THE EFFECTS OF LUTEIN AND ZEAXANTHIN SUPPLEMENTATION ON BRAIN MORPHOLOGY IN OLDER ADULTS

by

CATHERINE MATTOCKS MEWBORN

(Under the Direction of L. Stephen Miller)

ABSTRACT

A growing literature emphasizes the importance of lifestyle factors such as nutrition in successful aging. The current study examined if one year of supplementation with lutein (L) and zeaxanthin (Z), two nutrients with known antioxidative properties and cognitive benefits, impacted structural brain outcomes in older adults using a double-blind, randomized, placebo-controlled trial. Participants (20 male, 27 female) aged 65 – 87 years (M = 71.8 years, SD = 6.04 years) were randomized into supplement (N = 33) and placebo groups (N = 14). L and Z were measured as retinal and blood serum concentrations and structural brain outcomes, focusing on global and frontal-temporal lobe regions, were acquired using both MRI and DTI technologies. It was hypothesized that the supplement group would increase, maintain, or show attenuated loss in hypothesized regions-of-interest (ROIs) while the placebo group would show age-related declines in brain structural integrity over the course of the trial. While results showed age-related declines for frontal and temporal gray and white matter volumes and fornix white matter microstructure across both groups, only minimal differences were found between the supplement and placebo groups. However, exploratory analyses

showed that individuals who responded better to supplementation (i.e., showed greater increases in retinal L & Z concentrations) showed less decline in global and prefrontal gray matter volume than supplement “non-responders.” While results suggest that one year of L and Z supplementation has limited effects on structural brain outcomes, it is likely not harmful and appears to provide benefits for other aspects of cognitive and brain health that could improve or extend quality of life for older adults. Additionally, there may be a sub-sample of individuals for whom supplementation of L and Z provides greater benefits.

INDEX WORDS: lutein, zeaxanthin, carotenoids, white matter, diffusion tensor imaging, structural imaging, DTI, MRI, aging, older adults, randomized controlled trial, RCT

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B.A., Wake Forest University, 2013

M.S., The University of Georgia, 2016

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

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December 2019

ACKNOWLEDGEMENTS

This dissertation is the culmination of five years of work, and there are many people I would like to thank for their support and encouragement, not only during the completion of this project but also during the past five years. To my mentor, Steve Miller – you have been such a positive force during my time in graduate school. Thank you for your support and guidance both professionally and personally. It would be hard to find a kinder and better mentor. To my committee, Mary Ann Johnson and Larry Sweet – you have been with me since the beginning and I cannot thank you enough for the consistency and wisdom you have provided throughout all my major accomplishments in graduate school. To my labmates in the Neuropsychology and Memory Assessment Lab, Doug Terry, Cutter Lindbergh, Marissa Gogniat, Talia Robinson, and Kharine Jean – you have made my time in graduate school productive, supportive, and fun. My research would be nothing without your help. To our collaborators, Randy Hammond and Lisa Renzi-Hammond – thank you for allowing me to be part of this wonderful collaboration and pursue my dream of nutrition research. To all my closet friends, EA, KBW, LB, JD, AW, CJ, LAM, AJ, JBW, EH, my cohort, and a few special UGA staff – you have kept me afloat when time were tough. And most of all to my parents, Noland and Denise Mewborn – you taught me the value of education, hard work, and perseverance. I could never thank you enough for everything you have done for me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cognitive Aging and Its Costs

Old age brings about cognitive decline across a range of domains, including attention, processing speed, memory, working memory, language, visual-spatial functioning, and executive functioning (e.g., Glisky, 2007; Madden, 1992; Meijer, van Boxtel, van Gerven, van Hooren, & Jolles, 2009; Schaie & Willis, 1996; Tucker-Drob, Johnson, & Jones, 2009). Cognitive declines are seen during normal aging, in healthy older adults, as well as in those with mild cognitive impairment (MCI) and more advanced dementias (e.g., Fillit et al., 2002; Schaie, Willis, & Caskie, 2004). These age-related cognitive declines are associated with decreased ability to function in daily life (e.g., Cahn-Weiner et al., 2007; Miller, Brown, Mitchell, & Williamson, 2013; Owsley, Sloane, McGwin, & Ball, 2002; Puente, Terry, Faraco, Brown, & Miller, 2014; Royall et al., 2007; Tucker-Drob, 2011) and lowered mood and psychological distress (e.g., Couture, Lariviere, & Lefrancois, 2005). Furthermore, age-related cognitive decline represents a significant economic burden for individuals, families, and society through lost wages, hours spent providing informal, unpaid care, increased utilization of high-cost healthcare (e.g., nursing home care), and increased insurance claims (Alzheimer's Association, 2017; Barnes & Yaffe, 2011; Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007; Hurd, Martorell, Delavande, Mullen, & Langa, 2013). Recent estimates suggest that the total yearly cost of dementia in the United States is $259 billion with

costs expected to rise to more than $1.1 trillion by 2050 (Alzheimer's Association, 2017). Additionally, an estimated 5.5 million Americans are currently living with Alzheimer's disease, and the incidence is expected to double by 2050, with close to half of patients needing to utilize a high and costly level of care (Alzheimer's Association, 2017).

Previous research suggests that up to half of Alzheimer's disease cases might be preventable by addressing risk factors such as poor diet and physical inactivity, which contribute to diabetes, hypertension, obesity, and depression (Barnes & Yaffe, 2011). If prevention and intervention efforts could delay disease onset and progression by just one year, estimates suggest that there would be nearly 9.2 million fewer cases of Alzheimer's disease worldwide by 2050, which would represent a significant decline in healthcare utilization and costs (Barnes & Yaffe, 2011; Brookmeyer et al., 2007).

There is a strong connection between brain structure, cognition, and risk of developing dementia (e.g., Bartzokis, 2004; Lu et al., 2013; Madden et al., 2009; Sullivan, Marsh, Mathalon, Lim, & Pfefferbaum, 1995; Ziegler et al., 2010). Specifically, gray matter atrophy (e.g., Brickman et al., 2006; Hua et al., 2008; Kohama, Rosene, & Sherman, 2012; Leow et al., 2009), white matter atrophy (e.g., Brickman et al., 2006; Chen et al., 2009; Kohama et al., 2012; van Es et al., 2006), increased white matter hyperintensity or lesion burden (e.g., Aggarwal et al., 2012; Arvanitakis et al., 2016; Marchant et al., 2013), and decreased white matter microstructure through demyelination and axonal damage (e.g., Bennett & Madden, 2014; Brickman et al., 2012; Gold, Powell, Xuan, Jicha, & Smith, 2010; Kennedy & Raz, 2009; Lee et al., 2012) all predict poorer cognitive functioning across domains for healthy older adults, individuals with MCI, and individuals with Alzheimer's disease (Hua et al., 2008; Huang, Polk, Goh, & Park, 2012;

Kohama et al., 2012; Liao et al., 2010). Nutrition has been proposed as a factor that can preserve brain structure in aging through reduction of mechanisms that lead to neuropathology, such as oxidative stress and inflammation (e.g., Abdollahi, Moridani, Aruoma, & Mostafalou, 2014; Heneka et al., 2015; Poulose Miller, & Shukitt-Hale, 2014). Preserved brain structure, in turn, affects cognitive performance and rates of cognitive decline (Reuter-Lorenz & Park, 2014). Thus, the current study analyzed the impact of a nutritional intervention (supplementation of lutein and zeaxanthin) on brain structure in older adults. We focused on two major metrics of brain structure using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI): 1) brain volume (i.e., gray matter, white matter, ventricular, and white matter lesion volume) and 2) white matter tract integrity (i.e., degree of diffusion anisotropy).

Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) technology was developed in the 1980s and provides a non-invasive way to study the mechanisms of cognitive and brain aging (Lockhart & DeCarli, 2014). MR images are produced by exposing a patient to a strong magnetic field that aligns hydrogen nuclei (i.e., protons) in the direction of the magnetic field (Weishaupt, Kochli, & Marincek, 2003). Following proton alignment, radiofrequency (RF) pulses are applied to “excite” the protons and change, or “flip,” their orientation. After the removal of the RF pulse, protons relax to their original orientation and emit a signal that is detected by a receiver coil. Different types of tissue respond differently to excitation and have different relaxation times (i.e., the time it takes a proton to return from an excited to non-excited state). Thus, tissue types (e.g., gray matter, white

matter, cerebral spinal fluid) can be distinguished by their different signals, which result in varying levels of brightness on MR images.

MRI provides better spatial resolution and tissue contrast, and thus greater sensitivity to detect subtle anatomical differences, while avoiding many of the risks associated with other neuroimaging technologies (i.e., computerized tomography, or CT scans) such as exposure to harmful radiation (Shenton et al., 2012). Studies have shown that MRI is more sensitive to detect subtle white matter injuries, hemorrhage, and edema compared to CT scans (Johnston, Ptito, Chankowsky, & Chen, 2001; Niogi & Mukherjee, 2010). MRI technology has been used extensively in samples of older adults to determine the structural brain changes that can be expected during normal and pathological aging (e.g., Aggarwal et al., 2012; Arvanitakis et al., 2016; Fjell & Walhovd, 2010).

Gray Matter Volume

Gray matter volume declines with advancing age in adults (Raz, Gunning-Dixon et al., 2004; van Es et al., 2006). Total brain and gray matter volume increase during childhood, peak around adolescence, and then slowly decline thereafter (Courchesne et al., 2000; Ge et al., 2002; Smith, Chebrolu, Wekstein, Schmitt, & Markesbery, 2007). The rate of gray matter volume decline is estimated to be about between 0.2% and 1% per year in adults older than 60 years (Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003; Smith et al., 2007). In adulthood, global gray matter volume is negatively correlated with age, and these correlations are seen in all four lobes of the brain, in subcortical nuclei, and in the cerebellum (Abe et al., 2008; Allen, Bruss, Brown, & Damasio, 2005; Smith et al., 2007).

While age-related decline in gray matter volume is evident across the whole brain, frontal lobe decline is faster and more significant than decline in other regions (Allen et al., 2005; DeCarli et al., 1994; Jernigan et al., 2001). More specifically, significant age-related gray matter volume decline has been seen healthy older adults in the prefrontal and orbitofrontal cortex (Canu et al., 2011; Lemaitre et al., 2005; Misra, Fan, & Davatzikos, 2009; Raz et al., 1997; Raz, Gunning-Dixon, et al., 2004; Salat et al., 2004; Salat, Kaye, & Janosky, 1999), superior, inferior, and middle frontal gyri (Brickman et al., 2006; Canu et al., 2010; Ly et al., 2014; Salat et al., 2009; Ziegler et al., 2010), primary motor cortex, including the pre-central gyrus (Raz, Gunning-Dixon, et al., 2004; Salat et al., 2004; Ziegler et al., 2010), and the anterior cingulate cortex (Brickman et al., 2006; Canu et al., 2010; Jernigan et al., 2001; Resnick et al., 2003; Tisserand et al., 2002; Ziegler et al., 2010).

Studies show that temporal lobe reductions are the second greatest following frontal lobe decline in healthily aging individuals (Cowell et al., 1994; DeCarli et al., 1994). More specifically, the inferior, superior, and middle temporal gyri (Allen et al., 2005; Brickman et al., 2006; Canu et al., 2010; Ly et al., 2014; Raz et al., 1997; Raz et al., 2005; Raz, Rodrigue, Head, Kennedy, & Acker, 2004; Resnick et al., 2003; Salat et al., 2009; Ziegler et al., 2010), hippocampus, entorhinal cortex, and amygdala (Allen et al., 2005; Canu et al., 2010; Canu et al., 2011; Hua et al., 2008; Jernigan et al., 2001; Raz et al., 2005; Raz, Rodrigue et al., 2004; Walhovd et al., 2005) are vulnerable to age-related decline in healthy older adults. Individuals with more advanced neurodegenerative pathology show more significant decline in temporal and posterior

brain regions than healthy controls (e.g., Canu et al., 2010; Canu et al., 2011; Misra et al., 2009; van Es et al., 2006).

These findings are consistent with the "retrogenesis hypothesis" of aging, which states that neural structural decline occurs in the reverse order of neural development, so that late-developing anterior structures decline before early-developing posterior structures (Alves et al., 2015; Brickman et al, 2012). In other words, age-related neural decline proceeds in an anterior-to-posterior gradient of greater-to-lesser decline (Alves et al., 2015; Brickman et al., 2012). While this theory was developed in the context of research on age-related white matter deterioration, the pattern holds for gray matter decline as well. Late developing structures, such as those in the frontal lobe, decline before early-developing structures, such as visual and sensory-motor areas.

White Matter Volume

Like gray matter, total white matter volume declines in adulthood with advancing age. However, the trajectory of neural development and subsequent decline is slightly different for white matter compared to gray matter. White matter volume follows a quadratic trajectory across the lifespan, rapidly increasing from childhood to adolescence and then slowly continuing to increase until middle age before declining in older adulthood (Allen et al., 2005; Courchesne et al., 2000; Ge et al., 2002; Kochunov et al., 2011). White matter decline begins later than gray matter decline, around middle age versus late adolescence; however, some studies suggest that the rate of white matter decline exceeds that of gray matter decline, ranging from 0.4% to 2.3% per year in older adults (Brickman et al., 2006; Jernigan et al., 2001; Resnick et al., 2003; Salat et al., 1999). A strong negative correlation between age and white matter volume has been

supported through MRI and post-mortem studies of tissue samples, which have found declines not only in total white matter volume but also in the number and length of myelinated axons (Guttman et al., 1998; Kochunov et al., 2009; Marner, Nyengaard, Tang, & Pakkenberg, 2003; Meier-Ruge, Ulrich, Bruhlamann, & Meier, 1992; Piguet et al., 2009; Tang, Nyengaard, Pakkenberg, & Gundersen, 1997).

In the frontal lobe, volumetric declines are seen more specifically in subcortical white matter that supports the prefrontal and orbitofrontal cortex (Canu et al., 2010; Canu et al., 2011; Raz, Gunning-Dixon, et al., 2004; Raz et al., 2005; Salat et al., 2009; Salat et al., 1999), medial frontal cortex (Salat et al., 2009), inferior, middle, and superior frontal gyri (Wang, Guo, Qi, Yao, & Li, 2010; Salat et al., 2009), cingulum (Brickman et al., 12006; Canu et al., 2010; Canu et al., 2011; Salat et al., 2009; Wang et al., 2010), and pre-central gyrus (Salat et al., 2009). These findings are supported through both cross-sectional and longitudinal studies.

Age-related atrophy is also found in the subcortical white matter that supports the inferior, middle, and superior temporal gyri (Brickman et al., 2006; Canu et al., 2011; Jernigan et al., 2001; Salat et al., 2009; Wang et al., 2010), temporal horn (Misra et al., 2009), and temporal pole (Salat et al., 2009) in healthy older adults. Further declines are seen in subcortical parahippocampal and entorhinal cortex white matter (Salat et al., 2009). As with gray matter decline, research has supported the retrogenesis hypothesis for white matter deterioration in aging. Frontal lobe white matter atrophy occurs first and is the most significant, followed by temporal lobe atrophy and other posterior regions (Bartzokis, Beckson, Nuechterlein, Edwards, & Mintz, 2001; Brickman et al., 2006; Raz, Rodrigue, et al., 2004; Raz et al., 2005).

Ventricular Volume

In addition to changes in gray and white matter volume, changes in ventricular volume are also common in aging populations. Ventricular enlargement is seen among aging individuals, regardless of dementia status (Jack et al., 2008), and previous research estimates that the mean rate of ventricular expansion is 2.9% per year (Raz & Rodrigue, 2006). While studies have found ventricular enlargement among cognitively healthy older adults, MCI and Alzheimer's disease patients show greater ventricular volume changes than healthy controls, and the rate of ventricular enlargement may also increase as age increases (Apostolova et al., 2012; Carmichael et al., 2007; Driscoll et al., 2009; Jack et al., 2004; Nestor et al., 2008; Raz & Rodrigue, 2006). The earliest and most significant ventricular changes are seen in the frontal horn and temporal horns of the lateral ventricles but can extend to the body and occipital horns with progressive disease pathology (Apostolova et al., 2012; Thompson et al., 2004). Ventricular enlargement is associated with other biomarkers of disease state (e.g., CSF beta amyloid and tau protein levels) and cognitive performance, even after controlling for the effects of age, sex, and education (Chou et al., 2009; Chou et al., 2010). Thus, ventricular volume may be a useful marker of age-related neural pathology to consider in addition to measures of gray and white matter volume.

White Matter Lesion Volume

White matter lesions (WMLs) are common in adulthood (Arvanitakis et al., 2016; DeCarli et al., 2005; Jernigan et al., 2001; Kochunov et al., 2007). On MR images, WMLs are detected as signal abnormalities and may appear either brighter (i.e., "hyperintense") or darker (i.e., "hypointense") than the surrounding white matter, for T2-

weighted and T1-weighted MR images, respectively (Arvanitakis et al., 2016; Park & Reuter-Lorenz, 2009). Etiology of WMLs is non-specific and could be due to a range of factors, including ischemia, small vessel disease, traumatic brain injury, inflammation, reduced cerebral perfusion, transient ischemic attack (TIA), and stroke (Breteler et al., 1994; Burton et al., 2004; de Leeuw et al., 2001; Gouw et al., 2011; Pantoni & Garcia, 1995; Pantoni, Garcia, & Gutierrez, 1996; Raz & Rodrigue, 2006). WMLs not only result from cerebrovascular events but they also predict risk of future cerebrovascular events, including stroke and development of vascular and mixed dementias (Bombois et al., 2008; Debette & Markus, 2010). WMLs are viewed as markers of damaged white matter, including demyelination and gliosis, as well as potential accumulation of vascular amyloid pathology (Arvanitakis et al., 2016; Gouw et al., 2011; Hedden et al., 2012).

Both volume and total number of WMLs increase linearly with age (DeCarli et al., 2005; Kochunov et al., 2007; Raz & Rodrigue, 2006), although WML burden may be greater in individuals with significant cardiovascular risk factors (e.g., stroke, TIA, hypertension, carotid atherosclerosis) (de Leeuw et al., 2001; Raz & Rodgrigue, 2006). WMLs are found both in healthy older adults and individuals with MCI and more advanced neurodegenerative diseases (Bennett & Madden, 2014; DeCarli et al., 1995; Gunning-Dixon & Raz, 2000; Kochunov et al., 2007; Marchant, et al., 2013). Although WMLs are found throughout cortical and subcortical regions (Jernigan et al., 2001), burden is typically highest in the cortex, particularly in the frontal lobes (DeCarli et al., 1995; Gunning-Dixon & Raz, 2000; Pfefferbaum et al., 2000; Raz & Rodrigue, 2006; Ziegler et al., 2010) and periventricular areas (Bennett & Madden, 2014). WML burden

may progressively advance toward posterior areas in older individuals and patients with higher cardiovascular risk profiles (Raz & Rodrigue, 2006; Raz et al., 2003).

