Published in final edited form as:

Int J Psychophysiol. 2017 August ; 118: 1–8. doi:10.1016/j.ijpsycho.2017.05.005.

From Neuro-pigments to Neural Efficiency: The Relationship between Retinal Carotenoids and Behavioral and Neuroelectric Indices of Cognitive Control in Childhood

Anne M. Walk¹, Naiman A. Khan^{1,2}, Sasha M. Barnett³, Lauren B. Raine⁴, Arthur F. Kramer⁴, Neal J. Cohen^{5,6}, Christopher J. Moulton⁷, Lisa M. Renzi-Hammond⁸, Billy R. Hammond⁸, and Charles H. Hillman^{4,9}

¹Department of Kinesiology and Community Health University of Illinois

²Division of Nutritional Sciences University of Illinois

³Department of Food Science, Washington State University

⁴Department of Psychology Northeastern University

⁵Beckman Institute of Science and Technology

⁶Department of Psychology University of Illinois, Urbana, IL

⁷Abbott Nutrition

⁸Behavioral and Brain Sciences, University of Georgia

⁹Department of Health Science Northeastern University

Abstract

Lutein and zeaxanthin are plant pigments known to preferentially accumulate in neural tissue. Macular Pigment Optical Density (MPOD), a non-invasive measure of retinal carotenoids and surrogate measure of brain carotenoid concentration, has been associated with disease prevention and cognitive health. Superior MPOD status in later adulthood has been shown to provide neuroprotective effects on cognition. Given that childhood signifies a critical period for carotenoid accumulation in brain, it is likely that the beneficial impact would be evident during development, though this relationship has not been directly investigated. The present study investigated the relationship between MPOD and the behavioral and neuroelectric indices elicited during a cognitive control task in preadolescent children. 49 participants completed a modified flanker task while event-related potentials (ERPs) were recorded to assess the P3 component of the ERP waveform. MPOD was associated with both behavioral performance and P3 amplitude such that children with higher MPOD had more accurate performance and lower P3 amplitudes. These

Corresponding author: Naiman A. Khan, Department of Kinesiology & Community Health, 313 Louise Freer Hall, 906 South Goodwin Avenue, University of Illinois, Urbana, IL 61801, nakhan2@illinois.edu.

Conflict of Interest Statement

Christopher Moulton is a research scientist employed by Abbott Nutrition. No other authors have any conflicts of interest to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

relationships were more pronounced for trials requiring greater amounts of cognitive control. These results indicate that children with higher MPOD may respond to cognitive tasks more efficiently, maintaining high performance while displaying neural indices indicative of lower cognitive load. These findings provide novel support for the neuroprotective influence of retinal carotenoids during preadolescence.

Keywords

macular pigment optical density; carotenoids; lutein; event-related potentials; executive control

1. INTRODUCTION

The rise of childhood and adult obesity to epidemic proportions across the globe has fueled increasing concerns of the detrimental influence of western diet patterns, often deplete of green leafy vegetables, on not only physical, but also cognitive health. Carotenoids, a class of compounds found in richly colored plants, are of particular interest because epidemiological evidence demonstrates that greater intake of carotenoids offers protection against age-related macular degeneration, cancer and cardiovascular and neurodegenerative diseases (Krinsky & Johnson 2005). Lutein and zeaxanthin are xanthophyll carotenoids that are found in high concentrations in the macular pigment (MP) of the retina (Johnson, 2014; Vishwanathan & Johnson, 2013). Lutein, in particular, is known to accumulate across all cortices and brain membranes (see Erdman et al., 2015 for a review) and has been implicated in the prevention of eye diseases (Ozawa et al. 2012; Vishwanathan & Johnson 2013) as well as preservation of cognitive abilities in older adults (Renzi et al. 2014; Vishwanathan, Iannaccone, et al. 2014). The preferential accumulation of lutein in the brain is surprising since xanthophylls only contribute to 20% of dietary carotenoid intake while comprising over 70% of carotenoid accumulation in the brain. However, the neurocognitive implications of greater lutein accumulation in neural tissue remain understudied, particularly in childhood.

It has long been established that lutein and zeaxanthin are related to eye health, and are the only two carotenoids found to preferentially accumulate in the macula. Lutein and zeaxanthin have been related to decreased risk of macular degeneration (Snodderly 1995; Tan et al. 2008) and have been shown to be related to better visual processing. For example, Stringham and Hammond (2008) supplemented the diets of healthy young adults with lutein and zeaxanthin for six months. As a result of the intervention, participants' macular pigment was shown to be more spatially dense, and they showed improved performance on visual tasks when visual conditions were adverse (i.e., visual glare). It is theorized that these benefits are derived from the anti-oxidative properties of lutein and zeaxanthin, which protect against unstable molecules at the macula (Pintea et al. 2011; Beatty et al. 2000). Lutein and zeaxanthin have also been shown to act as filters for the shortest wavelengths of light that cause oxidative stress at the macula (Junghans et al. 2001).

More recently, reports have shown a relationship between lutein and cognitive and brain health (e.g., Johnson, 2014). Lutein and zeaxanthin are found in neural tissue in both the

geriatric (Craft & Dorey 2004) and pediatric (Lieblein-boff et al. 2015; Vishwanathan, Kuchan, et al. 2014) brain, and it is believed that the anti-oxidative properties of lutein may be advantageous at the neural level as well as the macular level (Johnson, 2014). Importantly, it has been shown that in non-human primates, lutein concentrations in MP are directly related to concentrations found in neural tissue.

Attention has recently turned to the possible beneficial effects of lutein on cognition, although these reports have mostly conceptualized lutein as a protective agent that combats cognitive decline in older age. For example, lutein has been shown to correlate with many measures of cognition in healthy older adults (Vishwanathan, Iannaccone, et al. 2014), adults with advanced macular degeneration (Kelly et al. 2015), and those with mild cognitive impairment (Renzi et al. 2014). Verbal fluency and memory have been shown to improve after supplementation of lutein and DHA in healthy older women (Johnson et al., 2008).

