MACULAR PIGMENT OPTICAL DENSITY AND ACADEMIC ACHIEVEMENT AMONG PREADOLESCENT CHILDREN

BY

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THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Nutritional Sciences in the Graduate College of the University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

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Abstract

Lutein has been shown to preferentially accumulate in the macula of the neural retina as macular pigment and across all brain cortices of infants and adults. This preferential accumulation in the brain has led to the hypothesis that lutein may positively impact cognition, possibly through its anti-inflammatory and/or antioxidant properties. Both macular pigment optical density (MPOD) – a non-invasive measure of retinal lutein and correlate of brain lutein – and dietary lutein supplementation have been associated with better cognitive function among adult populations. However, the reliability of heterochromatic flicker photometry (HFP) to measure MPOD has only recently been demonstrated to be a moderately reliable technique in preadolescent children. Therefore, to date, the relationship between MPOD and cognitive performance has not been directly investigated in children. Accordingly, the main objective of this thesis was to investigate the relationship between MPOD, assessed and averaged over two time points using HFP, and academic performance among 8-10-year-olds (N = 56). Additional objectives were to investigate how MPOD relates with dietary intake of lutein and zeaxanthin, and to determine if lutein and zeaxanthin intake would mediate the relationship between MPOD and academic performance. Academic performance was assessed using the Kaufman Test of Academic and Educational Achievement II (KTEA). Habitual dietary intake of lutein and zeaxanthin was measured using the average of 3-day food records. The results for the main objective of this thesis indicated a significant correlation between MPOD and the KTEA composite measures of achievement (r = 0.40, P < 0.01), reading (r = 0.28, P = 0.04), math (r = 0.35, P < 0.01), and written language (r = 0.41, P < 0.01), but not with reading fluency (r = 0.22, P = 0.11). Further analysis with stepwise hierarchical regression models was conducted with sex, intelligence quotient (IQ), whole body percent fat, and fat free mass VO₂max included in the
initial step. Subsequent addition of MPOD into the model was conducted to determine the contribution to the academic measures following adjustments of related variables. The addition of MPOD did not statistically improve the explained variance for the reading or reading fluency composite scores. However, the adding of MPOD in the model did explain additional variance for the achievement composite standard scores ($\Delta R^2 = 0.10, P < 0.01$), math composite standard scores ($\Delta R^2 = 0.07, P = 0.02$), and the written language composite standard scores ($\Delta R^2 = 0.15, P < 0.01$). Further, the composite measures were decomposed to investigate whether the subtests all had similar relations with MPOD as their composite scores. The results indicated that all subtests were consistent with their composite measures. For the additional objectives the results showed the dietary consumption of lutein and zeaxanthin was positively correlated with MPOD ($r = 0.39, P = 0.02$). Lutein and zeaxanthin intake was significantly related with the written language composite score ($r = 0.53, P < 0.01$), but not with any of the other academic composite scores. Stepwise regressions completed in a subset of children to assess if lutein and zeaxanthin intake would mediate the relationship between MPOD and academics, showed that MPOD’s relationship to academics still remained following adjustment for dietary intake. The accumulation of this evidence indicates that macular lutein is associated with superior performance on academic measures, particularly in math and written language while dietary intake had weaker relationships. This is the first study to demonstrate that retinal lutein and zeaxanthin, measured as MPOD, is related to academic achievement in children. This has implications as the macular pigment can be influenced by the diet in most of the population. However, this conclusion is tenuous and should be investigated further by conducting placebo-controlled interventions.
Acknowledgements

I would like to thank Dr. Charles Hillman for acting as my adviser, and for his committed guidance and assistance during the research and preparation of my thesis. I would also like to thank Dr. Naiman Khan for serving as chair of my graduate committee and providing helpful suggestions and comments on this project. I also wish to thank Dr. John Erdman for serving on my graduate committee. Thanks are also extended to my fellow graduate students, technicians, and undergraduate students who contributed their time and effort to this project. Additional thanks goes out to my fiancé, family, and friends for their continued support during this step in my education.
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CHAPTER 1 – INTRODUCTION

Carotenoids are a family of fat-soluble plant pigments that have received considerable investigation for their beneficial effects on a variety of chronic diseases (1). Interestingly, though there are over 700 carotenoids in nature, only about 40 are found in the typical diet and of those 40, only 20 can be found in human blood and tissue (2, 3). The carotenoids, from the xanthophyll class, lutein, zeaxanthin, and meso-zeaxanthin accumulate at the macula to the exclusion of all other carotenoids (4, 5). Collectively, they are known as the macular pigment. Lutein and zeaxanthin are entirely of dietary origin, while meso-zeaxanthin is believed to be present due to the bioconversion of lutein (4). Dietary sources of lutein and zeaxanthin include fruits and vegetables of various colors as well as eggs (6).

Lutein and zeaxanthin have been demonstrated to be of importance to retinal health (7). The macular pigment filters damaging blue light, wavelengths between 430 and 490nm with a maximum absorption at 465nm (8), and it has antioxidant properties that protect the retina from photo-oxidative damage (9-11). Macular pigment optical density (MPOD) has been associated with faster visual processing speed (12), reduced glare (13), better visual performance when ambient illumination is low (14), improved contrast sensitivity (15), reduced symptoms of visual fatigue (16), and increased neuronal signaling efficiency in the eye (17).

In non-human primates, MPOD has been found to serve as a good proxy for the amount of lutein and zeaxanthin in the brain (18), thus allowing MPOD to be used as a biomarker of lutein and zeaxanthin concentration in the brain. Recent studies in both the elderly and infants have found that much of the concentration of carotenoids in the brain is dominated by lutein. Lutein has been found to preferentially accumulate in the infant brain, accounting for 59% of total carotenoids in the brain while only constituting 12% of the infants’ carotenoid intake (19).
The relative contribution of lutein to the total carotenoids in infant brains is almost two-fold greater than in adults, accounting for 59% vs. 34%, respectively (19, 20), suggesting a selective neuroprotective role of lutein in early neural development.

Many direct and indirect measures of macular pigment optical density have been associated with better cognitive function among older adults (20-24). In a large prospective cohort, older adults consuming the highest amounts of vegetables had a slower rate of cognitive decline over 6 years, and green leafy vegetables had the strongest relationship (21). Six of eight measured cognitive functions, including measures of global cognition, verbal learning and fluency, recall, processing speed, and perceptual speed, were significantly correlated with MPOD (22). On the other hand, in this study the relationship with serum lutein and zeaxanthin was not as consistent to the cognitive measures, and serum levels only related to verbal fluency (22). Another study demonstrated that lower MPOD values were significantly associated with worse performance on tests of global cognitive function, processing speed, prospective memory, and executive function (23). In the Georgia Centenarian Study postmortem brain samples that contained higher concentrations of lutein and zeaxanthin were associated with better cognitive function at the time of death (20). Finally, lutein supplementation has been shown to improve verbal fluency among older women (24). Given this accumulating evidence for lutein’s impact on cognitive function of the elderly, and since lutein is found at higher relative concentrations in the infant brain than the elderly brain, it is a natural extension to ask the scientific question of whether the relationship between MPOD and cognitive abilities exists during childhood.

Performance on standardized academic achievement tests have been demonstrated to have reliable relationships with many facets of life, including academic and job performance (25). Since the implementation of the No Child Left Behind (NCLB) Act of 2001, schools have
been under increasing pressure by federal law to deliver on academic milestones, prompting many schools to alter nutrition during the days before standardized testing to boost short-term performance (26). Thus, with the external pressure on schools to provide students with a basic academic skill set and the known relationships between current academic performance and future success in life, it is crucial to provide evidence-based dietary guidance to support children’s abilities for long-term scholastic success. Although a growing body of literature supports the role that overall diet quality and breakfast consumption have on improved academic performance (27, 28), the influence of habitual intake of specific nutrients on academic success remains largely unknown.

MPOD is a tool for examining the habitual intake of lutein and zeaxanthin as it is more of a stable measure of intake than serum or blood, and it allows for a better representation for long-term intake of these nutrients (22). Accordingly, the major aim of this thesis was to determine whether MPOD was associated with academic performance. Secondary aims were to: (1) determine if dietary intake of lutein and zeaxanthin are related to MPOD measures, and (2) assess whether the relationship between MPOD and academics is mediated by dietary intake of lutein and zeaxanthin. Due to MPOD having significant positive associations with cognition in adults, and infants having higher brain levels of lutein as percent of carotenoids than the elderly, we hypothesized that higher MPOD would be associated with superior performance on standardized academic achievement tests among a sample of preadolescent children (8-9-year-olds). Further, we hypothesized that dietary measures would relate to MPOD, as they have in some previous adult studies (29), and that dietary intake would partially mediate the relationship between MPOD and academics, as intake of dietary lutein and zeaxanthin has been shown to contribute to MPOD in most of the population (30). The findings from these objectives are
important because they may shine light on whether current dietary intake, or potential previous intakes and early life factors, impact macular pigment in preadolescence. Additionally, these objectives contribute to how these factors impact performance on academic measures. To date there is no Daily Reference Intake (DRI) for lutein and/or zeaxanthin. Thus, the more studies that are completed showing the significance of these xanthophylls, the closer the support will be for completing the DRI process.
CHAPTER 2 – REVIEW OF THE LITERATURE

Given the available literature for lutein and zeaxanthin, diet relationships with cognition in children, and macular pigment optical density (MPOD) relationships to diet and cognition in adults, it is necessary to review the literature-base to provide a foundation for the present thesis. Specifically, this review will provide an overview of lutein and zeaxanthin. Next, the literature investigating dietary factors and their relationships with cognition in children will be reviewed to demonstrate why a noninvasive technique to measure MPOD in children would be advantageous. Then, an overview of MPOD history, the relationship between MPOD to diet, and finally, MPOD associations with cognition in adults will be discussed.

**Lutein and Zeaxanthin**

*Structure and Sources*

Carotenoids are richly colored molecules, and they are responsible for the yellow, orange, and red colors found in many plants (31). There are more than 700 carotenoids found in nature (3). Among these, only two accumulate in the macular pigment, and thus this review will only focus on those two pigments, lutein and zeaxanthin (32). Lutein and zeaxanthin are structural isomers. They have the chemical formula C₄₀H₅₆O₂. When examining their structures, lutein and zeaxanthin are the dihydroxy derivatives of α-carotene and β-carotene, respectively (33). While both α-carotene and β-carotene act as provitamin A carotenoids, lutein and zeaxanthin do not despite their structural similarities (34). All carotenoids are hydrocarbons, and they are divided into two subgroups: carotenes, which are oxygen free, and xanthophylls that are oxygenated. Both lutein and zeaxanthin are xanthophylls (35). Due to the presence of their hydroxy groups xanthophylls are more polar than carotenes (33). As a result of lutein and zeaxanthin possessing chiral centers, they can exist in many stereoisomeric forms. The *trans* form of xanthophylls
dominants in all foods over the \textit{cis} form, however when examining processed foods a higher level of \textit{cis} can be found than when examining fruits and vegetables, which may be due to isomerization during food processing (36). Isomerization is most impacted by thermal processing, however exposure to light and oxygen and even organic acid release through slicing and juicing can cause isomerization to occur (37). Within plants only one major stereoisomer of lutein, and one of zeaxanthin, is found due to stereospecific biosynthesis (38). Whether the carotenoid exists in the \textit{cis} versus the \textit{trans} form may impact its bioavailability in humans, however little research has investigated this for lutein and zeaxanthin.