Clinically, WML volume is associated with poorer cognitive and neurological functioning (Aggarwal et al., 2012; Arvanitakis et al., 2016; DeCarli et al., 1995; de Groot et al., 2000; Gunning-Dixon & Raz, 2000; Marchant et al., 2013; Ritchie et al., 2015). WML volume is also correlated with other metrics of neural structural integrity, such as gray and white matter volume and white matter diffusivity (Alves et al., 2012; DeCarli et al., 2005; O'Sullivan et al., 2004). However, studies show that WML burden predicts cognitive impairment above and beyond other significant factors, such as age, education, and gray matter volume (Arvanitakis et al., 2016). Thus, WML volume is an important factor to consider in the study of cognitive and brain aging, in addition to total brain, gray, and white matter volume (Ziegler et al., 2010).

Diffusion Tensor Imaging (DTI)

Diffusion tensor imaging (DTI) was introduced in the early 1990s as an alternative method for studying white matter structural integrity (Assaf & Pasternak, 2008). It has been used extensively to study white matter structure in a range of healthy and diseased populations, including aging and dementia, multiple sclerosis, stroke, schizophrenia, HIV infection, epilepsy, amyotrophic lateral sclerosis (ALS), and chronic alcohol dependence (Assaf & Pasternak, 2008; Kubicki et al., 2002; Lim & Helpern, 2002). DTI measures the diffusion of water molecules in different areas of the brain to estimate the orientation and coherence of white matter fiber tracts. While conventional MRI technology is sufficient for studying gross neuroanatomical changes, such as volume loss, ventricular enlargement, and white matter lesions, DTI provides the

capability of studying microstructural changes in white matter that may not be visible during inspection of conventional MRI (Nusbaum, Tang, Buchsbaum, Wei, & Atlas, 2001). Research has established that white matter microstructural alterations are detectable using DTI even in areas of "normal-appearing" white matter, without the presence of significant atrophy or white matter lesions (Abe et al., 2008; Canu et al., 2011; Chanraud, Zahr, Sullivan, & Pfefferbaum 2010; Lebel et al., 2012; Liao et al., 2010; O'Sullivan et al., 2001; Sullivan & Pfefferbaum, 2003). However, DTI and traditional MRI metrics (e.g., gray and white matter volume, cortical thickness, and white matter lesion burden) are positively correlated (Alves et al., 2012; Fjell et al., 2008; Kochunov et al., 2007; Lee et al., 2012).

DTI uses diffusion-weighted images acquired across time and along various orientations to determine the direction and magnitude of water diffusion in different regions of the brain. Generally, diffusion is described as isotropic (i.e., water moves equally in all directions) in areas of cerebral spinal fluid (CSF) and anisotropic (i.e., water moves primarily along one direction) in areas of white matter, where the structure of axons and myelin sheaths constrains water to diffuse primarily parallel to the tract (Beaulieu, 2002; Chanraud et al., 2010). Anisotropy of water in gray matter falls somewhere between that of CSF and white matter (Beaulieu, 2002; Chanraud et al., 2010; Helenius et al., 2002). Analysis of the diffusion-weighted images provides quantification of diffusion along three primary axes: v1, v2, and v3 (Melhem et al., 2002). Three eigenvalues ($\lambda 1$, $\lambda 2$, and $\lambda 3$) describe the strength of diffusion along the corresponding axis and can be combined to compute various metrics of diffusion and anisotropy (see Figure 1.1).

Four metrics of diffusion and anisotropy have been widely used in DTI research. Mean diffusivity (MD) is the average diffusivity along all three axes and is thought to reflect the average amount of diffusion present in a region (Bennett, Madden, Vaidya, Howard, & Howard et al., 2010). Higher MD values represent overall greater diffusion and isotropy (Pierpaoli & Basser, 1996). Fractional anisotropy (FA) is the diffusion of water along the primary axis (v1) relative to the other two axes (v2 and v3) and represents the ratio of diffusion that runs parallel versus perpendicular to a white matter tract (Chanraud et al., 2010). Higher FA values reflect more diffusion parallel than perpendicular to a tract; thus, higher FA values represent greater orientational coherence or anisotropy of a region (Caunraud et al., 2010; Pierpaoli & Basser, 1996). Both MD and FA are sensitive but non-specific measures of white matter microstructural integrity (Bartzokis et al., 2012). Alterations in MD and FA may occur due to several underlying etiologies, such as axonal lesions and demyelination (Assaf & Pasternak, 2008).

Two additional diffusion metrics can be used in combination with MD and FA to better determine the specific etiology of observed white matter microstructural changes. Radial diffusivity (RD) represents the average diffusion along the two secondary axes (v2 and v3) and, thus, represents the amount of diffusion perpendicular to a white matter tract (Chanraud et al., 2010). As perpendicular diffusion is limited in highly myelinated areas, increases in RD represent damage to or deterioration of the myelin sheath (Bennett et al., 2010). The association of RD with demyelination has been supported in both animal and human studies (Bennett et al., 2010; Beaulieu, Does, Snyder, & Allen, 1996; Fjell et al., 2008; Song et al., 2003; Song et al., 2002; Song et al, 2005).

Axial diffusivity (AD) reflects the amount of diffusion along just the primary axis (v1). Higher AD values signify overall greater amounts of diffusion parallel to a tract (Bennett et al., 2010). There is some evidence to suggest that higher AD values represent axonal damage (Alexander, Lee, Lazar & Field, 2007; Beaulieu, 2002; Beaulieu et al., 1996; Song et al., 2003) or shrinkage (Bennett et al., 2010). However, findings regarding AD changes in aging populations are inconsistent, with some studies reporting that AD increases with advancing age (Bennett et al., 2010; Inano, Takao, Hayashi, Abe, & Ohtomo, 2011) and others reporting that it decreases (Bennett et al., 2010; Fjell et al., 2008). Additionally, RD changes are more commonly and consistently seen in aging research (Bennett & Madden, 2014), and some evidence suggests that alterations in summary measures such as FA are due to RD rather than AD changes (Bartzokis et al., 2012; Davis et al., 2009; Delano-Wood et al., 2012; Fjell et al., 2008; Lebel et al., 2012). Thus, RD may be a better, more reliable, and validated metric to use in studies of cognitive and brain aging.

Global Diffusivity

Like white matter volume, DTI metrics change throughout the course of development and follow a quadratic trajectory across the lifespan. FA increases throughout childhood and adolescence, plateaus around middle adulthood, and declines during older adulthood (Bartzokis et al., 2012; Chen, Li, & Hindmarsh, 2001; Kochunov et al., 2011; Kochunov et al., 2012; Lebel et al., 2012; Sexton et al., 2014; Yap et al., 2013). In general, healthy older adults have lower global FA, higher global MD, and higher global RD than younger adults, and age is significantly correlated with all these diffusion metrics (Abe et al., 2008; Bennett & Madden, 2014; Chen et al., 2001; Engelter,

Provenzale, Petrella, DeLong, & MacFall, 2000; Inano et al., 2011; O'Sullivan et al., 2001; Pfefferbaum, Adalsteinsson, & Sullivan, 2005; Sullivan & Pfefferbaum, 2003). Longitudinal studies suggest that significant changes in diffusion metrics, ranging from about 0.5% to 3.5%, can be seen in as little as one year, even within healthy older adult populations (Barrick, Charlton, Clark, & Markus, 2010; Teipel et al., 2010). As previously stated, reported findings of AD changes with aging are inconsistent (e.g., Bennett & Madden, 2014). In studies of pathological aging, MCI patients had lower FA, and higher MD, RD, and AD than healthy controls, while Alzheimer's disease patients had lower FA, and higher MD, RD, and AD than both MCI patients and healthy controls (Alves et al., 2012; Engelter et al., 2000; Head et al., 2004). Thus, just as with total brain, gray, and white matter volume, DTI shows worsening white matter microstructure with the progression of age and disease pathology (Alves et al., 2012).

Regional Diffusivity

Consistent with the retrogenesis hypothesis, many DTI studies have demonstrated that white matter microstructural changes proceed along an anterior-to-posterior gradient of decline during older adulthood (Bennett & Madden, 2014; Head et al., 2004; Lebel et al., 2012; Pfefferbaum et al., 2005; Sullivan, Rohlfing et al., 2010; Yap et al., 2013). Additionally, previous research suggests that thinly-myelinated and densely packed fibers, such as those found in the genu of the corpus callosum and other association tracts, are more vulnerable to age-related changes in diffusion than more thickly-myelinated projection tracts (Kochunov et al., 2012; Sullivan, Rohlfing, & Pfefferbaum, 2010).

Specifically, the greatest changes in diffusion metrics in healthy aging are seen in areas of frontal white matter and late-developing tracts such as the genu of the corpus callosum, fornix, and anterior cingulum (Ardekani, Kumar, Bartzokis, & Sinha, 2007; Bartzokis et al., 2012; Bennett et al., 2010; Head et al., 2004; Kochunov et al., 2012; Lebel et al., 2012; Lehmbeck, Brassen, Weber-Fahr, & Braus, 2006; O'Sullivan et al., 2001; Yap et al., 2013). Consistently, studies have shown that FA decreases and MD and RD increase in older adulthood in anterior white matter tracts (e.g., Abe et al., 2008; Alves et al., 2012; Barrick et al., 2010; Bennett et al., 2010; Burzynska et al., 2010; Canu et al., 2011; Inano et al., 2011).

With advancing age and neurodegenerative pathology (i.e., MCI and Alzheimer's disease), additional changes in diffusion metrics can be seen in areas of temporal and parahippocampal white matter and earlier-myelinating tracts such as the posterior cingulate, uncinate fasciculus, and superior longitudinal fasciculus (Head et al., 2004; Lebel et al., 2012; Liao et al., 2010). Other association tracts such as the body and splenium of the corpus callosum, external capsule, inferior longitudinal fasciculus, and frontal-occipital fasciculus show changes in normal and pathological aging, although these alterations are not as significant as the previously mentioned changes in the genu, anterior cingulum, and fornix (e.g., Alves et al., 2012; Bennett et al., 2010; Brickman at et al., 2012; Davis et al., 2009; H. Huang et al., 2012; Inano et al., 2011; Mella, de Ribaupierre, Eagleson, & de Ribaupierre, 2013; Voineskos et al,. 2012; Wegrzyn et al., 2013).

Factors that Impact Brain Aging

While there is significant evidence to support the general statement that cognitive functioning and brain structure decline over time with advancing age, the study of cognitive and brain aging is full of individual differences, which makes the task of predicting an individual's trajectory of decline very difficult (Habib, Nyberg, & Nilsson, 2007; Nyberg, Lovden, Riklund, Lindenberger, & Backman, 2012). For example, some older adults, often referred to as "Super Agers," do not display significant cognitive decline during older adulthood, but rather maintain a level of cognitive functioning that is comparable to middle-aged individuals, even into their eighth and ninth decades of life (e.g., Gefen et al., 2015; Habib et al., 2007; Harrison, Weintraub, Mesulam, & Rogalski, 2012; Rogalski et al., 2013). Even when cognitive decline is evident, such as in MCI or early dementia, it is very difficult to predict the rate at which an individual will decline; some older adults decline very rapidly whereas other decline slowly and may never progress from MCI to dementia (Boyle et al., 2013).

The strength of the association between brain structural changes and clinical impairment is also subject to individual differences (Stern, 2002). Two patients with the same lesion burden may display very different clinical presentations of cognitive and functional impairment (Stern, 2002, 2009). For example, some patients with significant brain pathology and damage may be asymptomatic and maintain their functioning until the point of death, whereas other patients with less severe pathology may be significantly impaired (Stern, 2002, 2009). In fact, indices of pathological burden (e.g., amyloid-beta accumulation, neurofibrillary tangles, gross and micro-infracts, and Lewy bodies)

account for less than 50% of the variation in the onset and rate of cognitive decline (Boyle et al., 2013).

Several theories have been proposed to explain the significant heterogeneity in cognitive and brain aging. One of these theories is the Scaffolding Theory of Aging and Cognition (STAC), which suggests that the aging brain develops compensatory strategies to preserve cognitive performance in the face of neural deterioration (Park & Reuter-Lorenz, 2009). This theory was developed to explain the commonly observed result in functional MRI (fMRI) studies that older adults show more neural activation while completing a cognitive task than younger adults, even if the accuracy of their performance is equivalent (Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Cappell, 2008). This observed "over activation" is taken as evidence that the brain engages in "compensatory scaffolding" to maintain cognitive functioning despite deteriorating neural structure (Park & Reuter-Lorenz, 2009). Although scaffolding is seen as adaptive, older adults who can maintain performance without such compensatory mechanisms could be considered to have greater "neural efficiency," a marker of aging well (Duverne, Habibi, & Rugg, 2008; Duzel, Schutze, Yonelinas, & Heinze, 2011; Josefsson et al., 2012; Park & Reuter-Lorenz, 2009).

More recently, the STAC model was revised (STAC-r) to include lifestyle factors that may influence compensatory processes and explain some of the heterogeneity in cognitive and brain aging (Reuter-Lorenz & Park, 2014). The model includes factors that deplete (e.g., APOE allele status, stress, vascular disease, low SES, depression, head trauma, and toxin exposure) or enhance (e.g., intellectual engagement, education, physical fitness, multilingualism, social/intellectual engagement, exercise, cognitive

training, meditation, and nutrition) brain structure, function, and compensatory scaffolding (Reuter-Lorenz & Park, 2014). While the association of many of these factors with brain structure and cognitive functioning has been confirmed through previous research (e.g., Bartzokis, 2004; Lee, Kim, & Back, 2009; Nyberg et al., 2012; Poulose et al., 2014), there is currently little understanding of the mechanisms by which lifestyle factors impact the trajectory of cognitive and brain aging (Reuter-Lorenz & Park, 2014). Understanding the mechanisms of action for the connection between lifestyle factors and brain agingmis essential for being able to harness these lifestyle factors as interventions that can potentially impact cognitive decline, brain structure and pathology, and neural efficiency.

Mechanisms of Action

The Free Radical/Oxidative Stress Theory of Aging was first proposed in 1956 as an explanation for how aging occurs at the molecular level (Harman, 1956), and it remains one of the most prominent theories of biological aging today (Abdollahi et al., 2014; Bokov, Chaudhuri, & Richardson, 2004). This theory proposes that oxidative stress causes damage to DNA, proteins, and lipids, and contributes to a range of issues such as neural inflammation, neurotoxicity, damage to glial cells, reduced cerebral perfusion, and accumulation of neural pathology, such as amyloid-beta accumulation, that is characteristic of many dementias (Bartzokis, 2004; Heneka et al., 2015; Lobo, Patil, Phatak, & Chandra, 2010; McGeer & McGeer, 2010; Ruitenberg et al., 2005). Thus, oxidative stress may be one of the main contributors to brain aging through the development of neuropathology (Abdollahi et al., 2014; Bokov et al., 2004) and, in fact, oxidative damage is observed in the brains of individuals with MCI and dementia

(Ancelin, Christen, & Ritchie, 2007; Guerreiro et al., 2007; Keller et al., 2005; Lobo et al., 2010). As the central nervous system (CNS) is one of the most metabolically active systems in the body, it requires a large amount of oxygen, which makes neurons and glial cells particularly vulnerable to destruction by oxidative damage and toxicity from nitric oxide (Bartzokis, 2004; McGeer & McGeer, 2010; Miller, Morel, Saso, & Saluk, 2014). Overall, oxidative stress and neural inflammation contribute to structural brain changes, or "brain aging," in older adults through breakdown of myelin, neuronal death, atrophy, ventricular enlargement, and development of neurodegenerative pathology (Bartzokis, 2004; Heneka et al., 2015; McGeer & McGeer, 2010; Noble, 2004).

Targeting factors that might mitigate oxidative damage and inflammation could be an effective strategy for preventing the onset of cognitive decline and slowing the progression from MCI to dementia (Abdollahi et al., 2014; Guerreiro et al., 2007; Rinaldi et al., 2003). Nutrients with antioxidant and anti-inflammatory properties, such as Vitamins A, C, and E, flavonoids, and carotenoids (e.g., lutein, zeaxanthin, lycopene, and alpha- and beta-carotene) are plentiful in a variety of foods, including walnuts, beets, tea, wine, and brightly colored fruits and vegetables (Ancelin et al., 2007; Clifford, Howatson, West, & Stevenson, 2015; Commenges et al., 2000; Gillette-Guyonnet et al., 2007; Perkins et al., 1999; Poulose et al., 2014; Rinaldi et al., 2003). In fact, some researchers suggest that primary prevention of many neurodegenerative diseases could be achieved by consuming a diet high in antioxidant and anti-inflammatory nutrients (Poulose et al., 2014). Antioxidant levels are lower in MCI and Alzheimer's disease patients compared to healthy controls (Rinaldi et al., 2003). Additionally, intake of flavonoids and carotenoids has been associated with reduced risk of indecent dementia

and slower rates of cognitive decline, above and beyond other risk factors such as age, sex, education, and weight (Ancelin et al., 2007; Commenges et al., 2000; Devore, Kang, Breteler, & Grodstein, 2012; Gomez-Pinilla, 2008; Kang, Ascherio, & Grodstein, 2005; Kuriyama et al., 2006). These positive effects are attributed to reduced inflammation, oxidative stress, and vascular burden, lower blood pressure, and increased cerebral perfusion (Commenges et al., 2000; Lundberg, Feelisch, Bjorne, Jansson, & Weitzberg, 2006). Thus, dietary interventions may be an effective way to preserve neural structure in older adults and to prevent the negative impacts of oxidation on cognitive and brain aging.

Nutritional Effects on Brain Aging

A growing literature provides evidence for the link between a healthy diet, nutrition, and positive neural effects, including preserved brain structural integrity, increased cerebral perfusion, and enhanced neural activation during task performance. For example, high fish consumption, lower meat intake, and high adherence to the Mediterranean diet are all correlated with larger total brain volume, global gray, and global white matter volume, as well as volume in specific regions of the cingulate, temporal and parietal lobes, hippocampus, precuneus, and orbitofrontal cortex (Gu et al., 2015; Raji et al., 2014; Titova et al., 2013; Witte et al., 2014). Intake of foods containing omega-3 fatty acids are also associated with greater global cortical thickness and cortical thickness of superior-frontal regions, orbitofrontal cortex, entorhinal cortex, and cingulate cortex (Gu et al., 2015; Mosconi et al., 2014).

