THE INDEPENDENT AND INTERACTIVE INFLUENCE OF LUTEIN AND CHOLINE ON COGNITIVE CONTROL

BY

CAITLYN EDWARDS

DISSERTATION

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Doctoral Committee:

Associate Professor Ryan Dilger, Chair
Assistant Professor Naiman Khan, Director of Research
Associate Professor Aron Barbey
Assistant Professor Hannah Holscher
ABSTRACT

Objectives

Diet quality plays a role in progressive physical, as well as mental, co-morbidities, particularly among those with elevated weight status. Therefore, research exploring cost-effective, practical, and sustainable strategies to promote healthy weight status as well as healthy cognitive and mental health status are of great interest to public health agencies and individuals alike. Dietary intake as well as serum and cortical accumulation of the dietary components lutein and choline have been linked with benefits for cognition across the lifespan. This Dissertation work utilized three aims to investigate the impact of both independent and interactive dietary consumption and biomarker concentrations of these components in a young-to-middle aged sample of adults with overweight or obesity. Aim 1 utilized a cross-sectional sample as well as a randomized controlled trial to examine the impact of dietary, serum, and retinal lutein concentrations on cognitive control. Aim 2 examined the impact of dietary choline intake on cognitive control. Lastly, Aim 3 examined the interactive nature of dietary intake of lutein and choline, serum lutein concentrations, and choline metabolites on cognitive control.

Methods

The Persea Americana for Total Health (PATH) Study was a 12-week randomized controlled trial aimed at assessing the impact of avocado consumption on primary outcomes of visceral adiposity and glycemic control. Secondary outcomes analyzed within this work were behavioral and neuroelectric indices of cognitive control, specifically selective attention, response inhibition, and cognitive flexibility. Adults with overweight and obesity (Body Mass Index [BMI] ≥ 25.0) between the ages of 25-45 years completed 7-day diet records, venous blood draws, heterochromatic flicker photometry for assessment of macular pigmentation (MPOD), the
Kaufman Brief Intelligence Test–2 (IQ), the Eriksen Flanker task, Oddball/No-go task, and a Switch task while undergoing electroencephalographic recording for event-related potential extraction (ERP). For intervention analyses, participants were randomized to a treatment group (N = 47) that received a 12-week daily meal with fresh Hass avocado or a control group (N = 37) that received an isocaloric meal (clinicaltrials.gov, NCT02740439). Baseline data, prior to randomization, was utilized for cross-sectional analyses. Cognitive outcomes included behavioral (accuracy and reaction time [RT]) as well as the N2 and P3 ERP components.

Results

Aim 1-a utilized partial correlations between MPOD and behavioral and neuroelectric outcomes of an Oddball Task to examine the impact of retinal xanthophyll accumulation (MPOD) on cognitive control. These cross-sectional analyses revealed that after controlling for sex and dietary lutein/zeaxanthin intake, higher MPOD was associated with lower N2 mean amplitude (\( \rho=-0.21, p=0.04 \)) and P3 peak latency (\( \rho=-0.27, p<0.01 \)) during target trials, as well as lower P3 peak amplitude (\( \rho=-0.22, p=0.03 \)) during standard trials. Improvements in behavioral outcomes were not observed (all \( p \)'s > 0.05).

Aim 1-b utilized intervention data from the PATH study to examine the impact of 12-week avocado consumption on behavioral and neuroelectric outcomes of a modified Eriksen Flanker, Oddball, and No-go task. These analyses revealed significant time x group interactions (F(1,79) = 26.98, \( p < 0.001 \), \( \eta^2_p = 0.26 \)) for serum lutein concentrations as well improvements in accuracy in both the congruent (change: 1.3\%, \( p=0.05 \); 95\% CI:−3.02 to 2.3) and incongruent (change: 3.0\%, \( p< 0.01 \); 95\%CI: 1.1 to 5.0) Flanker task conditions, indicating that the group consuming avocado exhibited increased concentrations of serum lutein as well as overall
improvement on a selective attention task. Improvements in MPOD, on other cognitive tasks, and neuroelectric indices of cognitive outcomes were not observed (all p’s>0.05).

Aim 2 utilized hierarchical linear regression analyses to examine the impact of dietary choline intake on behavioral and neuroelectric outcomes of a modified Eriksen Flanker Task. These cross-sectional analyses revealed that after controlling for age, sex, BMI, IQ, and overall diet quality, higher choline intake was associated with lower P3 peak amplitude ($\beta=-0.25$, $p<0.01$) during incongruent trials. Relationships with behavioral outcomes were not observed (all p’s>0.05).

Aim 3 examined the independent and potentially interactive effects of dietary lutein, dietary choline, serum lutein concentrations, MPOD, plasma phosphatidylcholine (PC) and free choline concentrations on cognitive control. These analyses utilized hierarchical linear regression analyses controlling for sex, age, IQ, BMI, and household income. A multiplicative interaction term of dietary lutein and dietary choline intake improved models for RT in all trials of a cognitive flexibility task: NonSwitch RT ($\Delta R^2=0.10$ $p=0.03$), Switch RT ($\Delta R^2=0.10$ $p=0.05$), and Global Switch Cost ($\Delta R^2=0.08$ $p=0.05$) during the switch task. Plasma PC independently was associated with accuracy during a Switch task ($\beta=0.24$, $p=0.04$).

Conclusion

The dietary components lutein and choline have been hypothesized to play a protective role in cognitive health across the lifespan. This dissertation work demonstrated that independent as well as interactive consumption of both lutein and choline was associated with neurocognitive benefits for adults with overweight or obesity in middle-adulthood. This work represents additional evidence suggesting that foods high in lutein and choline may offer neuroprotective effects among persons with overweight and obesity.
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# TABLE OF CONTENTS

**CHAPTER 1: INTRODUCTION** ........................................................................................................... 1

**CHAPTER 2: REVIEW OF KEY LITERATURE** .................................................................................. 3

**CHAPTER 3: SPECIFIC AIMS AND HYPOTHESES** ......................................................................... 15

**CHAPTER 4: AIM 1-A: A CROSS-SECTIONAL EXAMINATION OF DIETARY AND RETINAL LUTEIN IN ADULTS WITH OVERWEIGHT AND OBESITY** .......................... 17

**CHAPTER 5: AIM 1-B: A RANDOMIZED CONTROLLED TRIAL EXAMINATION OF DIETARY, SERUM, AND RETINAL LUTEIN IN ADULTS WITH OVERWEIGHT AND OBESITY** .................................................................................. 37

**CHAPTER 6: AIM 2: A CROSS-SECTIONAL EXAMINATION OF DIETARY CHOLINE INTAKE IN ADULTS WITH OVERWEIGHT AND OBESITY** .......................... 72

**CHAPTER 7: AIM 3: A CROSS-SECTIONAL EXAMINATION OF INTERACTIONS BETWEEN DIETARY CHOLINE INTAKE AND BIOMARKER STATUS OF LUTEIN AND CHOLINE ON COGNITIVE CONTROL** .................................................................................. 93

**CHAPTER 8: CONCLUSIONS AND FUTURE DIRECTIONS** .......................................................... 119

**CHAPTER 9: REFERENCES** .......................................................................................................... 122
CHAPTER 1: INTRODUCTION

Recent work demonstrates that diet quality throughout the United States continuously fails to meet current dietary recommendations\(^1\). This is concerning, as diet quality has been demonstrated to play a role in progressive physical, as well as mental, co-morbidities. Currently, national dietary recommendations are developed based on comprehensive evidence for prevention of physical health related outcomes, with minimal consideration of the importance of diet quality for cognitive and mental health. Elucidation of the particular role of nutrient consumption on cognitive and mental health may provide a feasible strategy to preserving cognitive health across the life-span.

While improvements in diet quality and nutrient intake are recommended to all individuals, individuals with overweight and obesity may benefit disproportionately from these proposed dietary improvements. Defined as having a body mass index (BMI; kg/m\(^2\)) greater than or equal to 25.0, individuals with overweight and obesity are at higher risk of exhibiting deficits in cognition, particularly cognitive control, than their healthy weight peers, particularly during middle-adulthood\(^2\). Additionally, elevated weight status during middle-adulthood has been linked with a potential 35% increased risk of dementia in individuals with overweight, and 74% increased risk in individuals with obesity\(^3\). Intriguingly, these deficits in cognitive function have been observed independent of obesity’s clinical co-morbidities such as type 2 diabetes, alterations in glycemic control, and metabolic risk factors\(^4\). Therefore, research exploring cost-effective, practical, and sustainable strategies to promote healthy weight status as well as healthy cognitive
and mental health status across the lifespan are of great interest to public health agencies and individuals alike.

The work herein focuses on two particular dietary components of interest, the xanthophyll carotenoid lutein, and the vitamin-like component choline. Recent work has identified potential physical as well as cognitive benefits from increased consumption of these dietary components, but this work has yet to be done within a sample of adults with overweight or obesity. Therefore outlined in this dissertation are four examinations of the potential impact of dietary intake as well as biomarker concentrations of lutein and choline on cognitive control in young to middle aged adults with overweight or obesity.
CHAPTER 2: REVIEW OF KEY LITERATURE

Neurocognitive outcomes of cognitive control as a target for nutrition interventions in adults with overweight and obesity

Cognitive control, also known as executive function, is comprised of a set of interrelated, yet dissociable, higher order processes including inhibition (the ability to resist distractions and maintain attention), response inhibition (the ability to inhibit responses to stimuli), and cognitive flexibility (the ability to dynamically shift attention and alter response strategy to changing demands)\(^5\). These processes allow for the ability to interact with, as opposed to merely respond to, salient stimuli in our surrounding environments.

Cognitive control is frequently examined using tasks designed to assess behavioral outcomes such as accuracy and reaction time (RT). These behavioral outcomes have been demonstrated to be beneficially associated with aerobic activity and diet in human populations\(^6\)–\(^8\). While these behavioral benefits have been observed, further explanation of underlying inducting mechanisms of these benefits have been far less examined. Utilizing electroencephalographic (EEG) recording of neuroelectric potentials time-locked to task stimuli (event-related potentials [ERPs]) allows for exploration of the underlying neural mechanisms of mental chronometry potentially responsible for observed behavioral differences. EEG can reflect changes in cognitive effort irrespective of changes in performance. Specifically, potentials emitted at the level of the scalp can be used to quantify the magnitude of resources required (amplitude) and speed of processing necessary for cognitive processes (latency). In comparison to other currently available neuroimaging techniques, ERPs are considered the gold standard of temporal resolution. ERPs
allow for examination of ongoing brain activity with no delay, and studies have examined ERP potentials as early as 20ms post-stimulus onset.

Examination of responses to stimuli is made possible through the extraction of outcomes referred to as ERP components. An ERP component is defined as a voltage deflection that is produced when a specific neural process occurs in a specific brain region. Each task stimuli elicits a number of components, and when summed together, the components produce an ERP waveform (Figure 2.1). Two components of interest in the present work are the N200 (N2) and the P300 (P3, P3b). The N2 is a negative-going potential occurring approximately 200ms post-stimulus onset and is an index of response inhibition. The P300 (P3, P3b) is a positive-going potential occurring between 300-700ms post-stimulus onset as an index of context updating, resource allocation, and categorization. Longer latencies of the N2 and the P3 in response to visual stimuli have been positively associated with cognitive decline, with potential amelioration with positive health behaviors such as a healthy diet. Utilizing ERPs in conjunction with behavioral responses to explore the associations of dietary intake and biomarker concentrations may therefore explicate the dietary derived cognitive benefits previously observed in behavioral outcomes. Both latency and amplitude of the N2 and P3 component have been associated with weight status, and it has been demonstrated that individuals with overweight or obesity display larger N2 and P3 amplitudes than their healthy weight peers.
When interpreting ERP components, neural efficiency provides an explanation for differences observed. The neural efficiency hypothesis was first introduced by Haier et al. in 1988. In work utilizing utilized positron emission tomography Haier and colleagues observed that “for normal young adults for whom a cognitive task is difficult, more cortical activity throughout the brain is necessary to perform the task.” They postulated that among the individuals who performed worse on an abstract reasoning test, this recruitment of additional resources may be due to the inappropriate use of excess energy by each neuron and/or use of more neurons to perform the task. The result is thus an increase in cognitive resources utilized, lower task performance, and an overall inefficient approach to completing the task at hand. This theory has been thus replicated using EEG methodology. Neubauer et. al. report that in a Figure-spatial Posner task, those with higher figural-spatial ability need less brain activation to carry out a task and that task performance in brighter individuals is accompanied by a stronger increase in short-range communication between adjacent brain regions. This implies that efficient brain activation could be the result of better functional coupling of short-range connections. In the context of the ERP, there is general consensus that both the amplitude and latency of the N2 and P3 components can

![Figure 2.1. An example depiction of the time course of Event-Related Potentials post-stimulus onset (0 ms).](image)
be utilized as endogenous measures of processing efficiency. Latency, or onset of an ERP component, reflects the timing of mental processes. A decreased/faster latency on a task would therefore imply more efficient processing. Peak amplitude, or the largest deviation from baseline during the time-frame of a chosen component, reflects the intensity of processing. A larger peak amplitude in comparison to a smaller peak amplitude would imply the need for recruitment of additional resources\textsuperscript{15}.

\textit{Lutein and Zeaxanthin}

Lutein and zeaxanthin belong to the carotenoid class of dietary components. Carotenoids are plant pigments found in green leafy vegetables as well as eggs and avocados. Specifically, lutein and zeaxanthin are polar, oxygenated carotenoids referred to as xanthophylls. Lutein and zeaxanthin accumulate in many areas throughout the body, including but not limited to skin, adipose tissue, and serum, and they are able to cross the blood-brain interface to preferentially deposit within the macula of the eye and the brain\textsuperscript{16}. Lutein is the predominant carotenoid in the human brain, with levels in the infant brain comprising \(~59\%\) of total carotenoids, and levels in octogenarian brain samples comprising \(~34\%\) of total carotenoids\textsuperscript{17,18}. Lutein, alongside zeaxanthin and its retinal stereo-isomer, meso-zeaxanthin, accumulates in an extension of the central nervous system, the macula of the eye. Therefore, Macular Pigment Optical Density (MPOD), or retinal accumulation as an extension of brain accumulation, can be assessed using the validated and non-invasive measure of heterochromatic flicker photometry\textsuperscript{19}. MPOD levels are closely correlated to xanthophyll concentrations found in pediatric and geriatric human retina and brain samples, substantiating their status as biomarkers of retinal and neural tissue xanthophyll accumulation\textsuperscript{20,21}. Lutein quantification may also be done through serum samples. Serum lutein
may serve as a transient site of lutein until it is further able to be stored in peripheral tissues, and serum lutein may be a better marker of acute dietary lutein intake, while brain or retinal accumulation may be a better representation of chronic lutein intake\textsuperscript{18}.

Carotenoids, including xanthophylls, are not synthesized \textit{de novo}, and thus, humans must consume them from dietary sources in order to facilitate bodily accumulation. While a host of modifiable and non-modifiable factors may influence rate of lutein accumulation, as the human body is incapable of xanthophyll synthesis, levels quantified in bodily tissue are attributable to dietary intake\textsuperscript{16}. Lutein and zeaxanthin are polar, lipophilic molecules, most commonly found in plants such as fruits and vegetables, avocados, and egg yolks\textsuperscript{22}. Zeaxanthin, lutein’s structural isomer, is derived from similar food items, and thus lutein and zeaxanthin quantification from foods is aggregated in most nutrient databases. As the work herein utilized dietary records to assess xanthophyll intake, lutein and zeaxanthin will be cumulatively referred to as dietary lutein throughout.

\textit{Lutein and Cognition}

The eye is an extension of the neural system, and research has highlighted a role between visual health and cognitive health\textsuperscript{23–25}. Similarly, macular pigmentation, or MPOD, is hypothesized to play a role in cognitive and mental health\textsuperscript{26}. Previous work performed by our laboratory and others has indicated that higher accumulation of neural lutein – as assessed through retinal MPOD – is associated with cognition across the lifespan – with studies in childhood, young-to-middle adulthood, and older adulthood\textsuperscript{27–30}. While mechanistic explanations for these relationships are thus far sparse, theories point towards lutein’s role in increased neural processing speed and improved efficiency of attentional resource facilitation\textsuperscript{31}. Post-receptorally, lutein and
zeaxanthin are hypothesized to play various neuroprotective roles across the lifespan, possibly as antioxidants and anti-inflammatory agents. The brain has a high oxygen demand and is comprised of a large number of polyunsaturated fatty acids, thus making it particularly susceptible to oxidative damage. While the exact mechanisms of xanthophylls cognitive benefits are yet to be understood, it is widely accepted that the main role in the eye of retinal accumulation of xanthophylls is antioxidant, and anti-inflammatory\textsuperscript{32–34}. As levels in the retina and the brain have been demonstrated to be related, a likely theory is that xanthophylls in the brain play a role similar to their role in the retina. Additional support for xanthophylls role in inflammation draws from work in individuals with cardiovascular disease, and reports that xanthophyll status is inversely related with circulating inflammatory markers such as c-reactive protein and interleukin-6\textsuperscript{35,36}. As decrements in cognition have been linked with increased oxidative damage and inflammation, it is likely that lutein is a pertinent neuroprotective agent in this context\textsuperscript{37}. Another theory for the mechanism of xanthophyll action in the brain surrounds brain integrity\textsuperscript{38}. Loss of white matter integrity has been associated with a reduction in processing speed. This loss in integrity has also been associated with age, as well as diet. Supplementation of xanthophylls may thus promote increased brain integrity, facilitating neural efficiency.

\textit{Choline}

Intake of choline, a vitamin-like water-soluble nutrient, has been linked to cognitive improvements in human populations among both perinatal and geriatric populations\textsuperscript{39}. Humans are capable of \textit{de novo} choline biosynthesis through the phosphatidylethanolamine N-methyltransferase pathway, albeit in concentrations that are insufficient to maintain many biological processes\textsuperscript{40}. Thus, it is imperative that choline be consumed through diet. Common
foods rich in choline include eggs (147 mg/serving), soybeans (107 mg/serving), chicken breast (72 mg/serving), and beef (72 mg/serving). The Institute of Medicine recommends an Adequate Intake (AI) for choline of 425 mg/d for women and 550 mg/day for men. However, 90% of the US population does not meet this AI recommendation. Choline is vital for neurotransmitter synthesis (as the precursor to acetylcholine [ACh]), cell-membrane integrity and signaling function (through phosphatidylcholine [PC]), and methyl-donor metabolism (through the conversion of methionine to homocysteine), and lipid-cholesterol transport and metabolism. Higher choline intake has also been associated with lower whole body %fat and higher levels of lean mass amongst both animal and clinical models. Choline plays a vital role in central nervous system development and choline supplementation has been shown to benefit memory and brain development in human and pre-clinical models. Phosphatidylcholine is the most prevalent form of choline in the body and it forms essential structural components of cell membranes and myelin sheaths throughout the brain and the body.

**Choline and Cognition**

Choline plays a vital role in central nervous system development. Investigation into the proposed mechanisms of action for choline and its related metabolites for cognitive performance and maintenance are ongoing. Theories include: choline’s role as a methyl donor, and through DNA methylation, potential modulation of expression of genes involved in learning and memory processing, prevention against the pathogenetic phospholipid defects potentially specific to AD, and choline’s indirect reduction of plasma homocysteine concentrations. Circulating PC concentrations have been found to be lower in patients with AD, and alterations in peripheral and brain PC/lipid metabolism among patients with AD have been demonstrated. Adequate
concentrations of choline in the brain are believed to protect against age-related cognitive decline and certain types of dementia\textsuperscript{46}. These findings allude to a potential benefit of circulating PC for cognitive health, though testing of this relationship has been problematic due to heterogeneity of both results and testing methods. In elderly patients with AD, higher concentrations of free choline have been observed within the brain\textsuperscript{49}. These higher concentrations of free choline have been interpreted as a marker of neurodegeneration, in what has been referred to as “autocannibalism” – or the brains degenerating attempts to release choline from its largest storage site (PC) and compensate for the characteristic lack of free choline and ACh\textsuperscript{49}. Much of current AD treatment is based around this choline deficit, through the use of cholinergic agonists to promote choline production and preserve cell membrane integrity\textsuperscript{50}.

Regardless, much evidence for the imperative role of choline in brain development begins with prenatal development. Rodent research has demonstrated that rats born to choline-deficient mothers have alterations to hippocampal structure and function\textsuperscript{51,52}. Similar results have been shown in the piglet model, perhaps the animal model closest anatomically as well as physiologically to the human brain. Piglets born to sows on choline-deficient diets have been found to exhibit decreased total brain volume when compared to piglets born from choline-sufficient sows\textsuperscript{53}. These findings have been mirrored in recent human work as well. The closest replication of this animal work is evidenced through the Project Viva Study. Two iterations of literature have emerged from this largescale longitudinal study – one at years 3 of age and another at age 7. At age 3, there was no observed relationship between maternal choline intake and cognitive outcomes, yet at age 7 higher maternal choline intake was associated with improved visual memory\textsuperscript{54,55}. Published work conducted in other age groups suggests that high choline intake as well as choline
supplementation has benefits for memory and brain development, and higher phosphatidylcholine (PC) intake has been found to be associated with a lower risk of dementia\textsuperscript{56}. In the Framingham Offspring Study, Poly et al. reported that middle-aged, healthy individuals (mean age = 60.1 years) with higher choline consumption five years prior to fMRI testing showed an inverse relationship with choline intake and White Matter Hyperintensity (WMH), an associated marker of cognitive impairment and Alzheimer’s Disease\textsuperscript{46}. As white matter integrity has been associated with greater performance on cognitive variables such as reading, IQ, information processing, and attention, this again points to the neuroprotective role of choline in the neocortex across the lifespan\textsuperscript{57}. WMH has also been inversely associated with obesity, in particular, the metabolically active visceral adiposity has been positively associated with the increased presence of WMH\textsuperscript{58}. As choline intake has also been shown to be inversely related to whole body \%fat positively associated with higher levels of lean mass, increasing choline intake may be of particular importance to individuals with overweight or obesity.

\textit{Choline and lipid production}

Beyond the direct role of choline on ACh in the brain, choline acts as a regulator of hepatic lipid secretion. In animal studies, choline deficiency has been shown to induce fatty liver within hours of diet initiation\textsuperscript{59}. PC is necessary to form lipoprotein particles (particularly VLDL and LDL), and with insufficient dietary intake of choline, PC is not capable of being produced in quantities able to support lipoprotein synthesis. Thus, consequences are two-fold: there is intracellular accumulation of triglycerides within hepatic cells, and there is diminished transport of dietary components carried throughout the body on lipoproteins. One such dietary component that relies on lipoprotein synthesis for peripheral distribution is lutein.
Nutrient Interaction vs. Association

While comparably greater progress has been made in identifying single-nutrient benefits on cognitive health, research has presently frequently failed to take into account the interactive action and metabolism of nutrient combinations. It is not likely that the benefits of nutrition for cognitive health and cognitive decline are solely the result of small and summative independent nutrients. Determination of beneficial interactive potential may help inform food and dietary pattern level recommendations. Theoretically, and statistically, untangling the independent versus interactive impacts of nutrients though has been problematic. The term interactive means “when the effect of one explanatory variable depends on the particular level or value of another explanatory variable.” In comparison, the term association means “information about the value of one variable conveys information about the average value of another variable.” Put more simply, an interaction would be examining if the cognitive benefit of consuming high amounts of lutein is greater when consuming higher amounts of choline, or vice versa.

