Investigating the Relationships Among Self-Reported Carotenoid Intakes, Skin Carotenoid Concentrations and Cognitive Outcomes in Early Adolescents

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Authorization to Submit Thesis

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Abstract

Lutein and zeaxanthin are two carotenoids, known as the xanthophylls, which have recently garnered attention for their potential role in cognition. Within the brain, the xanthophylls are believed to be protective via their action as antioxidants. Previous research has indicated possible correlations between serum and macular carotenoid concentrations and cognitive outcomes, but results are generally mixed, particularly when referring to specific aspects of cognition. Measurement of carotenoids via resonance Raman spectroscopy of the skin is reliable, non-invasive and requires minimal training, but there is limited research examining the direct relationship between skin carotenoid concentrations and measures of cognition. Additional insight is needed on the nature of these associations in younger populations.

The purpose of this research was to investigate the relationships among self-reported carotenoid intake, skin carotenoid concentrations and cognitive outcomes in early adolescents. Thirty adolescents aged 11-14 years participated in the cross-sectional study. Dietary intake of lutein and zeaxanthin, lycopene, alpha and beta-carotene, cryptoxanthin, total carotenoids, and total fruits and vegetables were assessed from three days of 24-hour dietary recall data collected and analyzed using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, developed by the National Cancer Institute. Skin carotenoid concentrations were measured by resonance Raman spectroscopy. Assessments from the NIH Toolbox for Assessment of Neurological and Behavioral Function were administered to determine scores of executive function, episodic memory, working memory, attention, processing speed, and fluid cognition, all adjusted for age, gender, race, ethnicity, and parent education. Data analysis was conducted using SAS software and a significance value of $p \leq 0.05$. Pearson correlations were used to evaluate the relationship between variables with a normal distribution and Spearman correlations were used between variables not exhibiting characteristics of normality. No significant relationships were found between skin carotenoid concentrations and intakes of carotenoids, including lutein and zeaxanthin. A significant positive association was found between skin carotenoid concentrations and scores of working memory ($R^2 = 0.43$, $p=0.02$). A significant inverse relationship was noted between intakes of lutein and zeaxanthin and scores of working memory ($R^2 = -0.43$, $p=0.02$) and significant positive correlations were found between scores of episodic memory and intakes of cryptoxanthin (R^2 = 0.41, p=0.02), lycopene (R^2 = 0.40, p=0.03), total carotenoids (R^2 = 0.39, p=0.03), and total fruit and vegetable intake (R^2 = 0.38, p=0.04). Overall, skin carotenoid concentrations were positively associated with scores of working memory. Dietary intake of lutein and zeaxanthin was negatively associated with working memory scores and intakes of cryptoxanthin, lycopene, and total carotenoids were positively associated with episodic memory scores in adolescents aged 11-14 years. Dietary intake of specific carotenoids may have varied associations with specific domains of cognition. Larger sample sizes are needed to comprehensively evaluate these relationships in adolescent populations.

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Dedication

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List of Abbreviations

BMI	Body Mass Index
BSQ	Beverage and Snack Questionnaire
DCCS	NIH Toolbox Dimensional Change Card Sort Test
FFQ	Food Frequency Questionnaire
FICA	NIH Toolbox Flanker Inhibitory Control and Attention Test
HFP	Heterochromatic Flicker Photometry
HPLC	High-Performance Liquid Chromatography
LSWM	NIH Toolbox List Sorting Working Memory Test
MPOD	Macular Pigment Ocular Density
MPVUC	Macular Pigment Volume Under the Curve
NIH	National Institute of Health
PCPS	NIH Toolbox Pattern Comparison Processing Speed Test
PSM	NIH Toolbox Picture Sequence Memory Test
ROS	Reactive Oxygen Species
RRS	Resonance Ramen Spectroscopy
SAS	Statistical Analysis Software

Chapter 1: Research Purpose and Overview

Problem Statement

Carotenoids and specifically, the xanthophylls, lutein and zeaxanthin, have recently garnered attention for their potential role in cognition. Within the macula and the brain, the xanthophylls are protective via their action as antioxidants. Previous research on brain tissues has revealed that the xanthophylls account for ~66-77% of total carotenoid concentrations in the adult brain with lutein alone accounting for ~34% (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Johnson et al., 2013). In the infant brain, lutein concentrations have been noted to be even higher, comprising more than half of total carotenoids (Jia et al., 2017; Vishwanathan, Kuchan, Sen, & Johnson, 2014). The specific nature of the relationship between carotenoids and cognition has remained somewhat unclear in the literature. Previous research has indicated possible correlations between serum carotenoid concentrations and measures of macular carotenoids with cognitive outcomes, but results are often inconsistent and mixed when referring to specific aspects of cognition. Measurement of carotenoids via resonance Raman spectroscopy (RRS) of the skin is reliable, non-invasive and requires minimal training, but there is limited research examining the direct relationship between skin carotenoid concentrations and measures of cognition. Furthermore, there remains a need for more information on the nature of this association within younger populations. The greater concentrations of lutein in children may indicate that specific carotenoids have a particularly important role in the development of the pediatric brain. A better understanding of how certain carotenoids, in the skin and in the diet, may influence cognition in this population, could lead to research on dietary interventions designed to optimize cognitive development in children. Further research may have applications for developing dietary recommendations of carotenoids for children, or in the way that carotenoids are promoted in public health and nutrition education. The goal of this study is to examine how skin carotenoid concentrations measured via RRS, as well as dietary intake of specific carotenoids, including lutein and zeaxanthin, may relate to measures of cognition in a sample of early adolescents.

Statement of Purpose

The purpose of this study was to investigate the relationships among self-reported carotenoid intakes, skin carotenoid concentrations and cognitive outcomes in a sample of early adolescents.

Research Questions

Three different research questions were addressed in this thesis. The first research question was "in early adolescents, is there a significant correlation between skin carotenoid concentrations and measures of cognition (including composite scores of fluid cognition and individual scores of executive function, episodic memory, working memory, attention and processing speed)?" The second research question was "in early adolescents, is there a significant correlation between self-reported carotenoid intake (specifically lutein and zeaxanthin) and measures of cognition (including composite scores of fluid cognition and individual scores of executive function, episodic memory, working memory, attention and processing speed)?" The third research question was "in early adolescents, does a significant correlation exist between self-reported carotenoid intake and concentrations of skin carotenoids measured via RRS?"

Hypotheses

Regarding the first research question, it was hypothesized that skin carotenoid concentrations, measured via RRS, would have a positive and significant correlation with averaged composite scores of fluid intelligence as well as a correlation with individual measures of cognition including executive function, episodic memory, working memory, attention and processing speed. Second, it was hypothesized that self-reported carotenoid intake, specifically lutein and zeaxanthin intake, would have a significant and positive correlation with composite scores of cognition as well as with individual measures of cognition, including executive function, episodic memory, working memory, attention and processing speed. Finally, self-reported carotenoid intake, specifically of lutein and zeaxanthin, would have a significant and positive correlation with concentrations of skin carotenoids measured via RRS.

Limitations

This study has several limitations. Data collection occurred between the months of April and September, therefore spanning multiple seasons of the year. These seasonal factors may have led to variability in the concentrations of skin carotenoids and carotenoid intakes. Previous research has found a significant increase in skin carotenoid concentrations during summer and autumn months when compared to winter and spring months (Darvin et al., 2008). Additionally, different seasons may contribute to variations in availability and intake of seasonal fruits and vegetables therefore influencing carotenoid intake.

After being told about the purpose of the study and participating in the in-person visit, participants and parents/guardians may have, intentionally or unintentionally, changed their intake of carotenoid containing foods. To avoid this, researchers asked participants not to change their dietary behaviors for the duration of the study. Furthermore, participants may have responded differently to the 24-hour diet recall when performing the task at home rather than on site while supervised by a researcher. Researchers were trained with the purpose of only providing guidance for how to use the online dietary assessment tool and answer questions as needed.

Another limitation of this study is the use of skin carotenoid measurements and dietary intake of carotenoids as a possible biomarker for carotenoids in the brain. The proposed mechanism for the relationship between carotenoid status and cognition is the presence of carotenoids in the brain. Despite many previous studies finding a high correlation between skin carotenoids, serum carotenoids and macular pigment carotenoids, the use of skin measurements to find a connection to cognition is both a strength and weakness of this study. If results support a relationship between skin carotenoid measurements and cognitive outcomes, measurement of carotenoid status via the skin would provide an efficient research methodology requiring minimal training that can be used to assess populations throughout the lifespan. This remains a limitation because the measurement of carotenoids via the skin does not directly reflect the amount and distribution of carotenoids in the brain. These relationships may therefore be weaker when attempting to evaluate this correlation using this methodology.