Intake of omega-3 and omega-6 fatty acids and adherence to the Mediterranean diet are also associated with better white matter microstructure, defined as higher FA and

lower MD, RD, and AD, globally and in the corpus callosum, thalamic radiations, cingulum, fornix, frontal-occipital fasciculus, and uncinate fasciculus (Gu et al., 2016; Pelletier et al., 2015; Witte et al., 2014). Individuals with higher adherence to the Mediterranean diet also have reduced odds of developing cerebral infarcts or lesions, even after controlling for demographic and vascular risk factors (Scarmeas et al., 2011; Virtanean, Siscovick, Longstreth, Kuller, & Mozaffarian, 2008).

Antioxidant intake through consumption of pomegranate, grape, and beetroot juice may enhance neural activation during task performance and increase cerebral blood flow, particularly in areas of the frontal cortex such as the dorsolateral prefrontal cortex and anterior cingulate cortex (Bookheimer et al., 2013; Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2012; Presley et al., 2011; Rifkind et al., 2007). Another study found that a high flavonoid dietary intervention increased cerebral blood flow to the dentate gyrus compared to a control condition (Brickman et al., 2014).

As stated above, the hypothesized mechanism of action for the relation between diet and brain health is that intake of the antioxidant and anti-inflammatory nutrients found in many healthy foods reduces brain aging through reduction of oxidative damage and inflammation, protection against accumulation of neurodegenerative pathology (e.g., toxin protein aggregates), and enhancement of cerebral perfusion, which in turn preserves brain structural integrity and functioning (Abdollahi et al., 2014; Heneka et al., 2015; Lobo et al., 2010; Poulose et al., 2014; Rosano, Marsland, & Gianaros, 2012; Shaw, Schultz, Sperling & Hedden, 2015).

Lutein and Zeaxanthin

Lutein (L) and zeaxanthin (Z) are two nutrients in the carotenoid family that have powerful antioxidant and anti-inflammatory properties. L and Z are not produced endogenously in humans and must be obtained entirely through the diet; however, they are plentiful in a variety of foods, including spinach, egg yolks, tomatoes, mangos, dark, leafy greens, and other brightly-colored fruits and vegetables (Chug-Ahuja et al., 1993; Johnson, 2012; Khachik, Beecher, Goli, & Lusby, 1991; Mangels, Holden, Beecher, Forman, & Lanza, 1993; Rasmussen, Muzhingi, Eggert, & Johnson, 2012). Research has shown that high adherence to diets such as the Mediterranean diet are associated with significantly higher levels of carotenoids in blood serum, including L and Z (Beatty, Nolan, Kavanagh, & O'Donovan, 2004; Djuric, Ren, Blythe, VanLoon, & Sen, 2009; Trichopoulou, Benetou, et al., 2003).

Compared to other carotenoids, L and Z preferentially accumulate in the macular region of the retina (Bone, Landrum, Tarsis, 1985; Rapp, Maple, & Choi, 2000; Widomska & Subczynski, 2014), where they are known to protect against diseases such as age-related macular degeneration (AMD), the leading cause of blindness in most developed countries (The Eye Diseases Prevalence Research Group, 2004; Ma et al., 2012; SanGiovanni & Neuringer, 2012; Snodderly, 1995). Similarly, L and Z are the dominant carotenoids in the central nervous system (CNS) in both early- and late-life, where they account for 66-77% of the total carotenoid concentration in human brain tissue (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Johnson, 2012; Johnson, 2014; Johnson et al., 2013). By and large, L and Z are the only carotenoids capable of crossing the blood-retinal and blood-brain barriers, which accounts for their dominance in both

retinal and neural tissue (Snodderly, 1995). The selective uptake of L and Z into the retina and brain may be due to their high membrane solubility, which distinguishes them from other carotenoids and enhances their protective functions (Widomska & Subczynski, 2014). Higher concentrations of carotenoids in the brain have been linked to longevity and increased lifespan (Cutler, 1984). Furthermore, neural concentrations of L are significantly lower for individuals with cognitive impairment compared to healthy individuals (Johnson, 2012).

Mechanisms

High concentrations of L and Z are correlated with better cognitive and neural outcomes, as detailed below (e.g., den Heijer et al., 2001; Johnson, Chung, Caldarella, & Snodderly, 2008; Johnson et al., 2013; Lindbergh et al., 2017). Several mechanisms of action have been proposed to explain this connection. In the retina, L and Z bind with tubulin to stabilize cell membranes, prevent deterioration, and thus, prevent AMD (Bernstein, Balashov, Tsong, & Rando, 1997). Further, L and Z attenuate exposure to harmful blue-wave light, protecting against photo-oxidation and oxidative damage in the retina (Bian et al., 2012; Erdman et al., 2015; Johnson, 2014; Krinsky, 2002).

In the brain, as in the retina, L and Z become embedded in cell membranes, where they protect against lipid oxidation and inflammation (Erdman et al., 2015; Krinsky, 2002; Sujak et al., 1999; Widomska & Subczynski, 2014). Given their preferential accumulation in cell membranes, L and Z can influence membrane properties such as fluidity, ion exchange, oxygen diffusion, membrane stability, and inter-neuronal communication through enhanced signaling at gap junctions (Erdman et al., 2015; Stahl et al., 1997; Stahl & Sies, 2001). Accumulation of L and Z in myelin sheaths may help

maintain structural integrity of white matter and ensure efficient neural communication (Erdman et al., 2015).

Previous studies have shown that supplementation of lutein reduced inflammation and increased antioxidant potential in infants (Perrone et al., 2010; Rubin et al., 2012). Lutein protects against ischemic and hypoxic damage through its antioxidant, anti-apoptotic, and anti-inflammatory properties (Li et al., 2012). While lutein contributes to reduced ocular inflammation, studies have shown that it may also have a more systemic anti-inflammatory influence (Kijlstra, Tian, Kelly, Berendschot, 2012; Li et al., 2012). Overall, research suggests that L and Z may influence cognitive and brain aging by preventing neuronal damage, dysfunction, death, and disconnection (Erdman et al., 2015).

Measurement

L and Z are typically measured via retinal and blood serum concentrations. In the retina, L and Z concentrations can be obtained by measuring the density of the macular pigment layer, or macular pigment optical density (MPOD) (Neelam, Nolan, Chakravarthy, & Beatty, 2009; Renzi & Hammond, 2010). MPOD can be obtained through non-invasive heterochromatic flicker photometry (HFP) technology, which has been shown to be a reliable and valid measurement of MPOD, even in patients with AMD (Gallaher et al., 2007; Hammond & Wooten, 2005; Hammond, Wooten, & Smollon, 2005; Snodderly et al., 2004; Stringham & Hammond, 2008; Wooten & Hammond, 2005; Wooten, Hammond, Land, & Snodderly, 1999). Supplementation of L and Z increases MPOD levels, providing further validation for this measurement (Akbaraly, Faure, Gourlet, Favier, & Berr, 2007; Berendschot et al., 2000; Bovier, Renzi,

& Hammond, 2014; Hammond et al., 1997; Hammond et al., 2017; Johnson et al., 2000; Landrum et al., 1997). Given the strong connection between the retina and the CNS, MPOD is thought to be a useful biomarker for L and Z concentrations in brain tissue (Erdman et al., 2015; Johnson, 2012). Previous research has demonstrated that retinal concentrations of L and Z predict concentrations in the brain tissue across age ranges in infants, younger adults, older adults, and centenarians (Johnson, 2014; Tanprasertsuk et al., 2016; Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013; Vishwanathan, Schalch, & Johnson, 2016). In general, MPOD declines with age and advancing degenerative disease (Neelam et al., 2009; Renzi, Hammond, Dengler & Roberts, 2012).

Serum concentrations are another validated and widely-used measurement of L and Z. Dietary supplementation of L and Z increases serum concentrations (Akbaraly et al., 2007; Berendschot et al., 2000; Djuric et al., 2009; Hammond et al., 1997; Johnson et al., 2000; Vishwanathan, Gendron, Goodrow-Kotyla, Wilson, & Nicolosi, 2010; Wang et al., 2013). Research on changes in serum L and Z concentrations with age are mixed. Some studies suggest that serum concentrations of L and Z do not differ between younger and older adults (Cardinault et al., 2003), while others suggest that concentrations increase with age (Johnson, Maras, Rasmussen, & Ticker, 2010; Olmedilla-Alonso, Beltran-de-Miguel, Estevex-Santigao & Vuadrado-Vives, 2014). However, MCI and Alzheimer's disease patients have lower serum L and Z concentrations than healthy older adults (Nolan et al., 2015; Rinaldi et al., 2003). Additionally, individuals with lower serum concentrations of L and Z may be at increased risk to develop Alzheimer's disease in the future (Min & Min, 2014). Serum L and Z concentrations are highly affected by

recent food intake, which may explain the significant inter- and intra-individual differences found in previous studies (Beatty et al., 2004).

MPOD and serum concentrations of L and Z are positively correlated, but distinct measures (Beatty et al., 2004; Berendschot et al., 2000; Johnson et al., 2013; Mares et al., 2006). One major difference between the two is that MPOD is thought to reflect stable levels of L and Z, acquired over time, whereas serum concentrations represent the effects of recent dietary factors. While supplementation of L and Z increases both MPOD and serum concentrations, changes in MPOD tend to be more long-lasting. Following supplementation, increases in MPOD can last for several months after resuming a normal diet, whereas serum concentrations tend to return quickly to baseline after discontinuation of supplementation (Berendschot et al., 2000; Hammond et al., 1997; Johnson et al., 2000; Landrum et al., 1997).

Cognitive Effects

L and Z have been associated with cognition in both cross-sectional and longitudinal studies. Both MPOD and serum L and Z have been positively correlated with global cognition, memory, language, processing speed, reaction time, attention, reasoning, verbal learning and fluency, and visual-spatial abilities, even after controlling for age, sex, education, and cardiovascular disease risk factors (Ajana et al., 2018; Feeney et al., 2013; Johnson et al., 2013; Mewborn et al., 2018; Renzi, Dengler, Puente, Miller, & Hammond, 2014; Renzi & Hammond, 2010; Renzi, Iannaccone, Johnson, & Kritchesvsky, 2008; Vishwanathan et al., 2014). In a longitudinal study spanning nine years, decreased levels of serum L and Z were related to cognitive decline in global cognition, executive functioning, processing speed, and psychomotor speed (Akbaraly et

al., 2007). In centenarians, brain concentrations of L and Z were significantly related to pre-mortem cognition, including memory, verbal fluency, and dementia severity (Johnson, 2012; Johnson et al., 2013). Further, supplementation of L and Z has been shown to increase visual processing speed, visual-motor performance, and visual performance under glare conditions (Bovier et al., 2014; Stringham & Hammond, 2008).

To date, there have been few published randomized-controlled trials (RCTs) of L and Z supplementation. Two studies found that supplementation of L and/or Z improved verbal fluency, learning, and memory in middle-aged and older adults (Johnson et al., 2008; Power et al., 2018). Another study found positive effects of L and Z supplementation on visual memory, reasoning, and complex attention in younger adults (Renzi-Hammond et al., 2017). Additionally, older adults enrolled in the year-long, double-blind, placebo-controlled RCT of L and Z supplementation at the University of Georgia from which these dissertation data were taken showed improved performance on complex attention and cognitive flexibility tasks if they received the active L and Z supplement compared to the placebo (Hammond et al., 2017).

Neural Effects

As stated above, research has demonstrated that L and Z can traverse the blood-brain barrier and accumulate in brain tissue (Craft et al., 2004; Johnson, 2012; Johnson, 2014; Johnson et al., 2013; Snodderly, 1995). L and Z accumulate in both gray and white matter and are seen diffusely throughout the brain (Craft et al., 2004). Much of what is known about the relation between L and Z and the brain has been determined through post-mortem studies. In post-mortem infant brains, L and Z accumulation was found in the frontal, temporal, and occipital cortices as well as the hippocampus (Johnson, 2014).

Similarly, a study of centenarian brains showed L and Z accumulation across the frontal, temporal, occipital cortices, and the cerebellum (Johnson et al., 2013). Very few studies have compared the relative concentrations of L and Z across different brain regions, although one study did find that the frontal lobes had higher concentrations than the occipital lobe, and that the frontal lobes showed the most age-related decline in total carotenoid concentrations (Craft et al., 2004). Additionally, most studies regarding L and Z and the brain have been focused on areas of accumulation, rather than the relation of L and Z to metrics of neural integrity. However, one study found that higher levels of carotenoids were associated with less severe periventricular white matter lesions (den Heijer et al., 2001).

To date, only two published studies have tested the relation of L and Z to neural functioning *in vivo*, using neuroimaging technology. One study examined cross-sectional data from the aforementioned University of Georgia RCT and tested the relation of L and Z to neural activation during an fMRI-adapted verbal learning and memory task (Lindbergh et al., 2017). Results showed that greater L and Z concentrations (both MPOD and serum) were associated with less neural activation (i.e., "neural efficiency") in several brain regions known to be important for verbal learning and memory, including the inferior frontal gyrus, supramarginal gyrus, superior parietal lobule, planum polare, frontal and middle temporal gyri, and cerebellum (Lindbergh et al., 2017). Longitudinal results from this trial suggest that L and Z supplementation increased activation in the dorsolateral prefrontal cortex and anterior cingulate cortex during word-pair learning (Lindbergh et al., 2018).

To our knowledge, there is only one published study examining the relation of L and Z to brain structure *in vivo*. A cross-sectional examination from the University of Georgia RCT of the relation of L and Z to white matter microstructure using DTI showed that both MPOD and serum concentrations of L and Z were positively correlated with measures of white matter microstructure in major white matter tracts vulnerable to age-related decline, including the uncinate fasciculus and cingulum (Mewborn, Terry, Renzi-Hammond, Hammond, & Miller, 2017). Taken together, these findings suggest that L and Z may contribute to preserved brain structure and enhanced neural efficiency.

Current Study

The cognitive effects of L and Z have been well established, and there is a growing literature on the neural effects, particularly regarding neural functioning and neural efficiency. Recent RCTs have demonstrated that the effects of L and Z supplementation can be measured at a neural level using neuroimaging technology (e.g., Lindbergh et al., 2018). However, there remains limited literature on the structural brain effects of L and Z, and the one published study that examined the effect of L and Z on brain structure *in vivo* was cross-sectional (Mewborn et al., 2017). Thus, the overarching aim of the current study was to extend previous literature on the relation between L and Z and brain structure in older adults by using a double-blind, placebo-controlled RCT design to evaluate the impact of one-year of L and Z supplementation on several metrics of brain structure. Within the context of the overarching aim, the current study also had two specific aims to better understand if L and Z impact: 1) brain volume and 2) white matter tract integrity.

Specific Aims

Brain Volume. The first specific aim was to examine of supplementation of L and Z impacts brain volume, including gray and white matter volume, lateral ventricular volume, and white matter lesion (WML) burden in older adults. It is well known that gray and white matter volume decline globally and in specific regions (i.e., frontal) during older adulthood and that such declines can be observed in as little as one year (Barrick et al., 2010; Sullivan et al., 2010; Teipel et al., 2010). Additionally, ventricular enlargement, particularly of the lateral ventricle, is observed in cognitively healthy and impaired individuals, and has been significantly correlated with cognitive performance, biomarkers of disease progression (e.g., APOE-4 allele and CSF markers of beta-amyloid and tau proteins), and clinical presentation of dementia symptoms (e.g., Apostolova et al., 2012; Carmichael et al., 2007; Chou et al., 2009; Chou et al., 2010; Jack et al., 2008; Thompson et al., 2004). WMLs are also common in older adulthood, and WML volume tends to increase with advancing age in healthy older adults as well as patients with MCI and dementia (Bennett & Madden, 2014; DeCarli et al., 1995; Gunning-Dixon & Raz, 2000; Kochunov et al., 2007; Marchant, et al., 2013).

In line with the STAC-r theory (Reuter-Lorenz & Park, 2014), L and Z were expected to "enhance" neural structure through their antioxidant and anti-inflammatory functions, which serve to protect cell membranes against damage and deterioration. Thus, it was hypothesized that L and Z supplementation would positively relate to brain volume, such that the supplement group would increase, maintain, or attenuate loss of brain volumes over the course of the trial while the placebo group would show expected age-related declines in brain volume. Given that frontal areas are the most vulnerable to

age-related atrophy, followed closely by medial-temporal areas, more specific effects were hypothesized to be found in gray matter and subcortical white matter of the orbitofrontal cortex, prefrontal cortex, anterior cingulate cortex, hippocampus, and medial-temporal cortex. L and Z supplementation was hypothesized to negatively relate to ventricular and WML volume, such that members of the supplement group would maintain or attenuate increases of lateral ventricular and global WML volume, while members of the placebo group would show an age-related increase in lateral ventricular and global WML volume.

White Matter Microstructure. The second specific aim of the current study was to determine the impact of L and Z on white matter microstructure using DTI technology. White matter microstructure was defined as higher fractional anisotropy (FA), lower mean diffusivity (MD), and lower radial diffusivity (RD) (Chanraud et al., 2010). Previous literature has demonstrated that age is associated with increased diffusivity and decreased anisotropy globally and in specific white matter tracts, particularly in anterior white matter tracts such as the genu, fornix, and anterior cingulum (e.g, Bennett & Madden, 2014; Head et al., 2004; Kochunov et al., 2012). Accumulation of L and Z in myelin sheaths may help maintain structural integrity of white matter through prevention of oxidative and inflammatory damage (Erdman et al., 2015). Thus, it was hypothesized that L and Z supplementation would be positively associated with white matter microstructure, such that the supplement group would improve, maintain, or attenuate loss of global white matter microstructure and integrity of the genu of the corpus callosum, fornix, and anterior cingulum, while the placebo group would show expected age-related decline in white matter microstructure (i.e., lower FA, higher MD, and higher

RD) in these areas. AD was also measured but was not considered part of the primary, directional hypotheses given the inconsistencies reported in past literature regarding the directionality of AD changes with aging (e.g., Bennett et al., 2010; Fjell et al., 2008; Inano et al., 2011).

