NEUROCOGNITIVE EFFECTS OF LUTEIN AND ZEAXANTHIN IN OLDER ADULTS MEASURED WITH FUNCTIONAL MAGNETIC RESONANCE IMAGING

by

CUTTER AUGUSTUS LINDBERGH

(Under the Direction of L. Stephen Miller)

ABSTRACT

A growing body of literature suggests a relationship between nutrition and brain health, particularly in old age. The overarching goal of the present research project was to extend this literature, focusing specifically on the micronutrient lutein (L) and its isomer zeaxanthin (Z). L and Z are xanthophylls within the carotenoid family that accumulate preferentially in the macular region of the eye and have long been shown to benefit visual functions. More recently, L and Z have demonstrated a positive relation to cognition in young and older adults. The current randomized controlled trial aimed to investigate underlying neural mechanisms using both task-based and resting state functional magnetic resonance imaging (fMRI). It was hypothesized that L and Z supplementation in old age would enhance neural efficiency and benefit overt cognitive performance on an fMRI-adapted verbal learning task relative to placebo. Supplementation was also expected to improve *intra*-network integrity of default mode network (DMN) and reduce *inter*-network connectivity between DMN and other resting state networks. Participants were drawn from a community-dwelling older adult sample ($N$ = 75; mean age = 73 years) randomly assigned to receive a daily supplement containing L and Z (12 mg) or a

physiologically inert placebo for a period of one year. Results indicated that L and Z supplementation significantly influenced brain function relative to placebo, though not all effects were in the hypothesized direction. Task-based fMRI revealed increased blood-oxygen-level-dependent signal in left dorsolateral prefrontal cortex and anterior cingulate cortex during learning in the supplemented group ($p < .05$, family-wise-error corrected). L and Z supplementation also appeared to buffer decline in overt cognitive performance on the verbal learning task (Cohen's $d = .84$) but the effect was not statistically significant ($p > .05$). Resting-state fMRI yielded significantly increased *inter*-network connectivity (Cohen's $d = .89$) and no significant effect on *intra*-network connectivity. Taken together, these results suggest that L and Z may facilitate the aging brain's capacity for neuroplastic compensation by increasing prefrontal activation during cognitive task performance and enhancing integration between functionally segregated neural networks while the brain is at rest.

INDEX WORDS: Aging, Carotenoids, Cognition, Default mode network, Diet, FMRI, Functional connectivity, Learning, Lutein, Nutrition, Older adults, Resting state fMRI, Zeaxanthin

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## Overview of Project

Lutein (L) and its isomer zeaxanthin (Z) are two micronutrients that must be acquired through diet and have historically been studied in relation to eye health to prevent macular degeneration. A rapidly emerging literature suggests that these carotenoids have potential to benefit cognitive functioning as well, particularly in older adults. This is likely due to several unique properties shared by L and Z, including their preferential accumulation in human brain tissue, antioxidant and anti-inflammatory effects, and ability to enhance synaptic membrane structure and function while improving interneuronal communication. The present study represents the first randomized controlled trial (RCT) to investigate neural mechanisms underlying the relation of L and Z to cognition *in vivo* using functional magnetic resonance imaging (fMRI) in a sample of older adults. In a broader sense, this research expands the surprisingly sparse evidence base involving the critical relationship between nutrition and brain health, while evaluating the potential of dietary factors to promote neurocognitive function in the rapidly growing older adult segment of the population.

## Neurocognitive Aging

### Cognitive Changes

Characterizing the course of cognitive decline in old age has proven difficult for various reasons, including heterogeneous trajectories across different cognitive domains

(Mungas et al., 2010) and widespread individual differences (Stern, 2012), not to mention methodological challenges (e.g., the need for extended longitudinal assessment). Whereas some older adults decline slowly, others decline rapidly and ultimately progress to dementia (Boyle et al., 2013). Still other aging populations, often referred to as "Super Agers," evidence cognitive performance in their 80s and 90s that is largely indistinguishable from that of (middle-aged) individuals in the 50-65 year old range (Harrison, Weintraub, Mesulam, & Rogalski, 2012).

General patterns have emerged, however, such as the tendency for loss to occur in certain domains, including processing speed, working memory, attention, mental flexibility, and other executive functions (Buckner, 2004; Logie & Morris, 2014; Salthouse, 1996; Wecker, Kramer, Hallam, & Delis, 2005). By contrast, some cognitive abilities, most notably semantic memory or general knowledge about the world (e.g., vocabulary)—also referred to as "crystallized intelligence"—are preserved or perhaps even enhanced in old age (e.g., Baltes, Staudinger, & Lindenberger, 1999; Laver, 2009).

Memory appears to be particularly impacted during the aging process, including the ability to acquire and retain new verbal and non-verbal information (Nyberg, Lövdén, Riklund, Lindenberger, & Bäckman, 2012), with estimates placing the prevalence of age-associated memory impairments (subjective concern plus objective impairment on standardized tests) as high as 38.4% (Ward, Arrighi, Michels, & Cedarbaum, 2012). Memory performance is often conceptualized as the product of a multi-step process, including both encoding (i.e., the acquisition of new information) and retrieval (i.e., the recall of encoded information), and it appears that age-related memory difficulties involve difficulties at both of these stages (Craik & Rose, 2012).

Many of the cognitive changes seen in old age may commence as early as one's 20s, persisting at a gradual rate throughout adulthood in the absence of overt pathology (Salthouse, 2004). Rates of cognitive decline show increasing inter-individual variability with age (Mungas et al., 2010) and appear to follow non-linear trajectories, often markedly accelerating in the few years prior to death (Wilson et al., 2015).

The widespread variability in cognitive function observed among older adults likely reflects a complex and diverse range of underlying biological processes. For example, amyloid load, tangle density, macroscopic infarcts, microinfarcts, Lewy bodies, small vessel disease, hippocampal sclerosis, and TAR DNA-binding protein-43, to name a few, have all been shown to contribute to different forms of cognitive impairment in old age, both in isolation and combination (Corey-Bloom et al., 1997; Marchant et al., 2013; Schneider, Arvanitakis, Bang, & Bennett, 2007; Tremblay et al., 2011). Even when considered collectively, pathological indices corresponding to the most common causes of dementia, including Alzheimer's disease, cerebrovascular disease, and Lewy body disease (Schneider et al., 2007), have been found to account for only 41% of individual variability in rates of cognitive decline in late life over an 18-year period, thus leaving a majority of the variance unexplained (Boyle et al., 2013). While this finding may reflect important disease processes that have yet to be identified, it also reveals the important role that lifestyle factors play in influencing individual trajectories (Stern, 2012).

**Neural Changes**

Advances in neuroimaging modalities that permit *in vivo* evaluation of brain structure and function have contributed to a more comprehensive and mechanistic understanding of the cognitive aging process. In particular, magnetic resonance imaging

(MRI) technology, developed in the mid-1980s, has been applied extensively in aging populations to evaluate structural brain changes and the relation of these changes to cognitive functioning (Lockhart & DeCarli, 2014). MRI is a noninvasive neuroimaging technique that affords many advantages relative to other structural techniques (e.g., X-ray and computed tomography), including substantially superior spatial resolution and tissue contrast and thus greater sensitivity to more subtle anatomical changes (Shenton et al., 2012). Briefly, MRI involves exposure to a powerful, static magnetic field ($B_0$) that aligns the spin activity of subatomic hydrogen particles ("protons") within the human body along the direction of the field (Weishaupt, Kochli, & Marincek, 2003). Radiofrequency pulses are then applied to temporarily manipulate or "flip" the spinning proton orientations. Following removal of the radiofrequency field, the excited "spins" relax to their original orientation along $B_0$ while emitting energy that is detected by a receiver coil. Different properties of biological materials and tissues result in different responses during excitation and relaxation, which can ultimately be represented as varying levels of brightness on MRI images.

Structural MRI studies have revealed that aging, even in the absence of dementia, is associated with various brain changes, including ventricular system expansion and volumetric shrinkage, particularly in frontal cortex, temporal cortex, putamen, thalamus, and accumbens (Fjell & Walhovd, 2010). Although this pattern is characterized by substantial inter-individual heterogeneity, cortical thickness and subcortical volume evidence a 0.5-1% reduction, on average, each year in normal adult aging (Fjell & Walhovd, 2010). In turn, these neuroanatomical changes are significant mediators of cognitive decline in such domains as processing speed, executive functions, and memory

(Fjell & Walhovd, 2010). Other age-related neurological changes that have been revealed through structural MRI approaches include white matter deterioration (Arvanitakis et al., 2016) and infarcts (Aggarwal et al., 2012), which in turn correlate with cognitive loss in older adults.

In addition to structural MRI, functional MRI (fMRI) has increasingly been used to evaluate age-related brain changes (e.g., Dennis & Thompson, 2014). FMRI is similar to structural MRI in many respects, though the focus is on brain activity or "function" rather than brain anatomy. The most commonly used fMRI approach involves evaluating changes in blood-oxygen-level-dependent (BOLD) signal, which provides an indirect measure of neuronal activity based on metabolic changes associated with neuronal transmission relative to baseline (Ogawa et al., 1993). More specifically, when a particular brain area is "active" or engaged in a task, there are increases in cerebral blood flow and volume to that region accompanied by relative increases in the ratio of oxyhemoglobin to deoxy-hemoglobin (oxygen-bound and unbound hemoglobin, respectively; Mayer, Bellgowan, & Hanlon, 2015). Because hemoglobin-carrying oxygen and deoxygenated hemoglobin possess different magnetic properties, BOLD signal intensity changes (increases) relative to periods of brain inactivity, suggesting local neuronal signaling (Matthews & Jezzard, 2004).

Two forms of fMRI are task-dependent and resting state, each of which provides unique and complementary information. Task-dependent (or, "task-based") fMRI is collected while the subject engages in a task, often requiring the use of higher-order cognitive functions though a variety of other processes have been considered as well (e.g., emotion, sensation, perception, and motoric). The focus is thus on evoked brain

activity during task performance and—assuming an interest in cognition—information is provided regarding the relation between cognitive functioning and neural functioning, typically in isolated brain regions (Baldassarre & Corbetta, 2015). Considerable evidence has accumulated for age-related changes in task-evoked brain activity, with observations of both hyper- and hypo-activation, depending on such factors as the specific paradigm employed, aging population assessed, and brain region evaluated (Grady, 2012). Changes in brain activation as measured by fMRI predict cognitive functioning in late life and show differential patterns in healthy older adults relative to older adults with genetic risk for Alzheimer's disease (Bookheimer et al., 2000), subjective memory complaints (Erk et al., 2011), mild cognitive impairment, and dementia (Dickerson et al., 2005).

In contrast to task-dependent fMRI, resting state fMRI provides data regarding functional connectivity within and between large-scale networks of brain regions and is collected while subjects lay passively in the scanner with little to no visual stimulation (e.g., eyes shut or fixated on a static cross; Baldassarre & Corbetta, 2015). The focus of resting state fMRI is the brain's intrinsic activity at rest, which consumes a majority of the brain's metabolic resources (Raichle & Mintun, 2006) and is often measured by assessing temporal correlations between spontaneous BOLD signal fluctuations in different brain regions that are functionally related (Fox & Raichle, 2007). Traditional resting state approaches have revealed several (10-15) spatially distributed networks that exhibit synchronous deactivation during cognitive task performance and correlated activity at rest (Baldassarre & Corbetta, 2015), perhaps most notably the default mode network (DMN) comprised of posterior cingulate cortex, ventral anterior cingulate cortex, and supramarginal gyrus (Buckner, Andrews-Hanna, & Schacter, 2008).

Older adults with various neurodegenerative conditions show altered functional connectivity relative to normal aging (Seeley, Crawford, Zhou, Miller, & Greicius, 2009), and the aging process in general impacts both within and between network functional connectivity (Geerligs, Mauritis, Renken, & Lorist, 2014; Geerligs, Renken, Saliasi, Maurits, & Lorist, 2015). Relative to young adults, older adults show reduced connectivity within specific functional networks (e.g., DMN, as well as fronto-parietal control, dorsal attention, salience, and motor networks) and greater correlations of activity within functional networks to activity outside of functional networks (Ferreira & Busatto, 2013; Geerligs, Mauritis et al., 2014; Voss et al., 2010). These findings have been extended using brain-wide analytical approaches that have similarly demonstrated reduced modularity (i.e., relatively lower intra-network connectivity coupled with relatively higher inter-network connectivity) in older adults relative to young adults (Geerligs, Renken et al., 2015). The observed reduction in functional network specificity has been related to dedifferentiation theory, which holds that brain regions become less functionally specialized with advancing age (Baltes & Lidenberger, 1997). While this may reflect a compensatory process (discussed below), dedifferentiation in resting state networks has been described as a form of neural inefficiency, particularly because the brain's metabolic load is reduced when local networks exhibit high clustering and increased by the type of long range (inter-network) connections seen in old age (Geerligs, Renken et al., 2014; Meunier, Lambiotte, & Bullmore, 2010).

Intact functional connectivity is necessary for intact cognitive functioning (Biswal, Eldreth, Motes, & Rypma, 2010) and resting state network dysfunction has demonstrated a strong relation to cognitive dysfunction (Ferreira & Busatto, 2013;

Geerligs, Mauritis et al., 2014). This relation has been demonstrated in various ways, including in studies that investigate associations between connectivity levels within specific resting state networks and specific cognitive abilities. For example, reduced connectivity within DMN nodes has been related to worse performance on tests of memory, executive function, and processing speed in older adults (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Wang et al., 2010). This finding has been borne out in longitudinal designs, with reductions in DMN connectivity predicting memory decline over a six-year period in a combined sample of middle-aged and older adults (Persson, Pudas, Nilsson, & Nyberg, 2014).

Aggregate connectivity scores across multiple resting state networks have also been evaluated, yielding a relation between global connectivity levels and global cognitive functioning in old age (Shaw, Shultz, Sperling, & Hedden, 2015). Between-network connectivity has similarly been investigated in the context of age-related changes in cognition, specifically between "task-positive" networks, which are comprised of brain regions that typically show increased activity during cognitive task performance (e.g., fronto-parietal control network), and "task-negative" networks, which are comprised of brain regions that de-activate during cognitive task performance (e.g., DMN; Fox et al., 2005). The strength of the anti-correlation between task-positive and task-negative networks has been shown to decrease with age (Geerligs, Renken et al., 2015) and is related to worse cognitive (e.g., working memory) performance (Hampson, Driessen, Roth, Gore, & Constable, 2010). Decreased segregation of resting state brain networks (i.e., greater inter-network connectivity) has also been observed with advancing

age, which in turn predicts episodic memory dysfunction (Chan, Park, Savalia, Petersen, & Wig, 2014).

With respect to specific aging populations, dysfunction of resting state networks has been observed in older adults with mild cognitive impairment (Garces et al., 2014), individuals at genetic risk for Alzheimer's disease (Reiman et al., 2004), cognitively normal individuals with amyloid plaques (Sheline et al., 2010), and older adults with dementia (Greicius, Srivastava, Reiss, & Menon, 2004). Taken together, these resting state findings suggest that functional connectivity is altered in old age and the extent of these alterations has important clinical implications in both healthy and pathologically aging populations, possibly serving as a mechanism underlying cognitive decline.

## Scaffolding Theory of Aging and Cognition

The Scaffolding Theory of Aging and Cognition (STAC) has garnered attention and empirical support over the years to account for a given older adult's level of cognitive function and the considerable variability in cognitive status seen across different older adults (Park & Reuter-Lorenz, 2009). According to the model, the aging brain is impacted by different types and degrees of neural deterioration, including both age-related changes in brain structure (e.g., atrophy, cortical thinning, and white matter deterioration) and function (e.g., reduced specificity and dysfunction in resting state networks). To compensate, the brain engages in an adaptive process of "compensatory scaffolding," which functions to counteract structural and functional degradation by enlisting the support of additional neural circuitry to preserve cognitive function. Evidence for the compensatory scaffolding process is seen in studies showing recruitment of additional brain regions (e.g., prefrontal and parietal) in older adults relative to young

adults during cognitive task performance (Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008; Huang, Polk, Goh, & Park, 2012), as well as a tendency for bilateralization on tasks that tend to be lateralized earlier in the lifespan (Cappell, Gmeindl, & Reuter-Lorenz, 2010). This parallels work in young adults showing additional brain activity when acquiring new skills (Petersen, van Mier, Fiez, & Raichle, 1998) or faced with particularly challenging cognitive tasks (Reuter-Lorenz & Cappell, 2008). In other words, the brain possesses an ability to adaptively approach a given task by reorganizing the use of existing circuitry, whether to meet the needs of a particularly challenging task or to overcome neural insult associated with the aging process. How exactly this reorganization manifests itself in brain function is broadly defined under the STAC; compensatory recruitment models such as Hemispheric Asymmetry Reduction in Older Adults (HAROLD; see Cabeza, 2002), Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH; see Reuter-Lorenz & Cappell, 2008), and Posterior-Anterior Shift with Aging (PASA; see Davis et al., 2008) are all considered to fall under the STAC umbrella and provide examples of different scaffolding profiles.

Although scaffolding is considered an adaptive response to insult to maintain cognitive status or at least buffer against cognitive decline, older adults who are able to maintain a healthy, "younger" brain by avoiding age-related neuropathological processes tend to require less compensatory recruitment (Duzel, Schutze, Yonelinas, & Heinze, 2011; Josefsson, de Luna, Pudas, Nilsson, & Nyberg, 2012). These individuals are considered to have greater neurobiological efficiency given a reduced need to recruit additional neural resources to meet task demands (Duverne, Habibi, & Rugg, 2008). The notion that neurophysiological decline (e.g., gray matter atrophy) can contribute to brain

inefficiency has been demonstrated empirically and is exemplified by findings that greater age-related volumetric loss in left inferior frontal gyrus is associated with greater recruitment of right inferior frontal gyrus during language processing (Tyler et al., 2010). Cognitive performance in this older adult sample was found to be equivalent to a young adult comparison group, supporting the notion that the observed neural recruitment served a compensatory role (Tyler et al., 2010). This is not always the case, however, as sometimes an individual's compensatory scaffolding capacity is insufficient to overcome extensive age-related neurodegeneration, such that cognitive impairment remains apparent relative to healthy controls (Dickerson et al., 2005). It should also be noted that over-recruitment of task-irrelevant brain regions (including default mode regions) has been associated with poorer cognitive performance, possibly reflecting a misallocation of resources (Stevens, Hasher, Chiew, & Grady, 2008), general neural inefficiency (Morcom, Li, & Rugg, 2007), or both.

More recently, the original scaffolding model was revised (STAC-r) to place a stronger emphasis on "life-course" variables, consistent with findings that there are a host of factors beyond the aging process that shape brain structure and function (Reuter-Lorenz & Park, 2014). The term "neural resource enrichment" was thus incorporated to reflect lifestyle influences that have a positive effect on neurocognitive function, such as cognitive stimulation, social engagement, education level, nutrition, and physical fitness (e.g., Yaffe, Hoang, Byers, Barnes, & Friedl, 2014). As the name implies, neural resource enrichment is considered to lead to better cognitive outcomes by directly enhancing brain health through, for example, improved connectivity, increased cortical thickness, and greater synaptic complexity (e.g., Erickson et al., 2013). A less direct pathway has also

been acknowledged under the STAC-r, whereby life-course experiences increase resilience to neural degradation on a functional level by promoting the development of compensatory cognitive strategies (Reuter-Lorenz & Park, 2014). This is consistent with a body of literature demonstrating that factors such as educational and occupational attainment reduce the likelihood of developing dementia (Dekhtyar et al., 2015; Rentz, 2010; Stern et al., 1994). The relation of low educational attainment to increased risk of dementia prevalence (odds ratio: 2.61) and incidence (odds ratio: 1.88) has been confirmed in meta-analytic findings controlling for a variety of study and participant characteristics (Meng & D'Arcy, 2012).

The STAC-r shares many similarities with the concept of "reserve" advocated by Stern (2002, 2009, 2012), which was originally conceived to explain individual differences in clinical outcomes in response to age-related brain pathology. For example, an indirect relationship between level of cognitive function and degree of Alzheimer's disease brain pathology at autopsy has repeatedly been observed (e.g., Katzman et al., 1989). The reserve concept is traditionally divided into two major components, often referred to as "brain reserve" and "cognitive reserve" (Stern, 2012). Briefly, brain reserve refers to quantifiable properties of brain structure (e.g., intracranial volume, number of synapses, and dendritic density) that have been shown to account for individual differences in cognitive decline and dementia (e.g., Barulli & Stern, 2013; Mortimer, Snowdon, & Markesbery, 2003). Brain reserve has thus been described as more "passive" in nature given that once the ratio of pathological burden to protective brain quantities reaches a certain threshold, cognitive and functional impairment is inevitable (Barulli & Stern, 2013; Stern, 2009, 2012). By contrast, cognitive reserve focuses on cognitive

processes, such as efficiency, flexibility, and capacity, to explain individual differences in clinical manifestations despite similar levels of brain changes or pathology (Stern, 2012). Cognitive reserve is considered more "active" in nature given that it can be altered by a variety of experiences across the lifespan (e.g., leisure activity engagement, education level, and occupational complexity) and there does not appear to be a fixed, quantitative threshold at which point functional decline occurs (Barulli & Stern, 2013). Although cognitive and brain reserve were traditionally considered to make unique contributions to clinical outcomes, a blurring between the two constructs has increasingly been acknowledged based on findings that various lifetime activities, such as physical activity and cognitive stimulation, may lead to quantitative structural brain changes (Barulli & Stern, 2013; Brown et al., 2003).

Despite considerable overlap between the STAC-r and theories of reserve, a notable distinction is the relatively greater emphasis that STAC models place on the potential for neural enrichment to occur later in life with beneficial effects (Reuter-Lorenz & Park, 2014). This notion has gained some empirical support with interventions leading to improvements in cognition (Willis et al., 2006) and everyday function (Rebok et al., 2014) in older adult samples. However, it should be noted that the literature is often mixed on this topic and effect sizes tend to be modest. For example, a meta-analysis of 29 studies revealed that while aerobic exercise training significantly improves some aspects of cognitive function, particularly for older adults with mild cognitive impairment, summary effect sizes were small for attention and processing speed (Hedges' $g = 0.16$), executive functions ($g = 0.12$), and memory ($g = 0.13$), and non-significant for working memory (Smith et al., 2010). A meta-analysis of 10 studies evaluating the effect of

cognitive training in older adults similarly demonstrated small to medium effect sizes, with notably large confidence intervals, for recognition memory, working memory, processing speed, and global cognition (Kelly et al., 2014). Non-significant effect sizes were observed for outcome measures of immediate recall, delayed recall, and attention (Kelly et al., 2014). In addition, while a limited number of studies have found cognitive training transfer effects to other (non-trained) cognitive domains (e.g., Basak, Boot, Voss, & Kramer, 2008) and everyday functions (e.g., Edwards et al., 2008), generally speaking promising findings such as these have been rare (Barulli & Stern, 2013; Owen et al., 2010).

A very small number of studies have used neuroimaging tools to evaluate underlying neural mechanisms. While findings are preliminary and in need of replication, some evidence has accumulated to suggest that behavioral interventions may lead to changes in brain structure and function, indicating the potential for ongoing plasticity in old age (e.g., Anguera et al., 2013; Chapman et al., 2015; Engvig et al., 2010; Engvig et al., 2012; Lovden et al., 2010). Many of the observed effects on brain function have been interpreted as promoting neural efficiency by reducing hyper-active regions post-intervention, such that brain activity changes to more closely resemble that of young adults (Heinzel et al., 2014; Meinzer, Lindenberg, Antonenko, Flaisch, & Floel, 2013). Changes in resting-state fMRI activity have also been evaluated in at least one intervention study involving anodal transcranial direct current stimulation (Meinzer et al., 2013). In addition to bolstering cognitive performance in older adults, the intervention improved functional connectivity in frontotemporal, sensory-motor, and posterior regions, such that the overall connectivity pattern in the older adult group became more

similar to that of a young adult control group (Meinzer et al., 2013). The literature has not unanimously supported the possibility of intervention-related brain plasticity in late life, however, with some evidence indicating a lack of change in brain function (e.g., Dahlin, Neely, Larsson, Backman, & Nyberg, 2008) and brain structure (e.g., Heinzel et al., 2014) following cognitive training. In addition, effect sizes have ranged considerably from small (e.g., Engvig et al., 2012) to very large (e.g., Lovden et al., 2010). Given the equivocal and limited research base to date, several recent reviews have called for additional brain imaging studies to better understand this process while more fully characterizing the extent to which brain plasticity is preserved in old age (e.g., Duzel, van Praag, & Sendtner, 2016; Nyberg et al., 2012; Reuter-Lorenz & Park, 2014; Summers, Kang, & Cauraugh, 2016).

## Modifiable Factors Related to Late-Life Cognition

Effective treatments for age-related neurodegenerative diseases, such as Alzheimer's disease, have been elusive, in part due to irreversible neuronal damage and loss that occurs during often prolonged (e.g., 10-15 year) clinically "silent phases" leading up to disease onset (Marx, 2006). At present, available pharmacological treatments may modestly and temporarily improve cognitive symptoms but do little if anything to alter underlying pathobiological processes (O'Brien & Burns, 2011; Sala Frigerio & De Strooper, 2016) and often possess harmful side effects (e.g., gastrointestinal; Tricco et al., 2013). Consequently, there is increasing interest in modifiable lifestyle factors that may function to preserve cognitive functioning in late life (Yaffe et al., 2014). Evidence from cross-sectional, prospective, and RCT designs has amassed to support the role of various lifestyle factors in influencing cognitive function

and dementia prevalence in old age, including cardiovascular health, physical activity, cognitive stimulation, social engagement, sleep quality, and substance use (Bamidis et al., 2014; Jackson et al., 2016; Lehert, Villaseca, Hegervorst, Maki, & Henderson, 2015; Mattson, 2015; Prakash, Voss, Erickson, & Kramer, 2015; Rosano, Marsland, & Gianaros, 2012; Yaffe et al., 2014). While often considered in isolation, these factors have potential to act synergistically in ways that still remain to be fully understood and are currently an active area of research (Bamidis et al., 2014; Gomez-Pinilla, 2008; Ngandu et al., 2015; Praag et al., 2007). As discussed previously, however, the available evidence base is ambiguous as to whether such lifestyle activities confer neurocognitive benefits if undertaken only for discrete periods of time in late life (e.g., via interventions) or require long-term engagement across the lifespan (Barulli & Stern, 2013).