Regarding skin carotenoid measurement, another limitation is the change in measurement calculation that occurred approximately halfway through data collection due to changes in technical measurements via the S3 Scanner application. For the first 16 participants, hand scan measurements were provided rounded to the nearest one whole integer. For the second 14 participants, hand scan measurements were provided from the application rounded to the nearest thousand. These differences in RRS measurement rounding may have influenced the results of the study.

Finally, because of the homogenous demographic characteristics of this study's sample, results may not be generalizable to other populations of adolescents which may include more racial, ethnic, and socioeconomic diversity.

Chapter 2: Review of the Literature

Carotenoids and the Brain

Carotenoids consist of a large group of fat-soluble pigments that are found in plants and other organisms (Fiedor & Burda, 2014; Johnson, 2002). They are responsible for many of the bright red, orange and yellow colors that we see in various fruits and vegetables. Over 1,100 carotenoids have been identified in nature, but only approximately 50 are notably available in the human diet and only 20 have been found in human blood (Renzi-Hammond, Johnson, & Richer, 2018). In the context of human health, carotenoids play an important role in the prevention of disease and general maintenance of a healthy human body. Carotenoids are one of the most efficient scavengers of reactive oxygen species (ROS) in the cell (Fiedor & Burda, 2014). A shift in the balance between ROS generation and removal may lead to the overproduction of ROS which could result in an increased risk for a variety of chronic diseases (Fiedor & Burda, 2014). Please see reviews by Johnson (2002) and Fiedor and Burda (2014) for a more in depth description of the general role of carotenoids in human health and disease (Fiedor & Burda, 2014; Johnson, 2002). This review will focus on the influence that carotenoids may have on the human brain and, more specifically, cognition.

Lutein and zeaxanthin belong to a specific group of carotenoids called xanthophylls. These two carotenoids have garnered recent attention in the literature for their role in preventing eye-related disorders and possible influence in cognition (Fiedor & Burda, 2014; Johnson, 2012; Snodderly, 1995; Stringham, Johnson, & Hammond, 2019) . When consumed, lutein and zeaxanthin preferentially accumulate in the central retina forming the macular pigment. In the macula, these pigments help to prevent damage to photoreceptors by absorbing short-wavelength “blue” light (Erdman et al., 2015; Saint et al., 2018; Snodderly, 1995). Due to the high content of polyunsaturated lipids within retinal photoreceptors, the retina can also be susceptible to oxidative damage (Erdman et al., 2015). The macular carotenoids may play a role in minimizing potential oxidative damage within the retina through their action as antioxidants (Erdman et al., 2015). Lutein and zeaxanthin are also found to be two of the most abundant carotenoids in the tissue of the human brain. The mechanisms by which these carotenoids provide protection in the retina are suspected to provide similar protection in the brain (Erdman et al., 2015). Due to its lipid composition and high metabolic activity, the brain is also at risk for damage by free radicals and the xanthophylls are believed to be neuroprotective (Erdman et al., 2015). The most notable carotenoids found in the human brain include lutein, zeaxanthin, anhydrolutein, alpha-cryptoxanthin, beta-cryptoxanthin, alpha-carotene, cis- and trans-beta-carotene and cis- and trans-lycopene, with the xanthophylls comprising ~66-77% of the total carotenoids (Craft et al., 2004). Previous studies examining tissues from the occipital and frontal regions of the brain, have found that the frontal region contains significantly higher concentrations of total xanthophylls (Craft et al., 2004). More recent research has further confirmed the dominant presence of the xanthophylls in the elderly human brain, finding that lutein, zeaxanthin and cryptoxanthin represented 72% of the total concentration of carotenoids with lutein alone accounting for 34% (Johnson et al., 2013). In these samples, concentrations of lutein and zeaxanthin were found to be significantly greater in the cerebellum when compared to the frontal, occipital and temporal areas of the brain (Johnson et al., 2013). Lutein appears to have a dominant presence in the brain throughout the human lifetime. When compared to the brain of an adult, the relative contribution of lutein to total carotenoids is nearly twice the amount in a pediatric brain. In an infant, lutein accounts for more than half of the concentration of total carotenoids in the brain (Jia et al., 2017; Vishwanathan et al., 2014). These findings suggest a preference for lutein in the development of the infant brain (Johnson, 2014).

Methodology for the Measurement of Carotenoids

Multiple methods have been developed for the general measurement of carotenoids in the human body, including the eye, blood, and skin. Carotenoid concentrations, distribution, and measurement methods may vary depending on the part of the body being assessed. There are currently multiple ways to determine carotenoid levels in the human macula including high-performance liquid chromatography (HPLC) analysis and heterochromatic flicker photometry (HFP). HPLC measurement of macular carotenoids is notably time-consuming and tissue destructive (Conrady et al., 2017). Because of this, HFP has been more commonly used to measure macular pigment optical density (MPOD) non-invasively. HFP methodology still requires time as well as subject and researcher training to administer accurately (Conrady et al., 2017). Another, more recently developed method for measuring carotenoids in the macula, is imaging based technology. Through the use of reflectometry, autofluorescence attenuation or resonance Raman spectroscopy (RRS), specific instruments can obtain a high-resolution image of the eye providing data on the spatial distribution of macular carotenoids (Conrady et al., 2017).

HPLC has also been used to analyze serum samples in order to measure carotenoid concentrations in the blood. Some of the major plasma or serum carotenoids have been identified to be beta-carotene, alpha carotene, lycopene, cryptoxanthin and lutein/zeaxanthin (Parker, 1989). Serum analysis is specific but invasive due to the need for a blood draw as well as more time intensive blood analysis.

The development and use of RRS for the detection of carotenoids in human skin tissue has been somewhat recent. Carotenoid molecules can be excited when light overlaps their visible absorption bands. When in an excited state, they create a strong resonance Raman scattering response (Ermakov, Sharifzadeh, Ermakova, & Gellermann, 2005). This response allows for the differentiation of the characteristic vibrational energy levels of specific carotenoids in living human tissue. The RRS method requires specialized instruments but it provides a non-invasive and efficient way to quickly determine skin carotenoid content in a variety of populations with minimal administrator training. Assessment of skin carotenoid status via RRS has been found to be reproducible and valid (Mayne et al., 2013). Skin carotenoid levels may also be a better representation of long-term carotenoid status in contrast to serum levels ,which indicate more short-term dietary intake of carotenoids (Ermakov et al., 2005).

The carotenoids that have been identified via HPLC analysis as the most prevalent in human skin include lycopene, phytoene, the combined carotenes, lutein and zeaxanthin, and phytofluene (Hata et al., 2000). When combined, lycopene and the collective carotenes were found to make up ~60% of total carotenoid concentrations in the skin and the xanthophylls were found to comprise ~12% (Hata et al., 2000). Phytoene and phytofluene are not detected with Raman spectroscopy as they do not absorb the specific wavelength used with this method. Concentration of specific carotenoids varies depending on the part of the body being examined, with the palm region having the highest mean carotenoid concentration (Hata et al., 2000).

Previous studies have identified a significant correlation between RRS measurement of carotenoids in skin tissue and total serum carotenoids in adults (r=0.722) (Conrady et al., 2017) and in child populations ranging from 5-17 years (r=0.62) (Aguilar, Wengreen, Lefevre, Madden, & Gast, 2014; Nguyen et al., 2015). Recent research has also shown a correlation between skin carotenoid levels and carotenoid measurements via MPOD and macular pigment volume under the curve (MPVUC) methodologies (Conrady et al., 2017; Edwards et al., 2019). Measurement of macular pigments via newer, and potentially more specific methods, such as MPVUC, has been shown to have a slightly stronger correlation to RRS skin concentrations of carotenoids when compared to MPOD measurement (MPVUC 9°: r=0.663, MPOD 2°: r=0.629) (Conrady et al., 2017). Other studies have shown that children's self-reported fruit and vegetable intake and carotenoid consumption measured using food frequency questionnaires (FFQ) and 24-hr diet recall methods also correlate significantly with skin carotenoid levels (Aguilar, Wengreen, & Dew, 2015; Aguilar et al., 2014; Nguyen et al., 2015). An intervention study in 2016, which worked with children in the fourth grade, found that a change in reported dietary intake of carotenoids was correlated with a similar change in skin carotenoid levels (Beccarelli et al., 2017). Scarmo et al. (2012) also found a positive correlation between fruit and vegetable intake and skin carotenoid status in preschool children aged 3-5 years. This study was unique due to its use of a pre-school adapted liking survey and a modified food frequency screener in order to determine fruit and vegetable intake.