Potential interactive effects of lutein and choline

While current trends in research have explored the impact of single-nutrient intake on measures of physical and mental health, nutrients are not often consumed in isolation. Recent evidence has supported the beneficial role of nutrition in cognitive health, yet explanations of mode of delivery for nutrition are varied. The majority of nutrition research has focused on either the impact of a single nutrient or an entire food-source on physical, mental, and social health outcomes. The gap between these two methodologies is vast, and leaves many questions unanswered about the potential interactive capabilities of dietary components and whole food-sources on health.
related outcomes, particularly those cognitively-related. If nutrition is to be utilized as a means of cognitive maintenance and prevention of cognitive decline, further examination of these interactive capabilities is necessary.

One reason for identification of the potential interactions of lutein and choline is drawn from collaborative roles in absorption and circulation. To promote the bioavailability of xanthophylls, most randomized controlled trials promote consumption of lutein with a source of fat. Without a fatty matrix for the xanthophyll to adhere to, there is significantly decreased absorption. Much work has explored the impact of phospholipid (PL) type on absorption of carotenoids in vitro and in vivo. These studies have focused on PC and its hydrolyzed isomer, lyso-phosphatidylcholine (LysoPC). Within the small intestine, LysoPC is produced by reversible hydrolysis of PC by the pancreatic enzyme phospholipase-A2, and constitutes roughly 80% of the total phospholipids of duodenal content in humans. Work has demonstrated significantly increased carotenoid absorption in lysoPC compared to PC, and significantly increased absorption in PC compared to non-choline containing PL’s. Therefore, dual intake of dietary lutein and varying types of choline will promote maximal carotenoid absorption within the small intestine.

Within the brain, lutein serves as one of several anti-oxidants, and choline (derived from the diet or endogenous production) can serve as a precursor for the neurotransmitter ACh. ACh has been demonstrated to influence neuronal excitability, alter presynaptic release of neurotransmitters, and coordinate the firing of groups of neurons. In rodent models, high acetylcholinesterase (the enzyme that catalyzes breakdown of ACh) activity in the hippocampus and cortex has been associated with ethanol-induced memory impairments. Oral administration of lutein was demonstrated to attenuate both acetylcholinesterase activity and ethanol-induced memory impairment compared to a control group. Notably, a limitation of clinical research is the
inability to evaluate concentrations of cerebral ACh. It is possible that the benefits derived from combined consumption of lutein and choline are due to an increase in cerebral ACh production, driven perhaps by lutein’s attenuation of acetylcholinesterase activity and high amount of choline intake.

A single study on the potential interaction between lutein and choline and its subsequent effects on cognition has been conducted by Cheatham and et. al. They examined the relationship between choline, docosahexaenoic acid (DHA), and lutein on recognition memory in six-month old infants. Nutrient intake was assessed through analysis of breast milk, and recognition memory was assessed using a familiar/novel paradigm while infants underwent ERP testing. They observed that interactions between choline and DHA, and choline and lutein, were significant in predicting latency to peak amplitude in a recognition task while the dietary components in isolation were not. This work has yet to be extended beyond of the infant population and has not been expanded to cognitive control domains.

While understanding the individual contribution of dietary components to health related outcomes has been informative, understanding interactive effects may have increased translation. Thus, to expand on research already conducted, complementary nutrition research should focus on understanding the impact of interactions between nutrients. The work herein will elucidate the individual, as well as interactive, contributions of the dietary components lutein and choline on cognitive health.
CHAPTER 3: SPECIFIC AIMS AND HYPOTHESES

The dietary components lutein and choline have been hypothesized to play a protective role in cognitive health. Thus, work elucidating their independent and interactive impacts on cognition is warranted.

Aim 1

Aim 1-a: Cross-sectionally examine the role of retinal xanthophyll accumulation on behavioral and neuroelectric components of cognitive control in adults with overweight or obesity.

_Hypothesis:_ Individuals with higher retinal xanthophyll accumulation will exhibit higher behavioral performance and neuroelectric patterns, signifying greater neural efficiency.

Aim 1-b: Determine the effect of a 12-week daily Hass avocado randomized controlled trial on changes in serum lutein status, retinal xanthophyll concentration, and cognitive control in adults with overweight or obesity.

_Hypotheses:_

1. The treatment group receiving a daily meal with an avocado will exhibit increases in lutein status (i.e., serum and retina) and improvements in behavioral and neuroelectric indices of cognitive control, relative to the control group.

2. Increases in lutein status will be predictive of improvement in cognitive function.

Aim 2

Aim 2: Cross-sectionally examine the role of choline intake on behavioral and neuroelectric components of cognitive control in adults with overweight or obesity.
**Hypothesis:** Individuals with higher dietary choline intake will exhibit higher behavioral performance and neuroelectric patterns signifying greater neural efficiency.

**Aim 3**

**Aim 3:** Cross-sectionally examine the independent and interactive roles of choline and lutein intake and choline and lutein status on cognitive control in a sample of adults with overweight and obesity.

**Hypotheses:**

1. Individuals with higher dietary intake of lutein and choline will have higher retinal xanthophyll accumulation, and higher accumulation of choline metabolites (phosphatidylcholine and free choline).

2. The interactions between dietary intake of lutein and choline, and biomarker accumulation of these dietary components will be associated with benefits in cognitive control.
CHAPTER 4: AIM 1-A: A CROSS-SECTIONAL EXAMINATION OF DIETARY AND RETINAL LUTEIN IN ADULTS WITH OVERWEIGHT AND OBESITY

Abstract

Scope: Macular accumulation of xanthophyll carotenoids (lutein, zeaxanthin) is known to have neuroprotective potential, yet their influence on cognition among adults with overweight and obesity remains limited. This study examined the impact of macular xanthophylls on attentional resource allocation and information processing speed among adults with BMI ≥ 25 kg/m².

Methods and Results: Adults between 25-45 years (N=101) completed heterochromatic flicker photometry to determine macular pigment optical density (MPOD). Event-related brain potentials were recorded during a visual oddball task. Amplitude and latency of the N2 and P3 indexed attentional resource allocation and quantified information processing speed. Covariates included age, sex, education, intelligence quotient (IQ), %Fat (DXA) and dietary lutein and zeaxanthin (Diet History Questionnaire II). MPOD was inversely related to P3 peak amplitude during the standard trials and P3 peak latency during the target trials. Therefore, individuals with higher MPOD dedicated fewer attentional resources when attentional demands were low while exhibiting faster information processing speed when attentional demands were increased. Further, MPOD was inversely related to the N2 mean amplitude during target trials, signifying greater inhibitory control.

Conclusion: These findings are the first to link macular xanthophylls to neuroelectric indices of attentional and inhibitory control among adults with overweight and obesity.

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Introduction

Dietary quality amongst adults is continuously falling short of national recommendations\textsuperscript{67}. Concurrent with a failure to meet recommendations, the prevalence of overweight and obesity in the United States continues to rise\textsuperscript{68}. This is concerning, as diet quality is an essential component of both physical and mental health. Poor diet quality coupled with overweight and obesity is associated with a number of co-morbidities, including but not limited to: type 2 diabetes, cardiovascular disease, and cognitive decline. Recent work has indicated that diet, even after accounting for pertinent body composition and demographic variables, has an increasingly relevant role in cognitive health \textsuperscript{69}. Thus, further investigation of the interactions between diet and cognition amongst individuals with overweight and obesity is warranted.

A group of dietary components identified as carotenoids, in particular the xanthophylls lutein and zeaxanthin, have been demonstrated to be pertinent to cognitive health \textsuperscript{30,70}. Lutein, a non-polar, lipophilic molecule, is most commonly found in plants – primarily fruits and vegetables, as well as egg yolks \textsuperscript{22}. Zeaxanthin is derived from similar food items, and thus lutein and zeaxanthin quantification from foods is often aggregated across these xanthophylls. Importantly, lutein is able to cross the blood-brain barrier, and is the predominant carotenoid in the human brain, and has been shown to comprise nearly 77\% of infant brain carotenoid concentrations \textsuperscript{20}. Zeaxanthin can similarly cross the blood-brain barrier, yet has been shown to accumulate in smaller quantities \textsuperscript{21}. Conveniently, lutein also accumulates in the macula of the eye alongside zeaxanthin and its retinal isomer, meso-zeaxanthin \textsuperscript{21}. Thus, xanthophyll status can be assessed through self-report measures of dietary intake, while Macular Pigment Optical Density (MPOD), or retinal accumulation, can be assessed using the validated and non-invasive measure of heterochromatic flicker photometry. MPOD levels are closely correlated to xanthophyll
concentrations found in pediatric and geriatric human retina and brain samples, substantiating their status as biomarkers of retinal and neural tissue xanthophyll accumulation\textsuperscript{20,21}.

Preliminary research has indicated that higher accumulation of neural lutein – as assessed through retinal MPOD levels – is associated with cognition across the lifespan – with studies in childhood, young-to-middle adulthood, and older adulthood\textsuperscript{27-30}. While mechanistic explanations for these relationships are thus far sparse, theories point towards lutein’s role in increased neural processing speed and improved efficiency of attentional resource facilitation\textsuperscript{31}. Post-receptorally, lutein and zeaxanthin are hypothesized to play various neuroprotective roles across the life-span, primarily as antioxidants and anti-inflammatory agents. The brain has a high oxygen demand, and is comprised of a large number of polyunsaturated fatty acids, thus making it particularly susceptible to oxidative damage. While the exact mechanisms of xanthophylls cognitive benefits is yet to be understood, it is theorized that the main role of retinal accumulation of xanthophylls is antioxidant, and anti-inflammatory\textsuperscript{32-34}. As levels in the retina and the brain have been demonstrated to be related, a likely theory is that xanthophylls in the brain play a role similar to their role in the retina, though theories related to xanthophylls role in epigenetics and plasticity have been proposed. Additional support for xanthophylls role in inflammation draws from work in individuals with cardiovascular disease, and reports that xanthophyll status is inversely related with circulating inflammatory markers such as c-reactive protein and interleukin 6\textsuperscript{35,36}. As decrements in cognition have been linked with increased oxidative damage and inflammation, it is likely that lutein has a neuroprotective role in this context\textsuperscript{37}.

Consumption of lutein and zeaxanthin at all stages of life is important, with particular importance for individuals who may already be at risk for disruptions in cognitive function, such as individuals with overweight and obesity\textsuperscript{71}. Defined as having a body mass index (BMI; kg/m\(^2\))
greater than 25.0, individuals with overweight and obesity are at higher risk of exhibiting deficits in cognition, particularly cognitive control, than their healthy weight peers. Intriguingly, these deficits in cognitive function amongst individuals with overweight and obesity have been observed independently of obesity’s clinical consequences such as type 2 diabetes, alterations in glycemic control, and metabolic risk factors. Thus, the introduction of lutein and zeaxanthin into diet may serve as a relatively cost-effective approach to preventing, or delaying, cognitive impairments due to increased adiposity. An inverse relationship has been proposed between BMI and whole body percent fat (%Fat) and MPOD levels, though both study sample populations as well as results shown have been inconsistent. The present study is the first to attempt to examine this relationship in a sample comprised of solely individuals with overweight or obesity. Regardless, the growing population of individuals with overweight or obesity may exhibit benefit from increased lutein and zeaxanthin intake similar to their healthy weight peers.

The present study aimed to elucidate the relationship between macular xanthophylls and cognitive control in a population of young-to-middle aged adults with overweight and obesity. To assess cognition, event-related potentials (ERPs) were extracted from electroencephalographic recordings during a two-stimulus visual Oddball task. Cognitive control is a set of interrelated, yet dissociable, set of executive functions that form the foundation for higher-order behaviors such as goal-planning, reasoning, and delay of gratification. Attentional and inhibitory abilities, in particular, are vital as they allow for attention to relevant environmental stimuli and inhibition to irrelevant stimuli. ERPs are the gold standard for evaluating temporal resolution in response to visual stimuli. Thus, ERPs allow for examination of behavioral measures (i.e. accuracy and reaction time) as well the neuroelectric underpinnings that induce behaviors and allow for elucidation of the theories proposing a role for lutein in processing speed and efficiency.
Specifically, within the Oddball task, we investigated the N2 (N200) and P3 (P300, P3b) components. The N2 is a negative-going component occurring roughly 200-300 ms post-stimulus onset. The P3 is a positive-going component occurring roughly 300-700 ms post-stimulus onset. Longer latencies of the N2 and the P3 in response to visual stimuli have been positively associated with cognitive decline, with potential amelioration with positive health behaviors such as a healthy diet. Previous work has also indicated that adults with overweight and/or obesity exhibit poorer performance during attentional and inhibitory control tasks. However, the extent to which macular xanthophylls may provide neuroprotective effects – as assessed by ERPs – among adults with elevated weight status has not been directly investigated. Our central hypothesis was that individuals with higher macular xanthophyll concentrations – as assessed via MPOD – would exhibit neuroelectric patterns signifying greater neural efficiency as indicated by faster ERP component latencies and diminished amplitudes for the N2 and P3 components during a visual Oddball task.

**Experimental Section**

*Participants*

Adults between 25-45 years of age with a BMI > 25.0 kg/m² (N=101, mean = 32.78 ± 5.46) were recruited from the East-Central region of Illinois using university e-mails and flyers posted in community buildings frequented by the public. All subjects provided written informed consent prior to study participation. All procedures were administered in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Illinois. Participant exclusion criteria included BMI < 25 kg/m², pregnancy, history of neurological disease, history of chronic metabolic diseases, or non-normal or uncorrected vision based on the minimal 20/20 standard.
Anthropometrics and Adiposity

Height and weight measurements were completed to calculate BMI using the formula: weight [kg]/height [meters$^2$]. Measurements were conducted in triplicate while wearing lightweight clothing and no shoes, and the average was used for BMI calculation. A stadiometer (model 240; SECA, Hamburg, Germany) and a digital scale (WB-300 Plus; Tanita, Tokyo, Japan) were used to measure height and weight, respectively.

Adipose tissue, specifically, %Fat, was measured by dual energy x-ray absorptiometry (DXA) using a Hologic Horizon W bone densitometer (APEX Software version 5.6.0.5; Hologic, Bedford, MA). %Fat was computed using the standard Hologic software, as previously described$^7$. 

Dietary Lutein and Zeaxanthin Assessment

Habitual dietary consumption of lutein and zeaxanthin were quantified using the National Cancer Institute’s (NCI) Diet History Questionnaire II with portion sizes$^7$. The Diet History Questionnaire II is a self-report food-frequency questionnaire consisting of 142 items. Data were analyzed using National Cancer Institute’s Diet*Calc (Diet*Calc Analysis Program, Version 1.5.0.) software to quantify nutrient values. The primary variable of interest for the present study was the aggregate composite measure of lutein and zeaxanthin.

Intellectual Ability Assessment

The Kaufman Brief Intelligence Test, Second Edition (KBIT-2) was used to assess intelligence quotient (IQ). The KBIT-2 has been nationally normed for ages 4 – 90 to assess general intellectual abilities and has been shown to have comparable scores to other intelligence
scales\textsuperscript{79,80}. The KBIT-2 is comprised of three subtests. The verbal knowledge subtest includes 60 questions where the participant responds by choosing which image is most associated with the word or question spoken by the researcher. The riddle subtest consists of 48 riddles that have a single word response. The matrices subtest has 46 logic problems where the participant must choose which of 6 pictures is most associated with a single stimulus picture or which picture best completes a 2x2, 2x3, or 3x3 matrix. A summative IQ score is generated by combining the score of all three subtests, and normalizing for participant age.

\textit{Macular Pigment Optical Density}

MPOD was assessed via a customized hetero-flicker photometry (cHFP) technique administered using a macular densitometer (Macular Metrics Corporation, Rehoboth, MA, USA). The principles of this technique have been previously described. Briefly, the assessment was performed by trained members of the research staff. Participants were asked to view stimuli peaking at a measuring wavelength of 460 nm that flickers in counter phase with a 570 nm reference (flicker rate being optimized for the optimal width of the subject’s null zone). Participants were asked to adjust the radiance to identify a null flicker zone by indicating when they could no longer detect the flicker. The task is conducted while the stimulus is centrally-fixated (measuring macular pigmentation where it is most dense) and at 7 degrees in the para-fovea (where density is minimal). The MPOD score, with standard deviation, is calculated by subtracting the foveal from the parafoveal log sensitivity measurements after normalizing at 570 nm.
Attention and Neuroelectric Function

Cognitive control was assessed using a two-stimulus visual oddball task. During this task, participants viewed a series of large and small white circles on a black background presented serially with large circles (5.5 cm diameter) or smaller circles (3 cm diameter, **Figure 4.1**). The larger circles served as the “target” stimulus and were presented during 20% of the trials in a random order. The smaller circles served as the “standard” stimulus and were presented during the remaining 80% of trials. Participants were instructed using scripted and standardized language to respond to the target trials with a right button press. Additionally, participants were positioned at a standardized distance away from the stimuli presentation screen. Participants were presented with a practice block of 30 trials followed by 200 experimental trials. Stimuli were presented for 100 ms with a 1000 ms response window and an inter-trial interval of 2000 ms. This task has been described in detail previously.

**Figure 4.1.** Task stimuli and parameters for the Oddball task. Participants viewed a series of large and small circles presented serially with large “target” circles (5.5 cm diameter) or small “standard” circles (3 cm diameter). Participants completed a practice block of 30 trials followed by 200 experimental trials.
During completion of the oddball task, electro-encephalographic (EEG) activity was recorded via a Neuro-scan Quik-cap with 64 scalp electrodes arranged in the international 10-10 system. A midline sensor placed between Cz and CPz served as a reference and AFz served as the ground. Using a Neuroscan Synamps2 amplifier, continuous EEG signal was digitized at a sampling rate of 500 Hz, amplified 500 times to 70-Hz filter with a direct current and a 60-Hz notch filter. Electro-oculographic (EOG) activity was recorded with a set of four electrodes placed at the outer canthus of each eye and above and below the left orbit.

Offline, continuous data were re-referenced to the average mastoids. An independent components analysis (ICA) was used to systematically reject eye-blink artifacts from the data. Data were submitted to a 0.1 Hz high-pass filter before being submitted to the ICA. If a component identified during the ICA was correlated at or above 0.35 with the vertical EOG channel, it was considered an eye-blink and subsequently rejected. A time window of -200 to 1200 ms around stimulus onset was used for creating stimulus-locked epochs with a -200 to stimulus onset used for baseline correction. A 30-Hz zero phase shift low-pass filter was employed. ERP analysis was limited to trials with correct responses. Epochs were excluded if the moving window peak-to-peak amplitude exceeded ±100 μV using a 100 ms window and a 50 ms window step. ERP variables of interest were the N2 and P3 mean amplitude, peak amplitude, and peak latency. Mean amplitude refers to the average amplitude over a given time period (stated per component below) and peak amplitude refers to the crest of the waveform during a given time period. Electrode locations were determined following post-hoc analysis of maximal voltage localization. The N2 was maximal at FZ, and was defined as the average and localized amplitudes and corresponding latency 200-350ms post-stimulus onset (Figure 4.2). The P3 was maximal over a 6-sensor region of interest.
(ROI) comprised of C1, CZ, C2, CPZ, CP1, and CP2, and was defined as the localized peak and corresponding latency occurring between 350-750 ms after stimulus onset (Figure 4.3).

**Statistical Analyses**

Data were examined for normality using the Shapiro-Wilk test. Given the non-normalized distribution of cognitive task measures, non-parametric approaches were utilized to examine interrelationships between MPOD and cognitive task performance while adjusting for potentially confounding variables. First, variables thought to be predictive of MPOD and cognitive function including age, sex, socioeconomic status, education level, %Fat, IQ, and dietary lutein and zeaxanthin were initially subjected to Spearman’s Rho (ρ) bivariate correlations. Subsequently, variables found to be associated with either the cognitive task measures (i.e., behavioral performance, P3 amplitude and latency) or MPOD were adjusted for using partial correlational analyses. Further, for illustration purposes, participant cognitive task performance was assessed across the lowest and highest quartile scores for MPOD. All analyses were conducted using SPSS version 25 (IBM). Two-tailed tests are reported, with alpha of 0.05 for determining statistical significance.

**Results**

**Participant Characteristics**

Participant descriptive characteristics and task performance are summarized in Tables 4.1 and 4.2. Participants ranged in age from 25 to 45 years (Mean 34.98 ± 5.85 years). Females comprised the larger proportion of the study sample (70%) and the vast majority of the sample completed had completed a college degree (84%). Sixty one percent of the participants had
obesity (i.e., BMI ≥ 30 kg/m²) whereas 39% were of overweight status (i.e., BMI 25 to 29.9 kg/m²). Approximately 63% of the participants reported consuming less than 2000 µg/day of Lutein + Zeaxanthin. The average MPOD of our sample was 0.48 ± 0.26 log units.