While the evidence suggesting that lutein is related to brain and cognition is increasing, much less is known about these associations in children. The presence of lutein and zeaxanthin in the infant brain has been established. Vishwanathan et al. (2014) analyzed brain tissue from areas associated with vision, audition, memory, and executive function (occipital cortex, auditory cortex, hippocampus, and frontal lobe respectively) in 30 infant decedents. Xanthophylls were the only carotenoids found consistently in the samples, and lutein was found in the highest concentrations across all regions examined, indicating that lutein may be especially important for the infant brain. However, few studies to date have examined the relationship between lutein and cognition in living children. This is surprising, given that MP has been shown to influence visual function across the lifespan (e.g., Hammond, Fletcher, Roos, Wittwer, & Schalch, 2014; Stringham & Hammond, 2008) and visual functioning contributes to enriched sensory environments that likely affect cognitive and brain development (Johnson, 2014). Furthermore, it seems unlikely that a biological component like MP would affect cognition only in older adults, as biology is largely determined by natural selection via selective pressure, which is unlikely to emerge only after one is past reproductive age.

In addition, while evidence has linked lutein independently to the brain and to cognitive function, there is an absence of evidence linking lutein to brain-based measures of cognitive function such as fMRI or event-related potentials (ERPs). ERPs are a non-invasive measure of neural activity underlying cognitive task performance achieved through the averaging of event-locked electroencephalographic (EEG) signals. P3 is a widely studied ERP component thought to index attentional load (Wickens et al. 1983). The most common interpretation of P3 amplitude is that larger amplitudes indicate increased attentional allocation or resource engagement. P3 latency is thought to index neural processing speed, with shorter latencies indicative of faster, more efficient, processing (Polich 2007).

The aim of the present study was to explore the relationship between retinal carotenoids and cognitive control in preadolescent children. We examined children's cognitive performance (response accuracy and reaction time) during a modified flanker task as well as the peak amplitudes and latencies of the P3 elicited during the task. Abundant prior literature has used the flanker task to elicit P3 in many research contexts including investigations into

conflict monitoring and inhibition (Clayson & Larson 2011a; Clayson & Larson 2011b; Purmann et al. 2011; Groom & Cragg 2015), health-related behaviors (Hillman et al., 2014; Charles H. Hillman, Snook, & Jerome, 2003; Charles H Hillman, Buck, Themanson, Pontifex, & Castelli, 2009; Kamijo, Nishihara, Higashiura, & Kuroiwa, 2007; Pontifex & Hillman, 2007), child development (Brydges et al. 2014; Rueda et al. 2005; Ridderinkhof & van der Molen 1995; Ruberry et al. 2016), cognitive aging (Hillman, Belopolsky, Snook, Kramer, & McAuley, 2004; Hsieh, Liang, & Tsai, 2012; Korsch, Frühholz, & Herrmann, 2016; Reuter, Voelcker-Rehage, Vieluf, Parianen Lesemann, & Godde, 2016), and clinical diagnoses (Johnstone & Galletta 2013; Johnstone et al. 2009; Jonkman et al. 1999; Liu et al. 2011; Bartholow et al. 2003; Rusnakova et al. 2011; Racer et al. 2011). We assessed lutein levels via a measure of Macular Pigment Optical Density (MPOD) using customized hetero-flicker photometry (cHFP). cHFP is the most common in vivo method of assessing retinal carotenoids and is considered to be a reliable and valid measure of retinal carotenoids (Howells et al. 2011). Importantly, it is thought that there is a reliable relationship between retinal carotenoid levels and neural levels. Vishwanathan et al. (2013) fed rhesus monkeys pure carotenoid diets and subsequently assessed the relationship between the retinal and neural carotenoid levels and found significant relationships across several cortices.

Consistent with prior reports, it was expected that MPOD would be related with optimal task performance, such that children with higher MPOD values would exhibit higher accuracies and shorter reaction times. Since larger P3 peaks are often assumed to correspond to greater engagement of attentional load (e.g. Wickens et al., 1983) and earlier P3 latencies are associated with faster neural processing (e.g. Hoffman, Friedmann, Saltman, & Polich, 1999), it was also expected that higher MPOD values would be directly correlated with larger P3 peak amplitudes and earlier P3 latencies, as prior reports have shown larger P3 amplitudes associated with superior cognitive processing (Wickens et al. 1983; Polich 2007) and early P3 latencies with more efficient neural processing speed (e.g., Emmerson, Dustman, Shearer, & Turner, 1989; R. Johnson, Pfefferbaum, & Kopell, 1985; Polich, Ehlers, Otis, Mandell, & Bloom, 1986).

2. METHODS

2.1 Participants

Data were collected as a part of a larger, ongoing longitudinal study (FITKids, Hillman et al., 2014). Participants were preadolescent children recruited from East-Central Illinois. Participants were included in the present study if they successfully completed two pre-testing sessions, which included completion of demographic questionnaires, cognitive testing, and two separate and reliable MPOD assessments administered on non-consecutive days. Participant data was excluded if they responded correctly to fewer than 60% of the flanker trials ($N=7$). The remaining sample included 49 participants (31 females, Mean age = $8.69 \pm .54$). All participants provided written assent and their parents or guardians provided written consent prior to their participation, in accordance with the University of Illinois' Institutional Review Board and the Declaration of Helsinki.

2.2 Study Design

Participants underwent two testing sessions. On their first laboratory visit, legal guardians completed a questionnaire of demographic and health information while participants completed an MPOD assessment and an educational achievement test. On the second visit, participants completed a second MPOD assessment as well as a computerized flanker task, during which an electroencephalogram (EEG) was recorded.

2.3 MPOD Assessment

MPOD was measured via a customized hetero-flicker photometry (cHFP) technique and a macular densitometer (Macular Metrics Corporation, Rehoboth, MA, USA). cHFP is a non-invasive means of measuring MPOD *in vivo*. Participants are asked to judge the presence of a flicker of light in two varying wavelengths: one that is absorbed by the macular pigment (i.e., blue) and one that is not (i.e., green). When participants can no longer detect the flicker between the two disks of light, a null flicker zone has been detected. The task is repeated while participants view the flicker stimulus in their peripheral vision. Repetition of the task on a non-foveal, eccentric location of the retina serves as a reference, as macular pigment outside the fovea is low enough to be considered undetectable. The procedure used in the present study was similar to that used in Wooten et al. (1999) and in Stringham et al. (2008) with the exception that in the typical cHFP procedure, participants manipulate the radiance of the short-wave component (i.e., blue) to produce a null flicker zone themselves, while in our experiment with children, a trained examiner adjusted the test stimulus. This method is described in Renzi et al. (2015) and has been used with adult populations. For the present study, participants MPOD scores were averaged across the two testing days. Thus, any subsequent references to MPOD values refer to the average MPOD values of two testing sessions.

