studies did not consistently find a significant effect of inclusion of a high Omega-3 FA diet. Arendash, et al. studied the relationship of Omega-3 FA on cognition in double transgenic mice crossed with amyloid precursor protein (APP) and PS1 and non-transgenic mice.[143] The APP/PS1 mice were used because the double mutations are related to the occurrence of early-onset AD. Mice were randomly assigned to a high Omega-3 FA or standard diet group for 7 months and all were included in cognitive testing. There was no association between the concentration of Omega-3 FA and cognitive ability for either the AD transgenic mice or controls.[143] Mazereeuw et al. also conducted a meta-analysis looking at the impact of Omega-3 FA on cognitive performance. Review of randomized controlled trials reported that Omega-3 FA treatment had limited significant benefits in immediate recall. It was also suggested that Omega-3 FA may contribute to protecting the brain, but not actually improving cognitive performance.[144] The varying dosages of Omega-3 FA and longevity of research studies have also influenced the results of studies relating Omega-3 FA to cognitive ability[144,145]

Participants in our study consumed two eggs fortified with 660 mg Omega-3 FA and consumed a mean of 1708 $\pm$ 0.52 Omega-3 FA daily, which was significant over other groups. However, the 30-day duration of the study may have been too short to provide noticeable benefits. No serum analyses were conducted to confirm the presence of serum Omega-3 FA.

Other areas considered in the linear regression models that did not display a significant association included DHA and a family history of AMD.

Egg Consumption and VCP

Based on the IONSport pilot study and review of literature, we expected that subjects in the treatment groups that included egg yolks who consumed the highest levels of L, Z, choline, CHOL and Omega-3 FA would display a significantly greater serum response and overall VCP than the NEC or EW. The dietary treatment groups in this study were intentionally designed to provide distinctly different amounts of the key nutrients L, Z, choline, CHOL, and Omega-3 FA. Participants were asked to follow their usual diets other than the treatment group eggs provided to them. The NEC group was asked to omit eggs from their diet for 30 days. Based on submitted food logs, participants appeared to follow their dietary treatment by consuming the number of eggs assigned in their treatment groups. The groups consumed similar amounts of calories and macronutrients, and the O3E, EY, and WE treatment groups consumed the highest levels of L and Z in that respective sequence. Participants in the egg treatment groups were permitted to prepare the eggs based on their preferred method. This was permitted to reduce the potential for monotony associated with eating eggs for 30 days to enhance overall compliance. In retrospect, a standardized preparation method such as the requirement to consume scrambled eggs should have been designated since studies show that high heat preparation of eggs such as boiling, frying, and microwaving reduces xanthophyll levels.[79]

The WE treatment group had significant increases in L (P=0.02), Z (P=0.004) and L + Z (P=0.006). When comparing serum L and Z within the WE group, these participants had a greater increase in Z than L. This also increased the significance of the L + Z value. No other egg treatment group had significant differences from baseline to the end of the study.

The expectation of a significant improvement in serum L and Z levels and a greater variance in the participant serum levels based on their treatment group was only observed in the WE group. The average serum L and Z levels of all dietary treatment groups were within the usual ranges of 0.1 – 1.44 μmol/L for L but the mean for the treatment groups exceeded the normal range of 0.07-0.17 μmol/L for Z.[62] Our study was a four week study, which potentially could have hindered a significant difference in the serum L and Z levels. In review of other studies, this study appears to be one of the shortest in length. One study had subjects consume 2 and 4 egg yolks for five weeks each resulting in significant improvements in both serum L and Z with Z levels being higher than L. Study participants in this study also had a significant improvement in MPOD.[76] Another study had subjects consume 6 eggs with varying amounts of L and Z (total 331 μg vs. 964 μg) per egg weekly for 12 weeks. At the end of 12 weeks, there was a signficant increase in serum Z in both groups, but only a increase in L in the group that consumed the eggs with total 331 μg L and Z, but not the group consuming the eggs with 964 μg L and Z.[77] Again, there was a significant increase in MPOD in both groups from baseline to 12 weeks. It is interesting to note that the study observations

were similar to the present study (groups WE, O3E, and EY) as they also showed that serum Z had a greater improvement from baseline to the end of the study than did L.[76,77]

Another study that supported the need for a longer study and possibly higher levels of L and Z in the diet was one where participants were given varying levels of supplementation with the minimum being 6 mg L and 1 mg Z daily. This study did not show increases in serum L and Z until 2 weeks after baseline with peaks at 12 weeks.[73] In comparison to the 6 mg L and 1 mg Z used in this study, diet analysis showed 1498±927µg L+Z/day in the EY group and 2089±1824 µg L+Z/day in the O3E group (Table 10) respectively. Perhaps extending the length of our study with a higher quantity of L and Z may have generated a significant increase in serum L and Z and improvement in cognitive performance on the NT.