Table 4.1. Participant Characteristics and Task Performance

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.98 ± 5.85</td>
</tr>
<tr>
<td>Sex (F, M)</td>
<td>70, 31</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No College Degree, n (%)</td>
<td>16 (16)</td>
</tr>
<tr>
<td>Undergraduate College Degree, n (%)</td>
<td>37 (36)</td>
</tr>
<tr>
<td>Post Graduate Degree, n (%)</td>
<td>48 (48)</td>
</tr>
<tr>
<td>BMI</td>
<td>32.78 ± 5.46</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight, n (%)</td>
<td>39 (39)</td>
</tr>
<tr>
<td>Obese, n (%)</td>
<td>62 (61)</td>
</tr>
<tr>
<td>%Fat</td>
<td>39.4 ± 8.1</td>
</tr>
<tr>
<td>IQ</td>
<td>107.9 ± 12.4</td>
</tr>
<tr>
<td>MPOD</td>
<td>0.48 ± 0.26</td>
</tr>
</tbody>
</table>

MPOD, macular pigment optical density; IQ, intelligence quotient; BMI, body mass index
Table 4.2. Participant Task Performance and ERPs

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Accuracy, %</td>
<td>91.20 ± 11.22</td>
</tr>
<tr>
<td>Target Accuracy, %</td>
<td>87.53 ± 10.23</td>
</tr>
<tr>
<td>Target Reaction Time, ms</td>
<td>491.15 ± 62.18</td>
</tr>
<tr>
<td>Standard P3 ROI Peak Amplitude, µV</td>
<td>6.32 ± 5.03</td>
</tr>
<tr>
<td>Standard P3 ROI Mean Amplitude, µV</td>
<td>3.87 ± 4.42</td>
</tr>
<tr>
<td>Standard P3 ROI Peak Latency, ms</td>
<td>572.40 ± 119.92</td>
</tr>
<tr>
<td>Target P3 ROI Peak Amplitude, µV</td>
<td>12.70 ± 6.07</td>
</tr>
<tr>
<td>Target P3 ROI Mean Amplitude, µV</td>
<td>8.84 ± 5.64</td>
</tr>
<tr>
<td>Target P3 ROI Peak Latency, ms</td>
<td>562.77 ± 110.77</td>
</tr>
<tr>
<td>Standard N2 Peak Amplitude, µV</td>
<td>3.50 ± 3.88</td>
</tr>
<tr>
<td>Standard N2 Mean Amplitude, µV</td>
<td>-1.27 ± 3.84</td>
</tr>
<tr>
<td>Standard N2 Peak Latency, ms</td>
<td>260.97 ± 37.38</td>
</tr>
<tr>
<td>Target N2 Peak Amplitude, µV</td>
<td>2.99 ± 4.50</td>
</tr>
<tr>
<td>Target N2 Mean Amplitude, µV</td>
<td>0.27 ± 4.58</td>
</tr>
<tr>
<td>Target N2 Peak Latency, ms</td>
<td>261 ± 37.0</td>
</tr>
</tbody>
</table>

ROI, region of interest

**Bivariate Correlations**

Bivariate correlations are summarized in Table 4.3. Sex (females coded as 0 and males coded as 1) was inversely related to %Fat ($\rho=-0.74$, $P<0.01$) and positively related to accuracy during the target trials ($\rho=0.20$, $P=0.04$). Dietary lutein and zeaxanthin was positively related to accuracy during the standard trials ($\rho=0.20$, $P=0.05$) and MPOD ($\rho=0.22$, $P=0.03$). MPOD was inversely related to the N2 mean amplitude during the target trials ($\rho=-0.21$, $P=0.04$) and P3 peak latency during the Target trials ($\rho=-0.28$, $P<0.01$). Finally, there were no significant associations between age, %Fat, IQ, and MPOD and cognitive outcomes of interest (all $P$’s > 0.07).

**Partial Correlations**

Subsequent partial correlations were conducted to determine the relationship between MPOD and cognitive outcomes following adjustment of potential covariates observed to influence
either MPOD or cognitive outcomes based on the initial bivariate correlations (i.e., sex and dietary lutein and zeaxanthin). These covariates were applied to the N2 and P3 analyses. In regard to the N2, the relationship for mean amplitude in the target trials remained significant ($\rho=-0.21, P=0.04$). In regard to the P3, following adjustment for sex and dietary lutein and zeaxanthin, the negative association between MPOD and P3 peak amplitude during the standard trials persisted ($\rho=-0.22, P=0.03$). Similarly, the inverse association observed between MPOD and P3 peak latency during the target trials persisted, even after adjusting for covarites ($\rho=-0.27, P<0.01$). Therefore, individuals with higher MPOD exhibited faster neural processing speed during the target trials. Trends were observed between MPOD and P3 mean amplitude during the standard trials ($\rho=-0.18, P=0.07$) as well as the N2 peak amplitude ($\rho=-0.18, P=0.08$). Grand average ERP waveforms comparing the lowest and highest quartile of MPOD values are illustrated in Figure 4.2 and 4.3.

Discussion

A converging body of work implicates excess adiposity as a contributing factor to poorer cognitive function across the lifespan\textsuperscript{2,4}. However, the identification of dietary factors with the potential to provide neuroprotection among populations with elevated weight status has been limited. The present study is the first to examine relationships between macular xanthophyll accumulation and neuroelectric indices of attention and inhibition among in a sample comprised of only adults with overweight or obesity. Following adjustment for pertinent demographic and body composition variables, we observed statistically significant relationships between both the N2 and P3 ERP components in regard to amplitude and P3 latency. Given that no associations between MPOD and cognitive control were observed at the behavioral level (i.e., accuracy and reaction time), these findings provide support for the potential benefits of higher macular xanthophyll status for neural efficiency during cognitive task performance.
Table 4.3. Spearman’s Bivariate Correlations Between Subject Characteristics, Macular Carotenoids, and Attention

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Sex</th>
<th>Education</th>
<th>IQ</th>
<th>%Fat</th>
<th>L + Z</th>
<th>MPOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Education</td>
<td>-0.02</td>
<td>0.05</td>
<td>0.24*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IQ</td>
<td>-0.06</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>%Fat</td>
<td>0.08</td>
<td>-0.74**</td>
<td>-0.17</td>
<td>-23*</td>
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<tr>
<td>L + Z</td>
<td>-0.02</td>
<td>-0.05</td>
<td>0.09</td>
<td>0.03</td>
<td>0.02</td>
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<tr>
<td>MPOD</td>
<td>-0.07</td>
<td>0.06</td>
<td>0.19</td>
<td>0.18</td>
<td>-0.07</td>
<td>0.22*</td>
<td>-</td>
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<tr>
<td>Standard Accuracy</td>
<td>-0.08</td>
<td>0.02</td>
<td>0.13</td>
<td>0.16</td>
<td>-0.10</td>
<td>0.20*</td>
<td>0.16</td>
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<tr>
<td>Target Accuracy</td>
<td>0.08</td>
<td>0.20*</td>
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<td>0.12</td>
<td>-0.18</td>
<td>0.04</td>
<td>0.02</td>
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<tr>
<td>Target Reaction Time</td>
<td>-0.01</td>
<td>-0.09</td>
<td>0.08</td>
<td>0.07</td>
<td>-0.10</td>
<td>0.08</td>
<td>0.10</td>
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<tr>
<td>Standard P3 ROI Peak Amplitude</td>
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<td>0.02</td>
<td>-0.02</td>
<td>0.14</td>
<td>-0.04</td>
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<td>0.00</td>
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<td>0.16</td>
<td>-0.11</td>
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<td>-0.04</td>
<td>-0.15</td>
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<td>0.12</td>
<td>-0.03</td>
<td>-0.19</td>
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<tr>
<td>Target P3 ROI Peak Amplitude</td>
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<td>-0.07</td>
<td>0.01</td>
<td>0.05</td>
<td>0.14</td>
<td>0.10</td>
<td>-0.06</td>
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<tr>
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<td>-0.05</td>
<td>0.12</td>
<td>-0.09</td>
<td>-0.28**</td>
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<td>-0.12</td>
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<td>Standard N2 Mean Amplitude</td>
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<tr>
<td>Standard N2 Peak Latency</td>
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<td>-0.07</td>
<td>0.01</td>
<td>0.04</td>
<td>-0.03</td>
<td>-0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Target N2 Peak Amplitude</td>
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<td>-0.07</td>
<td>-0.14</td>
<td>0.214*</td>
<td>-0.03</td>
<td>-0.18</td>
</tr>
<tr>
<td>Target N2 Mean Amplitude</td>
<td>0.13</td>
<td>-0.05</td>
<td>-0.02</td>
<td>-0.11</td>
<td>0.19</td>
<td>-0.01</td>
<td>-0.210*</td>
</tr>
<tr>
<td>Target N2 Peak Latency</td>
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<td>0.00</td>
<td>-0.07</td>
<td>0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>0.10</td>
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</tbody>
</table>

*P<0.05
**P<0.01

L + Z, dietary lutein and zeaxanthin; ROI, region of interest
Figure 4.2. Waveform representations of the N2 Standard and Target Trials at electrode site FZ among the highest and lowest quartiles of MPOD scores, and representation of the maximal voltage peak over FZ.
Figure 4.3. Waveform representations of the P3 Standard and Target Trials in a region of interest (ROI; 6 electrode sites - C1, CZ, C2, CPZ, CP1, and CP2) among the highest and lowest quartiles of MPOD scores, and representation of the maximal voltage peak over the ROI.
A growing body of work supports the theory that higher neural lutein concentrations, assessed through MPOD, play a role in increased neural processing speed and efficiency of attentional resource facilitation. These results, as well as other findings from our research laboratory, support these claims of neural efficiency\(^2^7\). Many of the recent findings surrounding lutein and neuroefficiency are rooted in studies utilizing the fMRI technique to assess efficiency using blood-oxygen-level-dependent (BOLD) signaling. Colleagues Lindergh et. al. and Mewborn et. al. have demonstrated efficiency in the spatial dimension, noting increased efficiency in older adults in tasks of both verbal learning and visual-spatial processing, respectively\(^8^2,^8^3\). Relying on the BOLD signal allows for unmatched spatial resolution, and provides invaluable information on the regions of the brain recruited to complete a task. Yet, one limitation of fMRI is its lack of temporal resolution. Our current findings build on these, by demonstrating neural efficiency in the temporal dimension amongst individuals with overweight and obesity.

We observed lower mean (N2) and peak (P3) amplitudes with higher MPOD levels, as well as faster latency (P3) in an two-stimulus visual Oddball task. This task, in comparison to its auditory counterpart, relies on improved visual processing. Retinal xanthophyll accumulation has been shown to be related to lutein accumulation in samples of the visual cortex in older adults, and has been shown to improve components of visual processing such as contrast sensitivity, self-report measures of visual fatigue, and potentially even neuronal signaling efficiency in the eye\(^2^0,^8^4^–^8^6\). It is thus possible that improved visual processing underlies the efficiency relationships observed here – with initial efficient processing of visual stimuli we can see increased downstream efficiency of higher-order tasks. Important to note is the difference in size of the target and standard stimuli. While the same color scheme was used across trials (white stimuli on a black background)
it is possible that the larger, target circles, indeed require the use of higher amounts of MP and thus would be more easily, and perhaps more efficiently, identified in individuals with higher MPOD levels. Mean and peak amplitude of the N2 and P3 components reflect the amount of resources required for inhibition and categorization of stimuli, respectively. Mean amplitude refers to the average amplitude between two periods of time, while the peak amplitude refers to the highest point of the component waveform. It is generally interpreted that with higher observed P3 mean or peak amplitudes there is increased recruitment of neural resources. In the present study, we observed decreased neuroelectric activity amongst adults with higher MPOD levels, yet no relationship with behavioral responses. Thus, we can infer that individuals with higher xanthophyll accumulation are able to process stimuli more efficiently, i.e., using less resources, than their lower MPOD peers. A relationship between the latency of the P3 and higher MPOD levels was also observed, further indicating a role for MPOD in efficiency of processing speed. These results are important, as cognitive inefficiency, or the need for recruitment of excess resources, has been theorized to be a catalyst for cognitive decline\textsuperscript{87}. Often treatment for cognitive decline is not sought until overt behavioral differences are observed. These results highlight a role of lutein consumption in promoting neural efficiency across the life-span, and in adults with overweight and obesity.

Mechanistic explanations for observed neuroefficiency in both the spatial and temporal realm are currently lacking. Our sample was comprised of individuals with overweight and obesity who are at a heightened risk factor for increased systemic as well as neuro-inflammation\textsuperscript{88}. Systemic inflammation has also been associated with cognitive dysfunction, independently of obesity, indicating a pertinent role in the cognitive deficits associated with obesity\textsuperscript{71}. Xanthophyll carotenoids, owing to their molecular structures and membrane localization, can serve as efficient...
antioxidants in a variety of metabolic pathways\textsuperscript{89}. Increased fruit and vegetable intake, and subsequent increased xanthophyll carotenoid intake, has been shown to modulate inflammatory and oxidative stress pathways and reduce the risk of developing several co-morbidities associated with obesity such as type 2 diabetes, atherosclerosis, and cancer\textsuperscript{90}. MPOD levels have been previously shown to be inversely related to both BMI and %Fat, increasing the urgency in investigating the relationship between MPOD and cognitive control in this growing population. Intriguingly, this relationship was not observed in the current study. However, previous work has shown that relationships between obesity and MPOD are relatively small in magnitude \textsuperscript{73,91}. Further, it is possible that inclusion of adults with healthy weight status would have increased the likelihood of observing relationships between obesity and MPOD. This may also explain the lack of association between adiposity measures and cognitive function in the present study. Nevertheless, an important implication of the current work is that macular xanthophylls are predictive of neural markers of cognitive function and these effects may supersede those of fat mass, highlighting the importance of these understudied dietary factors for cognitive benefit.

Although the present work is the first to investigate the neuroelectric relationships between lutein and zeaxanthin and cognitive control amongst adults with overweight and obesity, it was not without limitations. This study was cross-sectional in nature, therefore, relationships observed cannot be used to determine directionality or causality. Further studies should investigate lutein and zeaxanthin supplementation to determine whether neural efficiency can be increased following xanthophyll consumption. This study was also conducted solely amongst adults with overweight and obesity, and lacked a healthy weight comparator sample. Finally, our results relied on self-report dietary intake data as well as MPOD in lieu or serum xanthophyll assessment. Future studies examining diet and MPOD in conjunction with biomarker data are thus warranted.
The aforementioned limitations notwithstanding, among a sample of young-to-middle aged adults, we demonstrated that macular xanthophylls were associated with mean and peak amplitude (N2, P3) and latency (P3) of neuroelectric indices underlying human behavior during a cognitive task, indicating a role of neural lutein accumulation in neural efficiency amongst adults with overweight and obesity. Therefore, these findings have relevance for public health given that consumption of fruits and dark green leafy vegetables – rich food sources of xanthophylls – consistently falls below recommended guidelines. Future experimental research is needed to elucidate vital questions regarding the importance of dose and duration of xanthophyll intake necessary for long-term benefit for neurocognitive health among at-risk populations such as those with overweight and obesity.

Acknowledgements

This work was supported by funds provided by the Department of Kinesiology and Community Health at the University of Illinois; the USDA National Institute of Food and Agriculture, Hatch project [Grant number 1009249]; and the Hass Avocado Board.
CHAPTER 5: AIM 1-B: A RANDOMIZED CONTROLLED TRIAL EXAMINATION OF DIETARY, SERUM, AND RETINAL LUTEIN IN ADULTS WITH OVERWEIGHT AND OBESITY

Abstract

Objectives: Excess adiposity increases risk for cognitive impairment. Consumption of avocado, a highly bioavailable source of the xanthophyll lutein, has been shown to improve retinal lutein accumulation and cognitive function. Thus, we evaluated the influence of avocado consumption on cognitive function and lutein status among adults with overweight and obesity using a randomized-controlled with matching design for pertinent study outcomes.

Methods: A cohort of 84 adults (25-45 years, 31 males) were randomized to a treatment group (N=47) that received a 12-week daily meal with fresh Hass avocado or a control group (N=37) that received an isocaloric meal (clinicaltrials.gov, NCT02740439). Serum lutein and macular pigment optical density (MPOD) were used to assess xanthophyll status. Attention and inhibition were assessed using the Flanker, Oddball and Nogo tasks with accompanying electroencephalographic (EEG) recording.

Results: Participants in the treatment group exhibited improvements in serum lutein and accuracy in the Flanker task. However, there were no relationships between performance and changes in lutein status, nor neuroelectric variables. No significant changes in MPOD were observed.

Conclusion: Daily avocado intake over 12 weeks, after controlling for covariates, improved attentional inhibition and increased serum lutein concentrations among adults with overweight and obesity.

obesity. However, the cognitive benefits were independent of changes in lutein concentrations. Additional work is necessary to determine non-carotenoid, or carotenoid interactive, dependent mechanisms by which avocados may influence cognitive function.

**Common abbreviations:** Body Mass Index (BMI), Macular Pigment Optical Density (MPOD), mono-unsaturated fatty acid (MUFA), poly-unsaturated fatty acid (PUFA), high-performance liquid chromatography (HPLC)

**Introduction**

A group of oxygenated plant pigments known as xanthophylls have been demonstrated to be pertinent to cognitive health across the lifespan \(^{30,70}\). Specifically, previous work has focused on lutein, a non-polar, lipophilic carotenoid, that is most commonly found in green leafy vegetables, fruits, such as avocados, and eggs \(^{22}\). Zeaxanthin, lutein’s structural isomer, is derived from similar food items in significantly smaller quantities, and thus lutein and zeaxanthin quantification from foods is generally aggregated. Lutein and zeaxanthin cross the blood-brain barrier to preferentially accumulate within the brain and the macula of the eye. Lutein is the predominant carotenoid in the human brain, having been shown to comprise 66-77% of total brain carotenoid concentrations \(^{20}\). Lutein’s specific mechanism of action within the brain is still undetermined, yet theories point towards lutein’s role as an anti-oxidant and anti-inflammatory agent, as well as a contributor to preservation of brain plasticity and efficient gap-juncture connectivity \(^{33}\). While the exact mechanisms by which these xanthophylls impart cognitive benefits is yet to be understood, it is known that retinal accumulation of these xanthophylls plays a role in blue light filtration, as well as acting as antioxidant and anti-inflammatory agents involved in protecting retinal tissue \(^{92}\).

Accumulation of lutein, zeaxanthin, and the intermediate meso-zeaxanthin within the macula is referred to as macular pigmentation, and levels of macular pigmentation have been
reported to be closely correlated to xanthophyll concentrations found in deceased pediatric and geriatric brain samples \(^{21}\). Heterochromatic flicker photometry can be used to non-invasively estimate retinal xanthophyll accumulation, referred to as Macular Pigment Optical Density (MPOD), thereby serving as a biomarker for neural xanthophylls \(^{20}\).

Improvements in MPOD, following avocado consumption, have been associated with improvements in cognitive function \(^{93}\). Approximately 90% of avocado carotenoids are the xanthophylls lutein, zeaxanthin (quantitatively combined), and cryptoxanthin. One half of a medium avocado (60g of edible portion) has been reported by the United States Department of Agriculture to contain from 185µg of the combined lutein and zeaxanthin up to 800-1110µg, depending on the time of harvest \(^{94}\). According to the Institute of Medicine, average daily lutein and zeaxanthin consumption for adults ages 19 to 30 was 2,032 µg/day \(^{95}\). Thus, the addition of one avocado daily could greatly enhance current lutein and zeaxanthin intake among the general population. The avocado provides an ideal food matrix for lutein and zeaxanthin bioavailability, as carotenoids and xanthophylls are more bioavailable when consumed in the presence of fat, and when consumed in food sources rather than obtained through supplementation \(^{16}\). It has been hypothesized that carotenoids remain within the enterocyte of the small intestine until long-chain fatty acids are available to package and transport the carotenoids through the lymphatic system. Avocados are thus a prime food ingredient for facilitating chylomicron formation and lutein transportation throughout the body. Due to the high presence of mono- and poly-unsaturated fatty acids (MUFAs, PUFAs; 6.6g and 1.24g per 60 g edible fruit, respectively) research has shown that carotenoid absorption from green leafy vegetables and salsa is enhanced with the addition of
avocado. Therefore, if increased consumption of lutein and zeaxanthin may benefit cognitive health, the avocado is an ideal method of dietary delivery.

While cognitive health is of global concern, individuals with overweight and obesity are at a greater risk for cognitive impairment. Defined as a Body Mass Index (BMI) $\geq 25.0$ kg/m$^2$, approximately 70% of Americans suffer from overweight or obesity. Numerous studies have linked mid-life obesity to later cognitive decline in the areas of intellectual functioning, psychomotor performance and speed, visual construction, concept formation, set shifting, and decision making, independent of obesity related physical comorbidities. Previous work has also indicated that adults with overweight and/or obesity exhibit poorer performance during attentional and inhibitory control tasks. Exact mechanisms of the influence of overweight and obesity on cognitive health have yet to be fully elucidated, but several theories have been proposed, including but not limited to, increased systemic and neural inflammation, alterations in communication between reward centers and control centers of the brain, and alterations in metabolic activity within brain cortices. Previously, lutein and zeaxanthin intake has been cross-sectionally associated with beneficial cognitive patterns amongst individuals with overweight and obesity. However, to our knowledge, intervention trials have not been undertaken to determine causality of these relationships. As elevated weight status has been associated with decrements in cognitive performance among populations with and without obesity-related comorbidities, this population may benefit from increased lutein intake, facilitated by avocado consumption.

In addition to examination of the impact of obesity on behavioral and self-report measures of cognitive health, it is important to examine the underlying neural mechanisms associated with
cognition. Assessment of executive function, or cognitive control, in individuals with overweight and obesity is important, as this population is at increased risk for cognitive deficits. Cognitive control processes allow for the formation of intentional and goal-directed behavior. Cognitive control can be indexed through behavioral measures (i.e. accuracy and reaction time [RT]) or neuroelectric measurement of event-related potentials (ERPs). ERPs refer to a subset of electroencephalographic activity that occurs in response to, or in preparation for, a stimulus or action. Evaluating ERPs in conjunction with behavioral measures allows for assessment of both the behavior induced in the cognitive task, as well as the potential neurocognitive underpinnings of said behavior. While ERPs have been related to obesity, there has also been research showing a potential role for amelioration of obesity-related cognitive deficits with beneficial dietary changes. Thus, individuals with overweight and obesity may disproportionately benefit from the addition of food items rich in lutein to their diet.

The primary aim of the present study was to determine the impact of 12-week daily consumption of fresh Hass avocado on behavioral and neuroelectric indices of cognitive control among persons with overweight and obesity. Additionally, we conducted secondary analyses to explore whether the cognitive benefits derived from daily avocado consumption were dependent on changes in circulating (i.e., serum) and retinal (i.e. MPOD) lutein concentrations. Our central hypothesis was that, relative to the control group, participants in the avocado treatment group would exhibit greater gains in behavioral performance and neuroelectric function during cognitive control tasks. Further, our secondary hypothesis was that changes in lutein status would correlate with benefits in cognitive control.
Methods

Dietary Intervention

The *Persea Americana* for Total Health (PATH) Study was a 12-week randomized control trial conducted in central Illinois (clinicaltrials.gov, NCT02740439) between 2016 and 2018 with the primary aims of assessing the impact of 12-week daily Hass avocado on measures of central adiposity and glycemic control. Randomization was completed by an algorithm designed by a member of the research team who was not involved in data collection (ADMC). Participants were assigned to groups using a pseudo-randomization procedure whereby they were matched on characteristics theorized to affect cognition including biological sex, age, and weight status, and then placed into the treatment or control groups using a random number table. The research team and coordinators were blinded to group allocation. Participants randomized to the treatment group consumed one meal a day with a Hass avocado, while participants randomized to the control group consumed an isocaloric meal matched for macronutrient composition without an avocado. As outlined in Table 5.1, the lutein/zeaxanthin content was 3 times higher in the intervention meals as it was in the control meals. Owing to the macronutrient composition of avocados, the treatment meals were also higher in total fiber (~4-fold) and lower in saturated fats and higher in monounsaturated fatty acids. Examples of study meals can be found in supplementary text. The study meals were provided on a 7-day menu cycle designed to be similar to a typical American diet, and meet the Acceptable Macronutrient Distribution Ranges (AMDR) set by the Institute of Medicine (45% carbohydrates, 40% fat, 15% protein). Calories per meal were based on the Harris Benedict equation, accounting for a sedentary level of activity. Female participants received 20% less of each item than the male participants. Meals were instructed to be consumed at any time during the day. Meal compilation occurred in a metabolic kitchen and each ingredient was weighed to the nearest gram. Participants completed meal consumption records and traveled to the
test site twice weekly to pick up study meals. Insulated meal coolers, ice packs, and information about food safety procedures were provided. **Figure 5.1** displays the full study cohort.

