A ROLE FOR NUTRITION IN HEALTHY BRAIN AGING

BY

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DISSERTATION

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ABSTRACT

Nutritional cognitive neuroscience is an emerging interdisciplinary field of research that seeks to understand nutrition's impact on cognition and brain health across the life span. While accumulating evidence indicates healthy cognitive aging is dependent upon both brain health and nutritional status, the relationship between nutrition, cognition, and brain structure and function remains to be elucidated. Methods that sensitively measure variability in nutritional status, cognition, and brain health are critical to clarifying the role of nutrition in healthy brain aging. This dissertation therefore employs a multidisciplinary approach that integrates cutting-edge techniques from nutritional epidemiology and cognitive neuroscience. The experiments presented herein define mediatory relationships between nutrition, cognition, and underlying brain structure and function in a sample of healthy older adults. This research program investigates the role of nutrition in healthy brain aging, applying methods that examine: (i) specific nutrient biomarkers, and (ii) a broader profile of nutrient biomarker patterns. Chapters two through four investigate the role of individual nutrients on cognitive performance and identify specific brain regions whose structural integrity mediates this relationship. Chapters five through seven broaden the scope of this investigation to assess the role of nutrient biomarker patterns in cognition and to examine their influence of specific structural and functional brain networks. Taken together, the results of this research program suggest that specific nutrients and nutrient groups may slow or prevent aspects of age-related cognitive decline by influencing particular age-related changes in brain health. This line of research strives to elucidate the nature of healthy cognitive aging and ultimately inform clinical nutritional interventions for healthy brain aging.

ACKNOWLEDGEMENTS

Though the name on the title page of this dissertation suggests otherwise, this work is the culmination of the efforts of many. I'd like to thank my advisor, Aron Barbey, for shaping my scientific mind, providing the resources for my graduate training, and perhaps most importantly, for the opportunity to make discoveries. I am also deeply grateful for my other mentors – Neal Cohen, Ryan Dilger, Janet Jokela, and Art Kramer – for their support throughout this journey and their invaluable contributions to this work. Of course, these experiments would not have been possible without my fellow and former labmates, who spent countless hours with participants throughout the past several years – Joey Operskalski, Kelsey Campbell, Michael Kruepke, Jack Kuhns, Nikolai Sherepa, Evan Anderson, Mickeal Key, and Kelly Hewes. In addition, the guidance from present and former post-docs, namely Erick Paul, Rachael Rubin, and Chris Zwilling, has been indispensable. Finally, thank you to the many others who I have failed to mention – your contributions are not forgotten (even if not explicitly stated).

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Last but certainly not least; I cannot express the depth of my gratitude to my partner in life, and sometimes in science, Austin Mudd. With all due respect to those mentioned above, you are one of the best things with which UIUC has presented me. I also cannot conclude without thanking my family, both old and new, and especially my mother for her unconditional support; my wonderful friends, particularly Kelsey Hassevoort for the distractions from work, both food and fitness-related; and the wise advisor from long ago who suggested I embark on this long and winding but ultimately worthwhile journey.

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CHAPTER 1: INTRODUCTION[1]

Scientific and technological innovation continues to advance our understanding of human health and disease, with recent discoveries providing insight into the therapeutic potential of an essential component of life: *nutrition*. At the frontiers of this effort, the interdisciplinary field of nutritional cognitive neuroscience (Zamroziewicz and Barbey, 2016) demonstrates that age and disease-related decline in cognition depends not only upon degeneration in brain structure and function, but also on dietary intake and nutritional status. Discovering the ways in which particular nutrients and dietary components influence specific aspects of brain structure and function to support cognition will have profound implications for understanding healthy brain aging and for treating age-related neurological disease. Given the rapidly expanding aging population, a successful strategy to promote healthy brain aging is of great interest to public health efforts and the United States economy. Between 2012 and 2050, the United States will experience significant growth in its older population, with the size of the population aged 65 and over almost doubling from an estimated 43.1 million in 2012 to 83.7 million in 2050 (Ortman and Guarneri, 2009). Diet and the many bioactive substances present in food represent a novel target for intervention that may promote healthy brain aging.

This dissertation applies an integrative framework that aims to synthesize research in nutritional epidemiology and cognitive neuroscience, incorporating: (i) methods for the precise characterization of nutritional health based on the analysis of nutrient biomarkers, (ii) cognitive measures that assess core facets of general intelligence, and (iii) indices of brain health derived

[1] Sections of this chapter appear in *Frontiers in Neuroscience* and are referred to in this dissertation as "Zamroziewicz & Barbey, 2016." This article is reprinted under the terms of the Creative Commons Attribution License. Zamroziewicz, M.K., Barbey, A.K. (2016). Nutritional cognitive neuroscience: Innovations for healthy brain aging. *Frontiers in Neuroscience* 10(240), 1-10. doi: 10.3389/fnins.2016.00240.

from high-resolution magnetic resonance imaging (MRI) of brain structure and function. Each of these experimental methods is reviewed in turn below.

Nutritional intervention as a strategy to promote healthy brain aging

Emerging evidence in nutritional cognitive neuroscience indicates that optimal nutrition may serve as a potential avenue to preserve cognitive function, slowing the progression of aging and reducing the incidence of debilitating diseases in healthy aging populations. Observational studies indicate that particular nutrients have beneficial effects on brain aging (reviewed in Vandewoude et al., 2016). The work within this dissertation investigates single nutrients and nutrient groups believed to act on the aging brain (**Table 1.1**).

Biochemical markers of dietary intake

The identification of neuroprotective nutrients is dependent upon accurate and sensitive measurement of diet, and innovative techniques from nutritional epidemiology can help to achieve this goal. Traditional research in nutritional epidemiology has examined food intake on the basis of self-reported dietary assessment methods such as food frequency questionnaires, 24 hour recall, and weighed food records (Zuniga and McAuley, 2015). Although these methods can be implemented in large samples with relative ease, they are associated with measurement error. Primary sources of error include energy expenditure under-reporting, recall errors, and difficulty assessing portion sizes (Bingham, 2002; Kipnis et al., 2003). Furthermore, cognitive decline (e.g., memory loss) may limit recall on self-reported dietary assessments, and therefore undermine the efficacy of nutritional assessment in older adults (Reuter-Lorenz and Park, 2010; Zuniga and McAuley, 2015). In addition, self-reported dietary data are known to be influenced

by age, gender, socioeconomic status, and education (Thompson and Subar, 2013). Finally, self-reported dietary assessment methods fail to account for variability in nutrient absorption (Scalbert et al., 2014).

Biomarkers of dietary exposure have been developed to circumvent the measurement errors of dietary assessment techniques (Combs et al., 2013). Biomarkers can provide measures of nutritional status and exposure to bioactive molecules in foods, and thus can be used as surrogate indicators of food intake (Potischman and Freudenheim, 2003). Biomarker measurement also permits the identification of nutrient deficiencies and therefore allows treat-to-target paradigms, rather than global dietary approaches (Combs et al., 2013).

Biomarkers can be analyzed from blood, urine, or tissue. In the context of this dissertation, all biomarkers are measured in blood plasma or serum. The concentration of a given marker reflects intake of a particular dietary component (Jenab et al., 2009; Zuniga and McAuley, 2015). Epidemiological studies have identified approximately 100 biomarkers that correlate with dietary intake, of which 33 are investigated in this work (**Table 1.2**; reviewed in Scalbert et al., 2014). These 33 biomarkers can be measured to estimate intake of a wide range of dietary components, including overall fruit and vegetable intake (Baldrick et al., 2011; Mennen et al., 2006), citrus fruits (Heinzmann et al., 2010; Lloyd et al., 2011a), cruciferous vegetables (Andersen et al., 2014; Edmands et al., 2011), salmon (Lloyd et al., 2011b), red meat (Cross et al., 2011; Stella et al., 2006), soy (Verkasalo et al., 2001), whole grain cereals (Andersson et al., 2011; Ross et al., 2012), coffee (Nagy et al., 2011; Rothwell et al., 2014), tea and wine (Hodgson et al., 2004; Mennen et al., 2006), food additives (Brantsæter et al., 2009), and food contaminants (Turunen et al., 2010). As a complement to self-reported methods, biochemical analyses of nutrient biomarkers can improve data validity by providing an objective and sensitive

assessment of a wide range of dietary components (Elmadfa and Meyer, 2014).

Nutrient biomarker patterns

Scientific advances in the characterization of dietary patterns and the analysis of nutrient biomarkers have led to new methods in nutritional epidemiology for the measurement of nutrient biomarker patterns. This approach applies principal component analysis to capture the effects of nutrients in combination, enabling discovery of patterns of nutrient biomarkers. This method detects nutrient biomarker patterns in plasma and therefore avoids methodological problems inherent in traditional food frequency questionnaires, such as faulty recall of dietary intake (Scalbert et al., 2014). Each nutrient biomarker pattern represents a linear combination of individual plasma nutrients that load heavily within each pattern. Each participant receives a standardized nutrient biomarker pattern score for each pattern, and this score can subsequently be used to assess the relationship between nutrient patterns, cognitive function, and brain health. Early applications of this method have revealed multiple nutrient patterns that influence cognition and brain aging, including a nutrient biomarker pattern composed of antioxidants C and E, B vitamins, and vitamin D associated with enhanced global cognitive function; and a nutrient biomarker pattern consisting of omega-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaeonic acid associated with white matter integrity (Bowman et al., 2012).

Age-related changes in cognitive health and the protective role of nutrition

A hallmark of cognitive aging is the deterioration of specific facets of intelligence during particular stages of life. For example, the capacity for adaptive reasoning and problem solving (also known as fluid intelligence) decreases systematically with age, whereas general semantic

knowledge (also known as crystalized intelligence) is largely preserved in older adulthood (**Figure 1.1**; Horn and Cattell, 1967; Schaie, 1994). Cognitive abilities that are known to decline with age, such as fluid intelligence, therefore provide an index of the rate of decline with age and enable the forecasting of cognitive impairments and susceptibility to clinical dysfunction with age (Johnson et al., 2007). A growing body of evidence suggests that certain nutrients may slow or prevent aspects of age-related decline (Bowman et al., 2013; Gu et al., 2016), and the incorporation of sensitive, neuropsychological assessments may aid in further delineating these relationships. **Table 1.3** details prior evidence which demonstrates the relationship between the nutrients and nutrient groups of interest and facets of intelligence.

Age-related changes in brain structure and function and the protective role of nutrition

Magnetic resonance imaging (MRI) enables the study of structural and functional brain changes that are associated with aging and the prediction of neuropathological processes in the aging brain (Buckner, 2004). Even within cognitively normal brains, neurodegenerative processes can be present and measured using MRI (Mungas et al., 2002; Rusinek et al., 2003; Wilson et al., 1999; **Figure 1.2**). Therefore, MRI can be applied to assess age-related changes in the brain, as well as the role of nutrients and nutrient patterns in slowing or preventing these changes.

Age-related changes in brain structure and the protective role of nutrition

Structural MRI enables high-resolution imaging of age-related changes in gray and white matter structure, for example, including: (1) total and regional brain volume (volumetry) and (2) integrity of white matter fiber tracts (diffusion tensor imaging). Application of these methods has

revealed the heterogeneous nature of brain aging. Although atrophy across the whole brain is evident with aging, these changes vary by region and tissue type.

Differential effects of aging are particularly evident in the cerebral cortex, in which the superior frontal, middle frontal, and superior parietal cortex are most susceptible to steady age-related atrophy (Lockhart and DeCarli, 2014). Other cortical regions have fluctuating rates of change, with some areas showing accelerated atrophy early in aging, others demonstrating accelerated atrophy late in aging, and others showing a combination of early and late acceleration (Claassen et al., 2016; Lockhart and DeCarli, 2014). Subcortically, the caudate nucleus, cerebellum, and hippocampus show susceptibility to age-related structural degeneration (Raz, 2005). Particular nutrients and nutrient groups may slow or prevent these age-related changes in gray matter (**Table 1.4**).

Another common age-related disruption to brain tissue is the deterioration of cerebral white matter, which occurs in the form of local lesions and long-range tract integrity loss (Lockhart and DeCarli, 2014). As white matter degenerates, the physical connections between brain regions are damaged, therefore resulting in reduced structural connectivity (Betzel et al., 2014). Water diffusion along white matter tracts can be measured to determine white matter integrity, with high directionality and low magnitude of water movement indicating preserved white matter structure (Filippi et al., 2013). These changes are thought to occur on an anterior to posterior gradient, wherein frontal regions show the greatest age-related decline, and a superior to inferior gradient, wherein superior tracts show the greatest age-related decline (Betzel et al., 2014; Sridharan et al., 2014; Storsve et al., 2016). Just as in gray matter, certain nutrients and nutrient groups may slow or prevent these age-related changes in white matter (**Table 1.4**).

Age-related changes in brain function and the protective role of nutrition

Functional neuroimaging (fMRI) has demonstrated that age-related decline in cognitive processes begins early – even when the prevalence of concomitant disease is low (Park and Reuter-Lorenz, 2009). Functional MRI measures the ratio of oxygenated to deoxygenated hemoglobin in the blood as a marker of change in neural activity related to cognitively demanding tasks or at rest (Lockhart and DeCarli, 2014). These changes in brain activity are known to reflect alterations in underlying neurotransmission and brain structure that are concentrated in the prefrontal and temporal cortices (Tomasi and Volkow, 2012).

Age-related changes in brain activity have been characterized in several ways - greater activity in prefrontal cortical regions and weaker activity in posterior regions (Davis et al., 2008; Stuss and Knight, 2013); reduced asymmetry in activity of the prefrontal cortex (Cabeza 2002); and changes in functional connectivity, which refers to the interactions between regions that work together as networks (Lockhart and DeCarli, 2014). Importantly, rather than changing interactions across lobes of the brain in a homogenous way, aging shows the strongest effects on functional connectivity between and within particular networks (Lockhart and DeCarli, 2014). Networks that are central to self-referential processing, cognitive control, and attention, consistently show decline in healthy aging (Betzel et al., 2014; Marstaller et al., 2015). Functional connections between regions within these networks are reduced with aging, suggesting that regions that work together become more weakly coupled and therefore decline in their ability to coordinate cognitive function (Andrews-Hanna et al., 2007). In addition, functional networks become less differentiated with age, as evidenced by decreased connectivity within networks (i.e., intranetwork connectivity) and increased connectivity between networks (i.e., internetwork connectivity; Betzel et al., 2014; Geerligs et al., 2015). Changes in neural

activity do not linearly relate to chronological age, and may therefore be mediated by environmental factors, such as diet (Geerligs et al., 2015). The handful of studies published on this topic indicate that particular dietary components may slow age-related changes in neural activity of specific regions, as measured by task-based fMRI, and functional connectivity across networks in the brain, as measured by resting-state fMRI (**Table 1.4**).

Limitations in understanding the impact of nutrition on the brain

Although evidence for the protective effects of nutrition on age-related cognitive decline remains promising, the findings to date are predominantly inconclusive. The inconsistencies are thought to arise from the limitations of previous work, including choosing sample populations that are not positioned to benefit from nutritional intervention, failure to sensitively measure effects of particular nutrients or nutrient patterns, failure to measure domain-specific declines in cognition, and failure to sensitively measure changes in brain health (Monti et al., 2015; Sheats et al., 2009).

An interdisciplinary approach to studying nutrition's impact on healthy brain aging

Accumulating evidence indicates that the effects of nutrition on brain health are complex and multifactorial, reflecting the influence of particular nutrients and nutrient patterns on specific aspects of brain aging. However, the relationship between nutrition and brain health has yet to be elucidated. Methods that sensitively measure individual variability in nutritional status, cognition, and brain health will pave the way toward understanding how nutrition can be used to benefit brain aging (Zamroziewicz and Barbey, 2016). Healthy older adults may provide key

information about the nutrients and nutrient patterns that underlie healthy brain aging (Monti et al., 2015) and are therefore the focus of this dissertation research.

This research program examines two key issues: (i) the relationships between particular nutrients and specific features of cognitive and brain health, and (ii) the relationships between nutrient patterns and cognition, along with the underlying structural and functional networks. In each experiment, a formal mediation analysis is conducted to model the three-way relationship between nutrition, cognition, and brain health, with the ultimate goal of evaluating whether features of brain health mediate the relationship between particular nutrients or nutrient groups and specific aspects of cognition (**Figure 1.3**).

This line of research addresses the following previously identified methodological limitations within nutritional neuroscience (Monti et al., 2015; Sheats et al., 2009; presented as limitation, approach):

1. Improper sample selection, by studying healthy adults in which nutrition is used to maintain health, rather than treat pathological disease.
2. Insensitive nutritional measures, by measuring blood biomarkers to study the effects of single nutrients and interactive nutrient patterns.
3. Coarse cognitive assessment, by measuring specific domains of cognition using standardized neuropsychological tests.
4. Insensitive neuroscience measures, by using high-resolution MRI to study the brain structures and functions that underlie cognitive performance.

Ultimately, this work aims to inform the development of nutritional interventions for healthy brain aging that account for individual variability in nutritional status, cognitive capacity, and

brain health (**Figure 1.4**). The final portion of the introduction provides an overview of the individual experiments carried out in light of these goals.

Prelude

Chapters two through seven provide reports on six completed experiments that aim to examine the role of nutrition in health brain aging. More specifically, chapters two through four explore the relationship between particular nutrients and specific features of brain aging, whereas chapters five through seven investigate the role of nutrient patterns in supporting structural and functional connections across the brain. Finally, chapter eight provides a discussion of the six experiments along with directions for future research.

Although evidence suggests that diet has a substantial influence on the aging brain, the question of how particular nutrients might relate to specific aspects of cognitive decline remains unanswered. Chapters two through four investigate the neural mechanisms that mediate the relationship between single nutrients and specific aspects of cognition in cognitively intact older adults. Chapter two identifies specific mediatory factors between nutrition, cognition, and brain health, demonstrating a novel structural mediation between plasma omega-3 polyunsaturated fatty acid levels and cognitive flexibility in 40 cognitively intact older adults (65 to 75 years old) with the APOE e4 polymorphism (Zamroziewicz et al., 2015). Chapter three provides further evidence that specific nutrients may slow or prevent decline in executive function by supporting brain structure, establishing a novel structural mediation between plasma phosphatidylcholine levels and cognitive flexibility in 72 cognitively intact older adults (65 to 75 years old; Zamroziewicz et al., 2016b). Lastly, chapter four demonstrates that these mediatory relationships are not limited to executive function by reporting a novel structural mediation between serum

lutein and crystallized intelligence in 75 cognitively intact older adults (65 to 75 years old; Zamroziewicz et al., 2016a). These three chapters demonstrate that the structure and function of particular brain regions supports the relationship between specific nutrients and cognitive function.

Accumulating evidence indicates that the effects of nutrition on brain health are complex and multifactorial, reflecting the influence of particular nutrient combinations on specific aspects of brain aging. Nutritional epidemiology has shown that diets are composed of many nutrients that have interactive effects, and has developed methods for deriving nutrient patterns that capture the robust effects of nutrient interactions. Cognitive neuroscience has shown that brain aging is a heterogeneous process characterized by widespread changes in structure and function, and has developed neuroimaging methods to measure these changes with high-resolution. Predictive nutrient patterns of healthy brain aging will emerge from the integration of methods that sensitively capture variability in both diet and brain aging (Zamroziewicz and Barbey, 2016). Thus, chapters five through seven examine the role of nutrient patterns in cognition and integrity of underlying structural and functional networks. In these chapters, nutrient patterns are measured using nutrient biomarker pattern analysis to capture the effects of nutrient biomarkers in combination. Networks in the brain are measured in terms of structure (i.e., gray matter structure of brain networks and white matter integrity across brain regions) and in terms of function (i.e., functional connectivity). Chapter five demonstrates novel evidence for particular omega-3 polyunsaturated fatty acid patterns in supporting fluid intelligence and its underlying structural gray matter circuit in 100 cognitively intact older adults (Zamroziewicz et al., 2017). Chapter six builds upon prior work to depict the relationship between patterns of omega-3 and omega-6 polyunsaturated fatty acids, memory, and underlying structural connectivity, as

measured by white matter integrity in 94 cognitively intact older adults (Zamroziewicz et al., 2018). Finally, chapter seven shows that patterns of monounsaturated fatty acids play a role in promoting general intelligence, and for the first time, that functional connectivity within a critical brain network may support the relationship between nutrition and cognition in 99 cognitively intact older adults. These three chapters demonstrate that the structure and function within brain networks supports the relationship between particular patterns of nutrients and cognitive function.

Taken together, this research program demonstrates that certain nutrients and nutrient groups may influence aspects of cognition by affecting particular features of brain health. This line of work strives to elucidate the role of nutrition in healthy brain aging and ultimately inform clinical nutritional interventions for healthy brain aging.

Tables and Figures

Table 1.1. Nutrient mechanisms: Nutrients and nutrient groups of interest and proposed mechanisms of action in brain

Dietary components	**Mechanisms**
Xanthophylls	Prevent oxidation (Widomska and Subczynski, 2014); prevent inflammation (Miller et al., 2014); influence membrane properties (Stahl and Sies, 2001)
Choline	Influences neuronal membrane properties; methyl donation; cholinergic transmission; lipid transport (reviewed in Zeisel, 2006)
Omega-3 polyunsaturated fatty acids	Produce anti-inflammatory eicosanoids; promote membrane fluidity; support neurotransmission; potent ligands for nuclear receptors (Schmitz and Ecker, 2008)
Omega-6 polyunsaturated fatty acids	Compete with omega-3 polyunsaturated fatty acids in terms of production and function (Schmitz and Ecker, 2008)
Monounsaturated fatty acids	Support metabolic function (Sartorius et al., 2012; Vessby et al., 2001)
Saturated fatty acids	Impair metabolic function (Tschritter et al., 2009)

Table 1.2. Nutrient biomarkers: Nutrients and nutrient groups of interest as measured by biomarkers in blood plasma and serum in this work

Nutrient category	**Nutrient biomarker**
Xanthophylls	Lutein
Choline metabolism	Phosphatidylcholine
Omega-3 polyunsaturated fatty acids	α-Linolenic acid (18:3n-3)
	Stearidonic acid (18:4n-3)
	Eicosatrienoic acid (20:3n-3)
	Eicosapentaenoic acid (20:5n-3)
	Docosapentaenoic acid (22:5n-3)
	Docosahexaenoic acid (22:6n-3)
Omega-6 polyunsaturated fatty acids	Linoleic acid (18:2n-6)
	γ-Linolenic acid (18:3n-6)
	Eicosadienoic acid (20:2n-6)
	Dihomo-γ-linolenic acid (20:3n-6)
	Arachidonic acid (20:4n-6)
	Docosadienoic acid (22:2n-6)
	Adrenic acid (22:4n-6)
Monounsaturated fatty acids	Myristoleic acid (14:1)
	cis-7-Hexadecenoic acid (16:1n-9)
	Palmitoleic acid (16:1n-7)
	Oleic acid (18:1n-9)
	cis-Vaccenic acid (18:1n-7)
	Gondoic acid (20:1n-9)
	Erucic acid (22:1n-9)
	Nervonic acid (24:1n-9)
Saturated fatty acids	Capric acid (10:0)
	Lauric acid (12:0)
	Myristic acid (14:0)
	Pentadecylic acid (15:0)
	Palmitic acid (16:0)
	Stearic acid (18:0)
	Arachidic acid (20:0)
	Behenic acid (22:0)
	Lignoceric acid (24:0)

Table 1.3. Nutrition and cognition: Summary of evidence examining role of nutrients and nutrient groups of interest in cognitive changes associated with brain aging

Cognition	Nutrient
Global cognition	Xanthophylls (Feeney et al., 2013, 2017; Johnson et al., 2013; Kesse-Guyot et al., 2012; Renzi et al., 2014; Vishwanathan et al., 2014a) Choline (Klavins et al., 2015; Mapstone et al., 2014; Schaefer et al., 2006) Omega-3 polyunsaturated fatty acids (Baierle et al., 2014; Eriksdotter et al., 2015; Nishihira et al., 2016) Monounsaturated fatty acids (Baierle et al., 2014; Naqvi et al., 2011; Solfrizzi et al., 1999, 2005) Saturated fatty acids (negative effect) (Baierle et al., 2014; Limbers and Young, 2015)
Executive functions	Xanthophylls (Feeney et al., 2013, 2017; Kesse-Guyot et al., 2013; Vishwanathan et al., 2014a) Choline (Nurk et al., 2013; Zamroziewicz et al., 2016b) Omega-3 polyunsaturated fatty acids (Baierle et al., 2014; Bowman et al., 2013; Zamroziewicz et al., 2015) Omega-3:omega-6 polyunsaturated fatty acid ratio (Baierle et al., 2014) Monounsaturated fatty acids (Baierle et al., 2014)
Intelligence (general, fluid, crystallized)	Xanthophylls (Johnson et al., 2008a; Kelly et al., 2015; Kesse-Guyot et al., 2012; Vishwanathan et al., 2014a) Omega-3 polyunsaturated fatty acids (Tan et al., 2012)
Memory	Xanthophylls (Feeney et al., 2013, 2017; Johnson et al., 2013; Kelly et al., 2015; Kesse-Guyot et al., 2014; Renzi et al., 2014; Vishwanathan et al., 2014a) Choline (Simpson et al., 2015) Omega-3 polyunsaturated fatty acids (Baierle et al., 2014; Yuan et al., 2016) Omega-3:omega-6 polyunsaturated fatty acid ratio (Baierle et al., 2014) Monounsaturated fatty acids (Baierle et al., 2014) Saturated fatty acids (negative effects; Baierle et al., 2014; Eskelinen et al., 2008; Oleson et al., 2016; Yuan et al., 2016)
Processing speed and attention	Xanthophylls (Feeney et al., 2013, 2017; Renzi et al., 2014) Choline (Naber et al., 2015; Nurk et al., 2013)

Table 1.4. Nutrition and brain health: Summary of evidence examining role of nutrients and nutrient groups of interest in structural and functional changes associated with brain aging

Neural component	**Dietary component**	**Neural component**	**Dietary component**
Whole brain measures		*Regional measures*	
Brain volume	Omega-3 polyunsaturated fatty acids (Tan et al., 2012)	Temporal cortex volume	Omega-3 polyunsaturated fatty acids (Conklin et al., 2007) Xanthophylls (Zamroziewicz et al., 2016a)
Cortical thickness	Choline (Zamroziewicz et al., 2016b)	Cingulate cortex volume	Omega-3 polyunsaturated fatty acids (Zamroziewicz et al., 2015)
Structural connectivity	Omega-3 polyunsaturated fatty acids (Chhetry et al., 2016; Witte et al., 2014) Omega-6 polyunsaturated fatty acids (Gu et al., 2016)	Frontal cortex volume	Omega-3 polyunsaturated fatty acids (Conklin et al., 2007)
Functional connectivity	Omega-3 polyunsaturated fatty acids (Grayson et al., 2014)	White matter lesions	Omega-3 polyunsaturated fatty acids (Bowman et al., 2013; Tan et al., 2012) Choline (Poly et al., 2011) Monounsaturated:saturated fatty acid ratio (Gardener, 2012)
		Neural activity	Omega-3 polyunsaturated fatty acids (Boespflug et al., 2016) Xanthophylls (Lindbergh et al., 2017) Monounsaturated fatty acids (Dumas et al., 2016; Sartorius et al., 2012) Saturated fatty acids (Sartorius et al., 2012)

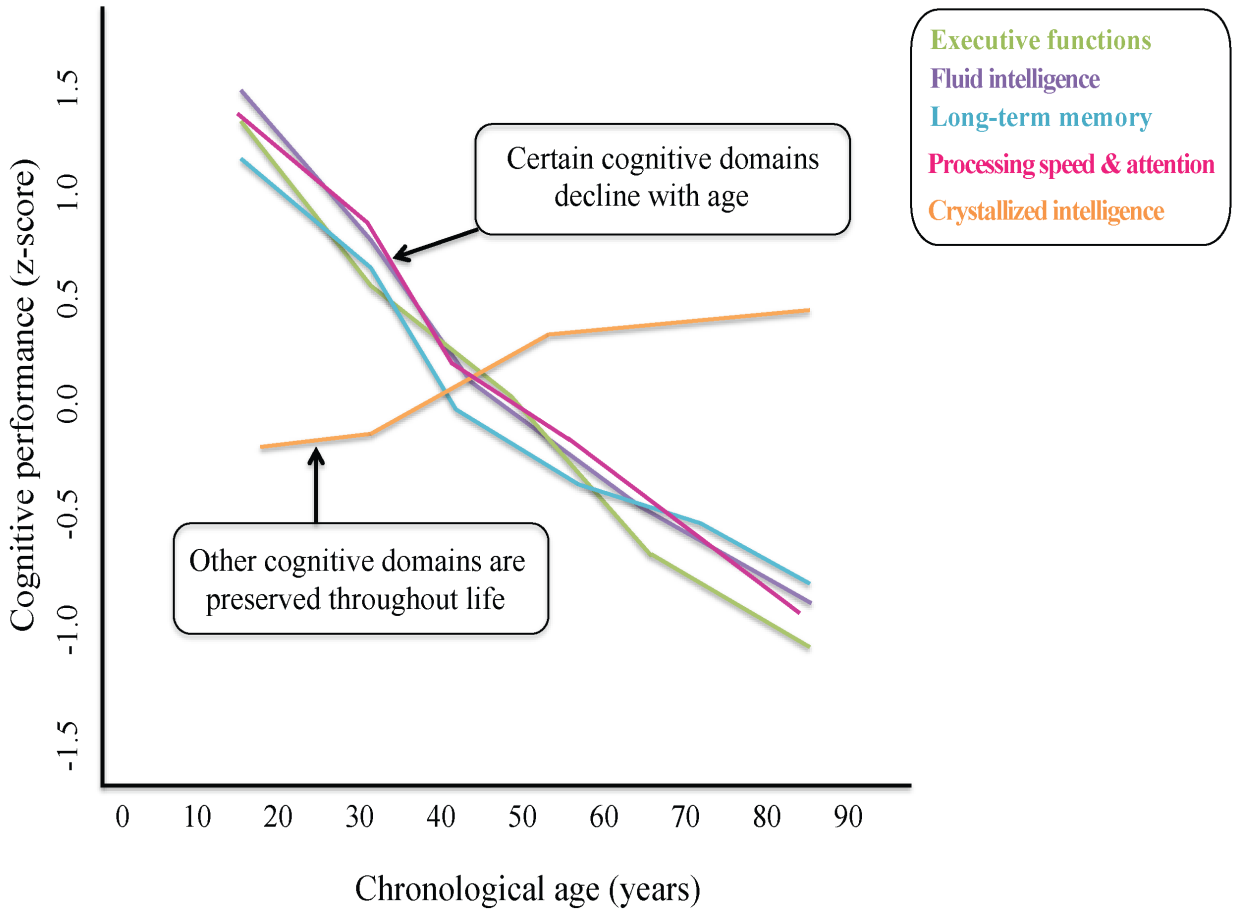

Figure 1.1. Age-related changes in cognition: Theories of cognitive decline demonstrate that cognitive function does not deteriorate uniformly across the lifespan, and the study of neuroprotective nutrients may benefit from implementation of this theoretical approach. It is well-known that crystallized intelligence, also known as general or semantic knowledge, is well-maintained into the sixth, seventh, and even later decades of life. Conversely, cognitive domains such as the executive functions, fluid intelligence, long-term memory, processing speed, and attention show approximately linear decreases with age. Therefore, the measurement of dysfunction in aspects of cognition that show steady, age-related decline, even when measurable deficits in general cognitive function are absent, may capture the continuum of normal aging or preclinical stages of dementia in a more sensitive manner. Adapted from Park and Reuter-Lorenz, 2009.