As the macular pigment is entirely from dietary origin (39) it is important to establish which foods provide a good source of the nutrients, lutein and zeaxanthin, that accumulate here. Lutein and zeaxanthin come into the diet from plant material by direct means, or indirectly in the form of metabolites in animal products such as eggs. These carotenoids are concentrated in green leafy vegetables, many colored fruits and vegetables, and eggs (6, 40). Even though eggs have lower levels of lutein and zeaxanthin present, as shown in Table 1, the nutrients are more bioavailable in this form than from spinach or supplements (40). Table 1, showing lutein and zeaxanthin content in foods, was generated using the USDA National Nutrient Database for Standard Reference Release 28 (41). Most databases combine lutein and zeaxanthin, thus in Table 1 the third column displays their sum. Additionally, even though the database gave one number per food type, carotenoids need to be represented as a range of values as the carotenoid content of foods is highly variable. It can be impacted by many factors such as genotype, season, geography, cultivation variation, stage of maturity at harvest, and postharvest storage conditions (37).
<table>
<thead>
<tr>
<th>Food</th>
<th>Serving</th>
<th>Lutein + Zeaxanthin (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale, frozen, cooked</td>
<td>1 cup</td>
<td>25.6</td>
</tr>
<tr>
<td>Spinach, canned</td>
<td>1 cup</td>
<td>22.6</td>
</tr>
<tr>
<td>Turnip greens, frozen, cooked</td>
<td>1 cup</td>
<td>19.5</td>
</tr>
<tr>
<td>Chard, swiss, cooked</td>
<td>1 cup</td>
<td>19.3</td>
</tr>
<tr>
<td>Collards, frozen, cooked</td>
<td>1 cup</td>
<td>18.5</td>
</tr>
<tr>
<td>Mustard greens, cooked</td>
<td>1 cup</td>
<td>14.6</td>
</tr>
<tr>
<td>Dandelion greens, raw</td>
<td>1 cup</td>
<td>7.5</td>
</tr>
<tr>
<td>Peas and carrots, frozen, cooked</td>
<td>10 oz</td>
<td>4.3</td>
</tr>
<tr>
<td>Zucchini, frozen, cooked</td>
<td>1 cup</td>
<td>4.2</td>
</tr>
<tr>
<td>Eggs, whole</td>
<td>1 cup, sifted</td>
<td>0.7</td>
</tr>
</tbody>
</table>

1Generated using the USDA National Nutrient Database for Standard Reference Release 28 (41)

Lutein is dominant over zeaxanthin in almost all foods, and ratios of 7:1 to 4:1 have been reported (33, 42). Indeed, lutein has been found to be the predominate xanthophyll in almost all fruits and vegetables (36). Exceptions to lutein being the predominate xanthophyll are corn, some corn products, orange peppers, nectarines, and some varieties of potatoes where zeaxanthin is found in higher amounts than lutein (36, 43, 44). Daily mean intakes of combined lutein and zeaxanthin vary with age, sex and ethnicity between 0.4-4 mg per day depending on the population observed (45-49). Additionally, within a population the individual daily intakes vary greatly as demonstrated by high standard deviations reaching up to 2.45 mg/day in one study (47). While there are currently no DRIs for lutein and zeaxanthin, the recommended daily intakes based on food intake for a potential health benefit to be observed is 6mg (50).
Absorption and Accumulation

To start the digestion process the carotenoid must be released from the food matrix and incorporated into a mixed micelle, comprised of dietary lipids and bile acids (51). This incorporation facilitates absorption into the intestinal mucosal cells. The process of assimilating into the mucosal cell is still being investigated, however, there is evidence of passive diffusion (52) and facilitated uptake via class B scavenger receptors on the membrane brush border such as scavenger receptor class B type 1 (SR-B1) (53) and cluster of differentiation 36 (CD36) (54). Next, within the intestinal mucosa carotenoids are incorporated into chylomicrons, which are triglyceride-rich lipoproteins that allow them to be released into the lymphatic system (51). As the chylomicrons are in circulation, lipoprotein lipase acts on them hydrolyzing them into chylomicron remnants (CRs) and apolipoprotein E (apoE) is acquired. The CRs must achieve a size small enough to enter the space of Disse in the liver where absorption occurs (55).

Additionally, apoE on the surface of the CR allows for the binding of the CR to hepatic low-density lipoprotein (LDL) receptors and the subsequent endocytosis of the remnant particle into hepatocytes (56). Additional mechanisms for entry of CR into hepatocytes include acquiring additional apoE that is secreted free into the space, and then removal directly by the LDL receptor-related protein (LRP), or the CR may be sequestered in the space by either the binding of apoE to heparan sulfate proteoglycans and/or binding of apoB to hepatic lipase. The sequestered particles are then able to be furthered metabolized resulting in apoE enrichment that allows for the transfer of the particle to either an LDL or LRP receptor for hepatic uptake (56). Within the liver carotenoids can be accumulated or repackaged and released as components of very low density lipoprotein (VLDL). As time progresses the polar xanthophylls, lutein and zeaxanthin, become more equally distributed between LDL and HDL (57).
The structure of carotenoids has some impact on their bioavailability. Due to their more polar nature, oxygenated carotenoids, the xanthophylls, have increased absorption over other hydrocarbon carotenoids. This polarity allows them to be incorporated into the outer portions of lipid micelles within the gastrointestinal tract, thus allowing for an easier uptake by the enterocyte membranes and ultimately chylomicrons, thus increasing their bioavailability (51). Indeed, the absorption of lutein was found to be five times higher than β-carotene (58).

In order for dietary carotenoids to be absorbed in the intestines they must be co-consumed with a fat source. The amount of fat needed to aid in the absorption seems to be low at about 3-5 g per meal, but depends on the physicochemical characteristic of the carotenoid ingested, for example from a food matrix versus a presolubilized/emulsified supplement (37, 59). Additionally, from examining multiple studies it appears that higher levels of co-consumed lipids seem to enhance carotenoid absorption (37).

The actual uptake of lutein and zeaxanthin after ingestion is impacted by many other dietary factors besides just the fat amount in the meal. In addition to the amount of fat, the source of fat can impact bioavailability. Certain chain lengths and degrees of saturation of fatty acids have been shown to be more effective at stimulating chylomicron secretion than others (60). In one study, dietary fats rich in saturated fatty acids led to a higher bioavailability of lutein and zeaxanthin than did dietary fats high in monounsaturated fatty acids or polyunsaturated fatty acids (61). The food matrix is a major determinant of carotenoid bioavailability. Processing of carotenoids, via mechanical homogenization or heat treatment, tends to increase their bioavailability by potentially disrupting the cellular structure and releasing the carotenoids from the food matrix (62). Dietary fiber may inhibit carotenoid utilization. One study found a decrease of 40-74% in absorption of lutein depending on the type of fiber consumed (63). It has been
suggested that the intracellular location, chloroplasts in leaves versus chromoplasts in fruits and other parts of the plant, may result in different bioavailability of carotenoids with the former speculated to be harder to disrupt the food matrix (64, 65). Interactions between carotenoids may impact their absorption due to potential competition to be incorporated into the micelle or exchanging the compounds between lipoproteins (66). It has been demonstrated that when β-carotene and lutein were ingested together the plasma levels of each were reduced compared to when they were ingested separately (67).

In addition to other dietary components consumed with carotenoids, personal characteristics may additionally impact absorption. Gastrointestinal malabsorption is a major issue that will severely limit carotenoid absorption, this could include intestinal parasites, steatorrhea, or fat malabsorption syndromes (68). Additionally, there are multiple genetic variations in genes that are involved in lipoprotein metabolism and lipid transfer that can affect the variability of plasma carotenoid concentrations in humans (69).

**Xanthophylls and the Macula**

When examining where carotenoids accumulate in the body, it has been established that the highest concentration of xanthophylls is in the retina, comprised of lutein and zeaxanthin to the exclusion of all other dietary carotenoids. Despite lutein and zeaxanthin being accumulated throughout the eye tissue, they are only optically dense within and around the fovea (70). Lutein is the major carotenoid in the peripheral retina, whereas zeaxanthin becomes more and more dominant approaching the foveal center (47). *Meso*-zeaxanthin levels decrease with increasing radial distance from the fovea (71). The concentration of lutein and zeaxanthin in the retina is about 10,000-fold higher than in the blood (72). This concentration level has led to the concept of a specific binding protein for these xanthophylls (73). Relatively recent research has revealed
a SR-B1 dependent mechanism for uptake of xanthophylls, opposed to β-carotene, by the cells of
the retina (74). Additional research has revealed other xanthophyll-binding proteins for the
transfer of lutein and zeaxanthin from the blood to the retina (75) including the Pi isoform of
 glutathione S-transferase (GSTP1) that has a high affinity for zeaxanthin (73) and a member of a
protein family collectively referred to as steroidogenic acute regulatory protein (StARD) as a
lutein binding protein (76). Tubulin has been identified as a less specific, but higher capacity
binding protein likely depositing carotenoids after specific uptake by other more specific binding
proteins (77).

In addition to the retina, lutein and zeaxanthin accumulate at high amounts in other
tissues. Another location where a large amount of carotenoids accumulate is in adipose tissue.
Indeed, having excess fat mass may interfere with the ability of lutein to accumulate in the eye as
the adipose tissue can serve as a ‘sink’ for lutein accumulation (78). Within the adipose tissue the
accumulation of carotenoids is site specific, and higher concentrations are found in the abdomen
than in the buttock or thighs (79). CD36 has been reported to be involved in the uptake of lutein
by the adipocytes and adipose tissue (80). The liver also has a large capacity for carotenoid
accumulation; as described above the chylomicron remnant particles are taken up via endocytosis
into hepatocytes and can remain there (56). Carotenoids accumulate at high levels in the skin.
Measurements using resonance Raman spectroscopy have revealed a non-uniform distribution in
the epidermis, with more accumulating where higher levels of sweat glands are present, as sweat
carries carotenoids to the skin surface (81).

**Xanthophylls and the Brain**

Finally, and perhaps of most importance to this current thesis, is the accumulation of
lutein in the brain, where it is preferentially accumulated over all of the other dietary carotenoids
In infants, lutein in the brain accounts for 59% of the total carotenoids despite it only constituting 12% of intake (19). Within the brain cortices of infants, lutein was found in significantly higher concentrations in the occipital cortex, hippocampus, frontal cortex, and prefrontal cortex, and was marginally higher in the auditory cortex (19). In the first study to investigate individual carotenoids and their contribution to the adult brain, the total concentration of carotenes was significantly less than the total concentration of xanthophyll carotenoids, which constituted 66-77% of the total carotenoids in both the frontal and occipital regions (82). Additionally, the total concentration of xanthophylls was significantly higher than carotenes in both the gray matter and in the white matter (82). In a subsequent adult study, xanthophylls accounted for 72% of the total carotenoids, of which lutein was found to account for 34% of carotenoids accumulated in the brain (20). Lutein in the adult brains was significantly higher than the other carotenoids even though β-carotene was the highest in the plasma (20).

MPOD may potentially be used as a biomarker to assess brain lutein and zeaxanthin status. In non-human primates, the only animals that possess a macula, lutein in the macula was positively related with lutein levels in the cerebellum, occipital cortex, and pons, while the relationship was marginally significant in the frontal cortex (18). Whereas, zeaxanthin in the macula was positively related to zeaxanthin levels in the cerebellum, frontal cortex, and pons and marginally significant for the occipital cortex. However, the relationships for zeaxanthin did not remain after adjustment for age, sex, and n-3 status, but the lutein relationships did (18). In humans, macular carotenoids in the retina were significantly related to their levels in the occipital cortex, but not in the hippocampus, and this relationship remained even after adjustment for age, sex, and cognitive status (83). The absence of the relationship in the hippocampus may be due to the small sample size (N=13) or potential pathological changes and...
damage, as 7 of the 13 subjects had Alzheimer’s disease prior to death and the hippocampus is the first brain region affected early on in the pathogenesis of the disease. Additionally, this study was the first to demonstrate that meso-zeaxanthin was absent in the human brain tissue analyzed (83). The associations of lutein and zeaxanthin in the macular region and brain seem plausible given that the retina is part of the central nervous system.