Cognition and Carotenoids

The specific relationship between carotenoid levels and cognition has remained somewhat unclear in the literature. There have been multiple studies examining carotenoid intake, serum carotenoid levels and MPOD and their relationships with various measures of cognition. While there is some evidence to suggest that there is a correlation between cognition and carotenoids, the results thus far have been mixed.

Dietary Intake of Carotenoids and Cognition

A systematic review in 2013 of prospective and longitudinal studies demonstrated mixed conclusions when examining the relationship between dietary carotenoid intake and cognitive function (Crichton, Bryan, & Murphy, 2013). Most studies identified in the review consisted of middle-aged to elderly adult populations and examined relationships specifically between cognition, dementia and beta-carotene. The authors identified seven longitudinal studies which showed no association between dementia risk and dietary beta carotene intake, two cross-sectional studies which found no associations between dietary carotenoids and cognitive outcomes, and two cross-sectional studies which found that higher intakes of beta carotene were associated with better cognitive function based on dementia screening questionnaires (Crichton et al., 2013). Interestingly, their review included no articles examining the relationship of lutein and zeaxanthin on cognition. A longitudinal study in France, looked at the correlation between fruit and vegetable intake in adults at baseline compared with cognitive performance 13 years later. They found that higher intakes of fruits and vegetables, fruits alone, vitamin C and vitamin E were correlated with better verbal memory performance but that higher intakes of fruits and vegetables and beta-carotene-rich fruits and vegetables were associated with poorer executive function (Péneau et al., 2011).

More recent research has begun to examine the impact of dietary supplementation of the xanthophylls on specific aspects of cognition. In a sample of young healthy adults aged 18-30 years, one year supplementation of lutein and zeaxanthin (10mg of lutein and 2mg of zeaxanthin) was significantly correlated with improved performance on visual memory tasks (Renzi-Hammond et al., 2017). Combined supplementation of lutein, zeaxanthin and mixed omega-3 fatty acids were also found to correlate with visual processing speed in a similarly aged sample (Bovier, Renzi, & Hammond, 2014). Power et al. (2018) found improvements in episodic memory tasks following supplementation of lutein, zeaxanthin and meso-zeaxanthin in a slightly older population (average age of 45.4 years). Another notable study utilized a high lutein dietary intervention to evaluate for changes in cognition in a sample of 48 healthy adults (average age of 62-63 years) (Scott, Rasmussen, Chen, & Johnson, 2017). In the intervention group, participants were instructed to consume one avocado per day and the control, one potato or one cup of chickpeas per day for a total of six months. Results found an increase in memory and spatial working memory for both groups but a significant improvement in sustained attention for the avocado group only.

The literature on the relationship between dietary intake of carotenoids and cognition in children appears to be limited. A study in Canada in 2014, which examined lutein intake and plasma levels in 160 children aged 5-6 years, found no significant relationship between lutein intake and cognitive test scores (Mulder et al., 2014). It should be noted that other researchers have critiqued the general conclusions made in the study due to the nourished status of the study population as well as the lack of specificity when using the Kaufman

Assessment Battery and Peabody Picture Vocabulary Test to measure outcomes of cognition (Hammond, 2014).

Macular Pigment Ocular Density and Cognition

Much of the research on the relationship between macular pigments and cognition has been performed with adult populations. In a study of younger adults aged 18-30 years, an improvement of MPOD status, over a one year period of time, was significantly associated with improved measures of visual memory, complex attention and reasoning ability tasks (Renzi-Hammond et al., 2017). Kelly et al. (2015) found a significant correlation between higher macular pigment levels, measured via customized HFP, and better performance on cognitive tasks evaluating phonemic fluency, attention switching and visual memory.

Other studies have shown somewhat mixed results regarding which aspects of cognition may be related to MPOD. When adjusting for age, sex and education only, a study of older adults in Ireland found a significant relationship between lower MPOD levels and poorer performance on tasks related to global cognition, memory (one task of six), executive function (one task of four), processing speed and sustained attention (Feeney et al., 2013). After adjusting for all confounding variables, which also included smoking, hypertension, cholesterol, body mass index (BMI), visual acuity, diabetes, age-related macular degeneration, depression scores, antidepressant use and problem drinking, the relationship only remained significant for one prospective memory task, one task of executive function and all processing speed tasks.

Only recently have researchers begun to investigate the possible relationship between carotenoids and cognition in child populations. A recent study in 2017 found that MPOD was significantly correlated with some aspects of performance on standardized cognitive assessments taken by adolescents aged 7-13 years (Saint et al., 2018). Results showed that MPOD was correlated specifically with global intelligence, executive processing and spatial abilities but was not correlated with cognitive efficiency, verbal learning and processing speeds or visual-auditory skills. Another study found that children with greater MPOD values had a significantly higher response accuracy when performing a flanker task for incongruent trials, suggesting a possible benefit in cognitive control processing during times of high demand (Walk et al., 2017). MPOD has also been found to have a positive correlation with academic performance in preadolescent children aged 8-10 years, specifically when referring to achievement scores, math and written language (Barnett et al., 2018).

Serum Carotenoids and Cognition

Ascertaining the relationship between serum carotenoids and cognition has proven to be more difficult. A notable portion of the literature has been unable to identify a significant relationship between serum levels of carotenoids and various measures of cognition (Kelly et al., 2015; Mulder et al., 2014). A prospective study published in 2008 examined the relationship between plasma carotenoids, tocopherols and retinols and cognitive function in older women. The study looked at serum levels when the women were in their mid-60s and compared this to cognitive testing 10 years later. No significant relationship was found between overall or individual serum levels and cognitive function or decline (Kang & Grodstein, 2008).

While previously in the minority, there have been a few recent studies showing correlations between serum carotenoid levels and cognitive function. In a population of centenarians and octogenarians, serum lutein and zeaxanthin concentrations were found to be correlated with multiple measures of cognitive performance (Johnson et al., 2013). Serum levels of beta-carotene were also significantly correlated to most measures of cognitive function (Johnson et al., 2013). Another recent study by Feeney et al. (2017), found that serum concentrations of lutein and zeaxanthin each showed a significant, positive and independent association with composite scores of global cognition, memory and executive function. Serum concentrations of zeaxanthin were additionally found to have a significant correlation with processing speed scores (Feeney et al., 2017). A study in 2018 found that increases in serum concentrations of lutein and meso-zeaxanthin were significantly related to improvements in memory tasks (specifically paired associated learning and verbal recognition memory tasks) (Power et al., 2018). In a sample of adult breast cancer survivors, researchers found that low serum carotenoid concentrations were associated with more self-reported cognitive complaints when compared with low-carotenoid and high-carotenoid control groups (Zuniga & Moran, 2018). Overall, it appears that there may be increasing evidence to suggest that serum levels of carotenoids, specifically of the xanthophylls, may have a positive relationship with various measures of cognitive performance. Given the high correlation of skin concentrations of carotenoids to serum concentrations, learning more about skin carotenoids may provide a non-invasive and low participant burden method to gain further insight into the complex relationship between carotenoids and cognition.

Skin Carotenoids and Cognition

Until very recently, the relationship between skin carotenoid status and cognition was unexplored in both adults and children. An abstract published by Edwards et al. (2019) highlighted an observational study examining the associations between skin carotenoids and academic achievement in a sample of children aged 7-12 years. Researchers found a significant positive relationship between skin carotenoid measurements and reading and math scores. These results provide preliminary evidence supporting a potential positive relationship between skin carotenoid status and measures of academic achievement in children.