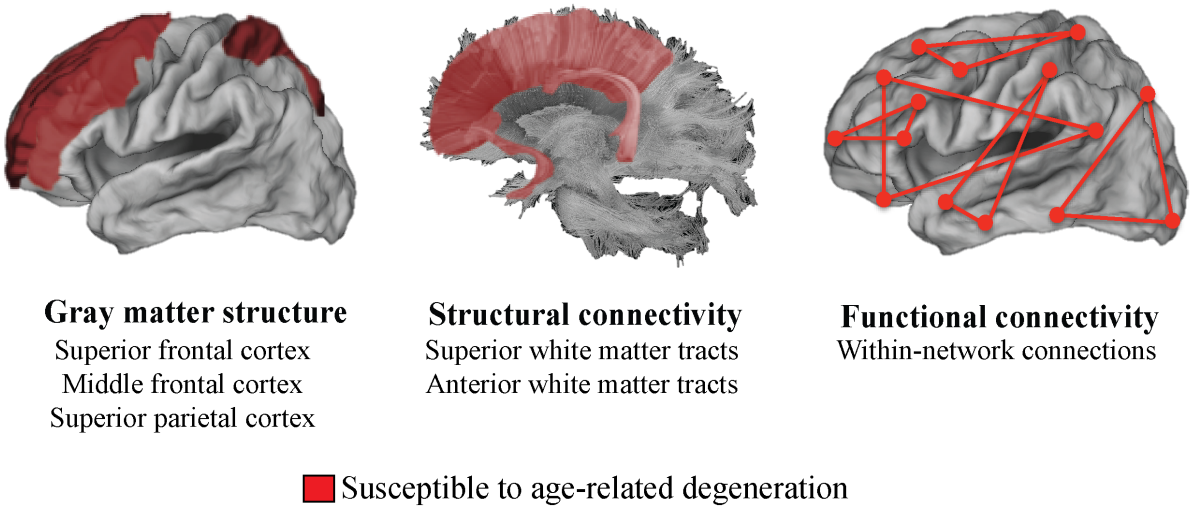

Figure 1.2. Age-related changes in brain health: Decline in brain structure and function does not occur uniformly across the lifespan. Gray matter atrophy varies by region, with areas such as the superior frontal, middle frontal, and superior parietal cortex showing susceptibility to early age-related atrophy. Structural connectivity, reflecting white matter tract integrity, varies by region, with superior and anterior regions showing susceptibility to age-related degeneration. In addition, functional connectivity between regions that work together as networks changes as a function of age, and is characterized by a weakening of within-network connections.

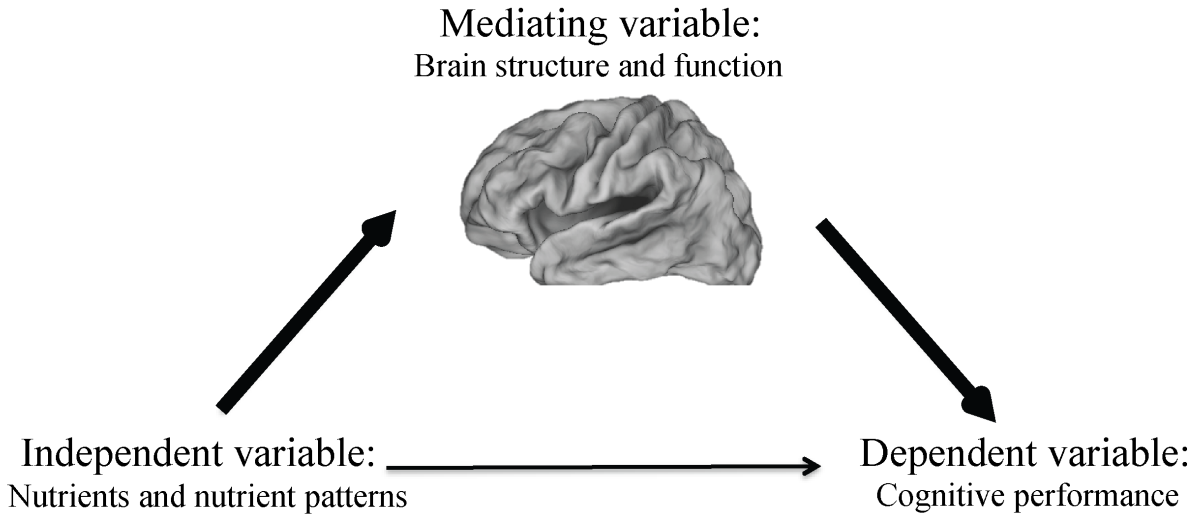

Figure 1.3. Mediation models: Mediation models are implemented to investigate the three-way relationship between nutrition, cognition, and brain health. The primary requirement for mediation is a significant indirect mediation effect, defined as the effect of the independent variable (nutrients and nutrient patterns) through the mediator (brain structure and function) on the dependent variable (cognitive performance).

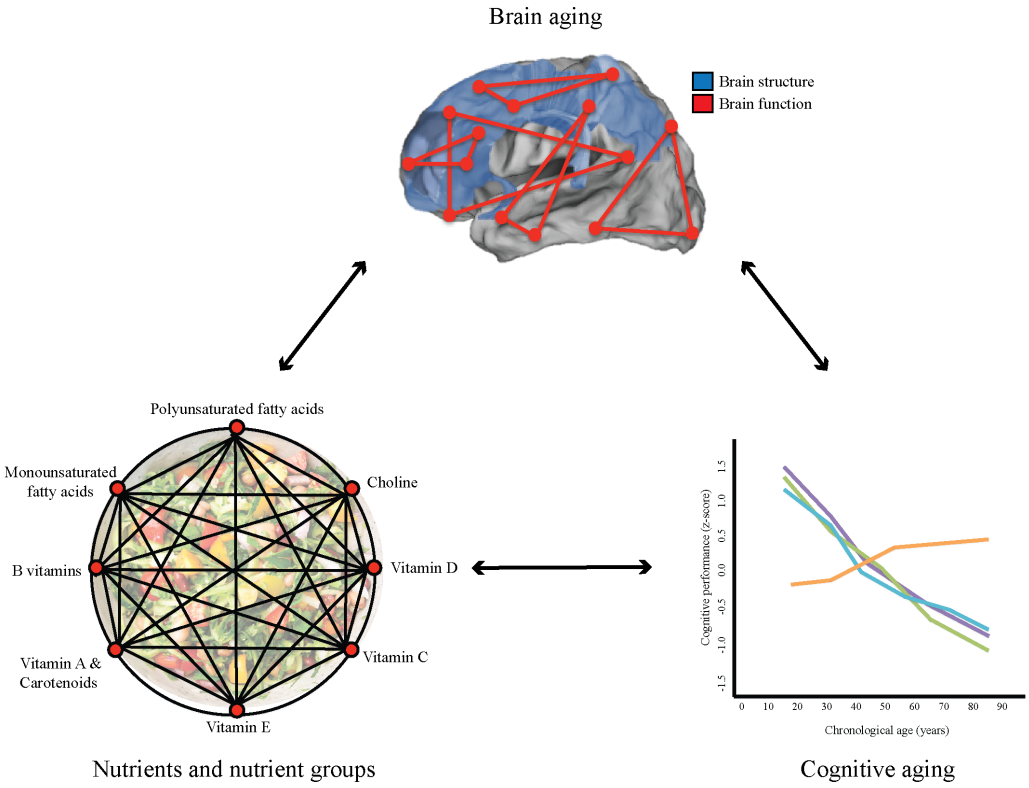

Figure 1.4. The relationship between diet, cognitive aging, and brain aging is multifaceted in nature: Nutrients and dietary components may have individual, interactive, or synergistic effects on cognitive and brain health. High-resolution neuroimaging methods can characterize the widespread changes in brain structure and function associated with age. The continuum of normal aging or preclinical stages of dementia can be captured by measuring dysfunction in aspects of cognition that show steady, age-related decline. In order to understand how nutrition can benefit cognitive and brain aging, each of these complex domains must be characterized through the use of precise methodology. Furthermore, these methods are well suited to identify whether particular nutrients may influence specific cognitive functions by targeting certain aspects of brain aging.

CHAPTER 2: ANTERIOR CINGULATE CORTEX MEDIATES THE RELATIONSHIP BETWEEN O3PUFAS AND EXECUTIVE FUNCTIONS IN APOE E4 CARRIERS[2]

Abstract

Although diet has a substantial influence on the aging brain, the relationship between biomarkers of diet and aspects of brain health remains unclear. This study examines the neural mechanisms that mediate the relationship between omega-3 polyunsaturated fatty acids (O3PUFAs) and executive functions in at-risk (APOE e4 carriers), cognitively intact older adults. We hypothesized that higher levels of O3PUFAs are associated with better performance in a particular component of the executive functions, namely cognitive flexibility, and that this relationship is mediated by gray matter volume of a specific region thought to be important for cognitive flexibility, the anterior cingulate cortex. We examined 40 cognitively intact adults between the ages of 65 and 75 with the APOE e4 polymorphism to investigate the relationship between biomarkers of O3PUFAs, tests of cognitive flexibility (measured by the Delis-Kaplan Executive Function System Trail Making Test), and gray matter volume within regions of the prefrontal cortex. A mediation analysis revealed that gray matter volume within the left rostral anterior cingulate cortex partially mediates the relationship between O3PUFA biomarkers and cognitive flexibility. These results suggest that the anterior cingulate cortex acts as a mediator of the relationship between O3PUFAs and cognitive flexibility in cognitively intact adults thought to be at risk for cognitive decline. Through their link to executive functions and neuronal

[2] This chapter appeared in its entirety in *Frontiers in Aging Neuroscience* and is referred to in this dissertation as "Zamroziewicz et al., 2015." This article is reprinted under the terms of the Creative Commons Attribution License. Zamroziewicz, M.K., Paul, E.J., Rubin, R.D., Barbey, A.K. (2016). Anterior cingulate cortex mediates the relationship between O3PUFAs and executive functions in APOE e4 carriers. *Frontiers in Aging Neuroscience* 7(87), 1-7. doi: 10.3389/fnagi.2015.00087.

measures of prefrontal cortex volume, O3PUFAs show potential as a nutritional therapy to prevent dysfunction in the aging brain.

Introduction

As the aged population expands, the economic burden of care and treatment of those with age-related health disorders also increases. The current and projected prevalence of Alzheimer's disease, for example, suggests an increase in the United States from 5.1 to 13.2 million by 2050 with healthcare expenditures related to this growth expected to surpass 1 trillion dollars (Alzheimer's Association, 2013). Therefore a successful strategy to promote healthy brain aging is of great interest to public health efforts and the United States economy. Diet and the many bioactive substances present in food represent a novel target for intervention that may promote healthy brain aging. Defining the precise mechanisms through which diet may impact brain health is a first step to developing successful dietary strategies against brain aging.

Accumulating evidence indicates that omega-3 polyunsaturated fatty acids (O3PUFAs) have a beneficial effect on cognitive aging. These long-chain polyunsaturated fatty acids serve as structural components of neuronal membranes and may have neuroprotective properties through anti-inflammatory, antioxidant, and energy metabolism pathways (Cunnane et al., 2009). However, the core brain regions that O3PUFAs may act upon are unknown. This study aims to investigate specific and sensitive neural mechanisms that mediate the beneficial effect of O3PUFAs on cognitive aging, and in particular, the brain regions that underlie the relationship between O3PUFAs and cognitive flexibility, a component of the executive functions. This investigation focuses on carriers of the APOE e4 allele to evaluate these relationships in cognitively intact aging individuals thought to be at risk for cognitive decline (Bell et al., 2012;

Davies et al., 2014; Jorm et al., 2007; Kozauer et al., 2008; Schiepers et al., 2012; Wisdom et al., 2011).

O3PUFAs have been to linked to superior cognitive performance, primarily in tasks that require executive functions (Bauer et al., 2014; Beydoun et al., 2008; Witte et al., 2014). Executive functions have been defined in many ways, but traditionally consist of planning and execution of goal-directed behaviors, abstract reasoning, and judgment (Stuss and Alexander, 2000). More recently, executive functions have been defined as the efficiency with which an individual applies his or her knowledge to cope with everyday life (Princiotta et al., 2014). The presence of executive dysfunction without measureable deficits in general cognition may represent the continuum of normal aging or a preclinical stage of dementia (Johnson et al., 2007). In particular, O3PUFAs have been directly linked to better performance on tasks that entail a particular component of the executive functions, namely cognitive flexibility (Bowman et al., 2013; Johnston et al., 2013). Cognitive flexibility refers to the ability to adjust to new demands or rules, and can be measured using task switching paradigms (Diamond, 2013).

Executive functions are implemented in the prefrontal cortex (PFC), and specific aspects of executive functions may be localized to particular sub-regions within the PFC (Barbey et al., 2012, 2013a, 2013b, 2013c, 2014a, 2014b). In general, higher gray matter volume in the PFC has been associated with better performance on tasks that elicit executive functions (Burzynska et al., 2012; Kochunov et al., 2009; Tu et al., 2012). More specifically, and of interest in this study, one of the anatomically distinct sub-regions of the ventromedial PFC known as the anterior cingulate is thought to underlie aspects of cognition known as attention, cognitive control, working memory, set maintenance, and goal directed behavior (Barbey et al., 2011; Nee et al., 2011). In fact, higher gray matter volumes in the anterior cingulate cortex have been related to the

cognitive flexibility component of the executive functions, as evidenced by performance on measures of task switching (Huster et al., 2009; Nee et al., 2011).

Given that O3PUFAs impact cognitive flexibility and that cognitive flexibility seems reliant upon the anterior cingulate cortex, we examined the role of regions within the PFC in mediating the relationship between O3PUFAs, as measured by blood biomarkers, and this particular component of the executive functions in cognitively intact aging individuals at risk for cognitive decline. In light of existing evidence, we predict that gray matter volume of the anterior cingulate would mediate the beneficial effect of O3PUFAs on cognitive flexibility.

Materials and methods

Participants

This cross-sectional study enrolled 95 elderly adults from Carle Foundation Hospital, a local and readily available cohort of well-characterized elderly adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination. Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for MRI. Of these 95 participants, only a subset (n=52) underwent genotyping of APOE alleles. Of this subset, 40 participants had the APOE e4 allele whereas 12 participants did not carry the APOE e4 allele. Our hypothesis focuses on APOE e4 carriers who may be at higher risk for cognitive decline and our sample

does not include many APOE e4 non-carriers, hence, only e4 allele carriers were included in the following analyses.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Nutrient biomarker acquisition and analysis

Fasting plasma was collected between 7:00 am and 12:00 noon Central Standard Time from January 2013 to May 2014. Gas chromatography equipped with a flame ionization detector quantified plasma fatty acid concentrations. Plasma lipids were measured with standard enzymatic methods (Bowman et al., 2011). The two fatty acids assessed in the current study include docosahexaeonic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), both of which are omega-3 polyunsaturated fatty acids. Participants were divided into low versus high DHA and EPA levels separately for each nutrient according to a median split. Low/high group assignment for DHA and EPA was the same for 39 of the 40 participants; therefore we computed a composite O3PUFA score by taking the average of DHA and EPA and then divided participants into low versus high O3PUFA levels based on the composite O3PUFA values (Bowman et al., 2013; Lotrich et al., 2013; McNamara et al., 2013).

APOE genotyping

APOE genotyping was determined using PCR (e4 carrier, y/n). The presence of the Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test (Hardy, 1908).

Neuropsychological tests

Executive function was measured by the Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test (Delis et al., 2006). This assessment yields a measure of executive function that can be isolated from underlying skills, including visual scanning, number sequencing, letter sequencing, and motor speed. In this task, participants alternate between multiple task goals (either number or letter sequencing), which elicits a specific type of executive function known as cognitive flexibility. The reported results from the D-KEFS Trail Making Test assess cognitive flexibility while controlling for motor speed and therefore provide a measure of cognitive flexibility that is not confounded by motor skill, which may be an important consideration in an elderly cohort.

Volumetric brain MRI

Volumetric analysis was performed on data from a 3D high-resolution (0.9 mm isotropic) T1-weighted scan using MPRAGE acquisition. Cortical reconstruction was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale et al., 1999; Dale and Sereno, 1993; Fischl et al., 1999a, 1999b, 2001, 2002, 2004a, 2004b; Fischl and Dale, 2000; Han et al., 2006; Jovicich et al., 2006; Reuter et al., 2010, 2012; Ségonne et al., 2004). All cortical reconstructions were manually checked for accuracy, as recommended by the software developers. This analysis focused on gray matter

volumes in the PFC provided by Freesurfer parcellation. These regions included the superior frontal cortex, rostral middle frontal cortex, the caudal middle frontal cortex, pars opercularis, pars triangularis, pars orbitalis, lateral orbitofrontal cortex, medial orbitofrontal cortex, precentral gyrus, paracentral gyrus, frontal pole, rostral anterior cingulate cortex, and caudal anterior cingulate cortex. In particular, the analysis sought to assess the specific and sensitive role of the rostral anterior cingulate, and exclude the involvement of other regions within the PFC.

Covariates

Covariates were included according to previous association with cognitive decline. These included age (continuous), gender (man/woman), education (nominal, year, fixed levels), income (nominal, five fixed levels), body mass index (BMI), and depression status (y/n). Although all participants had received a diagnosis of no depression at enrollment, the SF-36 Health Survey (Ware et al., 1993) revealed two participants with symptoms consistent with depression and so, in accordance with other studies, this was considered in the analysis as a covariate. PFC gray matter volume (continuous) was also included as a covariate in mediation analyses to assess the relationship between specific regions within the PFC, O3PUFA levels, and cognitive flexibility. These covariates were included in each of the four steps of the mediation analysis.

Statistical analyses

All statistics were performed in MATLAB v8.3.0.532 software. The variables of interest included the following: O3PUFA levels in blood, gray matter volumes of regions within the PFC provided by Freesurfer parcellation, and cognitive flexibility.

A formal mediation analysis was used in an effort to better understand the relationship between O3PUFA levels, gray matter volume of regions within the PFC, and cognitive flexibility using a four-step framework (Baron and Kenny, 1986). In each step of the mediation, a regression model was estimated and adjusted for all covariates listed above. The first step examined the relationship between O3PUFA levels and gray matter volumes in the PFC (**path a**). The second step examined the relationship between O3PUFA levels and cognitive flexibility (**path c**). The third step tested the direct pathway of mediation by including both gray matter volumes in the PFC and O3PUFA levels as independent variables, and cognitive flexibility as the dependent variable (**paths b and c**). The fourth step tested the indirect pathway of mediation, namely the interaction between paths a and b, using the Sobel z-test (**path c'**). A perfect mediation was indicated if the first three steps showed statistically significant relationships. A partial mediation was indicated if the first two steps showed statistically significant relationships, the third step showed non-significant relationships, and the Sobel z-test indicated a significant interaction between paths a and b.

Results

Participant characteristics

Participants had a mean age of 68.80 years and 72.5 percent of participants were females. Mean O3PUFA level in the low O3PUFA group was 102.30 nmol/mL and mean O3PUFA level in the high O3PUFA group was 216.00 nmol/mL. The mean MMSE score was 28.83 and the D-KEFS Trail Making Test cognitive flexibility score was 10.30 (**Table 2.1**). APOE genotype distribution did not deviate from Hardy-Weinberg equilibrium ($\chi_2 = 2.93$, $p>0.05$).

Plasma O3PUFA and cognitive flexibility, mediated by rostral anterior cingulate structure

The mediation analyses indicated that out of all regions within the PFC, gray matter volume of only the left rostral anterior cingulate partially mediates the relationship between O3PUFA blood levels and cognitive flexibility. Each relationship within the mediation is described below in a stepwise fashion.

First, O3PUFA levels associated with higher volume of the left rostral anterior cingulate cortex ($\beta = 428.6$, $p = 0.001$), with O3PUFA levels explaining 68.7% of the variation in cortical volume (**Figure 2.1 path a**). Second, O3PUFA levels marginally associated with better cognitive flexibility ($\beta = 1.968$, $p = 0.056$), with O3PUFA levels explaining 53.4% of the variance in cognitive flexibility (**Figure 2.1 path c**). Third, when adding both O3PUFA levels and left rostral anterior cingulate volume as independent variables, neither independent variable was a significant predictor of cognitive flexibility ($\beta = 0.002$, $p = 0.120$ for left rostral anterior cingulate volume; $\beta = 1.433$, $p = 0.209$ for O3PUFA levels), indicating the lack of a perfect mediation. Finally, the Sobel z-test showed a significant interaction between paths a and b ($z = 2.711$, $p = 0.007$), indicating that the volume of this brain region is a partial mediator of the relationship between O3PUFA levels and cognitive flexibility (**Figure 2.1**).

Discussion

This study found that volume of the left rostral anterior cingulate mediates the relationship between O3PUFA levels and cognitive flexibility. This is the first report of a specific and sensitive volumetric mediation between O3PUFA levels and a particular component of the executive functions, and is shown in an at-risk population of cognitively intact older adults. The individual relationships reported within the mediation, including those between

O3PUFA levels and cognitive flexibility, between O3PUFA levels and left rostral anterior cingulate, and between left rostral anterior cingulate and cognitive flexibility, are substantiated by prior findings described below.

The first relationship demonstrated a positive association between higher O3PUFA blood levels and larger volume in the rostral anterior cingulate cortex of the left hemisphere (**Figure 2.1 path a**). Past studies have also linked higher O3PUFA blood levels to superior anterior cingulate structure and function (Bauer et al., 2014; Conklin et al., 2007; McNamara et al., 2013). Second, higher O3PUFA levels associated with better cognitive flexibility (**Figure 2.1 path c**). In past work, higher O3PUFA intake has been linked to better performance on tasks of cognitive flexibility (Beydoun et al., 2008; Bowman et al., 2013; Witte et al., 2014). The indirect pathway of mediation indicated a mediatory effect of left rostral anterior cingulate gray matter volume on the relationship between O3PUFA blood levels and cognitive flexibility (**Figure 2.1 path c'**). Higher gray matter volumes in the anterior cingulate cortex have been linked to higher O3PUFA intake as well as superior cognitive flexibility, as evidenced by measures of task switching. The unilateral nature of this mediator is substantiated by prior work implicating the vulnerability of regions within the left hemisphere to Alzheimer's disease-related degeneration and associated cognitive impairments (Mosconi et al., 2014; Querbes et al., 2009; Risacher et al., 2010).

Cognitive aging literature indicates that there are two major postulated routes through which O3PUFAs might impact executive functions, namely the vascular and non-vascular hypotheses. In reporting a volumetric mediation between O3PUFA levels and cognitive flexibility, our study is the first to provide evidence for the non-vascular hypothesis within a formal mediation framework. While the vascular hypothesis suggests that reducing blood

pressure, lowering risk of thrombosis, reducing inflammation, and lowering serum triglyceride levels can prevent deterioration of the brain (Calder, 2004; Keli et al., 1994; Kelley et al., 2008), the non-vascular hypothesis states that cortical and subcortical volumes show reduction in healthy aging due to mechanisms not directly related to changes in vasculature. These effects are especially robust in the frontal cortex, and prevention or reduction of this atrophy can prevent cognitive decline (Salat et al., 2004). The non-uniform deterioration of both executive functions and PFC structure associated with aging support the notion that O3PUFAs may slow age effects on specific aspects of the executive functions and in particular regions within the PFC (MacPherson et al., 2002). Possible non-vascular mechanisms that may underlie this volumetric mediation include enhanced membrane and membrane-bound protein function, reduced amyloid-β production, reduced neuroinflammation and oxidative damage, increased levels of brain derived neurotrophic factor, and reduced excitotoxic omega-6 levels (Cole and Frautschy, 2010).

The partial nature of this mediation is supported by the specificity of this analysis, which honed in on one component of the executive functions and one region within the PFC. Brain aging is a dynamic, heterogeneous, and multi-faceted process (Koepsell and Monsell, 2012), and it is unlikely that the volume of a specific region within the PFC would completely and exclusively mediate the relationship between this vital nutrient for healthy aging and such an important cognitive function in aging. Rather than claiming that the rostral anterior cingulate is the sole mediator of the relationship between O3PUFAs and cognitive flexibility, this study is one of the first to begin defining specific and sensitive mediatory factors between nutrition and cognition.

The strength of this study includes the use of blood biomarkers to measure nutrition, which provided a more reliable assessment of dietary intake than that of food frequency

questionnaires. The potential limitations of this study include the cross-sectional design, relatively small sample size, and inability to assess this relationship in both carriers and non-carriers of the APOE e4 allele. Future longitudinal studies with larger samples size are needed to confirm these results.

Tables and Figures

Table 2.1. Characteristics of sample

Demographics	***n* = 40**
Age in years (M ± SD)	69 ± 3
Female (%)	73
Education (%)	13 high school degree
Some high school	0
High school degree	13
Some college	18
College degree	70
Income (%)	2(5.0) $15,000 - $25,000
< $15,000	0
$15,000 - $25,000	5
$25,000 - $50,000	20
$50,000 - $75,000	23
$75,000 - $100,000	20
> $100,000	33
Depression indicated (%)	5
Plasma nutrients	**(M ± SD, nmol/mL)**
Low O3PUFA	102.30 ± 25.76
High O3PUFA	216.00 ± 48.43
Cognition	**(M ± SD)**
Mini-Mental State Examination	28.83 ± 1.11
Cognitive flexibility score	10.30 ± 3.12
Gray matter volume	**(M ± SD, mm^3)**
Left frontal lobe	27400 ± 3240
Right frontal lobe	27197 ± 2984
Left superior frontal	19774 ± 2137
Right superior frontal	19245 ± 2203
Left rostral middle frontal	13769 ± 1756
Right rostral middle frontal	14367 ± 1959
Left caudal middle frontal	5764 ± 913
Right caudal middle frontal	5268 ± 953
Left pars opercularis	4291 ± 553
Right pars opercularis	3547 ± 542
Left pars triangularis	3183 ± 382
Right pars triangularis	3782 ± 560
Left pars orbitalis	2027 ± 262
Right pars orbitalis	2417 ± 375
Left lateral orbitofrontal	6744 ± 891
Right lateral orbitofrontal	6612 ± 844
Left medial orbitofrontal	4674 ± 710
Right medial orbitofrontal	4746 ± 697
Left precentral gyrus	12242 ± 1477
Right precentral gyrus	12044 ± 1526
Left paracentral gyrus	3105 ± 488
Right paracentral gyrus	3515 ± 430
Left frontal pole	815 ± 185
Right frontal pole	1123 ± 264
Left rostral anterior cingulate	2496 ± 475
Right rostral anterior cingulate	2098 ± 426
Left caudal anterior cingulate	1757 ± 353
Right caudal anterior cingulate	1864 ± 335

Abbreviations: mean (M), standard deviation (SD), omega-3 polyunsaturated fatty acids (O3PUFA)

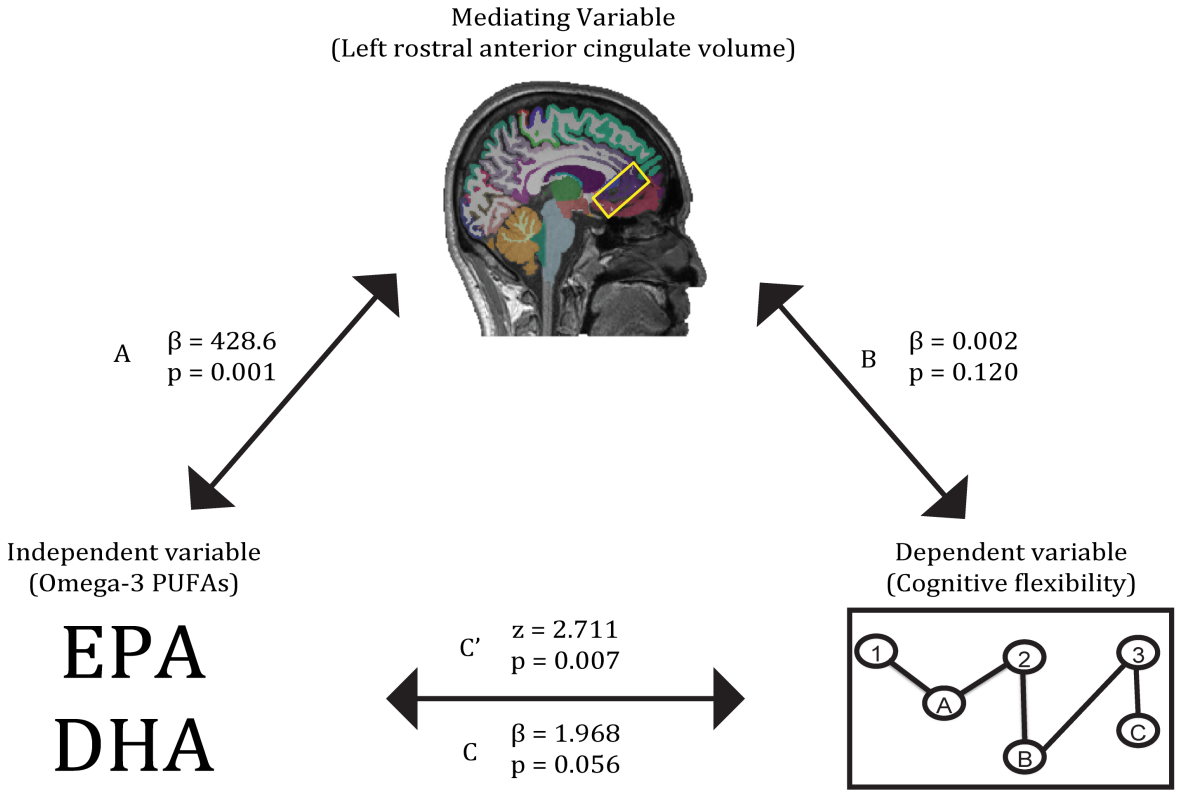

Figure 2.1. Mediation model statistics: Gray matter volume of the left rostral anterior cingulate partially mediates the relationship between O3PUFA blood levels and cognitive flexibility.

