THE RELATION BETWEEN THE MACULAR CAROTENOIDS AND COGNITIVE FUNCTION IN PRE-ADOLESCENT CHILDREN

by

SARAH SAINT HAMILTON

(Under the Direction of Janet E. Frick)

ABSTRACT

The macular carotenoids lutein (L) and zeaxanthin (Z) are obtained via diet and accumulate in the central retina where they are referred to as macular pigment. The density of this pigment (MPOD) has been positively correlated with cognitive functioning via measures of global cognition, processing speed, and visual-spatial abilities, among others. While improvements in cognitive function have been found in adults, much less is known about how L and Z intake may support or improve cognitive functioning during periods of rapid developmental change, such as childhood and pre-adolescence. This study examines the relation between MPOD and cognitive functioning in 7-13 year old children. MPOD was assessed using heterochromatic flicker photometry (HFP) and was used as the primary variable of interest. Temporal processing speed (measured via critical flicker fusion thresholds; CFF), psychomotor reaction time (fixed and variable; FRT and VRT) and coincidence anticipation timing (CAT) were also assessed. Woodcock-Johnson III composite standard scores (Brief Intellectual Ability, Cognitive Efficiency, Processing Speed, and Executive Processes) were used to assess cognitive functioning controlling for age. Both MPOD and CFF were significantly related to Executive

Processes, $r(49) = .264$, $p < .05$ and $r(47) = .243$, $p < .05$, respectively. CFF was also related to Processing Speed, $r(49) = .252$, $p < .05$, and Cognitive Efficiency, $r(49) = .286$, $p < .05$. FRT, VRT, and CAT performance were all related to age, therefore partial correlations controlling for age were calculated for these variables. FRT and VRT were significantly related to Executive Processes scores, $r(46) = -.260$, $p < .05$ and $r(46) = -.253$, $p < .05$, respectively. FRT was also related to Cognitive Efficiency, $r(48) = -.236$, $p < .05$, and number of missed trials at 20 mph on the CAT task was related to Processing Speed scores, $r(36) = -.301$, $p < .05$. Our findings support the idea that processing speed is a limiting factor for higher order cognitive functions and demonstrate that MPOD is similarly associated with cognitive functioning in childhood and pre-adolescence as it is in adulthood.

INDEX WORDS: Macular pigment, Lutein, CFF, Temporal vision, Cognition, Children

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by

SARAH SAINT HAMILTON

B. A., Manhattanville College, 2008

M. S. W., University of Georgia, 2010

M. S., University of Georgia, 2014

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SARAH SAINT HAMILTON

Major Professor:	Janet E. Frick
Committee:	Billy R. Hammond, Jr.
	Leann L. Birch

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2017

DEDICATION

For my spouse and my family, who have always encouraged me to push my limits and discover all that I am capable of.

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CHAPTER 1

INTRODUCTION

The carotenoids lutein (L) and zeaxanthin (Z) are found in highest concentrations in dark green leafy vegetables (e.g., kale and spinach) and, when present in the diet, accumulate in the central retina where they are referred to collectively as macular pigment. In the retina, these pigments serve as intraocular light filters, absorbing short wavelength "blue" light (peak absorption at 460nm) before it can reach the macula and damage the photoreceptors responsible for central vision. Like many naturally-derived compounds, however, the effects of L and Z on human biology are pleiotropic; emerging research, for instance, has demonstrated a relation between the macular carotenoids and cognitive performance in adults. There is reason to believe that these molecules may be particularly important for cognitive development in early life as well, but the relation between L and Z status and cognitive performance has not yet been studied in children. This will be the focus of the present study.

Lutein has been shown to affect neural activity in ways that protect the nervous system and enhance function. For example, L and Z are potent antioxidants and anti-inflammatory agents that help to protect the central nervous system from oxidative and inflammatory stress (e.g., Ozawa et al., 2012; Stahl & Sies, 2003). The brain and eye are particularly susceptible to free radical damage because they both have very high concentrations of polyunsaturated fatty acids and a high metabolic load. The presence of carotenoids in neural tissue also promotes the formation of gap junctions between neurons, which allow neurons to communicate laterally via direct ion exchange and electrical impulses (Stahl & Sies, 2001). This improvement in cell-to-

cell communication could lead to faster and more efficient processing within the visual system, as well as throughout the CNS (i.e. the neural efficiency hypothesis; Hammond & Wooten, 2005).

Measurement of L and Z in the retina can be done non-invasively using a psychophysical technique that capitalizes on lutein's absorption spectrum. L and Z status can also be measured via serum; however, serum levels are representative of the amount of L and Z that are circulating throughout the body and therefore change regularly based on recent dietary intake (Beatty, Nolan, Kavanagh, & O'Donovan, 2004). Macular pigment optical density (MPOD), on the other hand, is representative of the amount of L and Z that has been incorporated into retinal tissue, and thus, functions as a better biomarker for the amount of L and Z that has been incorporated into the brain, as well as L and Z intake over longer periods of time (Beatty et al., 2004; Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013).

Macular Carotenoids and Cognitive Functioning

The relation between MPOD and cognitive functioning has been studied using largely observational research designs (though a few randomized placebo-controlled trials have been completed) and there is relative consistency in findings across studies. For example, in a large population-based study of older adults in Ireland, Feeney and colleagues (2013) found that having low MPOD was associated with significantly lower performance on global measures of cognitive functioning (mini-mental status exam [MMSE] and Montreal Cognitive Assessment [MoCA]), as well as prospective memory and reaction time tasks. More recently, Kelly et al. (2015) compared MPOD and cognition in adult subjects with or without retinal disease and found significant relations between MPOD and cognitive functioning across the adult lifespan in both healthy and diseased groups. Specifically, both groups showed evidence of a positive

relation between MPOD and cognitive control (attention switching task), as well as visual memory and learning (paired associate learning task). Finally, functional magnetic resonance imaging (fMRI) research has supported the proposed negative relation between neural efficiency and MPOD in older adults using a verbal learning task; participants with higher MPOD required less brain activation to complete the task (Lindbergh et al., 2016).

