THE RELATIONSHIP BETWEEN MACULAR CAROTENOIDS AND COGNITIVE FUNCTION IN A YOUNG ADULT POPULATION

by

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I. LITERATURE REVIEW

Cognitive decline is common among adults over the age of 70 and is the leading cause of disability in older adults.[1,2] Adults suffering from cognitive decline are at risk for dementia and reduced quality of life and independence.[1] Previous research suggests that although it is important to promote cognitive health within this population, early intervention may be more important to help maintain brain health and prevent early cognitive decline.[3] There is significant research on modifiable lifestyle factors, such as diet and exercise, that impact the cognitive development of children, and it is known that cognitive health is central to their academic success.[4,5] However, cognitive function in young adults is equally important to examine, yet limited research in this population exists. Although young adults are at their cognitive peak, there is evidence that cognitive health can still be improved.[6,7] During aging, the ability to increase cognitive development declines substantially; therefore, promoting cognitive health early may help increase cognitive reserve and maintain cognitive function through aging.[7] In addition, improving cognitive function in young adults may improve their academic and career success.[5,8]

Lutein as an Antioxidant

There are a variety of factors that can promote cognition, such as physical activity, diet, and cognitive training.[7] A growing body of evidence suggests that higher intakes of fruits and vegetables may promote cognitive development and reduce the risk of cognitive decline in older adults.[9,10,11] A potential mechanism of the cognitive benefits of fruit and vegetable intake is explained by the antioxidant properties of nutrients such as carotenoids, vitamin C, and vitamin E, that are abundant in plant foods. Oxidative

stress and inflammation can promote neurodegeneration[12], and many cognitive diseases, such as dementia and Alzheimer's Disease (AD), have multiple causes including oxidative stress.[13] Therefore, dietary antioxidants may protect cognitive function through blocking the formation of free radicals, reducing oxidative stress, and ultimately slowing damage from neuroinflammation.[14]

Lutein is a non-provitamin A dietary carotenoid that is in a variety of fruits and vegetables, but is most abundant in dark green leafy vegetables such as kale and spinach.[15,16] Lutein is also found in corn, parsley, and egg-yolk.[16] Different from provitamin A carotenoids, lutein does not cleave into vitamin A molecules and is not considered an essential nutrient. Lutein accumulates in the brain and eye, specifically near the central part of the retina known as the macula during all stages of life. In fact, lutein is the most prominent carotenoid in the brain.[15] Lutein can protect against age-related macular degeneration (AMD), by absorbing blue light that can damage the eye.[14] Furthermore, high levels of oxidative stress and inflammation are found in patients in the early stages of AMD and lutein aids in eye health through blocking the formation of free radicals that may otherwise damage the eye. [12,14]

Macular Pigment Optical Density and Lutein Levels in the Brain

The carotenoids lutein, zeaxanthin, and meso-zeaxanthin accumulate in the macula and can be measured by macular pigment optical density (MPOD).[16] MPOD has been used as a biomarker for dietary intakes of lutein and zeaxanthin[17] and has been shown to be responsive to dietary supplementation of lutein and zeaxanthin.[18,19] Previous research has found that lutein concentrations in the brain are significantly correlated with concentrations in the retina.[15] In matching brain and retina tissue of deceased older

adults, retinal lutein was significantly correlated with lutein levels in the occipital cortex.[20] MPOD measures macular carotenoid levels in the retina, thus, MPOD can be a useful biomarker for determining lutein levels in the brain.[15]

MPOD and Cognitive Health in Older Adults and Children

Many studies have found lutein to be significantly associated with cognition and risk of cognitive decline in older adults. In healthy older adults, higher MPOD values, a biomarker of lutein, were significantly associated with higher recall, perceptual speed, global cognition, processing speed, verbal learning and fluency.[21] Moreover, in a randomized, double-blind, placebo controlled intervention with 51 healthy older adults, 1 year of lutein and zeaxanthin supplementation improved MPOD scores and cognitive performance in complex attention, cognitive flexibility, and executive function.[12] Other intervention studies on older adults have found similar results.[21, 22] The loss of cognitive function in older adults can be explained by loss of lutein throughout the lifespan.[15] Lutein is found in higher concentrations in an infant's brain.[15] Full-term infants are provided lutein through in utero accretion during the third trimester and research suggests that infants are able to maintain lutein levels without dietary intake of lutein after birth.[15] Lutein levels decrease as an infant ages giving way to the importance of dietary lutein intake to help maintain healthy levels in the retina and brain. In addition, maintaining high or improving MPOD levels in children is important. A study by Barnett el at. found positive associations between academic performance and MPOD[23] in preadolescent children, while controlling for other factors that may impact academic performance, such as aerobic fitness, adiposity, sex, and IQ.[23] Students with higher MPOD values had better academic performance, more specifically in written language and math.[23] Therefore,

academic performance may be improved through increased intake of lutein.[23] Academic success, along with career success is also important in young adults. As discussed, academic success may be improved through higher MPOD values as studies in older adults suggest that increasing lutein intake may improve recall and increase brain activity. [24] Therefore, lutein intake may improve intellectual wellness and cognitive factors important for career success, in addition to academic success.[24]

The Relationship Between Lutein, MPOD, and Cognitive Health in Young Adults

There is promising research concerning lutein, a dietary carotenoid containing antioxidant and anti-inflammatory properties that may promote cognition.[14,10,21] However, the majority of the research has been conducted in older adults and young children. Very few studies have examined the relationship between cognitive function and lutein in young adults. Therefore, promoting cognitive health through increasing lutein intake in a young adult population may improve cognitive function, which is important for school and career success and prevention of later life cognitive decline. A recent study by Renzi-Hammond et al. examined cognition and MPOD in healthy young adults (18-30 years old) that were provided lutein (10mg) and zeaxanthin (2mg) supplementation for a year.[3] The study was a double-masked, randomized, placebo-controlled trial design and MPOD and cognitive scores were measured every four months. The researchers found that supplementation of lutein and zeaxanthin significantly increased MPOD values and also led to significant improvements in reasoning ability, spatial memory, and complex attention.[3] Improving these different aspects of cognition in young adults may improve their academic and career success.[5,8] Although this study did find a correlation between cognitive function and MPOD, the

study did not account for other modifiable lifestyle factors that have been proven to impact cognition including adiposity, aerobic fitness, and diet.