<table>
<thead>
<tr>
<th>Table 5.1. Nutrient and food group comparison between study meals.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrient</strong></td>
</tr>
<tr>
<td>Calorie, kcal</td>
</tr>
<tr>
<td>Avocado, g</td>
</tr>
<tr>
<td>Total Fat, %</td>
</tr>
<tr>
<td>SFA, %</td>
</tr>
<tr>
<td>MUFA, %</td>
</tr>
<tr>
<td>PUFA, %</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
</tr>
<tr>
<td>Protein, %</td>
</tr>
<tr>
<td>Total Fiber, g</td>
</tr>
<tr>
<td>Soluble Fiber, g</td>
</tr>
<tr>
<td>Pectins, g</td>
</tr>
<tr>
<td>Insoluble Fiber, g</td>
</tr>
<tr>
<td>Lutein + Zeaxanthin, mcg</td>
</tr>
</tbody>
</table>
Compliance was assessed via weekly consumption records, which accounted for the percentage of the entire meal consumed, and the percentage of the avocado consumed among individuals in the intervention. Per protocol analyses was conducted among individuals who met the minimum compliance threshold of 80%.
Participants

Adults between 25-45 years of age with a BMI ≥ 25.0 kg/m² (N=163) were recruited from the East-Central region of Illinois using university e-mails and flyers posted in community buildings and buses frequented by the public. All subjects provided written informed consent prior to study participation. All procedures were administered in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Illinois. Participant exclusion criteria included BMI < 25 kg/m², pregnancy or lactation, history of neurological disease, history of chronic metabolic diseases, non-normal or uncorrected vision based on the minimal 20/20 standard, and food allergies or intolerances. Prior to testing, participants completed a health history and demographics questionnaire to confirm inclusion criteria and provide further health and demographic information. Based on this questionnaire, the highest level of education achieved was calculated from an 8-point scale, with 1 indicating less than 7th grade, and 8 indicating a doctoral degree or equivalent. Full baseline demographic information can be found in Table 5.2. Individuals were excluded from analyses if they were lost to follow-up (n=21), did not consume ≥ 80% of study meals (29), were unable to complete the study blood draw (25), or did not provide useable EEG data (4). Data from one individual in the avocado group was lost to an error during data collection for two of the cognitive tasks (1).
Table 5.2. Descriptive summary of PATH study participant characteristics at baseline and post-testing.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Testing</th>
<th>Post-Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Group</td>
<td>Avocado Group</td>
</tr>
<tr>
<td>N</td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>37</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>18F, 16M</td>
<td>23F, 15M</td>
</tr>
<tr>
<td>Age, years</td>
<td>34.0 (6.2)</td>
<td>34.6 (5.7)</td>
</tr>
<tr>
<td>Intelligence Quotient</td>
<td>109.2 (11.5)</td>
<td>110.9 (12.6)</td>
</tr>
<tr>
<td>Consumption, %</td>
<td>0.96 (0.05)</td>
<td>0.94 (0.05)</td>
</tr>
<tr>
<td>Highest Education, %</td>
<td>High School</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>College</td>
<td>44.1</td>
</tr>
<tr>
<td></td>
<td>Post-College</td>
<td>35.3</td>
</tr>
<tr>
<td>BMI², m/kg²</td>
<td>31.31 (5.49)</td>
<td>32.49 (5.83)</td>
</tr>
<tr>
<td></td>
<td>31.13 (5.82)</td>
<td>32.52 (5.69)</td>
</tr>
<tr>
<td>Whole Body % Fat, %</td>
<td>36.85 (8.61)</td>
<td>37.59 (7.67)</td>
</tr>
<tr>
<td></td>
<td>36.55 (8.79)</td>
<td>37.50 (7.50)</td>
</tr>
<tr>
<td>Macular Pigment Optical Density, MPOD</td>
<td>0.47 (0.19)</td>
<td>0.47 (0.22)</td>
</tr>
<tr>
<td></td>
<td>0.01-0.910</td>
<td>0.01-0.95</td>
</tr>
<tr>
<td>Serum Lutein, nm/L</td>
<td>0.14 (0.07)</td>
<td>0.12 (0.06)</td>
</tr>
<tr>
<td></td>
<td>0.027-0.357</td>
<td>0.048-0.324</td>
</tr>
</tbody>
</table>

²BMI = Body Mass Index
* Difference between pre- and post-testing is significant at the 0.05 level (2-tailed)
** Difference between pre- and post-testing is significant at the 0.01 level (2-tailed)
**Anthropometrics and Adiposity**

Height and weight measurements were completed to calculate BMI using the formula: weight [kg]/height [meters$^2$]. Measurements were conducted in triplicate while wearing lightweight clothing and no shoes, and the average was used for BMI calculation. A stadiometer (model 240; SECA, Hamburg, Germany) and a digital scale (WB-300 Plus; Tanita, Tokyo, Japan) were used to measure height and weight, respectively.

**Intellectual Ability Assessment**

The Kaufman Brief Intelligence Test, Second Edition (KBIT-2) was used to assess intelligence quotient (IQ). The KBIT-2 has been nationally normed for ages 4 – 90 years to assess general intellectual abilities and has been shown to have comparable scores to other intelligence scales $^{79,80}$. The KBIT-2 is comprised of verbal knowledge, matrices, and riddles subtests. A summative IQ score is generated by combining the score of all three subtests, and normalizing for participant age.

**Serum Carotenoid Assessment**

Serum lutein was assessed using high-performance liquid chromatography (HPLC) as previously described $^{105}$. Briefly, 250 µL of serum were mixed with an equal volume of ethanol containing 0.1% of butylated hydroxytoluene and vortexed. Serum carotenoids were extracted using 3 consecutive, 1 ml hexane extraction processes. Hexane layers were combined, dried under nitrogen, taken up into 90% MTBE, 8% methanol, and 2% ammonium acetate in water solution (1.5% solution) and then analyzed, in duplicate, for carotenoid concentrations using the Alliance HPLC system (e2695 Separation Module) equipped with 2998 photodiode array detector (Waters, Milford, MA, USA) and a reverse-phase C30 column (4.6 × 150 nm, 3 micron, YMC, Wilmington, NC, USA). Carotenoid standards were obtained from Carotenature (Ostermundigen, Switzerland).
For quantification, standard curves were run for each carotenoid, and serum carotenoid concentrations were quantified by use of a 2550 ethanol extinction coefficient in a 1% solution.

*Macular Pigment Optical Density*

MPOD was assessed via a customized hetero-flicker photometry technique administered using a macular densitometer (Macular Metrics Corporation, Rehoboth, MA, USA). The principles of this technique have been previously described [28]. Briefly, the assessment was performed by trained members of the research staff. The same eye was utilized for both pre and post testing assessments. Participants were asked to view stimuli peaking at a measuring wavelength of 460 nm that flickers in counter phase with a 570 nm reference (flicker rate being optimized for the optimal width of the subject’s null zone). The operator adjusted the radiance of the stimuli and participants were asked to identify a null flicker zone by informing the operator when they could no longer detect the flicker. The task is conducted while the stimulus is centrally-fixated (measuring macular pigmentation where it is most dense) and at 7 degrees in the para-fovea (where density is minimal). The MPOD score, with standard deviation, is calculated by subtracting the foveal from the parafoveal log sensitivity measurements after normalization at 570 nm.

*Flanker Task*

Attentional inhibition was assessed using a modified Eriksen Flanker task as previously described 106. The task order was fixed, with the Flanker task was presented first, followed by the Oddball, and then Nogo tasks. Responses were recorded on a 4-button response pad (Current Designs, Philadelphia, PA, USA). Briefly, participants viewed a series of 3-cm tall white arrows on a black computer screen in front of them. Using standardized and scripted language, participants were instructed to respond using a response pad to the direction of a centrally presented stimulus (arrow) amongst 4 flanking (2 arrows on each side of the central/target arrow), or distractor,
arrows. In the congruent trials, the flanking arrows face the same direction as the central arrow (>>>>>>, while in the incongruent trials the arrows surrounding the central stimuli are flanked by arrows facing the opposite direction (>><>>). The task began with 40 practice trials followed by two blocks of 100 randomized, and equiprobable, experimental trials. Stimuli were presented for 83 ms with a 1000 ms response window and jittered intertrial intervals of 1100, 1300, and 1500 ms. Behavioral measures of interest included mean accuracy and RT for overall, congruent, and incongruent trials.

Oddball Task
Response inhibition was assessed using a two-stimulus visual Oddball task. During the Oddball task, participants viewed a mixture of large (5.5 cm diameter) or smaller white circles (3 cm diameter) on a black computer screen. The larger circles served as the “target” stimulus and were presented during 20% of the trials in a random order. The smaller circles served as the “standard” stimulus and were presented during the remaining 80% of trials. Participants were instructed using scripted and standardized language to respond to the target trials with a right button press. Participants were presented with a practice block of 30 trials followed by 200 experimental trials. Stimuli were presented for 100 ms with a 1000 ms response window and an inter-trial interval of 2000 ms.

Nogo Task
During the Nogo condition participants were asked to respond to the frequent, small circle stimuli as targets, requiring them to inhibit responses to the established pre-potent response. Participants completed 30 practice “Nogo” trials followed by 200 experimental trials. Stimuli were presented for 100 ms with a 1000 ms response window and an inter-trial interval of 2000 ms. Measures of accuracy and RT for go and Nogo conditions were assessed.
**Event-related Potential Assessment**

EEG activity was recorded via a Neuro-scan Quik-cap with 64 passive, wet, sintered Ag/AgCl scalp electrodes arranged in the international 10-10 system. Electrooculographic activity was recorded with a set of four electrodes placed at the outer canthus of each eye and above and below the left orbit. A midline sensor placed between Cz and CPz served as a reference and AFz served as the ground. Online, using a Neuroscan SynampsRT amplifier, continuous EEG signal was digitized at a sampling rate of 500 Hz, amplified 500 times to an online low-pass 70-Hz filter with a direct current and a 60-Hz notch filter. Impedance values for all electrodes were maintained ≤10 kohms.

Signal processing and analysis were performed in Matlab (Mathworks, Natick, MA, version 2017b) using the EEGLab and ERPlab toolbox plug-ins. Offline EEG pre-processing was as follows: continuous data were re-referenced to the average of two mastoid electrodes. Next, all channels were plotted and underwent visual inspection for excessive noise by a trained member of the research staff. Channels shown to contain excessive noise after visual inspection were interpolated with an average of the nearest surrounding electrodes. An independent component analysis (ICA) was next used to systematically reject eye-blink artifacts from the data. Data were submitted to a 0.1 Hz high-pass butterworth filter before being submitted to the ICA. ICA and vertical eye sensor channel correlations greater than 0.35 were considered eye-blinks and were thus rejected. A time window of -200 to 1200 ms around stimulus onset was used for creating stimulus-locked epochs with a -200 to stimulus onset used for baseline correction. A 30-Hz (24 dB/octave) zero phase shift low-pass filter was employed. Artifacts were identified and rejected if a moving window peak-to-peak amplitude exceeded 100 µV, using a 100-ms window width, and 100-ms window step. Only correct trials from individuals who reported >50% of artifact-free trials
in all Flanker task trials and Target trials of the Oddball and Nogo task were included in analyses, as is consistent with previous literature in the field of ERPs and wellness 10,12.

Electrodes of interest were chosen based on a collapsed localizer technique. Evidence for selection was observed from maximal voltage localization of combined baseline and post-testing trials collapsed across task conditions. Post-hoc topographic average plots were constructed using a stylized topographic map plugin for EEGLAB/ERPLAB 110. Electrodes were consistent across tasks. From these topoplots. The N2 was maximal at FCZ, and was defined as the average and localized peak amplitudes and corresponding latency 150-300ms post-stimulus onset. The P3 component was defined as a region of interest comprised of C1, CZ, C2, CPZ, CP1, and CP2 with localized peak and corresponding latency occurring between 300-600ms post-stimulus onset.

Statistical Analyses
All analyses were conducted in SPSS (IBM SPSS Statistics version 24). Per protocol analyses were performed among all participants that reported ≥ 80% meal consumption. Intent to treat analyses can be found in supplementary material. Given that previously published research on avocado consumption effects on neuroelectric measures of cognitive control are lacking, an a priori power calculation using a moderate effect size (r=0.25), 2-sided α of 0.05, and 80% power, estimated that a sample size of 37 participants/group would be sufficient to address these study aims. All statistical analyses were conducted using a two-tailed family-wise alpha threshold of 0.05. Data were checked for normality using graphical methods (Q-Q plots) and the Shapiro-Wilk test. Initial Pearson product-moment correlation analyses were conducted between demographic variables as well as measures of adiposity and cognitive outcomes. Those found to be correlated with cognitive outcomes at baseline – sex (females coded as 0, males coded as 1), age, IQ, highest level of education, and BMI – were included as covariates in subsequent analyses. Analyses of
mean accuracy and mean RT were analyzed using unadjusted and adjusted repeated measures analyses of variance (ANOVAs/ANCOVAs). Behavioral analyses were modeled as 2 (group: control, treatment) x 2 (time: pre, post) x 2 (trial type i.e., congruent vs. incongruent or target vs. non-target). ERP and lutein status analyses were modeled as 2 (group) x 2 (time), and 2 (group) x 2 (time) x 2 (trial type) respectively. Any significant interaction effects observed were subsequently analyzed using paired t-tests and adjusted for multiple comparisons using Bonferroni correction.

Following initial modeling, changes in serum lutein concentrations, changes in MPOD, and changes in cognitive outcomes were correlated using Pearson product-moment correlations to determine associations between changes in lutein status and cognitive variables.

Results

Compliance and Weight Status

Self-reported dietary compliance for both groups was high, with overall compliance at 89.5%. After removal of individuals reporting consumption below 80%, Compliance values were 95% overall, with 94% reported compliance for the avocado treatment group, and 96% for the control group. There were no changes in BMI between pre- and post-testing in the control (p=0.3) nor treatment (p=0.8) group.

Markers of lutein concentrations

Unadjusted models for serum lutein concentrations revealed a main effect of time (F(1,82)=20.27, p<0.001, ηp²=0.20), no main effect of group (F(1,82)=0.39, p=0.54, ηp²=0.005), and an interaction effect of time x group (F(1,82)=28.40, p<0.001, ηp²=0.26). Following adjustment for sex, BMI, and age, there was no main effect of time (F(1,79)=0.25, p=0.62, ηp²=0.003) nor group (F(1,79)=0.87, p=0.35, ηp²=0.01) on serum lutein concentrations; however, there was a significant time x group interaction (F(1,79)=26.98, p<0.001, ηp²=0.26),
indicating that the avocado treatment increased serum lutein concentrations (change: 0.04 μmol/L, p=<0.001; 95% CI: 0.02 to 0.05;) compared to the control (p=0.3; Figure 5.2) in both the unadjusted and adjusted models.

Figure 5.2. Bar graphs of the pre and post testing values and standard error values (n=84) for serum lutein concentrations, highlighting statistically significant changes in the avocado group (change: 0.04μmol/L, p=<0.001) compared to the control (change: -0.005μmol/L, p=0.3).

Unadjusted models for MPOD revealed no main effect of time (F(1,82)=2.45, p=0.12, η²=0.03), group (F(1,82)=0.05, p=0.83, η²=0.001), nor interaction effect of time x group (F(1,82)=0.01, p=0.94, η²=0.00). After controlling for sex, BMI, and age, there was no main effect of time (F(1,79)=0.03, p=0.87, η²=0.00), group (F(1,79)=0.05, p=0.83, η²=0.001), nor interaction effect of time x group (F(1,79)=0.02, p=0.90, η²=0.00) on MPOD, indicating that neither the avocado nor the control group exhibited changes in MPOD over the course of the 12-week intervention (Figure 5.3).
At baseline, serum lutein concentrations and MPOD were correlated ($r=0.41$, $p<0.001$), yet the change in serum lutein concentrations and the change in MPOD throughout the study were not correlated ($r=0.09$, $p=0.5$). The baseline MPOD and change in MPOD were correlated ($r=0.38$, $p=0.001$), indicating that those with higher MPOD at baseline displayed smaller MPOD changes throughout the study period. Baseline serum lutein concentrations and the change in serum lutein concentrations were not correlated ($r=0.08$, $p=0.9$).

**Flanker Behavioral Performance**

Full pre- and post-testing data, as well as the number of ERP trials used in each task are outlined in Table 5.3. Unadjusted models for accuracy revealed no main effect of time ($F(1,82)=0.97$, $p=0.33$, $\eta_p^2=0.12$) nor group ($F(1,82)=2.96$, $p=0.09$, $\eta_p^2=0.04$). However, we observed a main effect of congruency ($F(1,82)=148.83$, $p<0.001$, $\eta_p^2=0.65$), and an interaction

![Figure 5.3. Bar graphs of the pre and post testing values for MPOD and standard error values (n=84) for MPOD, highlighting no group differences in values across a 12-week avocado intervention in either the control or intervention groups (all p's>0.8).](image-url)
effect of time \times group (F(1,82)=7.01, p=0.01, \eta^2=0.08). Adjusted models for accuracy revealed
no main effect of time (F(1,77)=1.17, p=0.28, \eta^2=0.02), group (F(1,77)=2.36, p=0.13, \eta^2=0.03),
nor congruency (F(1,77)=1.97, p=0.16, \eta^2=0.03), and a significant interaction effect of time \times

group (F(1,77)=8.29, p=0.005, \eta^2=0.10), indicating that individuals in the treatment group
improved in overall task accuracy (change: 2.2%, p=0.01; 95% CI: 0.5 to 3.9), whereas the control
group did not (p=0.2). Similarly, the treatment group exhibited improvements in both the
congruent (change: 1.3%, p=0.05; 95% CI: -3.02 to 2.3) and incongruent (change: 3.0%, p<0.01;
95% CI: 1.1 to 5.0) trial types, relative to the control group (congruent: p=0.10, incongruent:
p=0.60; Figure 5.4). Unadjusted analyses for RT revealed no main effect of time (F(1,82)=1.78,
p=0.19, \eta^2=0.02) or group (F(1,82)=0.46, p=0.50, \eta^2=0.006), a main effect of congruency
(F(1,82)=969.02, p<0.001, \eta^2=0.92), and an interaction effect of time \times group (F(1,82)=6.54,
p=0.01, \eta^2=0.07), indicating that individuals in the control group improved in RT during
incongruent trials (change: 25.5ms, p=0.003; 95% CI: 9.6 to 41.4) whereas the treatment group
did not (p=0.80). Adjusted analyses for RT revealed no main effect of time (F(1,77)=0.68,
p=0.41, \eta^2=0.01), group (F(1,77)=0.43, p=0.52, \eta^2=0.01), congruency (F(1,77)=1.85,
p=0.18, \eta^2=0.02), nor an interaction effect of time \times group (F(1,77)=3.47, p=0.07, \eta^2=0.04).
The average number of usable trials in the Flanker task were not different between pre-testing and post-testing, nor between intervention groups. Unadjusted analyses for N2 peak amplitude revealed a main effect of time ($F(1,82)=19.50$, $p<0.001$, $\eta_p^2=0.19$), no main effect of group ($F(1,82)=0.76$, $p=0.39$, $\eta_p^2=0.01$), a main effect of congruency ($F(1,82)=4.59$, $p=0.04$, $\eta_p^2=0.05$), and no interaction effect of time x group ($F(1,82)=0.00$, $p=0.97$, $\eta_p^2=0.00$). Adjusted analyses for N2 peak amplitude revealed no main effect of time ($F(1,77)=2.06$, $p=0.16$, $\eta_p^2=0.03$), group ($F(1,77)=1.00$, $p=0.32$, $\eta_p^2=0.01$), nor congruency ($F(1,77)=1.25$, $p=0.27$, $\eta_p^2=0.02$), and no interaction effect of time x group ($F(1,77)=0.05$, $p=0.83$, $\eta_p^2=0.00$). Unadjusted analyses for N2 peak latency revealed no main effects of time ($F(1,82)=0.00$, $p=0.99$, $\eta_p^2=0.00$), group ($F(1,82)=0.00$, $p=0.96$, $\eta_p^2=0.00$), nor congruency ($F(1,82)=0.18$, $p=0.68$, $\eta_p^2=0.00$), and no interaction effect of time x group ($F(1,82)=0.54$, $p=0.47$, $\eta_p^2=0.01$). Adjusted analyses for N2 peak latency revealed no main effects of time ($F(1,77)=0.82$, $p=0.37$, $\eta_p^2=0.01$), group ($F(1,77)=0.04$, $p=0.83$, $\eta_p^2=0.00$), nor congruency ($F(1,77)=0.18$, $p=0.68$, $\eta_p^2=0.00$), and no interaction effect of time x group ($F(1,77)=0.47$, $p=0.47$, $\eta_p^2=0.01$).
p=0.95, $\eta^2_{p}=0.00$), nor congruency ($F(1,77)=1.76$, $p=0.19$, $\eta^2_{p}=0.02$), and no interaction effect of time x group ($F(1,77)=0.07$, $p=0.80$, $\eta^2_{p}=0.00$).