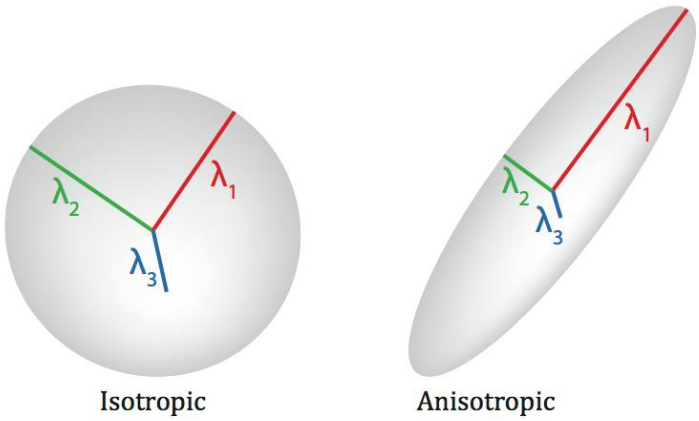

Figure 1.1 Schematic of a Diffusion Tensor.

CHAPTER 2

METHODS

Participants

Eighty-two community-dwelling older adults (aged 64 – 92 years) were initially screened for enrollment in a year-long randomized, double-blind, placebo-controlled trial evaluating the impact of lutein (L) and zeaxanthin (Z) supplementation on vision, cognitive functioning, and neural integrity. Recruitment lasted from August 2012 to August 2014, with follow-up lasting through October 2015. Potential participants were recruited via newspaper advertisements, flyers, and electronic media (e.g., listservs). Exclusion criteria included macular degeneration, corrected visual acuity worse than 20:40 (Snellen notation), carotenoid supplementation within the six-month period prior to enrollment (with the exception of multivitamins that contained less than 1 mg L+Z/day), gastric conditions known to impair absorption of nutritional supplements (e.g., gastric bypass or gastric ulcer), left-handedness, traumatic brain injury, previous history of stroke, dementia, Parkinson's disease or any other neurological condition known to impair cognitive function, and MRI incompatibility (e.g., cardiac pacemaker, aneurism clip).

Eligibility was assessed via a three-step process. First, interested participants completed a brief telephone screen to assess supplement use, history of neurological and ocular conditions, and MRI compatibility. If the participant passed the first step of eligibility screening, they were scheduled for a visit with the University of Georgia

Health Center for medical examination. If the study medical team confirmed good health and eligibility, the participant, along with a collateral informant, progressed to a visit with the University of Georgia Neuropsychology and Memory Assessment Laboratory and completed a clinical interview to assess dementia severity using the Clinical Dementia Rating Scale (CDR) (Morris, 1993). Participants were excluded from the study sample if they received a CDR global rating of 1 – 3, indicating mild to severe dementia. Participants were eligible for inclusion in the study sample if they received a CDR global rating of 0 or 0.5, indicating no cognitive impairment or mild cognitive impairment.

Following application of exclusion criteria and the completed eligibility screening process, 60 participants (23 male, 37 female), aged 65 – 92 years (M = 72.3 years, SD = 6.77 years) were included in the study sample and randomized into one of two groups: the active supplement group (N = 43) or the placebo group (N = 17). Of the 60 participants who were randomized, a total of three participants in the placebo group and eight participants in the active supplement group were lost to follow-up. Additionally, one participant who was randomized to the active supplement group withdrew from the study before supplementation due to family illness and death. Further, one participant who was allocated to the active supplement group showed worsening cognitive status through the year and discontinued the intervention due to concerns of dementia and not having the cognitive capacity to complete the program. Finally, one participant who was allocated to the placebo group was excluded from analysis as pre-intervention neuroimaging data were unavailable for this individual due to claustrophobia. Thus, the final study sample, including all intervention completers who had full neuroimaging datasets, totaled 47 participants (20 male, 27 female), aged 65 – 87 years (M = 71.8

years, SD = 6.04 years), with 33 participants in the supplement group and 14 participants in the placebo group. A visual depiction of the study screening, randomization, and intervention process, following the CONSORT guidelines (Schulz, Altman, & Moher, 2010), can be found in Figure 2.1.

Procedure

Data for the larger RCT were collected across 8 laboratory visits, in addition to the telephone screening and physical examination. Pre-intervention data were collected during three visits occurring within a two-week period which included vision testing, cognitive testing, measurement of retinal and serum L and Z concentrations, and acquisition of neuroimaging data.

Eligible participants were randomly assigned to groups using a 2:1 active supplement to placebo group ratio. Simple randomization was conducted by the study coordinator who was not involved in data collection. A set of numerical codes was generated to correspond with either the active supplement or the placebo group. The codes were placed in an opaque envelope and a unique code was randomly drawn for each participant. A master list of participant randomization was kept confidential by the study coordinator. All study personnel, including the staff who performed the vision, cognitive, and neuroimaging assessments, were blinded to participant randomization throughout the course of the trial. Blinding was broken only after data collection was complete and was necessary for statistical analysis of intervention effects.

Both the active supplement and placebo were provided by DSM Nutritional Products (Besel, Switzerland). The active supplement contained 10 mg L and 2 mg Z. The placebo was visually identical to the active supplement, and both the supplement and

placebo were contained in identical, opaque, sealed bottles with labels that were visually identical except for the randomization code on the label. Thus, participants were also blinded to intervention condition. Participants were instructed to take one tablet from the bottle daily with a meal for one year.

Participants completed follow-up visits at 4 months and 8 months to collect ongoing cognitive and vision data. Compliance to the intervention was monitored through twice monthly telephone calls and pill counts from bottles returned by the participants during follow-up visits. Participation could be discontinued if individuals reported non-compliance on four or more of the telephone check-ins; however, no participants were withdrawn from the study due to non-compliance. Post-intervention data were collected at 12 months and followed the same three visit acquisition procedure as the pre-intervention data collection. Of note, although the larger RCT included a more extensive battery of vision, cognitive, and neuroimaging measures, the current project focused only on the retinal and serum L and Z data together with structural neuroimaging data, collected at pre- and post-intervention visits.

Participants were compensated $300 for their time and effort, and payments were distributed across the four timepoints (i.e., pre-intervention, 4 months, 8 months, and post-intervention). The project was approved by the University of Georgia Institutional Review Board. All participants gave verbal and written consent prior to study enrollment, and the tenets of the Declaration of Helsinki were followed closely by all study personnel. The project was funded in part by Abbott Nutrition, Columbus, OH, USA.

Measures

Snellen Visual Acuity

Self-report of visual acuity was confirmed during the eligibility screening process using Snellen visual acuity testing as delineated in Levenson & Kozarsky (1990). Participants were eligible for the study if they had corrected visual acuity of 20:40 or better.

Clinical Dementia Rating Scale (CDR)

Dementia severity was assessed using the Clinical Dementia Rating scale (Morris, 1993) to confirm eligibility. The CDR is a semi-structured interview conducted with both participants and collateral informants. The interviewer rates an individual's abilities in six cognitive and functional domains: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Scores from each of these domains are combined to create a global rating of dementia severity ranging from 0 (no dementia) to 3 (severe dementia). A global score of 0.5 is often used as a proxy measure for mild cognitive impairment (MCI). Only older adults who received a global rating of 0 or 0.5 were eligible for the study.

Macular Pigment Optical Density (MPOD)

Retinal concentrations of L & Z were measured as macular pigment optical density (MPOD) and assessed using customized heterochromatic flicker photometry (cHFP). This method of data acquisition has been well-validated as an *in vivo* measure of macular pigment density (Hammond et al., 2005; Wooten & Hammond, 2005) and has been fully described elsewhere (e.g., Stringham & Hammond, 2008; Wooten et al., 1999). Briefly, participants were asked to view a disc that is composed of two

wavelengths of light: 460 nanometer (nm) shortwave “blue” light and 570 nanometer (nm) midwave “green” light. The two wavelengths of light are presented in square-wave, counter-phase orientation, which causes the disc to appear to “flicker.” The task was customized to individual participants based on their critical flicker fusion frequency (CFF) values, which were measured in the same session. Participants turned a knob to adjust the intensity of the 460 nm light until it appeared to match the luminance of the 570 nm light, causing the “flickering” to cease. This procedure was conducted in both the foveal and parafoveal regions of the retina. MPOD was calculated as the log of the intensity of 460 nm light required to match the 570 nm light in the fovea (where macular pigment is the densest) compared to the log of the intensity needed in the parafovea (where macular pigment is absent). MPOD data collection followed the same procedure at both pre- and post-intervention visits.

Serum Lutein and Zeaxanthin (Serum L & Z)

Seven milliliters (mL) of blood was collected by a certified phlebotomist to be used for the assessment of serum concentrations of L & Z. Full serum analytic methods can be found in Lindbergh et al. (2017). Briefly, following collection, samples were placed on ice and centrifuged for 15 minutes. Serum was collected and frozen in 1 mL cryotubes at -80° Celsius until analysis. Before analysis, serum data were extracted using standard lipid extraction methods. Carotenoid concentrations, including L and Z concentrations, were analyzed using a Hewlett Packard/Agilent Technologies 1100 series high performance liquid chromatography (HPLC) system with photodiode array detector (Agilent Technologies, Palo Alto, CA, USA). A 5 *u*m, 200 A° polymeric C_{30} reverse-phase column (Pronto-SIL, MAC-MOD Analytical Inc., Chadds Ford, PA, USA) was

used to separate the analytes. Serum L and Z were extracted separately and then combined to create an overall serum L & Z value (serum L levels, umol/L + serum Z levels, umol/L) for use in all statistical analyses (Lindbergh et al., 2017). Blood serum data collection followed the same procedure at both pre- and post-intervention visits.

Neuroimaging Acquisition

All images were acquired using a General Electric Signa HDx 3T MRI scanner (GE; Waukesha, WI, USA). A high-resolution 3D T1-weighted fast spoiled gradient echo (FSPGR) sequence was used to collect structural scans (TE = < 5 ms; TR = 7.5 ms; flip angle = 20°; 154 axial slices; slice thickness = 1.2 mm; FOV = 256 × 256 mm in a 256 x 256 matrix). These images provided coverage from the top of the head to the brainstem, with a total acquisition time of 6 minutes and 20 seconds.

Diffusion weighted imaging (DWI) scans were acquired axially using a single-shot diffusion-weighted spin echo-EPI sequence. Slices covered from the top of the head to the brainstem and were acquired aligned to the anterior commissure-posterior commissure line. Scan parameters included: TE = < 5 ms, TR = 15900 ms, 90° flip angle, 60 interleaved slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, FOV = 256 x 256 mm, parallel acceleration factor = 2, b-value: 1000, and 30 optimized gradient directions with three b0 images. Total scan time for the DWI acquisition was 9 minutes and 38 seconds.

Two pairs of magnitude and phase images were acquired for fieldmap-based unwarping of DWIs (TE_1 = 5.0 ms and TE_2 = 7.2 ms, TR = 700 ms, 60 slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, and FOV = 256 x 256 mm). Acquisition for each pair of images took approximately 2 minutes 20 seconds.

Several other scan sequences were collected as part of the larger study but were not included in the current analysis. Total scan time was approximately 1 hour and 15 minutes, including participant and scanner preparation and all scan sequences. Neuroimaging acquisition followed the same procedure at both pre- and post-intervention visits.

Neuroimaging Processing

Brain Volume

T_1-weighted 3D structural images were processed and segmented using FreeSurfer (v 6.0) (http://surfer.nmr.mgh.harvard.edu; Fischl, 2012; Fischl et al., 2002). The FreeSurfer processing pipeline consists of volume- and surface-based streams, which are fully described in Fischl et al. (2002) and Fischl et al. (2004). Due to the longitudinal design of the study, the FreeSurfer longitudinal processing stream was utilized (Reuter, Rosas, & Fischl, 2010; Reuter, Schmansky, Rosas, & Fischl, 2012).

The FreeSurfer longitudinal stream was designed to be unbiased with respect to timepoint by using a within-subject template for each individual that represents average subject anatomy across all timepoints (Reuter et al., 2010). This unbiased within-subject template is then used as a common starting point for the processing, registration, and segmentation of all timepoints, including baseline images (Reuter et al., 2010; Reuter et al., 2012). This method has been shown to reduce random variation in processing and to improve the robustness and sensitivity of the overall analyses, which is important in longitudinal studies that are often designed to detect small or subtle changes over time (Reuter et al., 2012). Additionally, this method has been shown to be reliable in both

healthy controls as well as individuals with neurodegenerative disease (Reuter et al., 2012).

The FreeSurfer longitudinal processing stream consists of three overall steps. First, all images from all timepoints are processed independently using the original cross-sectional processing stream, which includes motion correction (Reuter et al., 2010), intensity bias correction (Sled, Zijdenbos, & Evans, 1998), skull stripping via a hybrid watershed/surface deformation procedure (Segonne et al., 2004), and automated transformation to Talairach space (Talairach & Tournoux, 1988). The cross-sectional processing stream provides tentative image segmentation and surface reconstruction for each timepoint (Reuter et al., 2012). Next, an unbiased within-subject template is created from all timepoints to estimate mean subject anatomy; all timepoints are iteratively aligned to their median image with an inverse consistent robust registration method, resulting in a template image and simultaneous co-registration of all timepoints (Reuter & Fischl, 2011; Reuter et al., 2012). In the third and final step, longitudinal processing of each timepoint is initialized with information from both the within-subject template and cross-sectional results to reduce variability (Reuter et al., 2012). More specifically, longitudinal processing, much like the original cross-sectional processing, includes spatial normalization and intensity bias correction, skull striping, transformation to Talairach space, normalization, and atlas registration. Using the unbiased median images as a template, final full segmentation and surface reconstruction are performed (Reuter et al., 2012).

Cortical and subcortical labeling is based on a subject-independent probabilistic atlas and subject-specific measured values. The FreeSurfer atlas was built from a set of

subjects whose brains were manually labeled. Volume labels are mapped into Talairach space and surface labels are mapped into spherical space to achieve point-to-point correspondence for all subjects. In each voxel, a cortical or subcortical structural label is assigned, and the probability that a given voxel belongs to an anatomical label is calculated iteratively until results stabilize. This automated segmentation is insensitive to pathology and acquisition parameters (Fischl et al, 2004) and is statistically indistinguishable from manual segmentation (Fischl et al., 2002). Additionally, it has been shown to be sensitive to volumetric differences between groups (Morey et al., 2009).

White Matter Microstructure

Diffusion weighted images (DWIs) were preprocessed using the Oxford Centre's Functional MRI of the Brain (FMRIB) Diffusion Toolbox (FTD) (Beherens et al., 2003). Preprocessing followed a standard pipeline: first, images were corrected for head motion and eddy current distortions using the eddy current and motion correction tool and the first b0 image as a reference. Next, brain extraction was accomplished using the brain extraction tool (BET). Acquired magnitude and phase images were processed to create fieldmaps, which were then applied to the DWIs to correct for further distortions. Finally, the tool DTIFit was used to estimate diffusion tensors for each voxel.

Following preprocessing, Tract-Based Spatial Statistics (TBSS) (Smith et al., 2006) was used to optimize registration and create the mean FA, MD, RD, and AD images. TBSS is a tool within the FMRIB Software Library (FSL) (Smith et al., 2004) and was developed to address issues with inadequate registration of participant data into a common space and arbitrary decisions about the extent of spatial smoothing that are often

found in other voxel-based approaches (Smith et al., 2006). TBSS optimizes registration of images to standard MNI152 space by first identifying the participant with the most representative or "typical" images. This identification is accomplished by co-registering all participants' images to one another and identifying the individual with the minimum mean displacement relative to all other participants. Following identification of the most prototypical participant, all other participants' images are transformed into standard space by aligning them with the target participant's image and affine transforming the entire group into MNI152 standard space using the nonlinear registration tool FNIRT (Andersson, Jenkinson, & Smith, 2007a; Andersson, Jenkinson, & Smith, 2007b). Next, a mean FA image is created and thinned to make a mean FA skeleton, which represents the centers of all tracts common to the group of participants. Each subject's aligned FA data is then projected onto the skeleton and the resulting data can be used in cross-subject statistics. A similar procedure is used to create mean MD, RD, and AD image and skeletons. This procedure has been shown to be effective even with groups of differently aged individuals (e.g., Brickman et al., 2012) and groups that include both healthy individuals and those with neurodegenerative diseases or psychiatric disorders (Smith et al., 2006).

Statistical Analysis

As age and level of cognitive impairment are strong predictors of structural brain integrity in older adult populations (e.g., Abe et al., 2008; Allen et al., 2005; Alves et al., 2012; Bennett & Madden, 2014; Canu et al., 2010; Canu et al., 2011; Misra et al., 2009; Raz, Gunning-Dixon et al., 2004), both age and CDR score were used as covariates for all analyses.

Brain Volume

Following image processing, global gray matter, global white matter, lateral ventricular volume, and global white matter lesion (WML) volume (mm^3) were extracted. Additionally, the Desikan-Killiany Atlas (Desikan et al., 2006) was used to create binary masks and extract volumes for the following a priori regions-of-interest (ROIs): gray matter and subcortical white matter of the orbitofrontal cortex, prefrontal cortex, anterior cingulate cortex, medial temporal cortex, and hippocampus (see Figure 2.2). An automated measure of intracranial volume (ICV) calculated by FreeSurfer was used to correct volumes for differences in head size across participants. Volumes were adjusted according to the formula: normalized volume = raw volume – *b* (ICV x mean ICV), where *b* is the slope of the regression of an ROI volume on ICV. This normalization procedure is a common approach to correcting for differences in head size (Head, Rodrigue, Kennedy, & Raz, 2008; Kennedy et al., 2009).

Extracted whole brain and ROI volumetric measures were used in statistical analyses conducted using the Statistical Package for Social Sciences (IBM SPSS Version 24.0). To determine changes in global brain volume measures over time as a function of intervention condition, whole brain volumes (i.e., global gray matter, global white matter, lateral ventricular, and global white matter lesion volume) were entered into a series of two-way mixed ANCOVAs, with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the independent variables, ICV-corrected brain volumes as the dependent variables, and age and CDR score as the covariates.

For ROIs, a two-way mixed MANCOVA was performed with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the

independent variables, age and CDR score as the covariates, and ICV-corrected gray matter volume for orbitofrontal, prefrontal, anterior cingulate, and medial temporal cortex and the hippocampus as the conglomerate dependent variable. If the MANCOVA reached significance, follow-up two-way mixed ANCOVA analyses were conducted to determine volumetric changes in specific ROIs as a function of intervention condition and timepoint, controlling for age and CDR score. A second two-way mixed MANCOVA was performed for ICV-corrected subcortical white matter volume of the orbitofrontal, prefrontal, anterior cingulate, and medial temporal cortex, with planned follow-up two-way mixed ANCOVA analyses if the MANCOVA reached significance.