2.4 Demographic Measures

On the first day of testing, all participants' parent or legal guardian completed a series of health history and demographics questionnaires that included the participant's age, sex, socioeconomic status (SES), and pubertal timing. Participants' SES was assessed using a trichotomous index based on their parents' highest level of education, number of full time working parents, and participation in their school's free or reduced lunch program (Birnbaum et al., 2002). Pubertal timing was assessed using the Tanner Staging System (Tanner, 1962). Participants were assessed on their educational achievement using the Kaufman Test of Educational Achievement, 2nd edition (K-TEA, Kaufman & Kaufman, 2005).

2.5 Modified Flanker Task

Inhibitory control was assessed using a modified version of the Eriksen flanker task in which participants viewed a sequential array of visually presented stimuli (Figure 1). Stimuli were 2.5 cm illustrations of yellow fish, which were presented on a blue background for 200ms at a viewing distance of approximately 1m. The luminance was kept consistent between participants and set between 250 and 350 cd/m². Participants were asked to respond to the directionality of a centrally located target fish. The target fish was presented amid an array

of four task irrelevant distractor fish that either faced the same direction (congruent trials) or the opposite direction (incongruent) in reference to the target. Participants recorded their responses with a button press using a response pad (Current Designs, Philadelphia, PA). After receiving instructions, participants completed 50 practice trials, equally comprised of congruent and incongruent trial types presented in a random order. Then, two experimental blocks comprising 108 trials each were presented, equally comprised of congruent and incongruent trials as well as left and right target trials, presented in a random order. Participants had a response window of 1550ms, 1750ms, or 1950ms, which corresponded to variable trial times of 1600 (1400 ISI), 1800 (1600 ISI), and 2000ms (1800 ISI), respectively.

2.6 ERP Recording Technique

A Neuroscan Quik-cap was used to record electroencephalographic (EEG) activity from 64 electrode sites arranged according to the international 10-10 system (Chatrian et al. 1985). The reference electrode was a midline sensor placed between Cz and CPz. AFz served as the ground. Electro-oculographic (EOG) activity was monitored via electrodes placed above and below the left orbit and outer canthus of each eye. Inter-electrode impedance was kept below 10k Ω . Continuous data were digitized at a sampling rate of 500 Hz, amplified 500 times with a direct current to 70-Hz filter, and a 60-Hz notch filter using a Neuroscan Synamps2 amplifier. Offline, data were re-referenced to the average of the two mastoids. Data were submitted to an independent components analysis and subsequently rejected if identified components met or exceeded a .35 correlation with the vertical EOG channel. Data were filtered using a 30-Hz zero phase shift low pass filter. Stimulus-locked epochs were created from -200 to 1200 ms of each flanker trial and were baseline corrected using the -200 to 0 pre-stimulus interval. Trials on which participants committed a commission or omission error were excluded from ERP analysis. Epochs were also excluded from analysis if the moving window peak-to-peak amplitude exceeded ± 100 μ V using a 100 ms window and a 50 ms window step. The ERP variable of interest was the P3 interval mean amplitude, in which the mean amplitude was calculated within a 100 ms window surrounding the peak amplitude occurring within the latency window of 400–750 ms after stimulus onset (Pontifex et al. 2013). For correlation analyses, data was averaged over a 6-sensor region of interest (ROI) encompassing the topographic maxima (C1, Cz, C2, CP1, CPz, CP2).

2.7 Statistical Analyses

Statistical analyses were conducted using SPSS version 23 (IBM). To assess the relationship of our MPOD measure with the cognitive measures, bivariate correlations were run on MPOD, flanker reaction times, response accuracies, and the ERP interval mean amplitude collected at the P3 ROI for congruent and incongruent trial types. A .05 alpha was used as a cutoff for statistical significance, and two-tailed test values are reported. To aid interpretability of the correlations, bootstrapping was employed to calculate 95% confidence intervals. This was accomplished by re-sampling with replacement 1000 samples derived from the original sample.

In addition, a median split was performed on participants' MPOD values (Median = .568), resulting in 24 children in the High and Low MPOD groups. These groups were compared

across cognitive and ERP measures. One participant's score fell directly on the median and was excluded from all group comparisons. Flanker reaction time and accuracy were submitted to separate 2 (MPOD: High MPOD, Low MPOD) $\times$ 2 (Stimulus Type: Congruent, Incongruent) ANOVAs. P3 interval mean amplitudes from the ROI were submitted to a 2 (MPOD) $\times$ 2 (Stimulus Type) ANOVA. The Greenhouse Geisser F statistic was used for all ANOVAs to account for violation of sphericity, although original degrees of freedom are reported for simplicity. A critical value of .05 was used to determine statistical significance and partial eta squared is reported as an indicator of effect size. Follow-up univariate ANOVAs and t-tests were conducted as appropriate.

3. RESULTS

3.1 Demographics

A summary of participants' demographic data are presented in Table 1. Shapiro-Wilk tests indicated that within the full sample of participants, MPOD values and achievement scores were normally distributed (p 's $> .05$). Age was not normally distributed, indicating that our sample of participants was relatively young, even within the age range that was pre-specified for the study (8–10 years). No demographic variables were shown to significantly correlate with MPOD within the full sample (r 's $< .20$, p 's $> .084$).