**Functions**

Within the retina there are two types of photoreceptors, rods and cones. Rods are responsible for vision at low light levels, do not mediate color vision and have a low spatial acuity, while cones are active at higher light levels, are capable of color vision and are responsible for high spatial acuity. Despite the small size of the macula within the retina, it constitutes a large proportion of the projection onto the visual cortex, and joined with the very high density of cone photoreceptors, this area of the retina has the highest visual acuity (84). Therefore, it is not surprising the amount of research devoted to this region of the retina. The macular pigment is mostly accumulated in the inner Henle fiber layer (85). This layer is composed of the photoreceptor axons that overlay the photoreceptors. Having this location allows the macular pigments to filter out blue light before reaching the delicate structures such as the photoreceptors, the retinal pigment epithelium and the underlying choriocapillaris (7). In individuals with the average macular pigment levels, 20 to 40% of light at 460nm can be absorbed, whereas in those with higher than normal macular pigment levels up to 90% of light may be absorbed (47).

Blue light is of a short wavelength, and these short wavelength lights have been shown to be particularly damaging to the retina (86). Lutein and zeaxanthin have been well established as blue light filters. Lutein and zeaxanthin have fully conjugated double bonds along their
backbones, whereas their rings are only partially in conjugation (33). The pattern of conjugation is important as it determines the light-absorbing properties of the carotenoid, and additionally it can influence the antioxidant activity of carotenoids (33). Lutein has an absorption maximum of 445nm and zeaxanthin’s is 451nm, and as a result both carotenoids are efficiently absorbers of blue light (9, 87). Due to this high absorption, lutein and zeaxanthin are very effective at filtering the blue light prior to exposing delicate underlying tissues (9). It has been demonstrated that damage from acute exposure to blue light can be protected by the macular pigments (88). However, this acute exposure protection cannot yet be extrapolated to prove a protective role in low dose exposure to blue light.

Chromatic aberration occurs when light is not properly focused at the same point, which results in overlapping images and is commonly described as the occurrence of colored fringes and a loss of image sharpness. The presence of lutein and zeaxanthin, the yellow pigments in the macula, has been suggested to reduce the amount of blue fringes by absorbing some of the blue light (89, 90). However, it has not been well established the extent to which chromatic aberration may be limiting the acuity of the human eye.

A paper in 1920 discussed the possibility that the macular pigment could improve vision by improving contrast relations in the atmosphere (91). This idea was further developed by Wooten et al. (90) as a major new hypothesis, the visibility hypothesis, regarding how the macular pigment may improve visibility outdoors. This potential source of optical degradation is commonly overlooked when examining visual acuity and is sometimes referred to as blue haze. This blue haze is caused by small, suspended particles in the earth’s atmosphere that scatter short wavelength light more than other wavelengths resulting in a bluish veiling luminance. This haze can majorly impact how well and how far we can see targets outdoors. The macular pigment has
the ability to help improve the visual distortions caused by *blue haze* by absorbing the short wavelengths produced and allowing for increased contrast (90).

In addition to filtering out blue light, carotenoids have antioxidant properties. Lutein and zeaxanthin are effective at quenching reactive oxygen species (ROS). This has been demonstrated as oxidized byproducts of lutein and zeaxanthin have been identified within the human retina, which is indicative of a function as retinal antioxidants (11). Among ROS, carotenoids are the most effective at scavenging peroxyl radicals, which are generated during lipid peroxidation (33). Due to their lipophilic nature and scavenging peroxyl radicals, carotenoids are proposed to play an important role in protecting cellular membranes from oxidative damage (92). In addition to peroxyl radicals, carotenoids are efficient at physically quenching singlet oxygen (93), and this physically quenching by direct energy transfer allows for them to remain intact and be reused many times in quenching cycles (33, 93). Additionally, higher concentrations of zeaxanthin resulted in lower singlet oxygen quenching, suggesting an optimal concentration for carotenoids, specifically zeaxanthin, and that extra is not necessary beneficial (93). This concept was again demonstrated when skin fibroblasts were exposed to UVB light and an optimum concentration of lutein was found for protection. When concentration levels of lutein were below optimum less protection was found, whereas at higher levels prooxidant effects were observed (94).

Lutein and zeaxanthin have been shown to be more stable under photo-oxidative conditions than lycopene and β-carotene; when exposed to UV light in the presence of Rose Bengal (a singlet oxygen generator), when in the presence of a peroxyl radical initiator, when ‘bleached’ with hypochloric acid (NaOCL), and when exposed to natural sunlight, lutein and zeaxanthin were more resistant to degradation than lycopene and β-carotene (10). This slower
degradation under photo-oxidative states may be indicative of why the macular carotenoids accumulate in the retina over lycopene and β-carotene.

Due to their physicochemical properties, it is likely that the main task of carotenoids in the macula is to prevent damage from photo-oxidation. The functions described above point to the accomplishment of this in two main ways, blue light filtration and their antioxidant properties. Indeed, UV light exposure has been related to cataract formation and retinal degeneration (95). Additionally, there is increasing evidence that greater intake of the macular carotenoids, lutein and zeaxanthin, is inversely related with risk for age related macular degeneration (50, 96), which is the leading cause of blindness in developed countries (97).

Lastly, of most relevance to this thesis, is the potential contributions lutein and zeaxanthin may make to cognitive function. Many of the benefits observed in the eye may translate to the brain. Specifically, it is currently believed that carotenoids mediate cognitive function by mitigation of neuroinflammation (98). High plasma total carotenoid concentrations have been associated with reduced plasma interleukin-6 (IL-6) levels; IL-6 is an interleukin that can act as a pro-inflammatory cytokine or can have positive modulatory effects in the central nervous system likely dependent on its level of expression (98). Further, lutein may inhibit cytokine production via suppressing ROS stimulated nuclear factor kappa beta (NF-κβ) activation (99).

Lutein’s antioxidant properties are of importance in the brain as it is especially vulnerable to free radical damage due to its relatively low antioxidant content, high polyunsaturated fatty acid (PUFA) concentration, and high metabolic activity (100). Macular xanthophylls, lutein and zeaxanthin, were found to be about 14 times more concentrated in domains where docosahexaenoic acid (DHA), a polyunsaturated fatty acid, is present versus
domains where cholesterol and saturated lipids are present (101). This preferential location in domains formed from unsaturated lipids is ideal if they are to act as a lipid antioxidant. Also it is possible that these antioxidant and anti-inflammatory properties are particularly beneficial during childhood, since this period is characterized by extensive changes in both structure and function of the brain (102, 103). Furthermore, there may be a neuroprotective role of lutein in early neural development as the relative contribution of lutein to the total carotenoids in infant brains is almost two-fold greater than in adults, 59% versus 34% (19, 20).

Lutein and zeaxanthin have polar groups at each end that allow them to span membranes in lipid bilayers, while their non-polar counterparts, carotenes, are oriented rather randomly. This spanning configuration allows a nearly perpendicular orientation, enhancing their stability in membranes (104). Combining this property with their high solubility in membranes can strongly influence other membrane properties, such as fluidity, ion exchange, and oxygen diffusion (104), and may be contributing to their enhancement of interneuronal communication through gap junctions (105).

Future studies should investigate specific brain regions and membrane types in which lutein and zeaxanthin accumulate to better understand its functions as membrane composition varies among cell types and cellular compartments (106). As elaborated upon in a recent review by Erdman et al. (107), mitochondrial, nuclear, myelin, and neuronal plasma membranes have unique functions, many of which can determine cell viability. Determining which membrane types lutein and zeaxanthin accumulate in may give clues to its potential functions. As stated in the review, if found in neuronal plasma membranes it may influence cell survival signal transduction, in the mitochondria it may protect this organelle from damage, in the nucleus it may help with gene regulation and DNA damage that affect cell viability, and in myelin it may
be functioning to influence structural integrity and maintain proper communication between the neurons (107).

**Dietary Factors and their Relationships with Cognition in Children**

*Overall diet quality*

To date, most studies investigating nutrition and academic achievement have focused on hunger, malnutrition, micronutrient deficiency, and breakfast consumption (108). Few studies have focused on well-nourished children and the impact of nutrition on their academic achievement. One study did investigate overall diet quality and academic achievement in 5th graders from Nova Scotia, Canada (N=5,200), and found overall diet quality to be important to academic achievement, above and beyond socioeconomic factors (27). They utilized the Harvard Youth/Adolescent Food Frequency Questionnaire (YAQ) and calculated the Diet Quality Index-International (DQI-I) and the Healthy Eating Index (HEI) for composite measures of diet quality (109, 110). However, their academic measures were lackluster, as they used a dichotomous variable to measure academic achievement based on whether the children passed or failed one of two tests, a reading and writing assessment. Failing either test, or both tests, resulted in being rated as poor academic performance, while passing both was considered good academic performance (27). Therefore studies with better measures of academic achievement need to be implanted to expand upon their findings.

*Fruit and Vegetable Intake*

Within overall diet quality a Canadian study found that students with an increased intake of fruits and vegetables, known to be good sources of carotenoids, was positively associated with academic performance (27). This study did not account for health markers that may improve cognitive function, such as weight status and fitness, (111) while this thesis does account for
these markers. Additional studies that have accounted for health makers have shown that intake of dietary fibers, nutrients abundantly found in fruits and vegetables, is positively associated with cognitive control (112). An alternative explanation may be that high dietary fiber is an index of healthy eating, and therefore these results may reflect a more general benefit of diet quality on cognition.

**MPOD**

**History**

The first description of the macular yellow spot was in 1782 (113), and this was followed by the first literature review in 1798 (114). Macular pigment was originally described to contain xanthophylls, possibly lutein, by Wald in 1945 (8). Wald showed that these xanthophylls were concentrated within the macula. Additionally, Wald proved that this yellow pigment absorbed wavelengths between 430 and 490nm with a maximum absorption at 465nm. It was not until 1985 that the pigments in the eye were further identified as lutein and zeaxanthin (32). Later in 1993 an additional carotenoid was identified in the eye as meso-zeaxanthin (4). No significant amount of meso-zeaxanthin has been found in the diet, and it is not detectable in significant amounts in other human tissues or blood (4, 115); it has been proposed that this isomer is formed in the ocular tissue due to conversion of lutein (115).

**MPOD relation to Diet**

While the relationship between dietary intake and MPOD has been shown to be significant and positive in three studies, this result is not consistent within the literature (29). Lutein and zeaxanthin, the dietary carotenoids composing the macular pigment, can be measured by food frequency questionnaires, food records, and food recalls, but studies with these measures need to be interpreted with caution for a several reasons. These studies may introduce subject
recall bias, there could be possible digestive and absorptive idiosyncrasies among subjects, and using different sources of carotenoid data by investigators may introduce inconsistencies between studies (29). Additionally, subjects vary greatly in their ability to accumulate dietary lutein and zeaxanthin in the macula as macular pigment (30). Thus, it is of importance to be able to make reliable measures in neural tissues, if an aim of a study will examine cognition.