Flanker Task P3

Unadjusted analyses for P3 peak amplitude revealed no main effect of time ($F(1,82)=2.47$, $p=0.12$, $\eta^2_{p}=0.03$) nor group ($F(1,82)=0.00$, $p=0.98$, $\eta^2_{p}=0.00$), a main effect of congruency ($F(1,82)=18.94$, $p<0.001$, $\eta^2_{p}=0.19$), and no interaction effect of time x group ($F(1,82)=0.47$, $p=0.50$, $\eta^2_{p}=0.01$). Adjusted analyses for P3 peak amplitude revealed no main effect of time ($F(1,77)=1.81$, $p=0.18$, $\eta^2_{p}=0.02$), group ($F(1,77)=0.00$, $p=0.95$, $\eta^2_{p}=0.00$), nor congruency ($F(1,77)=2.06$, $p=0.16$, $\eta^2_{p}=0.03$), and no interaction effect of time x group ($F(1,77)=0.88$, $p=0.35$, $\eta^2_{p}=0.01$). Unadjusted analyses for P3 peak latency revealed a main effect of time ($F(1,82)=9.90$, $p<0.001$, $\eta^2_{p}=0.11$), no main effect of group ($F(1,82)=0.04$, $p=0.84$, $\eta^2_{p}=0.00$), a main effect of congruency ($F(1,82)=70.20$, $p<0.001$, $\eta^2_{p}=0.46$), and no interaction effect of time x group ($F(1,82)=0.09$, $p=0.76$, $\eta^2_{p}=0.00$). Adjusted analyses for P3 peak latency revealed a trend-level main effect of time ($F(1,77)=3.71$, $p=0.06$, $\eta^2_{p}=0.05$), no main effect of group ($F(1,77)=0.02$, $p=0.90$, $\eta^2_{p}=0.00$) or congruency ($F(1,77)=2.14$, $p=0.15$, $\eta^2_{p}=0.03$), and no interaction effect of time x group ($F(1,77)=0.54$, $p=0.46$, $\eta^2_{p}=0.01$). Flanker task N2 and P3 depictions are illustrated in Figure 5.5.
Table 5.3. Descriptive summary of PATH study participant characteristics and task performance at baseline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Testing</th>
<th>Post-Testing</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control Group</td>
<td>Avocado Group</td>
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<tr>
<td><strong>Flanker Task</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Accuracy, %</td>
<td>93.5 (4.7)</td>
<td>78.0-98.5</td>
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<tr>
<td>Overall RT1, ms</td>
<td>465.4 (51.1)</td>
<td>369.1-587.6</td>
</tr>
<tr>
<td>Congruent Accuracy, %</td>
<td>97.2 (3.5)</td>
<td>85.0-100.0</td>
</tr>
<tr>
<td>Congruent RT, ms</td>
<td>432.3 (50.7)</td>
<td>335.1-548.7</td>
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<tr>
<td>Incongruent Accuracy, %</td>
<td>89.4 (7.6)</td>
<td>67.0-99.0</td>
</tr>
<tr>
<td>Incongruent RT, ms</td>
<td>501.7 (51.8)</td>
<td>417.9-626.8</td>
</tr>
<tr>
<td>Usable ERP trials, #</td>
<td>186 (9.5)</td>
<td>155-197</td>
</tr>
<tr>
<td><strong>Oddball Task</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Accuracy, %</td>
<td>88.6 (11.19)</td>
<td>56.5-100.0</td>
</tr>
<tr>
<td>Non-Target Accuracy, %</td>
<td>88.9 (14.2)</td>
<td>50.0-100.0</td>
</tr>
<tr>
<td>Target RT, ms</td>
<td>491.7 (74.2)</td>
<td>354.5-736.8</td>
</tr>
<tr>
<td>Usable ERP trials, #</td>
<td>176.0 (24.1)</td>
<td>92.0-199.0</td>
</tr>
<tr>
<td><strong>NoGo Task</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Accuracy, %</td>
<td>85.7 (8.5)</td>
<td>64.5-98.5</td>
</tr>
<tr>
<td>Non-Target Accuracy, %</td>
<td>90.1 (8.3)</td>
<td>70.1-100.0</td>
</tr>
<tr>
<td>Non-Target RT, ms</td>
<td>457.9 (71.2)</td>
<td>331.9-617.8</td>
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<tr>
<td>Target Accuracy, %</td>
<td>68.5 (11.2)</td>
<td>50.0-97.5</td>
</tr>
<tr>
<td>Usable ERP trials, #</td>
<td>166.0 (20.4)</td>
<td>90.0-198.0</td>
</tr>
</tbody>
</table>

1RT = Reaction Time

* Difference between pre- and post-testing is significant at the 0.05 level (2-tailed)

** Difference between pre- and post-testing is significant at the 0.01 level (2-tailed)
Oddball Behavioral Performance

Unadjusted analyses for accuracy revealed a main effect of time \((F(1,81)=4.94, p=0.03, \eta^2=0.06)\), no main effect of group \((F(1,81)=0.36, p=0.55, \eta^2=0.00)\), a main effect of trial type \((F(1,81)=25.30, p<0.01, \eta^2=0.24)\), and no interaction effect of time x group \((F(1,81)=0.27, p=0.60, \eta^2=0.00)\). Adjusted analyses revealed no main effects of time \((F(1,76)=0.13, p=0.72, \eta^2=0.00)\), group \((F(1,76)=0.23, p=0.63, \eta^2=0.00)\), nor trial type \((F(1,76)=2.37, p=0.13, \eta^2=0.03)\).

**Figure 5.5.** Waveform depictions of a lack of significant differences \((n=84)\) between post testing values for a Flanker task for the a) N2 and b) P3 ERP components and the c) difference waves between control and intervention pre- and post-testing during a 12-week avocado intervention. Topoplots d) and e) represent the electrode sites of the collapsed (by trial, group, and timepoint) representations of the N2 and P3 components.
p=0.13, \eta^2_p=0.03), and no interaction effect of time x group (F(1,76)=0.99, p=0.32, \eta^2_p=0.01).

Unadjusted analyses for RT revealed a main effect of time (F(1,81)=21.13, p<0.001, \eta^2_p=0.21), no main effect of group (F(1,81)=1.25, p=0.27, \eta^2_p=0.02), a main effect of trial type (F(1,81)=45524.93, p<0.001, \eta^2_p=1.00), and no interaction effect of time x group (F(1,81)=0.00, p=0.95, \eta^2_p=0.00). Adjusted analyses for RT revealed no main effect of time (F(1,76)=1.12, p=0.29, \eta^2_p=0.02) nor group (F(1,76)=1.93, p=0.17, \eta^2_p=0.03), a main effect of trial type (F(1,76)=197.16, p<0.001, \eta^2_p=0.72), and no interaction effect of time x group (F(1,76)=0.16, p=0.69, \eta^2_p=0.00).

**Oddball N2**

Unadjusted analyses for N2 peak amplitude revealed a main effect of time (F(1,81)=44.63, p<0.001, \eta^2_p=0.36), no main effects of group (F(1,81)=1.21, p=0.28, \eta^2_p=0.02), nor trial type (F(1,81)=2.44, p=0.12, \eta^2_p=0.03), and no interaction effect of time x group (F(1,81)=1.94, p=0.17, \eta^2_p=0.02). Adjusted analyses for N2 peak amplitude revealed no main effect of time (F(1,76)=1.14, p=0.29, \eta^2_p=0.02), group (F(1,76)=0.89, p=0.35, \eta^2_p=0.01), nor trial type (F(1,76)=0.01, p=0.91, \eta^2_p=0.00), and no interaction effect of time x group (F(1,76)=3.04, p=0.09, \eta^2_p=0.04). Unadjusted analyses for N2 peak latency revealed a main effect of time (F(1,81)=8.22, p=0.01, \eta^2_p=0.09), no main effect of group (F(1,81)=0.02, p=0.89, \eta^2_p=0.00), a main effect of trial type (F(1,81)=11.88, p<0.001, \eta^2_p=0.13), and an interaction effect of time x group (F(1,81)=4.30, p=0.04, \eta^2_p=0.05). Adjusted analyses for N2 peak latency revealed no main effect of time (F(1,76)=0.58, p=0.45, \eta^2_p=0.01), group (F(1,76)=0.32, p=0.57, \eta^2_p=0.00), nor trial type (F(1,76)=1.41, p=0.24, \eta^2_p=0.02), and an interaction effect of time x group (F(1,76)=3.95,
p=0.05, \eta_p^2=0.05), indicating that the control group exhibited longer latencies (change: 24.2ms, p=0.02; 95% CI: 4.7 to 44.1) at post-testing in the Target trial types.

**Oddball P3**

Unadjusted analyses for P3 peak amplitude revealed no main effect of time (F(1,81)=0.30, p=0.59, \eta_p^2=0.00) nor group (F(1,81)=0.05, p=0.82, \eta_p^2=0.00), a main effect of trial type (F(1,81)=118.07, p<0.001, \eta_p^2=0.60), and no interaction effect of time x group (F(1,81)=0.34, p=0.56, \eta_p^2=0.00). Adjusted analyses for P3 peak amplitude revealed no main effect of time (F(1,76)=0.53, p=0.47, \eta_p^2=0.01), group (F(1,76)=0.23, p=0.63, \eta_p^2=0.00), nor trial type (F(1,76)=0.85, p=0.36, \eta_p^2=0.01), and no interaction effect of time x group (F(1,76)=0.52, p=0.48, \eta_p^2=0.01). Unadjusted analyses for P3 peak latency revealed no main effect of time (F(1,81)=2.31, p=0.13, \eta_p^2=0.03), group (F(1,81)=0.99, p=0.32, \eta_p^2=0.01), nor trial type (F(1,81)=1.11, p=0.30, \eta_p^2=0.01), and no interaction effect of time x group (F(1,81)=2.20, p=0.14, \eta_p^2=0.03). Similarly, adjusted analyses for P3 peak latency revealed no main effect of time (F(1,76)=2.32, p=0.13, \eta_p^2=0.03), group (F(1,76)=2.14, p=0.15, \eta_p^2=0.03), nor trial type (F(1,76)=2.05, p=0.16, \eta_p^2=0.03), and no interaction effect of time x group (F(1,76)=2.87, p=0.11, \eta_p^2=0.04). Oddball task N2 and P3 depictions can be seen in **Figure 5.6**.
Nogo Behavioral Performance

Unadjusted analyses for accuracy revealed no main effect of time (F(1,81)=2.60, p=0.11, \( \eta^2=0.03 \)) nor group (F(1,81)=0.41, p=0.52, \( \eta^2=0.01 \)), a main effect of trial type (F(1,81)=94.76, p<0.001, \( \eta^2=0.54 \)), and no interaction effect of time x group (F(1,81)=0.00, p=0.95, \( \eta^2=0.00 \)). Adjusted analyses for accuracy revealed no main effects of time (F(1,76)=2.83, p=0.10, \( \eta^2=0.04 \)), group (F(1,76)=0.21, p=0.36, \( \eta^2=0.00 \)), nor trial type (F(1,76)=0.04, p=0.94, \( \eta^2=0.00 \)).
p=0.84, $\eta_p^2=0.00$), and no interaction effect of time by group (F(1,76)=0.39, p=0.53, $\eta_p^2=0.01$).

Unadjusted analyses for RT revealed no main effect of time (F(1,81)=0.49, p=0.49, $\eta_p^2=0.01$) nor group (F(1,81)=0.29, p=0.59, $\eta_p^2=0.00$), a main effect of trial type (F(1,81)=42554.68, p<0.001, $\eta_p^2=1.00$), and no interaction effect of time x group (F(1,81)=1.19, p=0.28, $\eta_p^2=0.01$). Adjusted analyses for RT revealed no main effect of time (F(1,76)=0.07, p=0.79, $\eta_p^2=0.00$) nor group (F(1,76)=0.04, p=0.84, $\eta_p^2=0.00$), a main effect of trial type (F(1,76)=178.10, p<0.001, $\eta_p^2=0.70$), and no interaction effect of time x group (F(1,76)=0.64, p=0.43, $\eta_p^2=0.01$).

Nogo N2

Unadjusted analyses for N2 peak amplitude revealed a main effect of time (F(1,81)=41.56, p<0.001, $\eta_p^2=0.36$), no main effects of group (F(1,81)=0.02, p=0.89, $\eta_p^2=0.00$), nor trial type (F(1,81)=1.69, p=0.20, $\eta_p^2=0.02$), and no interaction effect of time x group (F(1,81)=0.14, p=0.71, $\eta_p^2=0.00$). Adjusted analyses for N2 peak amplitude revealed no main effect of time (F(1,76)=0.33, p=0.57, $\eta_p^2=0.01$), group (F(1,76)=0.00, p=0.95, $\eta_p^2=0.00$), nor trial type (F(1,76)=0.05, p=0.83, $\eta_p^2=0.00$), and no interaction effect of time x group (F(1,76)=0.05, p=0.82, $\eta_p^2=0.00$). Unadjusted analyses for N2 peak latency revealed a main effect of time (F(1,81)=4.49, p=0.04, $\eta_p^2=0.06$), no main effect of group (F(1,81)=0.28, p=0.60, $\eta_p^2=0.00$) nor trial type (F(1,81)=0.17, p=0.68, $\eta_p^2=0.00$), and no interaction effect of time x group (F(1,81)=1.19, p=0.28, $\eta_p^2=0.02$). Adjusted analyses for N2 peak latency revealed no main effect of time (F(1,76)=0.62, p=0.43, $\eta_p^2=0.01$), group (F(1,76)=0.19, p=0.66, $\eta_p^2=0.00$), nor trial type (F(1,76)=0.37, p=0.55, $\eta_p^2=0.01$), and no interaction effect of time x group (F(1,76)=1.08, p=0.30, $\eta_p^2=0.02$).
Nogo P3

Unadjusted analyses for P3 peak amplitude revealed no main effect of time (F(1,81)=3.37, p=0.07, \( \eta^2_p=0.04 \)) nor group (F(1,81)=0.09, p=0.76, \( \eta^2_p=0.00 \)), a main effect of trial type (F(1,81)=6.86, p=0.01, \( \eta^2_p=0.08 \)), and no interaction effect of time x group (F(1,81)=0.55, p=0.46, \( \eta^2_p=0.01 \)). Adjusted analyses for P3 peak amplitude revealed no main effect of time (F(1,76)=0.23, p=0.64, \( \eta^2_p=0.00 \)), group (F(1,76)=0.00, p=0.98, \( \eta^2_p=0.00 \)), nor trial type (F(1,76)=0.95, p=0.33, \( \eta^2_p=0.01 \)), and no interaction effect of time x group (F(1,76)=0.59, p=0.45, \( \eta^2_p=0.01 \)). Unadjusted analyses for P3 peak latency revealed no main effect of time (F(1,81)=0.14, p=0.71, \( \eta^2_p=0.00 \)) nor group (F(1,81)=0.05, p=0.82, \( \eta^2_p=0.00 \)), a main effect of trial type (F(1,81)=4.49, p=0.04, \( \eta^2_p=0.06 \)), and no interaction effect of time x group (F(1,81)=0.05, p=0.82, \( \eta^2_p=0.00 \)). Adjusted analyses for P3 peak latency revealed no main effect of time (F(1,76)=0.83, p=0.37, \( \eta^2_p=0.01 \)), group (F(1,76)=0.56, p=0.46, \( \eta^2_p=0.01 \)), nor trial type (F(1,76)=0.48, p=0.49, \( \eta^2_p=0.01 \)), and no interaction effect of time x group (F(1,76)=0.17, p=0.68, \( \eta^2_p=0.00 \)). Nogo task N2 and P3 depictions can be seen in Figure 5.7.
Correlations results

Bivariate correlations between change scores (post-testing minus pre-testing values) were conducted. There were no relationships between the change in serum lutein, nor change in MPOD, and changes in changes in cognition in either the behavioral or the neuroelectric outcomes (all p’s>0.2).

Figure 5.7. Waveform depictions of lack of significant differences (n=83) between post testing values for the Nogo Task for the a) N2 and b) P3 ERP components and the c) difference waves between control and intervention pre- and post-testing during a 12-week avocado intervention. Topoplots d) and e) represent the electrode sites of the collapsed (by trial, group, and timepoint) representations of the N2 and P3 components.
Discussion

An emerging body of literature indicates that obesity is a risk factor for poorer cognitive health among children and adults. Therefore, there is an increasing need for development of nutritional interventions with the potential to promote or prevent cognitive decline among persons with overweight or obesity. This 12-week randomized controlled trial investigated the impact of daily avocado consumption on measures of cognitive control and lutein status among young-to-middle-aged adults with overweight and obesity. Consistent with the a priori hypothesis, we observed that participants in the avocado treatment group exhibited significantly greater accuracy during the Flanker task in both the unadjusted and adjusted models, signifying improvement in attentional inhibition. However, there were no significant changes in response inhibition; therefore, the cognitive benefits of avocado consumption were domain specific and not generalized. Regarding secondary hypotheses pertaining to the role of lutein, we observed that the avocado treatment group exhibited a significant improvement in serum lutein status; however, there were no significant changes in retinal xanthophyll deposition. Further, the changes in serum lutein concentrations or retinal xanthophyll accumulation did not correlate to benefits in cognitive task performance. Therefore, the cognitive benefits were evident independent of changes in lutein status. These findings demonstrate, for the first time, that 12-week daily avocado consumption benefits cognitive control performance among individuals with overweight and obesity.

The xanthophyll carotenoids lutein and zeaxanthin have recently received considerable interest for their neurocognitive potential across the lifespan. Previous work has shown that supplementation with either lutein or zeaxanthin or a combination of the two xanthophylls can significantly improve several behavioral aspects of cognitive function. However, food-based
approaches for enhancing cognitive function have been limited. To the authors knowledge, only one previous study has examined the effect of avocado intake on xanthophyll status and cognitive function. In 2017, Scott and colleagues found that daily avocado intake over 6 months improved memory function among older adults. The authors were able to link improvements in MPOD with changes in cognitive outcomes; thereby, demonstrating a direct association between changes in MPOD and cognitive benefits.

Following comprehensive adjustment of covariates including adiposity, intellectual ability, and education level, the avocado treatment group exhibited significant improvements in accuracy during an attentional inhibition task. Intriguingly, these improvements were not correlated with observed changes in serum lutein, and were evident in the absence of changes in MPOD. Nonetheless, 12-week daily avocado consumption was associated with improvements in cognitive performance amongst an otherwise healthy cohort of young-middle-aged adults with overweight or obesity. While these improvements were not related to changes in serum lutein concentrations, these changes were only apparent in the avocado-consuming group, suggesting that the improvements observed can be attributed to a component of the avocado. These improvements were also seen in both the unadjusted and adjusted models, indicating that avocado consumption may be associated with cognitive health even after controlling for important pertinent covariates.

Avocados contain a plethora of beneficial dietary components, nutrients, and phytochemicals, including but not limited to unsaturated fatty acids, dietary fiber, folate, vitamin K, and potassium. Previous clinical trials have demonstrated that avocado consumption is associated with improvements in blood lipid profiles, decreased weight status, and more regular bowel movements. Therefore, avocado consumption may benefit cognitive function via indirect pathways.
involving improvements in digestive and metabolic health or direct mechanisms involving alterations in neural lipid and inflammatory profiles.\textsuperscript{114,115} These findings warrant further examination of the potential implications of other nutrients found in avocados on cognitive health.

Due to a lack of change in MPOD in the current sample, null results in regards to the neuroelectric findings are not entirely surprising. Previously, our laboratory has cross-sectionally evaluated MPOD and the N2 and P3 components in these tasks. These results demonstrated a relationship between MPOD and mean amplitude of the N2 and peak amplitude of the P3, yet no relationships between MPOD and behavioral measures have been previously demonstrated.\textsuperscript{100} Therefore, as changes in MPOD were not observed across the study period, previous findings supporting the role of MPOD and processing in the temporal realm are supported. In the Oddball task we observed an increased latency of the N2 in the control group, indicating a slowing of processing speed that was not observed in the avocado group. Previous work has pointed to the detrimental impact of the Western diet on cognitive function, specifically implicating saturated fats, as well as low fiber intake.\textsuperscript{116} It is possible that the addition of avocado to the current diet may have prevented this decrement amongst the treatment group.

Carotenoid status is not only dependent on diet but can also be influenced by various intra-subject factors including degree of adiposity and genetics.\textsuperscript{16} Of course, between-person differences in lutein bioavailability and metabolism exist, and thus the authors took care to include necessary and comprehensive covariates in all analyses. Within this study, we observed a 75\% increase in serum lutein concentrations amongst the participants in the avocado group, substantiating claims that while quantitatively relatively low in lutein concentration (537µg
compared to supplementation studies including gram dosages), the avocado fruit matrix is a highly bioavailable food source for lutein intake. In the 2017 study, Scott et. al. found that the addition of 135g of Hass avocado daily for 6-months improved both serum lutein concentrations and MPOD, though improvements in MPOD were observed in both the avocado and the control group. Increases in MPOD were not related to increased serum lutein concentrations. MPOD improvements were observed as early as 3-months in both groups, which is also the duration of the currently described trial. Possible explanations for the lack of improvements in MPOD in the current sample are three-fold: sample demographics, baseline MPOD levels, as well as the degree of MPOD eccentricity evaluated could have all played a role. The sample utilized by Scott and colleagues was comprised of older adults (mean age = 63.3 years) of healthy weight status (mean BMI = 24.1 kg/m²), whereas the present work included young-to-middle aged adults (mean age = 34.1 years) with overweight or obesity (mean BMI = 32.0 kg/m²). Differences in nutrient absorption, dietary patterns, as well as systemic inflammation could influence the efficacy of lutein transport and absorption, as well as the role that lutein plays within the body. Also, study participants in the study by Scott and colleagues had a baseline mean MPOD of 0.39, while the participants in the present study began the trial with a higher baseline retinal accumulation, with an average MPOD of 0.48. Given that the avocado treatment by Scott et al. resulted in an average MPOD of 0.49 at 6 months, it is possible that we observed a ceiling effect, where by participants in the present study had higher MPOD at enrollment with little room for improvement. It is also possible that a longer feeding period or higher avocado dose may be necessary to change MPOD status among persons with overweight and obesity. On the other hand, substantial increases in serum lutein concentrations were observed in both studies, thus substantiating theories that serum lutein serves as a transient biomarker of acute lutein intake. Similarly, it is possible that in this
sample of adults with overweight and obesity, and presumably more neural inflammation than a healthy weight adults, changes in xanthophyll accumulation in the brain may have occurred prior to any observable changes in MPOD. The assessment of MPOD in the present study was solely conducted at the central-eccentricity of 0.50°, while lutein is known to linearly increase with distance from the fovea. While previous work has demonstrated improvements in MPOD at this eccentricity with lutein supplementation, it is possible that changes in retinal status, and perhaps associated changes in behavioral performance, may have been evident outside of this point of reference.

A strength of this study included the reliance on a randomized-controlled study design to address the primary aims. We also employed, for the first time, the use of behavioral assessment in conjunction with the ERP technique to gain insights into the neural or mechanisms by which avocados impact brain function. Further, we used lutein biomarkers in serum and retina to test whether lutein status changes were necessary to derive cognitive benefits. However, this study was not without limitations. This study solely utilized a sample of individuals with overweight and obesity, limiting its translation to healthy weight individuals. Though, given that recent Center for Disease Control figures quantify the percentage of Americans with overweight or obesity as nearly 70% of the population, examining of beneficial effects of diet on cognition in this population is warranted and increasingly generalizable. This study also utilized a fixed order style of task presentation. While it may be argued that the Flanker task preceding the Oddball and Nogo tasks may have impacted the subsequent tasks, the stimuli between tasks were different, and practice blocks were given between each task. Future studies would also benefit from inclusion of other...
cognitive tasks and neuroimaging techniques e.g., magnetic resonance imaging to gauge the interaction between brain structural and functional changes and avocado intake.

In conclusion, this study utilized a randomized-control trial to investigate the impact of 12-week daily avocado consumption on measures of cognitive control and lutein status among a sample of individuals with overweight and obesity. We observed that avocado intake significantly increased serum lutein status over the control group in models both adjusted and unadjusted for pertinent covariates, while no differences were observed in macular pigmentation. The changes in serum lutein concentrations were not related to behavioral changes observed in the avocado group, and neuroelectric changes were not observed across either group. Future studies are needed to investigate the impact of longer-term daily avocado consumption on markers of macular pigmentation and cognitive health across the lifespan. Further, considering that the avocado is a fruit that is rich in several nutrients with neurocognitive potential e.g., fibers, monounsaturated fatty acids, and phytochemicals, additional work examining the influence of other nutrients in the avocado are needed to comprehensively characterize the cognitive benefits of daily avocado consumption.