3.2 Bivariate Correlations

The results of the bivariate correlation analyses are presented in Table 2. ERP waveforms for congruent and incongruent trials are presented in Figure 2. An average of 61.9 (SD = 14.0) congruent ERP trials and an average of 57.4 (SD = 15.4) incongruent trials were included in the ERP analyses. The topographical distributions are presented in Figure 3. The bivariate correlations indicated that flanker response accuracy was significantly related to MPOD values for incongruent trials ($r = .341$, $p = .017$, [CI = .124, .542]) but only moderately for congruent trials ($r = .243$, $p = .093$, [CI = .024, .454]). MPOD was not related to mean reaction time (r 's $< .106$, p 's $> .235$). Importantly, the confidence intervals for the significant and moderate correlations did not span 0, suggesting reliable moderate correlations. These results indicate that children with higher MPOD values were more likely to exhibit high performance on the flanker task and that this was particularly evident when greater levels of attentional control were demanded. However, they did not show a tendency to perform the task more quickly.

In addition, P3 mean amplitude was significantly correlated with MPOD values for incongruent trials ($r = -.30$, $p = .039$), indicating that children with higher MPOD values had systematically smaller P3 amplitudes. This finding was further supported by the confidence intervals, which suggested reliable moderate correlations (CI = $-.507$, $-.059$) between MPOD and P3 amplitude. However, MPOD was only moderately correlated with P3 mean amplitude ($r = -.27$, $p = .064$). It should be noted that the confidence intervals did not span 0, suggesting that while not significant, this correlation is reliable (CI = $-.468$, $-.065$). Collectively, the correlational analyses revealed an inverse pattern of association between flanker performance and P3 amplitudes, showing that children with higher MPOD scores were more likely to exhibit higher behavioral performance, but smaller P3 peak amplitudes.

Furthermore, this relationship was reliable for both congruent and incongruent trials, although more pronounced for incongruent trials.

3.3 Comparison across Median MPOD

Means and standard deviations for the performance and neuroelectric indices are provided in Table 3. The results of the analysis on response accuracy revealed a main effect of stimulus type ($F = 37.946, p = .000, \eta_p^2 = .452$), which was superseded by an interaction between MPOD x stimulus type ($F = 4.790, p = .034, \eta_p^2 = .094$). Decomposition of the interaction revealed that the accuracy of high and low MPOD groups were not significantly different for congruent trials ($F = .566, p = .456, \eta_p^2 = .012$), but were significantly different for incongruent trials ($F = 4.623, p = .037, \eta_p^2 = .091$), with the higher MPOD group exhibiting higher accuracy ($M = 82.242$) relative to the lower MPOD group ($M = 75.545$). The results of the analysis of the mean reaction times revealed no significant effects involving MPOD.

The analysis of P3 amplitudes revealed a significant effect of MPOD ($F(1, 46) = 5.287, p = .026, \eta_p^2 = .103$), indicating that participants with higher MPOD scores had smaller P3 amplitudes ($M = 14.247$) than participants with lower MPOD scores ($M = 18.535$). The results of the median split support the correlational analyses, providing further evidence that children with greater retinal carotenoids exhibit superior accuracy, but have smaller P3 amplitudes than those with lower levels of MPOD.

4. DISCUSSION

The aim of the present study was to explore the relationship between retinal carotenoids and cognitive and neural performance. The hypothesis that MPOD values would be positively related to flanker task performance was supported. Our data demonstrated moderate and reliable correlations between MPOD and response accuracy on both congruent and incongruent trials of the flanker task; however, this relationship was stronger for the incongruent trials. Thus, the beneficial effects of retinal carotenoids appear to have global benefits in cognitive control processing, though the greatest benefit is observed when cognitive control demands are higher. This response pattern has been shown in studies on a variety of health behaviors and dispositions such as in reports of metabolic syndrome risk (Scudder et al. 2015), acute exercise (Gothe et al. 2013), aerobic fitness (Drollette et al. 2014) and diet (Zuniga et al. 2015).

Our data did not show a significant relationship between MPOD and reaction time, indicating that the benefits of lutein on cognitive control have a preferential effect of accuracy as opposed to processing speed in children. Although we hypothesized that children with higher MPODs would respond more quickly to the task, other investigations of health related behaviors have shown similar preferential effects (e.g., Hillman, Buck, Themanson, Pontifex, & Castelli, 2009; Khan et al., 2015). This pattern may change over the course of development, as conflict monitoring improves with maturation (Rueda et al. 2004).

Contrary to our *a priori* hypothesis pertaining to the ERP findings, our correlational and group analyses indicate that MPOD was inversely related to P3 peak amplitudes in children. This was surprising, given that superior cognitive task performance is often accompanied by

larger P3 amplitudes. Indeed, this pattern has been shown in several reports of health-related behaviors in which higher fit individuals show larger P3 amplitudes compared to lower fit peers (e.g., Drollette et al., 2014; Hillman et al., 2009; Hillman et al., 2009; Pontifex et al., 2011). However, we believe the most parsimonious interpretation of our results involves further consideration of the cognitive processes thought to underlie P3 amplitude. A common interpretation of P3 amplitude is that it is an index of attentional resource allocation (Kok 2001; Polich 2007), a theory derived from findings that P3 amplitude is larger when participants are directed to attend to a target (Polich 1986), that P3 amplitude is enhanced when accurate cues are given about a target (Miniussi et al. 1999), and that P3 amplitude decreases with the addition of a secondary task (Nash & Fernandez 1996; Watter et al. 2001). Under this view, greater P3 amplitudes are indicative of greater allocation of attention or that the task necessitates a greater cognitive load. Along these lines, our results suggest that participants with higher MPOD values allocated fewer attentional resources to complete the flanker task compared to their low MPOD peers. Taken together with the behavioral performance data, these results suggest that the children with higher MPOD values completed the task more efficiently. That is, they showed superior performance, which was accompanied by a neural signature that indicated fewer cognitive resources were employed to successfully complete the task.