CHAPTER 3: INFERIOR PREFRONTAL CORTEX MEDIATES THE RELATIONSHIP BETWEEN PHOSPHATIDYLCHOLINE AND EXECUTIVE FUNCTIONS IN HEALTHY OLDER ADULTS[3]

Abstract

This study examines the neural mechanisms that mediate the relationship between phosphatidylcholine and executive functions in cognitively intact older adults. We hypothesized that higher plasma levels of phosphatidylcholine are associated with better performance on a particular component of the executive functions, namely cognitive flexibility, and that this relationship is mediated by gray matter structure of regions within the prefrontal cortex (PFC) that have been implicated in cognitive flexibility. We examined 72 cognitively intact adults between the ages of 65 and 75 in an observational, cross-sectional study to investigate the relationship between blood biomarkers of phosphatidylcholine, tests of cognitive flexibility (measured by the Delis-Kaplan Executive Function System Trail Making Test), and gray matter structure of regions within the PFC. A three-step mediation analysis was implemented using multivariate linear regressions and we controlled for age, sex, education, income, depression status, and body mass index. The mediation analysis revealed that gray matter thickness of one region within the PFC, the left inferior PFC (Brodmann's Area 45), mediates the relationship between phosphatidylcholine blood biomarkers and cognitive flexibility. These results suggest that the inferior PFC acts as a mediator of the relationship between phosphatidylcholine and cognitive flexibility in cognitively intact older adults. This report demonstrates a novel structural

[3] This chapter appeared in its entirety in *Frontiers in Aging Neuroscience* and is referred to in this dissertation as "Zamroziewicz et al., 2016b." This article is reprinted under the terms of the Creative Commons Attribution License. Zamroziewicz, M.K., Zwilling, C.E., Barbey, A.K. (2016) Inferior prefrontal cortex meditates the relationship between phosphatidylcholine and executive functions in healthy, older adults. *Frontiers in Aging Neuroscience* 8(226), 1-8. doi: 10.3389/fnagi.2016.00226.

mediation between plasma phosphatidylcholine levels and cognitive flexibility. Future work should examine the potential mechanisms underlying this mediation, including phosphatidylcholine-dependent cell membrane integrity of the inferior PFC and phosphatidylcholine-dependent cholinergic projections to the inferior PFC.

Introduction

A rapidly expanding older adult population has produced significant medical and economic demands for the treatment and care of individuals with age-related health disorders that continue to rise. The prevalence of Alzheimer's disease, for example, is projected to increase in the United States from 5.1 to 13.2 million by 2050, and associated healthcare expenditures are estimated to surpass 1 trillion dollars (Alzheimer's Association, 2013). Therefore, establishing a successful strategy to promote healthy brain aging is of great interest to public health efforts and the United States economy. Nutrition and the many bioactive substances present in the diet have been increasingly recognized as a promising target for intervention efforts to promote healthy brain aging (Zamroziewicz and Barbey, 2016). Identifying the means through which dietary intake may influence brain health will guide the development of successful dietary strategies for healthy brain aging.

Accumulating evidence suggests that phosphatidylcholine is a robust marker of age-related membrane degeneration and is associated with cognitive decline (Frisardi et al., 2011; Liu et al., 2014; Mapstone et al., 2014; Norris et al., 2015; Wurtman, 2015; Zeisel, 2006). Phosphatidylcholine is a phospholipid that carries a choline head group (Li and Vance, 2008). Phosphatidylcholine found in the blood may be derived from dietary sources, or may be endogenously synthesized by the phosphatidylethanolamine N-methyltransferase (PEMT)

pathway (Zeisel, 2006). Phosphatidylcholine serves a neuroprotective role by providing an essential component of neuronal membranes and a significant portion of the total choline pool, which contributes to forebrain cholinergic projections (Frisardi et al., 2011). However, the core brain regions upon which phosphatidylcholine may act are unknown. This study aims to investigate the neural structures that mediate the relationship between plasma phosphatidylcholine levels and an important aspect of cognitive aging, decline in a component of the executive functions known as cognitive flexibility.

Low plasma phosphatidylcholine levels are highly predictive of cognitive decline, and low levels of important components of phosphatidylcholine, including the long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA) and choline, are predictive of age-related decline in executive functions (Beydoun et al., 2007; Bowman et al., 2012; Naber et al., 2015; Nurk et al., 2013; Witte et al., 2014). Executive functions traditionally consist of planning and execution of goal-directed behaviors, abstract reasoning, and judgment, but also reflect the efficiency with which an individual applies his or her knowledge to cope with everyday life (Stuss & Alexander, 2000; Princiotta & Devries, 2014). Within the continuum of normal aging or preclinical stages of dementia, the presence of executive dysfunction may occur without measurable deficits in general cognition. Therefore, executive dysfunction may be a robust early marker of cognitive decline (Johnson et al., 2007). Importantly, components of phosphatidylcholine, including long-chain polyunsaturated fatty acids and choline, have been associated with prefrontal cortical integrity and forebrain cholinergic projections, respectively, suggesting a link between phosphatidylcholine and the PFC-driven executive functions (Kolisnyk et al., 2013; Zamroziewicz et al., 2015). More specifically, long-chain polyunsaturated fatty acids have been shown to influence cognitive flexibility, a component of the executive

functions (Bowman et al., 2013; Johnston, Deuster, Harris, MacRae, & Dretsch, 2013; Zamroziewicz et al., 2015). Cognitive flexibility refers to the ability to adjust to new demands or rules, and can be measured using task switching paradigms (Diamond, 2013).

Executive functions are implemented within the prefrontal cortex (PFC), and particular aspects of executive functions may be localized to specific sub-regions within the PFC (Barbey et al., 2012, 2013a, 2013b, 2013c, 2014a, 2014b). Larger gray matter thickness and volume in the PFC has been associated with better performance on tasks that elicit executive functions (Burzynska et al., 2012; Kochunov et al., 2009; Tu et al., 2012; Yuan & Raz, 2014). For example, the inferior PFC has been implicated in the cognitive control of memory, including semantic retrieval, recollection of contextual details about past events, resolution of proactive interference in working memory, and task switching (Badre and Wagner, 2007). The inferior PFC is particularly susceptible to age-related cortical thinning, and age-related changes in cholinergic projections (Aron et al., 2004; Fjell et al., 2009). Integrity of the left inferior PFC has been linked to cognitive flexibility, as measured by task switching paradigms (Aron et al., 2004).

In summary, prior research indicates that: (i) phosphatidylcholine is highly predictive of age-related cognitive decline; (ii) cognitive flexibility is an early marker of cognitive decline amenable to the effects of phosphatidylcholine components; and (iii) particular regions within the PFC, such as the inferior PFC, are critical for cognitive flexibility and susceptible to age-related degeneration. Therefore, we examined the role of regions within the PFC in mediating the relationship between plasma phosphatidylcholine and cognitive flexibility in cognitively intact aging individuals.

Materials and methods

Participants

This cross-sectional study enrolled 122 elderly adults from Carle Foundation Hospital, a local and readily available cohort of well-characterized elderly adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination (Folstein et al., 1975). Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for MRI. Of these 122 participants, 72 subjects had a complete dataset at time of data analysis, including neuropsychological testing, MRI, and blood biomarker analysis.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and the Carle Hospital Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Biomarker acquisition and analysis

Plasma was spiked with stable labeled internal standards of all the analytes, and extracted using the method modified from Bligh and Dyer (Bligh and Dyer, 1959). Samples were extracted with methanol/chloroform (2:1, v/v). The mixture was vortexed and left at -20°C overnight. At the end of the extraction with methanol/chloroform, samples were centrifuged and supernatants

transferred into new microcentrifuge tubes. Residues were re-extracted with methanol/chloroform/water (2:1:0.8, v/v/v). After vigorous vortexing and centrifugation, supernatants were collected and combined with the first extract. Water and chloroform were added into the resulting solutions to allow for phase separation. After centrifugation, the organic phase, which contains phosphatidylcholine, was 1:10 diluted with methanol and transferred into HPLC vials for instrumental analysis.

Quantification of the analytes was performed using liquid chromatography-stable isotope dilution-multiple reaction monitoring mass spectrometry (LC-SID-MRM/MS). Chromatographic separations were performed on an Atlantis Silica HILIC 3µm 4.6×50mm column (Waters Corp, Milford, USA) using a Waters ACQUITY UPLC system. The column was heated to 40°C, and the flow rate maintained at 1 mL/min. The mobile phases were: A - 10% acetonitrile/90% water with 10 mM ammonium formate and 0.125% formic acid, and B - 90% acetonitrile/10% water with 10 mM ammonium formate and 0.125% formic acid. For organic analytes, the gradient was at 5% A for 0.05min, to 20% A in 2.95 min, to 55% A in 0.05 min, at 55% A in 0.95 min, to 5% A in 0.05 min, and at 5% A for 2.95 min. The analytes and their corresponding isotopes were monitored on a Waters TQ detector using characteristic precursor-product ion transitions shown in the below table. Concentrations of each analyte in the samples were determined using the peak area ratio of the analyte to its isotope. MS parameters for phosphatidylcholine were as follows: precursor at 193 m/z, product at 193 m/z. Phosphatidylcholine levels were included in analyses as a continuous variable.

Neuropsychological tests

Executive function was measured by the Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test (Delis et al., 2006). This assessment yields a measure of executive function that can be isolated from underlying skills, including visual scanning, number sequencing, letter sequencing, and motor speed. In this task, participants alternate between multiple task goals (either number or letter sequencing), which elicits a specific type of executive function known as cognitive flexibility. The reported results from the D-KEFS Trail Making Test assess cognitive flexibility while controlling for number and letter sequencing trials and therefore provide a measure of cognitive flexibility that is not confounded by underlying cognitive abilities (i.e., number and letter sequencing) required by the task.

Volumetric brain MRI

Volumetric analysis was performed on data from a 3D high-resolution (0.9 mm isotropic) T1-weighted scan using MPRAGE acquisition. Cortical reconstruction was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale et al., 1999; Dale and Sereno, 1993; Fischl et al., 1999a, 1999b, 2001, 2002, 2004a, 2004b; Fischl and Dale, 2000; Han et al., 2006; Jovicich et al., 2006; Reuter et al., 2010, 2012; Ségonne et al., 2004). All cortical reconstructions were manually checked for accuracy, as recommended by the software developers. This analysis focused on gray matter thickness in the PFC provided by Freesurfer parcellation. These regions included the superior frontal cortex, rostral middle frontal cortex, the caudal middle frontal cortex, pars opercularis, pars triangularis, pars orbitalis, lateral orbitofrontal cortex, medial orbitofrontal cortex, precentral

gyrus, paracentral gyrus, frontal pole, rostral anterior cingulate cortex, and caudal anterior cingulate cortex.

Covariates

Covariates previously associated with cognitive decline (Coffey et al., 1998, 1999; Fotenos et al., 2008; Gunstad et al., 2008; Raz et al., 2010; van Tol et al., 2010) were tested, including age (continuous), gender (nominal, man/woman), education (ordinal, five fixed levels), income (ordinal, six fixed levels), body mass index (continuous, BMI), and depression status (nominal, yes/no). Although all participants had received a diagnosis of no depression at enrollment, the SF-36 Health Survey (Ware et al., 1993) revealed five participants with symptoms consistent with depression and so, in accordance with other studies, this was considered in the analysis as a covariate. PFC gray matter thickness (continuous) was also included as a covariate in mediation analyses to assess the relationship between specific regions within the PFC, plasma phosphatidylcholine, and cognitive flexibility. These covariates were included in each of the three steps of the mediation analysis.

Statistical analyses

A formal mediation analysis was used in an effort to better understand the relationship between phosphatidylcholine levels, gray matter thickness of regions within the PFC, and cognitive flexibility using a three-step framework. The goal of the mediation analysis was to understand whether the relationship between phosphatidylcholine levels and cognitive flexibility was mediated by gray matter thickness of regions within the PFC. The primary requirement for mediation is a significant indirect mediation effect, or the effect of the independent variable

(phosphatidylcholine) through the mediator (gray matter thickness of a PFC region) on the dependent variable (cognitive flexibility) (Zhao et al., 2010).

Statistics were performed in SPSS Statistical Packages version 23 (SPSS, Inc., Chicago, IL, USA), and mediation analyses were performed using the *indirect* macro designed for SPSS (Preacher and Hayes, 2008). Statistics were performed as follows:

1. In the first step, a regression model was used to characterize the relationship between phosphatidylcholine levels and gray matter thickness of regions in the PFC, controlling for the covariates listed above (**path a**).
2. In the second step, a regression model was used to characterize the relationship between phosphatidylcholine levels and cognitive flexibility, controlling for the covariates listed above (**path c**).
3. In the third step, the *indirect* macro was used to implement the bootstrapping method to estimate mediation effects. This analysis drew 1000 bootstrapped samples with replacement from the dataset to estimate a sampling distribution for the indirect and direct mediation effects, controlling for the covariates listed above. The indirect mediation effect refers to the pathway from phosphatidylcholine to gray matter thickness of a PFC region to cognitive flexibility (**path a to path b**). The direct mediation effect refers to the direct pathway from phosphatidylcholine to cognitive flexibility (**path c'**).

A statistically significant mediation that matches the hypothesized framework is indicated by: (i) an indirect mediation effect that does not include zero within 95% bias-corrected confidence intervals, and (ii) a direct mediation effect that does include zero within 95% bias-corrected confidence intervals (Zhao et al., 2010). Results are reported using unstandardized regression coefficients (β) and statistical significance (p) for each individual regression

relationship, and a 95% bias-corrected confidence interval (95% CI) for the direct and indirect effects of the mediation.

Results

Participant characteristics

Participants had a mean age of 69 years and 64 percent of participants were females. Education levels were reported as follows: 1 percent of participants completed some high school, 14 percent of participants received a high school degree, 17 percent of participants completed some college, and 68 percent of participants received a college degree. Annual household income levels were reported as follows: 1 percent of participants earned less than $15,000, 3 percent of participants earned $15,000 to $25,000, 17 percent of participants earned $25,000 to $50,000, 24 percent of participants earned $50,000 to $75,000, 22 percent of participants earned $75,000 to $100,000, and 33 percent of participants earned over $100,000. The mean phosphatidylcholine level was 2102 uM. The mean D-KEFS Trail Making Test cognitive flexibility score was 8. The mean gray matter thickness of the left PFC was 2.39 millimeters, and mean gray matter thickness of the left inferior PFC was 2.38 millimeters (**Table 3.1**).

Mediation results

The mediation analyses indicated that out of all regions within the PFC, gray matter thickness of only the left inferior PFC (pars triangularis, Brodmann area 45) mediated the relationship between phosphatidylcholine and cognitive flexibility, corresponding with prior work that suggests an influential role of this region. Each relationship within the mediation is described below in a stepwise fashion.

First, higher phosphatidylcholine associated with greater thickness of the left inferior PFC ($\beta = 0.001$ $p = 0.007$; **Figure 3.1; Figure 3.2 path a**). Second, higher phosphatidylcholine associated with better cognitive flexibility ($\beta = 0.002$, $p = 0.016$, **Figure 3.2 path c**). Third, the indirect pathway of meditation was significant (95% CI: 0.001 – 0.002, $\beta = 4.688$, $p = 0.047$, **Figure 3.2 path a-b**), but the direct pathway of mediation was insignificant (95% CI: -0.002 – 0.003, $\beta = 0.001$, $p = 0.089$, **Figure 3.2 path c'**). Therefore, the mediation indicated that gray matter thickness of the left inferior PFC fully mediated the relationship between phosphatidylcholine and cognitive flexibility (**Figure 3.2**).

Discussion

This study revealed that gray matter thickness of the left inferior PFC mediates the relationship between plasma phosphatidylcholine and cognitive flexibility. The mediation analysis provided a novel finding that links phosphatidylcholine to gray matter integrity of a specific cortical region and a particular component of the executive functions. The individual relationships reported within the mediation, including those between phosphatidylcholine levels and left inferior PFC (**Figure 3.2 path a**), between phosphatidylcholine levels and cognitive flexibility (**Figure 3.2 path c**), and between left inferior PFC and cognitive flexibility (**Figure 3.2 path b**), are each substantiated by prior findings reviewed in turn below.

The first relationship demonstrated a positive association between higher phosphatidylcholine levels and greater thickness in the inferior PFC of the left hemisphere. Past studies suggest that phosphatidylcholine plays a critical role in age-related changes in cortical integrity, and the inferior PFC, being a region that thins early in aging, may be particularly susceptible to these effects (Söderberg et al., 1990; Wurtman, 2015). More specifically,

phosphatidylcholine may contribute to structure and function of the inferior PFC via cholinergic projections, which enhance functional activity within this region (Berry et al., 2015; Blusztajn et al., 1987). Second, higher phosphatidylcholine levels are associated with better cognitive flexibility. Prior work demonstrates that higher phosphatidylcholine levels are related to slower cognitive decline, and components of phosphatidylcholine, including long-chain polyunsaturated fatty acids, such as DHA, and choline, are linked to superior performance on executive function tasks (Beydoun et al., 2007; Bowman et al., 2012; Hartmann et al., 2014; Mapstone et al., 2014; Naber et al., 2015; Nurk et al., 2013; Schaefer et al., 2006; Witte et al., 2014; Zamroziewicz et al., 2015). The indirect pathway of mediation indicated a mediatory effect of left inferior PFC gray matter thickness on the relationship between phosphatidylcholine levels and cognitive flexibility. Previous studies indicate that greater gray matter thickness within the inferior PFC contributes to superior cognitive flexibility, and that cholinergic transmissions, originating, for example, from phosphatidylcholine-derived choline, underlie activity within the inferior PFC during tasks of cognitive control (Berry et al., 2015; Blusztajn et al., 1987; Burzynska et al., 2012). The unilateral nature of this mediation is supported by prior work, which suggests that regions within the left hemisphere may be selectively susceptible to degeneration and cognitive impairment (Chételat et al., 2005; Mosconi et al., 2014; Querbes et al., 2009; Risacher et al., 2010).

Prior work indicates that the underlying physiological mechanisms of the relationship between phosphatidylcholine levels, cognitive flexibility, and cortical integrity of the inferior PFC may be three-fold. First, phosphatidylcholine may help slow or prevent age-related changes in cortical thickness by delivering two molecules that are critical for cortical integrity, including choline and long-chain polyunsaturated fatty acids (Cohen et al., 1995; Jerneren et al., 2015;

Söderberg et al., 1990; Zeisel, 2006). Second, the delivery of long-chain polyunsaturated fatty acids may help prevent inflammation in the brain (Wall et al., 2010). Third, delivery of choline contributes to acetylcholine synthesis, a neurotransmitter that has been implicated in set-shifting performance and projects to the inferior PFC via forebrain cholinergic transmissions (Berry et al., 2015; Blusztajn et al., 1987; Hasselmo and Sarter, 2011). Importantly, phosphatidylcholine-derived choline may be a primary contributor to the brain choline pool when age-related changes in brain choline uptake reduce extracellular choline supplies (Zeisel, 2006). Future mechanistic studies are needed to confirm underlying physiological mechanisms of the relationship between phosphatidylcholine levels, cognitive flexibility, and cortical integrity of the inferior PFC.

Research at the frontiers of nutritional cognitive neuroscience seeks to integrate methods that sensitively capture variability in nutritional intake, brain aging, and cognition, and in doing so, elucidate the neural structures that mediate the relationship between nutritional status and cognitive decline. This finding contributes to a growing line of evidence which suggests that particular nutrients may slow or prevent aspects of age-related cognitive decline by influencing specific features of brain aging (Bowman et al., 2012; Gu et al., 2016; Zamroziewicz et al., 2015). In the case of phosphatidylcholine, future studies are needed to assess the origins of plasma phosphatidylcholine, and whether dietary intake or endogenous synthesis preferentially contributes to the neuroprotective effect. Another promising direction for future work is to examine the interactive effects among nutrients through the use of nutrient biomarker pattern analysis – a technique that enables an investigation of the beneficial effects of broader nutrient profiles on healthy brain aging. Ultimately, this line of research can inform clinical investigations of comprehensive and personalized approaches to nutritional intervention that takes into account dietary patterns and individual variability in nutritional status and brain health.

The strengths of the present study include: (i) the use of blood biomarkers to measure physiological status of phosphatidylcholine, (ii) the use of structural magnetic resonance imaging to measure regional cortical integrity with high spatial resolution, and (iii) the assessment of a particular component of cognitive function known to be sensitive to age-related cognitive decline, rather than a global cognitive function measure with little variability in healthy aging adults. Directions for future research include (i) replication of results in a larger sample size, (ii) implementation of a longitudinal study to examine how changes in phosphatidylcholine levels relate to changes in executive functions and integrity of the prefrontal cortex, (iii) investigation of the physiological mechanisms proposed to underlie the relationship between phosphatidylcholine and PFC structure, (iv) examination of relationship between phosphatidylcholine, executive functions, and prefrontal cortex integrity in other models, including animal models and clinical populations, (v) elucidation of the relationship between phosphatidylcholine in plasma and cerebrospinal fluid, and (iv) examination of the origins of phosphatidylcholine in blood, as plasma phosphatidylcholine may be derived from the diet or de novo synthesis by the phosphatidylethanolamine N-methyltransferase (PEMT) pathway (Zeisel, 2006).

Tables and Figures

Table 3.1. Characteristics of sample

Demographics	***n* = 72**
Age in years (M ± SD)	69 ± 3
Female (%)	64
Education (%)	
Some high school	1
High school degree	14
Some college	17
College degree	68
Income (%)	
< $15,000	1
$15,000 - $25,000	3
$25,000 - $50,000	17
$50,000 - $75,000	24
$75,000 - $100,000	22
> $100,000	33
Depression indicated (%)	7
Plasma nutrients	**(M ± SD, uM)**
Phosphatidylcholine	2102 ± 400
Cognition	**(M ± SD)**
Cognitive flexibility score	8 ± 2
Gray matter thickness	**(M ± SD, mm)**
Left PFC	2.39 ± 0.08
Left inferior PFC	2.38 ± 0.13

Abbreviations: mean (M), standard deviation (SD), prefrontal cortex (PFC)

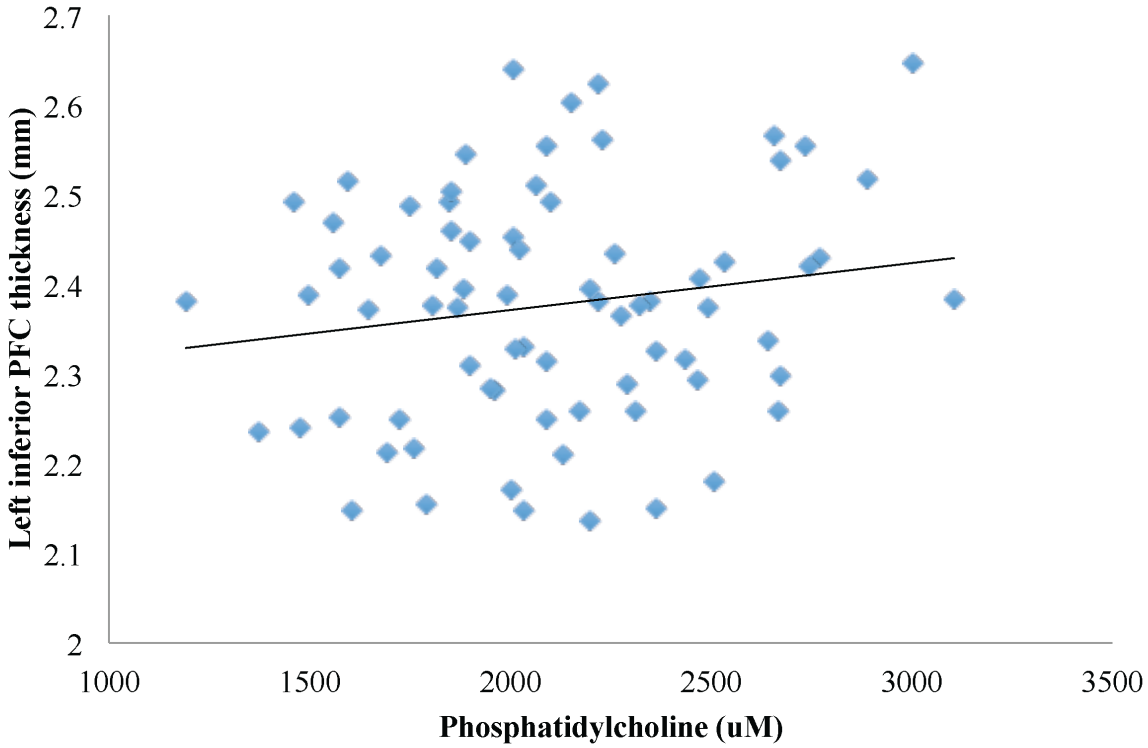

Figure 3.1. Mediation path a: A linear regression model was used to characterize the relationship between phosphatidylcholine levels and gray matter thickness of regions in the PFC. Phosphatidylcholine levels positively associated with gray matter thickness of the left inferior PFC ($\beta = 0.001$ $p = 0.007$).

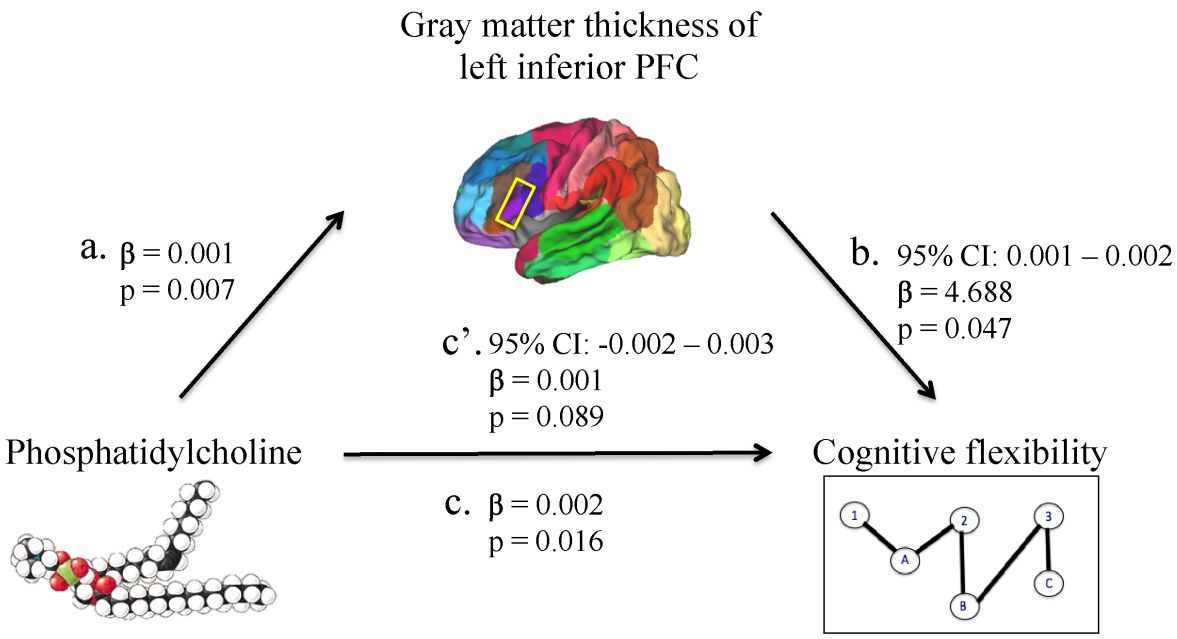

Figure 3.2. Mediation model statistics: Phosphatidylcholine levels positively associated with gray matter thickness of the left inferior PFC (**path a**). Phosphatidylcholine levels positively associated with cognitive flexibility (**path c**). The indirect pathway of mediation (i.e., the effect of phosphatidylcholine levels through gray matter thickness of the left inferior PFC on cognitive flexibility; **path a-b**) was statistically significant. The direct pathway of mediation (i.e., the effect of phosphatidylcholine levels directly on cognitive flexibility, accounting for the effect of gray matter thickness of the left inferior PFC; **path c'**) was not statistically significant. Therefore, gray matter thickness of the left inferior PFC fully mediated the relationship between phosphatidylcholine levels and cognitive flexibility.

CHAPTER 4: PARAHIPPOCAMPAL CORTEX MEDIATES THE RELATIONSHIP BETWEEN LUTEIN AND CRYSTALLIZED INTELLIGENCE IN HEALTHY OLDER ADULTS[4]

Abstract

Although diet has a substantial influence on the aging brain, the relationship between dietary nutrients and aspects of brain health remains unclear. This study examines the neural mechanisms that mediate the relationship between a carotenoid important for brain health across the lifespan, lutein, and crystallized intelligence in cognitively intact older adults. We hypothesized that higher serum levels of lutein are associated with better performance on a task of crystallized intelligence, and that this relationship is mediated by gray matter structure of regions within the temporal cortex. This investigation aims to contribute to a growing line of evidence, which suggests that particular nutrients may slow or prevent aspects of cognitive decline by targeting specific features of brain aging. We examined 76 cognitively intact adults between the ages of 65 and 75 to investigate the relationship between serum lutein, tests of crystallized intelligence (measured by the Wechsler Abbreviated Scale of Intelligence), and gray matter volume of regions within the temporal cortex. A three-step mediation analysis was implemented using multivariate linear regressions to control for age, sex, education, income, depression status, and body mass index. The mediation analysis revealed that gray matter thickness of one region within the temporal cortex, the right parahippocampal cortex (Brodmann's Area 34), partially mediates the relationship between serum lutein and crystallized

[4] This chapter appeared in its entirety in *Frontiers in Aging Neuroscience* and is referred to in this dissertation as "Zamroziewicz et al., 2016a." This article is reprinted under the terms of the Creative Commons Attribution License. Zamroziewicz, M.K., Zwilling, C.E., Barbey, A.K. (2016) Parahippocampal cortex mediates the relationship between lutein and crystallized intelligence in healthy, older adults. *Frontiers in Aging Neuroscience* 8(297), 1-9. doi: 10.3389/fnagi.2016.00297.

intelligence. These results suggest that the parahippocampal cortex acts as a mediator of the relationship between serum lutein and crystallized intelligence in cognitively intact older adults. Prior findings substantiate the individual relationships reported within the mediation, specifically the links between (i) serum lutein and temporal cortex structure, (ii) serum lutein and crystallized intelligence, and (iii) parahippocampal cortex structure and crystallized intelligence. This report demonstrates a novel structural mediation between lutein status and crystallized intelligence, and therefore provides further evidence that specific nutrients may slow or prevent features of cognitive decline by hindering particular aspects of brain aging. Future work should examine the potential mechanisms underlying this mediation, including the antioxidant, anti-inflammatory, and membrane modulating properties of lutein.