Intervention trials with L and Z have yielded similar findings. Johnson and colleagues (2008) supplemented healthy older women with L, docosahexaeonic acid (DHA, an omega-3 fatty acid), or a combination of L and DHA, and found cognitive benefits in all three groups, but especially the combined L and DHA group. Specifically, L and DHA each individually improved verbal fluency from baseline, but when combined, they improved participant performance on several delayed recall memory tests, on which performance typically declines with age (Harada, Natelson Love, & Triebel, 2013). In a randomized placebo-controlled trial, Bovier, Renzi and Hammond (2014) found significant improvements in neural processing speed as measured by critical flicker fusion (CFF) thresholds and coincidence anticipation timing (CAT) performance in both supplement groups (lutein plus high-dose zeaxanthin and omega-3 fatty acids, and high-dose zeaxanthin). CFF is a measure of temporal processing speed in the visual domain and represents the fastest frequency of a flickering stimulus that the visual system can resolve at 100% depth of modulation (stimulus completely on, then completely off, in a square-wave function). CAT is a measure of psychomotor speed and accuracy that requires hand-eye coordination to depress a button when a stimulus traveling at random speeds reaches a particular point on a track. In younger and older adults, temporal processing speed measured via CFF has been shown to be a strong indicator of executive functioning, even when controlling for age and global cognitive status (Mewborn, Renzi, Hammond, & Miller, 2015).

Macular Carotenoids and Temporal Processing Speed

A relation between MPOD and temporal processing speed (e.g., CFF) is particularly important given that it is widely thought that this visual measure is determined by cortical (not retinal) activity (see Skottun, 2013 for a review). For example, electroretinogram (ERG) and fMRI studies have demonstrated that the retina and visual cortex respond to flicker well past the point at which subjects report fusion (e.g., Carmel, Lavie, & Rees, 2006; Jiang, Zhou, & He, 2007), indicating that the perception of flicker is limited by higher-level cortical networks. This is why some have argued that temporal processing speed is a cognitive fundamental; faster processing is related to better functioning in many aspects of dynamic cognition such as fluency and short-term memory, and slower processing limits these higher level cognitive processes (e.g., Colombo, 1993; Salthouse, 1992). For example, Rose and colleagues (2011, 2012) have demonstrated in longitudinal studies that processing speed accounts for the differences in academic achievement in math and reading that exist between children who were born pre- versus full-term. When measured in the visual domain, temporal processing speed (i.e., CFF) correlates with L and Z status as measured by MPOD (Renzi & Hammond, 2010). In addition, supplementation with L and Z can significantly improve CFF in a relatively short period of time (e.g., 4 months in Bovier et al., 2014), providing a direct link between the macular carotenoids and temporal processing speed.

Macular Carotenoids and Neural Development

There is also reason to believe that L and Z may be particularly important during periods of rapid neural development early in life, yet this relation has yet to be studied directly. Lutein is the predominant carotenoid in the developing fetal and infant brain, despite relatively low dietary intake, and makes up 59% of the carotenoids in the infant brain (Vishwanathan, Kuchan, Sen, &

Johnson, 2014) compared to 34% in geriatric adults (Johnson et al., 2013). It has been suggested that such high concentrations of lutein in the developing brain is an indication that it may be necessary during periods of rapid neural development (e.g., Johnson, 2014). Indeed, recent work by Cheatham and Sheppard (2015) supports this link: infant recognition memory, tested via an event-related potential (ERP) odd-ball paradigm, was positively related to the amount of lutein and choline in their mother's breastmilk. Additionally, analyses of brain tissue collected from infants who died during their first 1.5 years of life reveal significantly lower concentrations of lutein and zeaxanthin in preterm infants compared to full-term infants, specifically in the prefrontal cortex, hippocampus, auditory cortex, and occipital cortex (Vishwanathan, Kuchan, et al., 2014). However, the relation between MPOD and cognition has not been directly tested in children at this point.

The Present Study

While L and Z status has been related to cognitive functioning in adults, much less is known about how L and Z intake may support or improve cognitive functioning during periods of rapid developmental change, such as childhood and pre-adolescence. This study directly tests whether MPOD relates to cognitive functioning in pre-adolescent children. Measurement of MPOD in children has been demonstrated to be possible with a moderate degree of reliability (Cronbach's $\alpha = 0.72$) using customized heterochromatic flicker photometry (cHFP; McCorkle et al., 2015) and was used as the primary independent variable of interest. Children's visual temporal processing speed was measured via CFF and visuo-motor integration abilities was measured via a coincidence anticipation timing (CAT) task.

Hypothesis 1: MPOD will positively correlate with measures of cognitive functioning.

Hypothesis 2: Temporal processing speed as measured via CFF will positively correlate with measures of cognitive functioning.

Hypothesis 3: CAT performance will positively correlate with measures of cognitive functioning.

CHAPTER 2

METHOD

Participants

Fifty-five children (45.5% female) were recruited from the Athens, GA community. Data from two of these children were ultimately excluded from all analyses because they did not have enough reliable data. Specifically, one 9-year-old male subject had been diagnosed with sensory processing disorder, which led to a great deal of difficulty in obtaining reliable data on any of the visual measures, and one 7-year-old male subject revoked assent before the first measure (MPOD) could be finished. This reduced the final sample to 53 children (47.2% female). These children ranged in age from 7 to 13 and were largely white (non-Hispanic; 77.4%) and from well-educated families (90.5% of children had at least one parent with some level of post-secondary education). See Table 1 for complete demographic information.