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Disclosure of interest

Funding for this study was provided by the Hass Avocado Board. The Hass Avocado Board had no contribution to the study analyses nor finalized manuscript.
CHAPTER 6: AIM 2: A CROSS-SECTIONAL EXAMINATION OF DIETARY CHOLINE INTAKE IN ADULTS WITH OVERWEIGHT AND OBESITY

Abstract

Objectives: Converging evidence indicates that obesity is associated with poorer brain health and cognitive function. However, it is not clear whether specific dietary factors may provide neuroprotective effects among individuals with overweight and obesity. Specifically, the essential nutrient choline may play an important neuroprotective role across the lifespan. Thus, it is concerning that only 1 in 10 adults meets the recommended intake for dietary choline. Further, there is a lack of research examining the impact of habitual choline intake on specific aspects of cognitive function among adults with overweight or obesity. Accordingly, the aim of this study was to examine the impact of choline intake on neurophysiological markers of attentional control among young and middle-aged adults with overweight or obesity.

Methods: 146 adults with BMI ≥ 25kg/m² (34.0 ± 5.9 years, 57 males) participated in the study. Behavioral performance (accuracy and reaction time) and neuroelectric indices (event-related brain potentials [ERPs]) of attentional inhibition were assessed during a modified Eriksen Flanker task. Specifically, amplitude and latency of the P3 waveform in a central-parietal region of interest (ROI) was used to index attentional resource allocation and information processing speed.

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respectively. Choline intake and overall diet quality (Healthy Eating Index [HEI-2015]) were assessed using 7-day diet records. General intellectual ability (IQ) was assessed using the Kaufman Brief Intelligence Test. Linear regression analyses were conducted to examine the relationship between habitual dietary choline intake and cognitive outcomes following adjustment of demographic factors, IQ, HEI-2015, and BMI.

**Results:** Only 19% of the participants reported meeting their daily recommended intake for choline. After controlling for covariates, choline intake was selectively associated with lower peak amplitude of the P300 waveform during incongruent task trials ($\beta = -0.25, p<0.01$). No significant relationships were observed for accuracy or reaction time.

**Discussion:** Given that individuals with greater choline intake exhibited lower P3 amplitude to achieve similar levels of behavioral performance, these results indicate that higher choline intake is associated with more efficient neural processing amongst adults with overweight and obesity, even after controlling for pertinent demographic factors, weight status, and overall diet quality. Future intervention studies are necessary to determine whether choline consumption provides neuroprotective effects for other facets of executive function across the lifespan among individuals with elevated weight status.
Introduction

Diet quality in the United States is continuously falling short of national recommendations. This failure to meet recommendations has been linked to the concurrent rise in the prevalence of obesity, with recent reports indicating that 70% of US adults are living with overweight or obesity. While diet is a known contributor to physiological health, it is also a pertinent contributor to brain and cognitive health. Better diet quality has been associated with improvements in memory, slowed cognitive decline, and even as a potential safeguard against Alzheimer’s Disease. With a perpetual failure to meet current dietary recommendations, as well as a continual prevalence of overweight and obesity, further research is warranted to understand the impact of nutritional intake on cognition among individuals with overweight and obesity.

Choline, an essential water-soluble nutrient, has been linked to cognitive improvements among perinatal and geriatric populations. Choline is vital for neurotransmitter synthesis (as the precursor to acetylcholine [ACh]), cell-membrane integrity (through phosphatidylcholine), and methyl-donor metabolism (through the conversion of methionine to homocysteine), and higher choline intake has been associated with lower whole body % fat and higher levels of lean mass. Choline can also be produced through de novo biosynthesis through the phosphatidylethanolamine N-methyltransferase pathway, albeit in concentrations that are insufficient to maintain many biological processes. Thus, it is imperative that choline be consumed through diet. Common foods rich in choline include eggs (147 mg/serving), soybeans (107 mg/serving), chicken breast (72 mg/serving), and beef (72 mg/serving). The Institute of Medicine recommends an Adequate Intake (AI) for choline of 425 mg/d for women and 550 mg/day for men. However, 90% of the US population does not meet this AI recommendation.
Choline plays a vital role in central nervous system development and choline supplementation has been shown to benefit memory and brain development in human and pre-clinical models\textsuperscript{44}. Phosphatidylcholine and sphingomyelin, the two most prevalent forms of choline in the body, form essential structural components of cell membranes and myelin sheaths throughout the brain and the body. Surprisingly, the relation of choline intake to executive function has been seldom studied. Executive function, often referred to as cognitive control, is comprised of a set of interrelated, yet dissociable, higher order processes including attention (the ability to resist distractions and maintain focus), response inhibition (the ability to inhibit responses to stimuli), and cognitive flexibility (the ability to dynamically shift attention and alter response strategy to changing demands)\textsuperscript{5}. These processes allow for the ability to interact with, as opposed to merely respond to, salient stimuli in the surrounding environment. Deficits in cognitive function, in particular executive function, have been often used as early markers of cognitive decline\textsuperscript{124}. Executive function can be indexed through behavioral measures (i.e. accuracy and reaction time [RT]) or the more sensitive neuroelectric measurement of event-related potentials (ERPs). ERPs refer to subset of electroencephalographic activity that occurs in response to, or in preparation for, a stimulus or response\textsuperscript{102}. Evaluating ERPs in conjunction with behavioral measures allows for assessment of both the behavior induced in the cognitive task, as well as the neurocognitive underpinnings of said behavior. More specifically, this study investigated the P3 (i.e. P300, P3b), a positive-going ERP component that occurs in adults 300-700 ms post-stimulus onset and is generated by several neural generators including the prefrontal cortex (PFC)\textsuperscript{125}. The P3 is associated with context updating, or revision of the mental representation induced by incoming stimuli, as well as attentional resource allocation\textsuperscript{126}. The peak amplitude of the P3 represents the amount of neuronal resources required for performing cognitive tasks that elicit higher attentional
demands, while the latency represents the speed of stimulus evaluation and classification. Thus, tasks that are less cognitively demanding and require fewer attentional resources will elicit smaller P3 amplitudes with decreased P3 latencies when compared to their more attention demanding counterparts. As elevated weight status has been associated with decrements in cognitive performance among populations with and without obesity-related comorbidities\textsuperscript{71,101}, this population may benefit from heightened habitual choline intake.

The PFC is comprised of cholinergic projects, stemming from the basal forebrain, as well as cholinergic targets, through ACh receptors\textsuperscript{44}. Thus, dietary choline consumption stands to impact the function of, and behaviors derived from, the PFC. This relationship has been demonstrated in animal models, as well as in older adult populations, yet there are few studies investigating these relationships in younger adults. Therefore, there remains limited data on the potential of choline to have neuroprotective effects across the lifespan and among populations with overweight and obesity. Accordingly, the aim of this study was to examine the impact of choline intake on attentional inhibition, a marker of executive function, in middle-aged adults with overweight or obesity. We hypothesized that individuals who consumed higher levels of dietary choline would exhibit improved behavioral performance on an attentional inhibition task as observed through higher accuracies and faster RTs. Additionally, we hypothesized that individuals with higher choline intake would exhibit more efficient neural processing, signified by lower P3 peak amplitude and faster P3 latency, when completing an executive function task.
Methods

Participants

Cross-sectional data were collected from 167 middle-aged adult participants with overweight and obesity (BMI ≥25kg/m²). Recruitment consisted of flyers and university mailings resulting in a population of both community and university members. To qualify for the study, participants had to provide all demographic data, complete the Kaufman-Brief Intelligence Test (KBIT-2), provide a readable electroencephalographic (EEG) recording, record their dietary intake for at least 7 days using a diet record, and be free of diagnosed neurological disorders and diseases (including ADD/ADHD and autism). All participants provided verbal and written informed consent in accordance with the University of Illinois’ Institutional Review board and the Declaration of Helsinki. After excluding participants who (a) had less than 7-days of recorded diet record entry (8), (b) were outliers in behavioral or dietary measures (10) (± 3 SD), or (c) had excessive noise in their EEG signal (3), we obtained 146 (34.04 ± 5.91 years, 57 male) participants available for further analyses. Complete group demographic characteristics are summarized in Table 1.

Testing Appointments

Participant testing was completed over two laboratory visits. On the first visit, informed consent was obtained, followed by administration of the KBIT-2, and height and weight assessments for Body Mass Index (BMI) calculation. On the second visit to the laboratory, participants completed cognitive testing. All ERP testing was conducted in the morning hours (between 6:00 am and 9:30 am) in the fasted state to avoid the confounding effects of acute macronutrient consumption on cognitive outcomes\(^\text{127}\). Participants were instructed at their Day 1
appointment as well as in an e-mail reminder to arrive to the laboratory for cognitive testing following a 10-hour fast of all food and beverage items apart from water. Compliance to this protocol was verbally assessed prior to cognitive testing and failure to comply resulted in rescheduling of the appointment. Following the cognitive testing, participants were educated on dietary record keeping by a staff member and sent home with a 7-day diet record that was returned at a later date.

**Anthropometric Measures**

To calculate BMI (kg/m²), height and weight were assessed using a stadiometer (model 240; SECA, Hamburg, Germany) and a digital scale (WB-300 Plus; Tanita, Tokyo, Japan). Participant height and weight were assessed while wearing light clothing and no shoes. The average of 3 measurements of height and weight were used for analyses.

**Habitual Diet Quality**

Diet analyses were conducted using the 2015 Nutrition Data System for Research (NDSR) software (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA). Only participants who completed at least 7 days of entry were included for further analyses. Average choline intake over the 7-day period was assessed using the averages of the NDSR Intake Properties Totals Output File. Healthy Eating Index (HEI-2015) was calculated to assess participants’ dietary patterns. HEI, developed as a measure of overall diet quality, scores individuals on a scale of 0-100 based on compliance to the recommendations of the 2015 Dietary Guidelines for Americans. The HEI contains 13 components, including nine adequacy (minimum
standard) and four moderation (maximum allowed) components. The component scores are then summed to calculate the total HEI-2015 score\textsuperscript{128}.

**Intelligence Quotient**

KBIT-2 is a test of general intellectual abilities that has been nationally normed for ages 4 – 90 years\textsuperscript{79}. The test is comprised of three subtests: Verbal Knowledge, Riddles, and Matrices. The Verbal Knowledge test includes 60 questions where the participant responds by selecting an image that is most associated with the word or question spoken by the researcher. The Riddle subtest consists of 48 riddles that have a single word answer. The Matrices test has 46 multiple choice problems where the participant must choose which of 6 pictures is most associated with the single stimulus picture or which picture best completes a 2x2, 2x3, or 3x3 matrix. In each of the subtests, correct answers are given a score of 1 and the total scores are converted into standard scores which have a maximum of 100. A composite score of the three subtests is then utilized as a measure of general intellectual abilities.

**Flanker Task**

Attentional inhibition was assessed using a modified Eriksen Flanker task. Participants viewed a series of arrows on a computer screen in front of them. Using standardized and scripted language, participants were instructed to respond using a response pad to the direction of a centrally presented stimulus (arrow) amongst four flanking, or distractor, arrows. In the congruent trials, the flanking arrows face the same direction as the central arrow (>>>>>>), while in the incongruent trials the arrows surrounding the central stimuli are flanked by arrows facing the opposite direction (>>>><>>). The congruent task trials have been demonstrated to require
significantly decreased attentional resources in young to middle aged adult’s samples. The task began with 40 practice trials followed by two blocks of 100 randomized, and equiprobable, experimental trials. Stimuli were presented for 83 ms with a 1000 ms response window and jittered intertrial intervals of 1100, 1300, and 1500. Behavioral measures of interest included mean accuracy and RT for both congruent and incongruent trials.

**ERP Assessment**

EEG activity was recorded via a Neuro-scan Quik-cap with 64 scalp electrodes arranged in the international 10-10 system as previously described. Electrooculographic activity was recorded with a set of four electrodes placed at the outer canthus of each eye and above and below the left orbit. A midline sensor placed between Cz and CPz served as a reference and AFz served as the ground. Using a Neuroscan SynampsRT amplifier, continuous EEG signal was digitized at a sampling rate of 500 Hz, amplified 500 times to an online low-pass 70-Hz filter with a direct current and a 60-Hz notch filter. Impedance values for all electrodes were maintained ≤ 10 kohms.

Offline, continuous data were re-referenced to the average mastoids. An independent components analysis (ICA) was used to systematically reject eye-blend artifacts from the data. Data were submitted to a .1 Hz high-pass filter before being submitted to the ICA. ICA and vertical EOG channel correlations greater than .35 were considered eye-blends and were thus rejected. -200 to 1200 ms around stimulus onset was used as a time window for creating stimulus-locked epochs with a -200 to stimulus onset used for baseline correction. A 30-Hz zero phase shift low-pass filter was employed.

Based on evidence observed from post-hoc topographic images, a region of interest (ROI) comprised of C1, CZ, C2, CPZ, CP1, and CP2 was utilized for P3 assessment. Topographic grand
average plots were constructed using a stylized topographic map plugin for EEGLAB/ERPLAB\textsuperscript{110}. Only correct trials from individuals who reported $>50$ usable trials in both congruent and incongruent trial types were used for analyses. Responses were considered valid if they occurred within a time window of 0-1200 ms post-stimulus onset. The P3 component was defined as the localized peak and corresponding latency occurring between 300-600 ms post-stimulus onset.

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS, version 24, IBM, Armonk, New York) software was used for all statistical analyses. Variables that did not meet normality distributions, with $p<0.05$ as assessed by Shapiro-Wilk, were log transformed. To examine the association between choline intake and attentional, inhibition hierarchical linear regression analyses were conducted. Step 1 consisted of variables that have been previously shown to be related to both choline intake (HEI-2015) as well as cognitive variables (Sex, Age, BMI, and IQ). Step 2 consisted of log transformed choline intake. The significance of the change in the $R^2$ value between the two steps was used to assess the independent contribution of choline intake for explaining variance in cognitive outcomes beyond that of the demographic factors, IQ, BMI, and HEI-2015. Significance was set at $p<0.05$. To depict neuroelectric waveforms and topoplots, and solely as a means of visual representation, a median split was conducted to assign participants as either “low” or “high” choline consumers. Independent t-tests were conducted to examine differences between these groups, with significance set at $p<0.05$. 

81
Results

Participant Characteristics

Participants spanned 25-45 years of age. Participants were adults with overweight (46%) or obesity (54%) (mean BMI = 32.49 ± 6.11 kg/m²). Of the 146 participants analyzed, average choline intake was 359.53 ± 134.74 mg/day. Only 18% of females, 22% of males, and 19% of our overall sample reported consuming choline adequate to meet their recommended AI. Consistent with previous findings, males were consuming significantly more choline than females (p<0.01). There were also differences in IQ (p<0.01) and BMI (p<0.01) between males and females. Total participant characteristics are outlined in Table 6.1.

<table>
<thead>
<tr>
<th>Value</th>
<th>Group</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>146</td>
<td>89</td>
<td>57</td>
</tr>
<tr>
<td>Age, y</td>
<td>34.04 ± 5.91</td>
<td>34.39 ± 5.78</td>
<td>33.48 ± 6.12</td>
</tr>
<tr>
<td>IQ</td>
<td>109.26 ± 12.68**</td>
<td>107.02 ± 12.24</td>
<td>112.75 ± 12.68</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32.49 ± 6.11**</td>
<td>33.7 ± 5.69</td>
<td>30.58 ± 6.31</td>
</tr>
<tr>
<td>HEI-2015³</td>
<td>50.1 ± 12.54</td>
<td>49.83 ± 11.93</td>
<td>50.51 ± 13.53</td>
</tr>
<tr>
<td>Choline, mg</td>
<td>359.53 ± 134.74**</td>
<td>313.27 ± 107.48</td>
<td>431.76 ± 141.93</td>
</tr>
<tr>
<td>Congruent Accuracy, %</td>
<td>96.55 ± 3.45</td>
<td>95.98 ± 4.02</td>
<td>97.44 ± 2.56</td>
</tr>
<tr>
<td>Congruent RT, ms</td>
<td>417.18 ± 51.13</td>
<td>418 ± 47.41</td>
<td>415.91 ± 56.86</td>
</tr>
<tr>
<td>Incongruent Accuracy, %</td>
<td>90.1 ± 6.75</td>
<td>89.41 ± 7.21</td>
<td>91.17 ± 5.86</td>
</tr>
<tr>
<td>Incongruent RT, ms</td>
<td>485.67 ± 52.02</td>
<td>488.24 ± 50.11</td>
<td>481.66 ± 55.07</td>
</tr>
</tbody>
</table>

1 Values are means ± SD unless otherwise stated  
2 Determined by the Center for Disease Control Body Mass Index (BMI) classifications  
3 Calculated using National Cancer Institute Healthy Index (HEI) 2015 Assessment  
* Significant differences between groups (p > 0.05)  
** Significant differences between groups (p > 0.01)

Behavioral Performance

Cognitive variables were used as dependent variables, while predictors in Step 1 consisted of BMI, Sex, Age, IQ, and HEI-2015, and Step 2 consisted of choline intake. In regard to accuracy, Step 1 produced a significant model for congruent and incongruent accuracy (p=0.04; p=0.01), but
a significant change in $R^2$ was not observed with the addition of choline intake in Step 2 (all p’s>0.05). In regard to RT, Step 1 also resulted in a significant model fit for congruent RT (p=0.03), but not for incongruent RT (p>0.05). Step 2 did not significantly change the $R^2$ for either congruent or incongruent trials. Representations of accuracy performance after conduction of a median split across low and high levels of choline consumption are depicted in Figure 6.1.

**P3 Amplitude and Latency**

P3 waveform and topographic plots are depicted in Figures 6.2 and 6.3. The average number of missing trials per participant was 3, out of 200, trials. Results of hierarchical linear regression analyses for all variables are displayed in Table 6.2. In regard to peak amplitude, Step 1 did not produce a significant model for congruent peak amplitude (p=0.09), but did for incongruent peak amplitude (p=<0.01). With the addition of choline intake in Step 2, there was no significant change in $R^2$ for congruent amplitude (p>0.05), but there was a significant $R^2$ change for incongruent amplitude ($\beta = -0.25$, p=<0.01). In regard to latency, Step 1 did produce a significant model (p=<0.01) for congruent latency driven by BMI ($\beta = 0.18$, p=0.04) and IQ ($\beta = -0.27$, p=<0.01). The addition of choline intake in Step 2 did not produce a significant $R^2$ change ($\beta = -0.02$, p=0.82). Neither Step 1 nor Step 2 produced a significant model for incongruent latency (all p’s > 0.05).
Table 6.2. Linear Regression between pertinent variables, choline intake, and Flanker ERP variables.

| Step and Variable | Congruent | | Incongruent | |
|-------------------|-----------|-----------------|-----------------|
|                   | Amplitude | Latency         | Amplitude       | Latency         |
|                   | β (SE)    | ΔR² | Model | P | β (SE) | ΔR² | Model | P | β (SE) | ΔR² | Model | P |
| Step 1            |           |     |       |   |         |     |       |   |         |     |       |   |
| BMI               | 0.00 (0.07) | 0.07 | 0.18* (0.88) | 0.00 (0.07) | 0.08 (0.85) |
| Sex               | -0.06 (0.81) | 0.15 | 0.10 (10.92) | -0.10 (0.82) | 0.13 (10.54) |
| Age               | -0.04 (0.06) | 0.13 (0.86) | 0.13 <0.01 | -0.14 (0.07) | 0.10* 0.01 | 0.00 (0.03) | 0.03 (0.03) |
| IQ                | 0.26** (0.03) | -0.27** (0.41) | 0.29** (0.03) | -0.09 (0.40) | -0.05 (0.39) |
| HEI-2015          | 0.03 (0.03) | -0.09 (0.41) | 0.03 (0.03) | -0.05 (0.39) | |
| Step 2            |           |     |       |   |         |     |       |   |         |     |       |   |
| Choline Intake    | -0.13 (2.50) | 0.01 | -0.02 (33.99) | -0.25** (2.48) | 0.00 (0.00) | 0.00 (0.00) |

SE = Standard Error
**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
Figure 6.1. a) Mean (SE) response accuracy and b) P3 peak amplitude for a median split of participants into low (248.75 ± 6.31 mg/day) and high (470.32 ± 10.96 mg/day) choline consumers by trial type.
Figure 6.2. a) Waveform depictions of a median split of choline consumers into low and high choline consumption of the compiled Flanker task trials, and b) a topographic representation of the 6 electrode sites (C1, CZ, C2, CPZ, CP1, and CP2) used in the P3 region of interest (ROI).
High Choline Consumers
(470.32 ± 10.96 mg/day)
(n = 73)

Low Choline Consumers
(248.75 ± 6.31 mg/day)
(n = 73)

Figure 6.3. Topographical distribution plots of the P3 amplitude for congruent and incongruent flanker trials, averaged from 300 to 600 milliseconds after stimulus onset.
Discussion

The aim of the present study was to examine the relationship between choline intake in middle-adulthood among individuals with overweight and obesity and attentional inhibition, a marker of executive function. No difference in behavioral measures were observed, yet individuals with greater choline consumption exhibited lower P3 peak amplitude during an attentional inhibition task. Thus, we are able to interpret that individuals with higher dietary choline intake were able to exhibit comparable behavioral performance while requiring less attentional resources. Interestingly, the positive relationships were only evident for the incongruent task condition, pointing to the selective benefits of dietary choline for cognitive processes requiring upregulation of attention or greater amounts of attentional inhibition.

Mechanistically, much is still to be learned about the role of choline in the human neocortex throughout the lifespan. Choline, when combined with acetyl coenzyme A via choline acetyltransferase, forms the neurotransmitter ACh. ACh plays an integral role in the regulation of cortical activity (including attention) across multiple timescales through its ability to change neuronal excitability, alter presynaptic release of neurotransmitters, and coordinate the firing of groups of neurons. The PFC as well as the anterior cingulate cortex, the proposed sites of P3 and attention origination, are deeply innervated with cholinergic projections, stemming primarily from the basal forebrain. The vital role of ACh on attention has been shown in pre-clinical literature with decrements in attention produced through cholinergic lesions and observations of increased levels of ACh in the PFC in response to attentionally demanding tasks. These results have been demonstrated particularly amongst the higher taxing, and higher attentional resource-demanding task trials. Surprisingly few human studies have examined behavior and choline or ACh status and even fewer have been conducted outside of perinatal or geriatric populations.
Within the current Flanker task design we presented a randomized mix of minimally (congruent) and higher (incongruent) taxing stimuli. While behavioral results were not observed in this study, consistent with previous literature, we did observe differential recruitment of attentional resources between the two trial types, as indexed by the P3 component.

This is, to the author’s knowledge, the first study to examine concurrent choline intake amongst humans and its association with neuroelectric markers of attention. ERPs are summative postsynaptic potentials and are elicited when large numbers of cortical pyramidal neurons fire in synchrony when processing stimuli\textsuperscript{102}. The magnitude of elicited voltages can be modulated by the presence and absence of neurotransmitters, such as ACh. Our results reveal evidence that higher choline consumption impacts the magnitude of the ERP potential, potentially by increasing the amount of cerebral ACh through greater available neurotransmitter substrate. The P3, as a resolute marker of attentional resource allocation, can be differentially interpreted. Often a higher P3 peak amplitude can be observed as improved attentional resource facilitation, while a lower P3 peak amplitude can be interpreted as an increased neural efficiency. As we observed no differences in behavioral performance, we can infer that individuals using fewer resources to reach the same level of performance as their lower-consuming peers are thus processing stimuli more efficiently.