Nonetheless, the collective impact of modifiable factors on cognitive decline is not trivial. For example, a 10 to 25% reduction in the prevalence of major risk factors for Alzheimer's disease—including diabetes, hypertension, obesity, smoking, depression, low educational attainment, and physical inactivity—would be expected to lower disease prevalence by up to 3 million cases worldwide (Barnes & Yaffe, 2011). Even if targeting lifestyle and health-related factors in old age would not prevent cognitive decline or dementia from eventually occurring, merely delaying progression could be enormously beneficial to the individual and society at large. For example, it has been calculated that the development of strategies to delay onset and slow progression of Alzheimer's disease by only one year would result in over 9 million fewer cases worldwide by 2050 (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). Considering that dementia costs society between $159 and 215 billion each year in the United States alone—and

these expenses are projected to increase 80% by 2040—modest delays in dementia onset could have substantial monetary benefits as well (Hurd, Martorell, Delavande, Mullen, & Langa, 2013). The ability to delay disease onset by 5 years has been estimated to reduce associated economic burden by 50% (Alzheimer's Association, 2015).

It should be noted that the models used to produce these estimates (e.g., Brookmeyer et al., 2007) incorporate death rates from Alzheimer's disease alongside other mortality factors common in aging populations (e.g., cancer), and thus disease altering treatments would not necessarily enhance life expectancies to a dramatic extent. For example, Brookmeyer et al. (2007) calculated late-stage Alzheimer's disease to add only 11% to background mortality rates based on prior literature. However, this also means that the development of treatments that modestly delay Alzheimer's disease onset and slow progression would preferentially result in fewer older adults living with advanced stages of the disease who tend to require the highest level of care (and monetary expense; Brookmeyer et al., 2007).

**Diet**

Despite longstanding popular opinion that "you are what you eat," nutrition has only recently begun to receive empirical attention as a modifiable and relatively inexpensive lifestyle factor with potential to bolster neurocognitive function in old age. Evidence has accumulated in recent years to suggest that healthy dietary patterns benefit brain and cognitive function in late life (Caracciolo, Xu, Collins, & Fratiglioni, 2014). The Mediterranean diet has been evaluated in particular, which primarily consists of high consumption of fruits, vegetables, legumes, cereals, unsaturated fatty acids (e.g., olive oil), and fish, low to moderate intake of dairy products and alcohol, and low consumption

of meat and saturated fatty acids (Trichopoulou, Costacou, Bamia, & Trichopoulou, 2003). Numerous studies, including RCTs (e.g., Martínez-Lapiscina et al., 2013), and systematic reviews (e.g., Chiu et al., 2014; Feart et al., 2015; Jackson et al., 2016) have concluded that Mediterranean diet adherence is associated with beneficial neurocognitive effects in older adult populations. A recent large-scale longitudinal study ($N$ = 960) demonstrated that the Mediterranean diet significantly reduces rates of cognitive decline in older adults over a 5-year period both globally and in specific cognitive domains, including episodic memory, working memory, semantic memory, visuospatial ability, and perceptual speed (Morris et al., 2015). Cognitive abilities of older adults in the highest tertile of diet adherence were found to equate to being 7.5 years younger in age (Morris et al., 2015). Meta-analytic results have corroborated these beneficial effects, demonstrating that older adults in the highest tertile of Mediterranean diet adherence are 33% less likely to evidence cognitive impairment relative to peers in the lowest tertile (Singh et al., 2014). Similar results were obtained in a separate meta-analysis, which found high adherence to a Mediterranean diet to be associated with significantly less risk of cognitive impairment (either mild or advanced) compared to low adherence (relative risk: 0.60; Psaltopoulou et al., 2013).

The relationship between more specific dietary features and cognitive aging has been evaluated as well. An association between vegetable consumption and cognitive functioning has gained support, with several prospective studies demonstrating slower rates of decline among middle-aged (Nooyens et al, 2011) and older adults who consume more vegetables (Kang, Ascherio, Grodstein, 2005; Lee, Kim, & Back, 2009; Morris, Evans, Tangney, Bienias, & Wilson, 2006). For example, in a sample of 3,718 older

adults followed over a 6 year period, Morris et al. (2006) found the rate of global cognitive decline among individuals in the highest quintile of vegetable intake to be -0.032 standardized units per year relative to -0.046 in the lowest quintile (i.e., a 30% difference in annual cognitive decline rates). Not all findings have been positive, however, with some well-powered longitudinal studies revealing no relationship between vegetable intake and cognitive function (e.g., Butchart et al., 2011; Rahman, Sawyer Baker, Allman, & Zamrini, 2007). In addition, effects may be limited to certain classes of vegetables, such as those that are green and leafy or cruciferous (Kang et al., 2005).

Evidence is less convincing for fruit consumption in general (Chen, Huang, & Cheng, 2012; Kang et al., 2005) though high intake of certain types of fruits, most notably berries, have been shown to delay cognitive aging by 2.5 years (Devore, Kang, Breteler, & Grodstein, 2012). In addition, both pomegranate supplementation (Bookheimer et al., 2013) and grape supplementation (Krikorian, Nash, Shidler, Shukitt-Hale, & Joseph, 2010) have been found to improve learning and memory in older adults with mild cognitive loss or age-related memory complaints, with effect sizes generally falling in the medium range. However, comparable interventions have yielded non-significant findings and small effect sizes (e.g., Crews et al., 2005). While a relatively recent systematic review of 19 epidemiological studies (11 longitudinal, 8 cross-sectional) and 6 dietary intervention studies concluded that the available literature generally suggests a positive association of fruit and vegetable consumption to cognition in older adults (Lamport, Saunders, Butler, & Spencer, 2014), additional RCTs are needed to determine the potential benefits of short-term engagement. A meta-analysis is

also in need to provide a summary effect size while systematically identifying sources of heterogeneity.

Nuts, especially walnuts (see Poulose, Miller, & Shukitt-Hale, 2014, for a review), have been investigated as another nutritional source to preserve or enhance brain function and cognition. In a large, population-based study of 2,613 men and women from 43-70 years of age, higher nut intake was cross-sectionally associated with better cognitive performance globally and in specific domains, including memory, processing speed, and cognitive flexibility when adjusting for age, sex, education, energy intake, consumption of other fruits and vegetables, cholesterol, blood pressure, waist circumference, smoking, physical activity, and general mental health ($\beta s$ all = 0.05; Nooyens et al., 2011). The difference in cognitive abilities between individuals in the lowest and highest quintile of nut intake was equivalent to 5-8 years of cognitive aging (Nooyens et al., 2011). In a cross-sectional study of 447 individuals (55-80 years of age), Valls-Pedret et al. (2012) observed walnut consumption to be associated with working memory ($\beta = 0.15$) upon adjusting for gender, age, education, body mass index, smoking, apolipoprotein E (APOE) status, physical activity, diabetes, hypertension, and hyperlipidemia. However, non-significant relationships were observed with measures of global cognitive ability, learning, and recall (Vall-Pedret et al., 2012). RCTs are lacking in older adults but at least one such study in young adults supplemented with 60 grams of walnuts daily for 8 weeks demonstrated a significant improvement in verbal reasoning relative to controls (Cohen's $d = 0.57$; Pribis et al., 2012). Taken together, there appears to be some preliminary evidence for a possible relation between nut consumption and cognitive function in late life, though the available evidence base is quite limited.

The extent to which diets rich in flavonoids—a class of polyphenolic compounds that are abundant in certain food products such as grapes, cocoa, and tea—buffer age-related cognitive decline has also received attention. Among older adult samples, flavonoid heavy diets have been associated with reduced incidence of cognitive impairment (multivariate-adjusted odds ratio = 0.46 for ≥ 2 cups of green tea per day; Kuriyama et al., 2006) and dementia (multivariate-adjusted relative risk = 0.49 for individuals in the two highest tertiles of consumption compared to the lowest; Commenges et al., 2000), even when adjusting for a variety of potential confounds, such as gender, education, weight, and Vitamin C levels. RCTs have demonstrated that cocoa flavanol supplementation improves cognitive functioning in healthy young (Scholey et al., 2010) and older adults in such domains as executive functioning and memory (Brickman et al., 2014; Mastroiacovo et al., 2015). However, non-significant findings have also been reported in older adults (e.g., Butchart et al., 2011) and effect sizes have ranged considerably from very small (Cohen's $d$ = 0.06) to large ($d$ = 0.86), likely related to widespread differences in cognitive assessments, intervention length, flavanol dosage, and other factors (Grassi, Ferri, & Desideri, 2016). Although a meta-analysis has yet to be conducted on this topic specifically, meta-analytic findings in 14 prospective cohort studies demonstrated a protective effect against cerebrovascular disease (relative risk = 0.95 for every 10 milligram per day increase in flavanol intake; Wang, Ouyang, Liu, & Zhao, 2014).

Several other specific dietary features have been evaluated in the context of age-related cognitive changes, such as alcohol consumption, certain herbs and spices, and omega-3 fatty acids (Chiu et al., 2014). For example, in a large ($N$ = 11,102) sample of

older adults followed across a 2-year period, moderate drinkers of any type of alcohol showed a reduced likelihood of cognitive decline relative to nondrinkers (relative risk = 0.85) after controlling for a variety of potential confounds (i.e., age, education, cardiovascular risk factors, physical activity, other features of diet, body mass index, drug use, smoking status, mental health, social integration, physical health, and baseline cognitive function; Stampfer, Kang, Chen, Cherry, & Grodstein, 2005). A meta-analysis of 15 prospective studies similarly observed a reduced risk of dementia in (generally modest) drinkers versus nondrinkers (relative risk = 0.53; Anstey, Mack, & Cherubin, 2009). Preliminary evidence suggests that the observed effects may be driven by red wine (e.g., Nooyens, Bueno-de-Mesquita, van Gelder, van Bostel, & Verschuren, 2014), though this conclusion has yet to gain a sufficient empirical base. Among the herbs and spices, ginseng in particular has demonstrated potential to benefit global cognitive functioning in pathologically aging older adults, including in a 24-week RCT (Cohen's *d* = 0.52; Heo et al., 2011). However, at least one meta-analysis has indicated that gingko is ineffective in preventing dementia onset (Yang et al., 2014). Omega-3 fatty acids, found in the highest concentrations in various fish species, have also shown some promise in benefiting cognitive function, particularly among older adults with mild levels of cognitive impairment (Cederholm, Salem, & Palmblad, 2013). Meta-analytic findings have confirmed modest effect sizes in select cognitive domains among mildly impaired older adults, including immediate recall (Hedges' $g$ = 0.16) as well as attention and processing speed ($g$ = 0.30; Mazereeuw, Lanctot, Chau, Swardfager, & Hermann, 2012). Non-significant effects were observed in healthy older adults and Alzheimer's disease patients (Mazereeuw et al., 2012).

Alongside findings that certain dietary features exert protective effects, research has begun to accumulate demonstrating the adverse effects of a poor diet on brain health and cognition in late life. In contrast to the Mediterranean diet described previously, individuals living in the United States and other Westernized societies appear to be particularly at risk due to a cultural tendency to consume large amounts of red meat, refined sugars, foods with high fat contents, and refined grains (Freeman, Haley-Zitlin, Rosenberger, & Granholm, 2013). In addition to contributing to obesity and poor general health, various features of the "Western diet," such as high levels of saturated and trans fatty acids relative to polyunsaturated fatty acids (e.g., omega-3s), have been proposed to adversely impact neurocognitive functioning (Freeman et al., 2013). Numerous studies using animal models have demonstrated that rodents fed diets characterized by high saturated fatty acid consumption (~40% of calories from lard) display worse cognitive performance relative to standard chow fed controls, including in the domains of learning and memory (e.g., Greenwood & Winocur, 1990; Molteni et al., 2004). Human studies have similarly demonstrated a relation between high-fat diets and global cognition in older adults, with individuals in the highest quintile of fat intake evidencing a 68% increase in annual decline rates (-0.058 standardized units/year) relative to the lowest quintile (-0.035 standardized units/year) over a 6 year period upon controlling for a host of demographic, cardiovascular, and dietary variables ($N$ = 2,560; Morris, Evans, Bienias, Tangney, & Wilson, 2004). After adjusting for age, sex, education, and total energy intake, greater intake of total fat, saturated fat, and cholesterol were independently associated with increased risk of dementia (risk ratios = 2.4, 1.9, and 1.7, respectively) in

a sample of 5,386 adults, aged 55 years and older, followed for an average of 2.1 years (Kalmijn et al., 1997).

In addition to fat consumption, insufficient intake of certain nutrients and vitamins (e.g., fiber, folate, vitamin C, beta-carotene, iron, and zinc) has been modestly associated with worse global cognitive status in older adults (Pearson *r*s range from 0.14 to 0.22; Ortega et al., 1997). Elevated blood glucose (measured in millimoles per liter) also appears to represent a risk factor, relating to greater rates of cognitive decline (measured in T-score units) in overall ability ($\beta$ = -0.71), perceptual speed ($\beta$ = -0.65), verbal ability ($\beta$ = -0.42), and spatial ability ($\beta$ = -0.51) in adults aged 50 years or older across a 16 year period ($N$ = 838; Seetharaman et al., 2015).

**Lutein and zeaxanthin.** The xanthophyll lutein (L) and its isomer zeaxanthin (Z) are among 40 micronutrients that belong to the carotenoid family, a group of over 750 organic pigments that occur naturally in the environment (Britton, Liaaen-Jensen, & Pfander, 2004; Khachik, Beecher, Goli, & Lusby, 1991). L and Z are not produced endogenously and thus must be derived from our diet, primarily through consumption of green leafy vegetables and fruits that are bright in color (Johnson, 2012; Mangels, Holden, Beecher, Forman, & Lanza, 1993). Some other food sources contain L and Z as well, such as egg yolks (Chung, Rasmussen, & Johnson, 2004). Given the widely studied relation between Mediterranean diet adherence and neurocognitive health, it is notable that plant foods central to the Mediterranean diet have been shown to significantly correlate with L and Z plasma concentrations in adult humans (Trichopoulou, Benetou et al., 2003). In addition, mean plasma carotenoid levels have been found to increase by 55%, on average, following 6 months of a Mediterranean diet relative to a diet-as-usual

condition, including a 41% increase in L and a 31% increase in Z (Djuric, Ren, Blythe, VanLoon, & Sen, 2009).

L and Z distribute throughout the body and are the most prevalent carotenoid in tissues of the central nervous system (CNS; Johnson, 2012). Of the 40 or so carotenoids typically present in human diet, L and Z, with their identical chemical compositions and highly similar structures (Krinsky, Mayne, & Sies, 2004), by and large are the only carotenoids capable of traversing the blood-retina barrier (Snodderly, 1995). L and Z accumulate in several regions of the eye (e.g., Rapp, Maple, & Choi, 2000) but are taken up most selectively in the central region of the retina, referred to as the macula (Bone, Landrum, & Tarsis, 1985). Pigment within the human macula consists of L and Z embedded in retinal tissue (Bone et al., 1985), as well as meso-Z, which is typically converted from L rather than acquired directly via diet (Johnson, Neuringer, Russell, Schalch, & Snodderly, 2005; Rasmussen, Muzhingi, Eggert, & Johnson, 2012). L and Z collect within the macula in about 500 fold concentrations higher than in circulating blood serum and protect the eye with their antioxidant properties by filtering blue-light (Snodderly, 1995) while increasing neuronal signal transduction efficiency (Stringham & Hammond, 2005). The importance of L and Z to eye (retinal) health is well established, particularly in protecting against macular degeneration (e.g., Ma et al., 2012; SanGiovanni & Neuringer, 2012), which is a major cause of visual loss in the older adult population (The Eye Diseases Prevalence Research Group, 2004).

In addition to their actions within the eye, L and Z accumulate preferentially in human brain tissue, including within the cerebellum and pons, as well as frontal, occipital, and temporal lobes (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Johnson et

al., 2013). Importantly, this is not to say that L and Z do not accumulate in other brain regions as well (e.g., parietal lobes) as available postmortem studies are limited in number and a fully comprehensive evaluation of L and Z levels throughout all neural tissues has yet to be conducted. Nonetheless, in neural regions for which data are available, L and Z have been found to account for two-thirds or more of total brain carotenoid concentrations (Craft et al., 2004). The observation that L and Z are the dominant carotenoids in brain tissue despite being underrepresented in diet relative to other carotenoids provides evidence for selective uptake into brain tissue (Johnson, 2014). The dominance of L and Z in neural tissue appears to hold true across the developmental spectrum, from infanthood to older adulthood (Johnson, 2014). L and Z were recently quantified in a sample of infants, with L accounting for 59% of total brain carotenoid levels (Vishwanathan, Kuchan, Sen, & Johnson, 2014). These findings and others have led to speculation that L and Z play a critical role in both retinal maturation and neural development more broadly (Hammond, 2008; Vishwanathan, Kuchan et al., 2014).

L and Z levels can be measured *in vivo* by evaluating the optical density of the macular pigment (MPOD) layer using non-invasive heterochromatic flicker photometry (Stringham et al, 2008). Briefly, this technique involves asking the subject to visually fixate on a stimulus comprised of a black dot situated within a flickering disk. The wavebands of LED-based light sources producing the stimulus are then manipulated until the subject can no longer perceive the flickering, at which point a precise MPOD measurement can be calculated. This technique is widely used to assess macular pigment and has been convincingly validated in a range of populations, including older adults

(e.g., Gallaher et al., 2007; Iannaccone et al., 2007; Mares et al., 2006; Snodderly et al., 2004; Wooten, Hammond, Land, & Snodderly, 1999). Postmortem studies have revealed that MPOD correlates with L and Z concentrations in brain tissue in both primates (Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013) and humans (Vishwanathan, Schalch, & Johnson, 2016), suggesting that it is a reliable proxy for brain levels. This is not surprising given that the retina is comprised of neural tissue and thus similar processes are likely involved in the uptake of L and Z in retina and brain (Vishwanathan et al., 2016). A limitation of the MPOD technique, however, is that its non-invasive nature does not allow for evaluation of the independent contributions of the three components that comprise macular pigment (i.e., L, Z, and meso-Z; Johnson, 2014) and MPOD may be more highly correlated with brain levels of L relative to Z (Vishwanathan et al., 2016). This may reflect findings that dietary consumption of L is typically at least 5 times higher than that of Z, despite these nutrients being in generally the same foods, and thus more L may be available to cross the blood-brain barrier to accumulate in brain tissue (Johnson, Maras, Rasmussen, & Tucker, 2010).

Another commonly used method for assessing L and Z *in vivo* involves directly measuring concentrations in serum. In contrast to MPOD measures, L and Z serum levels tend to be more heavily influenced by recent food consumption rather than long-term dietary habits, unless current eating behavior is representative of enduring patterns (Beatty, Nolan, Kavanagh, & O'Donovan, 2004). Consistent with this notion, several studies (e.g., Hammond et al., 1997; Johnson et al., 2000; Landrum et al., 1997) have demonstrated that serum L and Z levels evidence a rapid return to baseline levels following supplementation cessation whereas MPOD remains elevated for 3-4 months

post-intervention. In addition, serum levels tend to peak more quickly and plateau following initial L and Z supplementation while MPOD has been shown to continue to increase up to a month following supplement discontinuation (Berendschot et al., 2000). Nonetheless, both MPOD and serum measurements are sensitive to L and Z supplementation, even if they evidence different time courses (e.g., Hammond et al., 1997; Landrum et al., 1997; Rubin et al., 2012; Stringham & Hammond, 2008). Yet the slower biological turnover of L and Z in retinal tissue renders it a more appropriate proxy for lifestyle eating behavior (Beatty et al., 2004).

While it has long been established that L and Z benefit eye health, an accumulating evidence base suggests that these carotenoids have potential to benefit neurocognitive function as well. Cross-sectional studies using samples ranging in age from adolescents to older adults have demonstrated that higher levels of MPOD are associated with greater temporal processing speed within the visual system (Renzi & Hammond, 2010). This finding has been supported in RCTs, with 4 months of L and Z supplementation increasing visual processing speed and visual motor reaction time in healthy young adults, though effect sizes were small (Cohen's $d$ = 0.27 and 0.32, respectively; Bovier, Renzi, & Hammond, 2014).

A small but growing number of studies have investigated the relation of L and Z to cognition in older adults. In an initial, cross-sectional study using a sample of 118 older adults (76-85 years of age), retinal L and Z levels, as reflected by MPOD, significantly and positively correlated with processing speed, as well as indices of task accuracy and completion ability (Renzi, Iannacocone, Johnson, & Kritchevsky, 2008). Importantly, these relations remained significant upon controlling for age, sex, and

ethnicity. A large, population-based study ($N$ = 4,453) of adults aged 50 years or older demonstrated a significant and positive relation between MPOD and global cognitive functioning, as measured by the Mini-Mental State Examination ($\beta$ = 0.48) and the Montreal Cognitive Assessment ($\beta$ = 0.83), adjusting for age, sex, and education (Feeney et al., 2013). Higher MPOD was also related to better performance on tasks assessing prospective memory (odds ratio = 2.24), executive function (i.e., trail-making; $\beta$ = 0.09), and choice reaction speed ($\beta$ = 0.09) controlling for age, sex, education, smoking status, hypertension, cholesterol levels, body mass index, visual acuity, diabetes, and presence of age-related macular degeneration (Feeney et al., 2013). MPOD was additionally associated positively with sustained attention but only when adjusting for age, sex, and education (Feeney et al., 2013).

At least one study (Renzi, Dengler, Puente, Miller, & Hammond, 2014) has examined the relation between MPOD and cognitive function separately in cognitively intact older adults and in older adults with mild cognitive impairment. Upon adjusting for age, body mass index, gender, ethnicity, presence of Type II diabetes, family dementia history, supplement use, and education level, retinal L and Z levels positively correlated with visuospatial and constructional abilities in cognitively normal older adults (partial $r$ = 0.24). Interestingly, MPOD correlated positively not only with visuospatial and constructional abilities in older adults with mild cognitive impairment (partial $r$ = 0.36) but also with global cognition (partial $r$ = 0.44), language skills (partial $r$ = 0.44), and attention (partial $r$ = 0.43). These findings suggest that L and Z consumption may be especially beneficial for older adults in early stages of cognitive decline.

Vishwanathan, Iannaccone et al. (2014) investigated the relation of L and Z measured in both serum and retina (MPOD) to cognitive function in older adults. After adjusting for age, body mass index, educational attainment, and sex, MPOD was positively correlated with measures of verbal learning (partial $r = 0.26$) and recall (partial $r = 0.22$), verbal fluency (partial $r = 0.25$), processing speed (partial $r = 0.25$), perceptual speed (partial $r = 0.20$), and global cognition (partial $r = 0.27$). L and Z in serum was only related (positively) to verbal fluency, consistent with the notion that MPOD provides a better proxy for brain carotenoid levels and long term dietary activity.

To the author's knowledge, only one study (Johnson et al., 2013) has evaluated the relation of L and Z concentrations in postmortem brain tissue to premortem cognitive function in humans. In a subsample of decedents from the Georgia Centenarian Study who donated brain tissue for analyses ($n = 21$, mean age = 101), L and Z concentrations in cortex (averaged across frontal, temporal, and occipital lobes) were positively associated with global cognitive function, verbal fluency, and confrontation naming abilities assessed prior to death (partial $r$s adjusted for sex, education, diabetes, and hypertension = 0.49, 0.46, and 0.57, respectively). Given that the study authors restricted their analyses to frontal, temporal, and occipital cortex, it will be important for future studies to evaluate whether L and Z levels in other neural structures (e.g., parietal lobes) similarly show a relation to cognition. Premortem serum L and Z concentrations were available for a larger portion of the cohort ($n = 298$) and yielded significant positive relations with global cognition (partial $r = 0.13$), delayed recall (partial $r = 0.18$), verbal fluency (partial $r = 0.17$), and verbal reasoning (partial $r = 0.24$) when controlling for

age, sex, education, body mass index, smoking status, alcohol use, hypertension, and diabetes (Johnson et al., 2013).

An obvious limitation of many of the aforementioned studies is their cross-sectional nature and several recent reviews exploring the relation between L and Z and cognition have called for the need for RCTs (e.g., Erdman et al., 2015; Johnson, 2012; Johnson, 2014). This will be critical to answer such questions as whether low carotenoid levels contribute to cognitive loss or perhaps represent a consequence, either through neuropathological processes or lifestyle choices (e.g., poor nutritional decisions resulting from cognitive impairments; Johnson, 2012). In addition, RCTs will be necessary to determine whether the observed relation between L and Z and cognition is specific to these micronutrients or whether circulating L and Z levels merely serve as a proxy for a generally healthful diet. To the author's knowledge, only one such RCT exists (Johnson et al., 2008) in a rather small sample of older women (60-80 years of age) who were randomly assigned to receive 4 months of daily docosahexaenoic acid (DHA; a polyunsaturated fatty acid) supplementation ($n$ = 14), L supplementation ($n$ = 11), combined supplementation ($n$ = 14), or placebo ($n$ = 14). All active intervention groups showed significant gains in verbal fluency (Cohen's $d$ for L supplementation = 0.61) and the combined treatment group additionally evidenced improvements in learning and memory performance (Cohen's $d$ = 0.72). These results support the beneficial potential of L on cognitive functioning in older adults, though as noted by the study authors, replication is needed in larger samples receiving supplementation over longer periods of time.

**Possible underlying mechanisms**. As noted by Hammond (2015), the means by which L and Z—and diet more generally for that matter—influence brain function and cognition is an understudied area, largely because answering this question relies on collaboration between disciplines that historically have had little interface, such as psychology and nutrition. Several plausible mechanisms have been proposed, however, often based on our understanding of how these carotenoids preserve neural health in the retina (Erdman et al., 2015). Perhaps most notably, L and Z have strong antioxidant and anti-inflammatory properties that are likely at least partially responsible for their beneficial neurocognitive effects (Izumi-Nagai et al., 2007; Krinsky, 2002; Sasaki et al., 2009).