Introduction

As the older adult population expands, the economic burden of care and treatment of age-related health disorders also rises. Between 2015 and 2060, the United States will experience significant growth of its older population, with the size of the population aged 65 and over more than doubling from an estimated 46 million in 2015 to 98 million in 2060 (Mather et al., 2015). Therefore, successful strategies to promote healthy brain aging are of significant interest to public health initiatives in the United States.

Nutrition is a promising target for intervention efforts to support healthy brain aging (Zamroziewicz and Barbey, 2016). Accumulating evidence indicates that particular nutrients may slow or prevent aspects of age-related cognitive decline by targeting specific features of brain aging. Studies that couple neuroimaging techniques with neuropsychological testing provide insight into mechanisms of action through which particular nutrients might influence

specific aspects of age-related cognitive decline (Boespflug et al., 2016; Bowman et al., 2012; Gu et al., 2016; Zamroziewicz et al., 2015). While some nutrients may be effective at preventing late-life changes in the brain, other nutritional factors may accumulate across the lifespan and therefore confer neuroprotection in the aging brain (Coyle and Puttfarcken, 1993; Söderberg et al., 1990). Identifying the mechanisms through which nutrients provide neuroprotective effects will help guide the development of successful lifelong dietary strategies for healthy brain aging.

Carotenoids are naturally occurring pigments made by plants, and can only be acquired through the diet (Erdman et al., 2015). Xanthophylls are a subclass of carotenoids, which have a polar molecular structure and therefore possess unique membrane-spanning properties (Erdman et al., 2015; Widomska et al., 2016). As compared to the other dietary carotenoids, xanthophylls preferentially accumulate in neural tissue, with lutein accounting for the majority of carotenoid accumulation in the brain (Craft et al., 2004; Johnson, 2012; Johnson et al., 2013; Vishwanathan et al., 2014b; Widomska et al., 2016). As the most prevalent carotenoid in the brain, lutein is thought provide a variety of neuroprotective benefits. Candidate mechanisms of action are primarily based on the unique membrane spanning properties of lutein and include influencing membrane properties such as fluidity, interneuronal communication via gap junctions, ion exchange, oxygen diffusion, membrane stability, and preventing oxidation and inflammation (Erdman et al., 2015; Izumi-Nagai et al., 2007; Johnson, 2014; Krinsky, 2003; Paiva and Russell, 1999; Stahl and Sies, 1996, 2001; Widomska and Subczynski, 2014). Lutein accretes in the brain across the entire lifespan, and may therefore contribute to brain health in a lifelong manner (Bovier and Hammond, 2015; Lieblein-Boff et al., 2015; Renzi et al., 2014). Notably, lutein is selectively distributed in gray matter, and has been detected in the prefrontal cortex, the temporal cortex, and the hippocampus (Craft et al., 2004; Vishwanathan et al., 2014b). Blood levels of

lutein correlate with brain concentrations of lutein in older adults, suggesting that blood concentrations can serve a measure of lutein status in the brain (Johnson et al., 2013).

Lutein status has been linked to cognitive performance across the lifespan (Bovier and Hammond, 2015; Feeney et al., 2013; Johnson et al., 2013; Renzi et al., 2014; Vishwanathan et al., 2014a). Of particular interest, lutein levels have been linked to memory and general intelligence (Feeney et al., 2013; Johnson et al., 2013; Vishwanathan et al., 2014a), which are cognitive constructs closely related to crystallized intelligence. Crystallized intelligence refers to the ability to retrieve and use information that has been acquired throughout life (Horn and Cattell, 1966). Although most aspects of cognitive function undergo age-related decline, certain aspects of cognition—like crystallized intelligence—are spared and even show improvement with age (Craik and Bialystok, 2006; Park and Reuter-Lorenz, 2009). Measuring crystallized intelligence may therefore be a way to assess the lifelong impact of nutritional factors, rather than the immediate effects of nutrients on preventing age-related decline (Craik and Bialystok, 2006).

Crystallized intelligence is dependent upon the temporal cortex, and particular regions of the temporal cortex may play key roles in implementing this cognitive function (Barbey et al., 2012; Colom et al., 2009). In general, structural integrity of the temporal cortex is associated with performance on tasks of crystallized intelligence, but integrity of specific regions within the temporal cortex may underlie the preservation of this cognitive function in aging (Choi et al., 2008). For example, the parahippocampal cortex plays a role in mediating the storage of knowledge about objects (Aminoff et al., 2013; Ricci et al., 1999). This region of the temporal cortex shows resistance to age-related structural decline in the absence of neurodegenerative disease, unlike other regions within the temporal cortex that show significant cortical thinning

even in healthy aging (Jiang et al., 2014; Salat et al., 2004). Although the sparing of particular regions within the temporal cortex may be linked to the preservation of crystallized intelligence in healthy aging, the question remains: do particular neuroprotective nutrients like lutein underlie this sparing of cognition and brain health?

In summary, increasing evidence suggests that lutein may be a reliable nutrient biomarker for healthy brain aging: (i) among the carotenoids, lutein accounts for the majority of accumulation in the brain and provides unique neuroprotective benefits, (ii) lutein status has been linked to cognitive performance across the lifespan, and (iii) lutein is selectively distributed in gray matter of brain regions known to underlie the preservation of cognitive function in healthy brain aging. However, the core brain structures upon which lutein may act to preserve cognition have not been investigated. Prior research suggests that lutein plays a neuroprotective role across the lifespan, crystallized intelligence is resistant to age-related decline, and specific regions of the temporal cortex may underlie the preservation of crystallized intelligence. This study aims to explore the role of structures within the temporal cortex in mediating the relationship between lutein serum levels and crystallized intelligence in healthy older adults.

Materials and methods

Participants

This cross-sectional study enrolled 122 participants (ages 65-75) from Carle Foundation Hospital, a local and readily available cohort of well-characterized older adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination (Folstein et al., 1975). Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months,

and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for magnetic resonance imaging (MRI). Of these 122 participants, 76 subjects had a complete dataset at the time of data analysis, including neuropsychological testing, MRI, and serum analysis.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Serum acquisition and lutein analysis

Serum was prepared for extraction using 100 μL of sample and 0.5 mL 0.9% saline. Echinenone, in ethanol, was added as an internal standard (DSM Nutritional Products). The mixture was extracted by using 2 mL $CHCL_3$:CH_3OH (2:1, vol/vol). The mixture was vortexed and then centrifuged at 800g for 15 minutes at 4°C. The $CHCl_3$ layer was removed and evaporated to dryness under nitrogen. A second extraction was performed on the mixture using 3 mL hexane. The mixture was vortexed and centrifuged as above. The hexane layer was combined with the first extraction and evaporated to dryness under nitrogen. The residue was redissolved in 100 μL of ethanol, vortexed, and sonicated for 30 seconds. A 20 μL aliquot was used for HPLC analysis.

Lutein was measured using a reversed-phase, gradient HPLC system. The system consisted of a Waters Alliance 2695 Separation Module LC pump, auto-sampler, Waters 2996

Photo-Array Detector (Millipore, Milford, MA) and a semi-bore C30 column (3 μm, 150 x 4.6 mm, YMC, Wilmington, NC). The chromatographic separations were performed on a Waters Alliance 2695 (Millipore, Milford, MA) system using a UV detector and Waters Empower Pro software. The flow rate was 0.4 mL/min and the gradient elution used two mixtures of methanol, tert-butyl methyl ether, and water (mixture A: 83/15/2; v/v/v), mixture B: 8/90/2, v/v/v). The gradient procedure was: 0 to 1 minute 100% A, 1 to 8 minute linear gradient to 70% A, 8 to 13 minutes 70% A, 13 to 22 minute linear gradient to 45% A, 22 to 24 minutes 45% A, 24 to 34 minutes linear gradient to 5% A, 34 to 38 minutes 5% A, 38 to 40 minutes linear gradient to 100% A, and 40 to 50 minutes 100% A.

The method yielded adequate separation of lutein, which was then quantified at 445 nm. Quantification was determined from peak areas in the HPLC chromatograms calibrated against known amounts of standards. Concentrations were corrected for extraction and handling losses by monitoring the recovery of the echinenone internal standard. The lower detection with this method is 0.2 pmol. A composite lutein score was computed by summing across all-*trans* lutein and *cis*-lutein.

Neuropsychological tests

Crystallized intelligence was measured by the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II) (Wechsler, 1999). This assessment measured crystallized intelligence by way of a verbal comprehension index, which was the product of two subtests: a vocabulary subtest and a similarities subtest. In the vocabulary subtest, participants were asked to verbally define vocabulary words (i.e., What does lamp mean?) that became progressively more challenging. In the similarities subtest, participants were asked to relate pairs

of concepts (i.e., How are a cow and bear alike?) that became progressively more challenging. These subtests were compiled into a verbal comprehension index, which provided a measure of acquired knowledge, verbal reasoning, and attention to verbal information, and therefore served as a marker of crystallized intelligence.

Volumetric brain MRI

Volumetric analysis was performed on data from a 3D high-resolution (0.9 mm isotropic) T1-weighted scan using MPRAGE acquisition. Cortical reconstruction was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale et al., 1999; Dale and Sereno, 1993; Fischl et al., 1999a, 1999b, 2001, 2002, 2004a, 2004b; Fischl and Dale, 2000; Han et al., 2006; Jovicich et al., 2006; Reuter et al., 2010, 2012; Ségonne et al., 2004). All cortical reconstructions were manually checked for accuracy, as recommended by the software developers. The volumetric analyses focused on gray matter volume in the temporal cortex provided by Freesurfer parcellation. Regions of interest included the superior temporal cortex, middle temporal cortex, inferior temporal cortex, banks of the superior temporal sulcus, fusiform cortex, transverse temporal cortex, entorhinal cortex, temporal pole, and parahippocampal cortex.

Covariates

Covariates were included according to previous association with cognitive decline. These included age (continuous), gender (nominal, man/woman), education (nominal, five fixed levels), income (nominal, six fixed levels), body mass index (continuous, hereafter BMI), and

depression status (nominal, yes/no). Although all participants had received a diagnosis of no depression at enrollment, the SF-36 Health Survey (Ware et al., 1993) revealed four participants with symptoms consistent with depression, and in accordance with other studies, this was considered in the analysis as a covariate. Temporal cortex gray matter volume (continuous) was also included as a covariate in mediation analyses to assess the relationship between specific regions within the temporal cortex, serum lutein, and crystallized intelligence. These covariates were included in each of the three steps of the mediation analysis.

Statistical analyses

A formal mediation analysis was used in an effort to better understand the relationship between serum lutein, gray matter volume of regions within the temporal cortex, and crystallized intelligence using a three-step framework. The primary requirement for mediation is a significant indirect mediation effect (Zhao et al., 2010), defined as the effect of the independent variable (lutein) through the mediator (gray matter volume of regions within the temporal cortex) on the dependent variable (crystallized intelligence) (**Figure 4.1**).

Statistics were performed in SPSS Statistical Packages version 23 (SPSS, Inc., Chicago, IL, USA), and mediation analyses were performed using the *indirect* macro designed for SPSS (Preacher and Hayes, 2008). In the first step, a regression model was used to characterize the relationship between serum lutein and gray matter volume of regions within the temporal cortex (**path a**), controlling for the covariates listed above and applying a false discovery rate (FDR) correction for multiple comparisons ($q < 0.05$, one-tailed). In the second step, a regression model was used to characterize the relationship between serum lutein and crystallized intelligence (**path c**), controlling for the covariates listed above. In the third step, the *indirect* macro was used to

implement the bootstrapping method to estimate mediation effects. This analysis drew 1000 bootstrapped samples with replacement from the dataset to estimate a sampling distribution for the indirect and direct mediation effects, controlling for the covariates listed above. The indirect mediation effect refers to the pathway from serum lutein to gray matter volume of temporal cortex regions to crystallized intelligence (**path a-b**). The direct mediation effect refers to the direct pathway from serum lutein to crystallized intelligence (**path c'**). A statistically significant full mediation that matches the hypothesized framework is indicated by: (i) an indirect mediation effect that does not include zero within 95% bias-corrected confidence intervals, and (ii) a direct mediation effect that does include zero within 95% bias-corrected confidence intervals. A statistically significant partial mediation, (i.e., the mediation does not account for all possible mediators) is indicated by: (i) an indirect mediation effect that does not include zero within 95% bias-corrected confidence intervals, and (ii) a direct mediation effect that does not include zero within 95% bias-corrected confidence intervals (Zhao et al., 2010). Results are reported using unstandardized regression coefficients (β) and statistical significance (p) for each individual regression relationship, and a 95% bias-corrected confidence interval (95% CI) for the direct and indirect effects of the mediation.

Results

Participant characteristics

Participants had a mean age of 69 years and 67 percent of participants were females. The mean lutein level was 454 pmol/mL. The mean MMSE score was 29 and the Wechsler Abbreviated Scale of Intelligence crystallized intelligence score was 111 (**Table 4.1**).

Serum lutein and crystallized intelligence, mediated by parahippocampal structure

The mediation analyses indicated that out of all regions within the temporal cortex, gray matter thickness of only the right parahippocampal cortex (Brodmann's Area 34) mediated the relationship between serum lutein and crystallized intelligence. Each relationship within the mediation is described below in a stepwise fashion.

First, higher serum lutein levels were associated with larger volume of one region within the temporal cortex, the right parahippocampal cortex (β=0.336, p=0.003, **Table 4.2**). Therefore, the relationship between serum lutein concentration and gray matter volume of the right parahippocampal cortex was considered in the context of the mediation model (β=0.336, p=0.003, **Figure 4.2 path a**). Second, higher serum levels of lutein associated with superior crystallized intelligence (β=0.018, p=0.002, **Figure 4.2 path c**). Third, mediation effects were estimated for the right parahippocampal cortex. The indirect pathway of mediation was significant (95% CI: 0.002 – 0.013, β=0.016, p=0.006, **Figure 4.2 path a-b**), and the direct pathway of mediation was significant (95% CI: 0.0007 – 0.023, β=0.012, p=0.037, **Figure 4.1 path c'**). Therefore, the right parahippocampal cortex partially mediated the relationship between serum lutein and crystallized intelligence (**Figure 4.2**).

Discussion

This study revealed that gray matter volume of the right parahippocampal cortex mediates the relationship between serum lutein and crystallized intelligence. This report provides a novel link between lutein, gray matter volume of a specific cortical region, and crystallized intelligence. The individual relationships reported within the mediation, including those between serum lutein levels and parahippocampal cortex (**Figure 4.2 path a**), between serum lutein levels

and crystallized intelligence (**Figure 4.2 path c**), and between parahippocampal cortex and crystallized intelligence (**Figure 4.2 path b**), are each supported by prior findings reviewed in turn below.

Prior findings support each relationship reported within the mediation model. The first relationship demonstrated a positive association between serum lutein and gray matter volume of the parahippocampal cortex in the right hemisphere. Four lines of evidence support this finding: (i) serum lutein correlates with brain concentrations of lutein in older adults, (ii) lutein preferentially accumulates in brain tissue, (ii) xanthophylls are selectively distributed in gray matter, as opposed to white matter, and (iii) lutein is found in the temporal cortex (Craft et al., 2004; Johnson, 2012; Johnson et al., 2013; Vishwanathan et al., 2014b). Second, higher serum levels of lutein were associated with higher scores of crystallized intelligence. Prior work demonstrates that intake of green leafy and cruciferous vegetables – both major dietary sources of lutein - is positively associated with cognitive function (Kang et al., 2005; Morris et al., 2006). Indeed, levels of macular pigment optical density (MPOD), a biomarker of lutein concentrations in the brain, has been reported to be significantly related to cognitive function in three independent research groups (Feeney et al., 2013; Renzi et al., 2014; Vishwanathan et al., 2013, 2014a, 2016). Among the carotenoids, lutein most consistently associates with measures of intelligence and memory (Johnson et al., 2008b, 2013). Third, the indirect pathway of mediation indicated a mediatory effect of right parahippocampal gray matter volume on the relationship between lutein and crystallized intelligence. Previous studies indicate that greater temporal cortex gray matter volume correlates with general intelligence, and the parahippocampal cortex in particular shows activity during tasks that require auditory-verbal recall (Colom et al., 2009; Grasby et al., 1993). Furthermore, healthy aging is characterized by the preservation of

crystallized intelligence as well as structural integrity of the parahippocampal cortex (Horn and Cattell, 1966; Jiang et al., 2014; Salat et al., 2004). The unilateral nature of this mediation is supported by work suggesting that the right hemisphere is more resistant to age-related and disease-related neurodegeneration than the left hemisphere (Chételat et al., 2005; Mosconi et al., 2014; Querbes et al., 2009; Risacher et al., 2010).

The underlying physiological mechanisms of the relationship between serum lutein levels, crystallized intelligence, and cortical integrity of the parahippocampal cortex are three-fold. First, lutein is differentially localized to membrane domains high in polyunsaturated fatty acids, and is therefore well positioned to prevent oxidation of vulnerable lipids (Widomska and Subczynski, 2014). Second, by preventing lipid oxidation, lutein may help preserve long-chain polyunsaturated fats, such as docosahexaenoic acid (DHA), for cleavage and conversion into anti-inflammatory compounds (Miller et al., 2014). Third, lutein is a polar and soluble molecule, and can therefore span the membrane and influence membrane properties, such as fluidity, ion exchange, oxygen diffusion, membrane stability, and interneuronal communication via gap junctions (Stahl and Sies, 2001). Importantly, these neuroprotective effects are relevant across the lifespan, and may therefore be particularly important for the preservation of cognitive functions that are enhanced with increasing age, such as crystallized intelligence (Bovier and Hammond, 2015; Horn and Cattell, 1966; Lieblein-Boff et al., 2015).

The partial nature of this mediation is supported by the specificity of this analysis, which focused on one aspect of cognition and its underlying cortical structures. Given that lutein is thought to influence a wide range of biological processes in the brain, it is unlikely that the role of lutein in the aging brain is limited to crystallized intelligence and the parahippocampal cortex (Erdman et al., 2015). Rather than claiming the parahippocampal cortex as the sole mediator of

the relationship between lutein and crystallized intelligence, this study suggests that preserving structural integrity of the parahippocampal cortex is one mechanism through which lutein contributes to the preservation of cognitive function. These findings add to a growing line of evidence which suggests that particular nutrients may slow or prevent aspects of age-related cognitive decline by targeting specific features of brain aging (Bowman et al., 2012; Gu et al., 2016; Zamroziewicz et al., 2015). In the case of lutein, future studies are needed to assess whether lutein is uniquely protective of crystallized intelligence and temporal cortex structure, or whether other carotenoids contribute neuroprotective effects as well. Another promising direction for future work is to examine the interactive effects among nutrients through the use of nutrient biomarker pattern analysis – a technique that enables an investigation of the beneficial effects of broader nutrient profiles on healthy brain aging. Ultimately, this line of research can inform a comprehensive and personalized approach to nutritional intervention that takes into account dietary patterns and individual variability in nutritional status and brain health.

The strengths of the present study include: (i) the use of blood biomarkers to measure physiological status of lutein, (ii) the use of structural magnetic resonance imaging to measure regional cortical integrity with high spatial resolution, and (iii) the assessment of a particular component of cognitive function known to be highly variable across individuals, rather than a global cognitive function measure with little variability within a sample. The limitations of this study include: (i) relatively small sample size, (ii) cross-sectional design, (iii) lack of MPOD measurement to better reflect lutein concentrations in neural tissue (Vishwanathan et al., 2013, 2016), and (iv) lack of assessment of other carotenoids. Thus, directions for future research include: (i) larger sample sizes to confirm these results, (ii) longitudinal studies to investigate the relationship between serum lutein and crystallized intelligence in different stages of life, (iii)

MPOD measures of lutein in neural tissue, and (iv) assessment of other carotenoids to determine potential neuroprotective benefits.

Tables and Figures

Table 4.1. Characteristics of sample

Demographics	***n* = 76**
Age in years (M ± SD)	69 ± 3
Female (%)	67
Education (%)	
Some high school	1
High school degree	15
Some college	16
College degree	68
Income (%)	
< $15,000	1
$15,000 - $25,000	3
$25,000 - $50,000	17
$50,000 - $75,000	22
$75,000 - $100,000	24
> $100,000	33
Depression (%)	5
Serum nutrients	**(M ± SD, pmol/mL)**
Lutein	454 ± 275
Cognition	**(M ± SD)**
Mini-Mental State Examination	29 ± 1
Crystallized intelligence score	111 ± 14
Gray matter volume	**(M ± SD, mm^3)**
Left temporal lobe	6670 ± 645
Right temporal lobe	5458 ± 599
Left superior temporal	10889 ± 1482
Right superior temporal	10858 ± 1502
Left middle temporal	9593 ± 1418
Right middle temporal	10724 ± 1445
Left inferior temporal	10105 ± 1606
Right inferior temporal	9665 ± 1401
Left banks of the superior temporal sulcus	2229 ± 464
Right banks of the superior temporal sulcus	2179 ± 409
Left fusiform	9281 ± 1445
Right fusiform	8973 ± 1397
Left transverse temporal	1102 ± 210
Right transverse temporal	836 ± 164
Left entorhinal	1779 ± 388
Right entorhinal	1729 ± 369
Left temporal pole	2464 ± 383
Right temporal pole	2247 ± 342
Left parahippocampal	2067 ± 318
Right parahippocampal	1914 ± 258

Abbreviations: mean (M), standard deviation (SD)

Table 4.2. Linear regression models: Serum lutein levels and association with crystallized intelligence

Cortical Region Volume	**β**	**p**
Left superior temporal	-0.424	0.318
Right superior temporal	-0.378	0.364
Left middle temporal	-0.809	0.084
Right middle temporal	0.421	0.356
Left inferior temporal	0.922	0.093
Right inferior temporal	0.186	0.695
Left banks of the superior temporal sulcus	0.013	0.943
Right banks of the superior temporal sulcus	-0.051	0.713
Left fusiform	-0.318	0.442
Right fusiform	-0.328	0.432
Left transverse temporal	-0.086	0.393
Right transverse temporal	-0.171	0.03
Left entorhinal	0.331	0.037
Right entorhinal	-0.148	0.151
Left temporal pole	0.265	0.095
Right temporal pole	0.133	0.457
Left parahippocampal	0.106	0.443
Right parahippocampal	0.336	0.003*

*$p < 0.05$, FDR-corrected

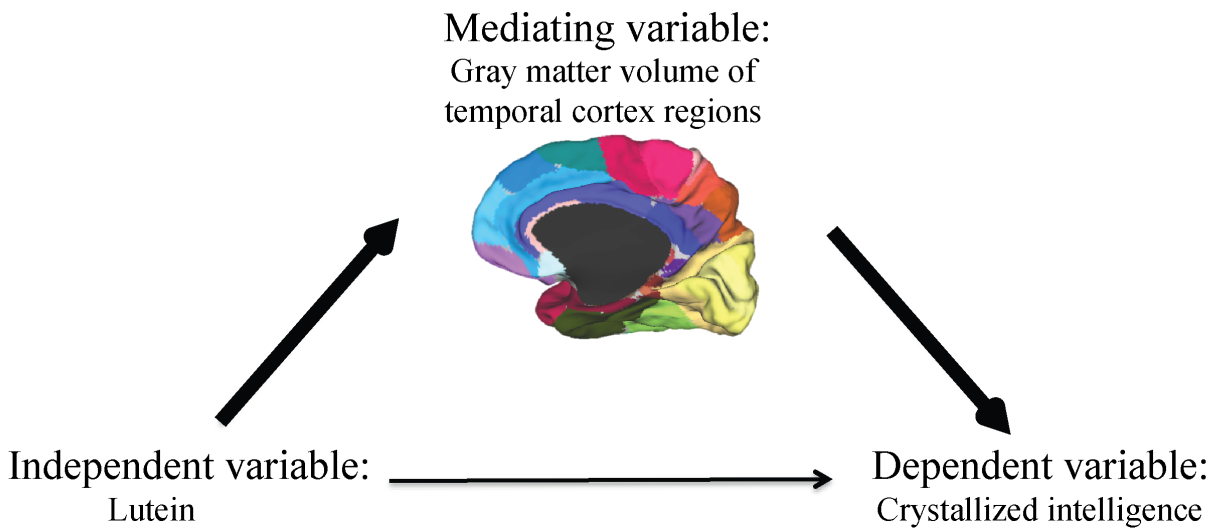

Figure 4.1. Proposed mediation model: The primary requirement for mediation is a significant indirect mediation effect, defined as the effect of the independent variable (lutein) through the mediator (gray matter volume of regions within the temporal cortex) on the dependent variable (crystallized intelligence).

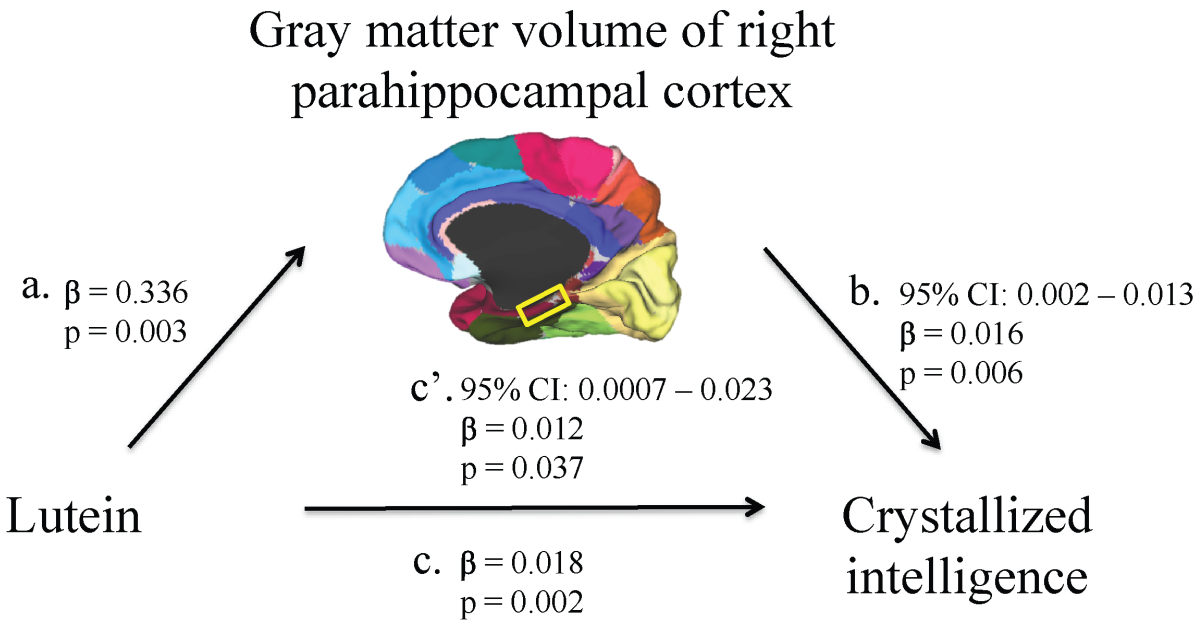

Figure 4.2. Mediation model statistics: Serum lutein concentrations positively associated with gray matter volume of the right parahippocampal cortex (**path a**). Serum lutein positively associated with crystallized intelligence (**path c**). The indirect pathway of mediation (i.e., the effect of serum lutein through gray matter volume of the right parahippocampal cortex on crystallized intelligence; **path a-b**) was statistically significant. Likewise, the direct pathway of mediation (i.e., the effect of serum lutein on crystallized intelligence; **path c'**) was statistically significant. Therefore, gray matter volume of the right parahippocampal cortex partially mediated the relationship between serum lutein and crystallized intelligence.

CHAPTER 5: DETERMINANTS OF FLUID INTELLIGENCE IN HEALTHY AGING: OMEGA-3 POLYUNSATURATED FATTY ACID STATUS AND FRONTOPARIETAL CORTEX STRUCTURE[5]

Abstract

Accumulating evidence indicates that cognitive decline depends not only upon changes in brain health, but critically, also upon nutritional status. Decline in fluid intelligence, one of the most debilitating aspects of cognitive aging, has been linked to omega-3 polyunsaturated fatty acid status; however, it is not known whether this phenomenon results from specific omega-3 polyunsaturated fatty acids acting on particular aspects of brain health. Therefore, this study aims to explore whether particular patterns of omega-3 polyunsaturated fatty acids influence fluid intelligence by supporting specific neural structures. We measured six plasma phospholipid omega-3 polyunsaturated fatty acids, fluid intelligence, and regional gray matter volume in the frontal and parietal cortices in 100 cognitively intact older adults (65 to 75 years old). A four-step mediation analysis was implemented using principal component analysis and multivariate linear regressions, adjusted for age, gender, education, and body mass index. The mediation analysis revealed that one pattern of omega-3 polyunsaturated fatty acids, consisting of alpha-linolenic acid, stearidonic acid, and eicosatrienoic acid, was linked to fluid intelligence, and that total gray matter volume of the left frontoparietal cortex fully mediated the relationship between this omega-3 polyunsaturated fatty acid pattern and fluid intelligence. These data demonstrate

[5] This appeared in its entirety in *Nutritional Neuroscience* and is referred to in this dissertation as "Zamroziewicz et al., 2017." This is the authors accepted manuscript of an article published as the version of record in *Nutritional Neuroscience* 11th May 2017 (http://www.tandfonline.com/ http://dx.doi.org/10.1080/1028415X.2017.1324357), and has been reprinted with permission. Zamroziewicz, M.K., Paul, E.J., Zwilling, C.E., Barbey, A.K. (2017) Determinants of fluid intelligence in healthy aging: omega-3 polyunsaturated fatty acids status and frontoparietal cortex structure. *Nutritional Neuroscience*. doi: 10.1080/1028415X.2017.1324357

that fluid intelligence may be optimally supported by specific omega-3 polyunsaturated fatty acids through preservation of frontoparietal cortex gray matter structure in cognitively intact older adults. This report provides novel evidence for the benefits of particular omega-3 polyunsaturated fatty acid patterns on fluid intelligence and underlying gray matter structure.