Often, when assessing cognition, decrements are not detected until overt behavioral changes can be observed and this is frequently too late for proper treatment. The lack of differences in behavioral outcomes observed here highlights the necessity of utilizing behavioral measures in conjunction with more sensitive performance characterization techniques, such as the ERP.

Choline intake also impacts the brain structurally. In the Framingham Offspring Study, Poly et. al. reported that middle-aged, healthy individuals (mean age = 60.1 years) with higher choline consumption five years prior to fMRI testing showed an inverse relationship with choline
intake and White Matter Hyperintensity (WMH), an associated marker of cognitive impairment and Alzheimer’s Disease\textsuperscript{46}. As white matter integrity has been associated with better performance on cognitive variables such as reading, IQ, information-processing, and attention, this again points to the neuroprotective role of choline in the neocortex across the lifespan\textsuperscript{57}. Based on our results, it is possible that this neuroprotection may be characterized by neural transmission and efficiency. Therefore, examining dietary components, such as choline, that may increase neural efficiency, may prove to be beneficial for neuroprotection as well as quality of life throughout the lifespan.

Interestingly, WMH has also been associated with obesity, in particular the metabolically active visceral adiposity has been positively associated with the increased presence of WMH\textsuperscript{58}. Choline intake has also been associated with lower levels of whole body % fat in middle-aged adults\textsuperscript{43}. Thus, our sample of individuals with overweight and obesity is particularly susceptible to cognitive benefits from improved diet quality. In this sample, we observed longer latencies on the congruent version of the task among individuals with greater weight status \((r=0.16, p=0.05)\), yet adiposity did not serve as a contributing variable in the incongruent trial types. Even so, while the study of obesity on cognition is not to be discounted, these results highlight the importance of studying obesity within the context of diet and other lifestyle factors.

There has been research conducted within animal models that suggests a role of maternal choline intake on cognitive ability throughout the lifespan\textsuperscript{132}. Among animal models, high maternal choline intake in-utero has been linked with higher subsequent measures of visuospatial memory across the lifespan, perhaps due to the choline’s fundamental contributions to brain formation and neural development\textsuperscript{45,53}. In human work, the closest replication of this animal work is evidenced through the Project Viva Study. Two iterations of literature have emerged from this large-scale longitudinal study – one at years 3 of age and another at age 7. At age 3 there was no
observed relationships between maternal choline intake and cognitive outcomes, yet at age 7 higher maternal choline intake was associated with improved visual memory\textsuperscript{54,55}. These studies investigated the impact of maternal intake and did not assess choline intake at the time of cognitive testing. Therefore it is difficult to determine the potential differential impact of maternal and concurrent choline intake in these studies. Maternal intake was not assessed in the present study, and it is thus impossible to know whether the effects seen here are driven by early maternal intake, or by current choline intake. Nonetheless, there is a current lack of research investigating the role of either maternal or concurrent choline in middle adulthood.

It is important to note that 81\% of our participants did not meet AI recommendations for choline consumption, with males consuming more than females, findings which align with national intake patterns\textsuperscript{41}. Nevertheless, our study was able to highlight differences in P3 amplitude even at suboptimal levels of choline intake. Further research is needed to support increasing choline consumption amongst this age group for quality of cognitive life and, as our population was of childbearing age, for quality of life for future generations.

This study derived its strengths from its novel use of behavioral assessment in conjunction with the ERP technique in relation to dietary choline intake. Of note, this is not common within the field of nutritional neuroscience. Interestingly, the beneficial influence of choline intake was only evident for the neuroelectric measures, emphasizing the need to utilize both standard behavioral outcomes with neurophysiological data when studying dietary choline’s implications for cognitive function. Albeit, this study was not without limitations. First, this study was solely comprised of a sample of adults with overweight or obesity, limiting its translation to healthy weight individuals. Given that recent Center for Disease Control figures quantify the percentage of Americans with overweight or obesity as nearly 70\% of the population, evaluation of the effects
of choline intake in this population is warranted and generalizable\textsuperscript{119}. Second, our study was cross-sectional in nature and, thus, we can only infer associations as opposed to causality. Third, our study did not include the use of biomarkers to assess choline status alongside dietary intake. Future studies are needed to investigate relationships between choline status and cognitive outcomes. Nevertheless, these associations highlight the need for dietary interventions aimed at increasing choline intake across the lifespan.

This study observed positive associations between neural efficiency, as indexed through the P3 component, and dietary choline intake amongst individuals with overweight and obesity. Further, this work represents, to our knowledge, the first study to link dietary choline intake with cognitive outcomes in young to middle-aged adults. As only 1 in 10 adults in the US are currently consuming adequate choline, this study highlights a pressing need for future studies aimed at further investigating the link between choline intake and executive function, and improving dietary choline intake across the lifespan.

**Disclosure of interest**

The authors have no conflicts of interest to declare.
CHAPTER 7: AIM 3: A CROSS-SECTIONAL EXAMINATION OF INTERACTIONS BETWEEN DIETARY CHOLINE INTAKE AND BIOMARKER STATUS OF LUTEIN AND CHOLINE ON COGNITIVE CONTROL

Abstract

**Background:** Independent consumption and biomarker status of the xanthophyll carotenoids lutein+zeaxanthin and the dietary component choline have been linked to benefits in cognition. However, knowledge on the interactive influence of these dietary components on cognitive function is sparse.

**Objective:** We examined cross-sectional associations between dietary intakes and biomarkers of lutein+zeaxanthin and choline with cognitive flexibility among adults with overweight and obesity.

**Design:** 80 young-to-middle-aged adults with overweight and obesity (BMI ≥25.0 kg/m²), completed 7-day diet records, venous blood draws, heterochromatic flicker photometry for assessment of macular pigmentation, the Kaufman Brief Intelligence Test – 2 for assessment of intelligence quotient (IQ) and a Switch task of cognitive flexibility while undergoing electroencephalographic recording for event-related potential (ERP) extraction. Multiplicative interaction terms were calculated to assess interactive capabilities. Hierarchical linear regressions, controlling for age, body mass index, sex, annual household income, and IQ, were
utilized to assess independent and interactive contributions of dietary and metabolite data on Switch task outcomes.

**Results:** Higher intake of lutein+zeaxanthin, and choline was associated interactively, but not independently, with faster reaction time (RT), even after controlling for pertinent covariates. Dietary intake of lutein+zeaxanthin and choline was associated with serum lutein concentrations, but not with plasma choline metabolites nor macular pigmentation. Plasma phosphatidylcholine (PC) concentrations were associated with higher accuracy in Switch trials, while no other biomarkers were associated with cognitive outcomes. Dietary intake and biomarker data were not related to the N2 nor P3 ERP component.

**Conclusions:** Individuals with greater dietary intake of choline and the xanthophylls lutein+zeaxanthin exhibited faster RT during the cognitively demanding trials of a cognitive flexibility task. Higher circulating PC was associated with higher response accuracy. The benefits derived from interactive consumption and PC on cognitive flexibility were evident without differences in the N2 or P3 component, suggesting alternative neural benefits of xanthophyll and choline consumption on cognitive function.

**Introduction**

Recent evidence has supported the beneficial role of nutrition on cognitive health, particularly among the 70% of Americans currently suffering from overweight or obesity \(^3\). The majority of nutrition research has focused on the impact of a single nutrient on mental and
cognitive health. This approach limits the understanding of the potential interactive capabilities of dietary components on cognitive function. Therefore, additional research examining the interactive influence of nutrients with potential complementary influences on cognitive function is needed.

Two dietary components that have been both inversely associated with adiposity and related to cognitive health are the xanthophyll carotenoids lutein+zeaxanthin and the nutrient choline. Lutein is a fat-soluble molecule found primarily in plant pigments. In the majority of nutrient databases lutein is aggregated with its lesser occurring stereo-isomer, zeaxanthin. Therefore, the term lutein will be used throughout to describe aggregated lutein+zeaxanthin intake. Lutein accumulates preferentially within the eye and the brain in related increments allowing for a non-invasive assessment of cortical lutein accumulation through the measurement of macular pigment optical density (MPOD). Choline is a nutrient found primarily in fish, poultry, and egg yolks and is involved in neurotransmitter synthesis (as the precursor to acetylcholine [ACh]), cell-membrane integrity, and signaling function (through phosphatidylcholine [PC]). Both lutein and choline are diet-dependent – carotenoids are not synthesized de novo, and while humans are capable of de novo choline synthesis, it is not synthesized in sufficient amounts. Approximately 90% of the US population does not meet the recommended adequate intake for choline (425 mg/d for women and 550 mg/day for men). While there are no dietary reference intakes for lutein consumption, average adult consumption is approximately 1-2 mg/day, with efficacious benefits suggested at 6 mg/day. Lutein and choline have exhibited interactive roles in absorption and circulation; in vitro as well as in vivo work has demonstrated that carotenoid absorption is enhanced with not only co-consumption of lipids, but is further enhanced through co-consumption of PC.
Independently, both lutein and choline intake have been related to beneficial cognitive control, or the processes that allow humans to engage in higher-order functioning including reasoning, problem-solving, and planning. Cognitive flexibility is perhaps the most demanding component of cognitive control. Cognitive flexibility builds upon lower-facets of cognition, namely inhibitory control and working memory, to allow for flexibility, spontaneity, and adaption to our ever-changing environments. Cognitive flexibility is frequently assessed using tasks designed to assess behavioral outcomes such as accuracy and reaction time (RT), and these behavioral outcomes have been shown to benefit from aerobic activity and diet in human populations, as well as choline supplementation in rodent models. While these behavioral benefits have been observed, further explanation of underlying inducting mechanisms of these benefits are less understood. Utilizing electroencephalographic recording of potentials time-locked to task stimuli (event-related potentials [ERPs]) allows for exploration of the neural mechanisms of mental chronometry potentially responsible for observed behavioral differences. Specifically, potentials emitted at the level of the scalp (ERP’s) can be used to quantify the magnitude of resources required, and speed of processing necessary, for cognitive processes. The N200 (N2), a negative-going potential occurring approximately 200ms post-stimulus onset is an index of response inhibition, while the P300 (P3, P3b) is a positive-going potential occurring between 300-700ms post-stimulus onset as an index of context updating, resource allocation, and categorization. Utilizing ERPs in conjunction with behavioral responses to explore the associations of dietary intake and status may therefore explicate the dietary derived cognitive benefits previously observed in cognitive flexibility tasks.

While it is assumed that all individuals may cognitively benefit from a healthy diet, individuals with overweight and obesity may benefit disproportionately. Despite this knowledge,
the interactive influence of lutein and choline on cognition has only once been studied once, and not in a sample older than infancy. Therefore, this study aimed to examine the associations between dietary intake of lutein and choline, biomarker concentrations of serum lutein and MPOD, plasma free choline and PC concentrations, and their individual and interactive impacts on cognitive flexibility among a sample of middle-aged adults with overweight and obesity. We hypothesized that individuals with higher consumption and biomarker concentrations of these components would exhibit interactive benefits for cognitive flexibility.

**Methods**

**Subjects**

Participant characteristics are described in Table 7.1. Baseline data from 80 adults enrolled in a larger clinical trial (NCT02740439), with body mass indices (BMI) ranging from 25.0 to 57.7 kg/m² were utilized. To qualify for the study, participants had to have a BMI > 25.0 kg/m², be between 25-45 years of age, successfully complete study sample collections, and be free of diagnosed neurological disorders. Participants were recruited using flyers posted in community settings, e-mails sent to University employees, as well as word-of-mouth recruitment, and were compensated with gift cards upon completion of all study procedures. All participants provided verbal and written consent in accordance with the University of Illinois’ Institutional Review Board and the Declaration of Helsinki.

**Testing Appointments**

During the first laboratory visit, participants provided demographic data including household income, completed the Kaufman Brief Intelligence Test II (KBIT), and height and weight measurements were recorded for BMI assessment. In between visits one and two, participants completed a 7-day dietary record to quantify dietary component intake. During the
second laboratory visit, cognitive testing was conducted following a 10-hour fast in the morning hours between 6:00am and 9:00am to reduce the potentially confounding effects of acute meal consumption on cognitive performance\textsuperscript{127}. Following cognitive testing a fasted venous blood draw was performed for collection of serum and plasma samples for assessment of serum lutein concentrations and plasma choline metabolites.

**Anthropometric Measures**

To calculate BMI (kg/m\(^2\)), height and weight were assessed using a stadiometer (model 240; SECA, Hamburg, Germany) and a digital scale (WB-300 Plus; Tanita, Tokyo, Japan). Participant height and weight were assessed while wearing light clothing and no shoes. The average of 3 measurements of height and weight were used for analyses.

**Intelligence Quotient**

KBIT-2 is a test of general intellectual abilities that has been nationally normed for ages 4 – 90 years \textsuperscript{79}. The test is comprised of three subtests: Verbal Knowledge, Riddles, and Matrices. A composite score of the three subtests is calculated and utilized as a measure of general intellectual abilities.

**Serum Carotenoid Assessment**

Serum lutein concentrations were assessed using high-performance liquid chromatography (HPLC). This technique has been previously described \textsuperscript{105}. Briefly, 250 µL of serum were mixed with an equal volume of ethanol and vortexed. Serum carotenoids were extracted using 3 consecutive, 1 ml hexane extraction processes. Hexane layers were combined, dried under nitrogen, taken up into a water solution and then analyzed, in duplicate, for carotenoid concentrations using the Alliance HPLC system (e2695 Separation Module) equipped with 2998 photodiode array detector (Waters, Milford, MA, USA) and a reverse-phase C30 column (4.6 ×
150 nm, 3 micron, YMC, Wilmington, NC, USA). Carotenoid standards were obtained from Carotenature (Ostermundigen, Switzerland). Standard curves were run for each carotenoid, and serum carotenoid concentrations were quantified by use of a 2550 ethanol extinction coefficient in a 1% solution.

**Plasma Choline and Choline Metabolite Assessment**

Phosphatidylcholine (PC) was extracted from 100 µL of plasma and was quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) according to the method of Koc et. al.\(^{136}\) with modifications based on available instrumentation\(^{137}\). Free choline was measured by LC-MS/MS as previously described\(^{138}\). Briefly, 100 µL of 0.1% formic acid in acetonitrile and 5 µL of internal standard mix was added to 50 µL of plasma. Internal standard mix contained choline D13 (CDN Isotopes). After vortexing and centrifugation, 5 µL of clear supernatant was injected on Syncronis Silica 150 x 2.1, 5u column with matching guard column (ThermoFisher Scientific). Metabolites were separated under isocratic conditions using 19% of 15 mM ammonium formate with 0.1% formic acid and 81% acetonitrile with flow of 0.5mL/min. Assay impression was less than 5% for each metabolite based on in-house human plasma controls.

**Macular Pigment Optical Density (MPOD)**

MPOD was assessed via a customized hetero-flicker photometry (cHFP) technique administered using a macular densitometer (Macular Metrics Corporation, Rehoboth, MA, USA). The principles of this technique have been previously described\(^ {136–138}\). Briefly, participants were asked to view stimuli peaking at a wavelength of 460 nm that flickers in counter phase with a 570 nm reference. Trained members of the research staff adjusted the radiance of the stimuli and participants were asked to identify a null flicker zone by informing the operator when they could no longer detect the flicker. The task was conducted while the stimulus was both centrally and
parafoveally fixated. The MPOD score, with standard deviation, was calculated by subtracting the foveal from the parafoveal log sensitivity measurements after normalization at 570 nm.

**Switch Task**

Cognitive Flexibility was assessed using a Switch task that has been described previously. Briefly, participants completed two blocks of homogeneous trials, in which they learned and practiced two basic rule sets with a test block of 90 jittered trials with an inter-stimulus interval (ISI) of either 1600, 1800, or 2000 ms. One subsequent heterogeneous condition was then completed, where participants were required to flexibly switch between the previously learned rule sets for a practice block of 50 trials, and 200 trials of randomized stimuli with similarly jittered ISI. A “Switch” trial was defined as a trial in which the previous stimuli belonged to a different mental rule set than the one presented. A “NonSwitch” trial was defined as a trial in which the previous stimuli belonged to the same mental rule set as the stimuli presented. Measures of accuracy and RT for homogeneous and heterogeneous blocks, and for both Switch and NonSwitch trials of the heterogeneous block, were assessed. Local Switch Cost for accuracy was calculated as NonSwitch accuracy – Switch accuracy, and for RT as Switch RT – NonSwitch RT. Global Switch Cost for accuracy was calculated as overall accuracy of the homogenous trials – overall accuracy of the heterogeneous trials, and for RT as overall RT of the heterogeneous trials – overall RT of the homogenous trials.

**Event-related potential Analysis**

EEG activity was recorded via a Neuro-scan Quik-cap with 64 scalp electrodes arranged in the international 10-10 system. Electrooculographic activity was recorded with a set of four electrodes placed at the outer canthus of each eye and above and below the left orbit. A midline
sensor placed between Cz and CPz served as a reference and AFz served as the ground. Further explanation of reduction procedures have been previously published.

Based on evidence observed from post-hoc topographic images, sensor FCZ was utilized for N2 assessment, and a 6-sensor region of interest (ROI) comprised of C1, CZ, C2, CPZ, CP1, and CP2 electrodes was utilized for P3 assessment. Only correct trials from individuals who reported >50% usable trials in both homogenous and heterogeneous trial types were used for analyses. The N2 component was defined as the localized peak and corresponding latency occurring between 150-300 ms post-stimulus onset, and the P3 component was defined as the localized peak and corresponding latency occurring between 300-600 ms post-stimulus onset. Peak amplitude was measured as a change score from the pre-stimulus baseline and peak latency was defined as the time point of the maximum amplitude.

**Dietary Intake**

Analyses of 7-day dietary records were conducted using the 2015 Nutrition Data System for Research (NDSR) software (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA). Diet records were administered and entered into NDSR by trained members of the research staff. Within NDSR, as with most nutrient databases, lutein and zeaxanthin are aggregated. Thus, lutein intake refers to the joint intake of lutein and zeaxanthin. Choline intake in NDSR refers to the combined intake of free choline, phosphocholine, PC, glycerophosphocholine, as well as sphingomyelin. Average lutein and choline intake over the 7-day period was assessed using the averages of the NDSR Intake Properties Totals Output File (04).

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS, version 24, IBM, Armonk, New York) software was used for all statistical analyses. Variables that did not meet criteria for
normality, as assessed through graphical methods (Q-Q plots) or with $p<0.05$ as assessed by Shapiro-Wilk, were log transformed and normality was reassessed. Independent t-tests were conducted to examine differences between males and females, with significance set at $p<0.05$.

Initial Pearson product-movement bivariate correlations were conducted between dietary components (lutein, choline), serum lutein concentrations, MPOD, and plasma concentrations of free choline and PC. To assess the interactions of independent variables, interaction terms were calculated as the product of the two independent variables of interest. Interaction terms produced were 1) dietary lutein x dietary choline, 2) plasma PC x plasma free choline, 3) plasma PC x MPOD, and 4) plasma PC x serum lutein. Potential multicollinearity was evaluated utilizing variance inflation factors with a cut-off >2. Variables that displayed multicollinearity were mean centered by subtracting the sample mean from each variable, and the centered variables were used in further analyses. To address the differences in individual and interactive contributions of independent variables on measures of cognitive flexibility, data were subjected to hierarchical linear regression analyses, with cognitive flexibility outcomes as dependent factors. Step 1 consisted of pertinent demographic and weight status variables (age, sex, BMI, IQ, and household income), and Step 2 contained two independent variables of interest as well as the interaction term. $\Delta R^2$ and $\Delta$Akaike Information Criteria (AIC) were used to assess model fit. To determine the unique contribution of each predictor variable, the squared semipartial correlations were utilized. For models in which the centered interaction term was significant simple slopes models were produced. The simple slope procedure takes the low value of a variable (in this case the lowest tertile of dietary choline intake), the mean value of a variable (the second tertile of dietary choline intake), and a high value of a variable (the third tertile of dietary choline intake) and determines whether or not the slope of the first independent variable changes significantly with respect to the
Results

Correlations between Dietary Intake and Biomarkers

Full descriptions of dietary intake and biomarker concentrations are depicted in Table 7.1. Full bivariate correlation results are described in Table 7.2. Dietary lutein intake was correlated with dietary choline intake (r=0.35, p<0.01) and serum lutein concentrations (r=0.42, p<0.01). Dietary choline intake was correlated with serum lutein concentrations (r=0.35, p<0.01). MPOD was correlated with serum lutein concentrations (r=0.32, p<0.01). Neither plasma PC concentrations, nor plasma free choline concentrations, were correlated with dietary measures of either lutein or choline, nor measures of serum lutein nor MPOD concentrations (all p’s>0.05).

Cognitive task performance

Age, sex, BMI, IQ, and household income were entered into Step 1 for each model. Full regression values including standardized regression coefficients, independent variable significance, ΔR², model significance, and squared semipartial correlations can be found in Tables 7.3 and 7.4.