We are not the first to report this pattern of findings. Daffner et al.(2011) used an n-back task to elicit P3 in younger and older adults. They found that in both age groups, participants that performed well on the most difficult task condition (2-back) showed smaller P3 amplitudes compared to low performers when the task was not demanding (i.e. on the 0-back task). Additionally, both high and low performers had similar accuracy in the low demand condition. Interestingly, as task demands increased, high performers showed an increase in amplitude whereas low performers showed a decrease in amplitude. Thus, the typical trend of high performers showing higher amplitudes was only present when the task was most difficult. The authors interpreted the lower amplitudes in the low attentional demand task to be a marker of greater neural efficiency, with high performing participants retaining neural resources in the low demand task, and up-regulating these resources as demands increased. We believe that a similar underlying mechanism may be involved in these data. Though the high and low MPOD groups differed in response accuracy for incongruent trials, the cognitive load may not have been sufficiently high to render a larger P3 among the high MPOD group (which in this case also constituted higher performers). Under this view, we may expect to see higher amplitudes emerge for high MPOD children with a task involving higher cognitive demands. Of course, we can only speculate since the prior study (Daffner et al. 2011) was conducted on adults and not child participants. However, this may be a fruitful avenue for future research.

It should also be noted that a similar pattern of results is more commonly seen in other ERP components. For example, studies have shown that ERN amplitudes increase when participants are highly motivated (Pailing & Segalowitz 2004), while studies on health related behaviors are mixed with some reports of healthier individuals exhibiting higher (e.g. Kamijo et al., 2014) and lower (e.g. Pontifex et al., 2011) ERN amplitudes. The difference in ERN amplitudes may be tied to motivational constructs such as externalizing behavior and trait anxiety (Olvet & Hajcak 2008; Hajcak & Foti 2008). Furthermore, ERN amplitudes

have been shown to vary depending on whether speed or accuracy is emphasized in the instructions (Themanson et al. 2008). While P3 has not been shown to be affected by individual differences like externalization and anxiety, these problems illustrate the complexity of measuring the relationship between an index of health and neuroelectric activity. It is possible that there may be individual differences mediating our effects that were not measured in our sample. Further, it has been shown that children show a response patterns to tasks of inhibition that varies maturationally (Davidson et al. 2006), suggesting that modulations in underlying ERP amplitudes may be affected by response sets.

These novel data provide evidence that greater MPOD status is related to cognitive performance in preadolescent children. Furthermore, we are the first to provide evidence directly linking lutein to the underlying neural correlates of cognitive performance. While this finding stands in contrast to a previous report regarding lutein and cognition in children (i.e. Mulder et al., 2014), that study based the analysis on plasma lutein and self-reported dietary intake of lutein while our study used MPOD, a retinal measure which may be more sensitive to cognitive measures than serum lutein. Kelly et al.(2015) found that in adults, both macular pigment and serum lutein were associated with cognitive outcomes, but only the associations with macular pigment remained after controlling for relevant characteristics like sex, age, diet, and education level.

If, as our data suggest, lutein actively changes brain function, it begs the question of what mechanism(s) may underpin such an effect. Much of the work on carotenoids has focused on their role as antioxidants and anti-inflammatories (Ozawa et al. 2012). Although this reduces the likelihood of accumulated damage, it can also, more acutely, change other aspects of physiology. For instance, inflammation is known to modulate the expression of brain-derived neurotrophic factor (BDNF) (Calabrese et al. 2014). Lutein, as an anti-inflammatory compound, could lower systemic inflammation increasing production of BDNF in the brain. Indeed, recent data has shown that supplementing lutein increases the amount of systemic BDNF in healthy younger adults (Stringham, Holmes, & Stringham, 2016). Metabolomic analysis of infant brain showed lutein activity in areas that are key to nutrition such as occipital and frontal cortex and hippocampus (Lieblein-boff et al. 2015). As an antioxidant, lutein has been shown to reduce apoptotic death of dopaminergic neurons (Nataraj et al. 2015). In further support of this postulation, a study in Rhesus monkeys recently demonstrated that lutein co-localizes with docosahexaenoic acid (DHA) in brain regions known to underlie cognitive control in rhesus monkeys (Mohn et al. 2016). Taken together, the idea that lutein could have immediate effects on the efficiency of neural processing is reasonable.

The findings presented here are foundational and there are several ways in which future work could extend the present findings. One limitation to the present study is that we did not include a report of dietary intake. As such, it is possible that the neurocognitive findings ascertained from our data may be the result of overall diet quality or an unmeasured factor that underlies both cognition and diet quality, such as parental involvement or responsibility. However, many studies reporting dietary intake use self-reported measures that are subject to measurement error, while our study uses a direct index of diet, in that lutein is plant pigment derived from foods that are known to be healthful including fruits and dark green leafy

vegetables. Furthermore, the relationship between diet quality and cognitive outcomes has been previously established across the lifespan (Wengreen & Neilson 2009; Neumann et al. 2003; Florence et al. 2008). We propose that rather than viewing our findings as a completely disparate line of inquiry, our data serves as complementary to this previous work, offering a possible mechanism (i.e. retinal lutein) by which the relationship between diet and neurocognitive growth is possible. In addition, our study is cross-sectional in its design. Therefore, it is impossible to make any claims about causation from our data alone. Although previous work has shown that supplementation of lutein enhances cognitive function (Bovier et al. 2014; Schalch 2014), additional interventions are needed to determine if supplementation would enhance the neural components underlying cognitive function, and to what extent supplementation is beneficial in children. Additionally, apart from the few studies that have examined lutein in neural tissue, little is known about how it affects cognition at different stages in development. Cross-sectional and longitudinal data of lutein and cognition across the lifespan would aid in interpreting our results in light of typical developmental trajectories. Finally, examination of retinal carotenoids across cognitive domains and developmental ages is necessary. While our data offer unique insight into one aspect of executive control, more work utilizing inhibition, memory, and learning tasks as well as other attentional paradigms would extend and generalize our findings.

In conclusion, the study of retinal lutein has become the focus of several recent investigations into cognitive and brain health. While the work in older adults is promising, we offer the first data to support a relationship between retinal carotenoids and cognitive function in children. Furthermore, this study is the first to investigate lutein in relation to any brain-based measure of cognition. Our data suggest that lutein is indeed related to both cognitive control performance and its underlying neural correlates in children such that those with higher MPOD values show better performance but with neural markers of lower cognitive load. The benefits of lutein to neural efficiency should be investigated in the future across the lifespan using a variety of cognitive and brain-based methods as well as dietary interventions.