White Matter Microstructure

Following processing in FSL and TBSS, the Johns Hopkins University (JHU) ICBM-DTI-81 White Matter Atlas (Hua et al., 2008; Mori et al., 2008) was used to label sections of the skeletonized FA, MD, RD, and AD images corresponding to the major white matter tracts in both hemispheres. A binary mask was created for global white matter (i.e., all white matter tracts in the atlas) and for each ROI (i.e., genu of the corpus callosum, fornix, and anterior cingulum) (see Figure 2.3). These masks were applied to the skeletonized images to extract the average FA, MD, RD, and AD values from within each ROI. When appropriate, right and left hemisphere values were added to create a single mean value for each ROI, which was used in statistical analysis.

Statistical analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Version 24.0). Changes in global white matter microstructure were analyzed using a series of two-way mixed ANCOVAs with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the independent

variables, global diffusivity values (i.e., global FA, global MD, global RD, and global AD) as the dependent variables, and age and CDR score as the covariates. A series of two-way mixed MANCOVAs were conducted to determine changes from pre-to-post intervention for the ROIs, with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the independent variables, age and CDR score as the covariates, and white matter microstructure of the genu of the corpus callosum, fornix, and anterior cingulum as the conglomerate dependent variable. If the MANCOVA reached significance, follow-up two-way mixed ANCOVA analyses were conducted to determine diffusion changes in specific ROIs as a function of intervention condition and timepoint, controlling for age and CDR score. Analyses for each parameter (i.e., FA, MD, RD, and AD) were performed separately.

Intervention Response

To confirm intervention effectiveness, paired-samples t-tests were conducted in both the supplement group and placebo group to assess statistically significance changes in retinal (i.e., MPOD) and serum concentrations of L and Z over the course of the intervention. Exploratory analyses were then conducted to determine whether there were any brain changes corresponding to increased L and Z concentrations, regardless of group membership. Across both groups, individuals were classified as “responders” if they increased L and Z concentrations over the course of the trial and “non-responders” if they decreased or showed no change in L and Z concentrations over the course of the trial. Change scores (post-intervention minus pre-intervention) were calculated for brain volume and white matter microstructure measures for each participant. Then, linear regressions were conducted in the combined group and in the supplement and placebo

groups separately with age and CDR score as the covariates, response status (i.e., responder vs. non-responder) as the independent variable, and change scores for brain volume and white matter microstructure measures as the dependent variables to determine whether changes in structural brain outcomes differed between those classified as responders vs. non-responders.

Power Analysis

A sensitivity analysis was conducted in G*Power 3.0.10 (Faul, Erdfelder, Buchner, & Lang, 2009) to determine the minimum effect size that would be detectable given the sample size, alpha-level, and power. The following parameters were used: F tests for repeated measures ANOVA: $\alpha = 0.05$, power $(1 - \beta) = .80$, total sample size = 47, number of groups = 2, repetitions = 2, correlation among repeated measures = .90 (which was the average correlation between pre- and post-intervention measures for brain volume and white matter microstructure), and nonsphericty correction $\epsilon = 1$. Between-factors effects, within-factors effects, and within-between interaction were assessed separately.

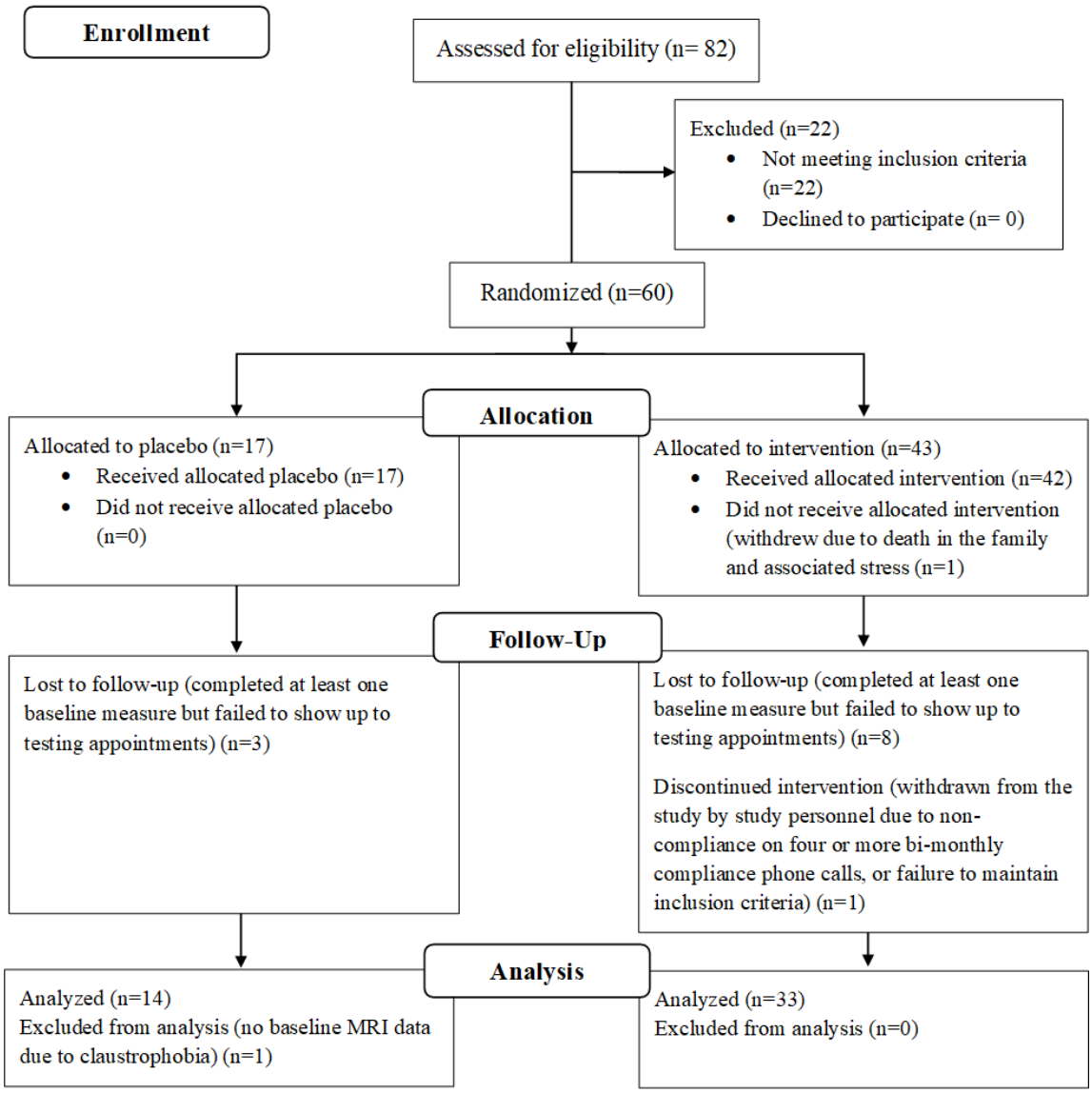

Figure 2.1 CONSORT Flow Diagram.

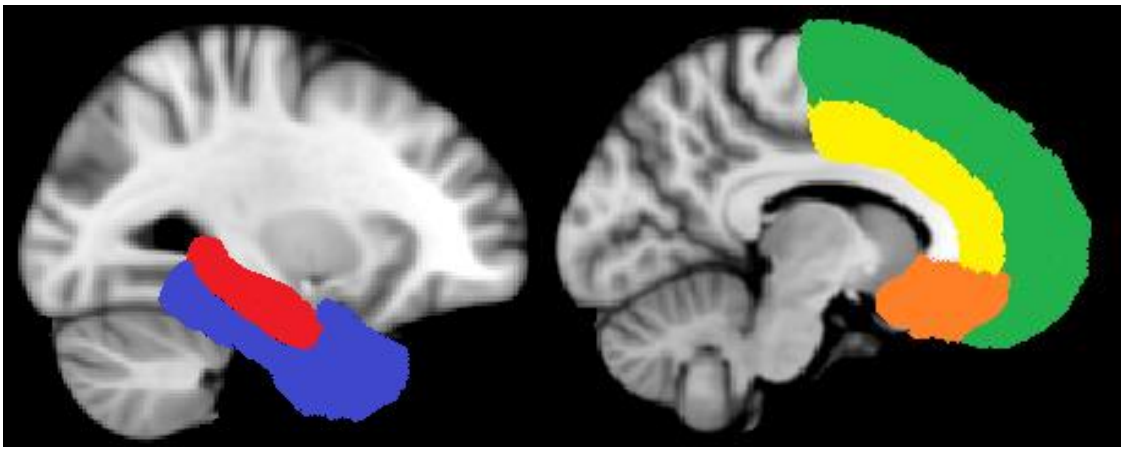

Figure 2.2 Volumetric Regions of Interest (ROIs). The figure depicts the masks used for volumetric ROI analyses for the hippocampus (red), medial temporal lobe cortex (blue), prefrontal cortex (green), anterior cingulate cortex (yellow), and orbitofrontal cortex (orange). Masks are superimposed on a T_1-weighted template in MNI space provided in FMBRIB's Software Library (FSL).

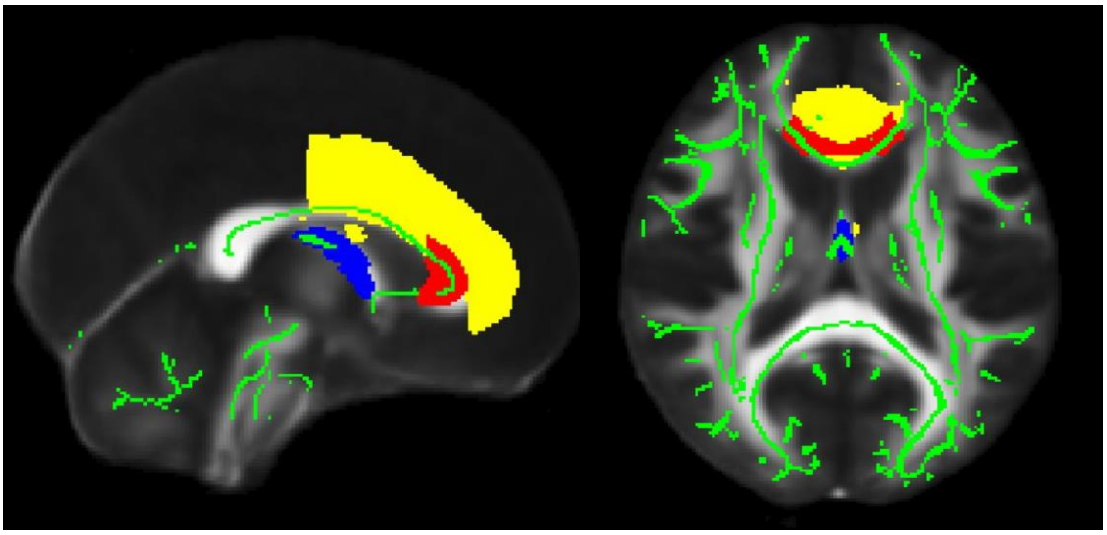

Figure 2.3 White Matter Microstructure Regions of Interest (ROIs). The figure depicts the masks used for white matter microstructure ROI analyses for the genu (red), anterior cingulum (yellow), and fornix (blue) in the sagittal view (left) and axial view (right). Masks are superimposed on a single-subject diffusion weighted template in MNI space provided in FMRIB's Software Library (FSL). The mean skeleton for the sample is overlaid on the single-subject diffusion-weighted template image in green.

CHAPTER 3

RESULTS

Demographic characteristics of the sample can be found in Table 3.1. Independent samples t-tests confirmed that the supplement group and placebo group did not significantly differ at pre-intervention on age, education level, baseline MPOD or serum L & Z concentrations. Chi-square tests also confirmed that the two groups did not significantly differ at pre-intervention in terms of sex or level of cognitive impairment (CDR score). Similarly, there were no significant differences between participants who completed the study (N = 47) and participants who attrited (N = 13) with respect to age, sex, education, baseline MPOD and serum L & Z concentrations, and cognitive impairment (CDR score).

Brain Volume

Global Brain Volume

Pre- and post-intervention values for both global and regional brain volumes can be found in Table 3.2. When controlling for age and cognitive impairment (CDR score), there were no significant main effects of group for any of the global measures, including global gray matter, global white matter, lateral ventricular volume, and white matter lesion (WML) volume. Additionally, there were no significant main effects of time, although qualitatively, both groups showed expected age-related changes (i.e., decreased global gray and white matter volume and increased lateral ventricular and WML

volume). There were no significant group*time interactions for any measures of global brain volume.

Regions-of-Interest Volume

A two-way mixed MANCOVA showed a significant main effect of time for gray matter ROIs (i.e., gray matter volume of the prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, medial temporal lobe, and hippocampus) ($F = 2.60$, $p = .040$), but no significant main effect of group or group*time interaction was observed. Qualitatively, both groups showed an average decrease in prefrontal, anterior cingulate, and hippocampal volume, the supplement group showed an average decrease in orbitofrontal and medial temporal lobe volume, and the placebo group showed an average increase in orbitofrontal and medial temporal lobe volume; however, these changes were not statistically significant. When conducting planned follow-up ANCOVAs, none of the ROIs alone showed significant changes over time, between groups, or significant group*time interactions.

Similarly, a two-way mixed MANCOVA showed a significant main effect of time for white matter ROIs (i.e., subcortical white matter volume of the prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, and medial temporal lobe) ($F = 3.76$, $p = .011$), but no significant main effect of group or group*time interactions. Qualitatively, both group showed expected age-related decreases in subcortical prefrontal, orbitofrontal, and anterior cingulate white matter volume, while the supplement group showed a decrease and the placebo group showed an increase in subcortical medial temporal white matter volume. However, follow-up ANCOVAs showed that only changes over time in the anterior cingulate cortex, regardless of group

status, were individually significant (F = 5.71, p = .021). No other significant main effects of time, main effects of group, or group*time interactions were observed.

White Matter Microstructure

Global Integrity

Pre- and post-intervention values for both global and regional white matter microstructure can be found in Table 3.3. When controlling for age and cognitive impairment (CDR score), results showed a significant main effect of time for global FA (F = 7.14, p = .011). Contrary to hypotheses, both groups showed a significant increase in global FA. Qualitatively, the supplement group showed an average increase in global MD, RD, and AD, consistent with expected age-related changes, while the placebo group showed a decrease in all these measures; however, these changes were not significant. Additionally, no significant group differences or group*time interactions were observed.

Integrity of Regions-of-Interest

Two-way mixed MANCOVAs for white matter microstructure ROIs (i.e., genu of the corpus callosum, fornix, and anterior cingulum) showed a significant main effect of time for FA (F = 4.88, p = .005), but no significant main effects of group or group*time interactions. Qualitatively, both groups showed an increase in FA in the anterior cingulum, although the supplement group showed expected age-related decreases in genu and fornix FA while the placebo group showed increases in those areas. Follow-up ANCOVAs showed that only changes over time in the anterior cingulum (F = 7.53, p = .009) and fornix (F = 8.23, p = .006), regardless of group status, were individually

significant. No other significant main effects of time, main effects of group, or group*time interactions were observed.

The supplement group showed expected age-related increases in MD in all ROIs, while the placebo group showed a decrease in anterior cingulum MD. Both groups showed expected age-related increases in RD in the genu and fornix; contrary to hypotheses both groups also showed a decrease in RD in the anterior cingulum. Finally, the supplement group showed increases in AD in all ROIs, while the placebo group showed a decrease in anterior cingulum AD. However, no analyses with MD, RD and AD were significant.

Intervention Response

Confirmation of Intervention Efficacy

Pre- and post-intervention values for both MPOD and serum L & Z concentrations can be found in Table 3.4. Paired-samples t-tests confirmed that the supplement group showed a significant increase in both MPOD ($t = 2.25$, $p = .030$) and serum L & Z concentrations ($t = 9.74$, $p < .001$) while the placebo group did not show any significant changes in MPOD ($t = .788$, $p = .445$) or serum L & Z ($t = .174$, $p = .865$). However, there was heterogeneity in both groups, with some individuals in the placebo group showing increased MPOD and serum concentrations and some individuals in the supplement group appearing to fail to respond to the intervention (i.e., showing decreased MPOD and serum concentrations). Thus, exploratory analyses were undertaken to determine if there were any brain changes corresponding to increased L and Z concentrations, regardless of group membership. As only one individual in the

supplement group showed decreased serum L & Z concentrations, MPOD was used as the measure of "intervention response" in the following analyses.

Brain Volume

Across both groups, those classified as responders (i.e., increased MPOD from pre-to-post intervention) (N = 32) showed significantly less decline in prefrontal cortex gray matter volume (ΔR^2 = .102, F = 5.16, p = .028) than those classified as non-responders (i.e., no change or decreased MPOD from pre-to-post intervention) (N = 19), after controlling for age and CDR score. In the supplement group alone, those classified as intervention responders (N = 23) showed significantly less decline in total gray matter volume (ΔR^2 = .120, F = 4.60, p = .041) and prefrontal cortex gray matter volume (ΔR^2 = .154, F = 5.72, p = .023) than those classified as non-responders (N = 10), after controlling for age and CDR score. However, in the placebo group alone, there was no significant difference in any brain volume measures between individuals who showed increased MPOD (N = 9) versus those who showed stable or decreased MPOD (N = 5).

White Matter Microstructure

There were no significant differences in global or region white matter microstructure measures between those classified as responders (i.e., showed an increased in MPOD) versus those that were classified as non-responders (i.e. showed no change or decrease in MPOD) in either the supplement or placebo groups individually, or across both groups combined.

Power Analysis

A sensitivity analysis conducted in G*Power 3.0.10 showed that the sample (N = 47) was well powered ($1 - \beta = .80$) to detect a large between-between factors effect (f =

0.4), a small within-factors effect (f = .09), and a small within-between factors interaction (f = .09) at significance level $\alpha = .05$.

Table 3.1

Pre-Intervention Characteristics

	% or M (SD)		
	Supplement (N = 33)	**Placebo (N = 14)**	**Overall Sample (N = 47)**
Age (years)	72.4 (6.27)	70.4 (5.43)	71.8 (6.04)
Sex (% female)	51.5%	71.4 %	57.4%
Race (% Caucasian)	100%	100%	100%
Education (years)	16.6 (3.31)	16.7 (3.02)	16.6 (3.19)
Cognitive Impairment (%)			
No Impairment (CDR = 0)	87.9%	100%	91.5%
Mild Impairment (CDR = 0.5)	12.1%	0%	8.5%

Note: CDR = Clinical Dementia Rating Scale (Morris, 1993).