Additionally, previous reports indicate differences in the L and Z serum and MPOD measures. Serum L and Z levels reflect acute carotenoid intake and are variable based on individual consumption patterns, demographic differences, and lifestyle.[97,99,101] MPOD measures the MP and appears to be a more accurate assessment of L and Z since it is not impacted by acute dietary changes and has been shown to be elevated for a longer time (approximately 100 days). Additionally, MPOD is reflective of L and Z brain concentrations.[88,96,97,99]

Another hypothesis of our study was those who consumed the highest levels of L and Z would also have greater VCP. While our study did not show a significant difference among the treatment groups, there was an improvement in VCP in all groups (+37%, $P<0.01$). It was noted that men had a significantly higher VCP (NT

mean=1.02±0.33) than women (0.88±0.27, *P*=0.02). Several studies have reported the positive association between L and Z and cognitive performance.[94,97,100,120] Additionally, studies have reported the that CT has improved VCP in varying age groups, including older persons.[125-127] Extending the study length with increased L and Z may have resulted in a significant improvement in VCP.

Factors Impacting the Bioavailability of Lutein and Zeaxanthin

In consideration of L and Z intake, it must be noted that varying factors have been reported to influence serum L and Z and MPOD levels supporting individual variability of xanthophyll absorption. Smoking is one lifestyle behavior that has been associated with reduced MPOD, which may be associated with increased inflammation.[46] However, we only had one participate in our study that used nicotine.

Individual adiposity including percent body fat and BMI have been associated with a lower MPOD was another factor that influenced MPOD.[101] More specifically, those with a BMI >29 and body fat of >27% had the lowest MPOD levels when compared to those with a lower BMI and percent body fat.[102] Of the participants in this study, 72% of them had either a BMI >29 or body fat of >27%. The gender median body fat (27% men, 37% women) was a significant predictor of VCP. In addition to xanthophylls being stored in the macula, L and Z are also stored in adipose tissues. Although the mechanism for storage of L and Z in adipose tissues is not well understood, the transporters, specifically CL36 has been associated with transport of L into adipose tissue. [86] One theory is that adipose tissue could compete with the retina for the xanthophylls, which would reduce the MP and MPOD. Factors that appear to

influence carotenoid storage in adipose tissues include higher quantity of storage in the abdomen when compared to the buttocks or thigh, food intake and gender. There appears to be a positive correlation with lutein storage in adipose tissues and MPOD in men.[159]

Another theory that could influence the association between adiposity and MPOD is the individual's dietary intake. Poor food choices are often associated with higher BMI and fat accumulation. Modifying dietary habits could also potentially modify MPOD.[12]

Ethnicity has been reported to appear to impact serum xanthophyll's and MP. Some studies have linked Caucasians to having a higher risk of AMD than blacks while other studies suggest blacks have a lower MPOD. [104-106] Our study was primarily Caucasian men and women so while their ethnicity may have impacted their serum xanthophyll level, it is unlikely that ethnicity affected variability presently. Additionally, genotypes were reported to impact MP absorption and stability.[104] Genetic variants from CD36, β-carotene monooxygenase BCMO1 and ABCG8 were shown to influence MPOD and plasma L by 38% and 25%.[104,107]

Egg Consumption and Serum Lipid Levels

Due to the increase in egg consumption in our study, we evaluated particpant lipid levels at baseline, 15 and 30 days. Our goal in doing this was to show that eating EY daily for 30 days would not have a significant negative impact on serum biochemical markers including CHOL, CE, Apo B, triglycerides (TG), and lipoprotein levels. There was not a significant change for most lipid levels. The extensive serum analyses conducted included CHOL, CE, Apo B, TG, LDL-C, HDL-C, LDL-C/HDL-C ratio, and

LDL and HDL subfractions using Lipoprotein Density Profiling and Density Gradient Ultracentrifugation and Fluorescence Spectrometry. Of these, only TG and CE had significant changes from baseline to the end of the study. Based on the two-sample t test, the EY group was the only treatment group that had a significant change in CE (P<0.006) from baseline to the end of the study. Similarly, the EY treatment group had a significant change in TG (P<0.003) from baseline to the end of the study. The O3E group also had a significant change in TG (P<0.02) from baseline to the end of the study. While the EY group did have a significant increase in CE and TG over a 30-day period, it is important to note that this group consumed four egg yolks daily to create a differential in the diet profile. This is not the usual number of eggs eaten daily so this would be a limited concern for the general population.