Acknowledgments

Funding Sources

This work was supported by the National Institutes of Health (HD069381); Abbot Nutrition through the Center for Nutrition, Learning, and Memory (CNLM); and USDA National Institute of Food and Agriculture under the Transdisciplinary Obesity Prevention Program grant (2011-67001-30101 to L.B.R.).

We thank Jeanine Benskin for her tireless efforts coordinating the FITKids randomized control trial.

References

- Bartholow BD. Effects of alcohol consumption and alcohol susceptibility on cognition: A psychophysiological examination. *Biological Psychology*. 2003; 64(1–2):167–190. [PubMed: 14602361]
- Beatty S. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of Ophthalmology*. 2000; 45(2):115–134. [PubMed: 11033038]
- Bovier ER, Renzi LM, Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on neural processing speed and efficiency. *PloS one*. 2014; 9(9):e108178. [PubMed: 25251377]

- Brydges CR. Predictive validity of the N2 and P3 ERP components to executive functioning in children: a latent-variable analysis. *Frontiers in human neuroscience*. 2014; 8:80. [PubMed: 24600376]
- Calabrese F. Brain-derived neurotrophic factor: A bridge between inflammation and neuroplasticity. *Frontiers in Cellular Neuroscience*. 2014; 8(430)
- Chatrian G, Lettich E, Nelson P. Ten Percent Electrode System for Topographic Studies of Spontaneous and Evoked EEG Activities. *American Journal of EEG Technology*. 1985; 25(2):83–92.
- Clayson PE, Larson MJ. Conflict adaptation and sequential trial effects: Support for the conflict monitoring theory. *Neuropsychologia*. 2011a; 49(7):1953–1961. [PubMed: 21435347]
- Clayson PE, Larson MJ. Effects of repetition priming on electrophysiological and behavioral indices of conflict adaptation and cognitive control. *Psychophysiology*. 2011b; 48(12):1621–1630. [PubMed: 21806636]
- Craft N, Dorey CK. Carotenoid, tocopherol, and retinal concentrations in elderly human brain. *The Journal of Nutrition, Health, and Aging*. 2004; 8(3):156–162.
- Daffner KR. Mechanisms underlying age- and performance-related differences in working memory. *Journal of Cognitive Neuroscience*. 2011; 23(6):1298–314. [PubMed: 20617886]
- Davidson MC. Development of cognitive control and executive functions from 4 to 13 years: Evidence from manipulations of memory, inhibition, and task switching. *Neuropsychologia*. 2006; 44(11):2037–2078. [PubMed: 16580701]
- Drollette ES. Acute exercise facilitates brain function and cognition in children who need it most: An ERP study of individual differences in inhibitory control capacity. *Developmental Cognitive Neuroscience*. 2014; 7:53–64. [PubMed: 24309300]
- Emmerson RY. P3 latency and symbol digit performance correlations in aging. *Experimental Aging Research*. 1989; 15(3):151–159. [PubMed: 2638634]
- Erdman JW. Lutein and brain function. *Foods*. 2015; 4:547–564. [PubMed: 26566524]
- Florence MD, Asbridge M, Veugelers PJ. Diet quality and academic performance. *Journal of School Health*. 2008; 78(4):209–215. [PubMed: 18336680]
- Gothé N. The Acute Effects of Yoga on Executive Function. *Journal of Physical Activity and Health*. 2013; 10:488–495. [PubMed: 22820158]
- Groom MJ, Cragg L. Differential modulation of the N2 and P3 event-related potentials by response conflict and inhibition. *Brain and Cognition*. 2015; 97:1–9. [PubMed: 25955278]
- Hajcak G, Foti D. Errors Are Aversive Defensive Motivation and the Error-Related Negativity. *Psychological Science*. 2008; 19(2):103–108. [PubMed: 18271855]
- Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Investigative Ophthalmology and Visual Science*. 2014; 55(12):8583–8589.
- Hillman CH, Buck SM. Aerobic Fitness and Cognitive Development: Event-Related Brain Potential and Task Performance Indices of Executive Control in Preadolescent Children. *Developmental Psychology*. 2009; 45(1):114–129. [PubMed: 19209995]
- Hillman CH. Effects of the FITKids randomized controlled trial on executive control and brain function. *PEDIATRICS*. 2014; 134(4):e1063–e1071. [PubMed: 25266425]
- Hillman CH. Physical activity and executive control: Implications for increased cognitive health during older adulthood. *Research quarterly for exercise and sport*. 2004; 75(2):176–85. [PubMed: 15209336]
- Hillman CH, Pontifex MB. The effect of acute treadmill walking on cognitive control and academic achievement in preadolescent children. *Neuroscience*. 2009; 159(3):1044–1054. [PubMed: 19356688]
- Hillman CH, Snook EM, Jerome GJ. Acute cardiovascular exercise and executive control function. *International Journal of Psychophysiology*. 2003; 48(3):307–314. [PubMed: 12798990]
- Hoffman LD. Neuroelectric assessment of nutrient intake. *International Journal of Psychophysiology*. 1999; 32(2):93–106. [PubMed: 10380944]