Table 3.2

Brain Volume

	M (*SD*) in mm^3					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Gray Matter						
Global	589258 (41075)	583090 (43892)	598888 (26886)	595608 (27435)	592127 (37387)	586819 (39829) *
Regions-of-Interest (ROIs)						
Prefrontal	102435 (10156)	101378 (10354)	105206 (7147)	104517 (6733)	103260 (9372)	102313 (9460)
Orbitofrontal	23839 (2388)	23809 (2208)	23430 (1645)	23590 (1719)	23717 (2184)	23744 (2058)
Anterior Cingulate	7989 (1319)	7910 (1447)	7887 (1055)	7864 (1102)	7958 (1236)	7896 (1341)
Medial Temporal	27361 (2589)	27234 (2558)	27063 (2342)	27234 (2658)	27212 (2496)	27234 (2559)
Hippocampus	8681 (989)	8407 (1050)	9144 (743)	8962 (886)	8819 (939)	8572 (1027)
White Matter						
Global	437384 (74298)	432185 (81221)	427438 (40081)	423846 (39899)	434422 (65691)	429701 (71090) *
Regions-of-Interest (ROIs)						
Prefrontal	84364 (8774)	83736 (10028)	83119 (7414)	82317 (7317)	83994 (8332)	83313 (9247)
Orbitofrontal	18494 (2083)	18421 (2345)	18141 (1484)	18083 (1531)	18389 (1915)	18313 (9247)
Anterior Cingulate	9228 (902)	9160 (951)	9336 (696)	9294 (699)	9260 (840)	9200 (878)*
Medial Temporal	17092 (2499)	16955 (2736)	16803 (2039)	16961 (2015)	17006 (2353)	16957 (2521)
Lateral Ventricle	33250 (15696)	35066 (16500)	28855 (11638)	30229 (12088)	31941 (14622)	33625 (15352)
White Matter Hypointensities	7759 (14588)	8090 (16083)	5543 (4446)	5625 (4690)	7099 (12437)	7356 (13692)

Note: All volumes are corrected for intra-cranial volume (ICV) and have been rounded to the nearest mm^3. * indicates a significant change from pre-to-post intervention, $p < .05$, controlling for age and Clinical Dementia Rating (CDR) score.

Table 3.3

White Matter Microstructure

	M (*SD*)					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Fractional Anisotropy (FA)						
Global	.55076 (.02855)	.55257 (.03287)	.55384 (.01966)	.56195 (.02032)	.55167 (.02604)	.55537 (.02978)*
Regions-of-Interest (ROIs)						*
Genu	.61821 (.04982)	.61228 (.05182)	.63634 (.03231)	.63892 (.02889)	.62361 (.04573)	.62021 (.04750)
Anterior Cingulum	.62050 (.05117)	.62390 (.05587)	.63488 (.03001)	.64467 (.03492)	.62478 (.04606)	.63008 (.05107)*
Fornix	.37296 (.09130)	.37186 (.10075)	.37936 (.08800)	.37860 (.10308)	.37487 (.08942)	.37387 (.10037)*
Radial Diffusivity (RD)						
Global	.00053(.00005)	.00053 (.00061)	.00051 (.00003)	.00050 (.00003)	.00053 (.00005)	.00052 (.00006)
Regions-of-Interest (ROIs)						
Genu	.00050 (.00010)	.00052 (.00010)	.00047 (.00049)	.00047 (.00042)	.00049 (.00009)	.00050 (.00009)
Anterior Cingulum	.00053 (.00009)	.00052 (.00010)	.00049 (.00006)	.00048 (.00006)	.00052 (.00009)	.00051 (.00009)
Fornix	.00156 (.00040)	.00158 (.00043)	.00154 (.00047)	.00156 (.00045)	.00155 (.00041)	.00157 (.00043)
Mean Diffusivity (MD)						
Global	.00081 (.00005)	.00081 (.00005)	.00079 (.00003)	.00078 (.00003)	.00081 (.00042)	.00080 (.00005)
Regions-of-Interest (ROIs)						
Genu	.00086 (.00009)	.00087 (.00009)	.00083 (.00004)	.00083 (.00003)	.00085 (.00008)	.00086 (.00008)
Anterior Cingulum	.00090 (.00008)	.00090 (.00008)	.00087 (.00005)	.00085 (.00004)	.00089 (.00007)	.00088 (.00007)
Fornix	.00191 (.00036)	.00193 (.00038)	.00189 (.00044)	.00191 (.00039)	.00190 (.00038)	.00193 (.00038)
Axial Diffusivity (AD)						
Global	.00138 (.00004)	.00138 (.00005)	.00135 (.00004)	.00134 (.00003)	.00137 (.00004)	.00137 (.00005)
Regions-of-Interest (ROIs)						
Genu	.00156 (.00010)	.00158 (.00008)	.00155 (.00004)	.00155 (.00003)	.00156 (.00008)	.00157 (.00007)
Anterior Cingulum	.00164 (.00007)	.00166 (.00006)	.00161 (.00005)	.00160 (.00003)	.00163 (.00006)	.00164 (.00006)
Fornix	.00261 (.00029)	.00264 (.00301)	.00260 (.00038)	.00261 (.00030)	.00260 (.00032)	.00263 (.00030)

Note: * indicates a significant change from pre-to-post intervention, $p < .05$, controlling for age and Clinical Dementia Rating (CDR) score.

Table 3.4

Intervention Response – Change in L & Z Concentrations

	M (SD)					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
MPOD (o.d.)	.5185 (.1883)	.5945 (.2186)*	.4414 (.1397)	.4843 (.2005)	.4955 (.1774)	.5617 (.2173)*
Serum L & Z (umol/L)	.3041 (.1760)	1.175 (.45807)*	.3107 (.1258)	.2996 (.1490)	.3061 (.1610)	.8968 (.5644)*

Note: L = lutein. Z = zeaxanthin. o.d. = optical density. o.d. represents the log ratio of transmitted light passing through the macula.
* indicates a significant change from pre-to-post intervention, $p < .05$.

CHAPTER 4

DISCUSSION

Aging is associated with many changes, both cognitive and neural, that contribute to negative outcomes such as decreased functional independence, large personal and societal economic burden, and psychological distress for both aging individuals and their caregivers (Alzheimer's Association, 2017; Barnes & Yaffe, 2011; Couture et al., 2005; Puente et al., 2014; Tucker-Drob, 2011). Prior research suggests that incidents of dementia might be preventable by addressing lifestyle factors such as poor nutrition and physical inactivity which contribute to both cognitive and neural decline in older adults (Barnes & Yaffe, 2011). One of the most prominent theories of biological aging is the Free Radical/Oxidative Stress Theory of Aging (Harman, 1956), which states that oxidative stress causes damage to DNA and proteins, which then leads to neural inflammation, neurotoxicity, reduced cerebral perfusion, and disruption of neural structure and cognitive functioning (Abdollahi et al., 2014; Bokov et al., 2004). To combat the negative effects of oxidation, researchers have begun studying nutrients such as vitamins, flavonoids, and carotenoids for their potential in preventing and treating age-related cognitive and neural declines (e.g., Abdollahi et al., 2014; Guerreiro et al., 2007; Rinaldi et al., 2003).

The current study tested whether one year of supplementation of lutein (L) and zeaxanthin (Z), two carotenoids with known antioxidative properties and cognitive benefits, could prevent or slow age-related structural brain changes in a sample of

community-dwelling older adults. Using a randomized, double-blind, placebo-controlled trial, we hypothesized that older adults receiving supplementation of L & Z would increase, maintain, or show attenuated loss of brain volume and white matter microstructure in areas that are vulnerable to age-related decline (i.e., frontal and temporal gray and white matter regions) relative to older adults receiving a placebo. Structural brain outcomes were examined using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI).

Results showed that there were no differences in brain volume outcomes between the supplement and placebo groups. After controlling for age and level of cognitive impairment, there were no significant group differences or changes over time in global brain volume outcomes (i.e., global gray matter, global white matter, lateral ventricular volume, and white matter lesion volume). However, expected age-related declines were observed for frontal and temporal gray and white matter and particularly subcortical white matter of the anterior cingulate cortex, across both groups over the course of the trial. Average percent change for both global volumes and frontal-temporal volumes ranged from less than 1% to 3%, consistent with previous estimates of the average annual rate of gray and white matter volume decline in older adults (Brickman et al., 2006; Jernigan et al., 2001; Resnick et al., 2003; Salat et al., 1999; Smith et al., 2007).

Similarly, there were no significant differences in white matter microstructure outcomes between the supplement and placebo groups. Results did show an average increase in global FA, across both groups. Significant changes were also seen in anterior white matter tracts, including increased FA in the anterior cingulum and an expected, age-related decline in fornix FA. While it is encouraging to see improvements in global

and anterior white matter microstructure, it is important to note that these changes were seen in both the supplement and placebo groups and, thus, cannot be attributed to the intervention. Average percent change for both global and regional white matter microstructure ranged from less than 1% to around 2%, again consistent with estimates of annual changes in diffusion metrics in healthy older adult populations (Barrick et al., 2010; Teipel et al., 2010).

Although results confirmed that the intervention manipulation was effective (i.e., the supplement group showed a significant increase in retinal and serum L & Z concentrations while the placebo group did not), there was individual variability in both groups. This individual variability is not uncommon for nutritional intervention studies. There are many factors which may affect how one processes and absorbs nutritional supplements. For example, some studies have suggested that cholesterol levels and co-supplementation of other nutrients can impact the absorption and bioavailability of lutein and zeaxanthin (Sato et al., 2012; Tanumihardjo, Li, & Dosti, 2005). Additionally, the very nature of being in a nutritional study may cause some participants to choose healthier and more nutritious foods, whether consciously or unconsciously. Thus, exploratory analyses were conducted to determine whether individuals who responded better to the intervention, or otherwise increased their retinal L & Z concentration, showed better neural outcomes than those who had no change or decreased retinal L & Z. While there were no differences in terms of white matter microstructure outcomes, results showed that in the supplement group, intervention “responders” (i.e., increased retinal L & Z) had less decline in global gray matter and prefrontal gray matter volume than intervention “non-responders” (i.e., no change or a decreased retinal L & Z), while

there were no concurrent differences observed in the placebo group. These results suggest that lutein and zeaxanthin may slow age-related gray matter decline for some individuals who appeared to reap greater benefit from the supplementation regimen.

As with any study, the present trial had limitations. For example, a similar prospective study on the effects of Vitamin B12 on brain volume found significant results but followed the older subjects for five years as opposed to only one (Vogiatzoglou et al., 2008). Longer time periods may be necessary to allow enough sensitivity to discriminate how dietary components influence morphological loss. Our sample size was small and suffered from considerable attrition (approximately 22%). While we were adequately powered ($1 - \beta = .80$) to detect a small within-subjects effect and small within-between subjects interaction ($f = .09$), we were sufficiently powered to detect only a large between-subjects effect ($f = .40$), which may explain our lack of significant findings regarding group differences on our main outcomes. It may also be the case that the true population effect is even smaller than we were able to detect in our sample. For example, one study comparing individuals with low versus high adherence to a healthy diet found that the difference between groups in terms of global gray and white matter volume was significant, but very small ($f = 0.04$ and $f = -0.06$, respectively) (Gu et al., 2015). Our sample was homogenous in terms of background, consisting of 100% Caucasian and predominantly cognitively healthy and highly educated individuals. Research has shown that educated people also tend to have healthier diets (Akabaraly, Singh-Manouz, Marmot & Brunner, 2009), and our data support this trend. Baseline mean MPOD and serum L & Z values for our sample are slightly higher than other published data for older adults (Renzi et al., 2014; Renzi et al., 2012), suggesting

that our sample may have consumed a more nutritious diet than the general population at baseline, prior to supplementation. In this respect, it is encouraging that a small dietary change provided benefit to even a subsample of individuals in our study who were already well-nourished, educated, and affluent. Finally, as with any nutritional intervention, there was no true control condition; our entire sample has been exposed to both L and Z throughout their lifetimes, and even those participants in the placebo condition continued to consume a normal diet, which presumably contained some amount of L and Z, for the duration of the trial. It is possible that the biggest effects of L and Z on the brain could be driven by deficiency.

While the current study found that one year of supplementation with L and Z had limited effects on the structural brain integrity of community-dwelling older adults, there is a growing body of literature to suggest that these nutrients are important for other aspects of cognitive and brain health. Other analyses from the current RCT have shown that supplementation of L and Z benefited cognition, particularly complex attention and cognitive flexibility (Hammond et al., 2017), verbal learning (Lindbergh et al., 2018), and neural functioning in dorsolateral prefrontal and anterior cingulate areas that are vulnerable to age-related decline (Lindbergh et al., 2018). Emerging results suggest that L and Z supplementation may also benefit compensatory reorganization in aging brains through enhancing integration of functional brain networks (Lindbergh et al., manuscript submitted for publication). The importance of L and Z for cognitive and neural outcomes has been replicated cross-sectionally in other samples and by other researchers (Ajana et al., 2018; Craft et al., 2004; den Heijer et al., 2001; Feeney et al., 2013;

Johnson, 2012; Johnson et al., 2013; Vishwanathan et al., 2014) and is beginning to be confirmed longitudinally as well (e.g., Akbaraly et al., 2007; Johnson et al., 2008).

The current study used a novel approach to investigate in vivo structural brain outcomes of a year-long, double-blind, placebo-controlled trial of lutein (L) and zeaxanthin (Z) supplementation in older adults. While there were no significant differences between the supplement group and placebo group in terms global and frontal-temporal brain volume and white matter microstructure changes, exploratory analyses did suggest that there was a small group of individuals who reaped greater benefit from L and Z supplementation and who showed less decline in global and prefrontal gray matter volume than individuals who did not appear to benefit from the intervention. To that end, future studies could explore factors which may impact the efficacy of L and Z supplementation, such as vascular health, co-supplementation of other nutrients, and other factors known to interact with nutrient bioavailability and absorption. Replication of this study in a larger and more diverse sample may also determine whether there are some individuals who could benefit more from L and Z supplementation, such individuals who are more cognitively impaired or who may have had less access to nutritious diets across their lifetime. Factors such intervention dosage and particularly longer a duration should also be considered when determining intervention efficacy. Finally, future studies will need to determine the long-term effects of L and Z supplementation on outcomes such as the development of dementia and accumulation of brain pathologies. While L and Z supplementation over one year appears to have limited effects on structural brain outcomes in older adults, it is likely

not harmful and appears to provide benefits for other aspects of cognitive and brain health that could improve or extend quality of life for older adults.

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APPENDIX A

THE EFFECTS OF LUTEIN AND ZEAXANTHIN SUPPLEMENTATION ON BRAIN MORPHOLOGY IN OLDER ADULTS[1]

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[1]Mewborn, C.M., Lindbergh, C.A., Hammond, B.R., Renzi-Hammond, L.M., & Miller, L.S., Submitted to Journal of Aging Research, July 2019

Abstract

This study examined if supplementation with lutein (L) and zeaxanthin (Z) impacted structural brain outcomes in older adults using a year-long randomized controlled design. Participants aged 65-87 years (M=71.8, SD=6.04) were randomized into supplement (N=33) and placebo groups (N=14). We hypothesized the supplement group would increase, maintain, or show attenuated loss in global brain outcomes and frontal-temporal regions-of-interest while the placebo group would show age-related declines in brain structural integrity over the course of the trial. While we found age-related declines for frontal and temporal gray and white matter volumes and fornix white matter microstructure across both groups, only minimal differences were found between the supplement and placebo groups. However, exploratory analyses showed that individuals who responded better to supplementation had less decline in global and prefrontal gray matter volume than supplement "non-responders." Thus, results showed that one year of L and Z supplementation had overall limited effects on the structural brain integrity of older adults; however, there may be a subsample of individuals for whom supplementation provides greater benefits.

Introduction

Aging is associated with many changes, both cognitive and neural, that contribute to negative outcomes such as decreased functional independence, large personal and societal economic burden, and psychological distress for both aging individuals and their caregivers (e.g., Couture et al., 2005; Puente et al., 2014). One of the more common theories of biological aging is the Free Radical/Oxidative Stress Theory of Aging (Harman, 1956) which states that oxidative stress causes damage to DNA and proteins. In turn, this leads to neural inflammation, neurotoxicity, reduced cerebral perfusion, and disruption of neural structure and cognitive functioning (Abdollahi et al., 2014). To combat the negative effects of oxidation, researchers have begun studying nutrients such as vitamins, flavonoids, and carotenoids for their potential in preventing and treating age-related cognitive and neural declines. Intake of these nutrients, as well as healthy fatty acids and adherence to an overall healthy diet, have been associated with positive neural effects, including preserved gray and white matter volume, white matter microstructure, and lower risk of cerebral infarcts, even after controlling for demographic and vascular risk factors (e.g., Gu et al., 2015; Raji et al., 2014; Scarmeas et al., 2011).

Lutein (L) and zeaxanthin (Z) are two nutrients in the xanthophyll carotenoid family that have been shown to benefit cognitive and neural outcomes in older adults. Compared to other carotenoids, L and Z are the dominant carotenoids in the central nervous system (CNS) in both early- and late-life, where they account for 66-77% of the total carotenoid concentration in human brain tissue (Craft et al., 2004; Johnson et al., 2013). Although the cognitive effects of L and Z have been well established and there is a growing literature on the direct neural effects, particularly regarding neural functioning and neural efficiency, much of what is known about the relation between L and Z and

the brain has been determined through post-mortem studies (e.g., Craft et al., 2004; den Heijer et al., 2001; Johnson et al., 2013). Recent RCTs have demonstrated that the effects of L and Z supplementation can be measured at a neural level using functional neuroimaging technology (e.g., Lindbergh et al., 2018). However, there remains limited literature on the structural brain effects of L and Z, and the only published study that examined the effect of L and Z on brain structure in vivo was cross-sectional (Mewborn et al., 2017). Thus, the aim of the current study was to extend previous literature on the relation between L and Z and brain structure in older adults by using a randomized, double-blind, placebo-controlled trial design to evaluate the impact of L and Z supplementation on several metrics of brain structure.

Specifically, we examined if one year of supplementation of L and Z resulted in brain volume effects in older adults using magnetic resonance imaging (MRI) and white matter microstructure using diffusion tensor imaging (DTI). We examined global measures of brain volume (i.e., global gray and white matter volume, lateral ventricular volume, and white matter lesion (WML) volume) as well as specific regions-of-interest (ROIs) in the frontal and temporal lobes (i.e., prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, medial temporal cortex, and hippocampus), as it is well known that gray and white matter volume declines are typically seen first in anterior regions of the brain in healthily aging individuals (e.g., Abe et al., 2008; Salat et al., 1999). We also examined global white matter microstructure and integrity of several anterior white matter tracts (i.e., genu of the corpus callosum, fornix, and anterior cingulum) that are particularly vulnerable to age-related decline (e.g., Bennett & Madden, 2014; Head et al., 2004).