In the context of healthy metabolic activity, cellular environments are characterized by "redox balance," that is, general homeostasis between the effects of oxidizing agents (pro-oxidants) and anti-oxidizing agents (antioxidants; Clifford, Howatson, West, & Stevenson, 2015; Kannan & Jain, 2000). While low levels of reactive oxygen and nitrogen species, also referred to as pro-oxidants or free radicals, are necessary products of cellular metabolism and serve a variety of functions ranging from gene expression to cell proliferation, excess levels overtake endogenous antioxidant supplies and result in redox imbalance, oxidative stress, and ultimately cellular dysfunction (Clifford et al., 2015; Kohen & Nyska, 2002; Lobo, Patil, Phatak, & Chandra, 2010).

The CNS exhibits high metabolic demands, with the brain accounting for approximately 25% of the body's oxygen intake and 20% of the body's metabolic activity at rest despite representing only 2% of the body's total weight (Clark & Sokoloff,

1999; Poulose et al., 2014; Raichle & Mintun, 2006). The brain's low endogenous antioxidant content coupled with its high metabolic rate and large concentrations of (highly oxidizable) polyunsaturated fatty acids in neuronal membranes renders it particularly vulnerable to free radical attack in the event of redox imbalance (Johnson, 2014; Sopher, Fukuchi, Kavanagh, Furlong, & Martin, 1996). While the effects of free radicals are usually controlled, the aging process disrupts the equilibrium between the generation and quenching of free radicals, resulting in an increased susceptibility to oxidative stress (Poulose et al., 2014). In turn, oxidative stress has been shown to contribute to neuronal dysfunction and death, particularly among older adults (Coyle & Puttfarcken, 1993). It is well established that oxidative damage accumulates during aging and is related to cognitive decline (Bokov, Chaudhuri, & Richardson, 2004). Greater levels of plasma antioxidants (including L and Z) positively correlate with cognitive abilities in cognitively healthy older adults (Akbaraly, Faure, Gourlet, Favier, & Berr, 2007) while low levels are typically seen in conditions such as mild cognitive impairment (Keller et al., 2005; Rinaldi et al., 2003) and Alzheimer's disease (Mecocci et al., 2002; Rinaldi et al., 2003). The relation between oxidative stress and age-related neurodegenerative diseases appears to be causal in nature (Butterfield, Bader Lange, & Sultana, 2010).

Increases in oxidized lipids and free radicals have been shown to contribute to a cascade of neuroinflammatory events as well (Libby, 2002). Similar to pro-oxidants, inflammation is a necessary and adaptive response to tissue damage, infection, and other biologically harmful processes (Ricciotti & FitzGerald, 2011). Chronic inflammation, however, has been shown to result in significant cellular dysfunction within the organism

and plays a role in a variety of physiological disorders, ranging from cancer to heart disease (Ricciotti & FitzGerald, 2011). In a healthy organism, numerous regulatory processes are in place to manage the intensity and duration of the inflammatory response to acute ailments (Rosano, Marsland, & Gianaros, 2012). In old age, however, these regulatory mechanisms appear to be less functional, consistent with findings that inflammation is prolonged and associated with excess tissue damage relative to young adults in both peripheral and central immune systems (Krabbe, Pedersen, & Bruunsgaard, 2004; Rosano et al., 2012). Neuroinflammation has been associated with cognitive loss among healthy older adults (Teunissen et al., 2003) and inflammatory status is elevated in older adults with mild cognitive impairment (Guerreiro et al., 2007), Alzheimer's disease, and other neurodegenerative conditions relative to healthy controls (McGeer & McGeer, 2010). Importantly, emerging research strongly indicates that elevated neuroinflammatory status is not a mere corollary of cognitive decline but causally contributes to neurodegeneration (Heneka et al., 2015). The adverse effects on neurocognitive function likely occur through complex and varied pathways including but not limited to inflammatory cytokines, complement components, toxic free radicals, and microglia attack of pathological entities resulting in collateral damage to host neurons (McGeer & McGeer, 2010).

Given the harmful effects of prolonged oxidative stress and inflammatory response on neurocognitive functioning, as well as the vulnerability of the aging brain in particular to these effects, it seems likely that the observed relation of L and Z to cognition is at least partially attributable to their strong antioxidant and anti-inflammatory properties. As antioxidants, L and Z quench singlet oxygen molecules (i.e., high energy

oxygen) thereby preventing free radical attack and reducing damage from oxidative effects (Johnson, 2014). L and Z appear to reduce oxidation of vulnerable lipids in particular, such as the polyunsaturated fatty acid DHA (Widomska & Subczynski, 2014), which is especially noteworthy given that older adults with cognitive impairments show increased rates of DHA oxidation relative to cognitively intact counterparts (Miller, Morel, Saso, & Saluk, 2014). Reduced oxidation of such polyunsaturated fatty acids likely serves the dual benefits of improving the structure and function of the cell membranes which they comprise while increasing the availability of these polyunsaturated fatty acids for transformation into molecules with anti-inflammatory effects (Miller et al., 2014). L supplementation has been shown to increase plasma antioxidant capacity and reduce oxidative stress in RCTs in both newborns and adults (Perrone et al., 2010; Wang et al., 2013). While these studies did not evaluate impacts on cognition, a clear relationship has been established between antioxidant consumption and cognitive health (Perkins et al., 1999), including in clinical trials of healthy and mildly impaired older adults (Summers, Martin, Cunningham, DeBoynton, & Marsh, 2010).

L and Z's anti-inflammatory effects have been shown to occur through a variety of pathways, which have been explored primarily using animal models or *in vitro* (Kijlstra, Tian, Kelly, & Berendschot, 2012). As examples, L and Z successfully ameliorated inflammatory responses in cultured human retinal pigment epithelial cells by altering the expression of inflammation-related genes (Bian et al., 2012). L treatment has also been shown to reduce gliosis in murine models following hypoxic/ischemic injury and production of pro-inflammatory factors in Müller cells, which play a central role in retinal inflammation (Li et al., 2012). In addition, following inflammation-mediated

retinal neural damage in a mouse endotoxin-induced uveitis model, L administration was found to reduce downstream inflammatory cytokine signals and reactive oxygen species, ultimately buffering neural damage (Sasaki et al., 2009). L and Z have been shown to reduce inflammatory responses in a variety of other inflammation models as well, such as laser induced choroidal neovascularization and streptozotocin induced diabetes (for a review, see Kijlstra et al., 2012). While a paucity of research has evaluated L and Z's anti-inflammatory effects in humans, Rubin et al. (2012) conducted an RCT in preterm infants provided diets with or without L, lycopene, and β-carotene supplementation. Relative to controls, the supplemented group demonstrated significantly greater plasma L levels and reduced inflammatory markers, including plasma C-reactive protein levels.

Beyond their neuroprotective effects, L and Z may improve neurocognitive function by positively influencing cell membranes and communication. Indeed, L and Z have been shown to accumulate in cell membranes (Sujak et al., 1999) with their unique structure and high membrane solubility uniquely positioning them to span the lipid bilayer and influence its physical and dynamic properties (Erdman et al., 2015; Krinsky et al., 2004; Widomska & Subczynski, 2014). More specifically, L and Z improve membrane fluidity, permeability, stability, thickness, and ion exchange (Erdman et al., 2015; Krinsky et al., 2004; Widomska & Subczynski, 2014). Relatedly, L and Z may benefit cellular communication by altering the expression of relevant genes (e.g., connexin 43; Bertram, 1999) and enhancing inter-neuronal signaling at gap junctions (Stahl & Sies, 2001). These findings are noteworthy given that intact cognition is dependent upon successful cell-to-cell signaling, which in turn depends on cell membrane composition, as all signals must past through this structure. Aging has been shown to

contribute to adverse changes in neuronal membrane structure and function (e.g., reduced fluidity), and ultimately neuronal dysfunction, due to such factors as reduced polyunsaturated fatty acid concentrations (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002 ). L and Z hold potential to buffer or perhaps even prevent these age-related changes.

**Neuroimaging findings.** As discussed previously, advances in neuroimaging techniques have provided a means to evaluate the extent to which nutrition interventions improve brain function in human samples *in vivo*, while shedding light onto potential mechanisms underlying the observed relation between diet and cognition. To the author's knowledge, only one study (Lindbergh et al., 2016) has evaluated the relation of L and Z to cognition with neuroimaging technology. The sample in Lindbergh et al. (2016) was drawn from the current RCT sample but considered only cross-sectional data at baseline, prior to group assignment or supplementation. Using an fMRI-adapted verbal paired-associates task, Lindbergh et al. (2016) presented 43 community-dwelling older adults with unrelated word pairs across a series of learning and recall trials. Endogenous (i.e., pre-supplementation) L and Z levels were assessed via blood serum concentrations and MPOD. Greater natural L and Z status was negatively and significantly associated with BOLD signal in several brain regions activated during learning and recall, including left inferior frontal gyrus, insula, middle temporal gyrus, middle frontal gyrus, and central and parietal operculum. The observed results were interpreted to reflect a potential role of L and Z in promoting neural efficiency during cognitive performance, particularly in brain regions with established risk for age-related degeneration. Given the study's cross-sectional design, however, replication is warranted in an RCT prior to conclusions

regarding directionality of the observed relationship. In addition, it should be noted that L and Z levels did not significantly correlate with overt behavioral performance on the task (i.e., number of words successfully recalled) and the authors did not statistically examine the relationship of task-evoked brain activity to behavioral performance.

A limited but growing number of studies have employed MRI technology to investigate the nature of the relationship between brain health and diet more broadly. Structural MRI approaches have demonstrated that older adults with high Mediterranean diet adherence display substantially less cerebrovascular disease burden than those characterized by low adherence, with individuals in the highest tertile evidencing 36% reduced odds of cerebral infarct across a 6 year span (Scarmeas et al., 2011). This finding remained virtually unchanged with a number of covariates in the model, including age, education, caloric intake, body mass index, ethnicity, APOE genotype, smoking status, and physical activity. High Mediterranean diet adherence has also been associated with increased thickness of cortical regions with demonstrated vulnerability to Alzheimer's disease, including orbitofrontal cortex, entorhinal cortex, and posterior cingulate cortex, in a sample of middle-aged and older adults (Mosconi et al., 2014). This relationship remained significant when adjusting for gender, education, family history of Alzheimer's disease, APOE status, body mass index, insulin resistance scores, and hypertension presence. Adherence to a Mediterranean diet at baseline in a cohort of 70-year-olds, particularly the low meat consumption component, was related to larger total brain volume at a 5-year follow-up assessment, even after controlling for gender, energy intake, education, physical activity, cholesterol levels, body mass index, and systolic blood pressure (Titova et al., 2013). In a recent diffusion tensor imaging study, Pelletier

et al. (2015) found that Mediterranean diet adherence among older adults at baseline predicted white matter microstructure preservation an average of 9 years into the future. Of note, those in the top quintile of tissue integrity displayed the equivalent of a 10 year delay in cognitive aging (Pelletier et al., 2015). The relation of specific features of the Mediterranean diet to brain health in old age has been investigated as well. As examples, greater intake of fish and long-chain omega-3 polyunsaturated fatty acids have related positively to white matter integrity and gray matter volume, and negatively to cerebrovascular disease burden, in both cross-sectional (Raji et al., 2014; Virtanen, Siscovick, Longstreth, Kuller, & Mozaffarian, 2008) and RCT designs (Witte et al., 2014).

A relatively small number of studies have evaluated the relationship between nutrition and brain function using task-based fMRI. In young adults, acute supplementation (i.e., for 3 days and up to 45 minutes prior to scanning) with nitrate—a vasodilator found in green leafy vegetables—in amounts equivalent to a large plate of salad, was found to contribute to a smaller and faster BOLD response in visual cortex during a visual stimulation paradigm relative to controls, suggesting improved neurovascular coupling (Aamand et al., 2013). Five days of supplementation with flavanol-rich cocoa in a young adult sample was associated with an increase in BOLD signal intensity in prefrontal cortex, parietal cortex, cerebellum, and anterior cingulate cortex when making rapid judgments about letters (e.g., vowel or consonant) and numbers (e.g., even or odd) according to changing rules (Francis, Head, Morris, & Macdonald, 2006). The authors interpreted this finding to reflect the potential of dietary components such as flavanol to exert beneficial effects on neural functioning by

increasing cerebral blood flow to gray matter (Francis et al., 2006). A similar increase in BOLD signal was observed in dorsolateral prefrontal cortex during a working memory task in a young adult sample randomly assigned to receive either green tea extract or a placebo solution immediately prior to scanning (Borgwardt et al., 2012). By contrast, unhealthy dietary features administered to young adults immediately prior to fMRI acquisition, such as substances high in lipids or fructose, have been shown to reduce cerebral blood flow in a variety of brain regions, including thalamus, hippocampus, posterior cingulate cortex, fusiform gyrus, and visual cortex (Noseworthy, Alfonsi, & Bells, 2003; Page et al., 2013). Importantly, however, these studies either did not evaluate for potential cognitive effects (Aamand et al., 2013; Borgwardt et al., 2012; Noseworthy et al., 2003; Page et al., 2013) or did not observe significant changes in cognitive performance (Francis et al., 2006) related to the nutrition intervention.

Functional imaging has also been used to evaluate the relation between diet and brain function in older adult samples. Relative to controls placed on a two day low nitrate diet (consisting primarily of grains, meats, and dairy products), older adults in a high nitrate diet condition containing mostly fruits, leafy green vegetables, and beet juice for two days demonstrated increased regional cerebral perfusion in frontal lobe white matter, particularly in connections between dorsolateral prefrontal and anterior cingulate cortices (Presley et al., 2011). Although potential changes in cognition were not evaluated in this study, the authors noted that deep frontal lobe white matter is particularly at risk for age-related deterioration with associated loss in executive functions and other cognitive domains (e.g., Madden, Bennett, & Song, 2009). Increased blood flow to these regions was speculated to prevent such deterioration. In a sample of 32 older adults with age-

associated memory impairments randomly assigned to receive either antioxidant-rich pomegranate juice or a flavor-matched placebo solution on a daily basis for 4 weeks, increased activation was found in basal ganglia, thalamus, inferior frontal gyrus, middle frontal gyrus, occipital lobe, and fusiform gyrus during learning and memory performance in the experimental group relative to controls (Bookheimer et al., 2013). Compared to placebo, individuals in the pomegranate juice condition demonstrated significant behavioral improvements on a verbal memory task following the intervention (Cohen's $d = 0.90$). Brickman et al. (2014) administered either a low or high cocoa flavanol diet to a sample of 50- to 69-year-olds for 3 months using an RCT design. During an fMRI-adapted object recognition task, enhanced cerebral blood volume was observed in the dentate gyrus of the hippocampal formation post-intervention in the experimental group compared to controls (Brickman et al., 2014). A significant improvement in task-related reaction time was found in the high-flavanol group (Cohen's $d = 0.82$) though changes in memory retention were negligible (Cohen's $d = 0.06$). Most recently, a randomized, double-blind, placebo-controlled study demonstrated that 24 weeks of fish oil supplementation increases BOLD signal in the posterior cingulate cortex during working memory performance in older adults with subjective memory impairments (Boespflug, McNamara, Eliassen, Schidler, & Krikorian, 2016). Of note, this intervention was associated with a large positive behavioral effect on an *n*-back task for the 2-back condition (Cohen's $d = 0.94$) and 2-back performance significantly correlated with the observed changes in cingulate activation ($\beta = 0.35$).

Even fewer studies have been conducted to evaluate the extent to which diet impacts functional connectivity using resting state fMRI approaches, and to the author's

knowledge, no studies have been conducted in a purely older adult sample. In a relatively small group of young adults ($N$ = 12), Schmidt et al. (2014) employed a double-blind, counterbalanced, within-subject design to investigate the effects of green tea extract, administered immediately prior to scanning, on parieto-frontal connectivity during working memory performance. Relative to a placebo substance, green tea extract was found to enhance connectivity between right superior parietal lobule and middle frontal gyrus, which in turn correlated with improved performance on an *n*-back task ($r$ = 0.64). In addition, green tea extract enhanced task performance compared to the control condition (Cohen's $d$ = 1.23). By contrast, reduced intrinsic activity in hippocampus and inferior parietal cortex was observed in healthy young adults subject to 12 weeks of a saturated fatty acid-enriched diet relative to a monounsaturated fatty acid-enriched diet comparison group (Sartorius et al., 2013). Potential changes in cognitive performance were not examined in this study. Together, these findings suggest that not only do healthful diets have potential to improve functional connectivity but unhealthful diets may diminish connectivity.

More indirectly, obesity and insulin sensitivity have been related to abnormal functional connectivity in a variety of resting state networks in young adults, including the default mode and temporal networks (Kullman et al., 2012). Relative to healthy controls, late middle-aged adults with type 2 diabetes mellitus show weaker correlations among seed regions within the DMN, including posterior cingulate, middle temporal gyrus, inferior and medial frontal gyri, and thalamus (Musen et al., 2012). Given that obesity and type 2 diabetes mellitus are often associated with a poor diet, these findings

provide indirect evidence of a negative relation between unhealthful eating and intrinsic brain connectivity.

In summary, findings based on structural and functional MRI, including both task-dependent and resting state techniques, suggest that nutrition influences brain health in a variety of ways. However, many of these studies have been correlational in nature, which limits conclusions regarding directionality of observed relationships and the influence of uncontrolled extraneous variables. For example, it is plausible that individuals with cognitive impairments, and underlying neural dysfunction, are more likely to engage in unhealthy eating. While several RCTs have been employed in conjunction with task-based fMRI, many of these studies have considered only acute effects, with the substance of interest being administered for only a few days or even minutes prior to scanning. It is likely that the long and acute term impact of diet relate to brain function in different ways (Gomez-Pinilla, 2008). Only three RCTs have been conducted in older adult samples using task-based fMRI, which have ranged in supplementation duration from 1 to 6 months, and none have been conducted with L and Z. With respect to resting state fMRI, the available literature is even sparser. Although preliminary evidence suggests a relation between diet and functional connectivity, no studies have been conducted in older adult populations and the potential influence of L and Z on intrinsic brain activity remains completely unexplored.

## Present Study

The present study sought to expand the relatively limited but growing research base on the relation between diet and neurocognitive functioning in old age. FMRI techniques, including both task-based and resting state methodologies, were used in the

context of a double-blind, RCT to investigate neural mechanisms underlying the relation of L and Z to cognition that has recently emerged in the literature. To the author's knowledge, only one preliminary RCT (Johnson et al., 2008) investigating the effect of L, without Z, on cognition across 4 months of supplementation has been conducted, as described previously. In addition, only a single task-based fMRI study (Lindbergh et al., 2016), which was cross-sectional in nature, has evaluated the relation of L and Z to brain function in humans *in vivo*. While findings from these studies and others have been encouraging, the present RCT significantly extends the available literature by evaluating changes in brain and cognitive function in a sample of community-dwelling older adults randomly assigned to receive either L and Z supplementation or placebo across a period of 12 months.

This RCT had three major aims. The first was to characterize neural changes associated with L and Z consumption, if any, using task-based fMRI. A verbal learning and memory paradigm was employed in which participants were asked to learn and recall word pairs (Bookheimer et al., 2000). Within the framework of the STAC-r (Reuter-Lorenz & Park, 2014), L and Z were expected to function as "neural resource enrichment" due to their antioxidant and anti-inflammatory properties, as well as their positive effects on cellular membranes and communication, ultimately countering the effects of neurophysiological decline and reducing the associated need for compensatory scaffolding. Accordingly, it was expected that older adults receiving L and Z supplementation would evidence increased neurobiological efficiency during learning and recall as evidenced by reduced neural activity required to meet task demands relative to the control group. This hypothesis is consistent with the aforementioned line of

research demonstrating that older adults with less neuropathological burden tend to display greater neural efficiency and require less compensatory recruitment during cognitive performance relative to peers with higher levels of age-related deterioration (e.g., Duverne et al., 2008; Duzel et al., 2011; Morcom et al., 2007; Stevens et al., 2008; Tyler et al., 2010). It is further in agreement with cognitive intervention studies demonstrating that older adults display more "youthful" patterns of brain activation post-treatment relative to controls (e.g., Heinzel et al., 2014; Meinzer et al., 2013).

Based on regions-of-interest with demonstrated involvement in verbal learning and memory tasks in similar studies (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Cabeza & Nyberg, 2000; Clement & Belleville, 2009), the anticipated effects during learning trials were expected to predominate in medial temporal lobe, supramarginal and angular gyri, precuneus, dorsolateral and ventrolateral prefrontal cortex, anterior and posterior cingulate gyrus, Broca's area, cerebellum, and premotor areas. This activation pattern was hypothesized to be primarily left-lateralized given the language-based nature of encoding words pairs. A similar network of brain regions was expected to show effects during recall trials, though anterior prefrontal cortex and medial parieto-occipital regions (retrosplenial cortex and cuneus) were expected to evidence greater involvement given somewhat different processes involved in retrieval versus initial storage of information (Cabeza & Nyberg, 2000).

A second aim of the study was to replicate and extend previous findings showing a relation of L and Z to cognition in older adults. While a growing number of studies have demonstrated a positive correlation between L and Z levels and various aspects of cognitive function (e.g., Feeney et al., 2013; Renzi et al., 2008; Vishwanathan,

Iannaccone et al., 2014), these studies have been largely cross-sectional in nature, preventing conclusions regarding directionality and the ability to rule out extraneous factors that may be contributing to the observed relationship. The present study constitutes only the second RCT on this topic and has the advantage of including a prolonged, 1-year period of supplementation, thus enabling conclusions regarding longer-term cognitive effects. Consistent with the bulk of the available literature, L and Z supplementation was expected to benefit overt learning and recall performance on the fMRI-adapted verbal memory task relative to the placebo condition.

The third aim of the present study was to investigate functional connectivity changes associated with L and Z consumption using resting state fMRI. To the author's knowledge, this not only marks the first attempt to evaluate the relation, if any, of L and Z to intrinsic brain activity but also the first attempt to evaluate the relation between nutrition and brain health more broadly using resting state fMRI in an older adult sample. Based on findings that age-related neurodegenerative processes are related to altered functional connectivity both within and between resting state networks (Geerligs, Maurits et al., 2014; Geerligs, Renken et al., 2015), L and Z supplementation was expected to counter or buffer these changes, ultimately resulting in improved modularity (i.e., increased *intra*-network connectivity and reduced *inter*-network connectivity) relative to the control group. Given the well-established observation of changes in DMN activity associated with both healthy and pathological aging in the literature (Ferreira & Busatto, 2013; Garces et al., 2014), this network was the focus of our analyses.

# CHAPTER 2

# GENERAL METHOD

## Power Analysis

A power analysis was conducted to determine the sample size necessary to detect an effect if present. Although a behavioral effect of L and Z is included in the hypotheses, neuroimaging was the focus of the power analysis given that the primary aim of this study was to evaluate underlying neural mechanisms. In the absence of prior RCTs evaluating the relation of L and Z to brain function, the most similar studies available investigating the impact of nutritional interventions on fMRI signal in older adult samples were used to estimate an expected effect size (Boespflug et al., 2015; Bookheimer et al., 2013; Brickman et al., 2014). As noted previously, to the author's knowledge, researchers have yet to evaluate the neural effects of nutrition interventions in older adults using resting state fMRI and thus only task-based fMRI studies were considered.

Effect size metrics in regions-of-interest relevant to the present hypotheses were converted to Cohen's *d*, using the equation: $(M_1 - M_2) / s_{pooled}$, where $s_{pooled} = \sqrt{[s_1^2 (n_1 - 1) + s_2^2 (n_2 - 1)] / (n_1 + n_2)}$. The smallest calculated *d* value of 1.09 was selected for the power analysis, though it should be noted that the "large" magnitude of this effect may be positively biased due to its derivation from a limited sample of studies. In addition, data were unavailable for every region-of-interest in the present study, which may also contribute to effect size overestimation to the extent that non-analyzed brain regions in prior studies would have been associated with smaller effect sizes. Finally, the currently

available literature does not permit conclusions regarding expected effect sizes of nutrition interventions in aging populations for resting state fMRI.

With these caveats in mind, an aspirational power analysis was conducted in G*Power 3 using the following parameters: *t*-tests for independent means (two groups), *a priori* analysis, two-tailed, $\alpha = 0.05$, and power $(1 - \beta) = 0.90$ (Faul et al., 2007). Given that the group randomization ratio for the current study was not 1:1 (see below), the allocation ratio was set to 2. The power analysis indicated that a total sample size of 42 would be required to detect an effect at the desired level of power, with 14 individuals in the control group and 28 individuals in the experimental group.

## Participants

75 community-dwelling older adults (64-99 years of age) were recruited via newspaper advertisements, flyers, and electronic media (e.g., listservs) as part of a larger intervention study evaluating the relation between L and Z and neurocognitive function. Exclusionary criteria included left-handedness, traumatic brain injury, macular degeneration, gastric conditions that may interfere with supplement absorption, corrected visual acuity worse than 20:40, MRI incompatibility, Geriatric Depression Scale (GDS) total score > 19, or evidence of neurological disorder. Of the 75 participants who were eligible for inclusion following an initial phone screening, more thorough medical history review revealed that seven participants were incompatible with the MRI environment. Three more participants became uncomfortable within the scanner and requested to stop due to claustrophobia ($n = 2$) or sensitivity to noise ($n = 1$). An additional participant was unable to remain awake. 16 volunteers elected not to complete the study for various reasons, leaving 48 participants (experimental $n = 34$; control $n = 14$) with usable data at

baseline and post-intervention for resting state analyses. With respect to task-dependent analyses, data for one volunteer were lost and three volunteers were obviously unable to grasp the task, yielding a sample size of 44 (experimental $n = 30$; control $n = 14$).

## Procedures

Data for the present study were derived from a single-site, double-blind, RCT evaluating the relation of L and Z to neurocognitive function in community-dwelling older adults. Eligible participants were randomly assigned using a 2:1 experimental to control group ratio to receive either a supplement containing 10 mg of L and 2 mg of Z or a physiologically inert placebo of identical appearance. Participants were instructed to consume one pill per day with a meal for a period of 1 year.