Heterochromatic flicker photometry (HFP) has been demonstrated to be a reliable non-invasive measure of retinal lutein, a surrogate measure of brain concentrations of lutein (18), in both adults and children (116, 117). Thus, the use of the moderately reliable technique of heterochromatic flicker photometry in preadolescent children (117) is utilized in this current thesis to mitigate the potential issues introduced by dietary recall by providing an objective measure of MPOD.

**MPOD relation to Cognition**

Both MPOD and dietary lutein supplementation have been associated with better cognitive function among older adults (22-24). Within the study by Vishwanathan et al., six of the eight measured cognitive functions in healthy older adults with normal cognitive function were significantly correlated with MPOD measured via HFP. These included measures of global cognition, verbal learning and fluency, recall, processing speed, and perceptual speed after adjusting for age, BMI, education and sex (22). Another study again showed that lower MPOD measured via HFP was significantly associated with worse performance on tests of global cognitive function and processing speed; additionally lower MPOD was associated with worse executive function and prospective memory (23). In the Georgia Centenarian Study brain samples collected at postmortem that contained higher concentrations of lutein and zeaxanthin...
were associated with better cognitive function at the time of death (20). Finally, lutein supplementation has been shown to improve verbal fluency among older women (24).

To date, MPOD assessments are limited in preadolescents, and since lutein is the predominant carotenoid in the brain starting in early life (19), this is of concern. The scarcity of MPOD data in children has resulted in limited knowledge of the importance of dietary or brain lutein for optimal cognitive function and brain development (118, 119). Accordingly, the present thesis seeks to utilize HFP to measure MPOD and investigate how it relates to academic achievement in preadolescents.

**Hypotheses**

Due to the preferential accumulation of lutein in the brain over other carotenoids, and the beneficial functions of lutein and zeaxanthin, it was hypothesized that higher MPOD would be associated with superior performance on standardized academic achievement tests among a sample of preadolescent children. Dietary intake of lutein and zeaxanthin and MPOD measures were hypothesized to be positively correlated. Furthermore, for the subsample with diet data, it was hypothesized that the relationship of MPOD to academic performance would be mediated, in part, by diet.
Chapter 3 – METHODOLOGY

The present thesis applies the moderately reliable approach of measuring MPOD in preadolescent children by use of heterochromatic flicker photometry (117) and relate it to cognitive outcomes. Additionally, this thesis investigates how dietary intake of lutein and zeaxanthin, measured via 3-day food records, relates to MPOD in children and how diet may mediate the relationships of MPOD to academic measures. Accordingly, the research design and methods are described below.

Participants

Preadolescent children between the ages of 8 and 10 years from the East-Central Illinois community were recruited to participate in this study. Participants were excluded due to the presence of neurological disorders, physical disabilities, and psychoactive medication status. All participants had normal or corrected-to-normal vision. All participants provided written assent and their legal guardians provided written informed consent in accordance with the ethical standards and regulations of the Institutional Review Board (IRB) of the University of Illinois at Urbana-Champaign (Institutional Review Board number 12321).

This study utilized children from 2 different waves of the FITKids randomized controlled trial, an on-going physical activity intervention trail. All children (n = 49) from the 2015-2016 FITKids enrollment were included at their baseline measurement, prior to any intervention. Seven children from the 2014-2015 FITKids enrollment were included in the analysis at post intervention. These 7 children had the same examiners as the 2015-2016 FITKids, thus reducing variability stemming from the use of more than two examiners (117).
Procedure

All testing protocols were identical at baseline and post-intervention testing, thus including children from both time points should not be a confounding variable. Testing occurred across two separate days. On the first visit to the laboratory participants completed informed assent/consent, the Woodcock Johnson Tests of Cognitive Abilities to estimate intelligent quotient (IQ) (120), the Kaufman Test of Academic and Educational Achievement II (KTEA II) to assess scholastic achievement (121), had their height and weight measured, and completed a maximal oxygen consumption test (VO2max) to assess aerobic fitness (122). All cognitive testing took place prior to the cardiorespiratory fitness assessment to avoid any confounding effects of acute physical activity on cognitive performance (123). Concurrently, their legal guardian completed a preliminary screening, demographic and health history questionnaire, and pubertal timing scale (on behalf of their child) via the Tanner Staging Scales (124). From the information the parents provided, socioeconomic status (SES) was determined by creating a trichotomous index based on participation in a school meal-assistance program, maternal and paternal education levels, and the number of parents with full time employment. Before leaving the appointment participants were given food records to complete at home for three days, 2 week days and 1 weekend day, before their return to the lab. On the second visit, participants were fitted with an EEG cap and completed cognitive testing in a quiet chamber, and they completed an assessment of body composition. At both visits participants completed the MPOD assessment, and the average of the two values was used throughout this thesis.

MPOD

MPOD was measured using customized HFP (cHFP) and a macular densitometer (Macular Metrics Corporation, Rehoboth, MA USA) that was identical to a version described by
Wooten et al. (125) except that it did not allow for an assessment of the entire spatial profile. This procedure has been described previously (126). However, this study utilized a slightly varied form of the procedure typically described in adult studies. Unimpaired adults receive instruction from a trained examiner, and then they manipulate the radiance of the short-wave component of the test stimulus themselves (method of adjustment) to produce a null flicker zone.

In this study, the psychophysical technique was modified as described previously by Renzi et al. (127) for older adults with mild cognitive impairment. Briefly, instead of manipulating the radiance of the short-wave component themselves to find thresholds, the examiner manipulated the radiance of the short-wave component of the test stimulus while using simplified instructions also known as the method of limits. After the null zone was found via the method of limits, the method of constant stimuli was used to further narrow the range of the null zone.

The MPOD assessment started by determining a critical flicker frequency, which facilitates subject performance and reduces measurement error (126). An algorithm was established for each subsequent test to help the examiner know where to start the flicker frequency (126). Next, the participant views a disk that alternates between a blue wavelength (that is absorbed by the MP) and a green wavelength (which is not observed by the MP). This is done at two locations in the eye. First at a central (foveal) location, and then at a peripheral (parafoveal) location (7° eccentricity). The participant informs the examiner when they perceive the blinking to slow down to a stop, and thus a null flicker zone has been identified. If for either the foveal or parafoveal measurements the participant is unable to see a null flicker zone, or their null flicker zone was large, then the examiner adjusted the flicker frequency accordingly.

At both the foveal and parafoveal points the radiance of the blue light is adjusted by the examiner until a null flicker is reached. The difference between the required radiance at the
foveal and parafoveal points reflects the participants MPOD (23). This is due to the MP being at its highest density at the foveal, while at the parafoveal the MP is negligible (85). The calculation of MPOD is as follows: 

$$MPOD = -\log_{10} \left( \frac{R_f}{R_p} \right)$$

where $R_f$ is the radiance of the blue light needed for flicker null at the foveal location and $R_p$ is the radiance for a flicker null at the reference location in the parafoveal.

**Body Composition Assessment**

Participants height and weight were measured in stocking feet using a stadiometer and a Tanita WB-300 Plus digital scale (Tanita, Tokoyo, Japan), respectively. The mean of three measurements of height and weight were used for analyses. BMI was calculated by dividing body mass (kg) by height (m) squared ($((kg)/ht(m))^2$). Next, fat and muscle mass was measured using dual-energy X-ray absorptiometry (DXA) with a Hologic Discovery A bone densitometer (software version 12.7.3; Hologic, Bedford, MA).

**Academic Achievement Assessment**

Participants were administered the Kaufman Test of Academic and Educational Achievement II (KTEA II) (121). The comprehensive form was administered to determine a comprehensive achievement score. Academic outcomes included composite scores on math (math concepts and application and math computation subtests), reading (letter and word recognition and reading comprehension subtests), reading fluency (word recognition fluency and decoding fluency subtests), written language (written expression and spelling subtests), and the comprehensive achievement scores (reading composite, math composite, written expression subtest, and listening comprehension subtest). All scores reported herein are standard scores generated by using the age norms standard scores produced by KTEA II comprehensive norms (128).
The math concepts and application subtest was an 88-item subtest, which began with easier items that included basic math concepts such as comparing numbers and rounding numbers, and progressed to more difficult problems that required algebra, calculus, and trigonometry. The math computation subtest consisted of 72 items and asked participants to add, subtract, multiply, and divide whole numbers and fractions. Problems progressed in difficulty by involving exponents, decimals, negatives, and unknown variables. Participants had access to pencil and paper but were not allowed to use a calculator for the math subtests. The letter and word recognition subtest had participants pronounce words of gradually increasing difficulty. The reading comprehension subtest began with the participant reading a word and pointing to its corresponding picture. It progressed in difficulty by having the student perform the action of the word, and then answer literal or inferential questions about passages they read. For the word recognition fluency subtest the participant read isolated words as quickly as possible for one minute, and in the decoding fluency subtest they pronounced as many nonsense words as possible in one minute. For the written expression subtest the participant completed writing tasks in the context of an age-appropriate storybook format. In the spelling subtest the participant wrote words the examiner dictated from a steeply graded word list. In the listening comprehension subtest the participant listened to passages played from a CD and then orally responded to questions asked by the examiner (128).

**Diet assessment**

Three-day food records, with 2 week days and 1 weekend day, were used to determine dietary intake of lutein and zeaxanthin. The records were completed by the child with assistance from the parent. Both child and parent received instructions on how to correctly fill out the food records. Additionally, the records contained written instructions for recording food intake,
including portion size examples, how to describe food preparation methods, added fats, brand names, and ingredients of mixed dishes and recipes. The three days of intake were entered into Nutrition Data System for Research (NDSR 2014; Nutrition Coordinating Center, Minneapolis, MN, USA) software by trained staff. To investigate nutrient-level intakes the intake properties file from NDSR was utilized. The three days of intake were averaged together, and subsequently, these averages are used in the data analyses.

**Statistical Analysis**

A bivariate correlation was performed between dietary intake of lutein and zeaxanthin and MPOD. Next, bivariate correlations between MPOD, and lutein and zeaxanthin, with the academic composite scores were calculated.