Other Factors Contributing to Cognitive Health

Multiple studies have shown that aerobic fitness in all age groups promotes cognitive health and academic success.[5,8,25] Such studies, have found a variety of mechanisms through which aerobic activity aids in cognitive function, but overall a higher aerobic fitness level results in a number of responses in organs and muscles that alter and help regulate the structure and function of the brain.[8] On the other hand, adiposity is associated with lower cognitive function.[26,27,28] This is explained by the reduced integrity of white matter tracts with increased adiposity, which has been shown to cause executive and working memory dysfunction, less neural transmission speed, and reduced information processing.[28] Despite the strong and growing evidence that aerobic fitness is positively associated with cognitive function, brain volume, and brain connectivity in multiple age groups[29,30], many studies have not adequately controlled for fitness when examining the association between diet and cognitive performance. Individuals who consume a healthy diet often engage in other healthy lifestyle behaviors such as physical activity[31], and there is evidence of a significant relationship between diet quality and cardiorespiratory fitness.[32,33,34] Adherence to a healthy diet may be positively associated with fitness levels and lower adiposity, indirectly contributing to cognitive health. Thus, it is imperative to control for aerobic fitness and adiposity when examining the impact of diet on cognition. The purpose of this study was to determine if MPOD correlated with fruit and vegetable intake while controlling for body fat percentage. In addition, we aimed to determine if cognitive function, specifically cognitive domains

associated with fluid intelligence, was positively correlated with MPOD after considering body fat percentage and aerobic fitness level.

II. MACULAR CAROTENIODS AND COGNTIVE FUNCTION IN YOUNG ADULTS

Introduction

Older adults are at risk for cognitive decline which may lead to debilitating forms of dementia.[1] Those diagnosed with dementia may have reduced quality of life and independence, while close family members may be impacted though emotional distress and financial pressures.[1] Therefore, encouraging a healthy lifestyle to help promote cognitive function in older adults is important. However, early prevention may be more impactful to help slow the progression of cognitive decline in order to maintain brain health.[3] For example, mental performance decreases as we age, and research suggests that increasing cognitive reserve early may help maintain brain resources and slow down the progression of cognitive impairment.[35] Unfortunately, there are few studies that examine the young adult population and cognitive function since they are not going through a stage of cognitive decline. Although young adults are at their cognitive peak, studies show that cognitive health can still be improved.[6,7]

There is promising research suggesting that lutein may have protective effects that can slow the progression of cognitive decline. For example, lutein has been shown to improve MPOD and cognitive function, specifically fluid intelligence, in older adults and children.[3,12,21,36,37,38] This may be possible because lutein accumulates in the macular pigment and brain, and has protective antioxidant properties.[15]

There has only been one other study to examine MPOD and cognitive function in younger adults and a relationship was identified between MPOD and cognitive function. However, they did not control for aerobic fitness and adiposity, which are two factors that

impact cognitive health.[3,26,27,28] Therefore, the goal of this study was to examine the relationship between fruit and vegetable intake, MPOD, and cognitive function, while controlling for aerobic fitness and adiposity.

Methods

Seventy undergraduate students between the ages of eighteen and thirty were recruited from Texas State University to participate in the study. Students were recruited using the Psychology and Criminal Justice subjects pool which was managed through an online system. Interested students signed up to participate through the online portal and scheduled themselves for two, 60-minute visits. Additionally, fliers were posted throughout the Family and Consumer Sciences building on campus to aid with recruitment. Students without access to the online portal signed up for time slots through emailing the Principal Investigator (PI). Inclusion criteria for the study was as follows: students between the ages of 18 to 30 years, in good physical health, and have 20/20 vision with or without the use of glasses or contacts. Physical health for the aerobic fitness test was determined by the Screening Questionnaire for Research Involving Exercise, which assessed if the student was low risk based on recommendations by the American Heart Association (AHA) and American College of Sports Medicine (ACSM).[39] Therefore, eligibility for the study was determined upon completion of the consent form and screening questionnaire. During visit one, participants completed the consent form and the demographics, screening, and fruit and vegetable questionnaires. In addition, the participants performed the cognitive tests and MPOD. At visit two, BodPod testing and the aerobic fitness test were conducted. After completing each visit, participants received credit or ten dollars depending on if they signed up through the

online system or through the PI, respectively. Texas State University Institutional Review Board approved all testing procedures. A flowchart describing the study design is reported in Figure 1.