Measures

Macular Pigment Optical Density (MPOD). MPOD was measured using customized heterochromatic flicker photometry (HFP) via a Macular Densitometer™ (Macular Metrics Corporation, Rehoboth, MA, USA) as described in Wooten, Hammond, Land, and Snodderly (1999). Measurement of MPOD in children using HFP has been demonstrated to be possible with a moderate degree of reliability (Cronbach's $\alpha = 0.72$; McCorkle et al., 2015). Participants viewed a disc of blue (460 nm) and green (550 nm) LEDs alternating in square-wave counter phase. Short-wavelength "blue" light is highly absorbed by macular pigment, which ultimately reduces the amount of this light that reaches the photoreceptors of the macula. Green light is not

absorbed well by macular pigment, meaning that it can pass straight through to the photoreceptors of the macula without losing intensity. Therefore, it takes more blue light to achieve the same amount of photoreceptor stimulation in the macula as that produced by green light. When the two wavelengths differ in the amount of light that reaches the photoreceptors, the disc appears to flicker. The experimenter gradually adjusts the intensity of the blue light until the participant can no longer perceive flicker, which indicates that the intensity of the blue and green lights reaching the photoreceptors of the macula have become equivalent. Subjects with higher MPOD require more blue light to match the green and cancel the flicker. This procedure is conducted using 0.5° and 1° discs to measure MPOD at 30' and 60' retinal eccentricity, respectively. To measure MPOD at 120' and 210' retinal eccentricity, 2° and 3.5° ring-shaped (annulus) stimuli are used and the subject is instructed to fixate on a small black dot that is positioned directly in the center of the ring while making their judgments about when the ring stops flashing. These measurements are compared with measurements taken in the peripheral retina (7° retinal eccentricity; where there is no macular pigment) in order to factor out each individual's unique retinal sensitivity, and MPOD is calculated from the difference between these measurements. For the peripheral measure, a 2° disc positioned centrally and a red fixation LED positioned 7° nasally are used. The subject is instructed to fixate the red LED while making their judgment about whether the disc is flickering or not.

Temporal Vision. Participants' temporal contrast sensitivity was measured using a 1° target surrounded by a 5.5° background with a four arc-minute black circle separating the target and the surround to make the 1° target easier to fixate. Both target and surround are composed of 660nm light, which is a wavelength that is not absorbed by macular pigment and thus prevents individual differences in macular pigment optical density from confounding tCSF measurements.

Participants viewed the target through an eye-piece with a 3 mm artificial pupil. To begin, the flicker's depth of modulation was set to 100% and participants' CFF were measured with three ascending and three descending trials. Next, contrast sensitivity at 31.62 Hz, 25.12 Hz, 10.00 Hz, and 2.51 Hz was measured by reducing depth of modulation to zero and then slowly increasing it until the participant reported that they were able to detect flicker. This was completed five times per frequency and the average depth of modulation for each frequency was used to plot the participant's temporal contrast sensitivity function (TCSF) with the participant's CFF value as the 100% depth of modulation anchoring point. The area under the curve was then calculated as a reflection of the subject's sensitivity to flicker and contrast.

Reaction Time and Coincidence Anticipation Timing. Subject's visuo-motor reaction time was assessed using a wall-mounted track of white LEDs. Subjects stood or sat approximately 6 feet away from the device and pressed a button to respond to various task parameters. To measure participants' fixed position reaction time (FRT) the experimenter told the subject which LED would light up before starting the trials. Each trial started when the LED lit up and ended when the participant pressed the button, at which point the LED turned off. This continued for a total of 80 trials. To measure participants' variable position reaction time (VRT), the experimenter told the subject that any of the LEDs on the track may light up and that the subject should press the button as soon as they see any of the LEDs light up. One LED lit up in a randomly selected location along the track and as soon as the participant pressed their button to indicate that they had seen it, the LED turned off. This continued for a total of 80 trials. Finally, to measure participants' ability to time a motor movement to the arrival of a visual stimulus at a particular visual location (coincidence anticipation timing; CAT), the first LED on the track lit up to indicate to the subject that the trial was beginning and then the light appeared to travel

down the track at 5, 10, 15, or 20 mph on each trial. The participant was instructed to press their button as soon as they saw the light reach the target point (marked by a strip of white tape). This continued for a total of 60 trials. The lag time between trials on all three of these tasks was randomized.

Cognitive Testing. The Woodcock-Johnson III (WJ-III) Tests of Cognitive Abilities (Woodcock, McGrew, & Mather, 2001) was used to assess children's cognitive functioning. The WJ-III is a norm-referenced set of tests that was designed to measure intellectual abilities in 2 to 90+ year olds. The WJ-III was standardized on over 8,000 individuals who are representative of the demographics and communities of the general United States population (Mather & Woodcock, 2001). All subtests of interest for this study have median reliability scores of .8 or higher, with the exception of the Planning subtest (median reliability = .75; Mather & Woodcock, 2001). All Cognitive Performance Composite scores of interest have median reliability scores of .9 or higher (Mather & Woodcock, 2001).

Participants completed the following WJ-III subtests: Verbal Comprehension, Visual-Auditory Learning, Spatial Relations, Concept Formation, Visual Matching 2, Numbers Reversed, Decision Speed, Planning, and Pair Cancellation in one testing session. These tests are used to calculate participants' composite scores for Brief Intellectual Ability (BIA), Cognitive Efficiency, Processing Speed, and Executive Processes. One experimenter was responsible for testing all of the participants to reduce potential inter-rater reliability confounds. Participants were allowed to take breaks between subtests, and the order in which subtests were completed was altered as needed to maintain attention. For example, the Concept Formation subtest is particularly challenging and participants frequently feel cognitively fatigued by the time they get to it, so that subtest was sometimes moved to a later point in the testing session to allow

participants to recover their attention during the more hands-on tasks (e.g., Visual Matching 2, Decision Speed, Planning, and Pair Cancellation) before attempting the more challenging Concept Formation task. Completion of these WJ-III subtests took 90 minutes to two hours.