Following correlational analyses, the relationship between MPOD and academic performance was examined using multiple hierarchical linear regression analyses. First, confounding demographic and health variables determined via the bivariate correlations were included in step 1 of the final regression model predicting academic performance. Additionally, variables of a higher accuracy in their measurement of interest were chosen to be included in the modeling at step 1. For example, if both BMI and whole body percent fat correlated with the academic measures, then whole body percent fat would be included because it a direct measure of fat mass. Further, in instances where factors known to be related to sex, such as whole body percent fat or VO₂max, are significantly related but sex is not, then independent t-tests were performed to determine whether these measures varied between sexes. If cases where a difference was observed between sexes in the independent t-test, then sex was entered into step 1 of the model. Following adjustment of step 1 variables, MPOD outcomes were included in step 2 of the regression model. The change in variance of performance explained by MPOD on each
academic achievement variable in step 2 was examined. For the subsample regressions, step 1 remained the same as in the full sample, then in step 2 dietary intake of lutein and zeaxanthin was included, and in step 3 MPOD was added. The change in variance of performance explained by MPOD on each academic achievement variable in step 3 was examined to see how diet may mediate the relationships between MPOD and academics. The $\alpha$ level was set at 0.05 and SPSS 22 was used to perform all statistical analyses.
Chapter 4 – RESULTS

Participant Demographic Information

Table 2 presents participant characteristics, KTEA II academic performance standard scores of participants, MPOD, and dietary intake of lutein and zeaxanthin. To determine the relationship between dietary measure of lutein and zeaxanthin with the psychophysical measure (i.e., MPOD measured via HFP) a correlation between the two was performed. Figure 1 demonstrates this relationship.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full Sample</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>8.8 ± 0.1</td>
<td>8.7 ± 0.2</td>
<td>8.8 ± 0.1</td>
</tr>
<tr>
<td>Sex [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (30)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>39 (70)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IQ</td>
<td>112.8 ± 1.7</td>
<td>115.6 ± 3.1</td>
<td>111.5 ± 2.0</td>
</tr>
<tr>
<td>VO\textsubscript{2} max (mL*kg\textsuperscript{-1}*min\textsuperscript{-1})</td>
<td>43.0 ± 1.1</td>
<td>48.1 ± 2.1</td>
<td>40.8 ± 1.1</td>
</tr>
<tr>
<td>Fat Free Mass VO\textsubscript{2} max (mL*kgFFM\textsuperscript{-1}*min\textsuperscript{-1})</td>
<td>61.9 ± 1.0</td>
<td>64.9 ± 1.9</td>
<td>60.6 ± 1.1</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>18.7 ± 0.4</td>
<td>18.1 ± 1.0</td>
<td>18.9 ± 0.46</td>
</tr>
<tr>
<td>BMI-for-age percentile\textsuperscript{2}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight, BMI percentile &lt; 5 [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Normal weight, BMI percentile ≤5 and &lt;84.9 [n (%)]</td>
<td>32 (57)</td>
<td>12 (71)</td>
<td>20 (51)</td>
</tr>
<tr>
<td>Overweight, BMI percentile ≤85 and &lt;94.9 [n (%)]</td>
<td>14 (25)</td>
<td>3 (18)</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Obese, BMI percentile &gt; 95 [n (%)]</td>
<td>10 (18)</td>
<td>2 (11)</td>
<td>8 (21)</td>
</tr>
<tr>
<td>Whole Body % Fat (%)</td>
<td>31.3 ± 0.9</td>
<td>27.7 ± 1.5</td>
<td>32.8 ± 1.0</td>
</tr>
<tr>
<td>SES [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>22 (39)</td>
<td>5 (29)</td>
<td>17 (44)</td>
</tr>
<tr>
<td>Middle</td>
<td>19 (34)</td>
<td>6 (35)</td>
<td>13 (33)</td>
</tr>
<tr>
<td>High</td>
<td>15 (27)</td>
<td>6 (35)</td>
<td>9 (23)</td>
</tr>
<tr>
<td>Pubertal Timing [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1-2</td>
<td>51 (91)</td>
<td>17 (100)</td>
<td>34 (91)</td>
</tr>
<tr>
<td>Stage 2-3</td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Math Composite</td>
<td>108.3 ± 2.3</td>
<td>112.4 ± 4.4</td>
<td>106.6 ± 2.6</td>
</tr>
<tr>
<td>Reading Composite</td>
<td>111.5 ± 2.1</td>
<td>114.5 ± 4.0</td>
<td>110.2 ± 2.4</td>
</tr>
<tr>
<td>Reading Fluency Composite</td>
<td>111.1 ± 2.1</td>
<td>113.4 ± 3.9</td>
<td>110.2 ± 2.5</td>
</tr>
<tr>
<td>Written Language Composite</td>
<td>106.6 ± 2.5</td>
<td>105.3 ± 4.8</td>
<td>107.6 ± 3.0</td>
</tr>
<tr>
<td>Achievement Composite</td>
<td>109.5 ± 2.2</td>
<td>112.5 ± 4.0</td>
<td>108.1 ± 2.7</td>
</tr>
<tr>
<td>MPOD</td>
<td>0.64 ± 0.03</td>
<td>0.68 ± 0.05</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Lutein/ Zeaxanthin (mcg)\textsuperscript{3}</td>
<td>806.6 ± 63.0</td>
<td>1117.9 ± 177.2</td>
<td>728.7 ± 58.2</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are means ± SEM n = 56. IQ, intelligence quotient; VO\textsubscript{2} max, maximal oxygen uptake; SES, socioeconomic status; MPOD, macular pigment optical density.

\textsuperscript{2}Determined by the 2000 Centers for Disease Control and Prevention BMI-for-age growth charts

\textsuperscript{3}n = 35 (females: n = 28 and males: n = 7); n = 18 did not return food records and n = 3 were cut out due to being outliers
Bivariate correlations

Bivariate correlations between the achievement composite score and the demographic measures were performed. These correlations revealed that IQ ($r = 0.62, P < 0.01$), VO$_2$max ($r = 0.33, P = 0.01$), and fat free mass VO$_2$max ($r = 0.26, P = 0.05$) were positively correlated with the achievement composite score. BMI ($r = -0.37, P < 0.01$) and whole body percent fat ($r = -0.30, P = 0.03$) were negatively correlated with the achievement composite score. Age, sex, pubertal timing, and SES did not significantly correlate with the achievement composite score ($r \leq 0.21, P \geq 0.13$). Bivariate correlations between MPOD with the KTEA II academic composite scores as well as bivariate correlations between lutein and zeaxanthin with the KTEA II academic composite scores are shown in Table 3.

**Table 3.** Bivariate correlations between KTEA II performance and lutein and zeaxanthin intake among preadolescent children$^1$

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Achievement</th>
<th>Reading</th>
<th>Math</th>
<th>Written</th>
<th>Language</th>
<th>Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOD</td>
<td>0.40**</td>
<td>0.28*</td>
<td>0.35**</td>
<td>0.41**</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Lutein and Zeaxanthin$^2$</td>
<td>0.29</td>
<td>0.16</td>
<td>0.14</td>
<td>0.53**</td>
<td></td>
<td>0.23</td>
</tr>
</tbody>
</table>

$^1$All are academic achievement composite standard scores based on age norms. $^*P < 0.05$ $^**P < 0.01$ (two-tailed)

$^2$n = 35; n = 18 did not return food records and n = 3 were cut out due to being outliers
Bivariate correlations between MPOD and demographics revealed that age, sex, pubertal timing, SES, IQ, BMI, whole body percent fat, VO2max, and fat free mass VO2max had no significant correlations with MPOD ($r \leq 0.22$, $P \geq 0.10$). Bivariate correlations between lutein and zeaxanthin intake and demographics revealed that age, pubertal timing, SES, IQ, BMI, whole body percent fat, VO2max, and fat free mass VO2max had no significant correlations with lutein and zeaxanthin intake ($r \leq 0.28$, $P \geq 0.10$). However, sex did have a significant correlation ($r = 0.42$, $P = 0.01$) with lutein and zeaxanthin intake.

Hierarchical Regressions

The stepwise hierarchical regression models are summarized in Table 4 for the composite scores and Table 5 summarizes the decomposition of their subtests. As IQ, BMI, whole body percent fat, VO2max, and fat free mass VO2max were significantly correlated with the achievement composite score, these factors were considered for entry into step 1 of the model. Sex was entered, despite not being significantly correlated with the academic composite, due to whole body percent fat and fat free mass VO2max differing between genders in independent t-tests [whole body percent fat was significantly different for girls ($M = 32.8$, SE = 1.0) and boys ($M = 27.7$, SE = 1.5), $t(54) = 2.9$, $p = 0.01$; and fat free mass VO2max was significantly different for girls ($M = 60.5$, SE = 1.1) and boys ($M = 64.9$, SE = 1.9), $t(54) = -2.1$, $p = 0.04$]. Therefore, sex, IQ, whole body percent fat, and fat free mass VO2max were entered in step 1 of the regressions shown in Table 4 and Table 5. Subsequent addition of MPOD in step 2 was conducted to determine the contribution to the academic measures following step 1 adjustments. The addition of MPOD did not statistically improve the $\Delta R^2$ for the reading or reading fluency composite scores or any of their subtests (letter and word recognition, reading comprehension, word recognition fluency, and decoding fluency), nor did it improve the $\Delta R^2$ for
the listening comprehension subtest. However, the addition of MPOD resulted in a significant improvement in the model $\Delta R^2$ at step 2 for the achievement composite standard scores ($\Delta R^2 = 0.10$, $P = 0.002$), math composite standard scores ($\Delta R^2 = 0.07$, $P = 0.02$), and written language composite standard scores ($\Delta R^2 = 0.15$, $P = 0.001$), as well as the math subtests: math concepts ($\Delta R^2 = 0.05$, $P = 0.04$), and math computation ($\Delta R^2 = 0.09$, $P = 0.02$), and the written language subtests: written expression ($\Delta R^2 = 0.11$, $P = 0.008$), and spelling ($\Delta R^2 = 0.13$, $P = 0.004$).

Table 4. Summary of regression analyses predicting academic achievement composite standard scores

<table>
<thead>
<tr>
<th>Step and Variable</th>
<th>Achievement $\beta$</th>
<th>Reading $\beta$</th>
<th>Math $\beta$</th>
<th>Written Language $\beta$</th>
<th>Reading Fluency $\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta R^2$</td>
<td>$\Delta R^2$</td>
<td>$\Delta R^2$</td>
<td>$\Delta R^2$</td>
<td>$\Delta R^2$</td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>-0.04</td>
<td>-0.02</td>
<td>0.00</td>
<td>-0.12</td>
<td>-0.01</td>
</tr>
<tr>
<td>IQ</td>
<td>0.60*</td>
<td>0.57*</td>
<td>0.47*</td>
<td>0.47*</td>
<td>0.52*</td>
</tr>
<tr>
<td>Whole body % fat</td>
<td>-0.02</td>
<td>0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Fat Free Mass VO2</td>
<td>0.22</td>
<td>0.29*</td>
<td>0.24</td>
<td>-0.04</td>
<td>0.22</td>
</tr>
<tr>
<td>Step 2</td>
<td>0.10*</td>
<td>0.04</td>
<td>0.07*</td>
<td>0.15*</td>
<td>0.02</td>
</tr>
<tr>
<td>Average MPOD</td>
<td>0.32*</td>
<td>0.20</td>
<td>0.27*</td>
<td>0.40*</td>
<td>0.16</td>
</tr>
</tbody>
</table>

$^1$ *$P < 0.05$. IQ, Intelligence quotient.
Table 5. Summary of regression analyses predicting academic achievement subtest standard scores

<table>
<thead>
<tr>
<th>Composite Test</th>
<th>Reading</th>
<th>Math</th>
<th>Written Language</th>
<th>Reading Fluency</th>
<th>Subtest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtest</td>
<td>Letter and Word Recognition</td>
<td>Reading Comprehension</td>
<td>Math Concepts</td>
<td>Math Computation</td>
<td>Written Expression</td>
</tr>
<tr>
<td>Step 1</td>
<td>β</td>
<td>ΔR²</td>
<td>β</td>
<td>ΔR²</td>
<td>β</td>
</tr>
<tr>
<td>Sex</td>
<td>0.07</td>
<td>0.31*</td>
<td>-0.09</td>
<td>0.04</td>
<td>-0.02</td>
</tr>
<tr>
<td>IQ</td>
<td>0.47*</td>
<td>0.04</td>
<td>0.54*</td>
<td>0.56*</td>
<td>0.30*</td>
</tr>
<tr>
<td>Whole body % fat</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>-0.03</td>
<td>-0.04</td>
</tr>
<tr>
<td>Fat Free VO2</td>
<td>0.25*</td>
<td>0.26*</td>
<td>0.15</td>
<td>0.29*</td>
<td>-0.09</td>
</tr>
<tr>
<td>Step 2</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.05*</td>
<td>0.09*</td>
</tr>
<tr>
<td>Average MPOD</td>
<td>0.14</td>
<td>0.14</td>
<td>0.21</td>
<td>0.24*</td>
<td>0.30*</td>
</tr>
</tbody>
</table>

1 *P < 0.05. IQ, Intelligence quotient.
Hierarchical Regressions in the Diet Subsample

The stepwise hierarchical regression models in the subsample providing diet data, n=35, are summarized in Table 6 for the academic composite scores. Sex, IQ, whole body percent fat, and fat free mass VO$_2$max were entered in step 1 of the regressions. Subsequent addition of lutein and zeaxanthin intake in step 2 was conducted to determine the contribution to the academic measures following step 1 adjustments. The addition of lutein and zeaxanthin only improved the $\Delta R^2$ for the written language composite score ($\Delta R^2 = 0.24, P < 0.01$), with a trend for the overall academic achievement composite ($\Delta R^2 = 0.06, P = 0.09$). With the addition of MPOD in step 3 of the model, significant improvements in the model $\Delta R^2$ were observed for the achievement composite standard scores ($\Delta R^2 = 0.20, P < 0.01$), reading composite standard scores ($\Delta R^2 = 0.10, P = 0.04$), math composite standard scores ($\Delta R^2 = 0.20, P < 0.01$), and written language composite standard scores ($\Delta R^2 = 0.10, P = 0.02$), but not for reading fluency composite scores.