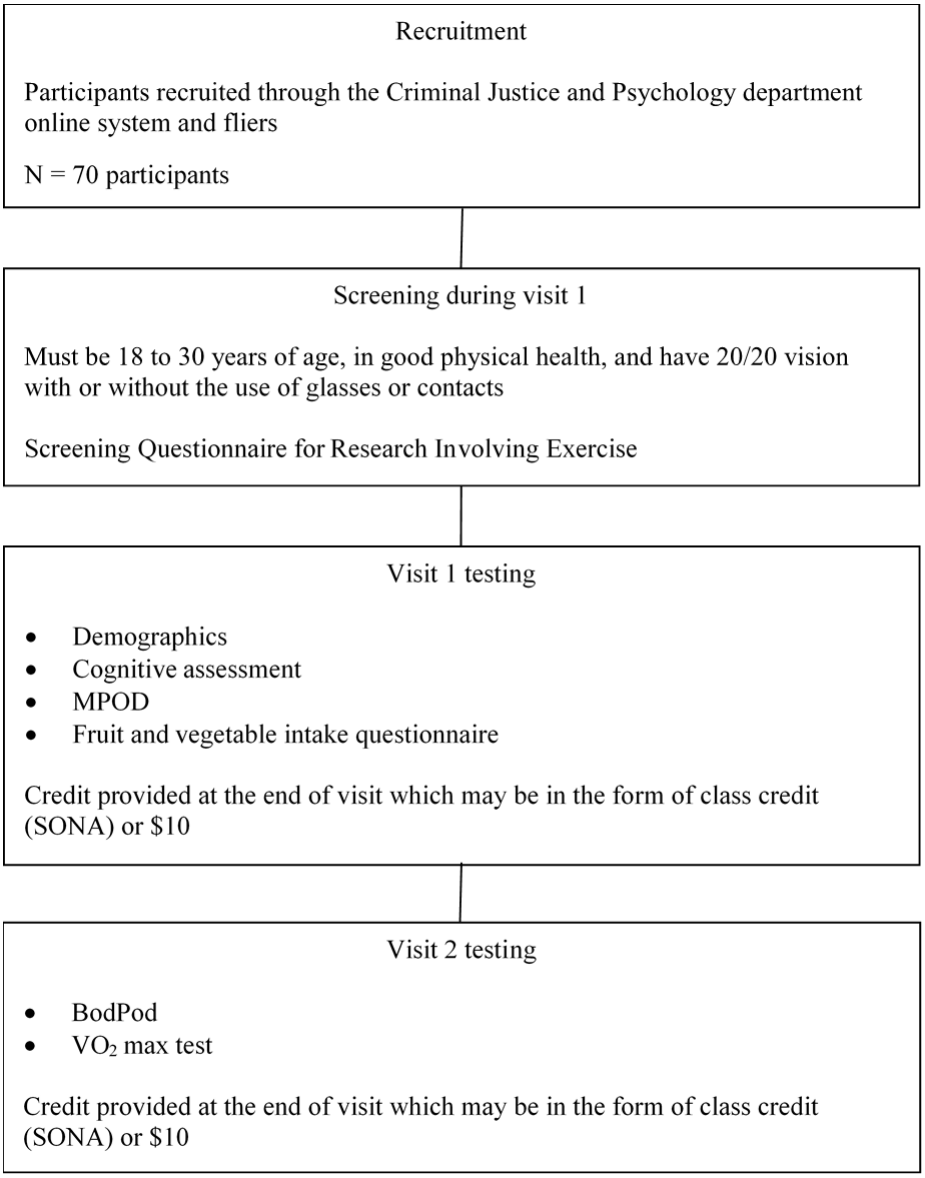

Figure 1. Study Design

Demographics

Once participants were determined eligible through the consent form and screening questionnaire, the participant was asked to fill out a demographics form. Demographics information related to race, gender, native language, education, and height (in.) and weight (lbs.) was measured using a Seca digital measuring tool.

Neuropsychological Assessment

The National Institutes of Health Toolbox for the Assessment of Neurological and Behavioral Function Cognition Battery provides a brief and comprehensive battery of tests that asses multiple cognitive domains. The battery includes eight tests that cover the following cognitive domains: executive function and attention, episodic memory, language, processing speed, and working memory. A more detailed list is shown in Table 1. Tests were administered on an iPad by trained research personnel. Fully corrected T-scores that account for age, education, gender, and race/ethnicity were used in the analysis.

Table 1. The National Institutes of Health Toolbox for the Assessment of Neurological and Behavioral Function Cognition Battery

NIHTB measure	Description	Cognitive domain	Length
Flanker Inhibitory Control and Attention	Participant must choose which way the middle arrow is pointing, while inhibiting their attention to other arrows that may point in a different direction.	Executive function and attention	3 minutes
Dimensional Change Card Sort	Participant must sort pictures based on color or shape, as indicated on the screen.	Executive function	4 minutes
Picture Sequence Memory	Participant must place pictures in the same order they were shown to them on the screen.	Episodic memory	7 minutes
Picture Vocabulary	Participant must select the picture that best matches the word said.	Language	4 minutes

Table 1. Continued

Oral Reading Recognition	Participant must pronounce the word or letter given to them as accurately as possible.	Language	3 minutes
Pattern Comparison Processing Speed	Participants must quickly choose if two pictures are the same or are not the same.	Processing speed	3 minutes
Oral Symbol Digit	Participants are given a key that matches numbers with symbols. Below the key are rows of symbols. Participants are asked to report which number matches each symbol.	Processing speed	3 minutes
List Sorting Working Memory	Participant recalls visual and orally presented stimuli.	Working memory	7 minutes

MPOD

MPOD was measured using the QuantifEye MPS II instrument (QuantifEye; MPS 9000 series: Tinsley Precision Instruments Ltd., Croydon, Essex, UK). To begin, the room was dimly lit and instructions were provided to the participant before each test. The participant was asked to look inside the instrument and concentrate on a dot that would begin to flicker. The participant was asked to press a response button when they saw the flicker. The participant performed a central and peripheral test. Each test took approximately 1-2 minutes to complete. QuantifEye uses heterochromatic flicker photometry to determine MPOD. The flicker is created by alternating blue and green lights and the blue light is absorbed by macular pigment (MP), while the green light is not. The peripheral test is used to correct for baseline flicker sensitivity that differs from person to person. Therefore, peripheral results were subtracted from the central test results to obtain an individual MPOD score for each participant. After each test, the software assessed the quality of the results and showed either acceptable, caution, or reject on the computer screen. The participant received two tries to achieve an acceptable test result if they were unable to get it during the first try.