CHAPTER 3

RESULTS

Analyses were completed using one-tailed tests, given the directional nature of all hypotheses. The standard scores for the WJ-III Brief Intellectual Ability, Processing Speed, Cognitive Efficiency, and Executive Processes composite measures were used in assessing specific components of cognitive functioning to control for age differences among subjects. Four of the children tested were born prematurely (< 37 weeks gestation). Given that prematurity has been linked to deficits in processing speed and academic achievement into adolescence (e.g., Rose et al., 2012; Rose, Feldman, Jankowski, & Van Rossem, 2011), independent-samples t-tests were conducted to determine whether any differences in cognitive or visual performance existed based on prematurity. No significant differences were found for any of the measures (visual or cognitive), therefore these children were kept in the data set. Outlier detection analyses (Tukey, 1977) were performed on all variables and revealed outliers in the CFF and TCSF data (an 8-year-old with a CFF of 13.53 and a 9-year-old with a CFF of 37.90 Hz and TCSF area under the curve of 1.157). These outliers were removed from CFF and TCSF analyses.

Prior to calculation of average VRT and FRT, RTs of < 10 ms or > 500 ms were removed from participant data, as they are indicative of either electronic errors or extreme failures of attention. Roughly halfway through data collection, the CAT and RT equipment began to exhibit button press failures (participant pressed button but it was not recorded by the computer) that became increasingly frequent. Fourteen subjects (out of 53) completed testing before this issue could be fixed, and thus their CAT data were removed prior to completing the relevant analyses.

The remaining 39 subjects were used for all CAT analyses. VRT and FRT data were available from 13 out of 14 of these subjects and thus were not excluded from analyses, given that any electronic button press failures would have almost certainly registered at >500ms and therefore would have been removed during the data cleaning process, as previously stated. Electronic button press failures during CAT testing would be impossible to distinguish from genuine misses given the nature of the task.

Finally, two subjects (7- and 10-years-old) were too fatigued to complete the Concept Formation subtest resulting in missing data for their BIA and Executive Processes composite scores, which require the Concept Formation test to be calculated. Additionally, one 7-year-old was too fatigued to complete the Spatial Relations subtest. Final *N*s and descriptive statistics for all measures can be found in Table 2.

H1: Macular Pigment and Cognition

MPOD was significantly related to the Executive Processes composite score (see Table 3 and Figure 1), as well as performance on the Spatial Relations subtest, $r(50) = .261$, $p = .031$, which is a measure of visual-spatial thinking abilities and is not a component of any of the composite scores. In addition, the relation between MPOD and performance on the Visual-Auditory Learning subtest (a measure of long-term memory storage and retrieval) approached significance, $r(51) = .22$, $p = .055$. None of the other cognitive composite measures (BIA, Verbal Learning, PS, and Cognitive Efficiency) were significantly related to MPOD (see Table 1; all *p*s > .10).

Previous research has found significant relations between MPOD and temporal vision (TCSF and CFF; Renzi & Hammond, 2010; Bovier & Hammond, 2015), as well as MPOD and CAT performance (absolute error at 5 mph and number missed at 15 mph; Bovier, Renzi, &

Hammond, 2014). In this sample, however, MPOD was also not significantly related to any of the temporal or anticipation/reaction time measures (CFF, TCSF, VRT, FRT, and CAT absolute error and number missed at all speeds), all *p*s > .10.

H2: Temporal Vision and Cognition

CFF was significantly related to the following WJ-III composite scores: Cognitive Efficiency, Processing Speed, and Executive Processes (see Table 3 and Figures 2-4). In addition, CFF was significantly related to the Spatial Relations subtest, $r(48) = .311$, $p = .014$, but not Visual-Auditory Learning, $r(49) = .170$, $p = .117$. TCSF area under the curve was not significantly related to any of the WJ-III composite scores or the Visual Auditory Learning and Spatial Relations subtests.

H3: Anticipation/Reaction Time and Cognition

Age was significantly related to performance on all three reaction time tasks (see Table 4); therefore partial correlations controlling for age were used in the following reaction time analyses. FRT and VRT were significantly related to WJ-III Executive Processes such that faster (shorter) reaction times on both measures were associated with higher standardized Executive Processes scores, and faster reaction times on the fixed measure (FRT) was associated with higher standardized Cognitive Efficiency scores (see Table 5).

None of the other cognitive measures were significantly associated with VRT or FRT performance; however, several showed trends toward being so. In particular, the relation between FRT and BIA approached significance, $r(47) = -.218$, $p = .066$, as did the relation between FRT and Verbal Ability, $r(49) = -.196$, $p = .084$. The number of missed trials on the most challenging speed (20 mph) of the CAT task was significantly related to scores on the WJ-III Processing Speed composite measure; better Processing Speed performance was associated

with fewer missed targets on the CAT task at 20 mph (see Table 5). In addition, the absolute error at 5 mph was significantly related to performance on the Spatial Relations subtest, $r(36) = -.281$, $p = .044$, such that better Spatial Relations performance was associated with less error (i.e., better performance) on the 5 mph CAT target. None of the other CAT measures were significantly associated with the cognitive measures or the Visual-Auditory Learning and Spatial Relations subtests, though the relation between Processing Speed and number of errors at 15 mph approached significance, $r(36) = -.255$, $p = .061$.

CHAPTER 4

DISCUSSION

The purpose of the present study was to directly test whether MPOD and measures of visual processing speed (CFF, reaction time, and anticipation timing) relate to cognitive functioning in pre-adolescent children, as they have been shown to in adults. MPOD is a measure of the amount of L and Z that has been incorporated into neural (retinal) tissue, and our findings showed that this measure was positively related to executive functioning and visual-spatial thinking abilities. In addition, the relation between MPOD and performance on a measure of long-term memory storage and retrieval approached significance ($p < .06$). These cognitive processes require a high level of efficiency in the frontal and/or occipital cortices, which are areas of the brain containing high concentrations of L and Z, when they are present in diet (Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013). These findings are in agreement with the results found in adult and aging populations, which support the neural efficiency hypothesis of lutein. Higher MPOD is related to more efficient neural functioning, whether that is demonstrated via fMRI in the form of less brain activation required to complete a task (Lindbergh et al., 2016), better cognitive control in an attention-switching task (Kelly et al., 2015), or higher executive functioning scores, as demonstrated in the present study.