Table 6. Summary of regression analyses predicting academic achievement composite standard scores in a subsample of children that returned dietary records

<table>
<thead>
<tr>
<th>Step and Variable</th>
<th>Achievement</th>
<th>Reading</th>
<th>Math</th>
<th>Written Language</th>
<th>Reading Fluency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B $\Delta R^2$</td>
<td>$\beta$</td>
<td>$\Delta R^2$</td>
<td>$\beta$</td>
<td>$\Delta R^2$</td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.42*</td>
<td>0.32*</td>
<td>0.38*</td>
<td>0.22</td>
<td>0.31*</td>
</tr>
<tr>
<td>IQ</td>
<td>0.56*</td>
<td>0.50*</td>
<td>0.43*</td>
<td>0.40*</td>
<td>0.44*</td>
</tr>
<tr>
<td>Whole body % fat</td>
<td>-0.10</td>
<td>0.05</td>
<td>-0.16</td>
<td>-0.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Fat Free Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2</td>
<td>0.15</td>
<td>0.17</td>
<td>0.28*</td>
<td>-0.08</td>
<td>0.27</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L and Z</td>
<td>0.06*</td>
<td>0.01</td>
<td>0.01</td>
<td>0.24*</td>
<td>0.05</td>
</tr>
<tr>
<td>Average MPOD</td>
<td>0.27*</td>
<td>0.14</td>
<td>0.11</td>
<td>0.56*</td>
<td>0.26</td>
</tr>
<tr>
<td>Step 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average MPOD</td>
<td>0.20*</td>
<td>0.10*</td>
<td>0.20*</td>
<td>0.10*</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$^1$*P < 0.05. $^tP < 0.10$. IQ, Intelligence quotient. L and Z, Lutein and Zeaxanthin intake.

$^2$n = 35; n = 18 did not return food records and n = 3 were cut out due to being outliers
CHAPTER 5 – DISCUSSION

The aim of the present thesis was to evaluate the relationships between dietary lutein and zeaxanthin intake, MPOD, and academic achievement measures among preadolescent children. The major finding was that children with higher MPOD values have superior performance on academic measures, particularly in math and written language. Given that MPOD was positively related to academic outcomes, even after the adjustment of sex, IQ, whole body percent fat, and fat free mass VO₂max, points to the importance of the habitual intake of lutein and zeaxanthin, as indirectly indicated by MPOD, for improved academic performance. This finding is important because macular lutein is modifiable and can be manipulated by dietary intake in most of the population (30).

The results of this thesis demonstrate that the associations of the various academic composite measures with MPOD were fairly consistent, whereas associations between academics and self-reported dietary intake of lutein and zeaxanthin were not as consistent. MPOD has been shown to be a stable measure of carotenoids embedded in the retina and has been demonstrated to be a better representation of long-term intake of lutein and zeaxanthin than serum (22). This may explain why MPOD was more consistent with academic measures than self-reported dietary intake of lutein and zeaxanthin. In an adult study, MPOD was related to six out of the eight cognitive measures, whereas serum concentrations of lutein and zeaxanthin were only positively related to one of the eight (22), thus demonstrating similar relationships to MPOD and measures that reflect recent nutritional intake of lutein and zeaxanthin (29) as our findings. Other factors contributing to the inconsistency of self-report diet records to cognitive measures is that their use may introduce subject recall bias, there could be possible digestive and absorptive idiosyncrasies
among subjects, and using different sources of carotenoid data by investigators may introduce inconsistencies between studies (29).

A positive relationship between dietary intake and MPOD was found in this sample. Such a finding is congruent with some, but not all, adult studies (29). This lack of consensus across studies is interesting as many factors impact the absorption of lutein and zeaxanthin into the blood (51). Further, some tissues may compete for the uptake of lutein and zeaxanthin, which may interfere with this relationship (78, 81). As this finding was observed in a small subset of the entire sample, those that returned diet records (n = 35), further study in larger samples of children is warranted to determine the robustness of this relationship. Contrary to our hypothesis, within the subsample, diet did not mediate the relationships of MPOD to academics. While this analysis was completed in a smaller sample size (n = 35 versus n = 56), it brings about the speculation that perhaps other early life factors, such as maternal diet, feeding methods, pre-term versus full term deliveries (19), may influence MPOD and in turn may be contributing to the seen relationships. Future studies should further this area of research by including these factors in their data analysis.

Lutein and zeaxanthin have received considerable attention for their impact on visual health, and more recently on cognitive health in the elderly (20-24). However, the influence of lutein and zeaxanthin on cognitive health in preadolescence has received little attention in comparison (118, 119). Although dietary carotenoids have not been investigated directly for their effect on academic performance, studies have been conducted on other dietary aspects and their impact on academic performance. Overall diet quality has been demonstrated to impact academic performance in children. A study completed in 5th graders showed a positive association of diet quality and academic performance (27). Within overall diet quality, it was found that students
with an increased intake of fruits and vegetables, sources high in carotenoids, was associated with their academic performance (27). Additionally, dietary fibers, nutrients found abundantly in fruits and vegetables, have been related to childhood cognitive function (112). After placement into a free school breakfast program that decreased nutritional risks in students, improvements in math grades were observed. (28). As dietary carotenoids are a hallmark of higher quality diets, our results are consistent with previous studies suggesting a role for diet in childhood cognitive function. This thesis does however add to the current literature, as it provides support for the neurocognitive potential of the macular carotenoids, even after adjusting for sex, IQ, whole body percent fat, and fat free mass VO₂max, measures that have been previously demonstrated to relate to academic achievement in preadolescence (111, 129-132).

The macular carotenoids, lutein and zeaxanthin measured via MPOD, demonstrated a significant relationship with academic performance among preadolescents congruent with our a priori hypothesis based on the preferential accumulation of lutein in the brain (19, 20). Significant associations have been shown between lutein and cognition in the elderly. MPOD has been shown to relate to cognition in adults (20, 22, 23). Additionally, supplementation with lutein and zeaxanthin has been shown to improve cognitive function in healthy older women (24).

Carotenoids may exert their effects through several mechanisms in the brain to improve cognitive function. The brain is especially vulnerable to free radical damage due to its relatively low antioxidant content, high polyunsaturated fatty acids (PUFA) concentration, and high metabolic activity (100). Carotenoids function as antioxidants and anti-inflammatory agents (133). These properties provide potential mechanisms for them to help improve cognition in
children, especially as their brains are still undergoing extensive development in both structure and function (102, 103).

Limitations of this study include the correlational study design. Thus, placebo-controlled interventions should be conducted to further investigate this relationship. Additionally, MPOD measurement via heterochromatic flicker photometry has been shown to only be moderately reliable in children, thus the use of a better validated technique, such as fundus reflectometry or fundus autofluorescence, would help to confirm these findings (116). However, both of these techniques do come with their own inherent limitations such as the need to dilate pupils, unpleasant light levels, the need to align the equipment precisely, and the higher cost of equipment (116). As such, further investigation into improving the reliability and validity of HFP in children should be a goal of future studies. Although not completely genetically controlled (134), there are some potential contributing genetic factors to the accumulation of lutein and zeaxanthin as macular pigments that is made clear from supplementation studies demonstrating that some people are non-responders to supplementation (30). Thus, another potential limitation of this thesis is not measuring genes that may contribute to variation in MPOD (135).

Due to the rapid rise in childhood obesity, links between the detrimental effects of excess fat mass, physical inactivity, and overall diet quality on childhood cognition are becoming clearer (27, 136-138). However, the effect of specific nutrients on cognition of children without nutritional deficiencies has not been thoroughly investigated. This thesis serves the purpose of linking the carotenoids lutein and zeaxanthin to academic performance of children. Diet recommendations for increasing foods which are high in these nutrients have been shown to have other health benefits (1). Thus, improved academic performance among preadolescents may be yet another beneficial aspect of increasing foods high in carotenoids. Especially as academic
performance influences future educational attainment and income, thus impacting the future health and quality of life for children (25).

Despite their importance in the diet, national food consumption data indicate that children in the United States consistently fail to meet their recommended servings of fruits and vegetables. Indeed, less than 50% of children meet their recommendations for fruits and less than 10% consume their recommended servings of vegetables (139). This under consumption of fruits and vegetables and in turn the overconsumption of foods high in calories, but low in nutritional value may be contributing to the rising childhood obesity rates. This is one factor contributing to the recent estimates that younger generations may lead shorter and less healthy lives than their parents, making this the first time in US history that such a trend has occurred (140). Therefore, public health initiatives should continue to promote fruit and vegetable intake as it has beneficial aspects across an array of health domains (1), and this work shows another potential benefit, improved academic performance among preadolescents, of consuming carotenoids on a regular basis. Additionally, as there is currently no Daily Reference Intake (DRI) for lutein or zeaxanthin, studies demonstrating their affects take them one step closer to being able to undergo the process for obtaining a DRI value. Once they have a DRI, then they can be incorporated into public health policy and the knowledge of their benefits will become more available for the general public.

Further consideration should be made to other early life factors that may be contributing to higher MPOD. As seen in the subsample of our population, diet did not mediate the relationship of academics to MPOD; other factors may be equally, or of more importance in determining children’s MPOD values. Future studies should continue to investigate potential contributing factors to continue to tell a fuller story on the relationships of MPOD to academics.
and other confounding variables that may be present. Then, these factors may be able to be focused on in early life to improve academic achievement in preadolescence.
CHAPTER 6 – CONCLUSIONS

Support for a positive relationship between dietary lutein and zeaxanthin intake and MPOD levels, as well as positive relationships between MPOD and academic achievement scores has been provided in this thesis work. As macular pigment levels serve as a biomarker for lutein and zeaxanthin concentration in the brain (18), the positive associations between MPOD and cognition found in this thesis are not entirely surprising. Additionally, as macular pigment can be impacted by diet in most of the population (30), dietary interventions that increase their concentrations are possible. If these interventions would help improve cognition in preadolescence, they may serve as a useful tool for improving academic performance. However, continued research is needed to gain a more causal understanding of the relationship between MPOD and cognition in children, including, but not limited to the inclusion of early life factors in data analysis.
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Pre-Participation Health Screening

Physical activity and fitness testing are safe for most children. To ensure safe participation for your child we would like to know some specific information about your child’s health before we include them in this study. In some cases, we simply need to know more information (e.g., that your child has a puffer for asthma) while in other situations, we might tell you that we’d like your child to see a physician before participating in the study. Please tick “☐ Yes” beside all items that apply to your child, and feel free to ask us to clarify if anything is unclear.