The study entailed 8 laboratory visits in total in addition to a physical examination with a collaborating health professional to verify appropriate health for the study, including supplementation history and presence of gastric conditions that may interfere with L and Z absorption. Following the physical examination, eligible participants completed three baseline visits occurring within a 2 week period, which included vision testing, cognitive testing, measurement of L and Z concentrations in macular pigment and serum, and acquisition of neuroimaging data. Participants were subsequently randomized to receive supplement or placebo. Bi-monthly contact was made to assess compliance, adverse events and concerns, and health changes that may render participants ineligible for ongoing participation. To additionally verify supplementation adherence, pill counts were conducted at 4 month, 8 month, and 12 month visits and participants were only provided with enough pills to last until their next visit. The 12 month assessment was

conducted across three visits that mirrored the baseline visits and consisted of the same measures. A breakdown of the study timeline is depicted in Table 3.1.

Participants were compensated with $300 for their time and effort with payments distributed across four time points (i.e., baseline, 4 months, 8 months, and 12 months). $100 was provided following completion of baseline visits and follow-up visits due to more extensive testing at these time points and $50 was provided at both 4 and 8 month visits. If participants required transportation to and from experimental sessions, $20 was provided to the collateral driver at each of the four time points.

The study was approved by the University of Georgia Institutional Review Board and the tenets of the Declaration of Helsinki were closely followed by all study personnel.

## Measures

As this study was part of a larger clinical trial, participants completed an extensive battery of behavioral and neuroimaging measures. The measures summarized below are restricted to aspects of the larger protocol that are relevant to the current analyses.

### Wechsler Test of Adult Reading

Premorbid intellectual functioning was estimated using the Wechsler Test of Adult Reading (WTAR), which takes approximately 5 minutes to administer and involves reading a list of 50 words with atypical grapheme to phoneme relationships (Wechsler, 2001). Word reading tests are often used for this purpose given high correlations with intelligence and stability in the face of cognitive decline resulting from brain injury or other neurodegenerative process (Wechsler, 2001; Willshire, Kinsella, & Prior, 1991).

The WTAR has been demonstrated as a valid and reliable measure, with test-retest correlations above 0.90 (e.g., Green et al., 2008; Whitney, Shepard, Mariner, Mossbarger, & Herman, 2010). Full-scale intelligence quotient (FSIQ) estimates were calculated using an algorithmic combination of WTAR performance (i.e., number of words correctly pronounced) and demographic (i.e., age, education, race, sex, and geographic region) variables (Wechsler, 2001).

**Geriatric Depression Scale**

The Geriatric Depression Scale (GDS) was used to screen out significant depressive symptomatology (Yesavage et al., 1983). The GDS is a self-report questionnaire consisting of 30 items with yes/no response options, yielding a total score ranging from 0 to 30. Higher scores correspond to greater levels of depression (0-9 = normal; 10-19 = mild; 20-30 = severe). Relative to depression measures constructed for younger adults, the GDS emphasizes cognitive and affective depressive symptoms while de-emphasizing somatic concerns that often accompany the aging process (e.g., sleep disturbances or sex drive changes; Smarr & Keefer, 2011). The GDS has been found to display good psychometric properties in older adult samples (Peach, Koob, & Kraus, 2001; Smarr & Keefer, 2011).

**Macular Pigment Optical Density**

Macular pigment optical density (MPOD) was measured using customized heterochromatic flicker photometry (cHFP), as described in detail elsewhere (e.g., Lindbergh et al., 2016; Stringham et al, 2008), to verify changes in L and Z status resulting from the intervention. Briefly, a macular densitometer (Macular Metrics; Rehoboth, MA) was used to present participants with a 1-deg visual stimulus that

consisted of two narrow-band LED-based light sources, peaking at 460 nm and 570 nm. The light sources were presented in square-wave, counter-phase orientation, which gave the appearance of flicker. Prior to measurement, each participant's critical flicker fusion frequency (CFF) was measured using only the mid-wave portion of the stimulus, so that the task could be customized to the individual viewer. Following determination of customized flicker sensitivity, participants were asked to fixate on a black dot displayed at the disk's core. The radiance of the lower (i.e., 460 nm) waveband was manipulated relative to the 570 nm component to assess the point at which flickering was no longer perceivable. This sequence was again conducted with a 2˚ target and fixation point at 7˚ nasally to allow a reference measurement in the parafovea (where MPOD approaches zero). The two loci (i.e., 30 minutes, derived from the 1-degree target, and 7˚ of retinal eccentricity) were then compared to provide the MPOD measurement at 30 minutes of retinal eccentricity.

## Neuroimaging

### Tasks

Participants engaged in a verbal learning task, conceptually derived from the Wechsler memory scale paired associates learning test (Wechsler, 2009) and similar to fMRI paradigms that have been published previously (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Braskie, Small, & Bookheimer, 2009). The basic goal of the task was to learn unrelated word pairs (e.g., "UP" and "FOOT"). E-Prime software (version 1.2, Psychology Software Tools, Inc., Pittsburgh, PA) was used to program and present the task in conjunction with MRI compatible goggles (Resonance Technology Inc., Northridge, CA). Participants provided responses using right and left index finger

buttons on Cedrus Lumina LU400 MRI compatible response pads (Cedrus, San Pedro, CA).

The task entailed 10 separate learning blocks, control blocks, retrieval blocks, and baseline fixation blocks (see Figure 3.1). During learning blocks, the first word of each pair was presented in isolation on the left side of the screen (1 second) followed by presentation of the second word on the right side of the screen, such that both words could be viewed side by side (2 seconds). There were 10 word pairs total, 5 of which were presented during each encoding block. Participants were told to respond with their right index finger whenever the second word in the pair appeared during learning trials, as a verification of attention. During retrieval trials, participants were presented with the first word in each pair (3 seconds) and asked to mentally recall the second word to avoid head motion, consistent with procedures used in analogous fMRI-adapted verbal learning paradigms (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013). Participants were instructed to make a right index finger press if they successfully retrieved the second word or a left index finger press to indicate unsuccessful retrieval (maximum score: 50). A control task interspersed learning and retrieval blocks, which mimicked the learning block except that Xs (i.e., "XXXXX") and Ys (i.e., "YYYYY") were presented in place of word pairs. Immediately following the scan, participants engaged in free recall (maximum score: 10) followed by cued recall (maximum score: 10) of the "second word" in each pair to assess task engagement within the scanner.

During resting state scans, participants were asked to remain awake and motionless while viewing a neutral screen through the MRI compatible goggles.

**Imaging Parameters**

Scans were acquired on a General Electric (GE; Waukesha, WI) 3 T Signa HDx MRI system. A high-resolution 3D T1-weighted fast spoiled gradient recall echo sequence was used to collect structural scans (TR = 7.5 ms; TE = < 5ms; FOV = 256 × 256 mm matrix; flip angle = 20°; slice thickness = 1.2 mm; 154 axial slices) with a total acquisition time of 6 minutes and 20 seconds. This protocol provided coverage from the top of the head to the brainstem and collected 176 images.

Task-dependent functional scans were aligned to each participant's AC-PC line and collected axially with a T2*-weighted single shot echo planar imaging (EPI) sequence (TR = 1500 ms; TE = 25 ms; 90° RF pulse; acquisition matrix = 64 × 64; FOV = 220 × 220 mm; in-plane resolution = 220/64 mm; slice thickness = 4 mm; 30 interleaved axial slices). Four dummy scans were acquired at the outset of the run and discarded. Total acquisition time was 12 minutes and 24 seconds. This EPI sequence consisted of 486 volumes and covered the cortical surface and a portion of the cerebellum.

Resting state functional scans were similarly aligned to each participant's AC-PC line, collected axially, and used a T2*-weighted single shot EPI sequence (TR = 5000 ms; TE = 25 ms; 90° RF pulse; acquisition matrix = 128 × 128; FOV = 220 × 220 mm; in-plane resolution = 220/128 mm; slice thickness = 2 mm; 60 interleaved axial slices). Acquisition time was 9 minutes and 25 seconds. 108 volumes were acquired that covered the cortical surface and a portion of the cerebellum.

Magnitude and phase images were also acquired, lasting 1 minute and 40 seconds each, for fieldmap-based unwarping (TR = 700 ms; TE = 5.0/7.2 ms; FOV = 220 × 220 mm matrix; flip angle = 30°; slice thickness = 2 mm; 60 interleaved slices).

## Data Analyses

### Task-Dependent

Functional neuroimaging data acquired during the verbal learning task were processed and analyzed using Statistical Parametric Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK). The dcm2nii conversion tool was used to convert data from GE DICOM to NIFTI format (Rorden, 2007). The preprocessing pipeline included slice time correction to address non-sequential, interleaved acquisition and realignment of functional images to the first volume of the functional run to adjust for head movement. To account for phase and magnitude variations during the scan, fieldmaps were used to realign and unwarp images. Co-registration of anatomical scans to the first image of the functional scan was followed by registration of anatomical and functional images to the Montreal Neurological Institute (MNI) template. The anatomical image was segmented to distinguish brain tissue (i.e., white and gray matter), cerebrospinal fluid, bone, non-brain soft tissue, and air. Deformation fields were applied to functional images to permit spatial normalization to MNI space. Finally, images were smoothed with a 6.75 mm FWHM Gaussian filter.

Following the above pre-processing steps, the General Linear Model (GLM; SPM12) was applied to create BOLD signal activation maps of encoding minus active baseline (i.e., the control task) and retrieval minus active baseline. A $p < .05$, family-wise-error (FWE) corrected statistical threshold with a minimum of eight contiguous

voxels was selected for analyses given optimal balance between Type I and II errors (Lazar, 2008).

Regions-of-interests (ROIs) were defined using the WFU PickAtlas version 3.0.5 (Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Burdette, & Kraft, 2003). Consistent with prior studies (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Cabeza & Nyberg, 2000; Clement & Belleville, 2009), ROIs during learning trials included medial temporal lobe, supramarginal and angular gyri, precuneus, dorsolateral and ventrolateral prefrontal cortex, anterior and posterior cingulate gyrus, Broca's area, cerebellum, and premotor areas. Recall trials consisted of these ROIs as well as anterior prefrontal cortex, retrosplenial cortex, and cuneus given somewhat different processes involved in retrieval versus initial storage of information (Cabeza & Nyberg, 2000).

To assess the anticipated effect of L and Z supplementation on brain activity, 2×2 flexible factorial models were used in second level analyses with group (control vs. experimental) as a between-subjects factor and time (baseline vs. post-intervention) as a within-subjects factor. A significant group × time interaction within the aforementioned ROIs would be consistent with the expectation that L and Z intake across a 1 year period alters neural activation during cognitive performance relative to placebo. Planned comparisons were used to more specifically characterize a significant interaction in terms of directionality (i.e., increases versus decreases in BOLD signal) in the supplemented and control groups following the intervention. All second level analyses were conducted separately for encoding trials and retrieval trials given non-identical neural underpinnings of these two cognitive processes (Cabeza & Nyberg, 2000).

Behavioral performance on the learning and memory paradigm was also assessed to help verify task engagement and evaluate for possible intervention effects. Within-scanner self-reported recall of the second word in each pair on the last two recall blocks (maximum score = 10), when learning is expected to be maximal, was calculated. These values were then correlated with actual recall immediately following the scan to evaluate performance validity. To determine whether L and Z exerted a positive effect on cognition relative to placebo, behavioral performance was also evaluated in the context of a 2×2 mixed design ANOVA to investigate the expected group (control vs. experimental) by time (baseline vs. post-intervention) interaction.

**Resting State**

To overcome issues recognized in the literature when using the traditional GLM-based subtraction approach to measure functional connectivity, such as obscuration of spatially overlapping regions and networks that serve meaningfully different functions (e.g., Logothetis, 2008), a novel computational framework involving sparse representations of whole-brain fMRI signals was employed (Lv et al., 2015a). At its core, this computational methodology, referred to as the Holistic Atlases of Functional Networks and Interactions (HAFNI) approach, involves aggregating hundreds of thousands of fMRI signals from the whole brain of each subject into a big data matrix (Lv et al., 2015b). The big data matrix is then factorized into an over-complete dictionary basis matrix (*D*) and a reference matrix (*A*) using a dictionary learning algorithm (Mairal, Bach, Ponce, & Sapiro, 2010). Over-complete dictionary basis matrix time series correspond to BOLD activities of a given brain network while its associated reference weight vector corresponds to the spatial map of the brain network. In this way, the spatial

overlap and interaction behavior of brain networks can be revealed by the decomposed reference weight matrix (Lv et al., 2015b), thus taking into consideration that a given brain region may serve multiple functional processes (Lv et al., 2015c) and its BOLD signal may include different components (Varoquaux, Gramfort, Pedregosa, Michel, & Thirion, 2011).

This data-driven strategy has yielded at least 32 spatially distributed and overlapping HAFNI components, including both task-evoked and resting state networks, which have been robustly reproduced across individuals using high-quality data from the Human Connectome Project (Lv et al., 2015b). Sparse-dictionary learning has demonstrated sensitivity to resting state changes associated with mild traumatic brain injury (Lv et al., 2016) and has been found to more closely correlate with DMN disruptions in older adults with mild cognitive impairment and Alzheimer's disease relative to traditional approaches (i.e., independent component analysis and seed-based approaches; Lee et al., 2016). Another advantage of the HAFNI approach is that it offers a very strict Type I error control rate relative to traditional fMRI analyses by reducing coefficients that are not consistently different between groups to zero (Lv et al., 2015c).

Sparse coding methodology was recently adapted (Lv et al., 2016) to permit group-wise, longitudinal analyses of changes in resting state networks and network-level interactions. Based on this framework and previous HAFNI publications (e.g., Lv et al., 2015c), pre-processing of resting state data for the present study followed a similar pipeline as described above in detail for task-based data, including skull removal, motion correction, slice time correction, spatial smoothing, and global drift removal. Pre-

processed volumes were then registered to MNI space and voxel signals from every subject were standardized with a mean of zero and a standard deviation of one.

Temporal concatenated sparse coding methodology (Lv et al., 2016) was implemented to extract whole-brain fMRI signals of each participant for aggregation into a 2D (voxel number × time series points) signal matrix ($S_x$). Signal matrices for individual participants were concatenated by group (i.e., control and supplement) and time point (i.e., baseline and post-intervention) resulting in four big matrices containing: (1) control group at baseline, (2) supplemented group at baseline, (3) control group post-intervention, and (4) supplemented group post-intervention. Dictionary learning and sparse coding (Mairal et al., 2010) was employed to factorize these big matrices into a time series signal dictionary matrix ($D$) and a coefficient matrix ($A$), which conserves the spatial voxel organization of $S_x$. In essence, the dictionary learning and sparse coding technique employs machine learning principles to algorithmically summarize and organize the hundreds of thousands of fMRI signals derived from the whole brains of individual participants, ultimately culling the data to permit efficient and meaningful downstream analyses (Mairal et al., 2010). This is conceptually similar to the function of a language dictionary in summarizing and organizing the meaning of words according to their core elements in a streamlined manner by focusing on reliably defining features.

Based on previous literature (e.g., Iraji et al., 2014; Lv et al., 2015a; Lv et al., 2015b; Lv et al., 2016), a range of potential dictionary sizes (20, 50, 100, and 500) were considered for our study sample. Consistent with procedures used elsewhere (e.g., Lee et al., 2016), the number of dictionary atoms that characterized DMN in a manner most consistent with the bulk of the neuroscience literature was selected for final analyses.

Because the dictionary learning and sparse coding process maintains the spatial organization of $S_x$ as well as information regarding group membership (control vs. supplement) and time point (i.e., baseline vs. post-intervention), it is possible to make group-wise level statistical comparisons. The hypothesis that L and Z supplementation would improve DMN modularity (i.e., reduce *inter*-network connectivity and increase *intra*-network connectivity) was tested in two ways. First, *inter*-network connectivity was evaluated by calculating interaction matrices for each participant comprised of correlations of DMN dictionary atoms to other learned resting state networks. Consistent with procedures specified by Lv et al. (2016), *t*-tests were then conducted to determine if network interactions differed across time points by group. More specifically, paired-sample *t*-tests were used to evaluate whether *inter*-network activity decreased in the supplemented group from baseline to post-intervention, as expected, while remaining generally constant in the placebo group, also as expected.

Second, the effects of L and Z supplementation on *intra*-network connectivity was tested by creating maps of activated voxels within DMN for the placebo and supplemented groups at baseline and post-intervention. Consistent with methods developed by Zhao et al. (2016), overlap rate similarity (ORS) was calculated between DMN and a well-established DMN template provided in the neuroscience literature (Smith et al., 2009). Paired-sample *t*-tests were used to determine whether ORS values changed, on average, from baseline to post-intervention. The supplemented group was expected to show a significant increase in DMN correspondence, indicative of improved *intra*-network connectivity, while the placebo group was expected to evidence comparable correspondence (i.e., no change) across the two time points.

# CHAPTER 3

# LUTEIN AND ZEAXANTHIN INFLUENCE BRAIN FUNCTION IN OLDER ADULTS: A RANDOMIZED CONTROLLED TRIAL[1]

[1] Lindbergh, C. A., Renzi-Hammond, L. M., Hammond, B. R., Terry, D. P., Mewborn, C. M., Puente, A. N., & Miller, L. S. Submitted to the *Journal of the International Neuropsychological Society*, 12/17/16.

## Abstract

The present study constitutes the first randomized controlled trial to investigate the relation of lutein (L) and zeaxanthin (Z) to brain function using functional magnetic resonance imaging (fMRI). It was hypothesized that L and Z supplementation in older adults would enhance neural efficiency (i.e., reduce activation) and cognitive performance on a verbal learning task relative to placebo. 44 community-dwelling older adults (mean age = 72 years) were randomly assigned to receive either placebo or L+Z supplementation (12 mg/daily) for one year. Neurocognitive performance was assessed at baseline and post-intervention on an fMRI-adapted task involving learning and recalling word pairs. Imaging contrasts of blood-oxygen-level-dependent (BOLD) signal were created by subtracting active control trials from learning and recall trials. A flexible factorial model was employed to investigate the expected group (placebo vs. supplement) by time (baseline vs. post-intervention) interaction in pre-specified regions-of-interest. L and Z appeared to buffer cognitive decline on the verbal learning task (Cohen's $d$ = .84). Significant interactions during learning were observed in left dorsolateral prefrontal cortex and anterior cingulate cortex ($p < .05$, family-wise-error corrected). However, these effects were in the direction of increased rather than decreased BOLD signal. Although the omnibus interaction was not significant during recall, within-group contrasts revealed significant increases in left prefrontal activation in the supplement group only. Taken together, the results suggest that L and Z supplementation benefits neurocognitive function by enhancing cerebral perfusion, even if consumed for a discrete period of time in late life.

## Introduction and Literature Review

The xanthophyll lutein (L) and its isomer zeaxanthin (Z) are among over 600 organic pigments within the carotenoid family (Britton, Liaaen-Jensen, & Pfander, 2004; Khachik, Beecher, Goli, & Lusby, 1991). L and Z are not produced endogenously and thus must be consumed through green vegetables, colored fruits, and other aspects of diet (Hammond et al., 1997; Malinow, Feeney-Burns, Peterson, Klein, & Neuringer, 1980). By and large, L and Z are the only carotenoids capable of traversing the blood-retina barrier (Bone, Landrum, & Tarsis, 1985). They accumulate in several regions of the eye (e.g., Bernstein et al., 2001) but are taken up most selectively in the central region of the retina, referred to as the macula (Bone et al., 1985). The importance of L and Z to eye health is well-established, particularly in protecting against age-related macular degeneration (Ma et al., 2012; SanGiovanni & Neuringer, 2012).

L and Z also accumulate preferentially in human brain tissue, including the cerebellum and pons, as well as frontal, occipital, and temporal lobes (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Johnson et al., 2013). Despite underrepresentation in diet relative to other carotenoids (Johnson, 2014), L and Z, together with other xanthophylls (e.g., ß-cryptoxanthin), account for two-thirds or more of total brain carotenoid concentrations (Craft et al., 2004).

Most studies determine L and Z levels by measuring blood serum concentrations or by evaluating the optical density of the macular pigment layer (MPOD) using non-invasive heterochromatic flicker photometry (Hammond, Wooten, & Smollon, 2005). Although both techniques have been widely validated, serum levels tend to be influenced by recent food consumption while MPOD reflects longer-term dietary habits and lifestyle

eating behaviors (Hammond et al., 1997). MPOD correlates with postmortem brain tissue concentrations in both primates and humans, and is generally considered the most reliable proxy for neural levels (Hammond et al., 2005; Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013; Vishwanathan, Schalch, & Johnson, 2016).

Several studies in humans and animals have shown a relationship between dietary intake of L and Z and MPOD. Rhesus monkeys raised on xanthophyll-free diets exhibit absent or severely depleted macular pigmentation (Malinow et al., 1980). In older women ($N$ = 1,698), self-reported dietary intake of L and Z positively correlated with MPOD, even after adjusting for various medical and lifestyle factors (Mares et al., 2006). Adults who augmented their diets with 60 grams of spinach daily for 15 weeks showed a 19% increase in MPOD on average (Hammond et al., 1997). L and Z supplements also significantly increase MPOD in both young and older adults (e.g., Landrum et al., 1997; Murray et al., 2013; Stringham & Hammond, 2008; Weigert et al., 2011). For example, Stringham and Hammond (2008) found 6 months of L and Z supplementation (12 mg/daily) to significantly increase MPOD in healthy adults (Cohen's $d$ = 0.97) and improve visual performance under glaring light conditions. Several other studies have similarly reported improvements in visual function resulting from L and Z supplementation (e.g., Kvansakul et al., 2006; Ma et al., 2009; Yagi et al., 2009), suggesting functional benefits of enhancing MPOD.

An emerging literature also suggests a relation of dietary intake of L and Z to cognition in older adults (for reviews, see: Erdman et al., 2015; Johnson, 2012; Johnson, 2014; Maci, Fonseca, & Zhu, 2016). For example, a carotenoid-rich dietary pattern in late middle age was associated with better episodic memory, verbal fluency, working

memory, and executive functioning 13 years into the future (Kesse-Guyot et al., 2014). Similarly, greater intake of vegetables containing L and Z has been longitudinally associated with slower rates of cognitive decline in older adults (Kang, Ascherio, Grodstein, 2005; Lee, Kim, & Back, 2009; Morris, Evans, Tangney, Bienias, & Wilson, 2006). L and/or Z concentrations in serum have been found to positively correlate with cognitive functions in older adults, including global cognition, delayed recall, verbal fluency, executive functions, sustained attention, psychomotor speed, and learning (Akbaraly, Faure, Gourlet, Favier, & Berr, 2007; Johnson et al., 2013; Kelly et al., 2015). Greater serum L is also associated with reduced risk for dementia and Alzheimer's disease mortality in older adults (Feart et al., 2015; Min & Min, 2014). MPOD positively correlates with cognitive functions in older adults, including visual learning, language skills, attention, visuospatial and constructional abilities, verbal learning and recall, prospective memory, executive functions, verbal fluency, processing speed, task accuracy, perceptual speed, and global cognition (Feeney et al., 2013; Kelly et al., 2015; Renzi, Dengler, Puente, Miller, & Hammond, 2014; Renzi, Iannacocone, Johnson, & Kritchevsky, 2008; Vishwanathan, Iannaccone et al., 2014). It is further noteworthy that Alzheimer's disease patients have lower L and Z levels relative to age-matched controls, as measured via serum and MPOD (Nolan et al., 2014).

Although studies investigating the correlation between L and Z and cognition typically control for potential confounds, several reviews have called for randomized controlled trials (RCTs) to determine whether low carotenoid levels contribute to cognitive loss or perhaps represent a consequence, either through neuropathological processes or poor nutritional decisions (Erdman et al., 2015; Johnson, 2012; Johnson,

2014). To the authors' knowledge, only one such RCT exists (Johnson et al., 2008) in a sample of older women randomly assigned to receive 4 months of daily DHA (a polyunsaturated fatty acid; $n = 14$), L ($n = 11$), combined supplementation ($n = 14$), or placebo ($n = 14$). The group receiving L showed significant gains in verbal fluency relative to placebo at 4 months ($d = 0.61$). The combined (DHA + L) group evidenced improvements in verbal fluency ($d = 0.90$), learning ($d = 0.70$), and memory ($d = 0.58$) at 4 months.

The means by which L and Z influence brain function is an understudied area (Zamroziewicz & Barbey, 2016). Several mechanisms have been proposed, largely based on how these carotenoids preserve neural health in the retina (Erdman et al., 2015). Perhaps most notably, L and Z have strong antioxidant and anti-inflammatory properties, which may counter the deleterious effects of oxidative stress and neuroinflammation in the context of age-related cognitive decline and dementia (Bokov, Chaudhuri, & Richardson, 2004; Butterfield, Bader Lange, & Sultana, 2010; Heneka et al., 2015; Rosano et al., 2012). Given that aging contributes to adverse changes in neuronal membrane structure and function (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002), L and Z may also benefit neurocognitive function through their positive effects on membrane fluidity, permeability, stability, thickness, and ion exchange (Erdman et al., 2015; Krinsky et al., 2004; Widomska & Subczynski, 2014). Relatedly, carotenoids such as L and Z may improve cellular communication by altering gene expression (e.g., connexin 43; Bertram, 1999) and enhancing inter-neuronal signaling at gap junctions (Stahl & Sies, 2001).

The present study was a 12-month RCT that sought to expand the research base on neural mechanisms underlying the relation of L and Z to cognition in community-dwelling older adults using functional magnetic resonance imaging (fMRI). Cross-sectional results from the study sample at baseline (Lindbergh et al., 2016) revealed a significant and negative relationship of pre-intervention L and Z levels (measured via serum and MPOD) to blood-oxygen-level-dependent (BOLD) signal in several brain regions during an fMRI-adapted verbal learning task. Lindbergh et al. (2016) interpreted the observed results to reflect a potential role of L and Z in promoting neural efficiency (the neural efficiency hypothesis; Renzi & Hammond, 2010) during cognitive performance, particularly in brain regions with established risk for age-related degeneration including left inferior frontal gyrus, insula, middle temporal gyrus, middle frontal gyrus, and central and parietal operculum. This interpretation was based in part on the revised Scaffolding Theory of Aging and Cognition (STAC-r), which emphasizes "life-course" variables, including nutrition, in shaping the structure and function of the aging brain (Reuter-Lorenz & Park, 2014). More specifically, Lindbergh et al. (2016) speculated that L and Z buffer age-related neuropathological processes and thus reduce the associated need for compensatory neural recruitment in response to cognitive challenge (Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2014). Although L and Z are not specifically mentioned in the STAC-r, this would be consistent with the more general construct of "neural resource enrichment" discussed by Reuter-Lorenz and Park (2014, p. 361), which encompasses various nutritional and lifestyle factors. As with most research on this topic, however, Lindbergh et al.'s (2016) conclusions were limited by the cross-sectional nature of the analyses. For example, endogenous L and Z levels may

simply proxy other aspects of a healthful diet or lifestyle (e.g., exercise; Bherer, Erickson, & Liu-Ambrose, 2013) that promote brain health in old age.