We hypothesized that L and Z supplementation would positively relate to brain structure, such that the L and Z supplement group would increase, maintain, or show attenuated loss of their brain volume and white matter microstructure over the course of the trial while the placebo group would show age-related declines in brain volume and white matter microstructure. L and Z supplementation was hypothesized to negatively relate to ventricular and WML volume, such that the supplement group was expected to maintain or attenuate increases of lateral ventricular and global WML volume, while the placebo group was expected to show age-related increases in lateral ventricular and global WML volume.

Method

Participants

Community-dwelling older adults were recruited for participation in a year-long randomized, double-blind, placebo-controlled trial evaluating the impact of lutein (L) and zeaxanthin (Z) supplementation on vision, cognitive functioning, and neural integrity. Recruitment methods included newspaper advertisements, flyers, and electronic media (e.g., listservs). Exclusion criteria included macular degeneration, corrected visual acuity worse than 20:40, xanthophyll carotenoid supplementation within the six-month period prior to enrollment (with the exception of multivitamins that contained less than 1 mg L+Z/day), gastric conditions known to impair absorption of nutritional supplements (e.g., gastric bypass or gastric ulcer), left-handedness, traumatic brain injury, previous history of stroke, dementia, Parkinson's disease or any other neurological condition known to impair cognitive function, and MRI incompatibility (e.g., cardiac pacemaker).

Sixty participants (23 male, 37 female), aged 65-92 years (M = 72.3 years, SD = 6.77 years), met inclusion criteria and were randomized into one of two groups: the active supplement group (N = 43) or the placebo group (N = 17). Of the 60 randomized participants, 47 participants (20 male, 27 female), aged 65-87 years (M = 71.8 years, SD = 6.04 years) completed the study, with 33 participants in the supplement group and 14 participants in the placebo group. A visual depiction of the study screening, randomization, intervention, and attrition can be found in Figure 1.

Procedure

Eligible participants were randomly assigned to groups using a 2:1 active supplement to placebo group ratio. Simple randomization was conducted by the study coordinator, who was not involved in data collection. A master list of participant randomization was kept confidential by the study coordinator. All study personnel, including the staff who performed the assessments, were blinded to participant randomization throughout the course of the trial. Blinding was broken only after all data collection was complete and then necessary for statistical analysis of intervention effects.

Both the active supplement and placebo were provided by DSM Nutritional Products (Besel, Switzerland). The active supplement contained 10 mg L and 2 mg Z. The placebo was visually identical to the active supplement, and both the supplement and placebo were contained in identical, opaque, sealed bottles with labels that were visually identical except for the randomization code on the label. Thus, participants were also blinded to intervention condition. Participants were instructed to take one tablet from the bottle daily with a meal for a period of one year.

Participants completed several pre-intervention visits to collect vision, cognition, and neuroimaging measures. Participants also completed follow-up visits at 4 months and 8 months to collect ongoing data. Compliance to the intervention was monitored through twice monthly telephone calls and pill counts from bottles returned by the participants during follow-up visits. Participation could be discontinued if individuals reported non-compliance on four or more of the telephone check-ins; however, no participants were withdrawn from the study due to non-compliance. Post-intervention data were collected at 12 months and followed the same acquisition procedure as the pre-intervention data collection. Of note, although the larger RCT included a more extensive battery, the current project focused only on the retinal and serum L and Z data together with the structural neuroimaging data, collected at pre- and post-intervention visits. Results from other outcomes can be found in Hammond et al. (2017) and Lindbergh et al. (2018).

Measures

Clinical Dementia Rating Scale (CDR). Dementia severity was assessed using the Clinical Dementia Rating scale (Morris, 1993) to confirm eligibility. The CDR is a semi-structured interview conducted with both participants and collateral informants. The interviewer rates an individual's abilities in six cognitive and functional domains. Scores from each of these domains are combined to create a global rating of dementia severity ranging from 0 (no dementia) to 3 (severe dementia). A global score of 0.5 is often used as a proxy measure for mild cognitive impairment (MCI). Only older adults who received a global rating of 0 or 0.5 were eligible for the study.

Macular Pigment Optical Density (MPOD). Retinal concentrations of L & Z were measured as macular pigment optical density (MPOD) and assessed using

customized heterochromatic flicker photometry (cHFP). This method of data acquisition has been well-validated as an in vivo measure of macular pigment density and has been fully described elsewhere (e.g., Wooten & Hammond, 2005; Wooten et al., 1999). Briefly, participants were asked to view a disc that is composed of two wavelengths of light (460 nanometer (nm) shortwave "blue" light and 570 nanometer (nm) midwave "green" light) that are presented in square-wave, counter-phase orientation, which causes the disc to appear to "flicker." The task was customized to individual participants based on their critical flicker fusion frequency (CFF) values, which were measured in the same session. Participants turned a knob to adjust the intensity of the 460 nm light until it appeared to match the luminance of the 570 nm light, causing the "flickering" to cease. This procedure was conducted in both the foveal and parafoveal regions of the retina. MPOD was calculated as the log of the intensity of 460 nm light required to match the 570 nm light in the fovea (where macular pigment is the densest) compared to the log of the intensity needed in the parafovea (where macular pigment is absent). MPOD data collection followed the same procedure at both pre- and post-intervention visits.

Serum Lutein and Zeaxanthin (Serum L & Z). Seven milliliters (mL) of blood was collected by a certified phlebotomist to be used for the assessment of serum concentrations of L & Z. Full serum analytic methods can be found in Lindbergh et al. (2017). Briefly, following collection, samples were placed on ice and centrifuged for 15 minutes. Serum was collected and frozen in 1 mL cryotubes at -80° Celsius until analysis. Before analysis, serum data were extracted using standard lipid extraction methods. Xanthophyll carotenoid concentrations, including L and Z concentrations, were analyzed using a Hewlett Packard/Agilent Technologies 1100 series high performance liquid

chromatography (HPLC) system with photodiode array detector (Agilent Technologies, Palo Alto, CA, USA). A 5 *u*m, 200 A° polymeric C_{30} reverse-phase column (Pronto-SIL, MAC-MOD Analytical Inc., Chadds Ford, PA, USA) was used to separate the analytes. Serum L and Z were extracted separately and then combined to create an overall serum L & Z value (serum L levels, umol/L + serum Z levels, umol/L) for use in all statistical analyses (Lindbergh et al., 2017). Blood serum data collection followed the same procedure at both pre- and post-intervention visits.

Neuroimaging Acquisition

All images were acquired using a General Electric Signa HDx 3T MRI scanner (GE; Waukesha, WI, USA). A high-resolution 3D T_1-weighted fast spoiled gradient echo (FSPGR) sequence was used to collect structural scans (TE = < 5 ms; TR = 7.5 ms; flip angle = 20°; 154 axial slices; slice thickness = 1.2 mm; FOV = 256 × 256 mm in a 256 x 256 matrix). These images provided coverage from the top of the head to the brainstem, with a total acquisition time of 6 minutes and 20 seconds.

Diffusion weighted imaging (DWI) scans were acquired axially using a single-shot diffusion-weighted spin echo-EPI sequence. Slices covered from the top of the head to the brainstem and were acquired aligned to the anterior commissure-posterior commissure line. Scan parameters included: TE = < 5 ms, TR = 15900 ms, 90° flip angle, 60 interleaved slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, FOV = 256 x 256 mm, parallel acceleration factor = 2, b-value: 1000, and 30 optimized gradient directions with three b0 images. Total scan time for the DWI acquisition was 9 minutes and 38 seconds.

Additionally, two pairs of magnitude and phase images were acquired for fieldmap-based unwarping of DWIs (TE_1 = 5.0 ms and TE_2 = 7.2 ms, TR = 700 ms, 60 slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, and FOV = 256 x 256 mm). Acquisition for each pair of images took approximately 2 minutes 20 seconds. Neuroimaging acquisition followed the same procedure at both pre- and post-intervention visits.

Neuroimaging Processing

Brain Volume. T_1-weighted 3D structural images were processed and segmented using FreeSurfer (v 6.0) (http://surfer.nmr.mgh.harvard.edu; Fischl et al., 2002). Due to the longitudinal design of the study, the FreeSurfer longitudinal processing stream was utilized, which includes motion correction, skull stripping, automated transformation to Talairach space, normalization, and atlas registration, with processing of each time point initiated from a within-subjects template that represents mean subject anatomy across time points (Reuter et al., 2012). The Desikan-Killiany atlas (Desikan et al., 2006) was used to extract region-of-interest (ROI) volumes (see Figure 2), and all volumes were corrected for intracranial volume (ICV) prior to statistical analysis, according to the formula: normalized volume = raw volume – *b* (ICV x mean ICV), where *b* is the slope of the regression of an ROI volume on ICV.

White Matter Microstructure. Diffusion weighted images (DWIs) were preprocessed using the Oxford Centre's Functional MRI of the Brain (FMRIB) Diffusion Toolbox (FTD) (Beherens et al., 2003). Preprocessing followed a standard pipeline, including head motion and eddy current correction, brain extraction, correction of distortion via fieldmap processing, and estimation of diffusion tensors for each voxel.

Following preprocessing, Tract-Based Spatial Statistics (TBSS) (Smith et al., 2006) was used to optimize registration and create the mean diffusion images which were thinned to create mean diffusion skeletons that represent the centers of all tracts common to the group of participants. The Johns Hopkins University (JHU) ICBM-DTI-81 White Matter Atlas (Mori et al., 2008) was used to create binary masks for each ROI (see Figure 3). Average diffusivity values were extracted from each skeletonized ROI and used in statistical analysis. When appropriate, right and left hemisphere values were added to create a single mean value for each ROI.

Statistical Analysis

As age and level of cognitive impairment are strong predictors of structural brain integrity in older adult populations (e.g., Abe et al., 2008; Bennett & Madden, 2014), both baseline age and CDR score were used as covariates for all analyses. Changes in structural brain outcomes over time as a function of intervention condition were determined using analysis-of-covariance (ANCOVAs). Global volumes (i.e., global gray matter, white matter, lateral ventricular, and white matter lesion volumes) and global diffusivity measures (i.e., global FA, RD, MD, and AD) were entered as dependent variables into a series of two-way mixed ANCOVAs with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the independent variables and baseline age and CDR scores as the covariates.

Region-of-interest (ROI) outcomes were similarly entered together in groups of two-way mixed MANCOVAs with intervention group (active supplement vs. placebo) and timepoint (pre- vs. post-intervention) as the independent variables and baseline age and CDR score as the covariates. The first group included ICV-corrected gray matter

volume for orbitofrontal, prefrontal, anterior cingulate, and medial temporal cortex and the hippocampus in the conglomerate dependent variable. The second group included ICV-corrected subcortical white matter volume of the orbitofrontal, prefrontal, anterior cingulate, and medial temporal cortex in the conglomerate dependent variables. The final group included white matter diffusivity values for the genu of the corpus callosum, fornix, and anterior cingulum in the conglomerate dependent variables. Analyses for each parameter (i.e., FA, MD, RD, and AD) were performed separately. If the MANCOVAs reached significance, follow-up two-way mixed ANCOVA analyses were conducted to determine changes in specific ROIs as a function of intervention condition and timepoint, controlling for baseline age and CDR score.

Results

Demographic characteristics of the sample can be found in Table 1. Independent samples t-tests confirmed that the supplement group and placebo group did not significantly differ at pre-intervention on age, education level, baseline MPOD, or serum L & Z concentrations. Chi-square tests also confirmed that the two groups did not significantly differ at pre-intervention in terms of sex or level of cognitive impairment (CDR score). Similarly, there were no significant differences between participants who completed the study (N = 47) and participants who attrited (N = 13) with respect to age, sex, education, baseline MPOD and serum L & Z concentrations, and cognitive impairment (CDR score).

Brain Volume

Pre- and post-intervention values for both global and regional brain volumes can be found in Table 2. When controlling for age and cognitive impairment (CDR score),

there were no significant main effects of group for any of the measures of global brain volume. Additionally, there were no significant main effects of time, although qualitatively, both groups showed age-related changes (i.e., decreased global gray and white matter volume and increased lateral ventricular and WML volume). There were no significant group*time interactions for any measures of global brain volume.

A two-way mixed MANCOVA showed a significant main effect of time for gray matter ROIs (i.e., gray matter volume of the prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, medial temporal lobe, and hippocampus) ($F = 2.60$, $p = .040$), but no significant main effect of group or group*time interaction were observed. When conducting planned follow-up ANCOVAs, none of the ROIs alone showed significant changes over time, between groups, or significant group*time interactions. Similarly, a two-way mixed MANCOVA showed a significant main effect of time for white matter ROIs (i.e., subcortical white matter volume of the prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, and medial temporal lobe) ($F = 3.76$, $p = .011$), but no significant main effect of group or group*time interactions. However, follow-up ANCOVAs showed that changes over time in the anterior cingulate cortex, regardless of group status, were individually significant ($F = 5.71$, $p = .021$). No other significant main effects of time, main effects of group, or group*time interactions were observed.

White Matter Microstructure

Pre- and post-intervention values for both global and regional white matter microstructure can be found in Table 3. When controlling for age and cognitive impairment (CDR score), results showed a significant main effect of time for global FA ($F = 7.14$, $p = .011$). Contrary to hypotheses, both groups showed a significant increase

in global FA. There were no significant effects for RD, MD, or AD, and no significant group differences or group*time interactions were observed.

Two-way mixed MANCOVAs for white matter microstructure ROIs (i.e., genu of the corpus callosum, fornix, and anterior cingulum) showed a significant main effect of time for FA ($F = 4.88$, $p = .005$), but no significant main effects of group or group*time interactions. Follow-up ANCOVAs showed that changes over time in the anterior cingulum ($F = 7.53$, $p = .009$) and fornix ($F = 8.23$, $p = .006$), regardless of group status, were individually significant. No other significant main effects of time, main effects of group, or group*time interactions were observed.

Intervention Response

Pre- and post-intervention values for both MPOD and serum L & Z concentrations can be found in Table 4. Paired-samples t-tests confirmed that the supplement group showed a significant increase in both MPOD ($t = 2.25$, $p = .030$) and serum L & Z concentrations ($t = 9.74$, $p < .001$) over the course of the trial while the placebo group did not show any significant changes in MPOD ($t = .788$, $p = .445$) or serum L & Z ($t = .174$, $p = .865$). However, there was heterogeneity in both groups, with some individuals in the placebo group showing increased MPOD and serum concentrations and some individuals in the supplement group appearing to fail to respond to intervention (i.e., showing decreased MPOD and serum concentrations). Thus, exploratory analyses were undertaken to determine if there were any brain changes corresponding to increased L and Z concentrations, regardless of group membership. As only one individual in the supplement group showed decreased serum L

& Z concentrations, MPOD was used as the measure of "intervention response" in the following analyses.

Across both groups, those classified as responders (i.e., increased MPOD from pre-to-post intervention) (N = 32) showed significantly less decline in prefrontal cortex gray matter volume (ΔR^2 = .102, F = 5.16, p = .028) than those classified as non-responders (i.e., no change or decreased MPOD from pre-to-post intervention) (N = 19), after controlling for baseline age and CDR score. In the supplement group alone, those classified as intervention responders (N = 23) showed significantly less decline in total gray matter volume (ΔR^2 = .120, F = 4.60, p = .041) and prefrontal cortex gray matter volume (ΔR^2 = .154, F = 5.72, p = .023) than those classified as non-responders (N = 10), after controlling for baseline age and CDR score. However, in the placebo group alone, there was no significant difference in any brain volume measures between individuals who showed increased MPOD (N = 9) versus those who showed stable or decreased MPOD (N = 5).

Finally, there were no significant differences in global or regional white matter microstructure measures between those classified as responders versus those that were classified as non-responders in either the supplement or placebo groups individually or across both groups combined.

Discussion

The current study tested whether one year of supplementation with lutein (L) and zeaxanthin (Z), two xanthophyll carotenoids with known antioxidative properties and cognitive benefits, could prevent or slow age-related structural brain changes in a sample of community-dwelling older adults. Using a randomized, double-blind, placebo-

controlled trial design, we hypothesized that older adults receiving supplementation with L & Z would increase, maintain, or show attenuated loss of brain volume and white matter microstructure in areas that are vulnerable to age-related decline (i.e., frontal and temporal gray and white matter regions) relative to older adults receiving a placebo.

Results showed age-related declines for frontal and medial-temporal gray and white matter and particularly subcortical white matter of the anterior cingulate cortex, across both groups over the course of the trial. However, L and Z did not appear to influence this loss. No significant group differences or changes over time were observed in global brain volume outcomes (i.e., global gray matter, global white matter, lateral ventricular volume, and white matter lesion volume). Additionally, no interactions between group and time were found for any of the brain volume measures. Average percent change for both global volumes and frontal-temporal volumes ranged from less than 1% to 3%, consistent with previous estimates of the average annual rate of gray and white matter volume decline in older adults (Jernigan et al., 2001; Salat et al., 1999).

We also did not find significant differences in white matter microstructure outcomes between the supplement and placebo groups. Results did show an average increase in global FA, across both groups. Significant changes were also seen in anterior white matter tracts, including increased FA in the anterior cingulum and an expected, age-related decline in fornix FA. While it is encouraging to see improvements in global and anterior cingulum white matter microstructure, it is important to note that these changes were seen in both the supplement and placebo groups and, thus, cannot be attributed to the intervention. Average percent change for both global and frontal-temporal white matter microstructure ranged from less than 1% to around 2%, again

consistent with estimates of annual changes in diffusion metrics in healthy older adult populations (Barrick et al., 2010; Teipel et al., 2010).

Although results confirmed that the intervention manipulation was effective (i.e., the supplement group showed a significant increase in retinal and serum L & Z concentrations while the placebo group did not), there was individual variability in both groups. This individual variability is not uncommon for nutritional intervention studies. There are many factors which may affect how one processes and absorbs nutritional supplements. For example, some studies have suggested that cholesterol levels and co-supplementation of other nutrients can impact the absorption and bioavailability of L and Z (Sato et al., 2012; Tanumihardjo et al., 2005). Additionally, the very nature of being in a nutritional study may cause some participants to choose healthier and more nutritious foods, whether consciously or unconsciously. Thus, exploratory analyses were conducted to determine whether individuals responded better to the intervention, or who otherwise increased their retinal L & Z concentration, showed better neural outcomes than those who showed no change or decreased retinal L & Z. While there were no differences in terms of white matter microstructure outcomes, results showed that in the supplement group, intervention "responders" (i.e., increased retinal L & Z) had significantly less decline in global gray matter and prefrontal gray matter volume than intervention "non-responders" (i.e., stable or decreased retinal L & Z), while there were no concurrent differences observed in the placebo group. These results suggest that L and Z may slow age-related gray matter decline for a subset of individuals who appeared to reap greater benefit from the supplementation regimen.