Fruit and Vegetable Intake Questionnaire

Participants completed a paper copy of the National Cancer Institute Fruit and Vegetable Intake Questionnaire (http://riskfactor.cancer.gov/diet/screeners/fruitveg/allday.pdf) at the end of visit one.[40] The questionnaire assessed the participant's fruit and vegetable intake within the past month by asking the frequency and amount of fruit or vegetable the participant consumed. The questionnaire included 16 questions, which research has shown to be less biased and more valid than shorter fruit and vegetable screeners.[40]

BodPod

The BodPod (COSMED©) was used to assess the participant's body fat percentage.[41] For testing, participants wore tight fitting clothing and a swim cap before stepping into the machine.

Aerobic Fitness

The aerobic fitness test was performed on a treadmill using the Bruce protocol.[42] Briefly, the test begins with a three-minute warmup which is followed by an increase in incline and speed every three minutes. Heart rate was recorded using a Polar H10 Heart Rate Monitor (HRM) that was secured to the participant's chest with a Bluetooth HRM Chest Strap. The participant's heart rate was recorded every minute during the test. During the test, expired air samples were taken at 30 second intervals until test termination due to exhaustion (determined by the participant) and/or symptom limitation. This was done to measure oxygen uptake (VO_2) (ParvoMedics True Max 2400). VO_2 peak is defined as the highest recorded VO_2 measurement when at least two of the three criteria are met: (1) plateau in VO_2 regardless of an increase in workload, (2) respiratory

exchange ratio ≥ 1.1, and (3) a heart rate within 10 beats per minute of age predicted maximum (i.e., 208-(0.7*age in years)).

Data Analysis

All analyses were performed using SPSS version 24. Variables that were not normally distributed were log transformed. For participant's demographic information, frequency for gender, race, and ethnicity was identified, while mean and standard deviation (SD) was determined for age and years of education. Descriptive statistics was used to determine minimum and maximum values, as well as mean and SD for MPOD, fruit and vegetable intake, body fat percentage, aerobic fitness, and cognitive tests. A multiple linear regression was performed to identify a relationship between fruit and vegetable intake and MPOD. Additionally, a multiple linear regression was used to identify a relationship between MPOD and fully-adjusted fluid intelligence cognitive scores. In both models, body fat percentage, a potential cofounding variable, was included. Gender was added as a covariate for MPOD and fruit and vegetable intake. Aerobic fitness could not be included as a covariate for MPOD and cognitive function as intended because there was not enough data available. For 4 of the participants the treadmill did not work properly, 11 did not show for visit 2, and for 33 participants VO_2 max was not reached or could not be determined. An ANOVA was performed to compare fruit and vegetable intake between low and high MPOD values. MPOD was split into two groups: (1) MPOD scores lower than 0.41; and (2) MPOD scores higher than and including 0.41. Although available, we did not divide the MPOD scores based on the national average of 0.33.[43] Our population included young adults, in contrast the national average value was derived from a population sample that included both young and older

adults.[43] The national average may be a lower score because older adults have lower MPOD.[17] Further, studies examining MPOD in young adults report higher averages among their population.[3,17] Therefore, MPOD scores were split at 0.41 because this was the median for our sample. A univariate ANOVA was used to compare fruit and vegetable intake as a function of MPOD group. Further, an ANOVA was performed to compare fluid cognitive tests between the same high and low MPOD groups.

Results

Participant Demographics

Participant demographics are provided in Table 2. There were 70 participants recruited for the study between the ages of 18 to 30 (Mean = 20.2, SD = 2.7). Among those recruited, a greater proportion of females (68.5%) participated in the study compared to males (31.4%). The study population was majority Caucasian (72.7%). Lastly, 40% of the population was Hispanic, which is more than usually seen in MPOD and cognitive studies.

Table 2. Participant Characteristics

Gender, *n* (%)	
Female	48 (68.5%)
Male	22 (31.4%)
Age, mean (SD)	20.2 (2.7)
Education in years, mean (SD)	14.2 (1.9)
Race, *n* (%)	
American Indian/Alaskan Native	-
Asian	1 (1.5%)
Native Hawaiian or other Pacific Islander	1 (1.5%)
Black or African American	16 (24.2%)
White	48 (72.7%)
Ethnicity, *n* (%)	
Hispanic	28 (40%)
Non-Hispanic	42 (60%)

Frequencies for Fruit and Vegetable Intake, Body Fat, Fitness Level, BMI, and MPOD

The frequencies for fruit and vegetable intake, body fat, fitness level, BMI, and MPOD are reported in Table 3. The majority of participants did not meet the USDA recommendations for fruit (16.1%) and vegetable intake (11.7%). The majority of the sample (69.4%) was considered lean; however, a high percentage also had excess body fat (25.4%). In addition, over a third of the population was overweight or obese (34.2%). The population was about evenly split between poor and good fitness level. Lastly, 40.9% of the population was above the national average for MPOD score.