The RCT design of the current investigation addressed limitations of cross-sectional analyses and permitted evaluation of L and Z's effects on the aging brain relative to placebo. An fMRI-adapted verbal learning and memory paradigm was employed in which participants were asked to learn and recall word pairs. Within the framework of the STAC-r (Reuter-Lorenz & Park, 2014), L and Z were expected to function as "neural resource enrichment," ultimately countering the effects of neurophysiological decline and reducing the need for compensatory scaffolding. Accordingly, it was expected that older adults receiving L and Z supplementation would exhibit increased neurobiological efficiency as evidenced by reduced neural activity (i.e., BOLD signal) required to meet task demands relative to controls. Based on regions-of-interest (ROIs) identified through prior literature (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Cabeza & Nyberg, 2000; Clement & Belleville, 2009), the anticipated effects during verbal learning were expected to predominate in medial temporal lobe, supramarginal and angular gyri, precuneus, dorsolateral and ventrolateral prefrontal cortex, anterior and posterior cingulate gyrus, Broca's area, cerebellum, and premotor areas. A similar network of brain regions was expected to show effects during verbal recall with the addition of anterior prefrontal cortex and medial parieto-occipital regions (retrosplenial cortex and cuneus) given somewhat different processes involved in retrieval versus encoding (Cabeza & Nyberg, 2000).

A secondary aim was to replicate and extend previous findings showing a positive relation of L and Z to cognition in older adults. Consistent with the bulk of the

available literature, it was hypothesized that L and Z supplementation would benefit performance on the fMRI-adapted verbal learning task in terms of number of words successfully recalled relative to placebo.

## Method

### Participants

75 community-dwelling older adults (64-86 years of age) were recruited via newspaper advertisements, flyers, and electronic media (e.g., listservs) as part of a larger intervention study. Exclusionary criteria included left-handedness, traumatic brain injury, macular degeneration, gastric conditions that may interfere with supplement absorption, corrected visual acuity worse than 20:40, MRI incompatibility, Geriatric Depression Scale (GDS) total score > 19, or neurological disorder. Of the 75 participants eligible for inclusion following an initial phone screening, more thorough medical history review revealed that seven participants were incompatible with the MRI environment. Three more participants became uncomfortable within the scanner and requested to stop due to claustrophobia ($n$ = 2) or sensitivity to noise ($n$ = 1). An additional participant was unable to remain awake. Sixteen volunteers elected not to complete the study for various reasons and behavioral data for one individual were lost due to technical malfunction. Three volunteers were obviously unable to grasp the fMRI task, yielding a final sample size of 44 (experimental $n$ = 30; control $n$ = 14) for analyses.

### Procedures

A single-site, double-blind, RCT design was employed. Eligible participants were assigned using a 2:1 experimental to control group ratio to receive either a supplement containing L (10 mg) and Z (2 mg) or a physiologically inert placebo of identical

appearance provided by DSM Nutritional Products (Basel, Switzerland). Randomization was performed by generating a set of numerical codes corresponding to either the active supplement or the placebo. These codes were placed in an opaque envelope and drawn for each participant by the study coordinator, who did not have any data collection responsibilities. Participants were instructed to consume one pill per day with a meal for one year. As noted previously, baseline (cross-sectional) fMRI findings from this study evaluating the relation of naturally circulating L and Z levels to neural activity, prior to supplementation, have been published elsewhere (Lindbergh et al., 2016). Lindbergh et al. (2016) used a largely overlapping though not completely identical subset of the sample in final analyses due to somewhat different variables of interest (see Lindbergh et al., 2016).

The RCT entailed eight laboratory visits in addition to a physical examination with a health professional to verify appropriate health for the study (see Table 3.1). Eligible participants completed three baseline sessions occurring within a two-week period, including vision testing, cognitive testing, measurement of L and Z concentrations in macular pigment, and acquisition of neuroimaging data. Bi-monthly contact assessed compliance, adverse events, and any health changes that could render participants ineligible for participation. Pill counts were conducted at 4 month, 8 month, and 12 month visits and participants were only provided with enough pills to last until their next visit. The 12 month (post-intervention) visit was completed across three sessions that mirrored the baseline visit.

Participants were compensated with $300 distributed across four time points (i.e., baseline, 4 months, 8 months, and 12 months). If participants required transportation to experimental sessions, $20 was provided to the collateral driver at each time point.

The study was approved by the University of Georgia Institutional Review Board and the tenets of the Declaration of Helsinki were closely followed by all study personnel.

## Measures

**Wechsler Test of Adult Reading**. Premorbid intellectual functioning was estimated using the Wechsler Test of Adult Reading (WTAR), which involves reading a list of 50 words with atypical grapheme to phoneme relationships (Wechsler, 2001). Full-scale intelligence quotient estimates were calculated using an algorithmic combination of WTAR performance (i.e., number of words correctly pronounced) and demographic (i.e., age, education, race, sex, and geographic region) variables (Wechsler, 2001).

**GDS**. The GDS was used to screen for significant depressive symptomatology (Yesavage et al., 1983). The GDS is a self-report questionnaire consisting of 30 items with yes/no response options, yielding a total score ranging from 0 to 30 (0–9 = normal; 10–19 = mild; 20–30 = severe).

**MPOD**. L and Z accumulate preferentially in the central area of the retina, referred to as the macula (Bone et al., 1985). Pigment within the human macula consists of L and Z embedded in retinal tissue (Bone et al., 1985). The density of the macular pigment layer (MPOD) is frequently used to index L and Z levels within the central nervous system (e.g., Vishwanathan et al., 2016) and can be measured noninvasively using customized heterochromatic flicker photometry (cHFP), a widely validated

technique (Stringham et al, 2008). The reader is referred to our published baseline findings for a detailed description of the cHFP procedure (Lindbergh et al., 2016), though it is important to note that Lindbergh et al. (2016) used MPOD as a *predictor* (i.e., independent variable) in the fMRI analyses. By contrast, in the present analyses, MPOD data are presented only as a *validity check* on our intervention to verify that our supplementation effectively increased L and Z status over the course of the study. Briefly, cHFP involved presenting a 1-deg visual stimulus consisting of two narrow-band light sources, peaking at 460 nm and 570 nm, using a macular densitometer (Macular Metrics; Rehoboth, MA). After determining customized flicker sensitivities, the lower (i.e., 460 nm) waveband radiance was manipulated relative to the 570 nm waveband to measure the point at which flickering could no longer be perceived. This sequence was conducted again with a 2˚ target and fixation point at 7˚ nasally to provide a parafoveal reference measurement where MPOD approaches zero. The two loci were then contrasted for an MPOD measurement at 30 minutes of retinal eccentricity.

**Neuroimaging**

**FMRI task**. Participants engaged in a verbal learning task, conceptually derived from the Wechsler memory scale paired associates learning test (Wechsler, 2009), similar to previously published fMRI paradigms (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Braskie, Small, & Bookheimer, 2009), and described in detail elsewhere (Lindbergh et al., 2016). The basic goal of the task was to learn unrelated word pairs (e.g., “UP” and “FOOT”). E-Prime software (version 1.2, Psychology Software Tools, Inc., Pittsburgh, PA) was used to program and present the task in conjunction with MRI compatible goggles (Resonance Technology Inc., Northridge, CA). Participants provided

responses using right and left index finger buttons on Cedrus Lumina LU400 MRI compatible response pads (Cedrus, San Pedro, CA).

The task entailed 10 each of learning blocks, control blocks, retrieval blocks, and baseline fixation blocks (see Figure 3.1). During learning blocks, the first word of each pair was displayed in isolation on the left side of the screen (1 second) followed by presentation of the second word on the right side, such that both words could be viewed side by side (2 seconds). There were 10 word pairs total with 5 pairs presented during each encoding block. During retrieval trials, participants were presented with the first word in each pair (3 seconds) and asked to mentally recall the second word to prevent head motion, consistent with procedures used in analogous fMRI-adapted verbal learning tasks (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013). Participants were instructed to make a right index finger press if they successfully retrieved the second word or a left index finger press to indicate unsuccessful retrieval. A control task interspersed learning and retrieval blocks, which mimicked the learning block except that "XXXXX" and "YYYYY" were presented in place of word pairs. Immediately post scan, participants engaged in cued recall of the "second word" in each pair (maximum score: 10) to help assess task engagement within the scanner.

**MRI acquisition.** Scans were acquired on a General Electric (GE; Waukesha, WI) 3 T Signa HDx MRI system. A high-resolution 3D T1-weighted fast spoiled gradient recall echo sequence was used to collect structural scans (TR = 7.5 ms; TE = < 5ms; FOV = 256 × 256 mm matrix; flip angle = 20°; slice thickness = 1.2 mm; 154 axial slices) with a total acquisition time of 6 minutes and 20 seconds. This protocol collected 176 images, providing coverage from the brainstem to the top of the head.

Functional scans were aligned to each participant's AC-PC line and acquired axially with a T2*-weighted single shot echo planar imaging (EPI) sequence (TR = 1500 ms; TE = 25 ms; 90° RF pulse; acquisition matrix = 64 × 64; FOV = 220 × 220 mm; in-plane resolution = 220/64 mm; slice thickness = 4 mm; 30 interleaved axial slices). Four dummy scans were collected at the outset of the run and discarded. The EPI sequence consisted of 486 volumes covering the cortical surface and a portion of the cerebellum with a total acquisition time of 12 minutes and 24 seconds. Magnitude and phase images were also acquired (1 minute and 40 seconds each) for fieldmap-based unwarping (TR = 700 ms; TE = 5.0/7.2 ms; FOV = 220 × 220 mm matrix; flip angle = 30°; slice thickness = 2 mm; 60 interleaved slices).

**Data Analyses**

Functional neuroimaging data were processed and analyzed using Statistical Parametric Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK). The dcm2nii conversion tool was used to convert data from GE DICOM to NIFTI format (Rorden, 2007). The preprocessing pipeline included slice time correction to address non-sequential, interleaved acquisition and realignment of functional images to the first volume of the functional run to adjust for head movement. To account for phase and magnitude variations across the scan, fieldmaps were used to realign and unwarp images. Co-registration of anatomical scans to the first image of the functional scan was followed by registration of anatomical and functional images to the Montreal Neurological Institute (MNI) template. The anatomical image was segmented into bone, air, cerebrospinal fluid, non-brain soft tissue, and brain tissue (i.e., white and gray

matter). Deformation fields were applied to permit spatial normalization to MNI space and images were smoothed with a 6.75 mm FWHM Gaussian filter.

Following the above pre-processing steps, the General Linear Model (SPM12) was applied to create BOLD signal activation maps of encoding minus control trials and recall minus control trials. A $p < .05$, family-wise-error (FWE) corrected statistical threshold with a minimum of eight contiguous voxels was selected for analyses given optimal balance between Type I and II errors (Lazar, 2008).

ROIs were defined using the WFU PickAtlas version 3.0.5 (Maldjian et al., 2003; Maldjian et al., 2004). Consistent with prior studies (e.g., Bookheimer et al., 2000; Bookheimer et al., 2013; Cabeza & Nyberg, 2000; Clement & Belleville, 2009), ROIs during learning trials included medial temporal lobe, supramarginal and angular gyri, precuneus, dorsolateral and ventrolateral prefrontal cortex, anterior and posterior cingulate gyrus, Broca's area, cerebellum, and premotor areas. Recall trials consisted of these ROIs as well as anterior prefrontal cortex, retrosplenial cortex, and cuneus (Cabeza & Nyberg, 2000).

To assess the anticipated effect of L and Z supplementation on brain activity, a 2×2 flexible factorial model was used in second level ROI analyses with group (placebo vs. supplement) as a between-subjects factor and time (baseline vs. post-intervention) as a within-subjects factor. Significant group × time interactions within the aforementioned ROIs would be consistent with the expectation that L and Z intake alters neural activation relative to placebo. Repeated measure within-group comparisons were used to more specifically characterize significant interactions within ROIs in terms of directionality (i.e., increases vs. decreases in BOLD signal). All second level ROI analyses were

conducted separately for encoding and retrieval given non-identical neural underpinnings of these two processes (Cabeza & Nyberg, 2000).

Behavioral performance was also assessed to help verify task engagement and evaluate for intervention effects. Within-scanner self-reported recall of the second word in each pair on the last two recall blocks (maximum score = 10), when learning is expected to be maximal, was calculated. These values were then correlated with actual cued recall assessed immediately post scan to evaluate performance validity. Behavioral performance was also subject to a 2×2 mixed design ANOVA to investigate the expected group (placebo vs. supplement) by time (baseline vs. post-intervention) interaction.

## Results

### Descriptive Statistics

Sample characteristics and (self-reported) dietary intake of vegetables, fruit, fish, and meat at baseline for both the placebo and supplement groups are presented in Table 3.2. Although there were no statistically significant differences between groups, the supplement group was somewhat older than the placebo group (small-to-medium effect size).

There were no significant differences between older adults who dropped out of the study ($N$ = 16) and those who completed the study ($N$ = 44) with respect to age, education level, premorbid intellectual functioning, sex, race, and dietary intake ($p$s > .05). In addition, MPOD and cognitive performance of individuals who completed the baseline vision ($N$ = 11) and fMRI ($N$ = 9) sessions but subsequently dropped were comparable to completers ($p$s > .05). .

**MPOD**

Table 3.3 presents MPOD values, which were used to verify a biological response to L and Z supplementation. Given that MPOD data were available at both 8 months and 12 months, we employed an average of these two time points to estimate L and Z changes over the course of the intervention. An average was employed to increase the stability of the measurement by doubling the number of observations, particularly given the relatively small number of individuals in the control group ($n$ = 14). Importantly, neither the supplement group nor the control group showed a significant change in MPOD between the 8 and 12 month time points. As expected, the supplement group evidenced a significant increase in MPOD across time [$t(29) = 2.46$, $p = .016$] while the placebo group's MPOD remained constant [$t(13) = .05$, $p = .961$]. The supplement group's MPOD was significantly greater than the control group's MPOD following the intervention [$t(42) = 2.44$, $p = .019$] despite the two groups showing comparable MPOD at baseline ($p > .05$).

We note that the same pattern of results is obtained if the data are analyzed without averaging across 8 and 12 month time points. That is, the supplement group's MPOD at 12 months showed a significant increase relative to baseline ($p = .03$, $d = 0.84$) while the placebo group's MPOD at 12 months does not significantly differ from baseline.

Zero-order bivariate correlations between MPOD and demographic variables are provided in Table 3.4. MPOD values at baseline were not significantly related to age, education, depressive symptomatology, or estimated intellectual functioning ($p$s all > .05).

**Whole-Brain Analyses**

Whole-brain analysis of the encoding minus control contrast in the pooled sample at baseline, independent of supplement status, is shown in Figure 3.2. Widespread activation was observed in brain regions typically associated with verbal learning in the literature, such as prefrontal (e.g., Broca's area), medial-temporal (e.g., hippocampus), and cerebellar regions with a general tendency for left-lateralization ($p < .05$, FWE-corrected, minimum eight contiguous voxels). Whole-brain analysis of the retrieval minus control contrast at baseline also revealed diffuse activation in brain areas commonly associated with verbal retrieval (see Figure 3.2), including prefrontal, medial-temporal, parieto-occipital, and cerebellar areas ($p < .05$, FWE-corrected, minimum eight contiguous voxels). Importantly, there were no significant differences in brain activation between supplement and control groups during either learning or retrieval trials at baseline. This finding remained with and without age as a covariate in the between group contrast.

**Effects of L and Z on fMRI performance**

**Behavioral.** The number of self-reported successful retrievals during the verbal learning task is presented by group and time point in Table 3.3. Importantly, the supplement and control groups showed comparable verbal learning performance at baseline ($p > .05$). The overall sample's average recall was 9.02 out of the 10 total word pairs at baseline and 8.61 out of 10 post-intervention. As expected, within-scanner recall significantly correlated with actual cued recall assessed immediately post scan at both time points (baseline $r = .47$, $p = .001$; post-intervention $r = .42$, $p = .005$), differing from one another by less than two words on average at baseline (mean difference = 1.61

words) and post-intervention (mean difference = 0.75 words). The observed congruence suggests that participants were actively engaged in the fMRI task and put forth reasonable effort.

A mixed design ANOVA did not yield a statistically significant group (supplement vs. placebo) × time (baseline vs. post-intervention) interaction for number of words recalled within the scanner [$F(1, 42) = 2.53$, $p = .119$], though the effect size was large ($d = .84$). Analysis of simple effects indicated that the supplement group maintained a similar level of performance at post-intervention relative to baseline [$t(29) = -.18$, $p = .856$, $d = .07$] while the placebo group showed a statistical trend toward decline [$t(13) = -1.87$, $p = .084$] characterized by a large effect size ($d = 1.04$).

**ROI analysis**. Following the encoding minus control contrast, the group (supplement vs. placebo) × time (baseline vs. post-intervention) interaction analysis yieleded significant clusters in left dorsolateral prefrontal cortex ($F = 21.34$, $p = .034$, FWE-corrected, cluster size: 498 voxels) and anterior cingulate cortex ($F = 19.63$, $p = .028$, FWE-corrected, cluster size: 146 voxels; see Figure 3.3). Suprathreshold activation was not found in any of the other ROIs though a cluster was approaching significance in right hippocampus ($F = 15.13$, $p = .054$, FWE-corrected, cluster size: 20 voxels). To characterize significant interactions, paired *t*-tests revealed increases in BOLD signal in the supplement group from baseline to post-intervention in left dorsolateral prefrontal cortex ($p = .014$, FWE-corrected) and anterior cingulate cortex ($p = .016$, FWE-corrected). Importantly, the placebo group did not show activation changes in these regions over the course of the study.

With respect to possible intervention effects during retrieval, a 2×2 flexible factorial model did not return significant interactions in the hypothesized ROIs (*p*s all > .05, FWE-corrected, minimum eight contiguous voxels). Exploratory paired *t*-tests did, however, yield significant increases in aspects of left dorsolateral prefrontal cortex ($t$ = 4.42, $p$ = .045, FWE-corrected, cluster size: 156 voxels) and anterior cingulate cortex ($t$ = 4.45, $p$ = .021, FWE-corrected, cluster size: 182 voxels) at post-intervention relative to baseline in the supplement group only (see Figure 3.4), consistent with findings during encoding. No other ROIs showed changes in BOLD signal that survived the FWE correction in exploratory analyses.

## Discussion

L and Z supplementation was found to significantly influence brain function on a verbal learning task relative to placebo. Rather than increasing neural efficiency as predicted, however, carotenoid consumption enhanced BOLD signal in aspects of select ROIs; significant clusters were observed in left dorsolateral prefrontal cortex and anterior cingulate cortex. A similar pattern emerged during verbal retrieval in exploratory within-group analyses but given non-significance in the omnibus interaction, these findings must be interpreted cautiously. It is noteworthy that a nearly significant effect was observed in a cluster within right hippocampus during learning, which suggests a possible influence of L and Z on brain function in this region as well. The hippocampal effect was in the hypothesized direction of reduced neural activity though conclusions are limited without having survived statistical correction for multiple comparisons.

On the surface, the observed increase in prefrontal BOLD signal seems inconsistent with cross-sectional findings from this sample at baseline, which showed a

negative relationship of endogenous L and Z levels to neural activity (Lindbergh et al., 2016). However, it is possible the baseline and longitudinal findings are actually complementary. Within the context of the STAC-r (Reuter-Lorenz & Park, 2014), high levels of L and Z consumption *over the course of a lifetime* may in fact buffer age-related neurophysiological decline and reduce the resulting need for compensatory scaffolding. This would be analogous to a "brain maintenance" effect as described by Nyberg Lövdén, Riklund, Lindenberger, and Bäckman (2012). Although Lindbergh et al. (2016) measured L and Z status at a single point in time, we speculate that this cross-sectional measurement at least to some extent reflects, and would correlate with, lifetime eating behaviors. This is consistent with empirical evidence suggesting that dietary patterns are relatively stable across time, at least from childhood through adulthood (e.g., Mikkilä, Räsänen, Raitakari, Pietinen, & Viikari, 2005). MPOD in particular, one of the predictors used by Lindbergh et al. (2016), has demonstrated considerable stability in adults over multiple year spans (e.g., Nolan et al., 2006). Accordingly, to the extent that cross-sectional L and Z levels (particularly MPOD) approximate lifetime eating behaviors, the Lindbergh et al. (2016) findings may reflect a lifespan neural efficiency effect. Of course, this interpretation relies heavily on the assumption that a "snapshot" measurement of L and Z status in old age meaningfully proxies L and Z intake across the lifespan. Longitudinal research will be required to evaluate this hypothesis, ideally focusing on changes in both MPOD and serum L and Z concentrations in relation to brain function. Such research may need to begin as early as childhood in light of recent findings that L and Z status in 7-10 year olds positively predicts hippocampal-dependent memory performance (Hassevoort et al., 2017).

Our current RCT findings neither support nor contradict the possibility of a lifespan neural efficiency effect but rather address a somewhat different question involving the neural effect of elevated L and Z intake circumscribed to a one year period in late life. Results suggest that L and Z consumed in this fashion increases cerebral perfusion and enhances neural response during cognitive performance. While further research is required to identify mechanisms underlying this effect, studies using animal models provide some insight. For example, antioxidants restore cerebral blood flow in rat models following traumatic brain injury (Bitner et al., 2012) and hypoxia-induced metabolic stress (Huang et al., 2013). Similar to other forms of insult, aging increases risk for cerebral hypoperfusion, which in turn is associated with cognitive impairment and dementia (D'Esposito, Deouell, & Gazzaley, 2003; Ruitenberg et al., 2005). L and Z supplementation may counter these effects by increasing blood flow to regions (e.g., frontal) at risk for poor cerebral perfusion and age-related deterioration.

The pattern of increased BOLD signal is consistent with a small number of RCTs that have examined fMRI changes related to other dietary factors. As examples, two days of high nitrate diet (Presley et al., 2011), four weeks of pomegranate juice consumption (Bookheimer et al., 2013), three months of high flavanol diet (Brickman et al., 2014), and six months of fish oil supplementation (Boespflug, McNamara, Eliassen, Schidler, & Krikorian, 2016) have all been found to enhance BOLD signal relative to control conditions. Pharmacological interventions (e.g., cholinesterase inhibitors) have similarly increased BOLD signal in older adults during memory performance (Goekoop et al., 2004; Saykin et al., 2004). Of note, several of these interventions observed BOLD signal increases in dorsolateral prefrontal cortex and anterior cingulate cortex specifically (e.g.,

Goekoop et al., 2004; Presley et al., 2011; Saykin et al., 2004), as was found in the present RCT.

Anterior cingulate cortex and dorsolateral prefrontal cortex comprise a closely related functional network (e.g., Fleck, Daselaar, Dobbins, & Cabeza, 2006; MacDonald, Cohen, Stenger, & Carter, 2000) and together mediate executive functions including attention, working memory, and cognitive control (Gasquoine, 2013). Left dorsolateral prefrontal cortex monitors and manipulates verbal information to support encoding processes (Barbey, Koenigs, & Grafman, 2013), and more generally subserves cognitive control processes specific to long-term memory formation (MacDonald et al., 2000; Niendam et al., 2012). This is particularly true when relationships must be built between items (e.g., word pairs; Blumenfeld, Parks, Yonelinas, & Ranganath, 2011) and dorsolateral prefrontal cortex activity positively correlates with success on relational encoding tasks (Blumenfeld & Ranganath, 2006). Anterior cingulate cortex is reliably activated on memory tasks as well, particularly when they are challenging or require a high level of attention to relevant stimuli (Cabeza & Nyberg, 2000; Niendam et al., 2012; Shenhav, Botvinick, & Cohen, 2013; Weissman, Gopalakrishnan, Hazlett, & Woldorff, 2005). Conflict detection and resolution are also rooted in anterior cingulate cortex, which may account for its observed role in memory decision-making (Fleck et al., 2006).

Although L and Z's behavioral effects on the verbal learning task were not statistically significant, it is notable that the effect size was large. The lack of significance may relate to inadequate power, which is a common phenomenon in fMRI studies that consider behavioral measures (Wilkinson & Halligan, 2004). Although conclusions must therefore be limited, our findings tentatively suggest that carotenoid supplementation in

old age helps maintain cognitive performance across time, possibly buffering against decline; enhanced cerebral perfusion in prefrontal regions offers a potential neural mechanism for this effect. Alternatively, L and Z may not be influencing learning and memory per se, but rather associated cognitive processes (e.g., attention). Finally, the possibility that the effects of L and Z supplementation on overt cognitive function in old age are actually quite modest, at least as measured in the current study, must also be acknowledged. A longer supplementation period may be required (i.e., > 1 year) or perhaps L and Z intake must be elevated across the lifespan to meaningfully influence cognition.

The present study has limitations. Our sample was racially homogenous, high functioning, and well educated. Future research is warranted to determine whether results generalize to populations characterized by greater diversity and cognitive variability. This is particularly important given that elevated socioeconomic position may reduce the strength of the relationship between dietary factors and cognition (Akbaraly, Singh-Manoux, Marmot, & Brunner, 2009). In light of the high average number of words recalled on the verbal learning task, which may indicate a ceiling effect, it will be important for future RCTs to consider changes using more sensitive neuropsychological measures and in other cognitive domains. The generalizability of our findings may also be influenced by the relatively high number of individuals who dropped out of the study (~27%), though it is encouraging that non-completers did not display any significant differences from completers on demographic and dietary factors. Finally, as with most nutrition studies, there was no true “control” group; L and Z are abundant in a variety of foods. This may have attenuated effects and contributed to the non-significant finding

with respect to overt cognition. In many respects, the observation of any brain effect beyond routine L and Z consumption in an intact and presumably well-nourished sample speaks to the robust potential for carotenoids to influence neurocognitive function.