- Howells O, Eperjesi F, Bartlett H. Measuring macular pigment optical density in vivo: A review of techniques. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2011; 249:315–347.
- Hsieh S, Liang YC, Tsai YC. Do age-related changes contribute to the flanker effect? *Clinical Neurophysiology*. 2012; 123(5):960–972. [PubMed: 21978651]
- Johnson EJ. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutritional Neuroscience*. 2008; 11(2):75–84. [PubMed: 18510807]
- Johnson EJ. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. *Nutrition Reviews*. 2014; 72(9):605–612. [PubMed: 25109868]
- Johnson R, Pfefferbaum A, Kopell BS. P300 and long-term memory: Latency predicts recognition performance. *Psychophysiology*. 1985; 22(5):497–507. [PubMed: 4048349]
- Johnstone SJ. Response inhibition and interference control in children with AD/HD: A visual ERP investigation. *International Journal of Psychophysiology*. 2009; 72(2):145–153. [PubMed: 19095016]
- Johnstone SJ, Galletta D. Event-rate effects in the flanker task: ERPs and task performance in children with and without AD/HD. *International Journal of Psychophysiology*. 2013; 87(3):340–348. [PubMed: 22841754]
- Jonkman LM. Perceptual and response interference in children with attention-deficit hyperactivity disorder, and the effects of methylphenidate. *Psychophysiology*. 1999; 36(4):419–29. [PubMed: 10432791]
- Junghans A, Sies H, Stahl W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Archives of Biochemistry and Biophysics*. 2001; 391(2):160–164. [PubMed: 11437346]
- Kamijo K. The interactive effect of exercise intensity and task difficulty on human cognitive processing. *International Journal of Psychophysiology*. 2007; 65(2):114–121. [PubMed: 17482699]
- Kamijo K. The negative association of childhood obesity to cognitive control of action monitoring. *Cerebral Cortex*. 2014; 24(3):654–662. [PubMed: 23146965]
- Kelly D. Cognitive function and its relationship with macular pigment optical density and serum concentrations of its constituent carotenoids. *Journal of Alzheimer's Disease*. 2015; 48:261–277.
- Khan NA. Dietary fiber is positively associated with cognitive control among prepubertal children. *The Journal of Nutrition*. 2015; 145:143–149. [PubMed: 25527669]
- Kok A. On the utility of P3 amplitude as a measure of processing capacity. *Psychophysiology*. 2001; 38:557–577. [PubMed: 11352145]
- Korsch M, Frühholz S, Herrmann M. Conflict-specific aging effects mainly manifest in early information processing stages-an ERP study with different conflict types. *Frontiers in Aging Neuroscience*. 2016; 8(MAR):1–12. [PubMed: 26858637]
- Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*. 2005; 26:459–516. [PubMed: 16309738]
- Lieblein-boff JC. Exploratory metabolomic analyses reveal compounds correlated with lutein concentration in frontal cortex, hippocampus, and occipital cortex of human infant brain. *PLOS One*. 2015:1–19.
- Liu T. Conflict control of children with different intellectual levels: An ERP study. *Neuroscience Letters*. 2011; 490(2):101–106. [PubMed: 21182895]
- Miniussi C. Orienting attention in time modulation of brain potentials. *Brain*. 1999; 122:1507–1518. [PubMed: 10430834]
- Mohn ES, et al. Lutein and DHA co-localize in cell membranes of brain regions controlling cognition in the rhesus macaque. *Experimental Biology* 2016. 2016; 30(1) The FASEB Journal. Supplement 689.2.
- Mulder, Ka, et al. Plasma lutein concentrations are related to dietary intake, but unrelated to dietary saturated fat or cognition in young children. *Journal of nutritional science*. 2014; 3:e11. [PubMed: 25191603]
- Nash AJ, Fernandez M. P300 and allocation of attention in dual-tasks. *International Journal of Psychophysiology*. 1996; 23:171–180. [PubMed: 8947783]

- Nataraj J. Lutein protects dopaminergic neurons against MPTP-induced apoptotic death and motor dysfunction by ameliorating mitochondrial disruption and oxidative stress. *Nutritional Neuroscience*. 2015:1–10.
- Neumann CG. Animal source foods to improve micronutrient nutrition and human function in developing countries: The impact of dietary intervention on the cognitive development of Kenyan school children. *Journal of Nutrition*. 2003; 133:3965S–3971S. [PubMed: 14672297]
- Olvet DM, Hajcak G. The error-related negativity (ERN) and psychopathology: Toward an endophenotype. *Clinical Psychology Review*. 2008; 28(8):1343–1354. [PubMed: 18694617]
- Ozawa Y. Neuroprotective effects of lutein in the retina. *Current Pharmaceutical Design*. 2012; 18:51–56.
- Pailing PE, Segalowitz SJ. The error-related negativity as a state and trait measure: Motivation, personality, and ERPs in response to errors. *Psychophysiology*. 2004; 41:84–95. [PubMed: 14693003]
- Pintea A. Xanthophylls protect against induced oxidation in cultured human retinal pigment epithelial cells. *Journal of Food Composition and Analysis*. 2011; 24:830–836.
- Polich J. Attention, probability, and task demands as determinants of P300 latency from auditory stimuli. *Electroenceph*. 1986; 63:251–259.
- Polich J. P300 latency reflects the degree of cognitive decline in dementing illness. *Electroencephalography and Clinical Neurophysiology*. 1986; 63:138–144. [PubMed: 2417814]
- Polich J. Updating P300: An integrative theory of P3a and P3b. *Clinical Neurophysiology*. 2007; 118:2128–2148. [PubMed: 17573239]
- Pontifex MB. Cardiorespiratory fitness and the flexible modulation of cognitive control in preadolescent children. *Journal of cognitive neuroscience*. 2011; 23(6):1332–1345. [PubMed: 20521857]
- Pontifex MB. Exercise improves behavioral, neurocognitive, and scholastic performance in children with Attention-Deficit/Hyperactivity Disorder. *The Journal of Pediatrics*. 2013; 162(3):543–551. [PubMed: 23084704]
- Pontifex MB, Hillman CH. Neuroelectric and behavioral indices of interference control during acute cycling. *Clinical Neurophysiology*. 2007; 118(3):570–580. [PubMed: 17095295]
- Purmann S. Adaptation to frequent conflict in the eriksen flanker task: An ERP study. *Journal of Psychophysiology*. 2011; 25(2):50–59.
- Racer KH. Attention network performance and psychopathic symptoms in early adolescence: An ERP study. *Journal of Abnormal Child Psychology*. 2011; 39(7):1001–1012. [PubMed: 21607659]
- Renzi LM. Neurobiology of aging relationships between macular pigment optical density and cognitive function in unimpaired and mildly cognitively impaired older adults. *Neurobiology of Aging*. 2014; 35(7):1695–1699. [PubMed: 24508218]
- Reuter E-M, et al. The P3 parietal-to-frontal shift relates to age-related slowing in a selective attention task. *Journal of Psychophysiology*. 2016; (August):1–18.
- Ridderinkhof KR, van der Molen MW. A psychophysiological analysis of developmental differences in the ability to resist interference. *Child Development*. 1995; 66(4):1040–1056.
- Ruberry EJ. Income, neural executive processes, and preschool children's executive control. *Development and Psychopathology*. 2016:1–12.
- Rueda MR. Development of the time course for processing conflict: An event-related potentials study with 4 year olds and adults. *BMC Neuroscience*. 2005; 5(39)
- Rueda MR, Posner MI, Rothbart MK. The Development of Executive Attention: Contributions to the Emergence of Self-Regulation The Development of Executive Attention: Contributions to the Emergence of Self-Regulation. *Developmental Neuropsychology*. 2004; 28(2):573–594.
- Rusnakova S. The executive functions in frontal and temporal lobes: A flanker task intracerebral recording study. *Journal of Clinical Neurophysiology*. 2011; 28(1):30–35. [PubMed: 21221007]
- Schalch W. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Investigative ophthalmology & visual science*. 2014; 55(12):8583–8589. [PubMed: 25468896]