Table 3. Frequencies for Fruit and Vegetable Intake, Body Fat, Fitness Level, BMI, and MPOD

Variables	N (%)
Met fruit and vegetable intake (n=68)	
Fruit intake	11 (16.1%)
Vegetable intake	8 (11.7%)
Body fat % (n=59)	
Risky (low body fat)	1 (1.6%)
Lean	41 (69.4%)
Excess fat	15 (25.4%)
Risky (high body fat)	2 (3.3%)
Fitness level* (n=22)	
Poor	12 (54.5%)
Good	10 (45.4%)
BMI	
Underweight	1 (1.4%)
Normal weight	45 (64.2%)
Overweight	17 (24.2%)
Obese	7 (10%)
MPOD	
Above national average	27 (40.9%)

*Includes participants who reached their VO_2 max.
N = number of participants who met the category for each variable.
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Descriptive Statistics for MPOD, Body Fat, Fruit and Vegetable Intake, and Aerobic Fitness

MPOD, body fat percentage, fruit and vegetable intake, and aerobic fitness level are reported in Table 4. The MPOD mean (Mean = 0.39, SD = 0.17) was above the national average of 0.33.[43] Participants reported consumption of approximately 1 cup of fruits and 1 cup of vegetables a day, indicating the sample did not meet USDA recommendations for fruit and vegetable intake. The mean body fat percentage for both males (Mean = 14.2, SD = 8.4) and females (Mean = 27.3, SD = 5.8) determined that the population was lean. The mean VO_2 max (ml/kg/min) for males (Mean = 47.6, SD = 16.5) and females (Mean = 35.4, SD = 3.7) indicated that both groups were considered above fair for their fitness level.

Table 4. Descriptive Statistics for MPOD, Body Fat Percentage, Fruit and Vegetable Intake, and Aerobic Fitness

Variable	Min – Max	Mean (SD)
MPOD (n=67)		
Absolute MP value right eye	0.02 – 0.94	0.39 (0.17)
Fruit and vegetable daily intake in cups (n=67)		
Fruit	0.0 – 6.5	1.0 (1.2)
Vegetable	0.0 – 6.0	1.0 (1.1)
Total	0.1 – 8.8	2.0 (1.9)
Body fat (%)		
Male (n=19)	3.0 – 31.0	14.2 (8.4)
Female (n=40)	17.5 – 41.9	27.3 (5.8)
Total (n=59)	3.0 – 41.9	23.1 (9.1)
Aerobic fitness (ml/kg/min) *		
Male (n=5)	30.3 – 72.8	47.6 (16.5)
Female (n=17)	27.3 – 42.6	35.4 (3.7)
Total (n=22)	27.3 – 72.8	38.1 (9.5)

*Only includes participants who reached VO_2 max.

Descriptive Statistics for Cognitive Performance

All scores are fully corrected T-scores that correct for age, gender, race/ethnicity, and education. The normative mean based on the T-score metric is 50 with a standard

deviation (SD) of 10. The Flanker Inhibitory Control and Attention test (Mean = 41.0, SD = 9.6) was roughly one standard deviation below the population mean. All other tests associated with fluid and crystallized cognition were approximately at the normative mean.

Table 5. Descriptive Statistics for Cognitive Performance (n=70)

Cognitive Test	Min-Max	Mean (SD)
Flanker Inhibitory Control and Attention	21-67	41.0 (9.6)
Dimensional Change Card Sort	29-74	50.0 (11.0)
Picture Sequence Memory	34-76	52.6 (11.1)
Picture Vocabulary **	31-76	53.8 (9.3)
Oral Reading Recognition **	37-85	57.9 (9.6)
Pattern Comparison Processing Speed	16-71	53.6 (12.0)
List Sorting Working Memory	28-64	48.4 (7.6)
Oral Symbol Digit *	75-143	98.8 (12.3)
Fluid Composite	24-70	48.5 (9.5)
Crystallized Composite**	34-74	56.5 (8.7)
Total Composite	34-71	52.9 (7.4)

* Raw score only (n=68)
**Crystallized cognition measures (All other tests are associated with fluid cognition)

Determining Correlation of Fruit and Vegetable Intake and MPOD and MPOD and Fluid Intelligence

Using multiple linear regression with body fat percentage and gender as covariates, total fruit and vegetable intake was not a significant predictor of MPOD, $F(2, 53) = 0.20$, $p = 0.81$, adj. $R^2 = -0.03$ (results not shown). Further, MPOD was not a significant predictor of any of the fluid intelligence performance variables (Table 6).

Table 6. MPOD and Fluid Intelligence

		Cognitive domains					
		Flanker Inhibitory Control and Attention	Dimensional Change Card Sort	Picture Sequence Memory	Pattern Comparison Processing Speed	List Sorting Working Memory	Fluid Composite
R^2		-0.022	-0.034	0.026	-0.009	0.013	-0.020
MPOD	B	-3.572	-2.366	9.013	-4.090	6.065	1.157
	SE B	7.343	8.634	8.670	9.675	5.860	7.401
	β	-0.065	-0.037	0.136	-0.056	0.136	0.021

Table 6. Continued

Body fat	B	-0.111	-0.049	0.276	0.219	0.156	0.136
	SE B	0.147	0.173	0.174	0.194	0.117	0.148
	β	-0.101	-0.038	0.208	0.151	0.175	0.123

Fruit and Vegetable Intake and Fluid Cognitive Scores Between High and Low MPOD Groups

An ANOVA was performed to assess differences between individuals with low (<0.41) and high (≥0.41) MPOD values. There was not a statistically significant difference in fruit and vegetable intake between the high and low MPOD groups (Table 7). There was a difference between high and low MPOD groups for the Picture Sequence Memory Test (Table 8). No other differences were found between the MPOD groups for the other fluid cognitive tests.