Limitations in Methodology

A limitation in the body of literature examining the relationship between carotenoids and cognition is the large variation in the way that cognitive performance is measured. The majority of studies discussed in this review have used a battery of cognitive assessments to measure different aspects of cognitive performance (Feeney et al., 2013; Kelly et al., 2015; Mulder et al., 2014; Power et al., 2018; Saint et al., 2018; Zuniga & Moran, 2018). Still other studies chose to focus on one specific aspect of cognition by using one assessment tool (Walk et al., 2017). A study in female older adults even used a telephone based cognitive assessment (Kang & Grodstein, 2008). Regarding studies working with pediatric populations, tools used to measure cognitive have included the Kaufman Assessment Battery (KABC-II), the Woodcock Johnson III Test of Cognitive Abilities and a modified flanker task assessment. Outside of the studies listed in this review, there exists many other ways of measuring cognitive performance in the individual. This variety in methodology may contribute to some of the inconsistency in specific results regarding the relationship between various carotenoids and cognitive outcomes.

Conclusion

Overall, there is still a need for more research examining the nuances in the relationship between carotenoids and cognitive outcomes, especially in pediatric populations. There is also minimal research that evaluates the potential correlation between skin carotenoid concentrations and elements of cognition directly. Historically, prior literature has focused specifically on the association between cognition and MPOD, but the procedures for obtaining MPOD measurements require time for researching training and may be more difficult and intimidating for younger populations. Measurement of skin carotenoid concentrations via RRS is non-invasive, quick, and requires minimal training for both the researcher and the participant. Skin carotenoid concentrations have also been shown to correlate well with MPOD measurements, serum carotenoid concentrations and carotenoid intakes. Therefore, the purpose of this research is to expand on the current literature evaluating the association between carotenoids and cognition and to identify the possible presence and nature of the specific relationship between skin carotenoid concentrations and cognitive outcomes in middle school aged children.

Chapter 3: Methods

Recruitment

Thirty adolescent participants were recruited via convenience-based sampling methods. Recruitment was performed via word of mouth, flyer advertisement in the local area of Moscow, Idaho and on social media websites, and via onsite promotion at local public locations and events, such as city parks and recreation facilities. Inclusion criteria specified that participants must be between 11 and 14 years of age. Individuals were encouraged not to participate if they had suffered a major illness within two weeks of data collection, but no formal exclusion criteria were enforced and therefore no interested individuals meeting inclusion criteria were excluded from the study.

Study Design and Protocol

This research was approved by the University of Idaho Institutional Review Board. The study was observational and cross-sectional in design. Data was collected during the months of April through September 2019. Participants were asked to attend one in-person visit on the University of Idaho campus. Prior to their visit, participants and/or their parent/guardian were emailed a copy of the informed consent form and a demographic and health questionnaire to review. These documents were either completed at home and brought to the in-person visit or completed at the start of the visit. In all cases, researchers answered questions as needed and briefly went over the protocol with participants and their parent/guardian prior to testing. The questionnaire contained demographic questions, including participant race, ethnicity, household income, and highest level of maternal education, as well as some health and lifestyle questions regarding physical activity, screen time, recent illness and sun exposure. After completing the informed consent and health and demographic questionnaire, researchers met one-on-one with participants to review the study protocol in detail and participants were asked to sign a child assent form. Researchers then administered the cognitive assessment, followed by the measurement of skin carotenoid concentrations. Finally, participants self-reported all beverage and food intake from the previous 24 hours via an online 24-hour dietary assessment tool. Participants were given a username and password in order to complete two additional 24-hour diet recalls on pre-assigned days at home. In total, the in-person visit took approximately two to three hours for each participant to complete. Participants were sent a $10 Amazon gift card via email as compensation for completing the study.

Cognitive Assessment

Cognitive testing was performed via the NIH Toolbox ® for Assessment of Neurological and Behavioral Function (NIH Toolbox). The NIH Toolbox comprises assessments for four domains of neurological and behavioral function which include cognition, motor, sensation, and emotion. The battery of tests utilized in this study consisted of five different testing instruments from the NIH Toolbox Cognition Battery used to assess specific domains of cognition, as well as overall fluid cognition, and required 30-45 minutes to complete. The NIH Toolbox Cognition Battery is a validated research tool used for populations aged 3-85 years (Mungas et al., 2013). The testing instruments comprised in the battery used in this study included the NIH Toolbox Flanker Inhibitory Control and Attention Test (FICA), providing a measure of executive function as well as attention, the NIH Toolbox List Sorting Working Memory Test (LSWM), providing a measure of working memory, the NIH Toolbox Dimensional Change Card Sort Test (DCCS), providing a measure of executive function, the NIH Toolbox Pattern Comparison Processing Speed Test (PCPS), providing a measure of processing speed, and the NIH Toolbox Picture Sequence Memory Test (PSM), measuring episodic memory (Bauer et al., 2013; Carlozzi, Tulsky, Kail, & Beaumont, 2013; Tulsky et al., 2013; Zelazo et al., 2013). Participants met one-on-one with trained researchers who administered the cognitive assessment, referred to as the "Brain Games." A five-minute break, or resting period, was required after the second testing instrument for all participants and additional breaks were provided as needed. An optional snack consisting of a granola bar or trail mix and water was offered to all participants prior to cognitive testing. Completion of all five testing instruments in the battery provided individual scores of executive function, episodic memory, working memory, attention and processing speed, as well as a fluid cognition composite score. Four types of scores are reported from the NIH Toolbox including Raw scores, Uncorrected Standard Scores, Age-Corrected Standard Scores, and Fully Corrected T-Scores, which are adjusted for key demographic variables including age, gender, race, ethnicity, and parent educational attainment.

The FICA test is used to measure a participant’s attention and inhibitory control. During the test, the participant is asked to focus on a given stimulus in the shape of an arrow while inhibiting attention to other stimuli flanking it. The stimulus may be congruent with other stimuli, pointing in the same direction, or incongruent with the stimuli, pointing in the opposite direction. Twenty trials are administered for participant’s aged 8-85 years. Scoring is based on both accuracy of responses and reaction time.

The LSWM test is a measure of working memory, requiring participants to utilize both information processing and storage. The application presents images of different foods and animals with accompanying audio recordings and written text indicating the noun associated with the image. The participant is asked to verbally repeat the items to the administrator in order from smallest to largest, first within a single concept dimension (food or animals) and then on two conceptual dimensions (foods, then animals). Scoring is based on the sum of the total number of correctly recalled items.

The DCCS test specifically measures cognitive flexibility within the executive function domain. Participants are presented with two target images that vary based on two dimensions (shape and color), then are asked to match a series of test pictures to the correct target pictures according to one of the dimensions (color or shape). The DCCS is scored based on a combination of accuracy and reaction time, similar to the Flanker test.

The Pattern Comparison test evaluates processing speed by requiring participants to determine if two side-by-side images are the same, or not the same. The participant has 90 seconds to respond to as many sets of images as possible. Scoring is based on the number of item pairs that were answered correctly within the allotted time.

The PSM test measures episodic memory by requiring participants to utilize acquisition, storage and effortful recall of novel information. The test displays a long series of images and activities which are presented both visually and verbally. After being presented with the full list of images ranging from 6-18 pictures, determined by age, participants are asked to recall the sequence of images in the correct order. Two trials of this task are given. The PSM test is scored based on the number of adjacent pairs placed correctly for each of the trials.

Dietary Assessment

Participants completed three self-reported 24-hour dietary recalls via the Automated Self-Administered 24-hour (ASA24; version 2018) Dietary Assessment Tool provided by the National Cancer Institute. The ASA24 is a public-access, web-based tool developed for researchers, clinicians, and educators and is based on the validated USDA Automated Multiple-Pass Method (Subar et al., 2012). The online diet assessment tool has been demonstrated to have more accuracy and less omissions with children aged 10 years and older when compared to those younger than 10 years of age, indicating that it is appropriate for use in early adolescent populations (Baranowski et al., 2012). Participants in the study completed the 24-hour diet recall on three nonconsecutive days, including two weekdays and one weekend day. The first recall was completed during the participant's in-person visit. Trained researchers introduced the online assessment tool and provided guidance and further instruction for participants as needed. For our sample, participants required 30-60 minutes to complete the in-person diet recall. After completing the first diet recall, participants were given a username and password in order to complete the following two 24-hour diet recalls on pre-assigned days at home. Dietary variables used in analysis included self-reported dietary intake of lutein and zeaxanthin (mcg), lycopene (mcg), alpha- and beta-carotene (mcg), cryptoxanthin (mcg), total carotenoids (mcg) and total fruits and vegetables (cups).