YES □ NO □

1. Your child has a diagnosed medical condition that prevents them from participating in intense exercise.

2. A doctor has ever told you that it would be unsafe for your child to do intense exercise.

3. Anyone in your child’s family has ever died of a sudden heart attack before the age of 35 years.

4. Anyone in your child’s family has ever been diagnosed with a serious heart condition before the age of 35 years.

5. Your child has high blood pressure.

6. Your child has asthma and uses a puffer or inhaler.

7. Your child has diabetes.

8. Your child has epilepsy (seizures).

9. Your child has unexplained fainting or dizziness, especially with activity.

10. Your child has unexplained chest pain, breathlessness, or tiredness with activity.

Pre-Participation Health Screening (Dr. Mark Tremblay, University of Ottawa)
Health History & Demographics Questionnaire

Please answer the following questions to the best of your ability.

<table>
<thead>
<tr>
<th>General Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What was your child’s date of birth? ________/<em><strong><strong><strong>/</strong></strong></strong></em></td>
</tr>
<tr>
<td>2. Was your child born before 37 weeks of pregnancy? □ Yes □ No</td>
</tr>
<tr>
<td>3. At what week of pregnancy was your child born? __________ weeks</td>
</tr>
<tr>
<td>4. What was your child’s birth weight? __________ lbs __________ oz</td>
</tr>
<tr>
<td>5. Did the mother of your child suffer from any medical condition while she was pregnant?</td>
</tr>
<tr>
<td>□ Yes □ No If yes, what condition?</td>
</tr>
<tr>
<td>6. What is your child’s current age? ______________</td>
</tr>
<tr>
<td>7. What is your child’s current (or recently completed) Grade Level? ______________</td>
</tr>
<tr>
<td>8. What is your child’s sex? □ Male □ Female</td>
</tr>
<tr>
<td>9. Which is your child’s dominant hand? □ Right □ Left □ No Preference</td>
</tr>
<tr>
<td>10. Is your child color blind? □ Yes □ No</td>
</tr>
<tr>
<td>11. Does your child wear contacts or glasses? □ Yes □ No</td>
</tr>
<tr>
<td>If yes, what was their prescription for?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Demographics</th>
</tr>
</thead>
</table>

55
1. Does your child live with their biological parents? □ Yes □ No

2. Does your child live in a single parent/guardian household? □ Yes □ No

3. Does your child live with their Mother or a Female guardian? □ Yes □ No

4. Does your child’s Mother/Female guardian work? □ Yes □ No

5. What is the highest level of education obtained by your child’s Mother/Female guardian?
   a) Did not complete high school  
   b) High School Graduate  
   c) Some College  
   d) Bachelor Degree  
   e) Advanced Degree

6. Does your child live with their Father or a Male guardian? □ Yes □ No

7. Does your child’s Father/Male guardian work? □ Yes □ No

8. What is the highest level of education obtained by your child’s Father/Male guardian?
   a) Did not complete high school  
   b) High School Graduate  
   c) Some College  
   d) Bachelor Degree  
   e) Advanced Degree

9. How many other children (under the age of 18) live with your child? _______________
   How old are they? _______________
   What is their sex? _______________

10. How many biological siblings does your child have? _______________

11. Does your child receive free or reduced-price school lunch? □ Yes □ No

12. Do you consider yourself to be Hispanic or Latino (A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race)? □ Yes □ No
13. What race / ethnicity do you consider your child?
   a) American Indian or Alaska Native  d) Native Hawaiian or other Pacific Islander
   b) Asian  e) White or Caucasian
   c) Black or African American  f) Mixed or Other

14. What is your approximate household income?
   a) <10,000  g) 61,000-70,000
   b) 10,000-20,000  h) 71,000-80,000
   c) 21,000-30,000  i) 81,000-90,000
   d) 31,000-40,000  j) 91,000-100,000
   e) 41,000-50,000  k) 100,000+
   f) 51,000-60,000

**Activities**

1. Does your child participate in musical activities?  □ Yes  □ No

   If yes:
   Does your child play an instrument?  □ Yes  □ No

   If so, what instrument(s)?

   Does your child participate in choir?  □ Yes  □ No

   How many hours a week does your child spend participating in musical activities?

2. Does your child participate in religious activities?  □ Yes  □ No

   If yes, how many hours a week does your child spend participating in religious activities?
3. Does your child participate in sports activities? □ Yes □ No

If yes:

Does your child participate in formal youth sports? □ Yes □ No

In what activities does your child participate?

4. Has your child attended regular afterschool care outside of your home in the last year?
□ Yes □ No

**Habits**

1. How much time does your child spend watching television on an average day during the **week**?
   - a) < 1 Hour per Day
   - b) 1 to 2 Hours per Day
   - c) 2 to 3 Hours per Day
   - d) 3 to 4 Hours per Day
   - e) 4 to 5 Hours per Day
   - f) 5 to 6 Hours per Day
   - g) 6 to 7 Hours per Day
   - h) 7 to 8 Hours per day
   - i) > 8 Hours per Day

2. How much time does your child spend watching television on an average day during the **weekend**?
   - a) < 1 Hour per Day
   - b) 1 to 2 Hours per Day
   - c) 2 to 3 Hours per Day
   - d) 3 to 4 Hours per Day
   - e) 4 to 5 Hours per Day
   - f) 5 to 6 Hours per Day
   - g) 6 to 7 Hours per Day
   - h) 7 to 8 Hours per day
   - i) > 8 Hours per Day

3. How much time does your child spend on a computer on an average day during the **week**?
   - a) < 1 Hour per Day
   - b) 1 to 2 Hours per Day
   - c) 2 to 3 Hours per Day
   - d) 3 to 4 Hours per Day
   - e) 4 to 5 Hours per Day
   - f) 5 to 6 Hours per Day
   - g) 6 to 7 Hours per Day
   - h) 7 to 8 Hours per day
   - i) > 8 Hours per Day

4. How much time does your child spend on a computer on an average day during the **weekend**?
   - a) < 1 Hour per Day
   - b) 1 to 2 Hours per Day
   - c) 2 to 3 Hours per Day
   - d) 3 to 4 Hours per Day
   - e) 4 to 5 Hours per Day
   - f) 5 to 6 Hours per Day
   - g) 6 to 7 Hours per Day
   - h) 7 to 8 Hours per day
   - i) > 8 Hours per Day
5. How much time does your child spend playing video games on an average during the week?
   a) < 1 Hour per Day
   b) 1 to 2 Hours per Day
   c) 2 to 3 Hours per Day
   d) 3 to 4 Hours per Day
   e) 4 to 5 Hours per Day
   f) 5 to 6 Hours per Day
   g) 6 to 7 Hours per Day
   h) 7 to 8 Hours per day
   i) > 8 Hours per Day

6. How much time does your child spend playing video games on an average during the weekend?
   a) < 1 Hour per Day
   b) 1 to 2 Hours per Day
   c) 2 to 3 Hours per Day
   d) 3 to 4 Hours per Day
   e) 4 to 5 Hours per Day
   f) 5 to 6 Hours per Day
   g) 6 to 7 Hours per Day
   h) 7 to 8 Hours per day
   i) > 8 Hours per Day

7. How much time does your child spend being physically active on an average during the week?
   a) < 1 Hour per Day
   b) 1 to 2 Hours per Day
   c) 2 to 3 Hours per Day
   d) 3 to 4 Hours per Day
   e) 4 to 5 Hours per Day
   f) 5 to 6 Hours per Day
   g) 6 to 7 Hours per Day
   h) 7 to 8 Hours per day
   i) > 8 Hours per Day

8. How much time does your child spend being physically active on an average during the weekend?
   a) < 1 Hour per Day
   b) 1 to 2 Hours per Day
   c) 2 to 3 Hours per Day
   d) 3 to 4 Hours per Day
   e) 4 to 5 Hours per Day
   f) 5 to 6 Hours per Day
   g) 6 to 7 Hours per Day
   h) 7 to 8 Hours per day
   i) > 8 Hours per Day

9. How much sleep does your child regularly get?
   a) < 5 Hours per Day
   b) 5 to 6 Hours per Day
   c) 6 to 7 Hours per Day
   d) 7 to 8 Hours per Day
   e) 8 to 9 Hours per Day
   f) 9 to 10 Hours per Day
   g) > 10 Hours per Day

10. How much sleep did your child get last night?
    a) < 5 Hours
    b) 5 to 6 Hours
    c) 6 to 7 Hours
    d) 7 to 8 Hours
    e) 8 to 9 Hours
    f) 9 to 10 Hours
    g) > 10 Hours
11. How many caffeinated soft drinks does your child regularly drink in a day?
   □ None       □ One       □ Two       □ Three or more

12. How many cups of tea does your child regularly drink in a day?
   □ None       □ One       □ Two       □ Three or more

13. How often would you rate your child’s stress level as HIGH?
   □ Occasionally □ Frequently □ Constantly

When was the last time your child:
   Had a caffeinated substance?
   Ate a meal or a snack?
   What did s/he have to eat?
   Exercised?
   What type of exercise?
   How long did s/he exercise for?
   How intense did s/he work out?

General Health

1. When was the last time your child saw a doctor? _____________________

2. Does your child have any allergies? □ Yes □ No

3. Does your child have any food allergies? □ Yes □ No
   Is your child allergic to milk? □ Yes □ No
   Is your child allergic to soy? □ Yes □ No

Please list any other allergies your child may have:___________________________
4. Is your child allowed to consume foods that contain animal products?
   □ Yes        □ No (my child follows a strictly plant-based/vegan diet)

5. Was your child breastfed?  □ Yes  □ No
   
   If yes, what was the duration of exclusive (no formula at all) breast feeding? ____ months
   
   At what age did your child stop drinking any breast milk? ____ months

6. At what age was infant formula introduced to your child? _______ months

7. How old was your child when he/she was first fed something (e.g., cereals, pureed foods, solid foods) other than breast milk or formula? _______ months

8. Has your child ever been diagnosed with dyslexia?  □ Yes  □ No

9. Has your child ever been diagnosed with an attentional disorder?  □ Yes  □ No

10. Has your child ever been diagnosed with asthma?  □ Yes  □ No

11. Is your child epileptic?  □ Yes  □ No

12. Is your child diabetic?  □ Yes  □ No
   
   If so please explain:

13. Has your child been diagnosed with any kind of cancer?  □ Yes  □ No
   
   If so please explain:

14. Does your child have hearing loss or wear a hearing aid?  □ Yes  □ No
15. Has your child been hospitalized within the last 6 months? □ Yes □ No
   If so please explain:

16. Has your child ever lost consciousness as a result of hitting their head? □ Yes □ No
   If yes:
   When did this occur?
   Where did s/he hit his/her head?
   How long was s/he unconscious?

17. Has your child ever lost consciousness as a result of any other type of injury or seizure? □ Yes □ No
   If yes:
   When did this occur?
   How long was s/he unconscious?

Medications/Supplements

Medications: Is your child presently taking or have they taken any of the following medications within the past two months? Please circle your answer.

- Aspirin, Bufferin, Anacin
- Blood Pressure pills
- Cortisone
- Cough Medicine
- Digitalis
- Hormones
- Insulin or Diabetic pills
- Iron or poor blood medications
- Laxatives
- Sleeping pills
- Tranquilizers
- Weight reducing pills
- Blood thinning pills
- Dilantin
- Allergy Shots
- Water pills
- Antibiotics
- Barbiturates
- Phenobarbital
- Thyroid medicine

Other(s): ____________________________________________

1. Does your child take Ginkgo Biloba supplements? □ Yes □ No
If yes:
When was the last time they took the supplement?
What dose of the supplement did they take?