Table 7. Fruit and Vegetable Intake Between High and Low MPOD Groups

	MPOD Group		
	Low (<0.41) n = 32	*High (≥0.41)* n = 32	p value
	M (SE)	M (SE)	
Total fruit and vegetable intake	1.4 (1.20)	1.2 (1.20)	0.724

Table 8. Fluid Cognitive Scores Between High and Low MPOD Groups

Cognitive Tests	**MPOD Group**		
	Low (<0.41) Mean = 0.24 ± 0.08 n = 32	*High (≥0.41)* Mean = 0.52 ± 0.12 n = 34	p value
	M (SE)	M (SE)	
Flanker Inhibitory Control and Attention	40.9 (1.7)	40.9 (1.6)	0.991
Dimensional Change Card Sort	50.5 (1.9)	49.7 (1.9)	0.774
Picture Sequence Memory	50.3 (1.9)	55.9 (1.8)	0.041*
Pattern Comparison Processing Speed	54.0 (2.1)	53.9 (2.1)	0.985
List Sorting Working Memory	48.7 (1.3)	48.7 (1.3)	0.993
Fluid composite	48.2 (1.6)	49.5 (1.6)	0.570

*Denotes a significant difference between MPOD groups ($p < 0.05$)

Discussion

Lutein and zeaxanthin are macular carotenoids that are in fruits and vegetables, and more abundant in green leafy vegetables such as kale and spinach.[38] These carotenoids accumulate in the central part of the retina where they make up the macular pigment (MP).[38] MPOD measures long-term intake of lutein and zeaxanthin within the MP and has been linked to cognitive function in children, young adults, and the elderly.[3,12,21,36,37,38] The aim of the present study was to identify a relationship between fruit and vegetable intake and MPOD, as well as MPOD and cognitive function in healthy young adults. Few studies have concentrated on the young adult population. Further, we attempted to extend the knowledge in this research through controlling for modifiable lifestyle factors that may impact MPOD and cognitive function. After controlling for modifiable lifestyle factors, we were unable to identify a relationship between fruit and vegetable intake and MPOD as well as MPOD and cognitive function. However, there was a significant difference for the Picture Sequence Memory test scores between the low and high MPOD groups.

Fruit and Vegetable Intake are Not Correlated with MPOD in Young Adults

The aim of examining the relationship between fruit and vegetable intake and MPOD was to determine if MPOD was a good noninvasive biomarker for fruit and vegetable intake. Previous studies have demonstrated a relationship between fruit and vegetable intake and MPOD in older populations.[17,44] However, a study by Alonso et al. including young (20 – 35 years) and older subjects (45 – 65 years) found no correlation between fruit and vegetable intake and MPOD in the younger subjects.[17] The younger participants in the study had a significantly lower intake of fruits and vegetables than the

older group. Consequently, there may not have been enough variation in fruit and vegetable intake to examine a relationship with MPOD. A study by Howells et al. did not find a significant correlation among young adults between fruit and vegetable intake and MPOD as well.[45] The sample size (117) for the study may have been too small to see a relationship. Similar to our study on young adults, we were unable to determine a relationship between fruit and vegetable intake and MPOD, most likely for similar reasons. Our population may have been too small, and the majority consumed less than the daily recommended amount of fruits (83.9%) and vegetables (88.3%). A study by Burke et al. may have corrected for low variation among fruit and vegetable intake.[44] The researchers identified a correlation between fruit and vegetable intake and MPOD in older adults by dividing individuals by their fruit and vegetable intake into low, medium, high, and very high groups.[44] Participants with low fruit and vegetable intake had the lowest MPOD scores, while participants with the highest fruit and vegetable consumption had the highest MPOD scores. Due to the lack of variation in fruit and vegetable intake in our study, it was difficult to detect a correlation between fruit and vegetable intake and MPOD. We used MPOD scores to split participants into low and high MPOD groups according to the sample median MPOD scores. However, unlike the study from Burke et al., fruit and vegetable intake were not different between groups. Regardless, considering that the majority of the population did not consume the daily recommended amount of fruits and vegetables, an intervention should be aimed towards young adults since adequate fruit and vegetable intake is associated with other important diet related diseases such as coronary heart disease and some cancers.[46,47]

MPOD is Not Correlated with Cognitive Performance in Young Adults

Intelligence can be measured as general intelligence or can be separated into its components, fluid and crystallized intelligence.[48] Our study examined fluid intelligence because it can be altered by modifiable lifestyle factors and tends to decline through aging, while crystallized intelligence is acquired throughout a lifetime.[48] In addition, we studied fluid intelligence because previous research shows that MPOD is related to fluid intelligence in children and older adults.[12,21,23,49] Inconsistent with this research, MPOD was not a significant predictor of fluid intelligence in our population, even after controlling for body fat. However, the scores for the Picture Sequence Memory test did differ between the low and high MPOD groups. One study in older adults found that higher MPOD was associated with improved verbal learning and fluency as well as processing and perceptual speed.[21] Similarly, another study in older adults found that lower MPOD was associated with poorer performance on cognitive tasks related to reaction time and prospective memory.[49] Although both studies did determine a correlation between MPOD and fluid intelligence, neither controlled for covariates that impact cognition, which includes body fat and aerobic fitness. Barnett et al. did control for body fat and aerobic fitness in a study that examined MPOD and academic performance in preadolescent children.[23] In this study, body fat and VO_2 max were associated with the achievement composite score that tested cognitive function. After the addition of MPOD into the model, there was a strong correlation between MPOD and math and written language scores. Although aerobic fitness was measured in our study, it was not included as a covariate because there was not enough data available. In our study, a smaller population and failure to control for aerobic fitness may have impacted

our ability to find a correlation between MPOD and cognitive function. Thus, separating MPOD scores into low and high values created enough variation to identify a difference between the MPOD groups for the Picture Sequence Memory test.