Skin Carotenoid Measurements

Participants' skin carotenoid concentrations were assessed using the Pharmanex BioPhotonic S3 Scanner from Nu Skin Enterprises® (Provo, UT). The S3 Scanner is a portable patented tool that non-invasively measures carotenoid concentrations in living human skin tissue via RRS. The use of RRS-based technology for the measurement of carotenoid status in human skin has been demonstrated to be both valid and reliable (Zidichouski, Mastaloudis, Poole, Smidt, & Reading, 2009). The patented methodology for RRS measurement of skin tissue in humans has been described in detail elsewhere (Bergeson et al., 2008; Zidichouski et al., 2009). Briefly, participants were asked to place their palm against the light window of the scanner and hold it there for 30 seconds. The scanner emits a safe, blue light onto the palm and then displays a score in Ramen intensity counts which correlates to carotenoid concentrations in the skin. One scanner was used for data collection with all participants and the scanner unit was calibrated prior to each participant visit. Each participant had their palm scanned three times in succession, with the average value of these three measurements used for data analysis.

Data Analysis

Data analysis was conducted using SAS software with a significance level of $p \leq 0.05$. The general characteristics of the sample population were evaluated by means of descriptive statistics. Variables were evaluated for characteristics of normality by visual evaluation and by calculating values of skewness and kurtosis. Spearman correlations were used to evaluate for associations between variable pairs not demonstrating characteristics of a normal distribution. These included relationships between individual nutrient intakes (lutein and zeaxanthin, lycopene, alpha- and beta-carotene, cryptoxanthin, total carotenoids, and total fruits and vegetables) and carotenoid skin concentrations, as well as between individual nutrient intakes and cognitive scores. Pearson correlations were used to evaluate for associations between variable pairs demonstrating characteristics of a normal distribution. These included relationships between all individual cognitive tests and skin carotenoid concentrations. Cognitive values used in data analysis consisted of Fully Corrected T-Scores which are adjusted within the NIH Toolbox application for age, gender, race, ethnicity, and parent educational attainment. These scores are relative to the national average after accounting for the previously mentioned variables based on a mean value of 50 and a standard deviation of 10.

Minimal literature exists investigating the direct relationship between skin carotenoids and cognition in populations of adults or children. This study was designed as a pilot. Previous research examining the relationship between carotenoid status and cognitive outcomes in child populations have included sample sizes ranging from 35-160 with the general sample size being approximately 50 (Barnett et al., 2018; Edwards et al., 2019; Mulder et al., 2014; Saint et al., 2018; Walk et al., 2017). Sample size for this study was based on convenience. After data analysis, Fisher’s Z Tests for Pearson Correlations were performed using correlation values from variable pairs exhibiting a significant association in order to determine power achieved.

Chapter 4: Results

Sample Demographics

Sample demographic characteristics are shown in Table 4.1. Thirty adolescents, 19 males (63%) and 11 females (37%), aged 11-14 years (M=12.467, SD=1.252) completed all required activities in the study. All participants reported ethnic and racial information as “Non-Hispanic” and “White.” Two participants (7%) also reported “American Indian or Alaska Native” racial identification in addition to “White.” Most participants reported a family household income over $74,000/year (n=21, 70%) and highest maternal education of at least a four-year degree (n=25, 83%).

Table 4.1: Demographic Characteristics

Sample Size	30
Age and Anthropometrics (Mean ± Standard Deviation)	
Age (years)	12.47 ± 1.25
Weight (kg)	53.14 ± 17.15
Height (cm)	161.17 ± 12.15
Gender (%)	
Male	63
Female	37
Highest Maternal Education (%)	
Some High School	0
GED or High School Diploma	3
Some College	3
2 Year Degree	10
4 Year Degree	63
Master’s Degree	10
Professional Degree	0
Doctoral Degree	10
Household Income (%)	
Less than $35,000/year	0
$35,000-$41,999/year	3
$42,000-$51,999/year	7
$52,000-$58,999/year	0
$59,000-$73,999/year	20
Over $74,000/year	70

Participants also completed a health questionnaire providing information on health-based statistics. None of the participants reported being in a smoking household. Participants indicated a wide distribution of sun exposure without sunscreen with 27% reporting less than one hour (n=8), 13% one-two hours (n=4), 23% three-four hours (n=7), 13% five-six hours (n=4), and 23% more than six hours (n=7). Regarding physical activity, 50% of the sample reported more than 60 minutes of moderate activity per day (n=15), 43% reported 30-60 minutes of moderate activity (n=13), and only 7% reported less than 30 minutes of moderate activity per day (n=2). Participants also reported time spent watching TV or movies, playing electronic games, or using a computer for something other than school related work. In this sample, 30% reported screen time as one hour or less (n=9), 33% reported 2 hours (n=10), 17% reported three hours (n=5), 17% reported four hours (n=5), and 3% reported five hours or more (n=1). Additionally, 23% of the participants reported suffering from cold, flu or allergy symptoms at the time of data collection (n=7).

Table 4.2 highlights descriptive statistics for the variables used in correlation analysis in the form of mean and standard deviation values. Fully adjusted cognitive scores were close to national averages (M=50, SD=10) based on age, education, race, ethnicity and parent education for the list sorting working memory test, dimensional change card sort test, pattern comparison processing speed test, and for fluid cognition. The flanker inhibitory control and attention test was notably lower than national averages and the picture sequence memory test was notably higher than national averages.

Table 4.2: Descriptive Characteristics

Variable	Mean ± Standard Deviation
Skin Carotenoid Level (RRS[a] Intensity Counts)	28324.43 ± 9224.92
Nutrients	
Calories (kcals)	2184.47 ± 690.65
Carotenes (alpha and beta) (mcg)	1578.98 ± 2012.54
Cryptoxanthin (mcg)	60.61 ± 67.29
Lycopene (mcg)	7468.76 ± 6358.53
Lutein and Zeaxanthin (mcg)	1035.07 ± 865.31
Total Carotenoids (mcg)	10143.42 ± 7887.86
Total Fruits and Vegetables (cups)	2.28 ± 1.50
Cognition Scores[b]	
Flanker Inhibitory Control and Attention (FICA)	39.50 ± 6.59
List Sorting Working Memory (LSWM)	53.83 ± 7.83
Dimensional Change Card Sort (DCCS)	49.63 ± 11.11
Pattern Comparison Processing Speed (PCPS)	48.80 ± 15.03
Picture Sequence Memory (PSM)	60.50 ± 12.99
Fluid Cognition	50.60 ± 11.50

[a] RRS indicates Resonance Ramen Spectroscopy

[b] Cognitive scores were fully-adjusted based on national averages (M=50, SD=10) for age, education, race, ethnicity and maternal educational attainment

Skin Carotenoids and Cognition

Table 4.3 shows Pearson correlation values and corresponding p-values used to determine the association between averaged skin carotenoid concentrations in RRS intensity counts and cognitive scores from each of the five testing instruments as well as the composite fluid cognition score. A significant positive correlation was noted between skin carotenoid counts and scores of working memory, based on the LSWM test ($R^2=0.43$, $p=0.02$). This correlation exhibited a statistical power of 0.681. To achieve a statistical power of 0.8, a sample size of 40 would be needed. No other significant relationships were found between skin carotenoid counts and cognitive scores.

Table 4.3: Pearson Correlations Between Averaged Skin Carotenoid Concentrations and Fully Corrected Cognitive Scores

Pearson Correlation Coefficients, N=30		
	RRS[a] Intensity Counts	
	R^2	P-Value
FICA[bg]	-0.04	0.83
LSWM[cg]	0.43	0.02
DCCS[dg]	-0.06	0.73
PCPS[eg]	-0.01	0.98
PSM[fg]	0.18	0.35
Fluid Cognition[g]	0.11	0.56

[a] RRS indicates Resonance Ramen Spectroscopy
[b] FICA indicates Flanker Inhibitory Control and Attention Test
[c] LSWM indicates List Sorting Working Memory Test
[d] DCCS indicates Dimensional Change Card Sort Test
[e] PCPS indicates Pattern Comparison Processing Speed Test
[f] PSM indicates Picture Sequence Memory Test
[g] Adjusted for age, education, race, ethnicity and maternal educational attainment

Dietary Intake of Carotenoids and Cognition

Spearman correlations were used to evaluate the relationships between averaged self-reported intakes of alpha- and beta-carotene, cryptoxanthin, lycopene, lutein and zeaxanthin, total carotenoids, total fruits and vegetables and cognitive scores of executive function, attention, working memory, processing speed, episodic memory and fluid cognition (Table 4.4). Self-reported intakes of lutein and zeaxanthin were found to have a significant negative correlation with cognitive assessment scores measuring working memory (R^2 = -0.427, p=0.019). Significant positive associations were found between scores measuring episodic memory and intakes of cryptoxanthin (R^2 = 0.411, p=0.024), lycopene (R^2 = 0.396, p=0.030), total carotenoids (R^2 = 0.395, p=0.031), and total fruits and vegetables (R^2=0.378, p=0.039). Figures 4.1, 4.2, 4.3, 4.4, and 4.5 demonstrate scatterplots showing the distribution of the relationship between significant variable pairs. These correlations exhibited a statistical power of 0.561-0.681. To achieve a statistical power of 0.8 for these correlations, a sample size of 40-52 would be needed. No significant relationships were noted between intakes of carotenoids and measurements of executive function, attention, processing speed or fluid cognition.