As with any study, the present trial had limitations. For example, a similar prospective study on the effects of Vitamin B12 on brain volume found significant results but followed the older subjects for five years as opposed to only one (Vogiatzoglou et al., 2008). Thus, longer time periods may be necessary to allow enough sensitivity to discriminate how dietary components influence morphological loss. Our sample size was small (e.g., the Vogiatzoglou et al. study tested 107 older adults) and suffered from considerable attrition (approximately 22%), which may have limited our power to detect certain group differences and intervention effects. Additionally, our sample was homogenous in terms of background, consisting of 100% Caucasian and predominantly cognitively healthy and highly educated individuals. Research has shown that more educated people also tend to have healthier diets (Akbaraly et al., 2009), and our data support this trend. Baseline mean MPOD and serum L & Z values for our sample were slightly higher than other published data for older adults (Renzi et al., 2014; Renzi et al., 2012), suggesting that our sample may have consumed a more nutritious diet than the general population at baseline, prior to supplementation. In this respect, it is encouraging that a small dietary change provided benefit to even a subsample of individuals in our study who were already well-nourished, educated, and affluent. Finally, as with any nutritional intervention, there was no true control condition; our entire sample has been exposed to both L and Z throughout their lifetimes, and even those participants in the placebo condition continued to consume a normal diet for the duration of the trial, which presumably contained some amount of L and Z that is naturally-occurring in many foods. It is possible that the biggest effects of L and Z on the brain could be driven by deficiency.

While the current study found that one year of supplementation of L and Z had limited effects on the structural brain integrity of community-dwelling older adults, there is a growing body of literature to suggest that these nutrients are important for other aspects of cognitive and brain health. Other analyses from the current RCT have shown that supplementation with L and Z benefited cognition, particularly complex attention, cognitive flexibility (Hammond et al., 2017), and verbal learning (Lindbergh et al., 2018), and neural functioning in dorsolateral prefrontal and anterior cingulate areas that are vulnerable to age-related decline (Lindbergh et al., 2018). The importance of L and Z for cognitive and neural outcomes has been replicated cross-sectionally in other samples and by other researchers (Ajana et al., 2018; Feeney et al., 2013; Johnson et al., 2013; Vishwanathan et al., 2014) and is beginning to be confirmed longitudinally as well (e.g., Akbaraly et al., 2007; Johnson et al., 2008).

The strength of this study lies in the novel approach used to investigate in vivo structural brain outcomes of a year-long, double-blind, placebo-controlled trial of lutein (L) and zeaxanthin (Z) supplementation in older adults. Exploratory analyses did suggest that there was a small group of individuals who reaped greater benefit from L and Z supplementation and who showed less decline in global and prefrontal gray matter volume than individuals who did not appear to benefit from the intervention. To that end, future studies could explore factors which may impact the efficacy of L and Z supplementation, such as vascular health, co-supplementation of other nutrients, and other factors known to interact with nutrient bioavailability and absorption. Replication of this study in a larger and more diverse sample may also determine whether there are some individuals who could benefit more from L and Z supplementation, such

individuals who are more cognitively impaired or have had less access to nutritious diets across their lifetime. Factors such as supplement dosage and particularly a longer duration should also be considered when determining intervention efficacy. Finally, future studies will need to determine the long-term effects of L and Z supplementation on outcomes such as the development of dementia and accumulation of brain pathologies. While L and Z supplementation over one year appears to have limited effects on structural brain outcomes in older adults, it is likely not harmful and appears to provide benefits for other aspects of cognitive and brain health that could improve or extend quality of lrife for older adults.

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Table 1

Pre-Intervention Characteristics

	% or M (SD)		
	Supplement (N = 33)	**Placebo (N = 14)**	**Overall Sample (N = 47)**
Age (years)	72.4 (6.27)	70.4 (5.43)	71.8 (6.04)
Sex (% female)	51.5%	71.4 %	57.4%
Race (% Caucasian)	100%	100%	100%
Education (years)	16.6 (3.31)	16.7 (3.02)	16.6 (3.19)
Cognitive Impairment (%)			
No Impairment (CDR = 0)	87.9%	100%	91.5%
Mild Impairment (CDR = 0.5)	12.1%	0%	8.5%

Note: CDR = Clinical Dementia Rating Scale (Morris, 1993).

Table 2

Brain Volume

	M (*SD*) in mm^3					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Gray Matter						
Global	589258 (41075)	583090 (43892)	598888 (26886)	595608 (27435)	592127 (37387)	586819 (39829)
Regions-of-Interest (ROIs)						*
Prefrontal	102435 (10156)	101378 (10354)	105206 (7147)	104517 (6733)	103260 (9372)	102313 (9460)
Orbitofrontal	23839 (2388)	23809 (2208)	23430 (1645)	23590 (1719)	23717 (2184)	23744 (2058)
Anterior Cingulate	7989 (1319)	7910 (1447)	7887 (1055)	7864 (1102)	7958 (1236)	7896 (1341)
Medial Temporal	27361 (2589)	27234 (2558)	27063 (2342)	27234 (2658)	27212 (2496)	27234 (2559)
Hippocampus	8681 (989)	8407 (1050)	9144 (743)	8962 (886)	8819 (939)	8572 (1027)
White Matter						
Global	437384 (74298)	432185 (81221)	427438 (40081)	423846 (39899)	434422 (65691)	429701 (71090)
Regions-of-Interest (ROIs)						*
Prefrontal	84364 (8774)	83736 (10028)	83119 (7414)	82317 (7317)	83994 (8332)	83313 (9247)
Orbitofrontal	18494 (2083)	18421 (2345)	18141 (1484)	18083 (1531)	18389 (1915)	18313 (9247)
Anterior Cingulate	9228 (902)	9160 (951)	9336 (696)	9294 (699)	9260 (840)	9200 (878)*
Medial Temporal	17092 (2499)	16955 (2736)	16803 (2039)	16961 (2015)	17006 (2353)	16957 (2521)
Lateral Ventricle	33250 (15696)	35066 (16500)	28855 (11638)	30229 (12088)	31941 (14622)	33625 (15352)
White Matter Hypointensities	7759 (14588)	8090 (16083)	5543 (4446)	5625 (4690)	7099 (12437)	7356 (13692)

Note: All volumes are corrected for intra-cranial volume (ICV) and have been rounded to the nearest mm^3. * indicates a significant change from pre-to-post intervention, $p < .05$, controlling for age and Clinical Dementia Rating (CDR) score.

Table 3

White Matter Microstructure

	M (*SD*)					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Fractional Anisotropy (FA)						
Global	.55076 (.02855)	.55257 (.03287)	.55384 (.01966)	.56195 (.02032)	.55167 (.02604)	.55537 (.02978)*
Regions-of-Interest (ROIs)						*
Genu	.61821 (.04982)	.61228 (.05182)	.63634 (.03231)	.63892 (.02889)	.62361 (.04573)	.62021 (.04750)
Anterior Cingulum	.62050 (.05117)	.62390 (.05587)	.63488 (.03001)	.64467 (.03492)	.62478 (.04606)	.63008 (.05107)*
Fornix	.37296 (.09130)	.37186 (.10075)	.37936 (.08800)	.37860 (.10308)	.37487 (.08942)	.37387 (.10037)*
Radial Diffusivity (RD)						
Global	.00053(.00005)	.00053 (.00061)	.00051 (.00003)	.00050 (.00003)	.00053 (.00005)	.00052 (.00006)
Regions-of-Interest (ROIs)						
Genu	.00050 (.00010)	.00052 (.00010)	.00047 (.00049)	.00047 (.00042)	.00049 (.00009)	.00050 (.00009)
Anterior Cingulum	.00053 (.00009)	.00052 (.00010)	.00049 (.00006)	.00048 (.00006)	.00052 (.00009)	.00051 (.00009)
Fornix	.00156 (.00040)	.00158 (.00043)	.00154 (.00047)	.00156 (.00045)	.00155 (.00041)	.00157 (.00043)
Mean Diffusivity (MD)						
Global	.00081 (.00005)	.00081 (.00005)	.00079 (.00003)	.00078 (.00003)	.00081 (.00042)	.00080 (.00005)
Regions-of-Interest (ROIs)						
Genu	.00086 (.00009)	.00087 (.00009)	.00083 (.00004)	.00083 (.00003)	.00085 (.00008)	.00086 (.00008)
Anterior Cingulum	.00090 (.00008)	.00090 (.00008)	.00087 (.00005)	.00085 (.00004)	.00089 (.00007)	.00088 (.00007)
Fornix	.00191 (.00036)	.00193 (.00038)	.00189 (.00044)	.00191 (.00039)	.00190 (.00038)	.00193 (.00038)
Axial Diffusivity (AD)						
Global	.00138 (.00004)	.00138 (.00005)	.00135 (.00004)	.00134 (.00003)	.00137 (.00004)	.00137 (.00005)
Regions-of-Interest (ROIs)						
Genu	.00156 (.00010)	.00158 (.00008)	.00155 (.00004)	.00155 (.00003)	.00156 (.00008)	.00157 (.00007)
Anterior Cingulum	.00164 (.00007)	.00166 (.00006)	.00161 (.00005)	.00160 (.00003)	.00163 (.00006)	.00164 (.00006)
Fornix	.00261 (.00029)	.00264 (.00301)	.00260 (.00038)	.00261 (.00030)	.00260 (.00032)	.00263 (.00030)

Note: * indicates a significant change from pre-to-post intervention, $p < .05$, controlling for age and Clinical Dementia Rating (CDR) score.

Table 4

Intervention Response – Change in L & Z Concentrations

	M (SD)					
	Supplement (N = 33)		**Placebo (N = 14)**		**Overall Sample (N = 47)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
MPOD (o.d.)	.5185 (.1883)	.5945 (.2186)*	.4414 (.1397)	.4843 (.2005)	.4955 (.1774)	.5617 (.2173)*
Serum L & Z (umol/L)	.3041 (.1760)	1.175 (.45807)*	.3107 (.1258)	.2996 (.1490)	.3061 (.1610)	.8968 (.5644)*

Note: L = lutein. Z = zeaxanthin. o.d. = optical density. o.d. represents the log ratio of transmitted light passing through the macula.
* indicates a significant change from pre-to-post intervention, $p < .05$.

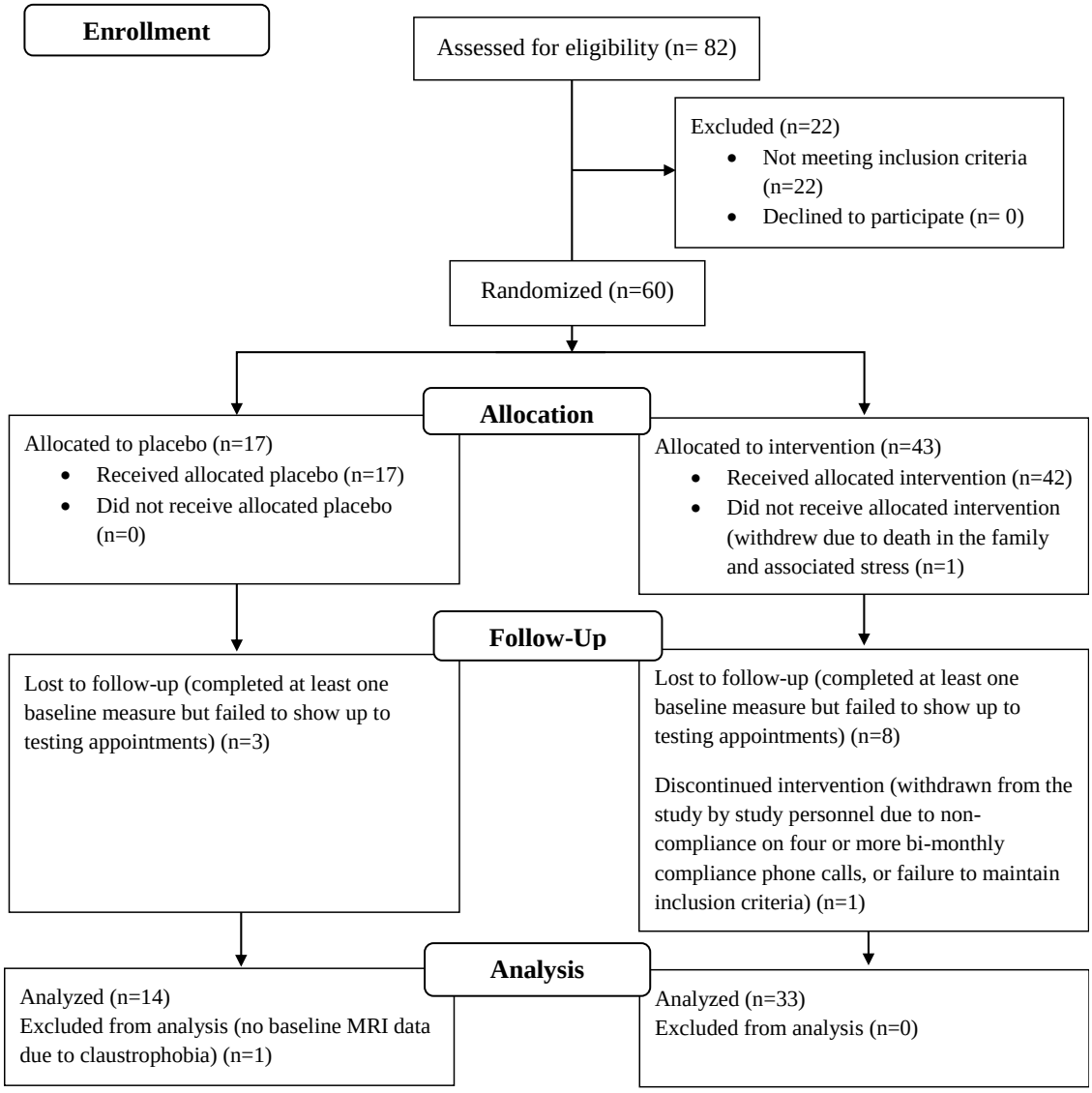

Figure 1 Participation Flow Diagram

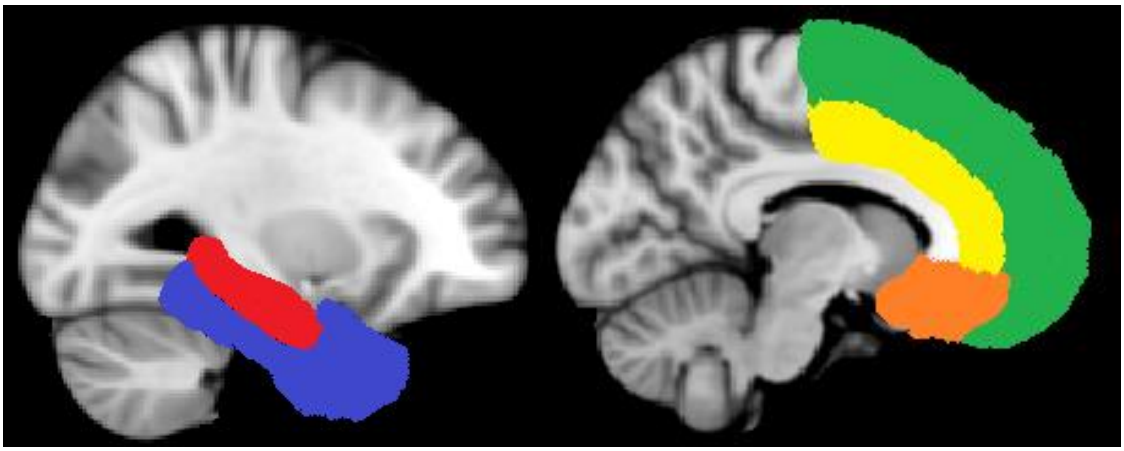

Figure 2 Volumetric of Interest (ROIs). The figure depicts the masks used for volumetric ROI analyses for the hippocampus (red), medial temporal lobe cortex (blue), prefrontal cortex (green), anterior cingulate cortex (yellow), and orbitofrontal cortex (orange). Masks are superimposed on a T_1-weighted template in MNI space provided in FMRIB's Software Library (FSL).

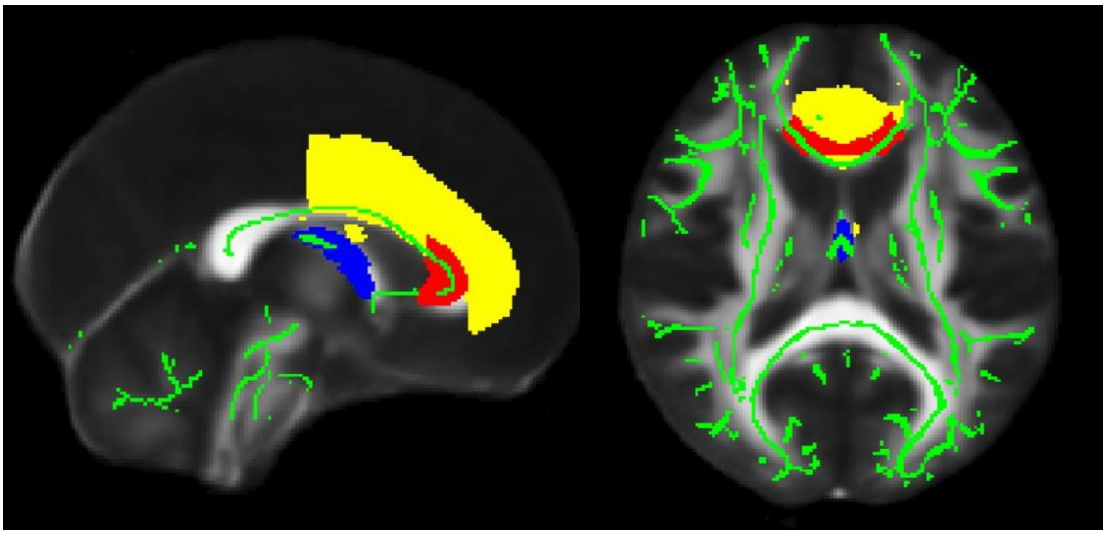

Figure 3 White Matter Microstructure Regions of Interest (ROIs). The figure depicts the masks used for white matter integrity ROI analyses for the genu (red), anterior cingulum (yellow), and fornix (blue) in the sagittal view (left) and axial view (right). Masks are superimposed on a single-subject diffusion weighted template in MNI space provided by Johns Hopkins University (JHU) in FMRIB's Software Library (FSL). The mean skeleton for the sample is overlaid on the single-subject diffusion-weighted template image in green.