Childhood and pre-adolescence are periods of rapid neural development and lutein is deposited in neural tissue at concentrations that are much higher than would be expected based on diet (Vishwanathan et al., 2014). As previously discussed, it has been suggested that this preferential deposition of L in the developing brain is an indication that it may be necessary

during periods of rapid neural development (e.g., Johnson, 2014). It may be that L is especially helpful during these periods because of its role in increasing gap junction and dendritic spine formation, or its role as an antioxidant, which would help to protect myelination processes from oxidative stress. If lutein supports faster neural processing via protection of myelination and more efficient neural communication via gap junction formation, it would be beneficial to have a steady supply in the brain during periods of rapid growth and development. Indeed, lutein makes up a much higher percentage of total brain carotenoids in infants compared to geriatric adults (59% vs. 34%; Johnson, 2014b; Vishwanathan et al., 2014), which makes sense given that aging is a developmental period associated with the slowing of processing speed and the recruitment of increasingly more brain tissue to complete cognitive tasks (i.e., neural inefficiency). Studies of older adults have supported this by demonstrating that higher MPOD is related to better performance on reaction time tasks (Feeney et al., 2013) and supplementation with L and DHA improves performance on a delayed recall memory task (Johnson et al., 2008).

CFF was also significantly related to several cognitive composite scores that require neural speed and efficiency (Cognitive Efficiency, Processing Speed, Executive Processes). This is not surprising, given that CFF is a measure of processing speed, which has been described as a cognitive fundamental, as previously discussed (e.g., Colombo, 1993; Salthouse, 1992). TCSF, however, was not significantly related to any of the cognitive measures, which is interesting given that CFF is a component of the full TCSF. This begs the question as to why CFF would be related to cognitive performance, but the overall TCSF would not. It could be that the speed at which visual information is processed is more of a limiting factor for cognitive performance than is contrast sensitivity during periods of rapid developmental change. It could also be that the CFF task places heavier demands on sensory processing than the lower frequency temporal contrast

sensitivity tasks do, and thus is able to more effectively distinguish cognitive performance in healthy young subjects when it is not diluted with these less challenging sensory tasks. If this is true, then CFF may be a more effective and efficient measure to use with young populations than the full TCSF.

Similarly, other measures of processing speed (reaction time and anticipation timing) were related to cognitive performance in this sample. These measures were also negatively related to participant age, which was to be expected given that it has long been known that psychomotor coordination improves with age in childhood and adolescence (e.g., Dunham, 1977; Thomas, Gallagher, & Purvis, 1981). Overall, cognitive performance was more consistently related to reaction time than anticipation timing abilities. Specifically, FRT and VRT were both negatively related to subjects' executive functioning abilities, and better performance on the FRT task was associated with higher levels of cognitive efficiency. Further, the only measure of CAT performance that was related to cognitive functioning was number of trials missed at 20 mph (the fastest and most challenging speed). For children and pre-adolescents especially, the CAT task at 20 mph becomes more like a pure reaction time task; the light travels so fast that a button press is required before the participant is consciously aware that it has reached the target. Again, these results point to speed of processing being a limiting factor for higher order cognitive processes.

Surprisingly, MPOD was not significantly related to any of the temporal (CFF or TCSF area under the curve) or reaction time/anticipation timing measures (VRT, FRT, CAT) in this study. As previously discussed, several studies have demonstrated significant relations between these variables. Bovier, Renzi and Hammond (2014) revealed that CFF and CAT performance improve with macular carotenoid supplementation, and Renzi and Hammond (2010) documented a link between MPOD and temporal vision (CFF and TCSF). Relations between MPOD,

temporal vision, and the reaction time/anticipation timing measures may have been attenuated in this sample for several reasons. First, a power analysis using criterion values of $\alpha = 0.05$ (one-tailed), $\beta = 0.25$, and an expected correlation of $r = .30$, revealed a need for a sample size of 59 in this study (Hulley, Cummings, Browner, Grady, & Newman, 2013). A Type II error rate of 25% is high and may have left us underpowered to detect relations that have been clearly demonstrated in the adult literature. Second, our sample exhibited relatively high MPOD and CFF values. The range of MPOD and CFF scores in Renzi & Hammond (2010) were 0.03-0.86 and 8.3-30 Hz, for example, whereas ours were 0.19-0.86 and 17.56-31.13 Hz, respectively. Range restriction may have also artificially attenuated the relations among visual and cognitive measures that were found in this study. Given that the beneficial effects of dietary carotenoids plateau once the body is replete, we would expect to see the largest differences in cases of low intake (e.g., MPOD of < 0.20), which are the cases specifically lacking in this sample. Further data collection may be necessary to increase statistical power and range of visual performance.

Finally, there may have been more measurement error associated with the measurement of MPOD and TCSF in children as compared to adults. Future studies should consider giving participants some practice trials on each device prior to the testing session. Children also varied widely, even within ages, in terms of how quickly their eyes fatigued from the vision tests and how fast their reaction time was in telling the experimenter that the flicker had stopped or started during the MPOD and TCSF tasks. Modifying the task might help with this; removing the anticipatory timing/reaction time requirements (i.e., waiting until they see the flicker stop to quickly respond in the MPOD task) and instead using discrete steps, randomized in size, in which the subject has to respond “yes” or “no” to whether the stimulus is flickering or not might lead to less error and fatigue.

Our findings demonstrate that MPOD is similarly associated with cognitive functioning in childhood and preadolescence as it is in adulthood and support the idea that processing speed is a limiting factor for higher order cognitive functions. This highlights the importance of diet in supporting cognitive development in children and pre-adolescents, given that supplementation with L and Z has been shown to improve processing speed even in healthy adult populations (Bovier & Hammond, 2015). By and large, children in the United States do not eat enough vegetables. Specifically, children under the age of 12 decreased their yearly vegetable intake between 2009 and 2015 by 12 servings per capita (Produce for Better Health Foundation, 2015), and the latest National Health and Nutrition Examination Survey (NHANES) fruit and vegetable intake report reveals that dark green vegetables were consumed by only 10.7% of US children between the ages of 6-11 on a given day in 2009-2010 (Nielsen, Rossen, Harris, & Ogden, 2014). This is concerning given that L and Z are found in highest concentrations in dark green leafy vegetables (e.g., spinach and kale). Future studies should attempt to increase L and Z intake in children via supplementation or dietary interventions to see if children exhibit the same positive benefits that adult supplementation studies have demonstrated (e.g., Bovier, Renzi, & Hammond, 2014; Johnson et al., 2008). If they do, this would further support the neural efficiency hypothesis and would provide an additional argument for interventions that could be used to improve children's fruit and vegetable intake, as well as perhaps the addition of L and Z to children's and adolescents' multi-vitamins.