Table 4.4: Spearman Correlations Between Nutrient Intakes and Fully Corrected Cognitive Scores

Spearman Correlation Coefficients, N=30							
		FICA[af]	LSWM[bf]	DCCS[cf]	PCPS[df]	PSM[ef]	Fluid Cognition[f]
Carotenes	R^2	-0.21	-0.32	-0.02	-0.03	0.23	-0.10
	P-Value	0.26	0.09	0.91	0.87	0.22	0.61
Cryptoxanthin	R^2	0.22	-0.21	0.11	0.10	0.41	0.17
	P-Value	0.25	0.28	0.58	0.59	0.02	0.36
Lycopene	R^2	0.13	0.00	-0.02	0.04	0.40	0.12
	P-Value	0.50	0.99	0.90	0.81	0.03	0.51
Lutein and Zeaxanthin	R^2	-0.04	-0.43	0.16	0.07	0.29	0.05
	P-Value	0.83	0.02	0.41	0.71	0.11	0.81
Total Carotenoid	R^2	0.04	-0.12	-0.00	0.06	0.39	0.09
	P-Value	0.84	0.52	0.99	0.74	0.03	0.64
Total Fruit and Vegetable	R^2	0.01	-0.26	0.26	-0.03	0.38	0.10
	P-Value	0.94	0.16	0.16	0.87	0.04	0.59

[a] FICA indicates Flanker Inhibitory Control and Attention Test
[b] LSWM indicates List Sorting Working Memory Test
[c] DCCS indicates Dimensional Change Card Sort Test
[d] PCPS indicates Pattern Comparison Processing Speed Test
[e] PSM indicates Picture Sequence Memory Test
[f] Adjusted for age, education, race, ethnicity and maternal educational attainment

Figure 4.1: Scatterplot of Lutein and Zeaxanthin Intakes and Working Memory Scores