Introduction

Nutrition has increasingly been recognized for its ability to help prevent and protect against disease, and at the frontiers of this effort is research within the emerging interdisciplinary field of nutritional cognitive neuroscience. This line of work demonstrates that cognitive decline depends not only upon changes in brain structure and brain function, but critically, also upon dietary intake and nutritional status (Zamroziewicz and Barbey, 2016). As the United States experiences rapid growth in the proportion of older adults, the search for effective strategies to promote healthy brain aging provides a catalyst for research to investigate the beneficial effects of nutrition on the aging brain.

In the absence of neurodegenerative disease, decline in fluid intelligence presents as one of the most debilitating aspects of cognitive aging (Schaie, 1994). Fluid intelligence refers to the intellectual abilities required for adaptive problem solving in novel situations, and reflects the capacity to creatively and flexibly grapple with the world in ways that do not rely on prior knowledge (Horn and Cattell, 1967). A fundamental issue in the study of cognitive aging has historically been whether fluid intelligence can be maintained into late adulthood (Tranter and Koutstaal, 2008). Indeed, recent evidence indicates that age-related decline in fluid intelligence is mediated by nervous system health, highlighting the potential for intervention by neuroprotective nutrients (Bergman and Almkvist, 2013).

Increasing evidence suggests that omega-3 (n-3) polyunsaturated fatty acids (PUFAs) benefit the aging brain (Parletta et al., 2013). PUFAs are known to contribute to structural integrity of neuronal membranes, control inflammation and oxidation, and promote energy metabolism (Cunnane et al., 2009). Omega-3 PUFAs have been linked to the preservation of cognitive functions vulnerable to age-related decline, including fluid intelligence (Tan et al., 2012). However, it is not known which brain structures n-3 PUFAs may act upon to support fluid intelligence, and whether particular patterns of n-3 PUFAs preferentially provide support.

Fluid intelligence engages a distributed brain circuit within the frontal and parietal cortex (Barbey et al., 2014b; Woolgar et al., 2015). Specifically, fluid intelligence is linked to structural integrity and neural activity within the lateral prefrontal and posterior parietal cortices, regions cumulatively referred to as the frontoparietal cortex (FPC) (Cole et al., 2012; Raz et al., 2008). Importantly, n-3 PUFAs slow age-related structural decline in the FPC (Titova et al., 2013; Witte et al., 2014), and in this way, could prevent age-related decline in fluid intelligence. Thus, the FPC plays a critical role in fluid intelligence and is amenable to n-3 PUFAs, making it a target region of interest for investigating the impact of n-3 PUFAs on the cognitive and neural mechanisms of fluid intelligence.

In summary, one of the most debilitating aspects of cognitive aging, decline in fluid intelligence and degeneration of the underlying FPC, may be ameliorated by n-3 PUFA intake. However, it is not known whether particular patterns of n-3 PUFAs influence core brain regions to support fluid intelligence. Therefore, this study aims to (i) identify nutritional biomarkers of fluid intelligence by empirically deriving patterns of n-3 PUFAs and (ii) distinguish the neural structures that mediate the beneficial effect of n-3 PUFAs on fluid intelligence.

Materials and methods

Study participants

This cross-sectional study enrolled 122 healthy elderly adult patients from Carle Foundation Hospital, a local and readily available cohort of well-characterized elderly adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination (Folstein et al., 1975). Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for magnetic resonance imaging (MRI). All participants were right handed with normal, or corrected to normal vision and no contraindication for MRI. Of these 122 participants, 22 participants did not have a complete dataset, which included neuropsychological testing, MRI, and blood biomarker analysis. Therefore, 100 participants were considered in the current analysis.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and the Carle Hospital Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Biomarker acquisition and analysis

Plasma lipids were extracted by the method of Folch, Lees and Sloane-Stanley (Folch et al., 1957). Briefly, the internal standard (25 μg each of PC17:0) was added to 200 μl of serum,

followed by 6 mL of choloroform:methanol:BHT (2:1:100 v/v/w). The protein precipitate was removed by centrifugation (2,500 g, 5 minutes, 4ºC). Then 1.5 mL of 0.88% KCl was added to the supernatant, shaken vigorously and the layers were allowed to settle for 5 minutes. The upper layer was discarded and 1 ml of distilled water:methanol (1:1 v/v) was added, the tube was shaken again and the layers allowed to settle for 15 minutes. The lower layer was transferred into a clean tube and evaporated to dryness under nitrogen. The phospholipid subfraction was separated by solid-phase extraction using aminopropyl columns as described by Agren, Julkunen and Penttila (Aryen et al., 1992). Then the phospholipid fraction was methylated by adding 2 ml of 14% BF3-MeOH and incubating at 95ºC for 1 hour (Morrison and Smith, 1964). The supernatant containing the fatty acid methyl esters (FAMEs) was dried down under nitrogen, resuspended in 100 μl of hexane, transferred into amber GC vials and stored at -20ºC until the time of analysis.

The phospholipid FAMEs were analyzed by a CLARUS 650 gas chromatograph (Perkin Elmer, Boston MA) equipped with a 100 m x 0.25 mm i.d (film thickness 0.25 μm) capillary column (SP-2560, Supelco). Injector and flame ionization detector temperatures were 250ºC and 260ºC, respectively. Helium was used as the carrier gas (2.5 mL/min) and the split ratio was 14:1. The oven temperature was programmed at 80ºC, held for 16 minutes and then increased to 180ºC at a rate of 5ºC/minute. After 10 minutes, the temperature was increased to 192ºC at a rate of 0.5ºC/minute and held for 4 minutes. The final temperature was 250ºC reached at a rate of 405ºC/minute and held for 15 minutes. Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc. MN) and expressed as absolute concentration (μmol/L). The plasma phospholipid lipids of interest were n-3 PUFAs, including α-linolenic acid (ALA, 18:3n-3), stearidonic acid (SDA, 18:4n-3), eicosatrienoic acid (20:3n-3, ETE),

eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3).

Nutrient biomarker pattern analysis of PUFAs

Nutrient biomarker pattern analysis was conducted in IBM SPSS statistical software, version 24 for Macintosh. Principal component analysis was used to identify nutrient biomarker patterns (NBPs) from the six n-3 PUFAs of interest. Of these, five n-3 PUFAs (ALA, ETE, EPA, DPA, and DHA) were non-normally distributed as indicated by Shapiro-Wilk test (all p-values<0.05), and therefore log-transformed to correct for skewness of variables and subsequently considered in the analysis. The appropriate rotation method was determined by examining the factor correlation matrix: varimax rotation was chosen for a correlation matrix with values less than 0.32 and direct oblimin rotation was chosen for a correlation matrix with values greater than 0.32 (Tabachnick and Fidell, 2007). Statistical validity of the factor analysis was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (≥ 0.50) (Kaiser, 1970) and Bartlett's Test of Sphericity ($p<0.05$) (Bartlett, 1950). The number of NBPs to be retained was determined by a combination of eigenvalues greater than 1.0, variance accounted for by each component, and scree plot inflection point. Interpretation of each factor was based on identifying biomarkers with an absolute loading value of greater than 0.50 on a NBP (i.e., identifying the dominant biomarkers contributing to each particular NBP). Each participant received a standardized NBP score for each pattern that corresponded to a linear combination of the nutrient biomarkers.

Neuropsychological tests

Fluid intelligence was measured by the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II) (Wechsler, 1999). This assessment measured fluid intelligence by way of a perceptual reasoning index, which was the product of two subtests: a block design subtest and a matrix reasoning subtest. In the block design subtest, participants were asked to reproduce pictured designs using specifically designed blocks as quickly and accurately as possible. In the matrix reasoning subtest, participants were asked to complete a matrix or serial reasoning problem by selecting the missing section from five response items. Subjects' raw scores were converted to normalized scaled scores and subsequently combined into a perceptual reasoning index, which provided a measure of nonverbal reasoning and fluid intelligence.

Volumetric brain MRI

Volumetric analysis was performed on data from a 3D high-resolution T1-weighted scan using MPRAGE acquisition (0.9 mm isotropic voxel; TR: 1900 ms, TI: 900 ms, TE: 2.32 ms, with GRAPPA and an acceleration factor of 2). Cortical reconstruction was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale et al., 1999; Dale and Sereno, 1993; Fischl et al., 1999a, 1999b, 2001, 2002, 2004a; Fischl and Dale, 2000; Han et al., 2006; Jovicich et al., 2006; Reuter et al., 2010, 2012; Ségonne et al., 2004). All cortical reconstructions were manually checked for accuracy, as recommended by the software developers. The volumetric analyses focused on gray matter volume in the FPC, given the role of this cortical region in fluid intelligence (Cole et al., 2012; Raz et al., 2008) and its sensitivity to n-3 PUFAs (Titova et al., 2013; Witte et al., 2014). As provided by Freesurfer parcellation, the FPC consisted of the following regions of interest:

superior frontal cortex, rostral middle frontal cortex, caudal middle frontal cortex, pars opercularis, pars triangularis, pars orbitalis, superior parietal cortex, supramarginal cortex, and precuneus (Colom et al., 2010; Vijayakumar et al., 2014). The volumetric analyses took into consideration total gray matter volume of the FPC as well as gray matter volume of individual regions within the FPC.

Covariates

Covariates were included according to previous association with cognitive decline (Coffey et al., 1998, 1999; Fotenos et al., 2008; Gunstad et al., 2008; Raz et al., 2010; van Tol et al., 2010). The covariates included age (continuous), gender (nominal, man/woman), education (nominal, five fixed levels), and body mass index (continuous). Volumetric analyses of the total FPC additionally accounted for intracranial volume (continuous), and volumetric analyses of individual regions within the FPC additionally accounted for total FPC volume (continuous) in an effort to isolate the contribution of each individual region.

Statistical analysis

A formal mediation framework was applied to: (i) identify predictive nutritional biomarkers of fluid intelligence, as derived by NBP analysis, and (ii) distinguish the neural structures that mediate the beneficial effect of n-3 PUFA patterns on fluid intelligence. First, regression models characterized the three relationships within the mediation framework: (i) the relationship between NBPs and fluid intelligence, (ii) the relationship between gray matter volume within the FPC and fluid intelligence, and (iii) the relationship between NBPs and gray matter volume within the FPC. Second, taking into account results of the regression analyses, a

mediation model assessed whether gray matter volume within the FPC mediated the relationship between NBPs and fluid intelligence (**Figure 5.1**). Statistics were performed as follows:

1. In the first step, one linear regression model was used to characterize the relationship between NBPs and fluid intelligence (**Figure 5.1 path a**). This analysis accounted for covariates listed above. The results of this regression model indicated independent variables for consideration in the mediation model.
2. In the second step, linear regression models were applied to characterize the relationship between each gray matter volume within the FPC, including total FPC volume and volume of individual regions within the FPC, and fluid intelligence **(Figure 5.1 path c**). This analysis accounted for covariates listed above and applied a false discovery rate (FDR) correction for multiple comparisons (q<0.05, one-tailed) (Benjamini and Hochberg, 1995). The results of these regression models indicated mediatory variables for consideration in the mediation model.
3. In the third step, linear regression models were used to characterize the relationship between NBPs and each gray matter volume within the FPC, including total FPC volume and volume of individual regions within the FPC (**Figure 5.1 path b**). This analysis accounted for covariates listed above and applied a false discovery rate (FDR) correction for multiple comparisons (q<0.05, one-tailed) (Benjamini and Hochberg, 1995). The results of these regression models further specified mediatory variables for consideration in the mediation model.
4. In the fourth step, the PROCESS macro designed for SPSS was applied to implement the bootstrapping method to estimate mediation effects (Preacher and Hayes, 2008). This analysis drew 1000 bootstrapped samples with replacement from the dataset to estimate a

sampling distribution for indirect and direct mediation effects, controlling for covariates listed above. The indirect mediation effect refers to the pathway from NBPs to gray matter volume within the FPC to fluid intelligence (**Figure 5.1 path b-c**). The direct mediation effect refers to the direct pathway from NBPs to fluid intelligence, accounting for the effect of gray matter volume within the FPC (**Figure 5.1 path a'**). As shown in **Figure 5.1**, the primary requirement for mediation is a significant indirect mediation effect (Zhao et al., 2010), or the effect of the independent variable (NBPs) through the mediator (gray matter volume within the FPC) on the dependent variable (fluid intelligence**)**. To further validate the proposed mediation model, an alternative mediation model, incorporating FPC as the independent variable, NBPs as the mediating variable, and fluid intelligence as the dependent variable, was also tested.

Results are reported using (i) R^2 and p for each model, (ii) unstandardized regression coefficients (β), unstandardized regression coefficient standard error (SE β), and p of each individual regression relationship, and (iii) a 95% bias-corrected confidence interval (95% CI) for the direct and indirect effects of the mediation. Significance was accepted at $p \leq 0.05$. A statistically significant mediation that matches the hypothesized framework is indicated by: (i) an indirect mediation effect that does not include zero within 95% CI, and (ii) a direct mediation effect that does include zero within 95% CI (Zhao et al., 2010).

Results

Participant characteristics

Participants (n=100) had a mean age of 69 years and 62 percent of participants were females (n=62). All other participant characteristics are reported in **Table 5.1**.

Nutrient biomarker patterns

Principal component analysis generated two NBPs (**Table 5.2**). The factor correlation matrix contained values greater than 0.32, therefore direct oblimin rotation was implemented. Statistical validity of the factor analyses was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (0.728) and Bartlett's Test of Sphericity ($p < 0.001$). Two NBPs were selected for retention because (i) after the second NBP extraction with principal component analysis, 71.8 percent of the total variance was accounted for in the original set of nutrient biomarkers, and (ii) inspection of the scree plot indicated that the inflection point occurred after the second NBP (**Figure 5.2**). Hereafter, the first NBP is described as product n-3 PUFAs (i.e., it is composed of downstream n-3 PUFAs, including EPA, DPA n-3, and DHA), and second NBP is described as precursor n-3 PUFAs (i.e., it is composed of three n-3 PUFAs that serve as precursors to EPA and DHA).

Nutrient biomarker patterns, fluid intelligence, and gray matter volume with the FPC

The mediation analyses indicated that fluid intelligence was linked to precursor n-3 PUFAs as well as total gray matter volume within the frontoparietal cortex, and furthermore, that total gray matter volume of the left frontoparietal cortex fully mediated the relationship between precursor n-3 PUFAs and fluid intelligence. Each relationship within the mediation is described below in a stepwise fashion:

1. Better fluid intelligence was predicted by higher precursor n-3 PUFAs ($R^2_{model}=0.133$, $p_{model}=0.035$; $\beta=3.236$, SE $\beta=1.544$, $p_{variable}=0.039$), but not higher product n-3 PUFAs (**Table**

5.3). Therefore, precursor n-3 PUFAs were considered as a candidate independent variable in the mediation model (**Figure 5.3 path a**).

2. Better fluid intelligence was predicted by larger volume of the left FPC (R^2_{model}=0.181, p_{model}=0.004; β=0.001, SE β<0.001, $p_{variable}$=0.009) and smaller volume of the right supramarginal cortex (R^2_{model}=0.220, p_{model}=0.001; β=-0.004, SE β=0.001, $p_{variable}$=0.006), but no other region of the FPC (**Table 5.4**). Therefore, left FPC and right supramarginal cortex were considered as candidate mediators in the mediation model (**Figure 5.3 path b**).
3. Higher precursor n-3 PUFAs also predicted larger volume of the left FPC (R^2_{model}=0.641, p_{model}<0.001; β=1494.073, SE β=557.356, $p_{variable}$=0.009), but no other region of the FPC (**Table 5.5**). Therefore, only left FPC was retained as a candidate mediator in the mediation model (**Figure 5.2 path b**).
4. The mediation model investigating the mediatory effect of the left FPC on the relationship between precursor n-3 PUFAs and fluid intelligence indicated a full mediation (R^2_{model}=0.30, p_{model}=0.001). The indirect pathway of mediation was significant (95% CI [0.178 – 2.741], **Figure 5.2 path b-c**; β=0.007, SE β =0.0003, $p_{variable}$=0.007, **Figure 5.2 path c**). However, the direct pathway of mediation was not significant (95% CI [-1.573 – 4.023], β =1.225, SE β=1.408, $p_{variable}$=0.387, **Figure 5.2 path a'**). Therefore, the mediation indicated that gray matter volume of the left FPC fully mediated the relationship between precursor n-3 PUFAs and fluid intelligence (**Figure 5.2**). Examination of an alternative mediation model, which incorporated FPC as the independent variable, NBPs as the dependent variable, and fluid intelligence as the dependent variable, yielded an insignificant indirect effect (95% CI [-0.0001 – 0.0003]) and significant direct effect (95% [0.0002 – 0.0013]). The alternative

mediation model did not present a statistically sound mediation approach and therefore confirmed the validity of the primary proposed mediation model.

Discussion

This study revealed fluid intelligence is predicted by specific n-3 PUFA patterns and FPC structure, and that FPC structure mediates the relationship between n-3 PUFA status and fluid intelligence. This report identifies a novel nutritional biomarker for fluid intelligence as well as a novel mediatory relationship between n-3 PUFAs, FPC structure, and fluid intelligence. The individual relationships reported within the mediation, including those between n-3 PUFAs and fluid intelligence (**Figure 5.3 path a**), between FPC and fluid intelligence (**Figure 5.3 path c**), and between n-3 PUFAs and FPC (**Figure 5.3 path b**), are each supported by previous work reviewed in turn below.

First, precursor n-3 PUFAs positively associated with fluid intelligence. Red blood cell phospholipid total n-3 PUFAs have been previously linked to intelligence in older adults (Whalley et al., 2008). More specifically, serum concentration of EPA, DPA n-3, and DHA has been linked to better performance on tests of frontal function in older adults (D'Ascoli et al., 2016; Tan et al., 2012); however, to our knowledge, no study has examined the effects of ALA or its immediate downstream products, including SDA and ETE, on intelligence or tests of frontal function in older adults. Importantly, ALA in serum (Yamagishi et al., 2017), red blood cell phospholipids (Kim et al., 2010), and plasma (Cherubini et al., 2007) has been linked to risk for dementia. Decline in fluid intelligence is a key feature of the cognitive changes that precede dementia (Schaie, 1994), thus ALA and its immediate downstream products, including SDA and ETE, could serve as predictive biomarkers for fluid intelligence.

Second, structural integrity of the frontoparietal cortex was linked to fluid intelligence**.** More specifically, gray matter volume of the left frontoparietal cortex positively predicted fluid intelligence. Evidence indicates that fluid intelligence relies on the structure and function of regions within the FPC (Barbey et al., 2014b; Cole et al., 2012; Woolgar et al., 2015). The unilateral effect is supported by prior work, which suggests that regions within the left hemisphere may be selectively susceptible to degeneration (Thompson et al., 2003). Conversely, gray matter volume of the right supramarginal cortex negatively predicted fluid intelligence. Although the supramarginal cortex is considered part of the frontoparietal cortex (Colom et al., 2010; Vijayakumar et al., 2014), neural activity in this region has been shown to decrease during tests of intelligence (Haier et al., 2003). In line with prior evidence, our results suggest that while the supramarginal cortex may contribute to the frontoparietal cortex as a whole, its individual contributions to intelligence are not congruent to that of the entire frontoparietal cortex.

Third, precursor n-3 PUFAs positively predicted structural integrity of the left frontoparietal cortex (**Figure 5.3, path b**). Higher red blood cell levels of DHA (Tan et al., 2012) combined EPA and DHA (Pottala et al., 2014), and ALA (Virtanen et al., 2013) have been linked to greater total brain volume and markers of reduced brain atrophy. In addition, supplementation of EPA and DHA has been shown to increase gray matter volume in the frontal and parietal cortices of the left hemisphere in healthy older adults (Witte et al., 2014). However, to our knowledge, no study has examined the effects of ALA or its immediate downstream products, including SDA and ETE, on FPC gray matter structure.

Lastly, gray matter volume of the left FPC fully mediated the relationship between precursor n-3 PUFAs and fluid intelligence. Thus, precursor n-3 PUFAs may influence fluid intelligence by promoting structural integrity of the left FPC. Each of the three relationships

within the mediation are supported by prior findings, described above, but the mediation analysis provides a novel link between particular n-3 PUFAs, a cognitive function that is particularly vulnerable to age-related decline, and an underlying neuroanatomical network. These findings contribute to accumulating evidence suggests that certain nutrients may slow or prevent aspects of age-related cognitive decline by influencing particular aspects of brain structure (Bowman et al., 2012; Gu et al., 2016; Zamroziewicz et al., 2015, 2016a, 2016b, 2018; Zamroziewicz and Barbey, 2016).

The predictive power of one NBP, the precursor n-3 PUFA pattern, has noteworthy implications for the neuroprotective potential of n-3 PUFAs on fluid intelligence. The precursor n-3 PUFA pattern is reflective of either metabolic processing of n-3 PUFAs or dietary intake of n-3 PUFA-rich oils, nuts, and seeds (James et al., 2003; Walker et al., 2013). Metabolic processing of n-3 PUFAs within the precursor n-3 PUFA pattern may be neuroprotective because ALA, SDA, and ETE are converted to EPA, and to a smaller extent, DHA. Although DHA is the most abundant long-chain n-3 PUFA in the brain (Kuratko and Salem, 2009), but both EPA and DHA have physiological effects that can improve brain health. These include reducing inflammation, reducing oxidative stress, reducing platelet aggregation, improving blood pressure, and improving arterial compliance (Mozaffarian and Wu, 2012). Alternatively, dietary consumption of precursor n-3 PUFAs may support neuronal health through the unique neuroprotective benefits of ALA and its immediate downstream products. Previous work has shown that phospholipid ALA may prevent brain atrophy (Virtanen et al., 2013) by providing glucose to the brain through efficient ketogenesis (Freemantle et al., 2006), increasing serotonin and dopaminergic neurotransmission in the frontal cortex (Delion et al., 1994), and increasing plasma levels of brain-derived neurotrophic factor, thereby indirectly promoting neurogenesis

and neuronal survival (Hadjighassem et al., 2015). Importantly, few studies have investigated the neuroprotective potential of n-3 PUFAs within the precursor n-3 PUFA pattern, and even fewer have derived empirical patterns of plasma phospholipid n-3 PUFAs. The methodology employed in the current study allowed for an unprecedented comprehensive assessment of nutritional status of n-3 PUFAs, and provided support for novel nutritional biomarkers of fluid intelligence and underlying cortical structure. Future mechanistic studies are needed to investigate whether precursor n-3 PUFAs are neuroprotective by way of conversion to EPA and DHA or whether these precursors possess unique neuroprotective benefits, as well as the endogenous and exogenous factors that contribute to the neuroprotective effects. Future longitudinal studies are also warranted to investigate the time scale on which precursor n-3 PUFAs influence fluid intelligence and underlying cortical structure. While measurement of n-3 PUFAs in plasma phospholipids reveals that short-term intake of these nutrients influences cognition and brain health, measurement of n-3 PUFAs in adipose tissue will indicate the neuroprotective effects of long-term n-3 PUFA intake (Arab, 2003).

The strengths of this study include: (i) the use of blood biomarkers to measure physiological status of n-3 PUFAs, (ii) the use of NBP analysis to empirically derive patterns of n-3 PUFAs (iii) the use of structural MRI to measure cortical integrity with high spatial resolution, and (iv) the assessment of a particular cognitive function that is known to be sensitive to age-related decline, rather than a global measure of cognitive function that presents with little variability in healthy aging adults. The limitations of this study include: (i) relatively small sample size (n=100), (ii) cross-sectional design, (iii) limited neuropsychological testing (i.e., only fluid intelligence), (iv) limited neuroimaging domains (i.e., only structural neuroimaging), (v) inability to explore mechanisms that support the relationship between precursor n-3 PUFAs

and FPC structure, (vi) inability to explore contributions of diet and metabolic processes to n-3 PUFA patterns, and (vii) isolation of a specific dietary component. Thus, directions for future research include: (i) replication of results in a larger sample, (ii) implementation of a longitudinal study to examine how changes in n-3 PUFAs relate to changes in fluid intelligence and integrity of the FPC, (iii) examination of other facets of cognitive function, (iv) investigation of other neuroimaging domains, such as white matter microstructure and functional activity, (v) examination of the mechanisms that support the relationship between precursor n-3 PUFAs and FPC structure, (vi) investigation of the relative contributions of diet and metabolic processes to n-3 PUFA patterns, (vii) examination of potential synergistic interactions between n-3 PUFAs and other known neuroprotective dietary components, such as antioxidant vitamins (i.e., carotenoids, vitamin E), that may reduce oxidation of ingested fatty acids and therefore optimize neuroprotective effects.

Research at the frontline of nutritional cognitive neuroscience suggests that certain nutrients may slow or prevent aspects of age-related cognitive decline by influencing particular age-related changes in brain structure (Bowman et al., 2012; Gu et al., 2016; Zamroziewicz et al., 2015, 2016a, 2016b, 2018; Zamroziewicz and Barbey, 2016). The present finding contributes to this research program, and provides a novel link between nutritional and neuroanatomical biomarkers for fluid intelligence in healthy older adults. Ultimately, this line of work can inform clinical studies of personalized and comprehensive approaches to nutritional intervention for healthy brain aging.

Tables and Figures

Table 5.1. Characteristics of sample

Demographics	***n* = 100**
Age in years (M ± SD)	69 ± 3
Female (%)	62
Education (%)	
Some high school	1
High school degree	11
Some college	18
College degree	70
Income (%)	
<$15,000	1
$15,000 - $25,000	4
$25,000 - $50,000	15
$50,000 - $75,000	23
$75,000 - $100,000	26
>$100,000	31
Body mass index (M ± SD)	26 ± 4
Depression indicated (%)	6
Plasma phospholipid nutrients	**(M ± SD, μmol/l)**
α-linolenic acid (18:3n-3)	5.2 ± 2.7
Stearidonic acid (18:4n-3)	2.4 ± 0.9
Eicosatrienoic acid (20:3n-3)	1.2 ± 0.5
Eicosapentaenoic acid (20:5n-3)	25.0 ± 17.8
Docosapentaenoic acid (22:5n-3)	23.0 ± 7.1
Docosahexaenoic acid (22:6n-3)	79.6 ± 33.4
Cognition	**(M ± SD)**
Fluid intelligence score	112 ± 14
Gray matter volume	**(M ± SD, mm^3)**
Intracranial	1447671.9 ± 149653.5
FPC (R, L)	80184.6 ± 7766, 80627.4 ± 7765.7
Superior frontal cortex (R, L)	19403.7 ± 2220.8, 19995.9 ± 2111.2
Rostral middle frontal cortex (R, L)	14689.5 ± 2047.5, 14219.0 ± 1840.3
Caudal middle frontal cortex (R,L)	5455.2 ± 983.8, 5807.3 ± 992.2
Paropercularis cortex (R, L)	3599.5 ± 549.6, 4275.3 ± 634.9
Parstriangularis cortex (R, L)	3774.7 ± 628.5, 3265.1 ± 480.4
Parsorbitalis cortex (R, L)	2430.2 ± 347.5, 2040.5 ± 270.5
Superior parietal cortex (R, L)	12389.2 ± 1538.2, 12243.7 ± 1320.2
Precuneus cortex (R, L)	9060.8 ± 1067.2, 8718.7 ± 1082.4
Supramarginal cortex (R, L)	9381.8 ± 1383.2, 10062.0 ± 1519.4

Abbreviations: mean (M), standard deviation (SD), right hemisphere (R), left hemisphere (L)

Table 5.2. Nutrient biomarker pattern construction: Pattern structure and variance explained[1]

Plasma phospholipid fatty acid	**NBP[2]**	
	1	**2**
α-linolenic acid (18:3n-3)	0.190	0.742*
Stearidonic acid (18:4n-3)	0.065	0.715*
Eicosatrienoic acid (20:3n-3)	-0.131	0.827*
Eicosapentaenoic acid (20:5n-3)	0.960*	-0.053
Docosapentaenoic acid (22:5n-3)	0.805*	0.143
Docosahexaenoic acid (22:6n-3)	0.902*	-0.028
Percent variance explained by each NBP	52.781	18.989
Cumulative percent variance explained with each extraction	52.781	71.770

Abbreviations: nutrient biomarker pattern (NBP)

[1] Extraction method: principal component analysis; rotation method: oblimin

[2] NBP interpretation based on strongest loading coefficients within each pattern

* Nutrients with absolute loadings $\geq$ 0.5 that are considered as dominant nutrients contributing to the particular nutrient pattern

Table 5.3. Linear regression models: n-3 PUFA patterns associated with fluid intelligence

NBP		**Fluid intelligence**
		Model 1[a]
NBP1	**β**	-0.645
	SE	1.604
NBP2	**β**	3.236*
	SE	1.544
Model	**R^2**	0.133*

Abbreviations: nutrient biomarker pattern (NBP)

[a] Model: fluid intelligence = NBP1 + NBP2 + age + gender + education + body mass index

* p<0.05, ** p<0.01, *** p<0.001

Table 5.4. Linear regression models: Gray matter regions associated with fluid intelligence

Region	Hemisphere	Fluid intelligence		
		β	**β SE**	**Model R^2**
FPC	**Left**[a]	0.001**#	<0.001**#	0.181**
	Right[a]	0.001	<0.001	0.153*
Superior frontal	**Left**[b]	-0.001	0.001	0.179**
	Right[c]	0.001	0.001	0.165**
Rostral middle frontal	**Left**[b]	<0.001	0.001	0.178**
	Right[c]	<0.001	0.001	0.153*
Caudal middle frontal	**Left**[b]	0.001	0.002	0.179**
	Right[c]	<0.001	0.002	0.153*
Parsopercularis	**Left**[b]	0.002	0.002	0.184**
	Right[c]	0.003	0.003	0.159*
Parstriangularis	**Left**[b]	0.003	0.003	0.187**
	Right[c]	0.001	0.003	0.154*
Parsorbitalis	**Left**[b]	-0.007	0.006	0.190**
	Right[c]	0.004	0.005	0.160*
Superior parietal	**Left**[b]	<0.001	0.002	0.178**
	Right[c]	0.001	0.001	0.154*
Precuneus	**Left**[b]	-0.001	0.002	0.180**
	Right[c]	0.001	0.002	0.156*
Supramarginal	**Left**[b]	<0.001	0.002	0.178**
	Right[c]	-0.004**#	0.001**#	0.220**

Abbreviations: frontoparietal cortex (FPC)

[a] Model: fluid intelligence = regional gray matter volume + age + gender + education + body mass index + intracranial volume

[b] Model: gray matter volume = regional gray matter volume + age + gender + education + body mass index + left FPC volume

[c] Model: gray matter volume = regional gray matter volume + age + gender + education + body mass index + right FPC volume