The majority of studies investigating MPOD and cognitive function include older adults. To our knowledge, only one other study has examined young adults 18-30 years of age and demonstrated that increased MPOD through supplementation improved cognitive function, specifically in spatial memory, reasoning ability, and complex attention.[3] In this study we identified a significant difference in the Picture Sequence Memory test scores, a measure of episodic memory, among low and high MPOD groups. Episodic memory is the memory of experiences including the time and place the event occurred.[50] Episodic memory is supported by many regions of the brain including the hippocampus, in addition to the parietal, temporal, and prefrontal cortices.[50] These regions of the brain are associated with cognitive decline. In addition, research shows that poor episodic memory is linked to increased risk for Alzheimer's disease.[51] Modifiable lifestyle factors can have an impact on these regions of the brain, thus effecting the risk of age-related decline in episodic memory. For example, education, physical activity, and smoking are all risk factors for episodic memory.[50,52] In addition, a study by Summers et al. showed that antioxidant supplementation can improve memory, including episodic memory, in older subjects.[52] The supplement used in the study consisted of a number of antioxidants including lutein. Lutein has been shown to accumulate in the hippocampus, a region of the brain that controls episodic memory, which supports our finding of greater episodic memory performance in high MPOD groups.[53] In fact, higher concentrations of lutein compared to other carotenoids are found in the hippocampus.[53] The hippocampus

is also a region of the brain that is sensitive to modifiable lifestyle factors.[51] Therefore, lutein status may be important to consider when evaluating episodic memory performance.

In our sample episodic memory was the only cognitive domain that was related to MPOD levels. Young adults are a difficult population to demonstrate cognitive change unlike older populations that may have nutrient deficiencies or progression towards cognitive impairment.[3] A study with young adults that was able to impact cognitive function in an otherwise healthy population through lutein and zeaxanthin supplementation, suggests that supplementation may be necessary to observe changes in MPOD and subsequent cognitive health benefits in young adults.[3] Most intervention studies, including the research on young adults, supplement with 10-12 mg/day of lutein+zeaxanthin; however, most adults in America only consume around 1-2 mg of lutein each day.[54] Considering that lutein is primarily available in fruits and vegetables[54], it is important to note that roughly 11% and 8% of our population met the daily recommendations for fruit and vegetable intake, respectively (Table 3). Therefore, the majority of our population had a low intake of fruits and vegetables and did not meet the daily recommendations, which may impact lutein and zeaxanthin dietary intake. In addition, the majority of MPOD scores for our population were around the national average. This did not provide enough variation in MPOD scores to establish a correlation with cognitive function. Therefore, supplementation may be useful to increase lutein and zeaxanthin dietary intake, consequently improving MPOD scores and allowing for more variation.

Strengths and Limitations

A strength of our study was that we controlled for body fat percentage. As previously stated, it is important to consider body fat when measuring cognitive function and MPOD because studies show they are significantly related.[23] Although aerobic fitness was not included as a covariate, we did acknowledge the importance of measuring VO_2 max and attempted to include it in our study. Lastly, 40% of our participants were Hispanic. Previous research on MPOD and cognitive function has been conducted in primarily Non-Hispanic Caucasians. Therefore, our study offered more diversity than previous studies.

A limitation for our research was that we had missing data, which reduced the power of the study. 15% of participants did not show to visit 2, therefore strategies are needed to improve adherence. In addition, we did not control for participant fatigue during administration of the cognitive tests. This may have impacted the participant's scores as research has shown that fatigue can impact psychomotor and cognitive function.[55] Another limitation was aerobic fitness was not included in the analysis due to a low number of participants who performed the fitness test and reached their VO_2 max. For future research, this will be important to include because aerobic fitness promotes functions associated with higher order regions that help with the control of cognition. Further, research has shown that individuals who are more active and fit can process information more quickly.[56] The fruit and vegetable intake questionnaire limited the evaluation of specific fruits and vegetables that contain lutein. Other studies used food frequency questionnaires or dietary recalls to get a better measure of typical consumption of lutein and zeaxanthin containing foods. In addition, a limitation for my study was that

we did not control for other nutrients that effect cognitive function. Studies have shown that omega-3 fatty acids and vitamins B6, B12, and folate impact cognitive function and brain atrophy.[57,58,59] The association between B6, B12, and folate and cognition can be explained by their role in homocysteine metabolism. Homocysteine is connected to cognitive impairment in high concentrations.[59] In addition, DHA makes up 30% to 40% of the polyunsaturated fatty acids in the grey matter of the cerebral cortex.[59] Therefore, it is important to control for these nutrients by using a more comprehensive assessment of dietary intake such as a food frequency questionnaire.

Overall the population chosen was not the healthy population we aimed to recruit; however, they are generally representative of the young adult population. In addition to low intake of fruits and vegetables, more than 25% of participants had excess fat or high body fat. Although we would expect MPOD scores to suffer from high body fat and low fruit and vegetable intake, almost half of the participants had an MPOD score around the national average.[48] MPOD scores above the national average were also seen in another sample of young adults.[3] Therefore, MPOD may not be as at risk for decline for younger populations. The frequency of high MPOD values in our sample did not provide enough variation to establish a relationship among MPOD values with many of our variables.

Conclusion

Episodic memory was the only cognitive domain that was associated with levels of MPOD within our sample. As stated previously, the hippocampus controls episodic memory and consists of high concentrations of lutein.[50,53] The hippocampus controls other cognitive domains and in a study with young adults more cognitive domains were related to MPOD.[3] Additionally, lutein accumulates in other regions of the brain that can

effect brain function.[53] Therefore, more research is needed to see which cognitive domains are most vulnerable to MPOD in young healthy adults.