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Table 1. Descriptive statistics for all participants.

	N (%)
Age (years)	
7	16 (30.2)
8	6 (11.3)
9	9 (17.0)
10	6 (11.3)
11	8 (15.1)
12	7 (13.2)
13	1 (1.9)
Sex	
Male	28 (52.8)
Female	25 (47.2)
Race	
White (Non-Hispanic)	41 (77.4)
Hispanic	1 (1.9)
>1 Race Listed	11 (20.8)
Parent Highest Education	
High School or less	3 (5.7)
College Degree (AS, BS)	19 (35.8)
Graduate Degree	29 (54.7)

Table 2. Descriptive statistics for all measures.

	Mean	SD	Range	*N*
Visual Measures				
CFF (Hz)	25.785	3.067	17.56-31.13	51
TCSF (area under the curve)	0.323	0.137	0.081-0.660	52
MPOD	0.487	0.175	0.190-0.863	53
Fixed Reaction Time (FRT; ms)	309.514	47.444	206.94-414.28	52
Variable Reaction Time (VRT; ms)	337.865	49.775	234.03-426.53	52
CAT Error – 5 mph (ms)	165.867	104.717	25.578-409.360	39
CAT Error – 10 mph (ms)	87.641	22.227	39.724-140.326	39
CAT Number Missed – 15 mph	7.372	3.686	1-15	39
CAT Number Missed – 20 mph	12.013	2.997	4.5-15	39
Cognitive Measures (All Standard Scores)				
WJ-III Composite Scores				
Brief Intellectual Ability (BIA)	109.37	13.260	81-137	51
Verbal Ability	111.68	13.119	77-144	53
Cognitive Efficiency	103.57	15.771	65-132	53
Processing Speed	99.26	17.729	62-151	53
Executive Processes	107.59	10.350	76-131	51
Select WJ-III Subtests				
Visual-Auditory Learning	100.58	13.060	75-132	53
Spatial Relations	108.31	13.292	72-132	52

Note. CFF = Critical Flicker Fusion; TCSF = Temporal Contrast Sensitivity Function; MPOD = Macular Pigment Optical Density; CAT = Coincidence Anticipation Timing.

Table 3. Correlations among visual and cognitive measures.

	BIA	Verbal Ability	Cognitive Efficiency	Processing Speed	Executive Processes
MPOD	.152	.021	.143	.080	.264*
	(*N* = 51)	(*N* = 53)	(*N* = 53)	(*N* = 53)	(*N* = 51)
CFF	.167	-.015	.286*	.252*	.243*
	(*N* = 49)	(*N* = 51)	(*N* = 51)	(*N* = 51)	(*N* = 49)
TCSF	.126	.068	.167	.004	.126
	(*N* = 50)	(*N* = 52)	(*N* = 52)	(*N* = 52)	(*N* = 50)

Note. Visual measures include Macular Pigment Optical Density (MPOD), Critical Flicker Fusion (CFF), and the Temporal Contrast Sensitivity Function (TCSF; area under the curve). Cognitive measures include the following WJ-III standard composite scores: Brief Intellectual Ability (BIA), Verbal Ability, Cognitive Efficiency, Processing Speed, and Executive Processes. The number of subjects who completed each set of measures is reported in parentheses below each correlation.

*$p < .05$

Table 4. Correlations among age and reaction time and anticipation timing measures.

	Age
Fixed Reaction Time ($N = 52$)	-.646**
Variable Reaction Time ($N = 52$)	-.610**
Coincidence Anticipation Timing ($N = 39$)	
Error – 5 mph	-.629**
Error – 10 mph	-.184
Missed – 15 mph	-.449**
Missed – 20 mph	-.731**

**$p < .01$

Table 5. Partial correlations (controlling for age) among reaction time and coincidence anticipation timing measures and cognitive measures.

	BIA	Verbal Ability	Cognitive Efficiency	Processing Speed	Executive Processes
FRT	-.218†	-.196†	-.236*	-.079	-.260*
	(*N* = 49)	(*N* = 51)	(*N* = 51)	(*N* = 51)	(*N* = 49)
VRT	-.194†	-.187†	-.139	-.029	-.253*
	(*N* = 49)	(*N* = 51)	(*N* = 51)	(*N* = 51)	(*N* = 49)
CAT					
Error – 5 mph	.068	-.195	-.071	-.143	.025
	(*N* = 37)	(*N* = 39)	(*N* = 39)	(*N* = 39)	(*N* = 37)
Error – 10 mph	.144	-.019	.112	.075	.234
	(*N* = 37)	(*N* = 39)	(*N* = 39)	(*N* = 39)	(*N* = 37)
Missed – 15 mph	-.046	.013	-.071	-.255†	.030
	(*N* = 37)	(*N* = 39)	(*N* = 39)	(*N* = 39)	(*N* = 37)
Missed – 20 mph	.020	.240	-.116	-.301*	.026
	(*N* = 37)	(*N* = 39)	(*N* = 39)	(*N* = 39)	(*N* = 37)

Note. FRT = Fixed Reaction Time, VRT = Variable Reaction Time, CAT = Coincidence Anticipation Timing. Cognitive measures include the following WJ-III standard composite scores: Brief Intellectual Ability (BIA), Verbal Ability, Cognitive Efficiency, Processing Speed, and Executive Processes.