* p<0.05, ** p<0.01, *** p<0.001, # p<0.05, FDR-corrected

Table 5.5. Linear regression models: n-3 PUFA patterns associated with gray matter structure of the frontoparietal cortex

Region	Hemisphere	NBP1		NBP2		
		β	β SE	β	β SE	Model R^2
FPC	**Left**[a]	-389.183	580.267	1494.073**#	557.356**#	0.641***
	Right[a]	-508.141	560.816	1424.340*	538.816*	0.673***
Superior frontal	**Left**[b]	-44.492	129.989	-213.372	128.783	0.754***
	Right[c]	-238.769	131.753	34.029	130.181	0.772***
Rostral middle frontal	**Left**[b]	107.278	125.255	12.413	124.093	0.700***
	Right[c]	82.595	153.719	-15.483	151.885	0.634***
Caudal middle frontal	**Left**[b]	-165.806	95.989	53.767	95.098	0.393***
	Right[c]	9.079	96.967	1.954	95.810	0.370***
Parsopercularis	**Left**[b]	23.717	67.597	59.900	66.970	0.265***
	Right[c]	83.108	53.686	15.063	53.046	0.381***
Parstriangularis	**Left**[b]	90.942	47.264	9.672	46.826	0.372***
	Right[c]	79.649	64.295	-37.704	63.528	0.321***
Parsorbitalis	**Left**[b]	15.187	28.148	-21.612	27.887	0.298***
	Right[c]	46.995	35.625	-27.554	35.200	0.318***
Superior parietal	**Left**[b]	102.797	92.112	56.757	91.257	0.684***
	Right[c]	42.707	122.006	-10.367	120.550	0.592***
Precuneus	**Left**[b]	-172.987	73.955	-91.045	73.269	0.697***
	Right[c]	-118.534	80.577	-6.624	79.616	0.630***
Supramarginal	**Left**[b]	43.363	103.833	133.520	102.870	0.697***
	Right[c]	13.211	121.662	46.687	120.211	0.498***

Abbreviations: nutrient biomarker pattern (NBP), frontoparietal cortex (FPC)

[a] Model: gray matter volume = NBP1 + NBP2 + age + gender + education + body mass index + intracranial volume

[b] Model: gray matter volume = NBP1 + NBP2+ age + gender + education + body mass index + left FPC volume

[c] Model: gray matter volume = NBP1 + NBP2+ age + gender + education + body mass index + right FPC volume

* p<0.05, ** p<0.01, *** p<0.001, # p<0.05, FDR-corrected

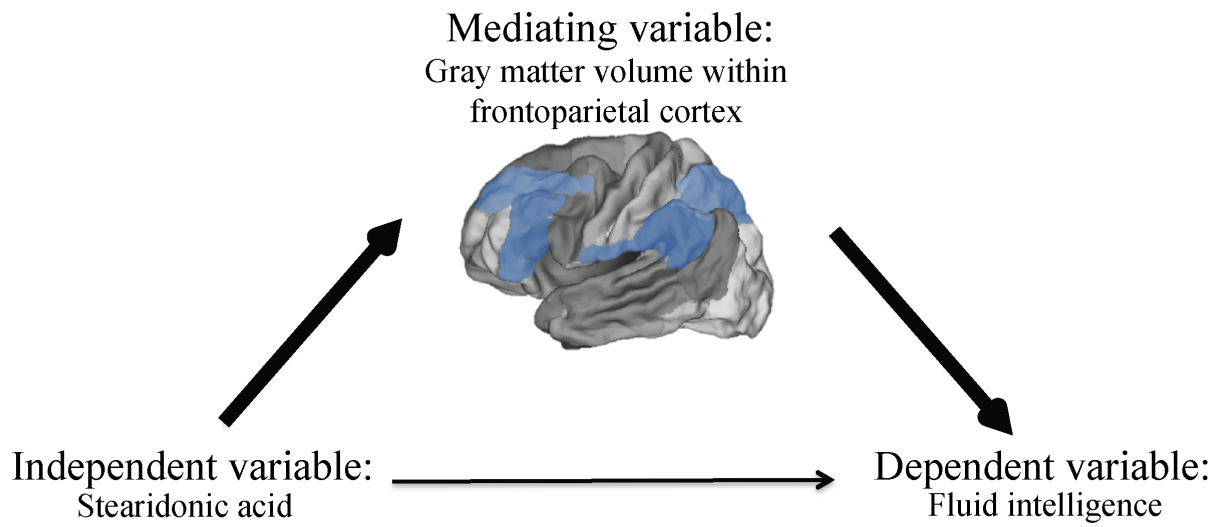

Figure 5.1. Proposed mediation model: The primary requirement for mediation is a significant indirect mediation effect, defined as the effect of the independent variable (nutrient biomarker patterns) through the mediator (gray matter volume within the frontoparietal cortex) on the dependent variable (fluid intelligence).

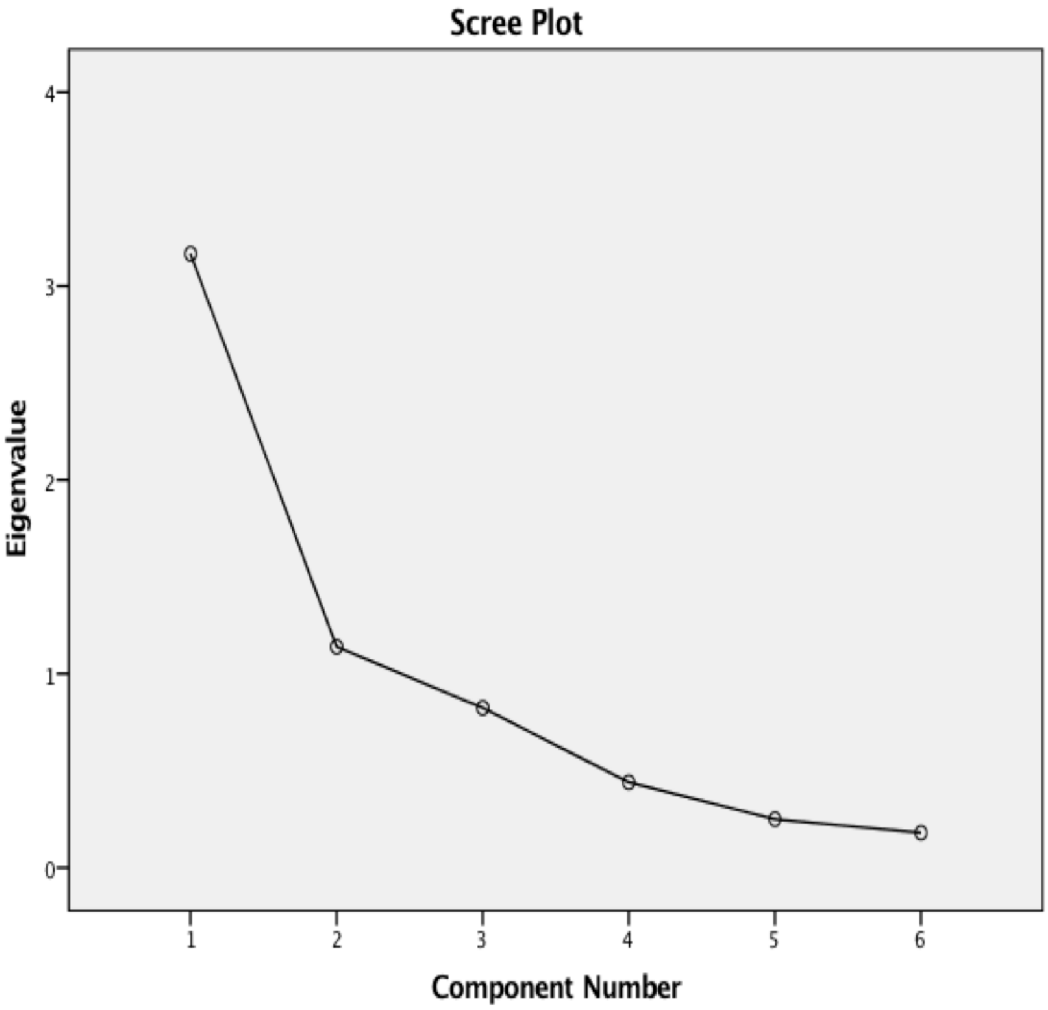

Figure 5.2. Scree plot: Inspection of the scree plot indicated that the inflection point occurred after the second component, or nutrient biomarker pattern, was extracted using a direct oblimin rotation.

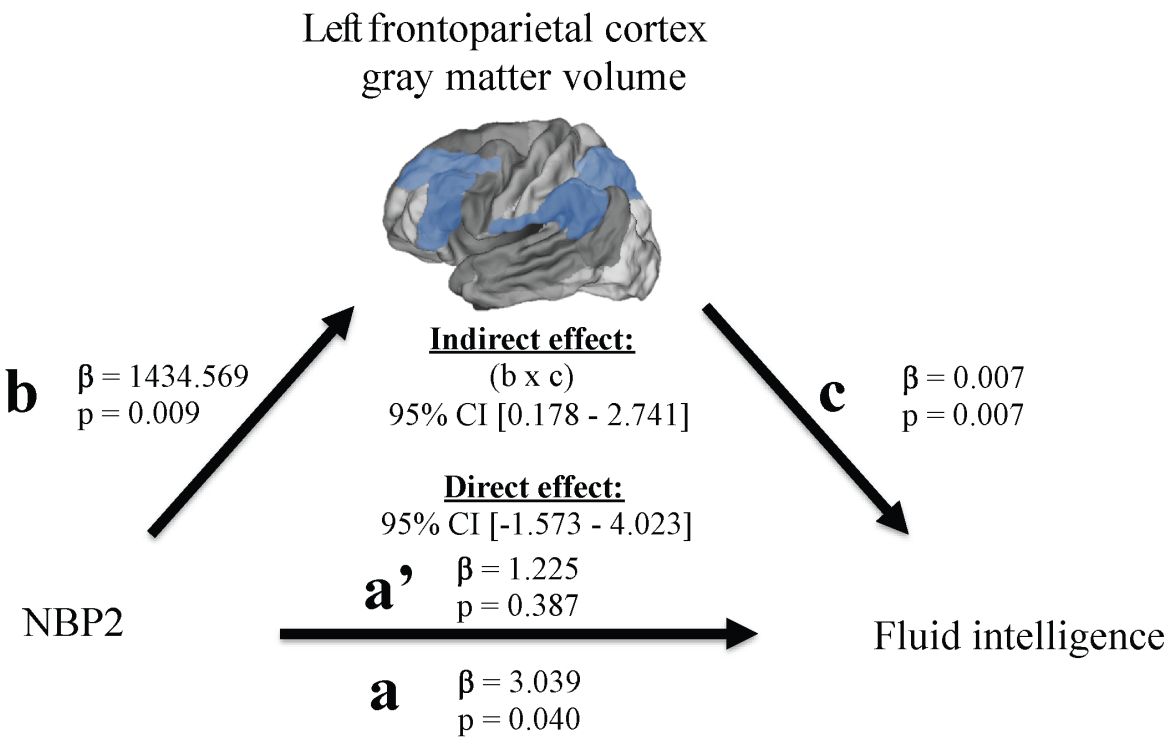

Figure 5.3. Mediation model statistics: A mediation model was used to characterize the relationship between nutrient biomarker pattern 2 (NBP2), left frontoparietal cortex gray matter volume, and fluid intelligence. NBP2 positively associated with fluid intelligence (**path a**). NBP2 positively associated with total gray matter volume of the left frontoparietal cortex (**path b**). The indirect pathway of mediation (i.e., the effect of NBP2 through total gray matter volume of the left frontoparietal cortex on fluid intelligence; **path b-c**) was statistically significant. The direct pathway of mediation (i.e., the effect of NBP2 on fluid intelligence, accounting for total gray matter volume of the left frontoparietal cortex; **path a'**) was not significant. Therefore, total gray matter volume of left frontoparietal cortex fully mediated the relationship between NBP2 and fluid intelligence.

CHAPTER 6: PREDICTORS OF MEMORY IN HEALTHY AGING: POLYUNSATURATED FATTY ACID BALANCE AND FORNIX WHITE MATTER INTEGRITY[6]

Abstract

Recent evidence demonstrates that age and disease-related decline in cognition depends not only upon degeneration in brain structure and function, but also on dietary intake and nutritional status. Memory, a potential preclinical marker of Alzheimer's disease, is supported by white matter integrity in the brain and dietary patterns high in omega-3 and omega-6 polyunsaturated fatty acids. However, the extent to which memory is supported by specific omega-3 and omega-6 polyunsaturated fatty acids, and the degree to which this relationship is reliant upon microstructure of particular white matter regions is not known. This study therefore examined the cross-sectional relationship between empirically-derived patterns of omega-3 and omega-6 polyunsaturated fatty acids (represented by nutrient biomarker patterns), memory, and regional white matter microstructure in healthy older adults. We measured thirteen plasma phospholipid omega-3 and omega-6 polyunsaturated fatty acids, memory, and regional white matter microstructure in 94 cognitively intact older adults (65 to 75 years old). A three-step mediation analysis was implemented using multivariate linear regressions, adjusted for age, gender, education, income, depression status, and body mass index. The mediation analysis revealed that a mixture of plasma phospholipid omega-3 and omega-6 polyunsaturated fatty acids is linked to memory and that white matter microstructure of the fornix fully mediates the

[6] This chapter appeared in its entirety in *Aging and Disease* and is referred to in this dissertation as "Zamroziewicz et al., 2018." This article is reprinted under the terms of the Creative Commons Attribution License. Zamroziewicz, M.K., Zwilling, C.E., Barbey, A.K. (2018) Predictors of memory in healthy aging: polyunsaturated fatty acid balance and fornix white matter integrity. *Aging and Disease* 9(1), 1-12. doi: 10.14336/AD.2017.0501.

relationship between this pattern of plasma phospholipid polyunsaturated fatty acids and memory. These results suggest that memory may be optimally supported by a balance of plasma phospholipid omega-3 and omega-6 polyunsaturated fatty acids through the preservation of fornix white matter microstructure in cognitively intact older adults. This report provides novel evidence for the benefits of plasma phospholipid omega-3 and omega-6 polyunsaturated fatty acid balance on memory and underlying white matter microstructure.

Introduction

Scientific and technological innovations in medicine continue to advance our understanding of human health and disease, with recent discoveries providing insight into an essential aspect of human biology: nutrition. At the frontiers of this effort, the interdisciplinary field of nutritional cognitive neuroscience (Zamroziewicz and Barbey, 2016) demonstrates that age and disease-related decline in cognition depends not only upon degeneration in brain structure and function, but also on dietary intake and nutritional status. Unraveling the ways in which particular nutrients and dietary components may influence specific aspects of brain structure and function to support cognition will have profound implications for understanding healthy brain aging and for treating age-related neurological disease. As the United States experiences rapid growth in the older adult population – and a corresponding increase in the medical and economic demands of treating individuals with age-related neurological disorders – effective medical and policy recommendations to promote healthy brain aging become increasingly important, providing a catalyst for research to investigate the beneficial effects of nutrition on the aging brain.

A large body of evidence demonstrates that omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have protective effects on the aging brain. PUFAs are known to contribute to neuronal membrane structural integrity, control inflammation and oxidation, and promote energy metabolism (Cunnane et al., 2009). High PUFA intake has been linked to better performance on tasks of memory in cross-sectional (Gu et al., 2016) and longitudinal (Eskelinen et al., 2008) studies. Importantly, age-related decline in memory precedes clinically detectable Alzheimer's disease, and thus presents as a potential preclinical marker of Alzheimer's disease (Caselli et al., 1999). However, it is not known whether particular patterns of PUFAs differentially influence memory.

Age-related and disease-related declines in memory are traditionally thought to rely upon hippocampal gray matter structure, but cutting-edge neuroimaging techniques suggest that memory decline may be better predicted by white matter microstructure (Carlesimo et al., 2010). Diffusion tensor imaging (DTI) is a sensitive neuroimaging technique that determines white matter microstructural integrity by measuring diffusion properties of water (Mori and Zhang, 2006). The most widely reported measure of DTI, fractional anisotropy (FA), indicates diffusion directionality and microstructural integrity (Pagani et al., 2008). Notably, high levels of n-3 PUFAs and omega-6 (n-6) PUFAs have been shown to slow age-related decline in FA across the brain (Gu et al., 2016). However, white matter microstructure does not decline in a uniform way (Bennett et al., 2009), and it is unclear whether PUFA patterns differentially support microstructure in particular regions and in turn preserve memory.

Recent findings from nutritional cognitive neuroscience further indicate that the balance between n-3 PUFAs and n-6 PUFAs has important implications for brain health (Loef and Walach, 2013). Gu and colleagues demonstrate that dietary patterns high in n-3 PUFAs and n-6

PUFAs may slow age-related decline in memory by preferentially promoting white matter microstructure (Gu et al., 2016). However, the extent to which particular combinations of n-3 PUFAs and n-6 PUFAs may protect against age-related decline in memory and regional white matter microstructure is unknown. Investigating this issue is critical in the continued effort to develop dietary recommendations that improve modern Western diets, which are known to have a higher ratio of n-6 PUFA to n-3 PUFA (15:1 to 20:1) than evolutionary diets (1:1 to 2:1) (Gómez Candela et al., 2011).

Therefore, the question remains: is memory dependent on specific patterns of n-3 PUFAs and n-6 PUFAs, and is this relationship reliant upon microstructure of particular white matter regions? This study investigates the influence of plasma phospholipid n-3 PUFA and plasma phospholipid n-6 PUFA patterns on memory and the extent to which regional white matter microstructure mediates this relationship in healthy older adults.

Materials and Methods

Participants

This cross-sectional study enrolled 122 healthy elderly adult patients from Carle Foundation Hospital, a local and readily available cohort of well-characterized elderly adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination (Folstein et al., 1975). Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for magnetic resonance

imaging (MRI). All participants were right handed with normal, or corrected to normal vision and no contraindication for MRI. Of these 122 participants, 94 subjects had a complete dataset at time of data analysis, including neuropsychological testing, MRI, and blood biomarker analysis.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and the Carle Hospital Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Plasma phospholipid PUFA acquisition

Plasma lipids were extracted by the method of Folch, Lees and Sloane-Stanley (Folch et al., 1957). Briefly, the internal standard (25 μg each of PC17:0) was added to 200 μl of serum, followed by 6 mL of choloroform:methanol:BHT (2:1:100 v/v/w). The protein precipitate was removed by centrifugation (2,500 g, 5 minutes, 4ºC). Then 1.5 mL of 0.88% KCl was added to the supernatant, shaken vigorously and the layers were allowed to settle for 5 minutes. The upper layer was discarded and 1 ml of distilled water:methanol (1:1 v/v) was added, the tube was shaken again and the layers allowed to settle for 15 minutes. The lower layer was transferred into a clean tube and evaporated to dryness under nitrogen. The phospholipid subfraction was separated by solid-phase extraction using aminopropyl columns as described by Agren, Julkunen and Penttila (Aryen et al., 1992). Then the phospholipid fraction was methylated by adding 2 ml of 14% BF3-MeOH and incubating at 95ºC for 1 hour (Morrison and Smith, 1964). The supernatant containing the fatty acid methyl esters (FAMEs) was dried down under nitrogen,

resuspended in 100 μl of hexane, transferred into amber GC vials and stored at -20ºC until the time of analysis.

The phospholipid FAMEs were analyzed by a CLARUS 650 gas chromatograph (Perkin Elmer, Boston MA) equipped with a 100 m x 0.25 mm i.d (film thickness 0.25 μm) capillary column (SP-2560, Supelco). Injector and flame ionization detector temperatures were 250ºC and 260ºC, respectively. Helium was used as the carrier gas (2.5 mL/min) and the split ratio was 14:1. The oven temperature was programmed at 80ºC, held for 16 minutes and then increased to 180ºC at a rate of 5ºC/minute. After 10 minutes, the temperature was increased to 192ºC at a rate of 0.5ºC/minute and held for 4 minutes. The final temperature was 250ºC reached at a rate of 405ºC/minute and held for 15 minutes. Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc. MN) and expressed as absolute concentration (μmol/L). The plasma phospholipid fatty acids of interest were n-3 PUFAs and n-6 PUFAs, listed in **Table 6.1**.

Nutrient biomarker pattern analysis of plasma phospholipid PUFAs

Nutrient biomarker pattern analysis was conducted in IBM SPSS statistical software, version 24 for Macintosh. Principal component analysis was used to identify nutrient biomarker patterns (NBPs) of n-3 PUFAs and n-6 PUFAs from the thirteen plasma phospholipid PUFAs listed in **Table 6.1**. Of these, nine plasma phospholipid PUFAs (γ-linolenic acid, eicosadienoic acid, dihomo-γ-linolenic acid, docosadienoic acid, adrenic acid, α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid) were non-normally distributed, as indicated by Shapiro-Wilk test (all p-values<0.05), and therefore log-transformed to correct for skewness of variables and subsequently considered in the analysis. The appropriate

rotation method was determined by examining the factor correlation matrix: varimax rotation was chosen for a correlation matrix with values less than 0.32 and direct oblimin rotation was chosen for a correlation matrix with values greater than 0.32 (Tabachnick and Fidell, 2007). Statistical validity of the factor analysis was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy ($\geq$0.50) (Kaiser, 1970) and Bartlett's Test of Sphericity ($p<0.05$) (Bartlett, 1950). Outliers were identified as participants with factor score values greater than 3.0 and removed from the dataset (Jolliffe, 2014). The number of NBPs to be retained was determined by a combination of eigenvalues greater than 1.0, variance accounted for by each component, and scree plot inflection point. Interpretation of each factor was based on identifying plasma phospholipid PUFAs with an absolute loading value of greater than 0.50 on a NBP (i.e., identifying the dominant plasma phospholipid PUFAs contributing to each particular NBP). Each participant received a standardized NBP score for each pattern that corresponded to a linear combination of the plasma phospholipid PUFAs.

Neuropsychological tests

Memory was measured by the Wechsler Memory Scale – Fourth Edition (WMS-IV) Older Adult Battery (Wechsler, 1999). This assessment measured memory by way of four indices: Auditory Memory Index, Visual Memory Index, Immediate Memory Index, and Delayed Memory Index. The Auditory Memory Index indicates a participant's ability to remember orally presented information. The Visual Memory Index indicates a participant's ability to remember visually presented information. The Immediate Memory Index indicates a participant's ability to recall visually and orally presented information immediately after it is presented. The Delayed Memory Index indicates a participant's ability to recall and recognize

visually- and orally-presented information after a 20 to 30 minute delay. Participants' raw scores on each subtest were converted to normalized scaled scores and subsequently combined into indices. Z-scores for each index were calculated and then averaged to create a composite memory score (Siedlecki et al., 2009).

Diffusion tensor imaging of white matter microstructure

Participants were scanned on a 3.0 T Siemens Magnetom Trio MRI system (Erlangen, Germany) using a 12 channel head coil. Whole brain diffusion tensor imaging was acquired with the following parameters: FOV = 240 x 240 mm; 72 slices, slice thickness = 2 mm; TE = 98 ms; TR = 10,000 ms; in-plane resolution = 1.875 x 1.875 mm; diffusion encoding directions = 30; b = 0 s/mm^2 and 1,000 s/mm^2. Data were processed using the University of Oxford's Center for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) release 5.0 (Smith et al., 2004) diffusion toolbox (FDT) (Behrens et al., 2003, 2007). Eddy current correction was accomplished using the eddy_correct tool and a diffusion tensor model was fit in each voxel using the DTIFIT tool, which generates fractional anisotropy (FA) values in every voxel. FA images were further processed using the FSL tract-based spatial statistics (TBSS) (Smith et al., 2006) toolbox, which projects each subjects' FA data onto a mean white matter skeleton, representing the white-matter tracts common to all subjects.

Mean FA within the white matter skeleton for specific regions of interest were calculated for each subject using the JHU ICBM DTI-81 atlas (Oishi et al., 2008). The regions of interest are listed in **Table 6.1**.

Covariates

Covariates were included according to previous association with cognitive decline (Gallucci et al., 2013; Hurst et al., 2013; Koen and Yonelinas, 2014; Pauls et al., 2013; Rönnlund et al., 2005; Wilkins et al., 2010). The covariates included age (continuous), gender (nominal, man/woman), education (nominal, five fixed levels), income (nominal, six fixed levels), body mass index (continuous, hereafter BMI), and depression status (nominal, yes/no). Although all participants had received a diagnosis of no depression at enrollment, the SF-36 Health Survey (Ware et al., 1993) revealed six participants with symptoms consistent with depression. Thus, in accordance with prior studies (Witte et al., 2014), this was considered in the analysis as a covariate.

Mediation analysis

A formal mediation analysis was conducted to model the relationship between NBPs, regional FA, and memory using a three-step framework, with the goal of evaluating whether regional FA mediated the relationship between NBPs and memory. The primary requirement for mediation is a significant indirect mediation effect (Zhao et al., 2010), or the effect of the independent variable (NBPs) through the mediator (regional FA) on the dependent variable (memory) (**Figure 6.1**).

Mediation analyses were performed using the PROCESS macro designed for SPSS (Preacher and Hayes, 2008). Statistics were performed as follows:

1. In the first step, a regression model was applied to characterize the relationship between NBPs and FA of white matter regions **(path a**). This analysis accounted for covariates listed

above and applied a false discovery rate (FDR) (Benjamini and Hochberg, 1995) correction for multiple comparisons ($q < 0.05$, one-tailed)

2. In the second step, a regression model was used to characterize the relationship between NBPs and memory (**path c**). This analysis accounted for covariates listed above.
3. In the third step, the PROCESS macro was applied to implement the bootstrapping method to estimate mediation effects. This analysis drew 1000 bootstrapped samples with replacement from the dataset to estimate a sampling distribution for the indirect and direct mediation effects, controlling for covariates listed above. The indirect mediation effect refers to the pathway from NBPs to regional FA to memory (**path a-b**). The direct mediation effect refers to the direct pathway from NBPs to memory, accounting for the effect of regional FA (**path c'**).

A statistically significant mediation that matches the hypothesized framework is indicated by: (i) an indirect mediation effect that does not include zero within 95% bias-corrected confidence intervals, and (ii) a direct mediation effect that does include zero within 95% bias-corrected confidence intervals (Zhao et al., 2010). Results are reported using unstandardized regression coefficients (β) and statistical significance (p) for each individual regression relationship, and a 95% bias-corrected confidence interval (95% CI) for the direct and indirect effects of the mediation.

Results

Participant characteristics

Participants had a mean age of 69 years and 61 percent of participants were females. All other participant characteristics are reported in **Table 6.1**.

Nutrient biomarker patterns of plasma phospholipid PUFAs

Principal component analysis generated three NBPs (**Table 6.2**). The factor correlation matrix contained values greater than 0.32, therefore direct oblimin rotation was implemented. Statistical validity of the factor analysis was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (0.762) and Bartlett's Test of Sphericity ($p<0.001$). One outlier was removed and the principal component analysis was rerun (n=95). Three NBPs were selected for retention because (i) after the third NBP extraction with principal component analysis, 69.21 percent of the total variance was accounted for in the original set of plasma phospholipid PUFAs, and (ii) inspection of the scree plot indicated that the inflection point occurred after the third NBP (**Figure 6.2**). The dominant plasma phospholipid PUFAs contributing to each NBP were those that had an absolute loading value of greater than 0.50 on a NBP. Thus, NBP1 consisted of five plasma phospholipid PUFAs, NBP2 consisted of five plasma phospholipid PUFAs, and NBP3 consisted of three plasma phospholipid PUFAs (**Table 6.2**). Hereafter, NBP1 is described as LCPUFA (long-chain polyunsaturated fatty acids; i.e., it is composed of two plasma phospholipid n-3 PUFAs and three plasma phospholipid n-6 PUFAs), NBP2 is described as n-6 PUFA (i.e., it is composed of four plasma phospholipid n-6 PUFAs and only one plasma phospholipid n-3 PUFA), and NBP3 is described as n-3 PUFA (i.e., it is composed of only plasma phospholipid n-3 PUFAs).

Mediation results

The mediation analyses indicated that FA of one region (fornix) fully mediated the relationship between one NBP (LCPUFA) and memory. Each relationship within the mediation is described below in a stepwise fashion.

1. Higher LCPUFA was associated with higher FA in the fornix (β=0.042, p<0.001; **Figure 6.3**), but no other NBP related to FA in any other region (**Table 6.3**). Therefore, the relationship between LCPUFA and fornix FA was considered in the context of the mediation model (**Figure 6.5 path a**).
2. Higher LCPUFA associated with a higher memory score (β=0.320, p=0.003; **Figure 6.4**), but no other NBP related to performance on memory (**Table 6.4**). Therefore, the relationship between LCPUFA and memory was considered in the context of the mediation model (**Figure 6.5 path c**).
3. The indirect pathway of mediation (the pathway from LCPUFA to fornix FA to memory) was significant (95% CI [0.003 – 0.133], **Figure 6.5 path a-b;** β=1.704, p=0.082; **Figure 6.5 path b**), but the direct pathway of mediation (the direct pathway from LCPUFA to memory, accounting for the effect of fornix FA) was not significant (95% CI [-0.004 – 0.388], β=0.192, p=0.055, **Figure 6.5 path c'**). Therefore, the mediation indicated that fornix FA fully mediated the relationship between LCPUFA and memory (**Figure 6.5**).

Discussion

This study revealed that memory is dependent upon a particular pattern of plasma phospholipid PUFAs, which comprised a mixture of plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs, and that white matter integrity of a specific white matter region, the fornix, mediates this relationship. This report provides a novel link between a

combination of plasma phospholipid PUFAs, white matter microstructure of one region, and memory. The individual relationships reported within the mediation, including those between plasma phospholipid PUFAs and white matter microstructure (**Figure 6.5 path a**), between plasma phospholipid PUFAs and memory (**Figure 6.5 path c**), and between fornix white matter microstructure and memory (**Figure 6.5 path b**) are each supported by previous work and are reviewed in turn below.

The first relationship demonstrated a positive association between LCPUFA and FA of the fornix. Although previous work has not directly examined the relationship between plasma phospholipid PUFAs and white matter microstructure of the fornix, several lines of evidence support this finding. Various diets and dietary components, including the Mediterranean diet (Pelletier et al., 2015), PUFAs (Gu et al., 2016; Witte et al., 2014), vitamin E (Gu et al., 2016), vitamin D (Moon et al., 2015), vitamin B1 (Dror et al., 2014), and vitamin B12 (Roy et al., 2015) have been linked to white matter integrity. The fornix in particular is vulnerable to the white matter atrophy induced by Alzheimer's pathology (Yasmin et al., 2009). However, loss of white matter integrity in the fornix is amenable to lifestyle factors (Xu et al., 2013), suggesting the potential for intervention with nutritional factors. This study indicates that LCPUFA is linked to a sensitive measure of fornix degeneration (Oishi and Lyketsos, 2014), measured by highly restricted diffusion within the fornix and represents superior myelination and a high number of myelinated nerve fibers (Salat et al., 2005).