Despite its limitations, the present study represents the first RCT to investigate the effects of L and Z supplementation on neural function *in vivo* during cognitive task performance. More broadly, our results add to the paucity of research that has evaluated the potential of nutrition—a modifiable and inexpensive lifestyle factor—to promote brain health, even if circumscribed to a discrete period of time in late life.

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Table 3.1

*Study Timeline*

| | *Pre-Screen* | *Baseline* | | | *4-mos* | *8-mos* | *Post-Intervention* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Health Center | V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 |
| Physical Exam | ✓ | | | | | | | | |
| Vision Testing | | | ✓ | | ✓ | ✓ | | ✓ | |
| Blood Draw | | | ✓ | | ✓ | ✓ | | ✓ | |
| Cog. Testing | | ✓ | | | ✓ | ✓ | ✓ | | |
| Neuroimaging | | | | ✓ | | | | | ✓ |
| Pill Counts | | | | | ✓ | ✓ | ✓ | | |

*Note*. Cog. Testing = cognitive testing, which included the Geriatric Depression Scale and Wechsler Test of Adult Reading at Visit 1 (V1). Mos = months. Baseline visits 1, 2, and 3 occurred within a two-week window, as did post-intervention visits 6, 7, and 8. The post-intervention visits occurred at 12 months. Vision testing included measurement of macular pigment optical density (MPOD).

Table 3.2

*Descriptive Statistics*

| | Placebo ($n$ = 14) | Supplement ($n$ = 30) | $p$ value ($t$-test or $\chi^2$) | Effect size (Cohen's $d$) |
|---|---|---|---|---|
| Age [*M* (SD)] | 70.43 (5.43) | 72.43 (6.48) | 0.321 | 0.33 |
| Race (% Caucasian) | 100% | 100% | -- | -- |
| Sex (% female) | 71.43% | 53.33% | 0.419 | 0.25 |
| Education [*M* (SD)] | 16.71 (3.02) | 16.73 (3.02) | 0.724 | 0.12 |
| WTAR [*M* (SD)] | 115.29 (7.44) | 114.43 (7.79) | 0.734 | 0.11 |
| GDS [*M* (SD)] | 1.71 (1.89) | 2.43 (3.09) | 0.427 | 0.27 |
| Dietary intake [*M* (SD)] | | | | |
| *Vegetable* | 14.25 (6.51) | 12.97 (5.55) | 0.505 | 0.22 |
| *Fruit* | 10.38 (4.88) | 12.59 (7.50) | 0.320 | 0.33 |
| *Fish* | 1.79 (1.25) | 1.67 (1.27) | 0.772 | 0.10 |
| *Meat* | 4.21 (2.12) | 4.20 (1.95) | 0.983 | 0.01 |

*Note*. GDS = Geriatric Depression Scale. *M* = mean. SD = standard deviation. WTAR = full-scale intelligence quotient predicted from the Wechsler Test of Adult Reading. Dietary intake represents self-reported servings of vegetables, fruits, fish, and meats per week.

Table 3.3

*Intervention Effects on MPOD and Cognition*

| | Variable | Baseline | Post-intervention | *p* value (*t*-test) | Effect size (Cohen's *d*) |
|---|---|---|---|---|---|
| Placebo | MPOD [*M* (SD)] | 0.44 (0.14) | 0.44 (0.19)* | 0.961 | 0.03 |
| (*n* = 14) | Recall [*M* (SD)] | 9.36 (0.75) | 8.21 (2.29) | 0.084 | 1.04 |
| Supplement | MPOD [*M* (SD)] | 0.54 (0.19) | 0.61 (0.22)* | 0.016 | 0.95 |
| (*n* = 30) | Recall [*M* (SD)] | 8.87 (1.50) | 8.80 (2.16) | 0.856 | 0.07 |

*Note*. *M* = mean. MPOD = macular pigment optical density. SD = standard deviation. Recall = number of words recalled on the final two blocks of the fMRI-adapted verbal learning paradigm (maximum = 10). * = average of 8 month and 12 month (post-intervention) time points.

Table 3.4

*Zero-Order Bivariate Correlations*

| | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| *1*. Baseline MPOD | -- | 0.12 (.423) | 0.12 (.448) | 0.10 (.509) | -0.02 (.900) |
| *2*. Age | | -- | -0.05 (.741) | -0.18 (.246) | -0.04 (.797) |
| *3*. Education | | | -- | 0.61 (<.001) | 0.08 (.602) |
| *4*. WTAR | | | | -- | -.12 (.429) |
| *5*. GDS | | | | | -- |

*Note*. Values are presented as Pearson's *r* (*p* value). GDS = Geriatric Depression Scale. MPOD = macular pigment optical density. WTAR = full-scale intelligence quotient predicted from the Wechsler Test of Adult Reading.

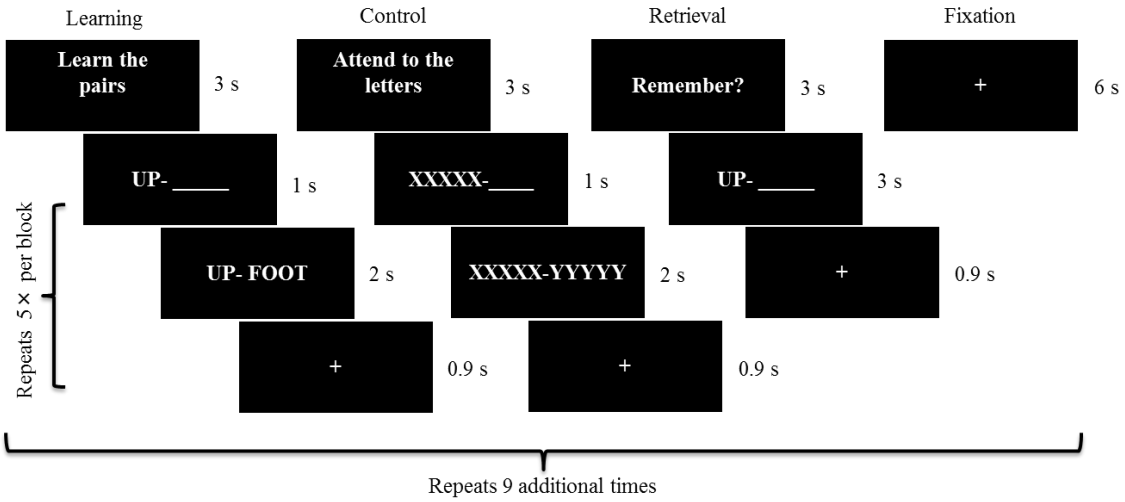
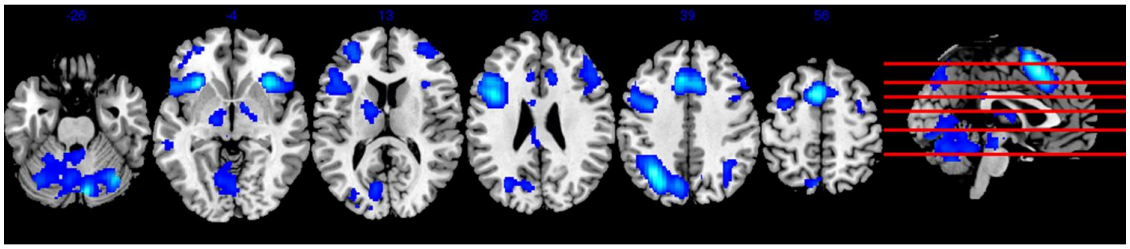



*Figure 3.1*. Visual schematic of the fMRI-adapted verbal learning task. Each block (i.e., learning, control, retrieval, and fixation) was presented 10 times. Five different word pairs were included within every learning block followed by five XXXXX – YYYYY pairings within every control block. Similarly, participants attempted to recall the second word of five different word pairs during each retrieval block.

(a)




(b)

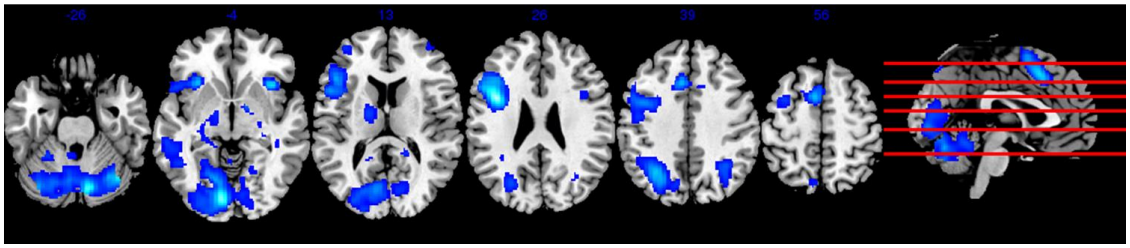
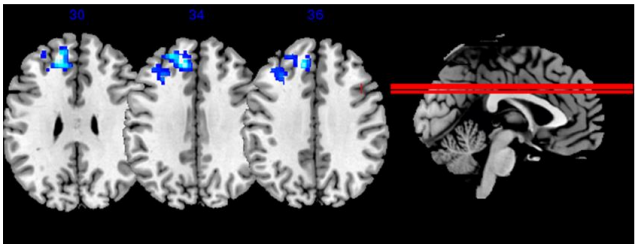



*Figure 3.2*. Whole-brain analyses. Panel (a) shows results from whole-brain analysis of the encoding minus control contrast at baseline in the pooled sample, independent of supplement status. Panel (b) similarly depicts whole-brain analysis of the recall minus control contrast in the pooled sample at baseline. Both panels (a) and (b) represent activity in MNI space superimposed on an anatomical template provided by MRIcron (http://www.mricro.com/mricron/install.html).

(a)




(b)

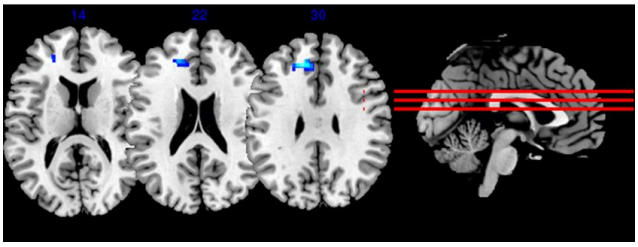
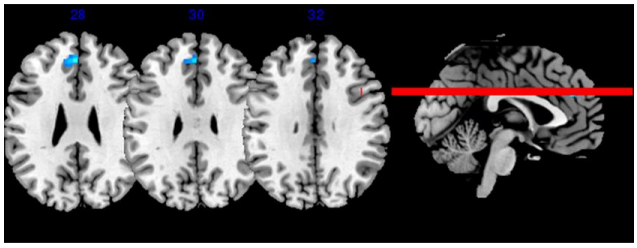



*Figure 3.3*. Intervention effects on brain function during verbal learning. Significant ($p$ < .05, FWE-corrected) group (supplement vs. placebo) × time (baseline vs. post-intervention) interactions in left dorsolateral prefrontal cortex [Panel (a)] and anterior cingulate cortex [Panel (b)] are depicted. Activity is in MNI space and superimposed on an anatomical template provided by MRIcron (http://www.mricro.com/mricron /install.html).

(a)




(b)

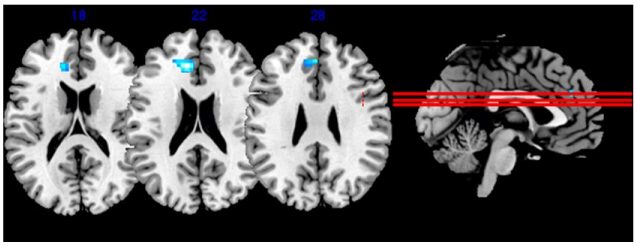



*Figure 3.4.* Intervention effects on brain function during verbal recall. Results from exploratory paired *t*-tests are shown. Significant ($p < .05$, FWE-corrected) increases in left dorsolateral prefrontal cortex [Panel (a)] and anterior cingulate cortex [Panel (b)] were observed in the supplement group at post-intervention relative to baseline. The control group did not show activation changes in these regions over the course of the study. Activity is in MNI space and superimposed on an anatomical template provided by MRIcron (http://www.mricro.com/mricron/install.html).

# CHAPTER 4

# THE EFFECTS OF LUTEIN AND ZEAXANTHIN ON RESTING STATE FUNCTIONAL CONNECTIVITY IN OLDER ADULTS: A RANDOMIZED CONTROLLED TRIAL[2]

[2] Lindbergh, C. A., Lv, J., Zhao, Y., Mewborn, C. M., Puente, A. N., Terry, D. P., Renzi-Hammond, L. M., Hammond, B. R., Liu, T., & Miller, L. S. To be submitted to *NeuroImage*.

## Abstract

The carotenoid micronutrients lutein (L) and zeaxanthin (Z) accumulate selectively in the macula of the eye and have long been shown to benefit visual health. A growing literature suggests cognitive benefits as well, particularly in older adults. The present randomized controlled trial sought to investigate neural mechanisms using resting state functional magnetic resonance imaging (fMRI). It was hypothesized that L and Z supplementation would (1) improve *intra*-network integrity of default mode network (DMN) and (2) reduce *inter*-network connectivity between DMN and other resting state networks. 48 community-dwelling older adults (mean age = 72 years) were randomly assigned to receive a daily L (10 mg) and Z (2 mg) supplement or a placebo for one year. Resting state fMRI data were acquired at baseline and post-intervention. A dictionary learning and sparse coding computational framework, based on machine learning principles, was used to investigate intervention-related changes in functional connectivity. DMN integrity was evaluated by calculating overlap with a well-established DMN template provided in the neuroscience literature. *Inter*-network connectivity was evaluated via time series correlations between DMN and nine other resting state networks. Contrary to expectation, results indicated that L and Z significantly increased rather than decreased *inter*-network connectivity (Cohen's $d = 0.89$). A significant *intra*-network effect on DMN integrity was not observed. Rather than restoring a "youth-like" pattern of intrinsic brain activity, L and Z may facilitate the aging brain's capacity for compensation by enhancing integration between networks that tend to be functionally segregated earlier in the lifespan.

## Introduction and Literature Review

Lutein (L) and its isomer zeaxanthin (Z) are xanthophyll micronutrients from the carotenoid family found in green leafy vegetables, bright fruits, and other aspects of diet (Britton, Liaaen-Jensen, & Pfander, 2004; Hammond et al., 1997; Khachik, Beecher, Goli, & Lusby, 1991; Malinow, Feeney-Burns, Peterson, Klein, & Neuringer, 1980). As the only carotenoids that cross the blood-retina barrier to accumulate selectively in the macula (the central region of the retina), L and Z have long been shown to benefit eye health and protect against age-related macular degeneration (Bone, Landrum, & Tarsis, 1985; Ma et al., 2012; SanGiovanni & Neuringer, 2012).

An individual's L and Z levels are commonly measured *in vivo*, either through blood serum concentrations or heterochromatic flicker photometry (Hammond, Wooten, & Smoolen, 2005). The latter technique is non-invasive and provides a measurement of the optical density of the macular pigment layer (MPOD). Although both approaches are widely used and validated, MPOD tends to better reflect long-term dietary patterns while serum L and Z concentrations fluctuate more with recent food consumption (Beatty, Nolan, Kavanagh, & O'Donovan, 2004; Hammond et al., 1997). MPOD is generally considered a more accurate representation of L and Z absorbed into the central nervous system and has been found to correlate with brain tissue concentrations assessed postmortem in humans and primates (Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013; Vishwanathan, Schalch, & Johnson, 2016).

L and Z have been found to selectively accumulate in cortical and subcortical brain tissue (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Johnson et al., 2013), which in turn has spurred research on possible cognitive benefits. In older adults, a growing

literature indicates that L and Z levels positively correlate with a range of cognitive functions, including visuospatial skills, learning, memory, language abilities, executive functions, processing speed, and global cognition (Feeney et al., 2013; Renzi, Dengler, Puente, Miller, & Hammond, 2014; Renzi, Iannacocone, Johnson, & Kritchevsky, 2008; Vishwanathan et al., 2014). However, the cross-sectional designs of these studies have limited conclusions regarding directionality and potential extraneous variables. It may be possible, for example, that cognitive loss leads to poor nutritional decisions or that elevated L and Z levels merely proxy other aspects of a healthy lifestyle. To the authors' knowledge, only one randomized controlled trial (RCT) exists (Johnson et al., 2008) in a relatively small sample of older adults ($N$ = 53). Results indicated that four months of combined L and DHA (a polyunsaturated fatty acid) supplementation leads to significant gains in learning (Cohen's $d$ = 0.70), memory (Cohen's $d$ = 0.58), and verbal fluency (Cohen's $d$ = 0.90) relative to placebo.

As noted in several reviews (e.g., Erdman et al., 2015; Johnson, 2012; Johnson, 2014; Zamroziewicz & Barbey, 2016), the identification of neural mechanisms underlying the relation of L and Z (and nutrition more broadly) to cognition in late life is an understudied area. Based on our understanding of how L and Z benefit neural health in the retina, these carotenoids may counter age-related cognitive decline through their strong antioxidant and anti-inflammatory properties (Bokov, Chaudhuri, & Richardson, 2004; Butterfield, Bader Lange, & Sultana, 2010; Heneka et al., 2015; Rosano, Marsland, & Gianaros, 2012). In addition, L and Z positively influence cell membrane stability, thickness, and ion exchange while improving cellular communication and gap junction signaling (Bertram, 1999; Erdman et al., 2015; Krinsky, Mayne, & Sies, 2004; Stahl &

Sies, 2001; Widomska & Subczynski, 2014). In turn, these actions may mitigate the deleterious effects of aging on neuronal membrane structure and function (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002).

The present study was a 12-month RCT that sought to extend the available literature on the important relationship between nutrition and brain health in old age by examining functional connectivity changes associated with L and Z supplementation relative to placebo. Cross-sectional functional magnetic resonance imaging (fMRI) findings from this study's sample at baseline (i.e., pre-supplementation) are reported elsewhere (Lindbergh et al., 2016) and represent the only published attempt to investigate neural mechanisms underlying the relation of L and Z to cognition *in vivo* using neuroimaging technology. A significant, negative relationship of L and Z levels to blood-oxygen-level-dependent (BOLD) signal during a verbal learning task was observed in multiple brain regions, including insula, inferior and middle frontal gyrus, middle temporal gyrus, and central and parietal operculum. The observed pattern was interpreted within the context of the Scaffolding Theory of Aging and Cognition (STAC; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2014) to suggest a possible role of L and Z in increasing neurobiological efficiency (i.e., the neural efficiency hypothesis; Renzi & Hammond, 2010).

To our knowledge, no studies have evaluated L and Z's neurocognitive effects using resting state fMRI. In contrast to task-based fMRI, resting state fMRI is collected while subjects lay passively in the scanner and provides data regarding functional connectivity within and between large-scale networks of brain regions (Baldassarre & Corbetta, 2015). Traditional resting state approaches have revealed several spatially

distributed networks that exhibit synchronous deactivation during cognitive performance and correlated activity at rest (Baldassarre & Corbetta, 2015), perhaps most notably the default mode network (DMN) comprised of posterior cingulate cortex, ventral anterior cingulate cortex, and supramarginal gyrus (Buckner, Andrews-Hanna, & Schacter, 2008). The aging process has been shown to reduce connectivity strength between seed regions (hubs) within resting state functional networks, such as between the anterior and posterior nodes of DMN (e.g., Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Sambataro et al., 2010). Increased correlative behavior between distinct functional networks that are typically segregated earlier in the lifespan has also been observed (Ferreira & Busatto, 2013; Geerligs, Mauritis, Renken, & Lorist, 2014; Geerligs, Renken, Saliasi, Maurits, & Lorist, 2015; Voss et al., 2010). Relatedly, older adults often fail to deactivate or suppress DMN during cognitive task performance and tend to show increased functional coupling (i.e., reduced anti-correlation) between DMN activity and task-positive networks relative to young adults (Park, Polk, Hebrank, & Jenkins, 2010; Spreng & Schacter, 2011). Such changes in DMN and other resting state networks have been associated with cognitive decline (Ferreira & Busatto, 2013; Persson, Pudas, Nilsson, & Nyberg, 2014; Wang et al., 2010) and are particularly exaggerated in pathological relative to normal aging (Garces et al., 2014; Greicius, Srivastava, Reiss, & Menon, 2004; Reiman et al., 2004; Sheline et al., 2010).

Although resting state fMRI holds considerable potential to contribute to our understanding of how nutrition influences brain function by moving beyond isolated neural structures to neural networks (Zamroziewicz & Barbey, 2016), very few studies have leveraged this approach. In young adults, acute administration of green tea extract

has been found to enhance parieto-frontal connectivity (Schmidt et al., 2014) whereas 12 weeks of an unhealthy saturated fatty acid-enriched diet reduced intrinsic activity in hippocampus and inferior parietal cortex (Sartorius et al., 2012). In older adults, two days of a high nitrate diet (mostly fruits, green leafy vegetables, and beet juice) improved cerebral perfusion in connections between dorsolateral prefrontal and anterior cingulate cortices relative to a low nitrate control condition (Presley et al., 2011). Taken together, these studies provide preliminary evidence that a healthful diet may improve functional connectivity while unhealthful dietary factors may diminish connectivity; however, additional research is clearly needed on this topic, particularly in older adults.

There are several available approaches for analyzing resting state fMRI data, including independent component analysis (ICA), seed-based techniques, and graph theoretic analyses (Baldassarre & Corbetta, 2015; Ferreira & Busatto, 2013). More recently, a novel computational methodology based on machine learning principles and referred to as Holistic Atlases of Functional Networks and Interactions (HAFNI) was developed and validated (Lv et al., 2015a). Relative to traditional methodologies that use the general linear model (GLM), the HAFNI approach characterizes spatial overlap and interaction behavior of brain networks (Lv et al., 2015b), thus taking into consideration that a given brain region may serve multiple functions (Lv et al., 2015c) and its BOLD signal may include different components (Varoquaux, Gramfort, Pedregosa, Michel, & Thirion, 2011). HAFNI employs a dictionary learning and sparse coding framework that has demonstrated sensitivity to resting state changes associated with mild traumatic brain injury (Lv et al., 2016) and more closely correlates with DMN disruptions in older adults with mild cognitive impairment and Alzheimer's disease than traditional approaches (i.e.,

ICA and seed-based; Lee et al., 2016). In addition, HAFNI has reliably reproduced at least 32 spatially distributed and overlapping functional networks using high-quality data from the Human Connectome Project (Lv et al., 2015b). In light of the strengths of the HAFNI approach, which may confer optimal sensitivity to subtle but meaningful network changes resulting from a nutritional intervention, this was the chosen methodology for the present study.

As discussed previously, age-related neurodegenerative processes are related to altered functional connectivity both within and between resting state networks, even among cognitively normal older adults (Ferreira & Busatto, 2013; Garces et al., 2014; Geerligs et al., 2014; Geerligs et al., 2015). DMN has received the most attention in the aging literature with evidence suggesting decomposition of connections between hubs within network as well as hyper-connectivity to other resting state networks (i.e., loss of segregation; Baldassarre & Corbetta, 2015; Sala-Llonch, Bartres-Faz, & Junque, 2015). The aim of the present RCT was to evaluate whether supplementation with L and Z—micronutrients with neurally-beneficial properties and apparent benefits to cognition—might counter or buffer these functional connectivity changes in older adults. It was hypothesized that consuming L and Z supplements for a one year period in late life would (1) improve *intra*-network integrity of DMN and (2) reduce *inter*-network connectivity between DMN and other resting state networks relative to a placebo control group. In essence, L and Z supplementation was expected to a restore a more "youth-like" pattern of intrinsic brain activity both within DMN and in the interactive behavior of DMN with other networks.

# Method

## Participants

An initial sample of 75 community-dwelling older adult volunteers (64-86 years of age) were recruited from the surrounding area to participate in a larger intervention study evaluating the relation between carotenoid consumption and various cognitive, neural, and visual outcomes. Exclusionary criteria consisted of left-handedness, traumatic brain injury, macular degeneration, gastric conditions impacting nutrition absorption, visual acuity poorer than 20:40 (corrected), MRI incompatibility, Geriatric Depression Scale (GDS) total score > 19, or neurological disorder. Although all participants in the initial sample passed a preliminary phone screen for study eligibility, more in depth review of medical histories resulted in the exclusion of seven participants due to MRI safety contraindications. During the MRI scan, data from an additional four participants were lost because of claustrophobia, noise sensitivity, and inability to stay awake. Over the course of the study, 16 volunteers decided to withdraw for various reasons. The final sample size for analyses was thus 48 (experimental $n = 34$; control $n = 14$).

## Procedures

The present study was a single-site, double-blind, RCT. A 2:1 ratio was used when randomly assigning participants to receive a lutein (10 mg) plus zeaxanthin (2 mg) supplement or a placebo of identical appearance. Participants were instructed to consume their pills once daily with a meal for a 12 month period.

The larger intervention study consisted of 8 laboratory visits as well as a physical examination with a collaborating health professional to confirm appropriate health and medical history for trial participation. Following the physical examination, participants

completed vision testing, cognitive testing, MPOD measurement, and neuroimaging across three baseline sessions within a two-week window. Randomized group assignment (supplement or placebo) was conducted upon completion of the baseline assessment. To verify compliance, pill counts were performed during study visits at 4 month, 8 month, and 12 month time points, and participants were supplied with only enough pills to last between visits. Bi-monthly contact was also made to evaluate compliance as well as relevant changes in health status or adverse events. The post-intervention visit at 12 months was divided into three sessions that replicated the baseline visit.

As compensation, participants were provided $300 divided among the baseline, 4 month, 8 month, and 12 month visits. If applicable, collateral drivers were offered $20 for transporting participants to each study session.

The University of Georgia Institutional Review Board fully approved this RCT and all study personnel upheld the Declaration of Helsinki tenets at all times.