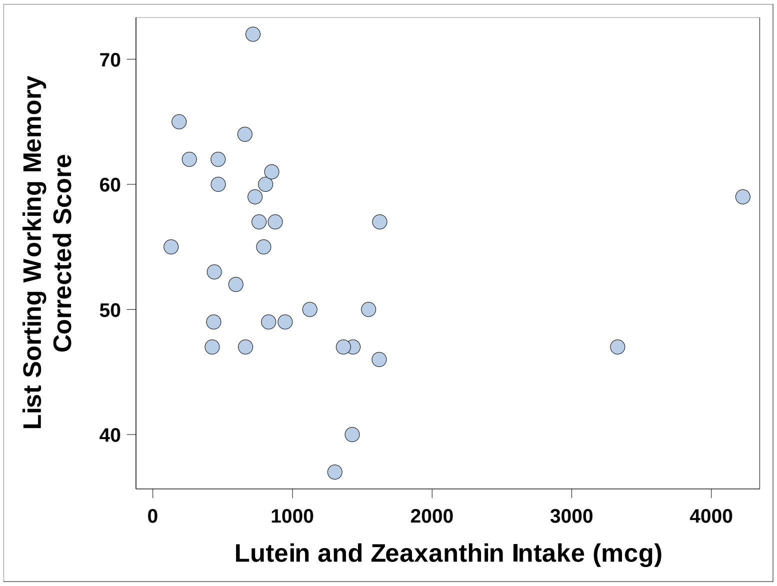


Figure 4.2: Scatterplot of Lycopene Intakes and Episodic Memory Scores

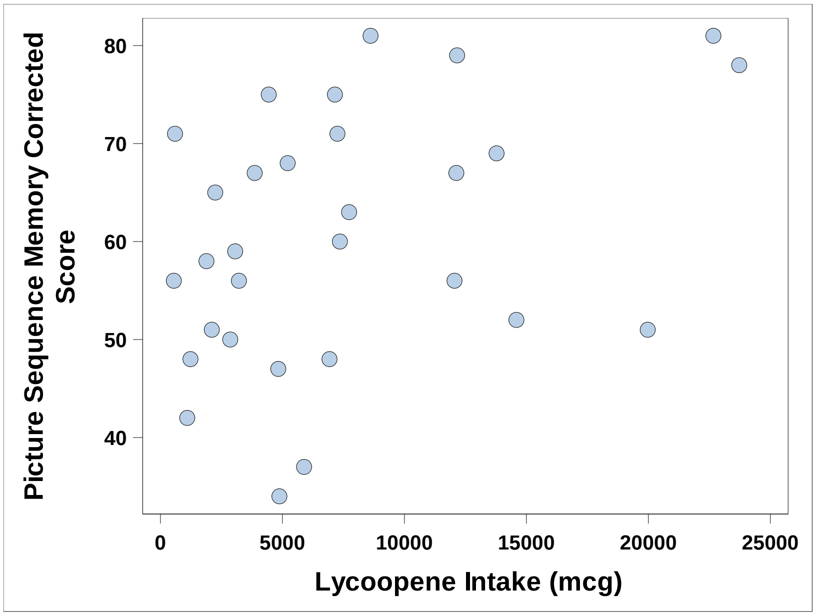


Figure 4.3: Scatterplot of Cryptoxanthin Intakes and Episodic Memory Scores

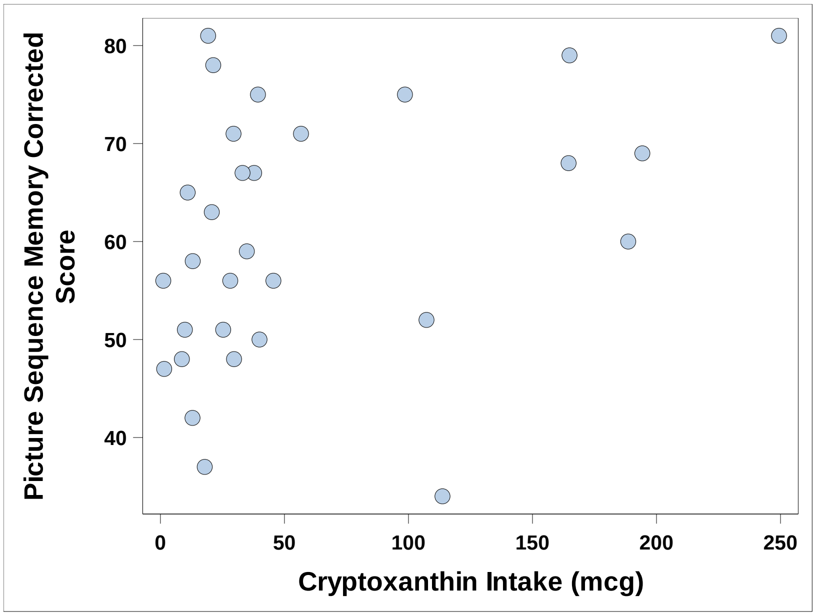


Figure 4.4: Scatterplot of Total Carotenoid Intakes and Episodic Memory Scores

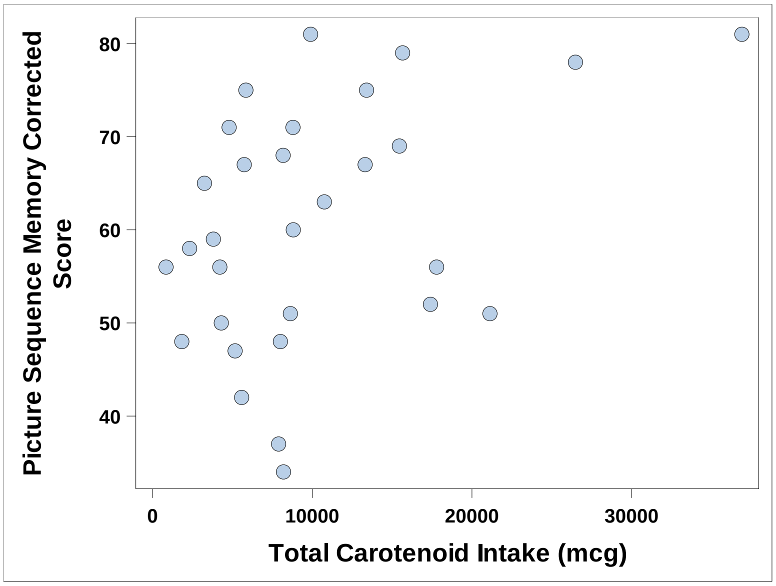
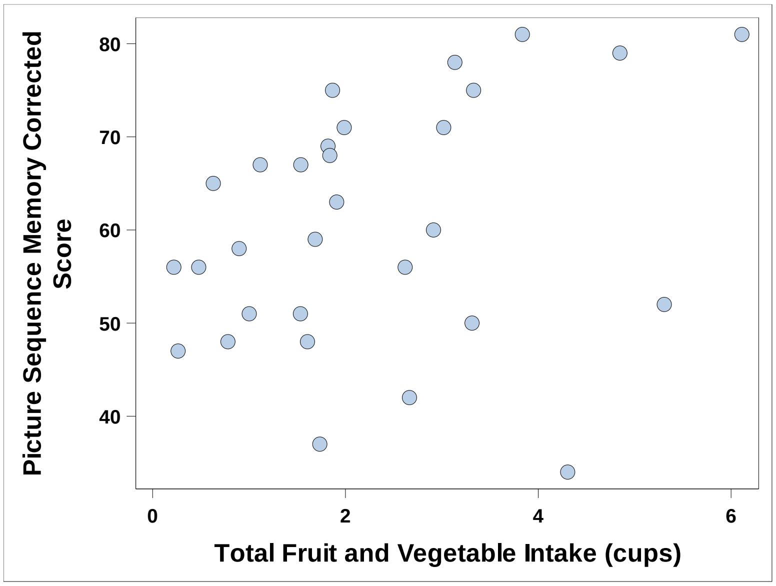


Figure 4.5: Scatterplot of Total Fruit and Vegetable Intakes and Episodic Memory Scores



Dietary Intake of Carotenoids and Skin Carotenoids

Table 4.5 shows Spearman correlations between averaged nutrient intakes and RRS intensity counts. No significant relationships were found between skin carotenoid concentrations, measured via RRS intensity counts, and averaged self-reported intakes of alpha- and beta-carotene, cryptoxanthin, lycopene, lutein and zeaxanthin, total carotenoids, and total fruits and vegetables.

Table 4.5: Correlations Between Skin Carotenoid Concentrations and Nutrient Intakes

Spearman Correlation Coefficients, N=30		
		RRS[a] Intensity Counts
Carotenes	R^2	-0.07
	P-Value	0.73
Cryptoxanthin	R^2	-0.05
	P-Value	0.81
Lycopene	R^2	0.09
	P-Value	0.62
Lutein and Zeaxanthin	R^2	0.01
	P-Value	0.95
Total Carotenoid	R^2	-0.01
	P-Value	0.95
Total Fruit and Vegetable	R^2	0.00
	P-Value	0.99

[a] RRS indicates Resonance Ramen Spectroscopy

Chapter 5: Discussion and Implications

Discussion

In a sample of early adolescents, dietary carotenoids and skin carotenoids had differential associations with cognitive assessments of executive function, attention, working memory, processing speed, episodic memory and fluid cognition. A significant positive correlation was found between skin carotenoid concentrations, measured in RRS intensity counts, and working memory scores. A significant inverse relationship was noted between self-reported intakes of lutein and zeaxanthin and scores of working memory and significant positive associations were found between scores of episodic memory and intakes of cryptoxanthin, lycopene, total carotenoids, and total fruit and vegetable intake. No significant relationships were found between skin carotenoid measurements and self-reported intakes of carotenoids.

This study found evidence supporting a positive relationship between skin carotenoid measurements and cognitive scores measuring working memory. Working memory references the ability to remember and manipulate information from a recent experience, often while performing complex tasks such as reasoning, comprehension or learning (Baddeley, 2010; Markowitz, Curtis, & Pesaran, 2015). Although no single region of the brain is responsible for memory based cognitive function, previous research has suggested that the prefrontal cortex plays a key role in the neural processes of working memory and episodic memory (Cohen et al., 1997; Lara & Wallis, 2015; Markowitz et al., 2015; Rugg, Otten, & Henson, 2002). As mentioned, previous researchers have reported concentrations of xanthophylls to be significantly higher in the frontal lobe when compared to other regions of the brain (Craft et al., 2004). This research suggests biological plausibility of a potential mechanism for these carotenoids to share a positive association with domains of cognition utilizing the prefrontal cortex, including working memory, episodic memory and executive function.

Previous studies in adult populations have also supported a positive relationship between carotenoid status and working memory. Power et al. (2018) reported improvements in scores of a paired associated learning task measuring episodic memory, and administered using the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge

Cognition, Cambridge, UK), after 12 months of carotenoid supplementation with lutein, zeaxanthin and meso-zeaxanthin. Kelly et al. (2015) also reported a significant positive correlation between levels of macular pigment and measures of visual and verbal memory and learning using the CANTAB in two groups of adults, including those with age-related macular degeneration and those with no retinal disease and low macular pigment levels. Finally, Feeney et al. (2013), reported the association of lower MPOD with poorer scores of prospective memory, although only one of six assessments used to evaluate memory was statistically significant. Research reporting a significant relationship between memory and carotenoid status in child populations appears to be more limited. In a sample of children aged 7-10 years, Hassevoort et al. (2017) found that MPOD contributed significantly to the amount of variance in measurements of relational memory even after accounting for other variables including IQ and aerobic fitness.

Only one other study has been identified examining the direct relationship between skin carotenoid status and cognition. Edwards et al. (2019) recently examined this relationship in a similar population of children 7-12 years old, but used academic achievement, measured using the Woodcock Johnson IV test, and a modified Eriksen flanker task, to evaluate for cognitive outcomes, finding a positive association with reading and math. Like the results found in our study, Edwards et al. (2019) did not find that skin carotenoids were significantly related to attention or interference control as measured by flanker interference scores.

The Woodcock Johnson IV contains three test batteries including the Woodcock-Johnson IV Tests of Cognitive Abilities, the Woodcock-Johnson IV Tests of Oral Language and the Woodcock-Johnson IV Tests of Achievement (Schrank & Wendling, 2018). The abstract by Edwards et al. (2019) only reports outcomes associated with the Woodcock-Johnson IV Test of Achievement, which contains tests of reading, mathematics, written language and academic knowledge and has been evaluated as a tool for assessing an individual’s academic abilities, strengths and weaknesses (Villarreal, 2015). These tests do not evaluate for specific cognitive abilities and therefore measure outcomes different from other cognitive assessment tools such as the NIH Toolbox. Because of the differences in measured cognitive domains, it is difficult to make further direct comparisons between this study and the study by Edwards et al. (2019).

These studies can be compared to similar research examining the relationship between carotenoid status, measured via MPOD and serum, and cognition in other pediatric samples. The results of this study and the study by Edwards et al. (2019) contradict the results of multiple studies indicating a significant positive relationship between carotenoid status and aspects of executive function and processing. In a sample of preadolescent children, Walk et al. (2017) found that MPOD was significantly associated with higher scores for incongruent trials of a modified flanker task but not scores of congruent trials or reaction time. Saint et al. (2018) found a significant positive relationship between MPOD and executive process scores based on the Woodcock-Johnson III Tests of Cognitive Abilities in children aged 7-13. The variability in the way that these cognitive domains are measured and defined may contribute to the inconsistency in results across different studies and samples. Additionally, the measurement of carotenoids via skin, while shown to correlate with other markers of carotenoid status such as MPOD and serum, does not provide a direct assessment of the carotenoid status in the brain and its potential influence on cognition, thereby potentially limiting the strength of the correlations found within this study. Research evaluating the relationship between cognition and carotenoid status, measured via MPOD, in pediatric populations, have also found a positive correlation with spatial abilities, global intelligence, math and written language (Barnett et al., 2018; Saint et al., 2018). Neither of these studies evaluated for memory based cognitive domains.