Dietary Intake

Multicollinearity between dietary and metabolite data was observed for all independent variables (all VIF’s>2.0, range=4.5-565.7). After centering of variables all issues of multicollinearity were resolved (all VIF’s<2.0, range=1.1-1.5). Step 2 included the addition of centered dietary lutein, centered dietary choline, and their multiplicative interaction term.
<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>80</td>
<td>48</td>
<td>32</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>34.7 ± 5.9</td>
<td>35.0 ± 6.1</td>
<td>34.3 ± 5.7</td>
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<tr>
<td><strong>Intelligence Quotient</strong></td>
<td>109.2 ± 11.8</td>
<td>107.9 ± 11.7</td>
<td>110.9 ± 11.9</td>
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<tr>
<td><strong>Body Mass Index, kg/m²,²</strong></td>
<td>33.0 ± 6.5</td>
<td>34.1 ± 6.5</td>
<td>31.4 ± 6.3</td>
</tr>
<tr>
<td><strong>Dietary Choline, mg/day</strong></td>
<td>353.3 ± 111.1**</td>
<td>317.6 ± 93.9</td>
<td>406.9 ± 114.6</td>
</tr>
<tr>
<td><strong>Dietary Lutein + Zeaxanthin, mg/day</strong></td>
<td>1.55 ± 1.13</td>
<td>1.54 ± 1.18</td>
<td>1.56 ± 1.07</td>
</tr>
<tr>
<td><strong>Serum Lutein, μmol/L</strong></td>
<td>0.12 ± 0.06</td>
<td>0.12 ± 0.05</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td><strong>Macular Pigment Optical Density</strong></td>
<td>0.44 ± 0.21</td>
<td>0.44 ± 0.20</td>
<td>0.45 ± 0.22</td>
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<tr>
<td><strong>Plasma Phosphatidylcholine, μmol</strong></td>
<td>2.15 ± 0.29</td>
<td>2.16 ± 0.30</td>
<td>2.13 ± 0.27</td>
</tr>
<tr>
<td><strong>Plasma Free choline, nmol</strong></td>
<td>8.12 ± 1.87*</td>
<td>8.45 ± 1.98</td>
<td>7.54 ± 1.55</td>
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<tr>
<td><strong>Homogeneous Accuracy, %</strong></td>
<td>95.0 ± 4.4</td>
<td>95.09 ± 3.92</td>
<td>94.90 ± 5.03</td>
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<tr>
<td><strong>Homogeneous RT, ms</strong></td>
<td>516.9 ± 69.2</td>
<td>514.8 ± 55.3</td>
<td>520.0 ± 86.9</td>
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<tr>
<td><strong>Heterogeneous Accuracy, %</strong></td>
<td>80.8 ± 10.6</td>
<td>80.4 ± 11.2</td>
<td>81.3 ± 9.9</td>
</tr>
<tr>
<td><strong>Heterogeneous RT, ms</strong></td>
<td>770.4 ± 100.9*</td>
<td>748.4 ± 98.8</td>
<td>803.5 ± 96.3</td>
</tr>
<tr>
<td><strong>NonSwitch Accuracy, %</strong></td>
<td>84.2 ± 11.0</td>
<td>83.6 ± 12.3</td>
<td>85.2 ± 8.9</td>
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<tr>
<td><strong>NonSwitch RT, ms</strong></td>
<td>720.7 ± 89.8**</td>
<td>699.1 ± 84.8</td>
<td>753.2 ± 88.4</td>
</tr>
<tr>
<td><strong>Switch Accuracy, %</strong></td>
<td>77.4 ± 11.5</td>
<td>77.3 ± 11.5</td>
<td>77.5 ± 11.5</td>
</tr>
<tr>
<td><strong>Switch RT, ms</strong></td>
<td>825.2 ± 122.7*</td>
<td>802.6 ± 122.5</td>
<td>859.1 ± 116.8</td>
</tr>
<tr>
<td><strong>Annual Household Income, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$10,000-$40,000</td>
<td>33</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>$41,000-$80,000</td>
<td>35</td>
<td>44</td>
<td>31</td>
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<tr>
<td>$81,000+</td>
<td>32</td>
<td>31</td>
<td>41</td>
</tr>
</tbody>
</table>

1 Values are means ± SD unless otherwise stated

² Determined by the Center for Disease Control Body Mass Index (BMI) classifications

* Significant differences between sexes (p > 0.05)

** Significant differences between sexes (p > 0.01)
Table 7.2. Bivariate correlations between dietary intake lutein and choline, serum lutein concentrations, phosphatidylcholine and free choline plasma concentrations, and macular pigment optical density (MPOD).

<table>
<thead>
<tr>
<th></th>
<th>Dietary Lutein</th>
<th>Dietary Choline</th>
<th>DIT</th>
<th>Serum Lutein</th>
<th>Phosphatidylcholine</th>
<th>Free choline</th>
<th>MPOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Lutein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dietary Choline</td>
<td>0.35**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DIT</td>
<td>0.93**</td>
<td>0.67**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum Lutein</td>
<td>0.42**</td>
<td>0.35**</td>
<td>0.47**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Free choline</td>
<td>0.12</td>
<td>-0.01</td>
<td>0.09</td>
<td>-0.04</td>
<td>-0.11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPOD</td>
<td>0.03</td>
<td>0.11</td>
<td>0.06</td>
<td>0.32**</td>
<td>-0.18</td>
<td>0.12</td>
<td>-</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
¹DIT=Dietary Interaction Term of Dietary Lutein x Dietary Choline
The addition of these variables improved the model for NonSwitch RT ($\Delta R^2=0.10$, $\Delta AIC=-3.99$, $p=0.03$), Switch RT ($\Delta R^2=0.10$, $\Delta AIC=-2.79$, $p=0.05$), and Global Switch Cost ($\Delta R^2=0.08$, $\Delta AIC=-2.74$, $p=0.05$). Dietary lutein and dietary choline were not independently related to measures of RT nor accuracy (all $p>0.05$); however, the dietary interaction term was associated with faster RT in both NonSwitch ($\beta=-0.27$, $p=0.02$) and Switch ($\beta=-0.29$, $p=0.01$) trials, as well as the Global Switch Cost ($\beta=-0.23$, $p=0.03$). Within these models, the dietary interaction term also accounted for the largest percentage of variance explained by the dietary contributors, at 6.4% for NonSwitch RT, 7.5% for Switch RT, and 4.8% for Global Switch Cost. Centered dietary lutein, centered dietary choline, and their interaction term were not related to accuracy in any task trials (all $p$’s $>0.05$). Figures 7.1-7.3 illustrate the plotted simple slope models of these interactions.

**PC and free choline**

For these analyses, Step 2 included the addition of centered plasma PC concentrations, centered plasma free choline concentrations, and their multiplicative interaction term (Table 7.4). The addition of these variables improved the model for Switch trial accuracy ($\Delta R^2=0.08$, $\Delta AIC=-2.10$, $p=0.05$). In this model, plasma PC concentrations were associated with higher task accuracy ($\beta=0.27$, $p=0.02$), while plasma free choline concentrations, and the interaction of plasma PC concentrations and plasma free choline were not significant (all $p>0.05$). PC concentration accounted for 6.6% of the variance explained by the metabolite contributors. Plasma PC, plasma free choline, and their interaction term were not related to RT in any task trials (all $p>0.05$).
Table 7.3. Linear Regression between pertinent covariates, dietary lutein, dietary choline, their interactive term, Switch task outcomes.

<table>
<thead>
<tr>
<th>Step and Variable</th>
<th>NonSwitch RT</th>
<th></th>
<th>Switch RT</th>
<th></th>
<th>Global Switch Cost RT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model</td>
<td>β</td>
<td>p</td>
<td>ΔR²</td>
<td>P</td>
<td>sr²</td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.29**</td>
<td>0.01</td>
<td>7.98</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td>-0.13</td>
<td>0.91</td>
<td>0.02</td>
<td>0.00</td>
<td>0.99</td>
</tr>
<tr>
<td>Intelligence Quotient</td>
<td></td>
<td>-0.11</td>
<td>0.33</td>
<td>1.09</td>
<td>-0.07</td>
<td>0.55</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>-0.07</td>
<td>0.56</td>
<td>0.39</td>
<td>-0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Household Income</td>
<td></td>
<td>0.26</td>
<td>0.03</td>
<td>5.60</td>
<td>0.36**</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centered Lutein</td>
<td></td>
<td>0.14</td>
<td>0.24</td>
<td>1.48</td>
<td>0.09</td>
<td>0.44</td>
</tr>
<tr>
<td>Centered Choline</td>
<td></td>
<td>0.13</td>
<td>0.32</td>
<td>1.06</td>
<td>0.07</td>
<td>0.55</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td>-0.27*</td>
<td>0.02</td>
<td>6.38</td>
<td>-</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Relationship is significant at the 0.01 level (2-tailed).
*Relationship is significant at the 0.05 level (2-tailed).
sr² = the squared semi-partial correlation, multiplied by 100
Table 7.4. Linear Regression between pertinent covariates, plasma phosphatidylcholine, plasma free choline, their interactive term, Switch task outcomes.

<table>
<thead>
<tr>
<th>Step and Variable</th>
<th>Homogeneous Accuracy</th>
<th>NonSwitch Accuracy</th>
<th>Switch Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model β</td>
<td>p</td>
<td>ΔR²</td>
</tr>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>-0.06</td>
<td>0.61</td>
<td>0.14*</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>-0.07</td>
<td>0.54</td>
<td>0.45</td>
</tr>
<tr>
<td>Intelligence Quotient</td>
<td>0.08</td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>Age</td>
<td>0.02</td>
<td>0.90</td>
<td>0.02</td>
</tr>
<tr>
<td>Household Income</td>
<td>0.35*</td>
<td>0.00</td>
<td>9.79</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centered Phosphatidylcholine</td>
<td>0.18</td>
<td>0.12</td>
<td>2.88</td>
</tr>
<tr>
<td>Centered Free Choline</td>
<td>0.02</td>
<td>0.86</td>
<td>0.04</td>
</tr>
<tr>
<td>Centered Interaction</td>
<td>0.13</td>
<td>0.25</td>
<td>1.57</td>
</tr>
</tbody>
</table>

**Relationship is significant at the 0.01 level (2-tailed).**
*Relationship is significant at the 0.05 level (2-tailed).

sr² = the squared semi-partial correlation, multiplied by 100
**PC and Serum Lutein concentrations**

For these analyses, Step 2 included the addition of centered plasma PC concentrations, centered serum lutein concentrations, and their multiplicative interaction term. The addition of these variables improved the model for Switch accuracy ($\Delta R^2=0.07$, $\Delta AIC=-2.10$, $p=0.05$). In the significant model for Switch accuracy, plasma PC concentrations were associated with higher task accuracy ($\beta=0.24$, $p=0.04$), while serum lutein concentrations, and the interaction of plasma PC and serum lutein concentrations was not significant (all $p>0.05$). Plasma PC concentration accounted for 5.5% of the variance explained by the metabolite contributors. Plasma PC concentration and serum lutein concentration were not related to RT in any task trials (all $p>0.05$).

**PC and MPOD**

For these analyses, Step 2 included the addition of centered plasma PC concentrations, centered MPOD, and their multiplicative interaction term. The addition of these variables improved the model for Switch accuracy ($\Delta R^2=0.07$, $\Delta AIC=-0.68$, $p=0.04$) and Local Switch Cost accuracy ($\Delta R^2=0.11$, $\Delta AIC=-4.16$, $p=0.03$). In the significant model for Switch accuracy, plasma PC concentrations were associated with higher accuracy ($\beta=0.28$, $p=0.02$) while MPOD, and the interaction term were not (all $p’s>0.05$). Plasma PC concentration accounted for 6.5% of the variance explained by these contributors. In the significant model for Local Switch Cost accuracy, plasma PC concentrations were associated with lower accuracy cost ($\beta=-0.28$, $p=0.02$) when alternating between NonSwitch and Switch trials, as was the interaction term ($\beta=-0.28$, $p=0.02$). Plasma PC concentrations accounted for 6.8% of the variance explained by these contributors, while the interaction term accounted for 6.1%. Plasma PC concentration and MPOD were not related to RT in any task trials (all $p>0.05$).
Event-Related Potentials

None of the models were predictive of N2 nor P3 peak amplitude or latency variables in either the homogenous or heterogeneous task conditions, nor in terms of Global or Local Switch Costs (all $p>0.05$). Therefore, while relationships were observed between variables of interest and behavioral accuracy and RT, there was a lack of a mechanistic explanation to be derived from underlying neuroelectric responses. Waveform depictions are presented in Figure 7.4.

Discussion

This study aimed to examine the associations between dietary intake of lutein and choline, biomarkers of serum lutein, macular pigmentation, plasma PC and plasma free choline, and their individual and interactive effects on cognitive flexibility among a sample of middle-aged adults with overweight and obesity. Dietary intake of lutein and choline was associated with higher serum lutein concentrations, and not with MPOD, plasma PC, or plasma free choline. Independently, dietary lutein and dietary choline were not related to RT nor accuracy in a cognitive flexibility task; however, even after controlling for pertinent demographic and weight status covariates, higher interactive consumption was associated with faster RT in the more cognitively demanding trial types. Among metabolites, PC concentrations were related to higher accuracy on Switch trial types, while neither serum lutein concentrations, MPOD, plasma free choline concentrations, nor their interactions with plasma PC concentrations, were related with cognitive flexibility. These results point to an interactive role of dietary intake of lutein and choline on cognitive flexibility for individuals with overweight and obesity.
In this study, we observed associations between dietary intake of the xanthophyll carotenoid lutein and the vitamin choline. Both dietary lutein and dietary choline were related to serum lutein concentrations, perhaps explained by simultaneous presence of these components in the gastrointestinal tract at time of digestion and the known interactive properties of absorption. Choline contributes to lipid transport throughout the body, particularly to lipoprotein production in the liver. It is possible that with higher choline intake there is increased lipoprotein production, which are the primary source of lutein transportation throughout the body, resulting in higher serum lutein concentrations. Dietary intake of lutein and choline were not related to choline metabolite concentrations, nor MPOD. Null associations between concurrent choline intake and metabolite status is supported in the literature, as choline serves a wide array of roles throughout the body. Serum lutein concentrations were correlated with MPOD, perhaps suggesting that serum lutein concentrations are more susceptible to acute dietary intake than retinal accumulation.

While both lutein and choline have been independently related to benefits for cognitive control, we did not observe benefits of individualized consumption in the present study. Further, faster processing speed, as evidenced by faster RT, was only observed with the interaction of the two components (i.e., lutein and choline). Within the brain, lutein serves as one of several antioxidants, and choline (derived from the diet or endogenous production) can serve as a precursor for the neurotransmitter acetylcholine (ACh). ACh has been demonstrated to influence neuronal excitability, alter presynaptic release of neurotransmitters, and coordinate the firing of groups of neurons. In rodent models, high acetylcholinesterase (the enzyme that catalyzes breakdown of ACh) activity in the hippocampus and cortex has been associated with ethanol-induced memory impairments. Oral administration of lutein attenuated both acetylcholinesterase...
activity and ethanol-induced memory impairment compared to a control group \(^6^5\). Notably, a limitation of clinical research is the inability to evaluate concentrations of cerebral ACh. It is possible that the benefits derived from combined consumption of lutein and choline are due to an increase in cerebral ACh production, driven perhaps by lutein’s attenuation of acetylcholinesterase activity and high amount of choline intake.

Within the human literature, to our knowledge, only one study has previously examined the interactive influence of these dietary components \(^6^6\). Among an infant cohort, Cheatham et. al. observed that higher combined intake of choline and lutein from breastmilk was predictive of faster latency to peak amplitude in a recognition memory task as assessed using ERPs. While the present study observed faster behavioral RT during a cognitive flexibility task, contrary to our hypotheses we did not observe relationships between peak amplitude nor latency of the N2 and P3 components and independent variables of interest. This lack of statistical significance provides information on the alternative neural mechanistic benefits that may occur with lutein and choline consumption in middle-age adults. ERPs are measurements of post-synaptic potentials from cortical pyramidal cells, emitted by cumulative changes in synaptic ionic activity due to the binding of neurotransmitters, such as ACh, to their receptors \(^1^0^2\). Cognitive flexibility, in particular, is reliant upon activation of mental rule-sets, which are contingent upon exogenous stimuli cuing (in this case, through the dashed or solid boxes). RT as measured by a behavioral response incorporates a number of cognitive processes, including but not limited to, response selection, response execution, and stimulus evaluation, and is not consistently related to latency or peak amplitude \(^1^4^4\). The N2 is most notably associated with the inhibition of response selection, while the P3 is indicative of stimulus evaluation and categorization. The present null relationships indicate that the mechanisms behind faster RT in this population may be driven by post-inhibitory or post-
categorization factors. Future studies utilizing follow-up methods of neuroimaging should thus be conducted.

In regard to the relationship between biomarker status and cognition, solely PC concentrations were related to benefits for cognitive flexibility, specifically accuracy within the Switch trials. It is important to note that within the present study, PC concentrations were assessed peripherally and were not derived from brain cortices. Nonetheless, circulating PC concentrations have been found to be lower in patients with Alzheimer’s Disease (AD), and alterations in peripheral and brain PC/lipid metabolism among patients with AD have been demonstrated. These findings allude to a potential benefit of circulating PC for cognitive health, though testing of this relationship has been problematic due to heterogeneity of both results and testing methods. In elderly patients with AD, higher concentrations of free choline have been observed within the brain. These higher concentrations of free choline have been interpreted as a marker of neurodegeneration, in what has been referred to as “autocannibalism” – or the brains degenerating attempts to release choline from its largest storage site (PC) and compensate for the characteristic lack of free choline and ACh. Much of current AD treatment is based around this choline deficit, through the use of cholinergic agonists to promote choline production and preserve cell membrane integrity. The present results are suggestive of a perhaps similar role for circulating PC in cognitive health in middle adulthood.

While this study derived its strengths from the use of neuroelectric and behavioral paradigms and utilized both dietary assessments as well as biomarkers of dietary intake to objectively measure serum lutein and choline concentrations, it is not without limitations. First, these results are cross-sectional, and thus relationships observed should be interpreted without the implications of causality. Second, this sample was comprised solely of adults with overweight and
obesity, and thus may not be extrapolated to those of healthy weight status. Though, given that 70% of the American population currently suffers from overweight or obesity, this sample is more generalizable than a healthy weight sample. The dietary data provided included aggregated quantification of dietary lutein and zeaxanthin intake, therefore we are unable to separate the impact of the two xanthophylls on cognitive function. Lastly, the dietary data provided was based on self-report measures. Though detailed instructions were given on proper dietary recording by trained members of the research staff, it is possible that participants may have over or underestimated foods and ingredients consumed.

Conclusion

After controlling for age, sex, BMI, IQ, and household income, middle-aged participants with overweight or obesity exhibited faster RT during a cognitive flexibility task with greater dietary consumption of lutein and choline. Consumption of these dietary components was also related to higher serum lutein concentrations, but was not related with MPOD, plasma PC concentrations, plasma free choline, nor measures of neuroelectric components. Future studies are necessary to further elucidate the potential interactive properties of dietary components such as lutein and choline to inform dietary recommendations for cognitive and brain health.
Figure 7.1. Simple slopes model of dietary lutein by choline interaction for reaction time (RT) in the NonSwitch Trials of a Switch Task. Negative values are indicative of faster RT. Choline intake was split into tertiles, with average tertile intake of 231.9 mg in the lowest tertile, 329.6 in the middle tertile, and 481.4 in the highest tertile.
Figure 7.2. Simple slopes model of dietary lutein by choline interaction for reaction time (RT) in the Switch Trials of a Switch Task. Negative predicted values are indicative of faster RT. Choline intake was split into tertiles, with average tertile intake of 231.9 mg in the lowest tertile, 329.6 in the middle tertile, and 481.4 in the highest tertile.
Figure 7.3. Simple slopes model of dietary lutein by choline interaction for reaction time (RT) in the Global Switch Trials of a Switch Task. Negative predicted values are indicative of faster RT. Choline intake was split into tertiles, with average tertile intake of 231.9 mg in the lowest tertile, 329.6 in the middle tertile, and 481.4 in the highest tertile.
Figure 7.4: Event-related potential waveform depictions of the P3 component. The sample underwent a median split of a multiplicative dietary lutein x dietary choline interaction term into Low and High groups for visualization of a) Switch Trials and b) NonSwitch trials. P-values reflect an independent samples t-test between the two groups.
CHAPTER 8: CONCLUSIONS AND FUTURE DIRECTIONS

The dietary components lutein and choline have been hypothesized to play a protective role in cognitive health across the lifespan, and have been cross-sectionally associated with decreased risk of dementia and AD\textsuperscript{47,48,145}. Dietary intake of beneficial dietary components may provide a feasible strategy to preserving cognitive health during across the lifespan, particularly among adults with overweight and obesity. Therefore, the specific aims of this work were to utilize a sample of adults with overweight and obesity to: 1) Cross-sectionally, and through a randomized controlled trial, examine the role of retinal xanthophyll accumulation and serum lutein status on behavioral and neuroelectric components of cognitive control, 2) Cross-sectionally examine the role of choline intake on behavioral and neuroelectric components of cognitive control, and 3) Cross-sectionally examine the independent and interactive roles of choline and lutein intake and choline and lutein concentrations on cognitive control. This was accomplished utilizing both baseline and intervention data from a large-scale clinical trial investigating the impact of avocado consumption on measures of behavioral and neuroelectric indices of cognitive control.

Summary of Aims and Findings

Aim 1. We demonstrated that, as hypothesized, higher MPOD was cross-sectionally associated with higher neural efficiency in both the N2 and P3 ERP components, though behavioral improvements were not observed. To assess the impact of lutein intake on MPOD and neuroelectric indices of cognitive control we conducted a 12-week avocado consumption randomized controlled trial. In this trial, we observed increases in serum lutein concentrations and improvements in selective attention performance in the intervention group, but no changes in MPOD nor neuroelectric markers of cognitive control in either group.
**Aim 2.** We demonstrated that higher dietary intake of choline was associated cross-sectionally to increased neural efficiency as indexed by the P3 component, though behavioral improvements were not observed.

**Aim 3.** We demonstrated that higher dietary intake of both lutein and choline, when compared to independent consumption of lutein and independent consumption of choline, was associated cross-sectionally with improvements in reaction time in a cognitive flexibility task, though associations with ERP components were not observed.

*Limitations and Future Work*

Plans for moving forward with this work are currently underway through additional data analyses by other team members, and through the initiation of an egg-based randomized controlled trial aimed at examining a whole-food source of lutein and choline intake on measures of cognitive control. While the work described thus far contributes to the growing literature emphasizing the importance of studying dietary components within the context of cognitive health, there is still research needed. Several limitations are worth considering when interpreting these findings, notably utilization of self-report data, the use of a sample of solely adults with overweight and obesity, and the cross-sectional nature of a number of the current findings.

There is a frequent prevalence of under and over reporting of self-report dietary intake, particularly among adults with overweight and obesity\(^146\). To address this concern, whenever possible the authors attempted to include a reliable biomarker of said dietary intake in analyses for validation (ex.: MPOD). The 7-day diet records used in Aims 2 and 3 were deployed by graduate students trained in diet record administration, were manually entered and then quality controlled.
by trained members of the research staff, and were then statistically analyzed for outliers \( \geq 3 \) standard deviations outside of group means. While the methods used to collect the presently presented data have been previously validated, moving forward with other methods of assessing dietary intake could provide insight, and confirmation of these results. Future work aiming to replicate these findings would benefit from using 7-day diet records in conjunction with novel technology.

While using the human model to examine the direct impact of a dietary components on cognitive outcomes has several significant strengths, one limitation is the lack of currently available preclinical techniques of testing these hypotheses. While there are excellent studies examining brain tissue samples in deceased pediatric as well as geriatric populations, currently there are no studies that have been able to directly examine the presence of, or potential actions of, lutein and choline in a middle-aged population\(^{17,147}\). Lutein and zeaxanthin do not accumulate within the retina of mice, pigs, or rodents; therefore, animal work has been largely limited to using non-human primate samples as primates do accumulate lutein and zeaxanthin in the retina\(^{20}\). This presents obstacles to understanding the mechanistic action that lutein or choline may have crossing the blood brain interface and accumulating and acting within brain cortices. Future work in non-human primates as well as development of additional methods of assessing nutrient biomarkers in living humans is thus warranted.

Lastly, while in Aim 3 we were able to observe statistically significant interactions between lutein and choline dietary intake and measures of response time, this was done cross-sectionally and analyses were post-hoc. Future replication work is needed both in cross-sectional work as well as randomized-controlled trials utilizing \textit{a priori} hypotheses about the interactive nature of dietary components in food items.
### CHAPTER 9: REFERENCES


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