Benefits of Egg Consumption

While eggs historically have been discouraged for their possible negative association with CVD and stroke risk, several large research studies have reported no significant associations between egg consumption, increased lipid levels and CVD.[141,142] Similarly, a meta-analysis did not show any connection between eggs and CVD in either gender.[142,143] Instead, some studies have shown that egg consumption can reduce mortality associated with strokes and can also positively contribute to enhanced glycemic control.[144,145] A study that added one boiled egg to the diets of hypercholesterolemic reported that men benefitted from the increase of L and Z by reducing the potential oxidation of LDL.[146]

Some researchers have recently refocused recommendations for egg consumption due to the recognition that some people are "hyper-responders" to dietary cholesterol while approximately 75% of persons do not negatively respond to a cholesterol-rich diet. Further, factors such as ethnicity, age, and weight status may affect the individual's response to egg consumption.[144,147] While eggs are high in cholesterol, they are also nutrient rich with beneficial bioactive nutrients such as L, Z, fat soluble vitamins and contribute to a beneficial increase in HDL-C.[144,147] Additionally, egg consumption has been associated with the improvement of MP due to its contribution of L and Z. [76]

Positive Impact of Eggs on HDL

LDL and HDL subfractions appear to be influenced by egg consumption[81-83] . LDL subfractions are classed and the classifications impact their oxidative properties. Similarly, the HDL is divided into subfractions, which potentially influences the oxidation of LDL subfractions. HDL subfractions are sequenced with HDL-3 which may inhibit dense LDL oxidation more than other HDL subfractions.[148] The association of egg consumption and the associated HDL benefits might be associated with the specific affinity of the xanthophyll's L and Z to HDL, and its increase associated with egg consumption.[81-83] We did not see any significant changes to HDL-C or its subfractions.

Omega-3 FA

Omega-3 FA is essential and must be obtained through dietary sources.[61] In addition to providing high biological value protein, L, Z, and choline, eggs are a good source of MUFA and PUFA EPA and DHA.[34] Recorded dietary intake showed that those in the O3E treatment group consumed significantly more Omega-3 FA than the

other groups. Based on the O3E consumption and studies supporting the role of Omega-3 FA, we expected participants in this group to minimally have an improved VCP over the NEC, EW, and WE treatment groups.[18,22,26,61] However, there was not a significant difference between treatment groups and there was a significant negative relation in the linear regression with Omega-3 FA and VCP.

Omega-3 FA supplementation in healthy individuals enhanced cognitive function[21,61] A meta-analysis with 34 randomized controlled trials that evaluated attention, processing speeds and reaction responses did not demonstrate significant relation between omega-3 supplementation and these cognitive measures.[153] This might have been due to the choice of Omega-3 FA supplement. Studies that used EPA-rich verses DHA-rich supplements had a more positive response to VCP. In two double-blind, crossover studies of healthy young adults using EPA-rich supplements, participants displayed significantly better choice reaction times when compared to the DHA-rich supplements.[154,155]

Choline and Omega-3 FA, both components of the egg yolk, were reported to have a synergistic impact. When evaluating the uptake of choline in retinal cells, it was noted that subjects supplemented with Omega-3 FA experienced enhanced cellular uptake of choline when compared to cells without Omega-3 FA supplementation. A deficiency of either choline or omega-3 fatty acids was reported to result in impaired visual development.[61]

Potential Benefits of Lutein and Zeaxanthin in Other Disease States

Lutein and Z are effective antioxidant and anti-inflammatory agents that protect to both the retina and brain. The retina is prone to oxidation due to its high oxygen content and PUFA concentration, and MP carotenoids appear to use their antioxidant and light-filtering properties to reduce the occurrence of ROS.[45] Additional research using the primate model identified trans-L throughout all brain regions and suggested it served as an antioxidant protecting DHA in the brain.[90]

Conditions such as AMD, DR, CVD, and metabolic syndrome have been associated with ROS and may benefit from the antioxidant and anti-inflammatory properties of L and Z. A systematic review looking at the potential benefits of L and Z concluded that higher serum L levels are generally linked to reduced CVD, however, the conclusions are based primarily from observational studies. Larger, long-term intervention studies are needed to assess further benefits of L and Z. [148]

Study Benefits

This study had a strong pilot study that provided positive preliminary data that was the basis of the design of this study. This study required 15 days of food logs instead of the traditional three food logs in most studies. While not all subject submitted 15 food logs, 97% of the participants submitted 11 or more food logs and 63 submitted 15 or more. The larger number of food logs provided broader perspective of the participant's routine food intake. The researchers provided virtual nutrition education and template handouts to enhance accuracy of food log records.