**Measures**

**Wechsler Test of Adult Reading**. The Wechsler Test of Adult Reading (WTAR) was administered to estimate full-scale intelligence quotients (Wechsler, 2001). The WTAR employs an algorithmic combination of the examinee's ability to read words with unusual grapheme to phoneme relationships and demographic variables (i.e., sex, age, race, education, and geographic region) to predict intellectual functioning.

**GDS**. The GDS, a 30 item self-report measure with a yes/no response format, was administered as a screener for significant depressive symptomatology (Yesavage et al., 1983). The total score ranges from 0 to 30, with higher scores indicating greater levels of depression (0–9 = normal; 10–19 = mild; 20–30 = severe).

**MPOD**. Customized heterochromatic flicker photometry (Stringham et al, 2008) was used to evaluate changes in MPOD resulting from L and Z supplementation. A detailed description of the exact procedure employed in this study has been published elsewhere (Lindbergh et al., 2016). Briefly, a macular densitometer (Macular Metrics, Rehoboth, MA) was used to present a 1˚ visual stimulus comprised of a 460 nm narrow-band light source and a 570 nm component. The 460 nm waveband was manipulated relative to the 570 nm component to assess the point at which it was no longer possible to perceive flickering. This sequence was repeated with a 2˚ target (fixation point = 7˚ nasally) to serve as a reference in the parafovea where MPOD approaches zero. An MPOD measurement at 30 minutes of retinal eccentricity was then calculated by contrasting the two loci.

**Neuroimaging**

**MRI acquisition**. Neuroimaging data were collected using a General Electric (GE; Waukesha, WI) 3 T Signa HDx MRI system. A high-resolution structural scan was collected with a 3D T1-weighted fast spoiled gradient recall echo sequence (TR = 7.5 ms; TE = < 5ms; FOV = 256 × 256 mm matrix; flip angle = 20°; slice thickness = 1.2 mm; 154 axial slices). The protocol, which had a total acquisition time of 6 minutes and 20 seconds, acquired 176 images from the top of the head to the brainstem.

Resting state functional scans were aligned to each participant's AC-PC line, collected axially, and used a T2*-weighted single shot EPI sequence (TR = 5000 ms; TE = 25 ms; 90° RF pulse; acquisition matrix = 128 × 128; FOV = 220 × 220 mm; in-plane resolution = 220/128 mm; slice thickness = 2 mm; 60 interleaved axial slices). Acquisition time was 9 minutes and 25 seconds. 108 volumes were acquired that covered

the cortical surface and a portion of the cerebellum. Participants were instructed to remain awake and motionless while viewing a neutral screen through MRI compatible goggles (Resonance Technology Inc., Northridge, CA).

**Data analyses**. Pre-processing of resting state fMRI data followed a standard pipeline (Lv et al., 2016) implemented in FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012), including skull removal, motion correction, slice time correction, spatial smoothing (FWHM = 5 mm), and global drift removal. Pre-processed volumes were registered to MNI space and voxel signals from every subject were normalized with a mean of zero and a standard deviation of one to prevent potential bias related to individual variability (Lv et al., 2015a; Lv et al., 2015b; Lv et al., 2015c; Lv et al., 2016).

A dictionary learning and sparse coding computational framework was applied to the pre-processed data, which has been validated and described in detail elsewhere (Ge et al., 2016; Lv et al., 2015a; Lv et al., 2015b; Lv et al., 2015c; Lv et al., 2016). To summarize, whole-brain fMRI signals of each participant were extracted and aggregated into a 2D (voxel number × time series point) signal matrix ($S_x$). A publicly available dictionary learning and sparse coding algorithm (Mairal, Bach, Ponce, & Sapiro, 2010) was subsequently employed to factorize the resulting data for each subject into a time series signal dictionary matrix ($D$) and a coefficient matrix ($A$). In essence, this technique applies machine learning and pattern recognition principles to "sparsely" summarize and organize the hundreds of thousands of whole-brain fMRI signals derived from individual participants. This is accomplished through implementation of an empirical cost function

that optimally culls the raw data without losing excessive information (Mairal et al., 2010):

$$f_n(D) \triangleq \frac{1}{n} \sum_{i=1}^{n} \ell(x_i, D)$$

$\ell$ represents a loss function to input signal that yields a "sparse" solution to $A_i$ while minimizing cost:

$$\ell(x_i, D) \triangleq \min_{\alpha_i \in \mathbb{R}^m} \frac{1}{2} \| x_i - D\alpha_i \|_2^2 + \lambda \| \alpha_i \|_1$$

Meanwhile, $D$ is constrained from arbitrarily large values by:

$$C \triangleq \{ D \in \mathbb{R}^{t \times m} \; s.t. \; \forall j = 1, \ldots m, \quad d_j^T d_j \leq 1 \}$$

The reader is referred to Mairal et al. (2010) for a detailed description of these functions and their associated variables, as well as the sparse coding procedure more generally. Ultimately, each column ($m$) within $D$ represents a learned functional network or "atom" (such that $m$ is much less than $n$, where $n$ = whole-brain fMRI signals from every voxel) while each row in $A$ maps the functional networks to their physical location within the brain by conserving the spatial voxel organization of $S_x$.

An important parameter to choose within this computational framework is the number of atoms, or $m$, in $D$. By definition, the larger the dictionary size, the greater the number of learned functional networks. Generally speaking, increasing the number of atoms more precisely fragments resting state networks into different components while reducing this parameter aggregates different components into the same resting state

network (Lee et al., 2016). Dictionary sizes have ranged considerably in previous studies, from as low as 15 (Lee et al., 2016) to as high as 600 (Lv et al., 2015b). In the absence of a widely accepted "gold standard," not to mention the likelihood that optimal dictionary sizes are somewhat idiosyncratic to study sample, we examined a range of dictionary size candidates that have been explored previously, including 20 (Lee et al., 2016), 50 (Lv et al., 2016), 100 (Lv et al., 2015b), and 500 (Lv et al., 2015a). Based on procedures used elsewhere (e.g., Lee et al., 2016), we selected the number of atoms that characterized DMN in a manner most consistent with the bulk of the neuroscience literature. This was accomplished quantitatively by calculating the correspondence of DMN "learned" at each candidate dictionary size to a widely accepted DMN template provided in the literature (Smith et al., 2009). More specifically, overlap rate similarity (ORS) was calculated for the pooled sample (i.e., averaged across all participants) using a previously published equation (Zhao et al., 2016) in which greater values correspond to greater agreement:

$$\text{ORS} = \sum_{k=1}^{|V|} \frac{min(V_k, W_k)}{(V_k + W_k)/2}$$

The numerator represents the minimum activation value within each pair of spatially corresponding voxels (*k*) between two volume maps, *V* and *W,* while the denominator represents the average activation value of each voxel pair. In this way, greater ORS values are indicative of greater network correspondence with respect to both spatial location and activation intensity. Here, *V* represents the Smith et al. (2009) DMN template while *W* represents the learned DMN within our pooled sample for each candidate dictionary size (i.e., 20, 50, 100, and 500). The dictionary size that optimally produced DMN in this fashion was selected for subsequent analyses.

After dictionary size selection, the ORS procedure was similarly implemented to quantitatively identify the dictionary atom corresponding to DMN at an individual subject level. In other words, for a given participant, the learned functional network that exhibited the greatest correspondence (i.e., the greatest ORS) with the Smith et al. (2009) DMN template was labeled DMN, consistent with prior work (Ge et al., 2016). The hypothesis that L and Z supplementation improves DMN modularity (i.e., increases *intra*-network integrity and reduces *inter*-network connectivity) was then tested in two ways. First, to evaluate potential effects of the intervention on DMN integrity, ORS values at baseline were compared to ORS values at post-intervention for each group (i.e., supplement and controls) using paired-samples *t*-tests. Second, changes in the interaction of each subject's learned DMN with other well-established resting state networks in the neuroscience literature were evaluated using procedures established and detailed elsewhere (Ge et al., 2016; Lv et al., 2016; Zhao et al., 2016). Briefly, dictionary atoms corresponding to several resting state networks, in addition to DMN, were identified for each subject at baseline and post-intervention by determining which atom exhibited the greatest ORS with nine well-defined resting state network templates (visual medial, visual occipital, visual lateral, cerebellum, sensorimotor, auditory, executive control, right frontoparietal, and left frontoparietal), also provided by Smith et al. (2009). In the rare cases (< 1%) in which the same dictionary atom for a given subject was quantitatively identified as the best representation of more than one resting state network (e.g., DMN and executive control), the data point was excluded from analyses. Pearson correlation coefficients were then calculated between the time series of each subject's learned DMN atom and the subject's other nine learned resting state networks. An

aggregate *inter*-network connectivity score was created by averaging across these correlations. Paired-samples *t*-tests could then be applied to determine whether global *inter*-network connectivity changed over the course of the study, by group.

## Results

### Descriptive Statistics

Sample characteristics are presented by group in Table 4.1. While no statistically significant differences between the placebo and supplement groups were observed (*p*s all > .05), it should be noted that individuals receiving L and Z were somewhat older than controls (small-to-medium effect size).

### MPOD

To assess changes in L and Z levels associated with the intervention, MPOD values at baseline were compared to MPOD averaged across subsequent (8 and 12 month) time points (see Table 4.2). We elected to use an average to double the number of data points and thereby maximize the reliability of the measurement, particularly in light of the relatively small cell size corresponding to the control group ($n$ = 14). As expected, the L and Z intervention significantly enhanced MPOD in the supplement group over the course of the study [$t(33) = 2.82$, $p = .008$] and this increase was characterized by a large effect size. The control group did not show changes in L and Z status, also as expected [$t(13) = .05$, $p = .961$]. We note that the same pattern of results is obtained if the data are not averaged across the 8 and 12 month time points. That is to say, the supplement group showed a significant increase in MPOD at 12 months relative to baseline [$t(33)=2.51$, $p = .017$] while the placebo group's MPOD at 12 months does not differ from baseline ($p > .05$).

The correlation of L and Z status to demographic variables at baseline is presented in Table 4.3. MPOD was not significantly related to age, education, depressive symptomatology, or estimated intellectual functioning (*p*s all > .05).

**Effects of L and Z on Intrinsic Brain Activity**

Although all four candidate dictionary sizes (20, 50, 100, and 500) produced a clearly recognizable DMN, the 500 component solution was quantitatively selected for analyses given that it yielded the greatest ORS (0.52) with DMN as established in the neuroscience literature (Smith et al., 2009). Consistent with prior work (Lee et al., 2016), we observed a tendency for increased noise in DMN as our potential dictionary sizes decreased. This is illustrated in a comparison of the 500 component solution with the 20 component solution (ORS = .40; see Figure 4.1).

**DMN integrity**. To assess for possible intervention effects on DMN, ORS values are provided by group and time point (baseline versus post-intervention) in Table 4.2. The control group exhibited a slight, non-significant decrease in DMN integrity over the course of the study [$t(13) = .257$, $p = .801$]. Contrary to expectation, the supplement group also displayed a non-significant reduction in DMN correspondence [$t(33) = .691$, $p = .494$], which was characterized by a small effect size.

**Between network interactions**. With respect to changes in DMN interaction behavior with other resting state networks, the control group displayed a non-significant increase in aggregate *inter*-connectivity correlation scores [$t(13) = .361$, $p = .724$] of small effect size magnitude (see Table 4.2). The supplement group also exhibited an unexpected and statistically significant increase in between network interactions over the course of the study [$t(33) = 2.55$, $p = .016$]. The magnitude of this change was

characterized by a large effect size. Qualitatively, eight out of nine between network correlations showed an increase in the supplement group at post-intervention relative to baseline, with the exception of DMN-cerebellum. Only five *inter*-network correlations increased in the control group (DMN-visual occipital, DMN-visual lateral, DMN-cerebellum, DMN-sensorimotor, and DMN-left frontoparietal).

## Discussion

Twelve months of L and Z supplementation significantly influenced resting state functional connectivity in an older adult sample relative to placebo. The effects, however, were limited to the interaction behavior of DMN with other resting state networks and were actually in the opposite direction as hypothesized; rather than reducing *inter*-network connectivity, L and Z enhanced correlations of DMN to other functional networks. It is notable that the size of this effect was large in magnitude.

Reduced specificity of functional brain networks in older adults has been interpreted within the context of dedifferentiation theory, which holds that the aging brain becomes less functionally specialized (Baltes & Lindenberger, 1997). On a behavioral level, dedifferentiation has been supported by research showing that distinct cognitive processes become increasingly correlated in late life (Li et al., 2004). Neurally, older adults exhibit less selective activation in parietal, frontal, visual, and other brain areas during performance on various cognitive tasks (Carp, Park, Hebrank, Park, & Polk, 2011; Carp, Park, Polk, & Park, 2011; Kalkstein, Checksfield, Bollinger, & Gazzaley, 2011; Park et al., 2004). Dedifferentiating processes generally carry a negative connotation and have been proposed to reflect increased “neural noise” in information processing

resulting from age-related changes in dopaminergic modulation, ultimately leading to less distinct cortical representations (Li, Lindenberger, & Sikström, 2001).

To the extent that increased interaction behavior between DMN and other resting state networks reflects dedifferentiation, the present results may draw into question whether L and Z supplementation in some way accelerates neural aging or is otherwise unfavorable. This interpretation seems quite unlikely, however, given the steadily growing evidence base indicating a positive relation of L and Z to neurocognitive function (Feeney et al., 2013; Johnson et al., 2013; Renzi et al., 2008; Renzi et al., 2014; Renzi & Hammond, 2010; Vishwanathan et al. 2014), including in RCTs (Bovier, Renzi, & Hammond, 2014; Johnson et al., 2008). In addition, L and Z possess several properties with well-established benefits to brain health, including their anti-inflammatory and antioxidant characteristics (Bokov et al., 2004; Butterfield et al., 2010; Heneka et al., 2015; Rosano et al., 2012), as well as their positive effects on cellular membranes and communication (Bertram, 1999; Erdman et al., 2015; Krinsky et al., 2004; Stahl & Sies, 2001; Widomska & Subczynski, 2014). Moreover, the increased *inter*-network connectivity observed in the present study did not occur in a random fashion, as might be expected if merely reflective of neural noise from deteriorating neuromodulatory mechanisms (Li et al., 2001). Instead, *inter*-network correlations systemically increased over the course of the intervention.

As demonstrated elsewhere (e.g., Carp, Gmeindl, & Reuter-Lorenz, 2010), it is important to balance dedifferentiation with theories of compensation. The latter assert that older adults reorganize and recruit additional neural circuitry to compensate for neurodegenerative processes (Sala-Llonch et al., 2015). In this context, increases in

neural activity can actually be considered an adaptive response to age-related insult and various theories have emerged to characterize the recruitment pattern, such as Hemispheric Asymmetry Reduction in Older Adults (HAROLD; Cabeza, 2002), Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH; Reuter-Lorenz & Cappell, 2008), and Posterior-Anterior Shift with Aging (PASA; Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). Numerous studies have supported these compensatory models, showing for example that older adults display: bilateralization on tasks that tend to be lateralized earlier in the lifespan (e.g., Cabeza et al., 2004); disproportionate increases in brain activation relative to young adults as task difficulty increases (e.g., Spaniol & Grady, 2012); and enhanced frontal activity in response to sensory processing deficits in occipitotemporal regions (e.g., Dennis, Daselaar, & Cabeza, 2007). Overarching aging models such as the STAC argue that such processes of reorganization are an essential means by which the aging brain combats neurofunctional deterioration to preserve cognitive performance and maintain functional ability (Reuter-Lorenz & Park, 2014). This is exemplified by Tyler et al.'s (2010) findings that activation in right inferior frontal gyrus increased with gray matter loss in left inferior frontal gyrus among older adults during a syntactic processing task. Importantly, older adults' overt cognitive performance was comparable to a young adult control group, suggestive of an underlying compensatory process (Tyler et al., 2010). Similarly, Duzel, Schutze, Yonelinas, and Heinze (2011) observed hyperactivation of prefrontal and parietal regions in a subset of older adults who displayed overtly similar memory performance to young adults.

Taken together with the available literature, which strongly suggests a positive role of L and Z in promoting brain health, these micronutrients likely play a role in facilitating adaptive compensatory processes when consumed for a circumscribed period of time in late life. While the bulk of the research base on age-related compensatory processes has involved task-based fMRI, a handful of studies have shed light onto how compensation may operate in intrinsic brain activity. For example, it has been demonstrated that older adults with mild cognitive impairment who have high cognitive reserve display significantly greater global functional connectivity than counterparts with low cognitive reserve (Franzmeier et al., 2016). The authors speculated that greater global functional connectivity might permit more flexible engagement of alternative brain networks during cognitive performance, particularly when neurodegenerative processes lead to local damage within a specific network such as DMN. Increased between-network functional connectivity has also been observed in cognitively normal older adults relative to young adults and is typically interpreted as compensatory (Betzel et al., 2014; Geerligs et al., 2014). More recently, beta-amyloid burden was found to correlate with between-network connectivity in older adults in ways that were thought to reflect compensatory reorganization, particularly given that this sample maintained normal cognition despite underlying brain pathology (Elman et al., 2016).

Given the susceptibility of DMN to neurodegenerative processes, even in very early stages (Sheline & Raichle, 2013), it seems probable that functional reorganization in late life to increase support from other brain networks would be advantageous. Using a combination of structural and functional connectivity methods, Betzel et al. (2014) demonstrated that information flow occurs along shorter, more efficient paths earlier in

the lifespan while shifting to multi-step paths along less directly connected nodes in older adults to adaptively support functional communication in the face of deterioration (Betzel et al., 2014). L and Z may augment such compensatory rewiring to preserve DMN function by facilitating interaction with brain regions and systems that tend to be functionally segregated earlier in the lifespan.

Although this interpretation suggests a compensatory effect rather than our originally hypothesized return to a more efficient, "youth-like" pattern, it is actually consistent with the small number of studies that have used task-based fMRI to evaluate neural mechanisms underlying nutrition interventions in older adults. As examples, supplementation for four weeks with pomegranate juice (Bookheimer et al., 2013), three months with flavanols (Brickman et al., 2014), and six months with fish oil (Boespflug, McNamara, Eliassen, Schidler, & Krikorian, 2016) have all been found to increase BOLD signal relative to control conditions while simultaneously improving aspects of cognitive task performance. It should be kept in mind, however, that nutritional behaviors over the course of a lifetime may exert a different effect on brain function than circumscribed interventions in old age, potentially promoting neural efficiency rather than compensatory increases in activation. This concept has been elaborated previously (e.g., Renzi & Hammond, 2010) and likely accounts for cross-sectional findings from this study sample at baseline suggestive of neural efficiency, which we believe to represent long-term sequelae of lifestyle eating routines (see Lindbergh et al., 2016).

There are limitations to the present study that warrant consideration. The sample was entirely Caucasian, high functioning, and likely possessed considerable reserve, which prohibits conclusions regarding the potential effects of L and Z in a more diverse

and cognitively impaired older adult population. In addition, as with the majority of nutrition-based RCTs, the "control" group was likely exposed to the intervention to varying degrees given that L and Z are present in a range of foods and no attempts were made to regulate routine dietary habits. On the other hand, it is promising that L and Z were able to exert any influence on intrinsic brain activity in individuals who were presumably already quite neurocognitively healthy and nutritionally well-nourished; even larger effects might be expected in a sample with more room for improvement. Yet there may also be a point along the decline continuum when nutrition ceases to be beneficial, similar to findings that compensatory processes eventually become overwhelmed with excessive brain pathology (Dickerson et al., 2005), and it will be important for future research to address this question.

A widely acknowledged issue in the resting state literature involves defining the number of nodes or otherwise making decisions regarding how precisely to functionally divide the cortex, which may alter resulting findings (Power et al., 2011). In the present study, we elected to use a quantitatively driven approach based on which dictionary size best captured DMN within our computational framework. It is possible that a different number of atoms would have yielded a somewhat different pattern of results. However, it is encouraging that previous studies have reported similar findings across different parcellation sizes in older adults (Cao et al., 2014) and our methodology successfully produced several resting state networks that are well established in the literature. In addition, a major strength of the dictionary learning and sparse coding technique is its ability to characterize spatially overlapping brain networks that serve different functions,

such that the BOLD activities of a single brain region involved in multiple processes are not obscured as can occur in GLM-based approaches (Logothetis, 2008).

Despite its limitations, the present study constitutes the first attempt to shed light onto functional connectivity changes associated with L and Z supplementation and has the advantage of a yearlong RCT design. It appears our original hypothesis—that L and Z would reverse the neural effects of aging, ultimately leading to a more "youth-like" pattern of intrinsic brain activity—was not specifically supported. Instead of serving as a nutritional "fountain of youth," L and Z supplementation may facilitate the aging brain's capacity for neuroplastic compensation by promoting greater functional integration between DMN and other resting state networks. In a broader sense, these results add to the limited research base investigating the important relationship between nutrition and brain health, and are among the very first to employ resting state fMRI. Healthful dietary factors such as the carotenoids L and Z, which are relatively inexpensive and without known side effects, hold high potential to positively influence brain function. This appears to be true even when consumed for an isolated period of time in old age.

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Table 4.1

*Descriptive Statistics*

| | Placebo group ($n$ = 14) | Supplement group ($n$ = 34) | $p$ value ($t$-test or $\chi^2$) | Effect size (Cohen's $d$) |
|---|---|---|---|---|
| Age [$M$ (SD)] | 70.43 (5.43) | 73.06 (6.48) | 0.188 | 0.43 |
| Race (% Caucasian) | 100% | 100% | -- | -- |
| Sex (% female) | 71.43% | 52.94% | 0.390 | 0.25 |
| Education [$M$ (SD)] | 16.71 (3.02) | 16.26 (3.19) | 0.655 | 0.15 |
| WTAR [$M$ (SD)] | 115.29 (7.44) | 114.00 (8.22) | 0.615 | 0.16 |
| GDS [$M$ (SD)] | 1.71 (1.89) | 2.76 (3.42) | 0.285 | 0.35 |

*Note*. GDS = Geriatric Depression Scale. $M$ = mean. SD = standard deviation. WTAR = full-scale intelligence quotient predicted from the Wechsler Test of Adult Reading.

Table 4.2

*Intervention Effects on MPOD and Functional Connectivity*

| | Variable | Baseline [*M* (SD)] | Post [*M* (SD)] | *p* value (*t*-test) | Effect size (Cohen's *d*) |
|---|---|---|---|---|---|
| Placebo | MPOD | 0.441 (0.14) | 0.444 (0.19)* | 0.961 | 0.03 |
| (*n* = 14) | DMN ORS | 0.249 (0.03) | 0.247 (0.03) | 0.801 | 0.14 |
| | Inter-network *r* | 0.043 (0.08) | 0.056 (0.09) | 0.724 | 0.20 |
| Supplement | MPOD | 0.500 (0.21) | 0.571 (0.23)* | 0.008 | 0.98 |
| (*n* = 34) | DMN ORS | 0.262 (0.03) | 0.259 (0.03) | 0.494 | 0.24 |
| | Inter-network *r* | 0.025 (0.09) | 0.073 (0.09) | 0.016 | 0.89 |

*Note*. *M* = mean. MPOD = macular pigment optical density. SD = standard deviation. Post = immediately post-intervention (12 months). DMN ORS = overlap rate similarity with a well-established default mode network template (Smith et al., 2009). Inter-network *r* = aggregate correlation (Pearson's) of DMN activity with visual medial, visual occipital, visual lateral, cerebellum, sensorimotor, auditory, executive control, right frontoparietal, and left frontoparietal resting state networks. * = average of 8 month and 12 month (post-intervention) time points.

Table 4.3

*Zero-Order Bivariate Correlations*

| | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| *1*. Baseline MPOD | -- | -0.04 (.813) | 0.17 (.245) | 0.21 (.144) | -0.08 (.597) |
| *2*. Age | | -- | -0.07 (.661) | -0.24 (.107) | 0.03 (.848) |
| *3*. Education | | | -- | 0.64 (< .001) | 0.19 (.186) |
| *4*. WTAR | | | | -- | -0.01 (.942) |
| *5*. GDS | | | | | -- |

*Note.* Values are presented as Pearson's *r* (*p* value) for the overall sample at baseline (*N* = 48). GDS = Geriatric Depression Scale. MPOD = macular pigment optical density. WTAR = full-scale intelligence quotient predicted from the Wechsler Test of Adult Reading.

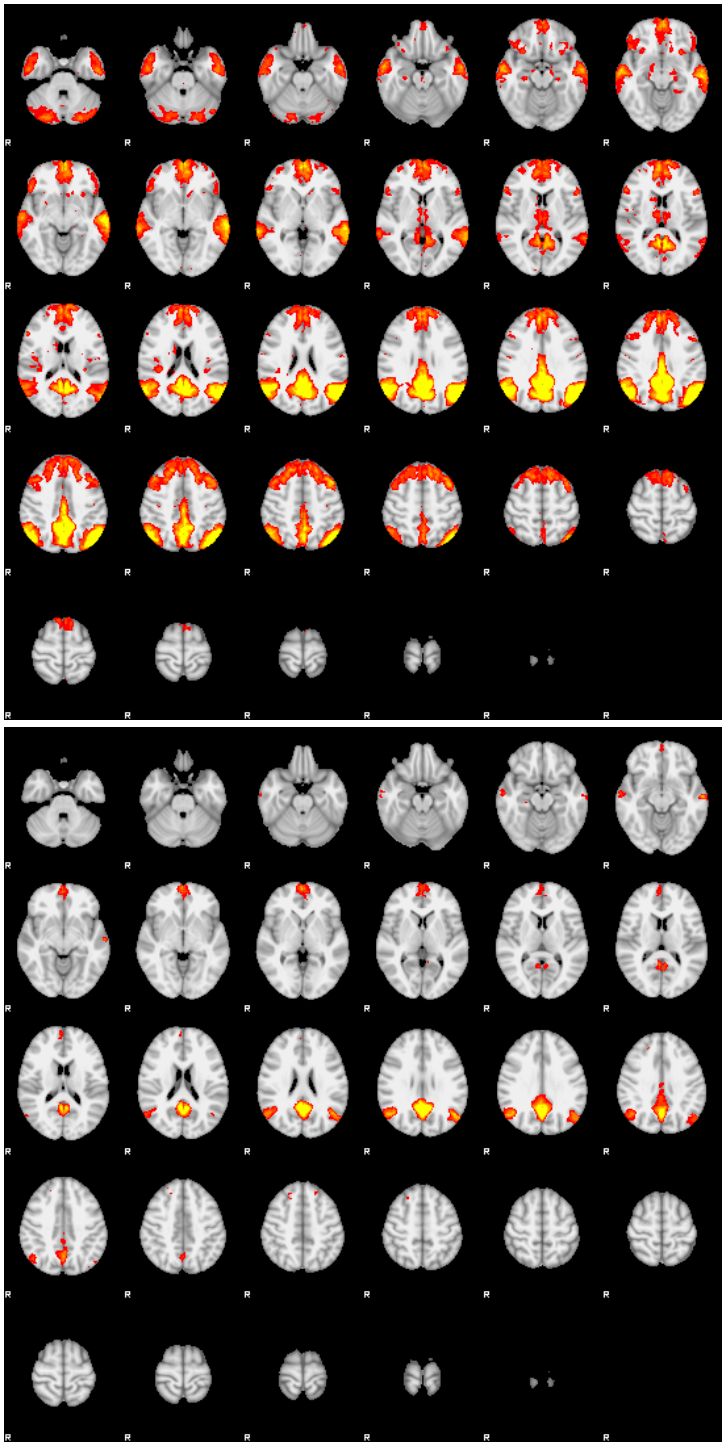

*Figure 4.1*. Default mode network (DMN) for two candidate dictionary sizes. Activity is averaged across all participants in the study sample at baseline and post-intervention. The 500 component solution (bottom panel) yielded the greatest overlap rate similarity (0.52) with DMN as established in the neuroscience literature (Smith et al., 2009) and was thus selected for use in our analyses. The 20 component solution (top panel) is presented for comparison purposes (overlap rate similarity = 0.40).