*$p < .05$, †$p \leq .10$.

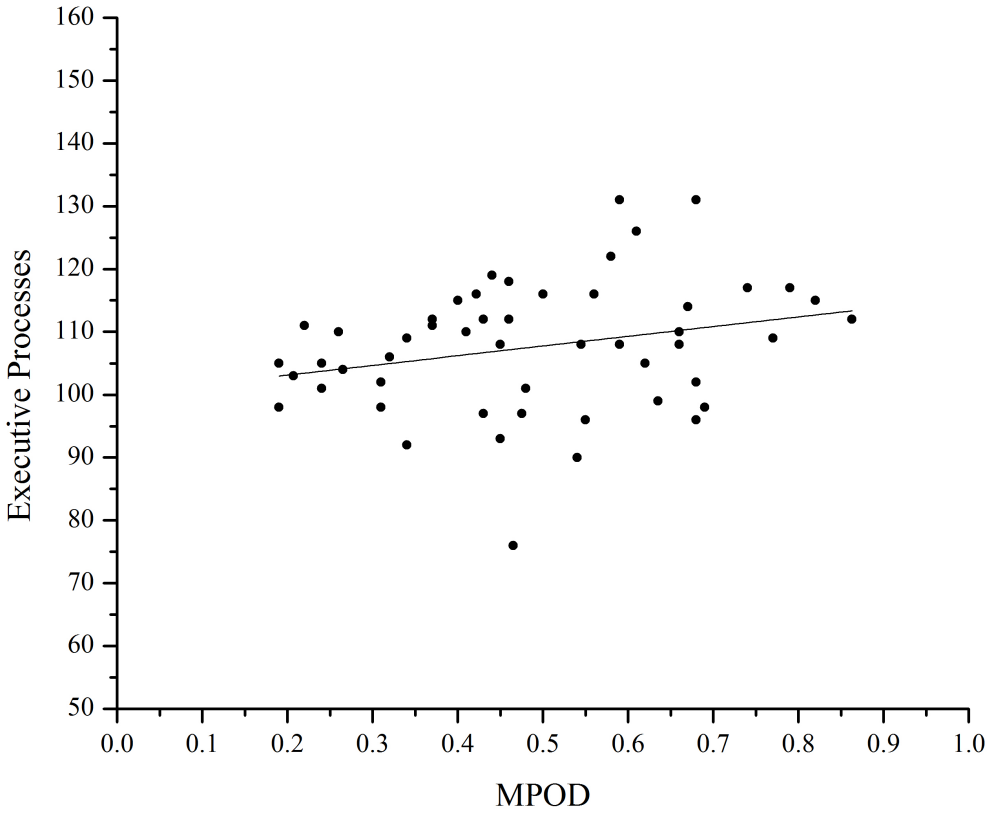

Figure 1. MPOD and WJ-III Executive Processes standard scores. The line represents the best fitting linear regression equation for the data, $y = 100.05 + 15.40x$. WJ-III standard scores reflect the subject's score in relation to same-age peers from the norming sample, with an average of 100 and standard deviation of 15.

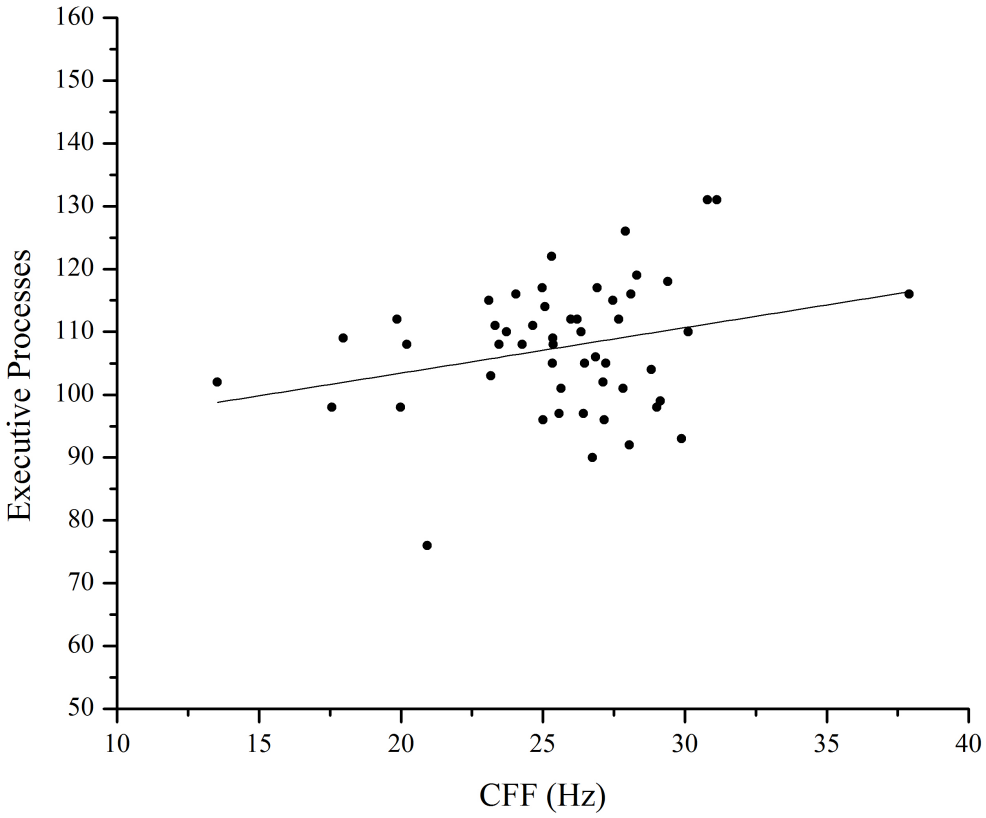

Figure 2. CFF and WJ-III Executive Processes standard scores. The line represents the best fitting linear regression equation for the data, $y = 89.03 + 0.72x$. WJ-III standard scores reflect the subject's score in relation to same-age peers from the norming sample, with an average of 100 and standard deviation of 15.