III. SUMMARY AND FUTURE DIRECTIONS

As stated previously, no relationship was found between fruit and vegetable intake and MPOD, as well as MPOD and cognitive function. Future studies should correct for the weaknesses that may have inhibited our ability to find a correlation between these two variables. Therefore, looking forward I would include a larger population. Based off a power analysis performed using the output from the Picture Sequence Memory test among the low and high MPOD groups, 128 participants would need to be recruited to achieve 80% power. The Fluid Composite results for the low and high MPOD groups indicated that a future study would need to include thousands of participants to reach 80% power. Therefore, a change in study design may be necessary for future studies. For example, to provide further variation among the sample, a targeted recruitment of high and low fruit and vegetable consumers may be needed.

I would also consider a different dietary approach that would determine lutein and zeaxanthin intake rather than fruit and vegetable intake. We originally chose the NCI fruit and vegetable intake questionnaire because of its ease of use. Other questionnaires that are longer or food records are burdensome for participants to complete. In addition, they include foods that do not contain lutein or zeaxanthin. On the other hand, studies have assessed lutein and zeaxanthin intake through food frequency questionnaires or 3-day food records, utilizing databases designed to calculate individual dietary carotenoid intake.[17,44,60] These studies were able to show a relationship between MPOD and dietary intakes of lutein and zeaxanthin in older adults. There are few studies investigating this relationship in young adults; therefore, it may be interesting to examine in order to

determine if MPOD is a good noninvasive biomarker for lutein and zeaxanthin intake rather than fruit and vegetable intake.

Further, the results from the cognitive assessment may not be an accurate measurement of the participants' cognitive status. Although the NIH Toolbox is a highly reliable objective cognitive battery that did allow us to compare our sample with the population norms, there were other factors that may have impacted the participant's scores. For example, fatigue can impact psychomotor and cognitive function.[55] Fatigue should be considered prior to testing as well as during testing, particularly for this toolbox that takes approximately 34 minutes to administer. For future studies, testing should be scheduled for same time of day to avoid inconsistencies among participants and fatigue should be measured before and after testing. Lastly, participants using cognitive brain game programs such as Luminosity or BrainHQ should be excluded from the study. Luminosity includes tasks that cover processing speed, working memory, flexibility, problem solving, and attention.[61] Many of these cognitive domains are also tested in neuropsychological assessments and research show that continuous use of Luminosity and other brain games can help improve scores on neuropsychological assessments.[61,62]

During the statistical analysis, I was unable to control for aerobic fitness. This is important to control for as research shows that fitness level has an impact on cognitive health.[29,30] The Bruce Protocol, although the gold standard to determine aerobic fitness, proved to be too difficult for our population to complete. This test is a direct measure of VO_2 max and requires the participant to work until volitional exhaustion. If the participant ends the test prior to reaching their VO_2 max, then the data is void. Therefore,

in the future VO_2 max should be measured using a different method. Calculating predicted VO_2 max may be a better measurement tool to ensure enough data is available for analysis. For example, an estimate of VO_2 max can be determined by a submaximal test. This test allows for a less strenuous environment that participants are more likely to complete successfully.[63]

In addition to all of these changes for a future cross-sectional study, I would also propose a study design of a randomized controlled trial that provided lutein and zeaxanthin supplementation. Supplementation may be necessary in order to demonstrate change in young adults since they are at their cognitive peak. Moreover, instead of concentrating purely on lutein and zeaxanthin, there are other nutrients that impact cognitive function. A study by Oulhaj et al. found that vitamin B6, B12, and folate supplementation in older adults with mild cognitive impairment and high baseline levels of omega-3 had improvements in episodic memory and general cognition.[58] In contrast, participants with low omega-3 status at baseline had no benefits after vitamin B supplementation. This may be possible through the homocysteine methylation cycle. In this cycle, B vitamins facilitate the conversion from phosphatidylethanolamine (PD) to phosphatidylcholine (PC), which is enriched in omega-3 fatty acids, and can then be transported to the brain. In addition, high levels of homocysteine, that results from low levels of vitamin B, inhibit the enzyme that converts PD to PC.[58] A separate study found that individuals with high omega-3 status at baseline that supplemented with B vitamins had a slower rate of brain atrophy compared to those that supplemented and had low baseline levels of omega-3.[57] These studies show that to improve cognitive function and slow the rate of cognitive decline, other nutrients need to be considered, and not just

studied in isolation. This could be in the form of controlling for vitamin B and omega-3 intake or including these nutrients in an intervention study in addition to lutein.

Conclusion

Although we did not identify a correlation between MPOD and cognitive function, this may have been due to the cross-sectional study design or, as stated earlier, there may be no significant relationship present unless participants consume at least 10mg of lutein a day, which is difficult to obtain from diet if individuals do not consume adequate fruits and vegetables. Young adults are a difficult population to demonstrate change unlike older populations that may have nutrient deficiencies or cognitive impairments. It may be important to consider that a relationship may not clearly exist among lutein, MPOD, and cognitive function in young adults that are at their cognitive peak. Additionally, although our findings conflict with several studies that have identified a relationship between MPOD and cognitive function, it is important to consider the possibility of publication bias. Button et al. discussed that neuroscience studies have a median statistical power of only 21%,[64] yet the majority of these papers report significant results. The power of this study was only 57%, however there were no statistically significant results found.

Our study is a needed contribution to the currently limited research on young adults related to MPOD and cognitive function. More research, however, is needed to identify other modifiable lifestyle factors to promote cognitive health. This is important in order to slow the progression of age related cognitive diseases and to potentially help young adults in their academic and career success.[7]

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