While a positive relationship between working memory scores and skin carotenoid measurement was found in this study, working memory scores exhibited a negative association with dietary intakes of lutein and zeaxanthin. Much of the literature has supported positive correlations between carotenoid intakes, especially of the xanthophylls, and aspects of cognition. The significant negative relationship found in this study is therefore surprising and perplexing. Furthermore, the scores measuring working memory, assessed by the LSWM test, are shown to have a negative relationship with most of the other nutrients evaluated, and while no others are significant, the association with the combined carotenes does approach significance (R^2=-0.32, p=0.09). Few other studies have reported a negative association between carotenoid intake and cognition. Péneau et al. (2011) reported that higher intakes of total fruits and vegetables and beta-carotene-rich fruits and vegetables were negatively associated with executive function in a sample of adults aged 45-60 years. Using principal component analysis, these researchers defined the executive function factor using three cognitive tests, two of which specifically measured working memory (forward digit span test and a backward digit span test). Based on this information, it could be suggested that this study also found a negative association between beta-carotene-rich vegetables and domains of working memory. These results may indicate that relationships between intakes of carotenoids and cognition may vary based on the domain being measured.

Other studies examining xanthophyll intake and its relationship with cognition have shown mixed results. Mulder et al. (2014) found no significant relationship between lutein intake and cognitive outcomes as measured by the Kaufman Assessment Battery in a sample of young children aged 5-6 years. In contrast, multiple intervention studies involving lutein supplementation have resulted in improvements in cognition in a variety of adult populations. In a sample of 49 women aged 60-80 years, Johnson et al. (2008) found improvements in verbal fluency after four months of lutein supplementation and improvements in rate of learning and some memory scores after receiving a combined supplement containing lutein and DHA. An intervention study of young healthy adults aged 18-32 years demonstrated improvements in visual processing speed after four months of combined supplementation of lutein, zeaxanthin, and omega-3 fatty acids (Bovier et al., 2014). In these intervention studies, xanthophyll supplements contained 8-12mg of lutein and 20-26mg of zeaxanthin per day. To put this in perspective, 100g of raw spinach, a higher dietary source of lutein, provides ~6.6mg of lutein, and orange pepper, a source of zeaxanthin, provides ~1.7mg of zeaxanthin per 100g (Perry, Rasmussen, & Johnson, 2009). The average intake of lutein and zeaxanthin in the United States is only approximately 1-2mg per day (Institute of Medicine, 2000). The average daily intake of the combined xanthophylls in this sample (~1mg) supports these estimates. These low intake values may limit the ability to find strong correlations between carotenoid intake and cognitive outcomes.

Results of this study also supported a significant positive relationship between scores of episodic memory and intakes of cryptoxanthin, lycopene, total carotenoids, and total fruits and vegetables. Research examining the correlations between cognition and carotenoid intakes outside of the xanthophylls appear to be more limited in number and lacking definitive conclusions. A review published in 2019, reported only four studies evaluating the relationship between lycopene and maintained cognition with three of those four reporting a significant positive association (Crowe-White, Phillips, & Ellis, 2019). Literature focusing specifically on memory related cognitive tasks and their relationship with other dietary carotenoids are even fewer in number. Kesse-Guyot et al. (2014) found that a high carotenoid dietary pattern, developed through a statistical analysis method called RRR and measured via 24-hour recalls, was positively associated with cognitive tests measuring episodic memory, semantic fluency, working memory and executive functioning, even after adjusting for multiple covariates including age, sex, education, BMI, occupational status, physical activity and history of diabetes, hypertension or cardiovascular disease. The high carotenoid dietary pattern analyzed in this study was most strongly correlated to serum levels of the carotenes, cryptoxanthin and lutein, and with dietary intakes of green fruits and vegetables, vegetable oils, orange fruits and vegetables, and soup. Investigating the relationships between intakes of other carotenoids, such as cryptoxanthin and lycopene, and memory based cognitive abilities may be an area of research worth further exploration.

Intakes of lutein and zeaxanthin were not found to be correlated with episodic memory in this study. This contrasts with an intervention study by Power et al. (2018) which found that 12 months of supplementation with lutein (10mg), zeaxanthin (2mg) and meso-zeaxanthin (10mg) resulted in significant improvements in episodic memory in a sample of adults. Again, the higher amounts of lutein and zeaxanthin provided in supplementation may provide a larger impact on cognitive improvements that aren’t seen in the typical American diet and weren’t seen in our sample (M=1.035mg, SD=0.865mg).

This research study found no clear evidence of a significant relationship between intake of carotenoids and skin carotenoid concentrations. These results notably contradict some of the recent literature exhibiting a significant association between skin carotenoid measurements and carotenoid intake in child populations (Aguilar et al., 2014; Beccarelli et al., 2017). Aguilar et al. (2014) reported a significant association between skin carotenoids and high carotenoid vegetable intake, as well as self-reported total fruit and vegetable intake in a sample of 45 children aged 5 to 17 years.. Both dietary variables were measured based on three 24-hour recalls and an FFQ developed from a modified version of a beverage and snack questionnaire (BSQ). Based on the 24-hour recall, intake of beta-carotene, alpha-carotene and total carotenoids were also significantly associated with skin carotenoid measurements. This study is one of the few to look specifically at carotenoid intake in addition to total fruit and vegetable intake. Unfortunately, it is difficult to discern a plausible explanation for the lack of evidence supporting this relationship in our sample.

One possible explanation for the lack of association is the potential for confounding variables influencing the accuracy of skin carotenoid measurement. After collecting data for 16 of the participants, the process of measuring skin carotenoid status changed slightly due to alterations in the S3 Scanner application. In the first half of participants, the application was able to round measurements to the nearest whole integer. During the second half of data collection, skin carotenoid measurements were rounded to the nearest thousand. The discrepancy between these two methods could have influenced the sensitivity and consistency of RRS measurements. Additionally, our sample displayed a wide variation in sun exposure. Sunlight exposure has been found to influence skin carotenoid levels, with higher exposure resulting in lower levels, independent of carotenoid intake and other dietary behaviors (Ermakov et al., 2005). Because of the small sample size participating in the study, feasibility of controlling for this variable in statistical analysis was limited. Therefore, the variation in the sample based on other behaviors, such as sun exposure, may have influenced the results in a way that couldn't be observed via direct correlations.

Other demographic variables may also point to a possible explanation for the contrasting results in our sample when compared to other recent research. The sample for this study was found to have a similar average value of RRS intensity counts when compared to samples in other studies examining child and adolescent populations with similar sample sizes (Aguilar et al., 2014; Beccarelli et al., 2017; Nguyen et al., 2015). Although, the higher standard deviation indicates that our sample had more variability than Aguilar et al. (2014). Regarding total carotenoid intake, the average value and standard deviation for our sample (M=10143.42mcg, SD=7887.86mcg) appeared to be notably larger than others in this population indicating a large range in intake values among our participants. Both Beccarelli et al. (2017) and Nguyen et al. (2015) reported an average total carotenoid intake of 6000-8000mcg with a standard deviation of 3000-7000mcg. The higher intakes and wider variation within our sample may be influencing the relationships being found, or not being found in the results of this study.

Next Steps

Further research in this area should look to replicating this observational study with larger sample sizes and a more diverse demographic to determine if these varied associations remain present. Evaluation of carotenoid status in the body may be improved by using methods of measurement providing a more direct connection to carotenoid status in the brain, such as the use of MPOD. If a significant positive association remains between carotenoid intake and aspects of cognition, such as episodic memory, intervention studies in child populations should be considered.

Conclusions

In a sample of early adolescents, no evidence was found supporting a significant relationship between skin carotenoids and dietary intake of carotenoids. Skin carotenoid concentrations were positively associated with scores of working memory. Dietary intakes of lutein and zeaxanthin were negatively associated with working memory scores and intakes of cryptoxanthin, lycopene, total carotenoids, and total fruits and vegetables were positively associated with episodic memory scores in early adolescents aged 11-14 years. Dietary intake of specific carotenoids may have varied associations with specific domains of cognition. Larger sample sizes are needed to comprehensively evaluate these relationships in child populations.

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