The second relationship indicated a positive association between LCPUFA and memory. High PUFA intake has been linked to better performance on tasks of memory in cross-sectional (Gu et al., 2016) and longitudinal studies (Eskelinen et al., 2008), but positive findings are not consistent (Sinn et al., 2012). Most studies have investigated the effects of n-3 PUFAs on

memory (Loef and Walach, 2013), but these findings suggest that a mixture of n-3 PUFAs and n-6 PUFAs may slow age-related decline in memory.

Third, FA of the fornix fully mediated the relationship between LCPUFA and memory. The fornix consists of bilateral white matter bundles that originate from the bilateral hippocampi, and represents a core element of the limbic circuit that is vulnerable to Alzheimer's disease (Oishi and Lyketsos, 2014). Fornix microstructure has been linked to conversion from healthy aging to cognitive impairment (Fletcher et al., 2013) as well as memory performance in cross-sectional and longitudinal investigations (Mielke et al., 2012). Given its vulnerability to Alzheimer's disease, and its role in memory, the fornix has been proposed as a target in novel therapies for Alzheimer's disease (Oishi and Lyketsos, 2014). The present findings support focusing on the fornix as a therapeutic target, and highlight the potential for nutritional interventions.

The predictive power of only one NBP, the LCPUFA pattern, suggests that particular physiological mechanisms may be important in the preservation of white matter microstructure and memory. The NBP analysis yielded three patterns of plasma phospholipid PUFAs: (i) the LCPUFA pattern consisted of both plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs that serve as precursors to long-chain PUFAs, (ii) the n-6 PUFA pattern primarily consisted of primarily plasma phospholipid n-6 PUFAs and one plasma phospholipid n-3 PUFA, and (iii) the n-3 PUFA pattern consisted of only plasma phospholipid n-3 PUFAs. All PUFAs can serve two basic functions: components of plasma membranes or precursors to prostanoids, which are vital pro- and anti-inflammatory regulatory factors (Skinner et al., 1993). Importantly, n-3 PUFAs and n-6 PUFAs share the same biosynthetic enzymes, and are therefore converted to long-chain PUFAs and prostanoids in a competitive manner (Crupi et al., 2013). A balance of n-

3 PUFAs and n-6 PUFAs is physiologically favorable because it allows for proportional production of long-chain PUFAs for integration into plasma membranes or conversion into prostanoids (Crupi et al., 2013). Therefore, the predictive power of the LCPUFA pattern suggests that it is a mixture of plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs possibly reflective of a balance of plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs, rather than the individual effects of only plasma phospholipid n-3 PUFAs or only plasma phospholipid n-6 PUFAs, that is robustly linked to memory and white matter microstructure in the aging brain. These findings imply that modern Western diets may be improved by balancing intake of dietary sources of n-3 PUFAs and n-6 PUFAs (Gómez Candela et al., 2011). Importantly, this study does not relate dietary intake of PUFAs to plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs, and future work is needed to determine how dietary intake of PUFAs may support memory and white matter microstructure in the aging brain.

The strengths of this study include: (i) the use of blood biomarkers to measure physiological status of plasma phospholipid PUFAs, (ii) the use of structural MRI to measure microstructural integrity with high spatial resolution, and (iii) the assessment of a particular aspect of cognition known to be sensitive to age-related decline and neural degeneration. Directions for future research include: (i) replication of results in a larger sample, (ii) assessment of dietary intake and its link to plasma phospholipid n-3 PUFAs and plasma phospholipid n-6 PUFAs (ii) determination of the optimal ratio of n-3 PUFAs to n-6 PUFAs, (iii) implementation of a longitudinal study to examine how changes in PUFA balance relate to changes in memory and white matter microstructure, (iv) examination of the mechanisms that support the relationship between PUFAs and fornix white matter microstructure, and (v) investigation of the

relationship between PUFA balance, cognition, and white matter microstructure in other model systems, including animal models and clinical populations.

Research at the frontline of nutritional cognitive neuroscience aims to incorporate sensitive measures of nutritional intake, brain structure and function, and cognition in an effort to demonstrate that cognitive aging is dependent not only on degeneration in brain structure and function, but also on nutritional status and dietary intake. In doing so, nutritional cognitive neuroscience strives to bridge the gap between largely disparate literatures of aging within the fields of nutritional epidemiology and cognitive neuroscience, and offer a novel perspective on healthy brain aging. Accumulating evidence suggests that certain nutrients may slow or prevent aspects of age-related cognitive decline by influencing particular age-related changes in brain structure (Bowman et al., 2013; Gu et al., 2016; Zamroziewicz et al., 2015, 2016a, 2016b, 2017; Zamroziewicz and Barbey, 2016). The present finding contributes to this research program, and provides novel evidence for the benefits of n-3 PUFA to n-6 PUFA balance on memory and underlying white matter microstructure. Ultimately, this line of work can inform clinical nutritional interventions for healthy brain aging.

Tables and Figures

Table 6.1. Characteristics of sample

Demographics	***n* = 94**	**Regional white matter FA**	**(M ± SD)**
Age in years (M ± SD)	69 ± 3	Corpus callosum genu	0.757 ± .0286
Female (%)	61	Corpus callosum body	0.754 ± 0.033
Education (%)		Corpus callosum splenium	0.845 ± 0.016
High school degree	12	Fornix	0.449 ± 0.010
Some college	18	Cerebral peduncle R	0.782 ± 0.019
College degree	70	Cerebral peduncle L	0.755 ± 0.022
Income (%)		Anterior internal capsule R	0.683 ± 0.023
$15,000 - $25,000	4	Anterior internal capsule L	0.659 ± 0.024
$25,000 - $50,000	15	Posterior internal capsule R	0.769 ± 0.028
$50,000 - $75,000	23	Posterior internal capsule L	0.726 ± 0.027
$75,000 - $100,000	27	Retrolenticular internal capsule R	0.715 ± 0.033
>$100,000	31	Retrolenticular internal capsule L	0.679 ± 0.027
BMI (M ± SD)	26 ± 4	Anterior corona radiata R	0.535 ± 0.032
Depression indicated (%)	6	Anterior corona radiata L	0.523 ± 0.032
Plasma phospholipid PUFAs	**(M ± SD, μmol/L)**	Superior corona radiata R	0.592 ± 0.029
Linoleic acid (18:2n-6)	601.2 ± 149.9	Superior corona radiata L	0.558 ± 0.027
γ-linolenic acid (18:3n-6)	2.7 ± 1.6	Posterior corona radiata R	0.573 ± 0.030
Eicosadienoic acid (20:2n-6)	9.2 ± 2.6	Posterior corona radiata L	0.588 ± 0.035
Dihomo-γ-linolenic acid (20:3n-6)	70.9 ± 25.7	Posterior thalamic radiation R	0.682 ± 0.040
Arachidonic acid (20:4n-6)	295.7 ± 66.6	Posterior thalamic radiation L	0.671 ± 0.036
Docosadienoic acid (22:2n-6)	0.3 ± 0.1	Sagittal stratum R	0.655 ± 0.035
Adrenic acid (22:4n-6)	10.5 ± 3.2	Sagittal stratum L	0.624 ± 0.030
α-linolenic acid (18:3n-3)	5.2 ± 2.5	External capsule R	0.567 ± 0.042
Stearidonic acid (18:4n-3)	2.3 ± 0.9	External capsule L	0.549 ± 0.031
Eicosatrienoic acid (20:3n-3)	1.2 ± 0.4	Cingulate part of cingulum R	0.631 ± 0.031
Eicosapentaenoic acid (20:5n-3)	24.7 ± 17.7	Cingulate part of cingulum L	0.669 ± 0.034
Docosapentaenoic acid (22:5n-3)	22.9 ± 6.9	Hippocampal part of cingulum R	0.759 ± 0.032
Docosahexaenoic acid (22:6n-3)	78.6 ± 32.4	Hippocampal part of cingulum L	0.719 ± 0.039
Cognition	**(M ± SD)**	Superior longitudinal fasciculus R	0.600 ± 0.030
WMS-IV AMI	113 ± 13	Superior longitudinal fasciculus L	0.568 ± 0.027
WMS-IV VMI	112 ± 12	Superior fronto-occipital fasciculus R	0.638 ± 0.042
WMS-IV IMI	115 ± 12	Superior fronto-occipital fasciculus L	0.586 ± 0.042
WMS-IV DMI	113 ± 13	Uncinate fasciculus R	0.589 ± 0.053
Composite memory score	113 ± 11	Uncinate fasciculus L	0.561 ± 0.048
		Tapetum R	0.664 ± 0.070
		Tapetum L	0.728 ± 0.096

Abbreviations: mean (M), standard deviation (SD), body mass index (BMI), polyunsaturated fatty acid (PUFA), Wechsler Memory Scale- Fourth Edition (WMS-IV), Auditory Memory Index (AMI), Verbal Memory Index (VMI), Immediate Memory Index (IMI), Delayed Memory Index (DMI), fractional anisotropy (FA), right hemisphere (R), left hemisphere (L)

Table 6.2. Nutrient biomarker pattern construction: Pattern structure and variance explained[1]

	NBP[2]		
Plasma phospholipid PUFAs	**1**	**2**	**3**
α-linolenic acid (18:3n-3)	0.780*		
Eicosadienoic acid (20:2n-6)	0.757*	0.328	
Eicosatrienoic acid (20:3n-3)	0.756*		
Linoleic acid (18:2n-6)	0.707*		
Docosadienoic acid (22:2n-6)	0.601*		
Adrenic acid (22:4n-6)		0.928*	
Arachidonic acid (20:4n-6)		0.767*	0.304
γ-linolenic acid (18:3n-6)		0.696*	
Dihomo-γ-linolenic acid (20:3n-6)	0.325	0.643*	
Stearidonic acid (18:4n-3)	0.378	0.606*	
Eicosapentaenoic acid (20:5n-3)			0.951*
Docosahexaenoic acid (22:6n-3)			0.910*
Docosapentaenoic acid (22:5n-3)		0.313	0.752*
Percent variance explained by each NBP	41.33	16.63	11.24
Cumulative percent variance explained with each extraction	41.33	57.96	69.21

Abbreviations: nutrient biomarker pattern (NBP), polyunsaturated fatty acid (PUFA)

[1] Extraction method: principal component analysis; rotation method: oblimin

[2] NBP interpretation was based on strongest loading coefficients within each pattern; only loadings with an absolute value ≥ 0.3 are shown in the table

* Nutrients with absolute loadings ≥ 0.5 that are considered as dominant nutrients contributing to the particular nutrient pattern

Table 6.3. Linear regression models: Nutrient biomarker patterns associated with regional fractional anisotropy

Regional FA	**LCPUFA**	**n6PUFA**	**n3PUFA**
Corpus callosum genu	0.001(0.823)	0.001(0.693)	-0.003(0.458)
Corpus callosum body	0.007(0.105)	0.005(0.221)	-0.001(0.708)
Corpus callosum splenium	-0.001(0.727)	0.001(0.533)	-0.001(0.731)
Fornix	0.042(<0.001)*	-0.008(0.426)	-0.024(0.021)
Cerebral peduncle R	<0.001(0.882)	-0.001(0.752)	-0.002(0.426)
Cerebral peduncle L	-0.002(0.427)	0.001(0.620)	-0.001(0.611)
Anterior limb of internal capsule R	<0.001(0.819)	0.004(0.103)	-0.004(0.166)
Anterior limb of internal capsule L	-0.002(0.434)	0.004(0.160)	<0.001(0.955)
Posterior limb of internal capsule R	-0.007(0.045)	0.001(0.735)	0.002(0.577)
Posterior limb of internal capsule L	-0.006(0.071)	0.001(0.647)	0.001(0.796)
Retrolenticular part of internal capsule R	-0.005(0.252)	-0.005(0.208)	0.007(0.074)
Retrolenticular part of internal capsule L	<0.001(0.965)	-0.003(0.300)	<0.001(0.953)
Anterior corona radiata R	0.004(0.306)	<0.001(0.976)	-0.006(0.101)
Anterior corona radiata L	0.004(0.280)	-0.002(0.654)	-0.003(0.437)
Superior corona radiata R	0.002(0.563)	-0.001(0.740)	<0.001(0.951)
Superior corona radiata L	<0.001(0.964)	0.001(0.721)	-0.001(0.696)
Posterior corona radiata R	0.001(0.817)	-0.002(0.646)	0.001(0.751)
Posterior corona radiata L	<0.001(0.989)	-0.002(0.607)	0.003(0.489)
Posterior thalamic radiation R	0.004(0.429)	-0.006(0.214)	-0.001(0.886)
Posterior thalamic radiation L	0.003(0.550)	-0.001(0.884)	>0.001(0.924)
Sagittal stratum R	<0.001(0.982)	-0.003(0.461)	0.002(0.665)
Sagittal stratum L	0.003(0.468)	0.002(0.643)	-0.007(0.044)
External capsule R	0.004(0.484)	0.003(0.443)	-0.011(0.017)
External capsule L	0.002(0.581)	0.002(0.609)	-0.003(0.435)
Cingulate part of cingulum R	0.008(0.051)	-0.003(0.306)	-0.004(0.264)
Cingulate part of cingulum L	0.004(0.343)	<-0.001(0.999)	-0.002(0.648)
Hippocampal part of cingulum R	-0.001(0.777)	0.001(0.882)	<0.001(0.977)
Hippocampal part of cingulum L	0.004(0.365)	-0.005(0.233)	-0.002(0.619)
Superior longitudinal fasciculus R	0.003(0.504)	-0.005(0.129)	-0.001(0.775)
Superior longitudinal fasciculus L	<0.001(0.987)	-0.001(0.800)	-0.001(0.773)
Superior fronto-occipital fasciculus R	0.003(0.559)	0.000(0.922)	-0.005(0.321)
Superior fronto-occipital fasciculus L	0.003(0.632)	-0.003(0.472)	-0.004(0.432)
Uncinate fasciculus R	0.007(0.324)	-0.001(0.846)	-0.010(0.123)
Uncinate fasciculus L	0.003(0.638)	-0.001(0.914)	-0.002(0.712)
Tapetum R	0.002(0.806)	-0.003(0.735)	0.003(0.716)
Tapetum L	-0.007(0.515)	-0.003(0.757)	-0.022(0.045)

Abbreviations: fractional anisotropy (FA), nutrient biomarker pattern 1 (LCPUFA), nutrient biomarker pattern 2 (n6PUFA), nutrient biomarker pattern 3 (n3PUFA), right (R), left (L)

Model: regional FA = LCPUFA + n6PUFA + n3PUFA + age + gender + education + income + body mass index + depression status

Results are presented as β(p)

* $p < 0.05$, FDR-corrected

Table 6.4. Linear regression models: Nutrient biomarker patterns associated with memory

NBP	**Composite memory score**
LCPUFA	0.320(0.003)*
n-6 PUFA	-0.126(0.191)
n-3 PUFA	-0.062(0.536)

Abbreviations: nutrient biomarker pattern (NBP), nutrient biomarker pattern 1 (LCPUFA), nutrient biomarker pattern 2 (n6PUFA), nutrient biomarker pattern 3 (n3PUFA)
Model: composite memory score = LCPUFA + n-6 PUFA + n-3 PUFA + age + gender + education + income + body mass index + depression status
Results are presented as β(p)
* $p < 0.05$

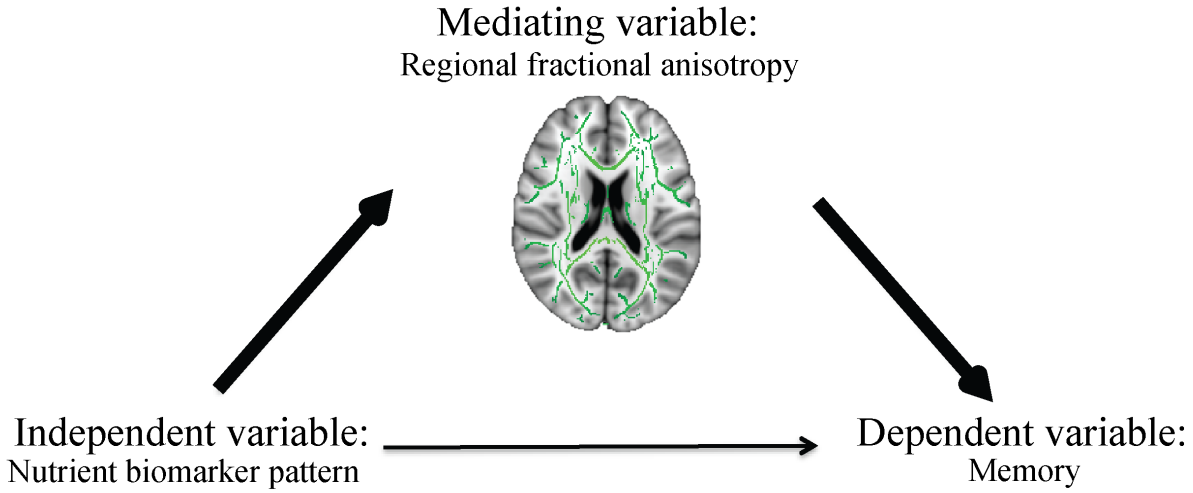

Figure 6.1. Proposed mediation model: The primary requirement for mediation is a significant indirect mediation effect, defined as the effect of the independent variable (nutrient biomarker pattern) through the mediation (fractional anisotropy in white matter regions) on the dependent variable (memory).

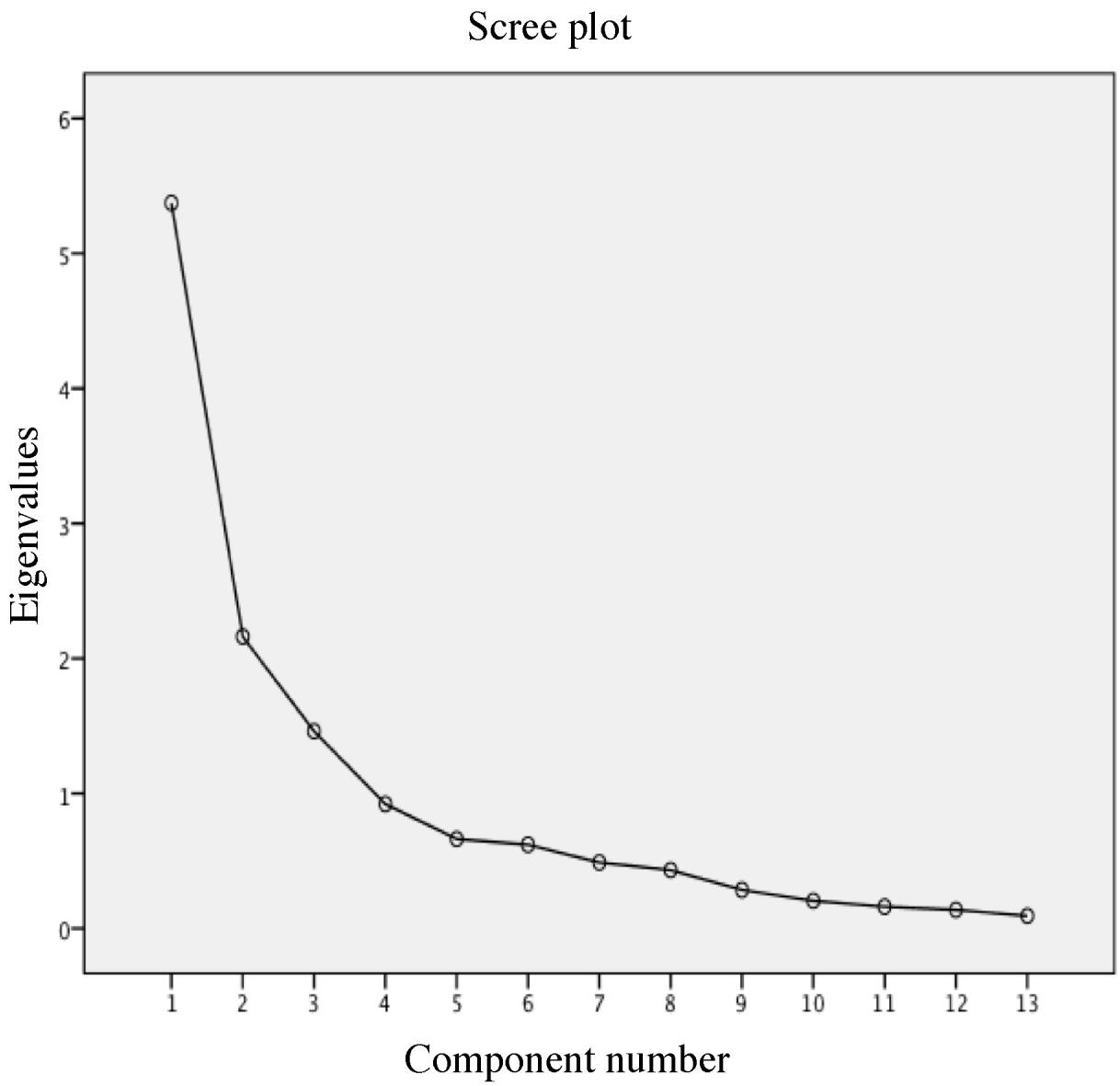

Figure 6.2. Scree plot: Inspection of the scree plot visually indicates which nutrient biomarker patterns explain the most variability in the data. A change in curvature, or inflection point, occurred after the third component, or nutrient biomarker pattern, was extracted. Thus, three components explained most variability in the data.

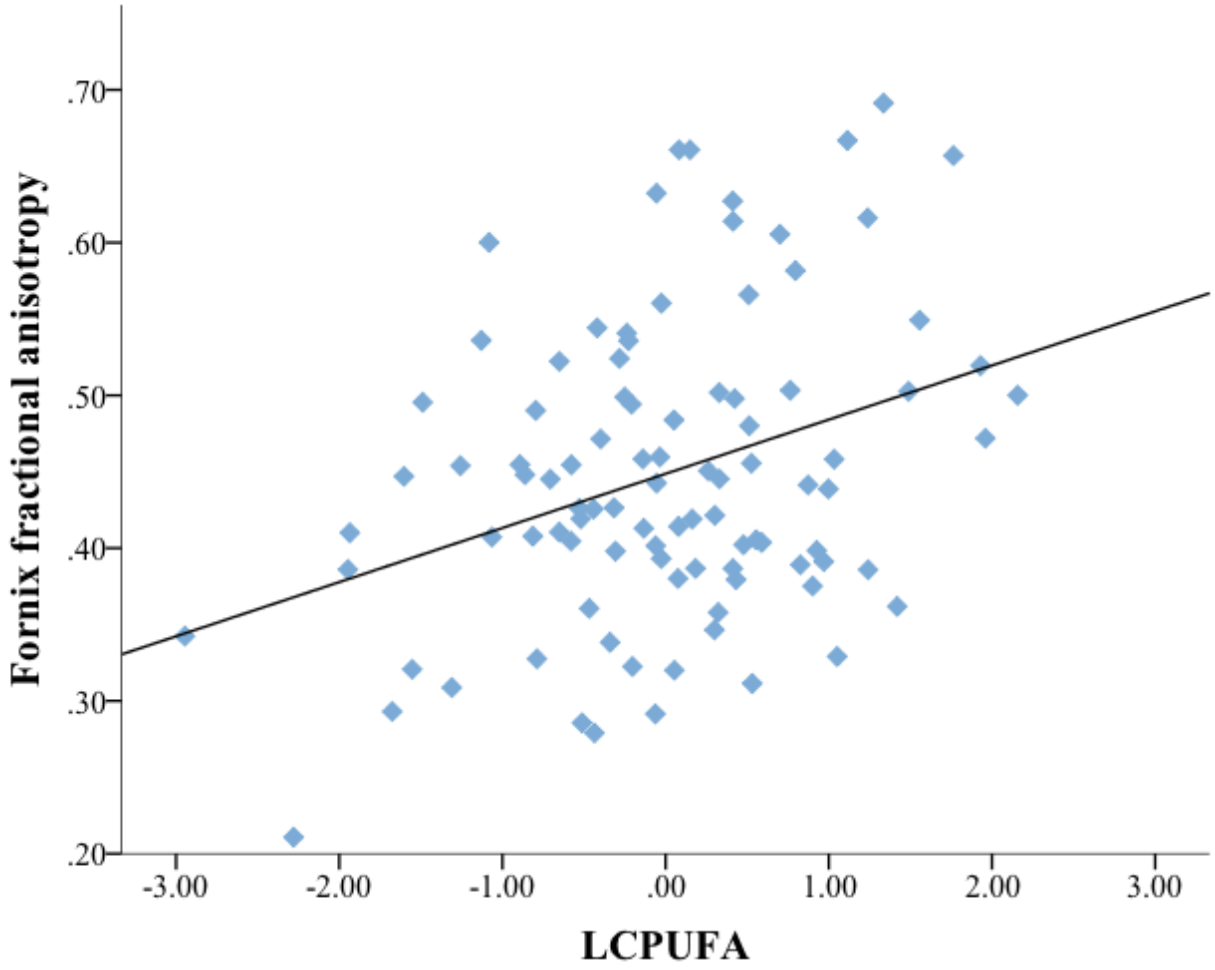

Figure 6.3. Mediation path a: Linear regression modeling showed that nutrient biomarker pattern 1 (LCPUFA) positively and reliably associated with fornix fractional anisotropy (β=0.042, $p<0.001$).

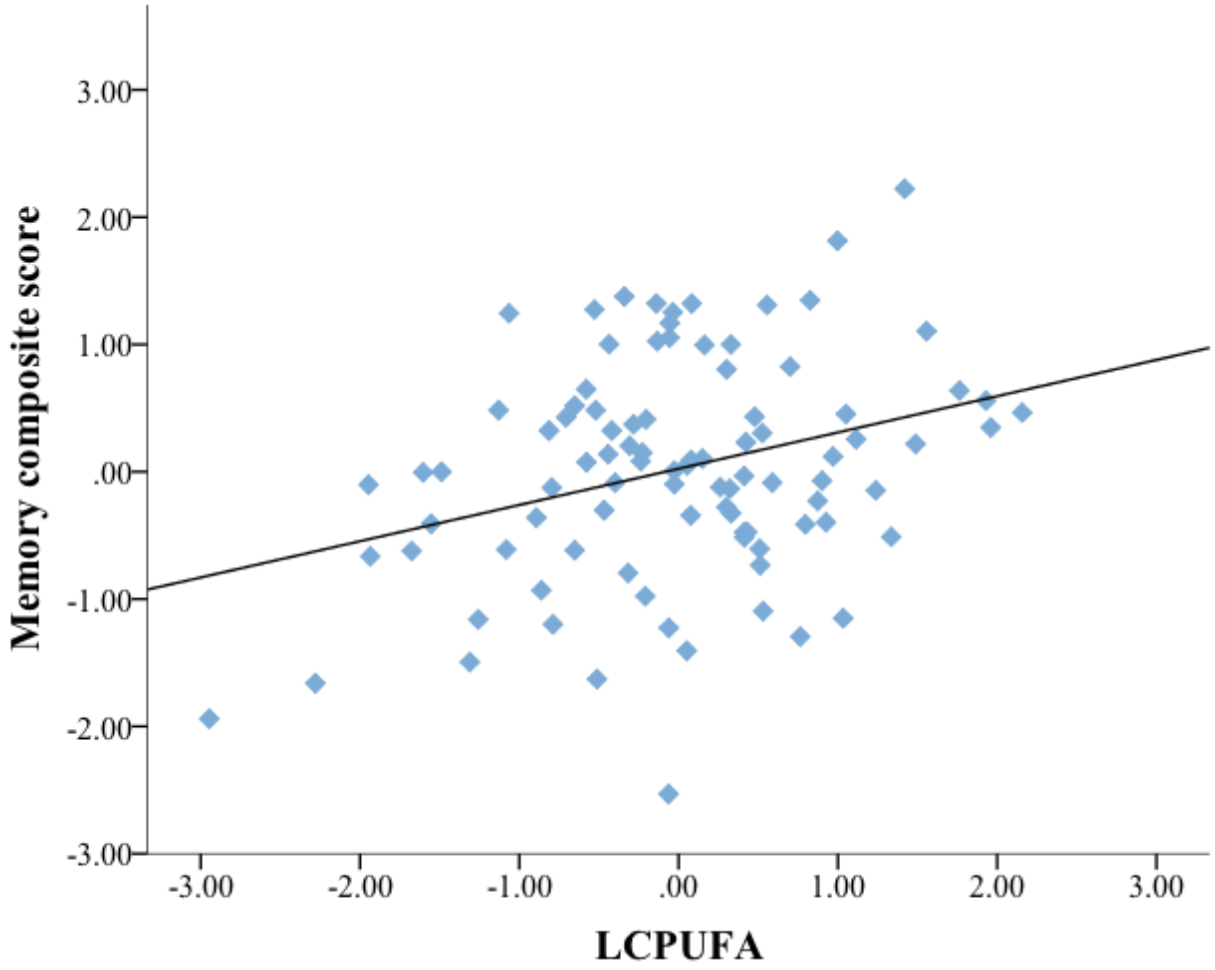

Fig 6.4. Mediation path c: Linear regression modeling showed that nutrient biomarker pattern 1 (LCPUFA) positively and reliably associated with memory (β=0.320, p=0.003).