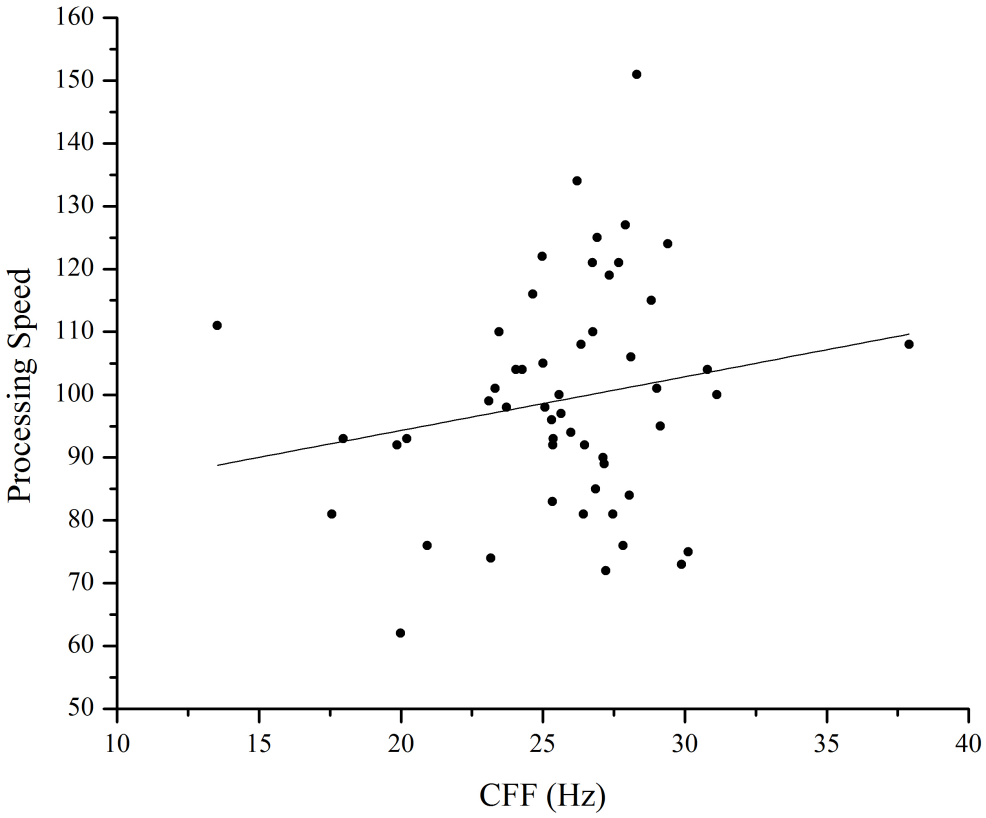

Figure 3. CFF and WJ-III Processing Speed standard scores. The line represents the best fitting linear regression equation for the data, $y = 77.22 + 0.86x$. WJ-III standard scores reflect the subject's score in relation to same-age peers from the norming sample, with an average of 100 and standard deviation of 15.

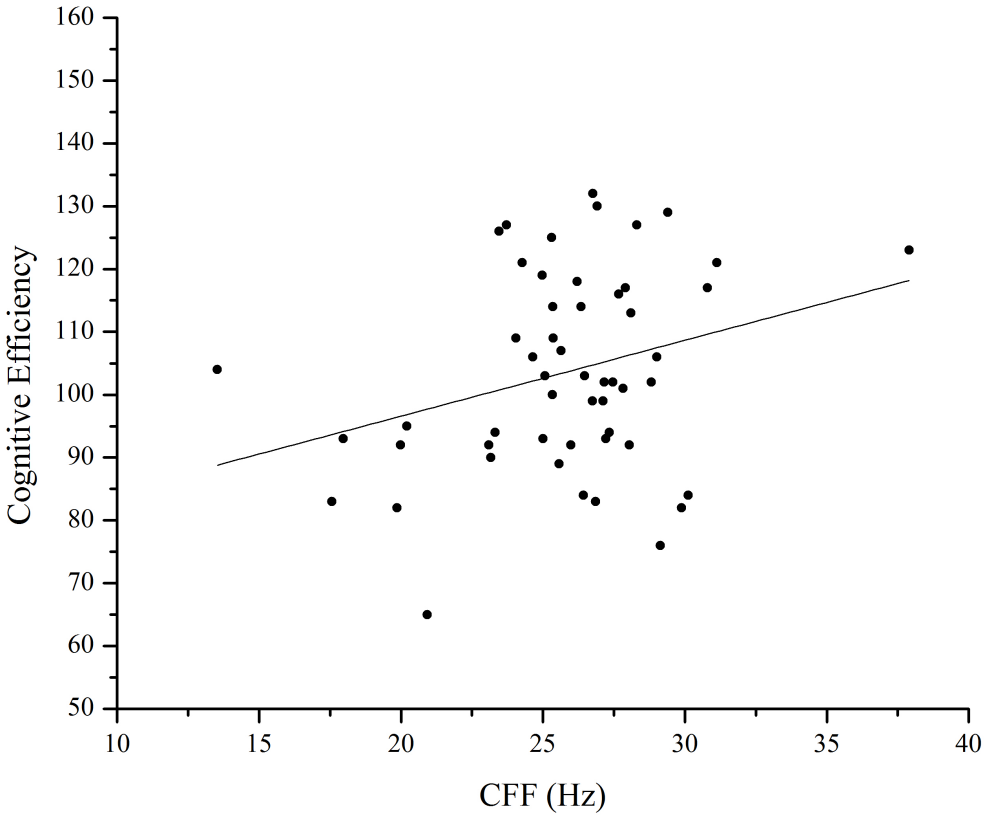

Figure 4. CFF and WJ-III Cognitive Efficiency standard scores. The line represents the best fitting linear regression equation for the data, $y = 72.48 + 1.21x$. WJ-III standard scores reflect the subject's score in relation to same-age peers from the norming sample, with an average of 100 and standard deviation of 15.