2. Does your child take Iron supplements?  □ Yes  □ No

If yes:
When was the last time they took the supplement?
What dose of the supplement did they take?

3. Does your child take any stimulants or sedatives?  □ Yes  □ No

If yes:
What do they take?
When was the last time they took it?
What dose of it did they take?

Cardiovascular Health

*Does your child have any of the following:*

1. □ Yes  □ No  Pain or discomfort in the chest, neck, jaw, arms, or other areas that may be related to poor circulation.

2. □ Yes  □ No  Heartbeats or palpitations that feel more frequent or forceful than usual or feeling that your heart is beating very rapidly.

3. □ Yes  □ No  Unusual dizziness or fainting.

4. □ Yes  □ No  Shortness of breath while lying flat or a sudden difficulty in breathing that wakes them up while sleeping.
5. □ Yes □ No  Shortness of breath at rest or with mild exertion (such as walking two blocks).

6. □ Yes □ No  Feeling lame or pain in the legs brought on by walking.

7. □ Yes □ No  A known heart murmur.

8. □ Yes □ No  Unusual fatigue with usual activities.

9. □ Yes □ No  Has any male in your immediate family had a heart attack or sudden death before the age of 55?

10. □ Yes □ No  Has any female in your immediate family had a heart attack or sudden death before the age of 65?

11. □ Yes □ No  Do you have family history of heart disease?

12. □ Yes □ No  Do you have family history of lung disease?

13. □ Yes □ No  Do you have family history of diabetes?

14. □ Yes □ No  Do you have family history of strokes?

15. □ Yes □ No  Has your child been diagnosed with a past or present cardiovascular disease?

16. □ Yes □ No  Does your child have any significant heart rhythm disorder?
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Has your child been diagnosed with hypertension?</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>□ Yes □ No</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>□ Yes □ No</td>
<td>Has your child been diagnosed with peripheral vascular disease?</td>
</tr>
</tbody>
</table>

**Other**

Is there anything else you feel we should know about your child’s current/past health?
Please circle the number that best describes your child’s home behavior over the last 6 months.

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fails to give close attention to details or makes careless mistakes in schoolwork.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2. Fidgets with hands or feet or squirms in seat.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3. Has difficulty sustaining attention in tasks or play activities.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4. Leaves seat in situations in which remaining seated is expected.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5. Does not seem to listen when spoken to directly.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6. Runs about or climbs excessively in situations in which it is inappropriate.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7. Does not follow through on instructions and fails to finish work.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>8. Has difficulty playing or engaging in leisure activities quietly.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>9. Has difficulty organizing tasks and activities.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10. Is “on the go” or acts as if “driven by a motor.”</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>11. Avoids tasks (e.g. homework) that require sustained mental effort.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>12. Talks excessively.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>13. Loses things necessary for tasks or activities.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>14. Blurs out answers before questions have been completed.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>15. Is easily distracted.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>16. Has difficulty awaiting turn.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>17. Is forgetful in daily activities.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>18. Interrupts or intrudes on others.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Tanner Staging Questionnaire

On each side of the line, please circle the number that best represents your child’s pubertal status.

1. The breasts are flat.
2. The breasts form small mounds.
3. The breasts form larger mounds than in 2.
4. The nipple and the surrounding part (the Areola) make up a mound that sticks up above the breast.
5. Only the nipple sticks out beyond the breast.

1. No hairs.
2. Very little hair.
3. Quite a lot of hair.
4. The hair has not spread over the thighs.
5. The hair has spread over the thighs.
Tanner Staging Questionnaire

On each side of the line, please circle the number that best represents your child’s pubertal status.

1. Scrotum and penis are the same size.

2. The scrotum has lowered a bit and the penis is a little larger.

3. The penis is longer and the scrotum is larger.

4. The penis is longer and wider; the scrotum is darker and bigger than before.

5. The penis and scrotum are the size and shape of an adult.

1. No hairs.

2. Very little hair.

3. Quite a lot of hair.

4. The hair has not spread over the thighs.

5. The hair has spread over the thighs.
Instructions for Recording the 3-Day Food Diary

1. Record everything you ate or drank during the 24-hour time period indicated (12:01 a.m. to midnight). Repeat this for a total of 3 days (2 week days, 1 weekend day).

2. To the best of your ability, describe combination or mixed dishes that were eaten. For example, what ingredients were included on that piece of pizza? Was it thick or thin crust? Include brand names if known.

3. Describe the amounts consumed in terms appropriate for that item. For example: ounces (cups) of milk, tablespoons of French dressing, slices of bread, pieces of fruit, etc. If you had a piece of pizza, how big was it in inches or sections, etc.? Record exact amounts to the best of your ability.

<table>
<thead>
<tr>
<th>Sample Breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raisin bran cereal</td>
</tr>
<tr>
<td>2% milk</td>
</tr>
<tr>
<td>Orange</td>
</tr>
<tr>
<td>Toast (whole wheat)</td>
</tr>
<tr>
<td>with butter</td>
</tr>
<tr>
<td>with strawberry jam</td>
</tr>
<tr>
<td>Black coffee</td>
</tr>
<tr>
<td>with sugar</td>
</tr>
</tbody>
</table>

4. Remember to include beverages, and anything you may add to them, such as milk or sweetener.

5. Remember to include anything added to a food after it is prepared, such as margarine, salt, catsup, mustard and the estimated amount.

6. If you need additional space, use the back of the paper or attach additional sheets.

7. Answer the question at the bottom of the day’s record. (Does this day’s record represent your usual food intake? ___ Yes ___ No). If your answer is no, explain why it wasn’t representative. Were you ill or are you on a special diet? Did you have unexpected guests and you took them out to dinner?
Portion Size Examples

**BASIC GUIDELINES**

1 cup = baseball
1/2 cup = lightbulb
1 oz or 2 tbsp = golf ball
1 tbsp = poker chip
3 oz chicken or meat = deck of cards
3 oz fish = checkbook

**GRAINS**

1 cup of cereal flakes = baseball
1 pancake = compact disc
1/2 cup cooked rice = lightbulb
1/2 cup cooked pasta = lightbulb
1 slice bread = cassette tape
1 bagel = 6 oz can of tuna
3 cups popcorn = 3 baseballs

**DAIRY & CHEESE**

1 1/2 oz cheese = 3 stacked dice
1 cup yogurt = baseball
1/2 cup of frozen yogurt = lightbulb
1/2 cup of ice cream = lightbulb

**FATS & OILS**

1 tbsp butter or spread = poker chip
1 tbsp salad dressing = poker chip
1 tbsp mayonnaise = poker chip
1 tbsp oil = poker chip

**FRUITS & VEGETABLES**

1 medium fruit = baseball
1/2 cup grapes = about 16 grapes
1 cup strawberries = about 12 berries
1 cup of salad greens = baseball
1 cup carrots = about 12 baby carrots
1 cup cooked vegetables = baseball
1 baked potato = computer mouse

**MEATS, FISH & NUTS**

3 oz lean meat = deck of cards
3 oz fish = checkbook
3 oz tofu = deck of cards
2 tbsp peanut butter = golf ball
2 tbsp hummus = golf ball
1/4 cup almonds = 23 almonds
1/4 cup pistachios = 24 pistachios

**MIXED DISHES**

1 hamburger (without bun) = deck of cards
1 cup fries = about 10 fries
4 oz nachos = about 7 chips
3 oz meatloaf = deck of cards
1 cup chili = baseball
1 sub sandwich = about 6 inches
1 burrito = about 6 inches
### 3 Day Food Diary

#### DAY 1

**Date** _________  **Day of the week:** ______________

<table>
<thead>
<tr>
<th>Time/Meal</th>
<th>Food, Beverage, Condiments</th>
<th>Amount Eaten (grams, cups, tsp, Tbsp, oz, etc)</th>
<th>Brand/Name How prepared</th>
<th>Home/Out</th>
<th>TV/Computer/Video Game</th>
</tr>
</thead>
<tbody>
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</table>

Circle One:  **Weekday**  **Weekend**

Does this day’s record represent your usual days’ intake? _____ yes _____ no

If no, explain why not ____________________________________________________________
### 3 Day Food Diary

- **Date**: __________
- **Day of the Week**: __________________
- **Circle One**: Weekday Weekend

**Does this day’s record represent your usual days’ intake?**

- [ ] yes
- [ ] no

If no, explain why not:

<table>
<thead>
<tr>
<th>Time/Meal</th>
<th>Food, Beverage, Condiments</th>
<th>Amount Eaten (grams, cups, tsp, Tbsp, oz, etc)</th>
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72
DAY 3
3 Day Food Diary

Date __________   Day of the week: __________________

Circle One:       Weekday    Weekend

<table>
<thead>
<tr>
<th>Time/Meal</th>
<th>Food, Beverage, Condiments</th>
<th>Amount Eaten (grams, cups, tsp, Tbsp, oz, etc)</th>
<th>Brand/Name How prepared</th>
<th>Home/Out</th>
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</table>

Does this day’s record represent your usual days’ intake? _____ yes _____ no
If no, explain why not _____________________________________________
MPOD

Study: ___________

Age: ________ Date: ___________ Length of Test: ________ Experimenter: _______

Indicate which eye the child is using: □ Left □ Right Point in Testing: _______

Has any eye injury occurred in the child’s past (i.e., anything that required a visit to ER or family physician)? □No □Yes: ___________

Determining Critical Flicker Fusion Frequency:

______ Hz ________ Hz ________ Hz ________ Hz ________ Hz Average CFF: ________

Suggested Conversion Guidelines:

<table>
<thead>
<tr>
<th>CFF</th>
<th>Foveal LFF</th>
<th>Parafoveal LFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-12</td>
<td>11-12</td>
<td>6</td>
</tr>
<tr>
<td>13-16</td>
<td>12-13</td>
<td>6-7</td>
</tr>
<tr>
<td>17-20</td>
<td>13-14</td>
<td>7-8</td>
</tr>
<tr>
<td>21-25</td>
<td>14-16</td>
<td>8-9</td>
</tr>
<tr>
<td>25+</td>
<td>16-17</td>
<td>9-10</td>
</tr>
</tbody>
</table>

Foveal measurement (Radiance values):

Starting LFF: ________ Ending LFF: ________

(“never stops flickering” = rate up; no flicker at points that vary by more than 100 = rate down)

(end with Trial 3 if average range of Trial 1-3 is < +/- 100)

<table>
<thead>
<tr>
<th>Trial 1: (start @ 3000, work down)</th>
<th>Trial 2: (start @ 0, work up)</th>
<th>Trial 3:</th>
<th>Trial 4:</th>
<th>Trial 5:</th>
</tr>
</thead>
<tbody>
<tr>
<td>________</td>
<td>________</td>
<td>________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

Parafoveal measurement (Radiance values): (should be between ~ 200 and 600)

Starting LFF: ________ Ending LFF: ________

(“never stops flickering” = rate up; no flicker at points that vary by more than 100 = rate down)

(end with Trial 3 if average range of Trial 1-3 is < +/- 100)

<table>
<thead>
<tr>
<th>Trial 1: (start @ 1000, work down)</th>
<th>Trial 2: (start @ 0, work up)</th>
<th>Trial 3:</th>
<th>Trial 4:</th>
<th>Trial 5:</th>
</tr>
</thead>
<tbody>
<tr>
<td>________</td>
<td>________</td>
<td>________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

Macular pigment optical density:

________

Standard deviation:

________

Notes (e.g., subject could not see disk, could not focus on red dot, etc.):