- Scudder MR. Cognitive Control in Preadolescent Children With Risk Factors for Metabolic Syndrome. *Health Psychology*. 2015; 34(3):243–252. [PubMed: 25133829]
- Snodderly MD. Evidence for protection against degeneration by carotenoids and antioxidant vitamins. *The American Journal of Clinical Nutrition*. 1995; 62(6 Suppl):1448S–61S. [PubMed: 7495246]
- Stringham JM, Hammond BR. Macular pigment and visual performance under glare conditions. *Optometry and Vision Science*. 2008; 85(2):82–88. [PubMed: 18296924]
- Stringham NT, Holmes PV, Stringham JM. Lutein supplementation increases serum brain-derived neurotrophic factor (BDNF) in humans. *Experimental Biology* 2016. 2016 The FASEB Journal, p. Supplement 689.3.
- Tan JL. Dietary antioxidants and the long-term incidence of age-related macular degeneration. *Ophthalmology*. 2008; 115(2):334–341. [PubMed: 17664009]
- Themanson JR, Pontifex MB, Hillman CH. Fitness and action monitoring: Evidence for improved cognitive flexibility in young adults. *Neuroscience*. 2008; 157(2):319–328. [PubMed: 18845227]
- Vishwanathan R, Kuchan MJ. Lutein and preterm infants with decreased concentrations of brain carotenoids. *Hepatology and Nutrition*. 2014; 59(5):659–665.
- Vishwanathan R. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutritional Neuroscience*. 2013; 16(1):21–29. [PubMed: 22780947]
- Vishwanathan R, Iannaccone A. Macular pigment optical density is related to cognitive function in older people. *Age and Ageing*. 2014; 43:271–275. [PubMed: 24435852]
- Vishwanathan, R., Johnson, EJ. Lutein and zeaxanthin and eye disease. In: Tanumihardjo, SA., editor. *Carotenoids and Human Health*. New York: Springer Science and Business Media; 2013. p. 215–235.
- Watter S, Geffen GM, Geffen LB. The n-back as a dual-task: P300 morphology under divided attention. *Psychophysiology*. 2001; 38:998–1003. [PubMed: 12240676]
- Wengreen H, Neilson C. Diet quality is associated with better cognitive test performance among aging men and women. *The Journal of Nutrition*. 2009; 139:1944–1949. [PubMed: 19675102]
- Wickens C. Performance of concurrent tasks: A psychophysiological analysis of the reciprocity of information-processing resources. *Science*. 1983; 221(4615):1080–1082. [PubMed: 6879207]
- Zuniga KE. Relationship between fruit and vegetable intake and interference control in breast cancer survivors. *European Journal of Nutrition*. 2015:1–8. [PubMed: 25296886]

Figure 1.
Sample of stimuli from the revised Flanker task.

Figure 2.
ERP waveforms for congruent and incongruent flanker trials.

Figure 3. Topographical distribution plots of the P3 amplitude for congruent and incongruent flanker trials, averaged from 400–750 milliseconds after stimulus onset.

Table 1

Participant Demographic Data

	Whole Sample (N=49)	High MPOD (N=24)	Low MPOD (N=24)
Age, years	8.69 (.08)	8.66 (.50)	8.73 (.60)
Female (n, %)	31, 63	15, 63	16, 67
Low SES (n, %)	19, 39	9, 38	10, 42
Achievement	108.37 (.15.88)	111.38 (19.61)	105.42 (11.08)
Pubertal Timing	1.33 (.05)	1.31 (.07)	1.33 (.06)
MPOD	.61 (.03)	.78 (.16)	.44 (.10)

SES, socioeconomic status; Achievement, Kaufman Test of Educational Achievement Composite standard score; Mean (Standard Deviations in parentheses) for all such values. No statistically significant differences were found between higher and lower MPOD groups on any demographic variables with the exception of MPOD ($F(1, 46) = 79.05, p = .000$).

Table 2

Bivariate Correlation Data

	Pearson <i>r</i>	<i>p</i> (2-tailed)	Correlation with MPOD 95% Confidence Interval	
			Lower	Upper
Mean RT				
Congruent	.106	.469	-.206	.408
Incongruent	.102	.483	-.220	.411
Accuracy				
Congruent	.243 *	.093	.024	.454
Incongruent	.341 **	.017	.124	.542
Mean Amplitude				
Congruent	-.266 *	.064	-.468	-.065
Incongruent	-.296 **	.039	-.507	-.059

* denotes statistical significance at $p < .10$;

** denotes statistical significance at $p < .05$.

Table 3

Descriptive Data on Flanker Task Performance and ERP Indices

	Whole Sample (N=49)	High MPOD (N=24)	Low MPOD (N=24)
Mean RT (ms)			
Congruent	573.6 (97.1)	592.1 (91.7)	553.5 (102.2)
Incongruent	623.3 (113.7)	641.6 (100.4)	602.3 (126.2)
Accuracy (%)			
Congruent	85.6 (8.6)	86.6 (9.2)	84.7 (8.1)
Incongruent	78.6 (11.3)	82.2 (12.2)	75.5 (9.1)
Mean Amplitude (μ V)			
Congruent	16.0 (6.5)	14.0 (5.3)	17.8 (7.1)
Incongruent	17.0 (7.6)	14.5 (5.8)	19.3 (8.5)

Standard deviations are reported in parentheses.