Another benefit of this study is that we collected a large amount of data that can be used to comprehensively examine factors associated to cognitive decline. In addition to the basic data on VCP using NT software, we collected the following on each participant: a baseline, midpoint and ending blood draw; physical activity (steps) data using an activity tracker; sleep patterns; hydration status; readiness to perform; and numerous diagnostics including BP, RHR, weight, body composition, oxygen saturation, and hand grip strength.

The opportunity to conduct multiple serum analyses is an additional benefit. In addition to analyzing the L and Z serum levels, we analyzed multiple lipid levels including LDL and HDL subfractions and several other high priority candidates that may influence cognitive performance. These analyses provide a broader prospective of the participant's overall health status.

Study Limitations

While there were several benefits to this research project, there are also some limitations. One limitation is the lack of information on the feed provided to the chickens who laid the eggs used in our study. We used standardized eggs for all participants according to the study design, but a more detailed analysis of the feed may be helpful in interpreting our results. The assessment parameters used to assess L and Z were limiting. While this study did serum analyses, measuring MPOD would have a more precise measure of chronic lutein intake. While food logs and serum analyses were used in combination to validate L and Z intake, serum L and Z levels reflect acute carotenoid intake and are variable based on individual consumption patterns, demographic

differences, and lifestyle while MPOD is reflective of chronic intake and proper absorption and target uptake.[101-106] In comparison to serum L and Z levels, MPOD is more stable and represents an accumulation of L and Z and MZ.[97,99] Using MPOD as a biomarker would provide a more accurate assessment at baseline. Once supplementation ceased, MPOD would also continue to be elevated for a longer time of approximately 100 days. Using MPOD would provide results that were not impacted by acute changes in dietary intake and were more reflective of the L and Z brain concetration.[23]

The last study limitation was the study population. Most participants were Caucasian, educated persons who had a past or current affiliation with Texas A&M University. We did not pay participants so that may have limited diversity of enrollment. Perhaps paying participants might have attracted persons with limited income to participate. Therefore, the results of this study are limited in generalization across diverse populations since L absorption is reported to be variable based on ethnicity and genetics.[104-107]

Next Steps

Our current study was 30 days in length. The duration of the study may have impacted the results as persons were only consuming the dietary treatment (1500-2000 µg/day) for two weeks prior to cognitive testing. They continued this same dietary pattern for the duration of the study. Lutein and Z are nutrients that provide many benefits. Generally, supplementation starts at 10 mg. Conducting another research study with L and Z supplementation at a minimum of 10 mg per day and providing guidelines of when to take the supplement and what foods to consume with the supplement. We

would use the NT for cognitive testing and restructure the study where we started recording NT speed threshold on the 6th session to enable the participant to become more acclimated to the process resulting in better scores. Another goal is to further examine the relation of L and Z with those who have a family history of AMD. Our study did not show a significant differece in performance between these two groups. However, the IONSport pilot study did have show a suggestive association of family history of AMD and VCP. Perhaps, we did not see a significant difference in our study since our study population was older and there was a significant negative relation with age and VCP. Additionally, we would like to look at the impact of obesity and diabetes on cognitive performance. The identified relation between adiposity and VCP is interesting due to the high incidence of obesity.

CHAPTER V CONCLUSON

There is global awareness of the increased growth in the aging population and the progressive decline in cognitive performance that often occurs with it. In addition to diminishing the quality of life for these individuals and their caregivers, there are extensive personal and societal costs. It is imperative that solutions be identified to diminish the consequences of this increasing concern.

The results of this study identified a significantly negative relation between aging and VCP. However, participants in all treatment and the NEC group improved VCP (+37%, $P<0.01$). Additionally, adiposity negatively impacted VCP. This study supports nutrition by maintaining a healthy body weight and cognitive training as potential resolutions to cognitive decline.

L and Z are nutrients that are antioxidants located in the macula and the brain. Eggs are an economical, high protein food that contains L and Z and other nutrients associated with cognitive performance. Our study used eggs as a model to increase the L and Z in the diets of our participants with the expectation that those who consumed highest levels of L and Z display highest VCP. Although we observed significant improvements in VCP, there were no differences between groups and there were no significant differences in lipid levels from baseline to the end of the study except in CE and TG levels in the EY treatment group. TG were also elevated in the O3E group.

Based on research on eggs, many studies have shown positive benefits associated with egg consumption including a positive impact on HDL and contributing positive nutrients to the diet such as L, Z, and choline.

The antioxidant and anti-inflammatory functional properties of xanthophylls appear to have the potential to positively impact conditions associated with oxidative stress. Combining the benefits of xanthophyll and the 3-D MOT cognitive training program provides a promising solution to the societal challenges of aging and cognitive decline as well as other conditions such as such as AMD, CVD and DM

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