# CHAPTER 5

# GENERAL DISCUSSION

As average lifespans increase, the need to identify factors that promote neurocognitive health in old age has intensified (Canudas-Romo, DuGoff, Wu, Ahmed, & Anderson, 2016; Crimmins, Zhang, & Saito, 2016). A growing literature suggests a positive relationship of nutrition to cognition in late life, though less research is available on underlying neural mechanisms (Zamroziewicz & Barbey, 2016). The emergence of neuroimaging techniques, most notably magnetic resonance imaging (MRI), has provided a noninvasive means of shedding light onto how dietary factors may influence brain structure and function *in vivo*. Although the research base is limited, particularly with respect to functional MRI (fMRI), adherence to healthful eating behaviors has been shown to positively relate to various indices of structural (Mosconi et al., 2014; Pelletier et al., 2015; Raji et al., 2014; Scarmeas et al., 2011; Titova et al., 2013; Virtanen, Siscovick, Longstreth, Kuller, & Mozaffarian, 2008; Witte et al., 2014) and functional (Boespflug, McNamara, Eliassen, Schidler, & Krikorian, 2016; Bookheimer et al., 2013; Brickman et al., 2014) brain health in older adults. To the author's knowledge, no studies have used fMRI to investigate the relation between nutrition and resting state functional connectivity in an older adult population.

The overarching goal of the current research project was to extend the literature on the relation between nutrition and brain function in old age, focusing specifically on lutein (L) and zeaxanthin (Z), two xanthophyll micronutrients within the carotenoid

family (Britton, Liaaen-Jensen, & Pfander, 2004; Khachik, Beecher, Goli, & Lusby, 1991). Historically studied within the context of eye health to prevent age-related macular degeneration (Ma et al., 2012), L and Z have been found to preferentially accumulate in brain tissue in both primates and humans (Craft, Haitema, Garnett, Fitch, & Dorey, 2004; Vishwanathan, Neuringer, Snodderly, Schalch, & Johnson, 2013). Although the research base is primarily correlative in design, L and Z levels have related positively to various cognitive functions in older adults, including processing speed, task accuracy, prospective memory, executive functions, attention, visuospatial skills, language abilities, learning, recall, and global cognition (Feeney et al., 2013; Johnson et al., 2008; Johnson et al., 2013; Renzi, Dengler, Puente, Miller, & Hammond, 2014; Renzi, Iannacocone, Johnson, & Kritchevsky, 2008; Vishwanathan et al., 2014). It has been speculated that these beneficial cognitive effects are at least partly attributable to L and Z's strong antioxidant and anti-inflammatory properties, as well as their positive effects on cell membranes and cellular communication (Johnson, 2012). This fits well with findings that the aging brain is particularly susceptible to oxidative stress, chronic neuroinflammation, and adverse changes to neuronal membrane structure and function (Bokov, Chaudhuri, & Richardson, 2004; Rosano, Marsland, & Gianaros, 2012). In essence, L and Z may counter or buffer these deleterious age-related processes, ultimately promoting neurocognitive health.

The present set of experiments sought to further advance our knowledge of neural mechanisms underlying the relation of L and Z to cognition using both task-based and resting state fMRI as part of a yearlong, double-blind, placebo-controlled randomized controlled trial (RCT). The first study employed task-based fMRI and aimed to illuminate

neural changes associated with L and Z supplementation relative to placebo while participants performed a verbal learning and memory task. A secondary aim of this study was to replicate and extend prior literature showing a positive relation of L and Z to cognition by evaluating possible intervention effects on overt learning and recall performance. The second study aimed to investigate potential effects of L and Z supplementation on functional connectivity using resting state fMRI.

The results from each of these studies have been discussed previously in Chapters 3 and 4. Major themes are expanded upon and integrated below.

## Task-Based FMRI

A year of supplementation with L and Z was hypothesized to increase neural efficiency during cognitive task performance relative to placebo (i.e., the neural efficiency hypothesis; Renzi & Hammond, 2010). This expectation was supported by aspects of the Scaffolding Theory of Aging and Cognition (STAC; Park & Reuter-Lorenz, 2009) implying that older adults with less neurophysiological decline require less compensatory recruitment of supplementary neural circuitry (Josefsson, de Luna, Pudas, Nilsson, & Nyberg, 2012) and thus exhibit greater neurobiological efficiency (Duverne, Habibi, & Rugg, 2008). More specifically, L and Z's neuroprotective (e.g., antioxidant and anti-inflammatory; Izumi-Nagai et al., 2007; Sasaki et al., 2009) and cellular enhancing effects (Erdman et al., 2015) were expected to counter age-related deterioration of brain structure and function, ultimately leading to a more efficient, "youth-like" pattern of BOLD signal activation during verbal learning and recall. A beneficial effect on overt cognitive performance in terms of number of words successfully recalled on the fMRI-adapted task was also hypothesized.

Interestingly, although L and Z supplementation was found to significantly influence task-related brain activation relative to placebo, the effects were in the opposite direction as hypothesized. Instead of increasing neural efficiency, L and Z enhanced BOLD signal in left prefrontal areas during verbal learning. Importantly, older adults receiving the supplement also better maintained overt cognitive performance on the task over the yearlong course of the intervention relative to controls. Although the behavioral effect of L and Z was not statistically significant, possibly related to a lack of power, it was characterized by a large effect size.

On the surface, L and Z's effects on brain activation seem to contradict cross-sectional findings from this same sample at baseline (i.e., pre-supplementation), which yielded a negative correlation of endogenous L and Z levels—measured via blood serum concentrations and macular pigment optical density (MPOD)—to BOLD signal during verbal learning and recall (Lindbergh et al., 2016). As elaborated in Chapter 3, however, we believe that the cross-sectional findings of reduced BOLD signal and the intervention findings of increased BOLD signal are actually complementary. In fact, this apparent "discrepancy" may prove critical in shedding light onto how nutrition influences brain function over the course of a lifetime (chronic effects) versus within the context of a time-limited intervention in old age (acute effects).

To understand how the chronic and acute effects of nutritional components may result in different neural outcomes, it is helpful to revisit the STAC model (Park & Reuter-Lorenz, 2009), which has since been updated (STAC-r; Reuter-Lorenz & Park, 2014) to incorporate research findings that a variety of factors across the lifespan influence the structure and function of the brain in old age (e.g., Yaffe, Hoang, Byers,

Barnes, & Friedl, 2014). These "life-course variables" (Reuter-Lorenz & Park, 2014, p. 360) include factors such as education level, physical activity, and—of particular relevance to the present study—nutrition.

While both shared and distinct mechanisms likely account for the relation of different life-course variables to neurocognitive health, the concept of "brain maintenance" is particularly relevant to considering how the chronic effects of diet may differ from its acute effects. Originally described by Nyberg, Lövdén, Riklund, Lindenberger, and Bäckman (2012) and subsequently incorporated within the STAC-r framework, brain maintenance involves the idea that the primary determinant of successful aging is simply the absence of brain pathology at various levels of the central nervous system (e.g., cellular, neurochemical, white matter, gray matter, and neural network). In essence, older adults who are able to avoid age-related neural decline require less compensatory reorganization, display similar patterns of brain activity to younger adults, and ultimately exhibit preservation of cognitive and functional abilities (Nyberg et al., 2012; Reuter-Lorenz & Park, 2014). This notion has been supported empirically by studies demonstrating that subsets of older adults exhibit comparable cognitive performance to young adults along with similar patterns of underlying brain structure and function. For example, Duzel, Schutze, Yonelinas, and Heinze (2011) identified a subgroup of older adults who displayed comparable long-term memory recollection performance to young adults as well as similar underlying neural activation profiles. The more the older adults' pattern of brain activity deviated from that of young adults, the more likely these individuals were to display impairments in memory coupled with lower gray matter densities and abnormalities in default mode network (DMN). Similarly,

Nagel et al. (2009) demonstrated that "high performing" older adults on a spatial working memory task displayed a "youth-like" pattern of load-dependent increases in BOLD response whereas "low performing" older adults did not. While longitudinal research is limited, older adults who were able to maintain global cognition across an approximately 10-year span avoided neurodegeneration of gray and white matter in certain brain regions, including medial temporal lobe and cingulate cortex (Rosano, Aizenstein et al., 2012).

The concept of brain maintenance overlaps considerably with the concept of brain reserve advocated by Stern (2012). The fundamental distinction is that brain reserve focuses on explaining inter-individual variability in cognitive loss according to a pathology-to-reserve ratio using quantifiable brain properties, such as intracranial volume and dendritic density (Barulli & Stern, 2013). Brain maintenance, on the other hand, emphasizes the actual conditions and factors—both genetic and environmental (e.g., nutritional)—that operate to preserve brain structure and function during the aging process (Nyberg et al., 2012). In other words, brain reserve focuses on accounting for how older adults cope with brain pathology once present whereas brain maintenance focuses on how to minimize neurophysiological decline on the front end (Nyberg et al., 2012).

Consumption of L and Z over the course of the lifespan, either as dietary supplements or through a diet rich in green leafy vegetables, brightly colored fruits, and other foods containing these carotenoids, is speculated to promote brain maintenance. Within the context of the STAC-r, it is possible that chronic L and Z intake functions as "neural resource enrichment" (Reuter-Lorenz & Park, 2014, p. 361), preserving and

enhancing various aspects of brain health, such as cell membrane integrity, cortical thickness, and structural connectivity. Consequently, high lifetime consumers of L and Z may be less susceptible to age-related neurophysiological deterioration, require less compensatory scaffolding during cognitive task performance, and overall maintain a more youthful, "neurally efficient" biological profile.

To the extent that L and Z measured cross-sectionally in our sample at baseline reflect lifestyle eating behaviors, the observed negative correlation with BOLD signal in various brain regions during verbal learning and recall may be consistent with a brain maintenance effect (Lindbergh et al., 2016). Although longitudinal studies testing this hypothesis are lacking, indirect support is provided by findings that higher concentrations of L in plasma predicted a reduced risk of dementia, and presumably underlying neural decline as well, at 10-year follow-up in old age (hazard ratio = .808; $N$ = 1,092; Feart et al., 2015). In addition, adherence to a Mediterranean-style diet, which contains substantial amounts of L and Z (Djuric, Ren, Blythe, VanLoon, & Sen, 2009; Trichopoulou et al., 2003), has been longitudinally associated with a number of positive neurologic outcomes in older adults, including a 36% reduction in odds of cerebral infarct across a six year span (Scarmeas et al., 2011), larger total brain volume five years into the future (Titova et al., 2013), and white matter integrity preservation at an average follow-up of nine years (Pelletier et al., 2015).

Of course, the brain maintenance interpretation of our baseline findings rests heavily on the assumption that diet exhibits sufficient stability for a snapshot measurement, at a single point in time, to serve as a reliable proxy for long-term (i.e., lifespan) eating behaviors. There is some evidence to support this possibility, such as

longitudinal findings from a sample of 1,037 individuals who tended to maintain consistent food behavior across a 21-year period from childhood through adulthood (Mikkilä, Räsänen, Raitakari, Pietinen, & Viikari, 2005). It is further noteworthy that the baseline findings employed MPOD as one of the predictors in the analysis, which is generally considered a long-term indicator of dietary consumption because it requires L and Z to be incorporated into central nervous system tissue (Beatty, Nolan, Kavanagh, & O'Donovan, 2004; Hammond et al., 1997). Average MPOD has demonstrated considerable stability in adults (23-51 years old) assessed on a monthly basis for two years (Nolan et al., 2006). In intervention studies, MPOD remains elevated for 3-4 months following supplementation cessation (e.g., Hammond et al., 1997; Johnson et al., 2000; Landrum et al., 1997) and has even shown lagged increases for at least one month post-intervention (Berendschot et al., 2000). Serum L and Z, another predictor in our baseline analyses, admittedly shows greater temporal variability and is considered a more acute index of recent dietary intake (Beatty et al., 2004; Hammond et al., 1997). Still, cross-sectional research has indicated that healthy older adults do not significantly differ from young adults in serum L concentrations and metabolically show similar responses to L consumption (Cardinault et al., 2003). Collectively, these findings suggest the possibility that L and Z assessed at a single point in time would correlate with life-course consumption patterns.

In contrast to the pattern of neural efficiency observed in our sample at baseline, one year of L and Z supplementation significantly increased BOLD signal during verbal learning in left prefrontal areas, including aspects of dorsolateral prefrontal cortex and anterior cingulate cortex. Given that supplementation also appeared to help preserve overt

cognitive performance relative to placebo, consistent with positive cognitive outcomes in a previous RCT (Johnson et al., 2008), L and Z may facilitate compensatory activation when consumed acutely as part of a late life intervention. Based on the STAC-r, neural resource enrichment can occur in an ongoing fashion throughout the lifespan, but may dynamically manifest in different ways according to current neurological status and needs (Reuter-Lorenz & Park, 2014). Accordingly, while the chronic effects of L and Z across the life-course may promote brain efficiency, the acute effects in old age appear to enhance neural response during cognitive performance. This may serve to compensate for aspects of age-related structural and functional deterioration that have been found to occur even in healthily aging populations (Fjell & Walhovd, 2010; Grady, 2012).

The interpretation that late life L and Z supplementation serves a role in compensatory facilitation is consistent with other nutrition interventions that have similarly observed enhanced BOLD signal relative to control conditions. As examples, pomegranate juice (Bookheimer et al., 2013), flavanols (Brickman et al., 2014), and fish oil (Boespflug et al., 2016) for supplementation periods ranging in duration from one to six months have each been found to increase brain activation in various regions while improving some aspects of cognitive performance, including verbal recall, reaction time, and working memory. More broadly, L and Z's effects on brain function fit with compensatory aging models that have received considerable support in the literature, such as Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH; Reuter-Lorenz & Cappell, 2008) and Posterior-Anterior Shift with Aging (PASA; Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). Importantly, these models hold that increased activation, particularly in frontal regions—including dorsolateral prefrontal cortex (e.g.,

Turner & Spreng, 2012) and anterior cingulate cortex (e.g., Ansado, Monchi, Ennabil, Faure, & Joanette, 2012)—is an adaptive response to age-related neural insult that allows the brain to maintain performance during cognitively challenging tasks.

Taken together, the baseline (cross-sectional) and longitudinal task-based fMRI findings suggest that the chronic and acute effects of L and Z, and perhaps other nutritional factors as well, are different but complementary. L and Z, when consumed in high levels over the life-course, may reduce the likelihood of age-related neurodegeneration and the associated need for compensatory scaffolding, ultimately leading to greater brain efficiency in late life. By contrast, when L and Z intake is elevated for a discrete period of time in old age, these carotenoid micronutrients enhance neural response during cognitive performance, possibly facilitating the aging brain's capacity for adaptive compensatory engagement. The extent to which these two different pathways lead to similar cognitive outcomes in old age remains to be investigated. However, the aging literature provides evidence that older adults can arrive at similar cognitive phenotypes through different underlying neural processes (Barulli & Stern, 2013). For example, as noted previously, Duzel et al. (2011) observed a subgroup of older adults who displayed similar long-term memory recollection to young adults as well as a "youth-like" pattern of underlying brain activity, likely due to a generally preserved neurobiology. Yet, Duzel et al. (2011) also identified a different subgroup of older adults to display preserved long-term memory accompanied by hyperactivity of prefrontal and parietal brain areas relative to young adults. Similar results have been reported elsewhere (e.g., Cabeza et al., 2004; Dennis, Daselaar, & Cabeza, 2007; Spaniol & Grady, 2012). As discussed in the STAC-r (see Reuter-Lorenz & Park, 2014), findings such as these

indicate that older adults can maintain cognitive status either through brain maintenance or compensatory reorganization, at least until compensatory efforts become overwhelmed with excessive brain pathology (Dickerson et al., 2005). L and Z may aid in both of these processes depending on whether consumed chronically across the lifespan or acutely in old age.

## Resting State FMRI

Unlike task-based fMRI, resting state fMRI is acquired while subjects are in a passive but alert state rather than actively performing a task. Temporal correlations in BOLD signal fluctuations are measured to provide information about functional connectivity within and between spatially distributed and overlapping networks of brain regions (Fox & Raichle, 2007). Accordingly, resting state fMRI sheds light onto intrinsic brain functions on a more global scale to complement task-based approaches, which tend to focus on evoked activities in isolated neural structures (Baldassarre & Corbetta, 2015). Despite the potential of resting state fMRI to offer important information about how nutrition influences brain function (Zamroziewicz & Barbey, 2016), no studies to the author's knowledge have investigated the effects of dietary factors on functional connectivity in an older adult sample. The present study sought to fill this gap in the literature, focusing specifically on L and Z.

Similar to our expectations for task-based fMRI, it was hypothesized that one year of supplementation with L and Z would counter age-related neurodegenerative processes to restore a more "youth-like" pattern of resting state functional connectivity relative to placebo. More specifically, older adults receiving the supplement were expected to show improved *intra*-network integrity of DMN and reduced *inter*-network connectivity of

DMN to other resting state networks derived from the neuroscience literature (Smith et al., 2009).

Although L and Z supplementation did not significantly influence *intra*-network integrity of DMN, it did globally alter *inter*-network interactions between DMN and other resting state networks. The effect, however, was in the opposite direction as expected; L and Z increased correlative behavior of DMN to other functional networks in the order of a large effect size.

As elaborated in Chapter 4, one possible interpretation of this pattern of findings is that L and Z augment dedifferentiating processes within the aging brain (Baltes & Lindenberger, 1997). Dedifferentiation is generally considered to negatively impact cognition due to age-related deterioration in specificity of neural representations and associated increases in neural noise, possibly resulting from dopaminergic system miss-modulation (Abdulrahman, Fletcher, Bullmore, & Morcom, 2015; Li, Lindenberger, & Sikström, 2001). Given the steadily growing research base showing a positive relation of L and Z to cognition in older adults [for a review, see Maci, Fonseca, and Zhu (2016)], as well as the observation that supplementation seemed to buffer cognitive decline on the verbal learning task in the current sample, we believe that the increase in *inter*-network interactions reflects an adaptive compensatory brain response facilitated by L and Z. In this way, the effect of L and Z on resting state functional connectivity would be consistent with the effect observed using task-based fMRI, which we also interpret as compensatory.

Although aging models such as the STAC-r posit that compensatory scaffolding extends to functional connectivity as measured using resting state fMRI (Reuter-Lorenz

& Park, 2014), the majority of the available research on age-related compensation has focused on task-based fMRI findings. This is demonstrated in the considerable attention that recruitment models emphasizing task-evoked brain activity have garnered in the literature, such as Hemispheric Asymmetry Reduction in Older Adults (HAROLD; Cabeza, 2002), CRUNCH (Reuter-Lorenz & Cappell, 2008), and PASA (Davis et al., 2008). The neural signature for compensatory activity while the brain is at rest is less well studied and characterized. However, an emerging literature suggests that older adults show increased between-network functional connectivity relative to young adults (Betzel et al., 2014; Geerligs, Maurits, Renken, & Lorist, 2014; Jockwitz et al., 2017; Voss et al., 2010). While this pattern is often interpreted as compensatory in nature (e.g., Betzel et al., 2014; Geerligs et al., 2014; Jockwitz et al., 2017), the possibility that it reflects dedifferentiation has also been entertained (e.g., Voss et al., 2010). Indirect support for the compensatory interpretation is provided by findings that enhanced *inter*-network interactions are seen in older adults who maintain a cognitively normal status (Betzel et al., 2014; Geerligs et al., 2014). In addition, indices of global connectivity are greater among mildly impaired older adults who have high cognitive reserve relative to those with low reserve (Franzmeier et al., 2016). While few studies have directly correlated specific cognitive abilities with between-network connectivity profiles, a large population-based study ($N$ = 711) observed greater working memory difficulties to predict a HAROLD-like pattern of reduced laterality in frontoparietal resting state networks, consistent with a compensatory effect (Jockwitz et al., 2017).

Unfortunately, the current state of the literature does not allow definitive conclusions regarding whether increased *inter*-network interactions in old age reflect

compensation versus dedifferentiation, or perhaps some combination; longitudinal studies will be required to determine if between-network changes causally contribute to cognitive decline or represent an adaptive compensatory response to help maintain functioning in the face of age-related neural insult. Results from the present study favor the compensatory hypothesis given that L and Z, agents with known cognitive benefits and neuroprotective properties, increased interaction behavior between DMN and several other resting state networks.

Understandably, Betzel et al. (2014) found greater levels of *inter*-network interactions in older adults to be less neurally efficient than the more functionally segregated profiles seen in young adults due to reliance on longer-range, multi-step connections. Despite the additional cost in neural resources, however, there appear to be functional advantages. Cross-network correspondence may adaptively support information flow along alternative pathways (Betzel et al., 2014) and permit flexible engagement of additional networks during cognitive performance when primary networks become compromised by age-related deterioration (Franzmeier et al., 2016). This may in part explain findings that resting state connectivity, and perhaps the aging brain's more general capacity for plastic reorganization of neural networks, accounts for increasingly more variance in cognitive function with advancing age (Tsvetanov et al., 2016).

It is important to note that the control group also exhibited increases in aggregate correlative behavior between DMN and other resting state networks over the yearlong course of the study, though the effect size was small. This is consistent with the perspective that healthy older adults, even in the absence of nutritional (or other) intervention, naturally reorganize resting state networks with advancing age, likely as a

form of compensatory scaffolding to cope with age-related neural decline (Geerligs et al., 2014). It may be particularly important for DMN to solicit support from other networks, given its centrality to intact cognition and susceptibility to early forms of neurodegenerative processes, including in cognitively normal older adults (Sheline & Raichle, 2013). L and Z supplementation, then, might best be thought of as facilitating the aging brain's natural capacity for adaptive plasticity in intrinsic neural network behavior. This provides a resting state analogue to the compensatory effect seen during verbal learning on task-based fMRI.

## Conclusions

The results from the current set of experiments indicate that one year of supplementation with the carotenoids L and Z significantly influence brain function in older adults relative to placebo. Effects were apparent using both task-based and resting state fMRI methodologies. Interestingly, the nature of L and Z's impact on brain function was in the opposite direction as hypothesized; rather than increasing neural efficiency and restoring a more "youth-like" pattern of brain activity, L and Z appeared to enhance the aging brain's capacity for compensatory scaffolding. More specifically, task-based fMRI findings revealed that supplementation increased BOLD signal in left prefrontal areas, including aspects of dorsolateral prefrontal and anterior cingulate cortices, while buffering cognitive decline on a verbal learning and memory task. Although the influence on overt cognition was not statistically significant, it was characterized by a large effect size. In a complementary fashion, resting state fMRI revealed that L and Z supplementation increased aggregate interactive behavior between DMN and other resting state networks. Taken together, the task-based and resting state fMRI findings

suggest that L and Z, when consumed in the context of a late life intervention, do not function as a "fountain of youth" by reversing the neurobiological consequences of aging. Instead, these micronutrients appear to facilitate the older adult's natural ability to plastically reorganize brain activity in response to age-related neurodegeneration, supporting neural processing in ways that may ultimately be more functional even if somewhat less efficient.

Future research is warranted to more comprehensively map the observed neural effects of L and Z onto cognitive outcomes, using more sensitive measures and evaluating domains beyond verbal learning and recall. The use of structural MRI techniques, such as diffusion tensor imaging, will also be important to provide complementary data regarding possible changes in brain structure. Additional RCTs are recommended to evaluate neural mechanisms underlying other dietary factors that have shown promise in promoting successful aging (e.g., walnuts; Poulose, Miller, & Shukitt-Hale, 2014), as well as non-nutritional interventions, such as cognitive training and exercise programs (Duzel, van Praag, & Sendtner, 2016; Kelly et al., 2014). The potential for synergistic effects should also be considered, both at the cognitive and neural levels, including possible interactions with genetic factors (Nyberg et al., 2012). Longitudinal research would be beneficial to better understand how nutrients such as L and Z influence brain health across the lifespan, including the possibility of different effects in individuals of different ages and neurobiological statuses. More broadly, the aging literature would benefit from research that enables more confident distinction between dedifferentiation and compensation, particularly with respect to how these processes are manifested in intrinsic, "task-free" brain activity and are amenable to intervention.

With these considerations in mind, the present research project extends the evidence base on the relationship between nutrition and brain health in old age. L and Z, two carotenoid micronutrients with established benefits to cognition in the literature, appear to facilitate the aging brain's capacity for plastic reorganization in response to age-related neural decline. As organic pigments, L and Z are easy to access and without known side effects, which make them strong candidates for promoting neurocognitive health in the older adult segment of the population.

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