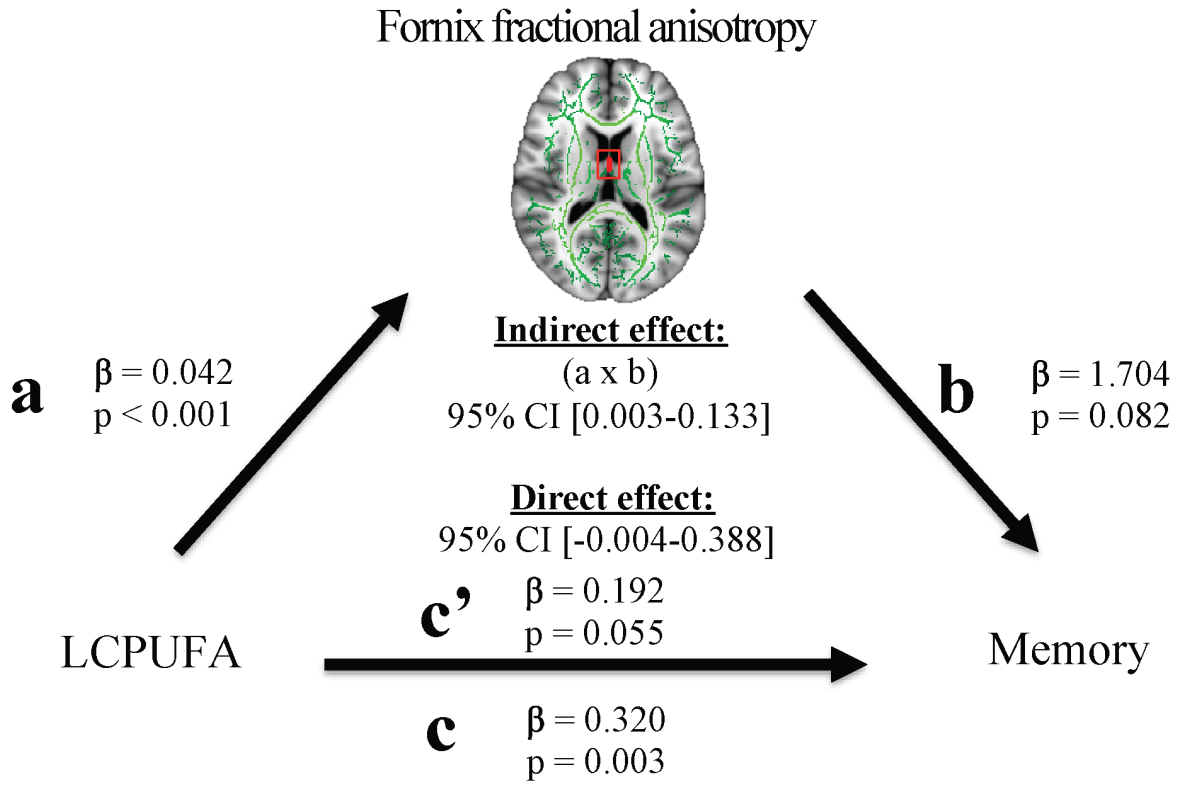

Fig 6.5. Mediation model statistics: Nutrient biomarker pattern 1 (LCPUFA) positively associated with fractional anisotropy of the fornix (**path a**). LCPUFA positively associated with memory (**path c**). The indirect pathway of mediation (i.e., the effect of LCPUFA through fornix fractional anisotropy on memory; **path a-b**) was statistically significant. The direct pathway of mediation (i.e., the effect of LCPUFA on memory, accounting for fornix fractional anisotropy; **path c'**) was not significant. Therefore, fornix fractional anisotropy fully mediated the relationship between LCPUFA and memory.

CHAPTER 7: NUTRIENT BIOMARKERS OF GENERAL INTELLIGENCE AND OPTIMAL BRAIN NETWORK ORGANIZATION

Abstract

Recent evidence demonstrates that cognitive function depends not only upon integrity in brain structure and function, but also on dietary intake and nutritional status. General intelligence enables adaptive reasoning and problem solving skills and relies upon the efficient communication of information within intrinsic connectivity networks of the brain. General intelligence is strongly predictive of real world occupational, social, and health outcomes, motivating the importance of understanding how its underlying brain mechanisms are influenced by nutrition. Importantly, the ratio of monounsaturated to saturated fatty acids has been linked to cognitive and brain function, but it is not known how these fatty acids may influence the intrinsic connectivity networks that support intelligence. This study therefore examined the extent to which intrinsic connectivity networks underlying general intelligence are influenced by patterns of monounsaturated and saturated fatty acids. We measured general intelligence, small world propensity (i.e., a sensitive measure of favorable connective architecture) of seven intrinsic connectivity networks, and seventeen plasma phospholipid monounsaturated and saturated fatty acids. A mediation analysis was implemented using (i) principal component analysis to empirically derive patterns of fatty acids and (ii) multivariate linear regressions to investigate relationships between general intelligence, small world propensity of intrinsic connectivity networks, and nutritional status (adjusted for age, gender, education, income, depression status, and body mass index). The mediation analysis revealed that small world propensity within an intrinsic connectivity network underlying general intelligence, the dorsal attention network, was

promoted by one pattern of fatty acids composed of only monounsaturated fatty acids. Furthermore, small world propensity in the dorsal attention network fully mediated the relationship between the monounsaturated fatty acid pattern and general intelligence. These results suggest that in healthy older adults, the efficiency of functional organization within a core network underlying general intelligence is amenable to the effects of monounsaturated fatty acids. This report provides an unprecedented connection between nutritional status and functional network efficiency, and further supports the notion that specific aspects of diet may support intelligence by influencing brain network organization.

Introduction

Recent discoveries in nutritional epidemiology and cognitive neuroscience provide insight into the therapeutic potential of nutrition for enhanced cognitive performance and brain health across the lifespan (reviewed in Zamroziewicz and Barbey, 2016). The interdisciplinary field of nutritional cognitive neuroscience is at the forefront of this effort and demonstrates that cognitive function depends not only upon integrity in brain structure and function, but also on dietary intake and nutritional status. Unraveling the ways in which particular nutrients and dietary components may influence specific aspects of brain structure and function to support cognition will have profound implications for understanding the nature of brain health and for treating neurological disease.

General intelligence enables adaptive reasoning and problem solving skills (Barbey et al., 2012, 2013a, 2013b, 2014b; Colom et al., 2010) that are important in everyday decision making (Gottfredson, 1997) and are strongly predictive of occupational attainment, social mobility (Strenze, 2007) and job performance (Gottfredson, 1997). Thus, understanding the brain

mechanisms underlying this general mental ability, and how they may be modulated by lifestyle factors, such as nutrition, may provide significant individual and societal benefits (Colom et al., 2010).

A large body of neuroscience evidence indicates that variation in the synchronization or efficiency of communication between brain regions reliably predicts individual differences in general intelligence (reviewed in Deary et al., 2010). Intrinsic connectivity networks, the fundamental organizational units of human brain architecture (Laird et al., 2011), display consistent spatial patterns of functional connectivity at rest that reflect information processing capabilities (Beckmann et al., 2005; Damoiseaux et al., 2006; Lowe et al., 1998; Raichle et al., 2001). Individual differences in intelligence have been related to traditional measures of resting state connectivity in neural networks involved in self-referential mental activity (i.e., default mode network), attentional control processes (i.e., dorsal attention network), and task-set maintenance (i.e., cingulo-opercular network) (Pamplona et al., 2015; Santarnecchi et al., 2015; Smith et al., 2015; Song et al., 2009; Wang et al., 2011; Yuan et al., 2012). Further, recent evidence suggests a key role for connections between prefrontal and parietal cortices that comprise the dorsal attention network (Hearne et al., 2016). Advances in neuroimaging provide a new tool that implements graph theory principles to measure and assess intrinsic connectivity networks (Watts and Strogatz, 1998). Graph theory metrics enable a precise characterization of the complex network organization of the brain (Bullmore and Sporns, 2009), with efficient information flow across functional brain networks representing a small-world organization defined by a high level of local clustering and a short path length between brain regions (van den Heuvel et al., 2008; Sporns and Zwi, 2004). In particular, small world propensity presents as an accurate measure of small world architecture that is impervious to nuisance variables but

sensitive to critical variables of small world organization (Muldoon et al., 2016). Importantly, diet and nutrients have been shown to improve brain function (Boespflug et al., 2016; Dumas et al., 2016; Konagai et al., 2013; Wiesmann et al., 2016); however, no study has investigated the influence of nutrition on small world propensity of the intrinsic connectivity networks that support cognition.

The Mediterranean diet is a well-recognized dietary pattern thought to promote healthy brain aging (Feart et al., 2015). Indeed, adherence to Mediterranean-style diets has been shown to support cognitive performance (Hardman et al., 2016) as well as brain structure and function (Matthews et al., 2014; Staubo et al., 2017). Scientific advances in the characterization of dietary patterns and measurement of nutrient biomarkers have led to a new methodology in nutritional epidemiology for the measurement of nutrient biomarker patterns (NBPs). In this approach, nutrients are measured by way of biochemical markers in the blood (i.e., nutrient biomarkers) and principal component analysis is applied to empirically derive patterns of nutrients, referred to as NBPs. This method is highly sensitive to variability within a restricted set of variables (i.e., 5-10 variables per observation; Osborne and Costello, 2004). Thus, in this study, we use NBP analysis to investigate one of the core components of the Mediterranean diet with high sensitivity and specificity: the monounsaturated fatty acid to saturated fatty acid ratio. This fatty acid ratio has been linked to cognitive function (Samieri et al., 2013; Solfrizzi et al., 1999, 2006) as well as brain function (Dumas et al., 2016). In fact, the monounsaturated fatty acid to saturated fatty acid ratio is thought to be one of the driving factors of the metabolic benefits of the Mediterranean diet on the brain (Aranceta and Pérez-Rodrigo, 2012; Dyson et al., 2011; Evert et al., 2013). However, no study has investigated how patterns of monounsaturated fatty acids and saturated fatty acids affect the intrinsic connectivity networks that underlie cognitive function.

In summary, general intelligence is an important predictor of real-world decision making and relies upon the functional integrity of underlying neural networks. The monounsaturated fatty acid to saturated fatty acid ratio, a core component of the Mediterranean diet, is known to promote cognition and brain structure, but how these fatty acids support the intrinsic connectivity networks that underlie general intelligence remains unknown. Therefore, this study applies cutting-edge methodologies from nutritional epidemiology and cognitive neuroscience to explore how nutrient profiles of monounsaturated fatty acids and saturated fatty acids impact small world propensity of intrinsic connectivity networks that support general intelligence in a sample of cognitively intact, older adults.

Materials and Methods

Participants

This cross-sectional study enrolled 122 healthy elderly adult patients through Carle Foundation Hospital, a local and readily available cohort of well-characterized elderly adults. No participants were cognitively impaired, as defined by a score of lower than 26 on the Mini-Mental State Examination (Folstein et al., 1975). Participants with a diagnosis of mild cognitive impairment, dementia, psychiatric illness within the last three years, stroke within the past twelve months, and cancer within the last three years were excluded. Participants were also excluded for current chemotherapy or radiation, an inability to complete study activities, prior involvement in cognitive training or dietary intervention studies, and contraindications for magnetic resonance imaging (MRI). All participants were right handed with normal, or corrected to normal vision and no contraindication for MRI. Of these 122 participants, 99 subjects had a complete dataset at time of data analysis, including neuropsychological testing, MRI, and blood biomarker analysis.

Standard protocol approval and participant consent

This study was approved by the University of Illinois Institutional Review Board and the Carle Hospital Institutional Review Board and, in accordance with the stated guidelines, all participants read and signed informed consent documents.

Neuropsychological tests

General intelligence was measured by the Wechsler Abbreviated Scale of Intelligence – second edition (WASI-II) (Wechsler, 1999). This assessment measured general intelligence by way of an estimated intelligence quotient score, which was the product of two subtests: a vocabulary subtest and a matrix reasoning subtest. In the vocabulary subtest, participants were asked to verbally define vocabulary words (i.e., What does lamp mean?) that became progressively more challenging. In the matrix reasoning subtest, participants were asked to complete a matrix or serial reasoning problem by selecting the missing section from five response items. Subjects' raw scores were converted to normalized scaled scores and combined into a perceptual reasoning index, which provided a measure of general intelligence.

Functional magnetic resonance imaging of small world propensity of intrinsic connectivity networks

All MRI data processing was performed using FMRIB Software Library (FSL) tools available in FSL version 5.0 (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The high-resolution T1 Magnetization-Prepared Rapid Gradient-Echo (MPRAGE) was brain extracted using the Brain Extraction Tool (BET) (Smith, 2002). FMRIB's Automated Segmentation Tool (FAST)

segmentation (Zhang et al., 2001) was performed to delineate gray matter, white matter, and cerebral spinal fluid (CSF) voxels. The resting-state fMRI data were pre-processed using the FSL FMRI Preprocessing and Model-Based Analysis (FEAT) analysis tool (Jenkinson et al., 2012; Satterthwaite et al., 2012). Pre-processing entailed: slice timing correction, motion correction, spatial smoothing (3 mm full width at half maximum kernel), nuisance signal regression (described below), temporal bandpass filtering (0.009 - 0.1 Hz), linear registration of functional images to structural images, and non-linear registration of structural images to the MNI152 brain template (2 mm isotropic voxel resolution).

Nuisance variables were modeled via General Linear Modeling (GLM) analyses to remove spurious correlations, noise introduced by head motion, and variables of no interest. These included head motion correction parameters (using the extended 12 motion parameters estimated in FEAT preprocessing), modeling of individual volume motion outliers estimated using DVARS (outliers flagged using the boxplot cutoff 1.5 x interquartile range; Power et al., 2012), and averaging of mean white matter and cerebrospinal fluid signals across all voxels identified from the segmentation of the high resolution MPRAGE. The fully preprocessed resting state fMRI data was the residual obtained from fitting these nuisance variables in the GLM framework. The residuals were transformed into normalized MNI152 space and re-sampled to 4 mm isotropic voxels.

The network measure of small world propensity (Muldoon et al., 2016) was investigated to quantify the extent to which functional brain networks exhibited high local clustering and short path lengths that promote efficient information flow within coupled neural systems. Small world propensity can be computed for weighted networks, thereby taking into account differential connectivity strengths that might have important implications for brain health. In our

study, we generated small world propensity measures for seven intrinsic connectivity networks: default mode network, frontoparietal network, dorsal attention network, ventral attention network, limbic network, somatomotor network, and visual network. These intrinsic connectivity networks were previously identified based on the clustering of functionally coupled regions (Yeo et al., 2011). Nodes were defined at each intrinsic connectivity network by applying a parcellated brain mask and identifying center of mass coordinates of brain parcels, which overlapped with intrinsic connectivity network regions. We applied Craddock's 800 brain parcel mask (Craddock et al., 2012) on the intrinsic connectivity networks as this provided whole brain coverage and maintained sufficiently high resolution for conducting network analysis on the intrinsic connectivity network nodes. The total number of nodes within each intrinsic connectivity network that were common across all 99 subjects were as follows: 55 in the visual network, 45 in the somatomotor network, 52 in the dorsal attention network, 33 in the ventral attention network, 39 in the limbic network, 47 in the frontoparietal network, and 91 in the default mode network.

For each intrinsic connectivity network, we generated subject-wise weighted connectivity matrices reflecting pairwise correlations between the mean time course of resting state blood oxygen level-dependent (BOLD) fMRI signal extracted from Craddock's brain parcels whose center of mass corresponded to the intrinsic connectivity network nodes. These correlations were Fisher's *Z*-transformed and then converted into standard normal *Z*-scores through multiplication with their standard deviation approximated as $\sigma = 1/\sqrt{(n-3)}$, where n is number of samples comprising the BOLD signal. Next, a Bonferroni-corrected statistical *Z*-threshold was applied to identify significant positive correlations ($p<0.05$) (Fox et al., 2009; Murphy et al., 2009). The thresholded *Z*-scores were rescaled to represent connection weights ranging from 0 to 1. Small world propensity was then calculated from these weighted connectivity matrices based on the

fractional deviation between a network's clustering coefficient , C_{brain}, and characteristic path length, L_{brain}, from both lattice ($C_{lattice}$, $L_{lattice}$) and random (C_{random}, L_{random}) networks having the same number of nodes and same degree distribution (Muldoon et al., 2016).

Nutrient biomarker acquisition

Plasma lipids were extracted by the method of Folch, Lees and Sloane-Stanley (Folch et al., 1957). Briefly, the internal standard (25 μg each of PC17:0) was added to 200 μl of serum, followed by 6 mL of choloroform:methanol:BHT (2:1:100 v/v/w). The protein precipitate was removed by centrifugation (2,500 g, 5 minutes, 4ºC). Then 1.5 mL of 0.88% KCl was added to the supernatant, shaken vigorously and the layers were allowed to settle for 5 minutes. The upper layer was discarded and 1 ml of distilled water:methanol (1:1 v/v) was added, the tube was shaken again and the layers allowed to settle for 15 minutes. The lower layer was transferred into a clean tube and evaporated to dryness under nitrogen. The phospholipid subfraction was separated by solid-phase extraction using aminopropyl columns as described by Agren, Julkunen and Penttila (Aryen et al., 1992). Then the phospholipid fraction was methylated by adding 2 ml of 14% BF3-MeOH and incubating at 95ºC for 1 hour (Morrison and Smith, 1964). The supernatant containing the fatty acid methyl esters (FAMEs) was dried down under nitrogen, resuspended in 100 μl of hexane, transferred into amber GC vials and stored at -20ºC until the time of analysis.

The phospholipid FAMEs were analyzed by a CLARUS 650 gas chromatograph (Perkin Elmer, Boston MA) equipped with a 100 m x 0.25 mm i.d (film thickness 0.25 μm) capillary column (SP-2560, Supelco). Injector and flame ionization detector temperatures were 250ºC and 260ºC, respectively. Helium was used as the carrier gas (2.5 mL/min) and the split ratio was

14:1. The oven temperature was programmed at 80ºC, held for 16 minutes and then increased to 180ºC at a rate of 5ºC/minute. After 10 minutes, the temperature was increased to 192ºC at a rate of 0.5ºC/minute, held for 4 minutes. The final temperature was 250ºC reached at a rate of 405ºC/minute and held for 15 minutes. Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc. MN) and expressed as absolute concentration (μmol/L). The plasma lipids of interest were saturated fatty acids and monounsaturated fatty acids, listed in **Table 7.1**.

Nutrient biomarker pattern analysis of fatty acids

Nutrient biomarker pattern analysis was conducted in IBM SPSS statistical software, version 24 for Macintosh. Principal component analysis was used to identify NBPs of fatty acids from the seventeen fatty acids listed in **Table 7.1**. Of these, twelve fatty acids (capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, myristoleic acid, palmitoleic acid, cis-7-hexadecenoic acid, cis-vaccenic acid, oleic acid, gondoic acid, erucic acid) were non-normally distributed as indicated by Shapiro-Wilk test (all p-values<0.05) and therefore log-transformed to correct for skewness of variables and subsequently considered in the analysis. The appropriate rotation method was determined by examining the factor correlation matrix: varimax rotation for a correlation matrix with values less than 0.32 and direct oblimin rotation for a correlation matrix with values greater than 0.32 (Tabachnick and Fidell, 2007). Statistical validity of the factor analysis was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy ($\geq$0.50; Kaiser, 1970) and Bartlett's Test of Sphericity (p<0.05; Bartlett, 1950). Outliers were identified as participants with factor score values greater than 3.0 and removed from all analyses (Jolliffe, 2014). The number of NBPs to be retained was determined by a combination of eigenvalues

greater than 1.0, variance accounted for by each component, and scree plot inflection point. Interpretation of each factor was based on identifying biomarkers with an absolute loading value of greater than 0.50 on an NBP (i.e., identifying the dominant biomarkers contributing to each particular NBP). Each participant received a standardized NBP score for each pattern that corresponded to a linear combination of the nutrient biomarkers.

Covariates

Covariates were included according to previous association with cognitive decline (Keon & Yonelinas, 2014; Ronnlund et al., 2005; Hurst et al., 2013; Gallucci et al., 2013; Pauls et al., 2013; Wilkins et al., 2010). The covariates included age (continuous), gender (nominal, man/woman), education (nominal, five fixed levels), income (nominal, six fixed levels), body mass index (continuous, hereafter BMI), and depression status (nominal, yes/no). Although all participants had received a diagnosis of no depression at enrollment, the SF-36 Health Survey (Ware et al., 1993) revealed five participants with symptoms consistent with depression. Thus, in accordance with prior studies (Bowman et al., 2012, 2013; Witte et al., 2014), this was considered in the analysis as a covariate.

Statistical analysis

A formal mediation analysis was conducted to model the relationship between small world propensity of intrinsic connectivity networks, general intelligence, and NBPs of monounsaturated and saturated fatty acids. The analysis used a four-step framework with the ultimate goal of evaluating whether small world propensity of intrinsic connectivity networks mediated the relationship between NBPs of monounsaturated and saturated fatty acids and

general intelligence. The primary requirement for mediation is a significant indirect mediation effect (Zhao et al., 2010), or the effect of the independent variable (NBPs) through the mediator (small world propensity of intrinsic connectivity networks) on the dependent variable (general intelligence) (**Figure 7.1**).

1. In the first step, regression models were applied to identify the intrinsic connectivity networks that predict general intelligence (**path b**). Two linear regression models were fit. Model one included small world propensity of seven intrinsic connectivity networks, age, gender, and education, all entered simultaneously. Model two was further adjusted for income, BMI, and depression status simultaneously. Model one aimed to avoid over-fitting and is reported in-text (Babyak, 2004), whereas model two aimed to comprehensively account for covariates that have been previously associated with cognitive decline.
2. In the second step, regression models were applied to characterize the relationship between NBPs and small world propensity of the intrinsic connectivity networks that underlie general intelligence (**path a**). Only the intrinsic connectivity networks that related to general intelligence in step one were considered. Two linear regression models were fit for each intrinsic connectivity network. Model one included each NBP, age, gender, and education, all entered simultaneously. Model two was further adjusted for income, BMI, and depression status simultaneously. As previously, model one avoided over-fitting and is reported in-text, and model two comprehensively accounted for covariates.
3. In the third step, regression models were applied to characterize the relationship between NBPs and general intelligence (**path c**). Two linear regression models were fit. Model one included each NBP, age, gender, and education, all entered simultaneously. Model two was further adjusted for income, BMI, and depression status simultaneously. As previously,

model one avoided over-fitting and is reported in-text, and model two comprehensively accounted for covariates.

4. In the fourth step, the PROCESS macro was applied to implement the bootstrapping method to estimate mediation effects (Preacher and Hayes, 2008). This analysis drew 1000 bootstrapped samples with replacement from the dataset to estimate a sampling distribution for the indirect and direct mediation effects, controlling for age, gender, education, income, BMI, and depression status. The indirect mediation effect refers to the pathway from NBPs to small world propensity of intrinsic connectivity networks to general intelligence (**path a-b**). The direct mediation effect refers to the direct pathway from NBPs to general intelligence, accounting for the effect of small world propensity of intrinsic connectivity networks (**path c'**).

Results are reported using (i) R^2 for each model, (ii) unstandardized regression coefficients (β), unstandardized regression coefficient standard error (SE β), and p of each individual regression relationship, and (iii) a 95% bias-corrected confidence interval (95% CI) for the direct and indirect effects of the mediation. Significance was accepted at $p \leq 0.05$ and a false discovery rate (FDR) correction for multiple comparisons (Benjamini and Hochberg, 1995) was applied ($q < 0.05$, two-tailed). A statistically significant mediation that matches the hypothesized framework is indicated by: (i) an indirect mediation effect that does not include zero within 95% CI, and (ii) a direct mediation effect that does include zero within 95% CI (Zhao et al., 2010).

Results

Participant characteristics

Participants had a mean age of 69 years and 63 percent of participants were females. All other participant characteristics are reported in **Table 7.1**.

Nutrient biomarker patterns

Principal component analysis generated four NBPs (**Table 7.2**). The factor correlation matrix contained values greater than 0.32, therefore direct oblimin rotation was implemented. Statistical validity of the factor analyses was confirmed via the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (0.780) and Bartlett's Test of Sphericity ($p<0.001$). One outlier was removed from the dataset. Four NBPs were selected for retention because (i) after the NBP extraction with principal component analysis, 71.4 percent of the total variance was accounted for in the original set of nutrient biomarkers, and (ii) inspection of the scree plot indicated that the inflection point occurred after the fourth NBP (**Figure 7.2**). Hereafter, NBP1 is described as SFA (i.e., it is composed of saturated fatty acids and one monounsaturated fatty acid, all derived or *de novo* synthesized from dietary sources of saturated fatty acids), NBP2 is described as SEED OIL (i.e., it is composed of saturated fatty acids and one monounsaturated fatty acid, all primarily derived from seed oils), NBP3 is described as MIXED (i.e., it is composed of saturated and monounsaturated fatty acids derived from various sources), and NBP4 is described as MUFA (i.e., it is composed of only monounsaturated fatty acids). Importantly, SEED OIL and MUFA have negative loading coefficients, therefore a higher factor score in these two NBPs is indicative of lower levels of nutrients within the patterns.

Mediation results

The mediation analysis indicated that small world propensity of the dorsal attention network fully mediated the relationship between MUFA and general intelligence. Each relationship within the mediation is described below in a stepwise fashion.

1. Small world propensity of the dorsal attention network (β=32.222 SE β=11.844, p=0.008) and frontoparietal network (β=42.555 SE β=19.357, p=0.031) predicted general intelligence (R^2=0.347, **Table 7.3**). Results remained significant after accounting for all covariates and because all variables of interest were included in the same model, no correction for multiple comparisons was needed (**Table 7.3**). Thus, the dorsal attention network and frontoparietal network were considered in step two.
2. Higher small world propensity of the dorsal attention network was associated with lower levels of fatty acids within SFA (β=-0.025 SE β=0.012, p=0.034) and higher levels of fatty acids within MUFA (β=-0.044 SE β=0.012, p=0.001), but no NBP reliably associated with small world propensity of the frontoparietal network (R^2=0.158, **Table 7.4**). After accounting for all covariates, results remained significant, but after correcting for multiple comparisons, only MUFA remained a significant predictor (**Table 7.3**). Thus, the dorsal attention network was considered in the context of the mediation model (**Figure 7.3 path a-b**).
3. General intelligence was predicted by MUFA (β=-3.832 SE β=1.399, p=0.007), but not by any other NBP (R^2=0.302, **Table 7.5**). Results remained significant after accounting for all covariates and correcting for multiple comparisons (**Table 7.3**). Thus, MUFA was considered in the context of the mediation model (**Figure 7.3 path c**).
4. The indirect pathway of mediation (the pathway from MUFA to dorsal attention network small world propensity to general intelligence) was significant (95% CI [-1.802, -0.011], **Figure 7.3 path a-b**), but the direct pathway of mediation (the direct pathway from MUFA

to general intelligence, accounting for the effect of small world propensity of the dorsal attention network) was not significant (95% CI [-4.751, 0.220], β=-2.266 SE β=1.251, p=0.073, **Figure 7.3 path c'**). Therefore, the mediation indicated that small world propensity of the dorsal attention network fully mediated the relationship between MUFA and general intelligence (R^2=0.377, **Figure 7.3**).

Discussion

This study revealed that an intrinsic connectivity network supporting general intelligence, the dorsal attention network, is influenced by monounsaturated fatty acids, and furthermore, that small world propensity of the dorsal attention network fully mediates the relationship between monounsaturated fatty acids and general intelligence. This report provides an unprecedented link between nutritional status, functional connectivity within intrinsic connectivity networks, and cognition. The individual relationships reported within these analyses, including those between small world propensity of intrinsic connectivity networks and general intelligence (**Figure 7.3 path b**), between monounsaturated fatty acids and small world propensity of intrinsic connectivity networks (**Figure 7.3 path a**), and between monounsaturated fatty acids and general intelligence (**Figure 7.3 path c**) are each supported by prior findings and reviewed below.

First, small world propensity of the dorsal attention network and frontoparietal network predicted general intelligence. Previous work supports the notion that functional connectivity within both the frontoparietal cortex and the dorsal attention network underlie general intelligence. In regard to cognitive function, the dorsal attention network serves externally-directed attention, whereas frontoparietal control network serves a pivotal gate-keeping role in goal-direction attention (Spreng et al., 2013). Traditionally, the frontoparietal network is thought

to be responsible for intelligence (Colom et al., 2010). However, with age, regions that are typically connected to the frontoparietal cortex, including the lateral and rostral prefrontal cortex, are reassigned to the dorsal attention network (Grady et al., 2016). Importantly, tasks of general intelligence are known to recruit the lateral prefrontal cortex (Duncan et al., 2000) along with prefrontal regions that comprise the dorsal attention network (Hearne et al., 2016). Furthermore, performance on tasks of intelligence is dependent upon organization of functional brain networks (van den Heuvel et al., 2008; Song et al., 2008), and age-related disorganization in these networks has been linked to worse cognitive performance (Damoiseaux, 2017). Thus, our findings are in line with previous evidence, which suggests a role for the dorsal attention network and frontoparietal network in general intelligence in older adults.

Second, small world propensity of the dorsal attention network was reliably linked to higher levels of fatty acids within the MUFA pattern. Previous evidence indicates that dietary intervention can indeed improve functional connectivity (Wiesmann et al., 2016). In fact, monounsaturated fatty acids have been shown to oppose the detrimental effects of saturated fatty acids on brain function (Dumas et al., 2016). Furthermore, dietary patterns that include high levels of monounsaturated fatty acids are known to increase metabolism in regions within the dorsal attention network (Berti et al., 2015). Therefore, our findings provide further support for the beneficial role of monounsaturated fatty acids on brain function.

Third, higher levels of fatty acids within the MUFA pattern predicted superior general intelligence. It has long been postulated that nutrition could at least in part explain secular increases in intelligence (Lynn, 1990). In older adults, fatty acid status has been linked to slower cognitive decline (Whalley et al., 2004). More specifically, high levels of monounsaturated fatty acids have been shown to improve overall cognitive status (Solfrizzi et al., 2006) as well as the

trajectory of cognitive decline (Samieri et al., 2013). To our knowledge, no study has previously investigated the relationship between fatty acid status and general intelligence. Our results suggest that monounsaturated fatty acids influence an aspect of cognition that supports everyday decision making.

Lastly, small world propensity of the dorsal attention network fully mediated the relationship between the MUFA pattern and general intelligence. The mediation results are supported by the three lines of evidence outlined above, but importantly, offer a novel perspective on the relationship between nutrition, cognition, and brain health. These findings indicate that general intelligence is not only dependent on underlying intrinsic connectivity networks, but also on nutritional status. To our knowledge, no study has previously assessed the link between nutritional status and intrinsic connectivity networks that underlie cognitive function.

The predictive power of monounsaturated fatty acids may be indicative of particular physiological mechanisms. One mechanism thought to underlie the benefits of nutrition, and particularly monounsaturated fatty acids, in the brain is the support of insulin sensitivity (Sartorius et al., 2012; Vessby et al., 2001). Type 2 Diabetes Mellitus is known to increase risk for Alzheimer's disease, but even in individuals without clinical diabetes, blood glucose levels are positively associated with accelerated cognitive decline (Crane et al., 2013). Furthermore, abnormal insulin signaling associated with metabolic dysfunction within the central nervous system has been shown to induce cognitive dysfunction (Manschot et al., 2006) as well as changes in functional connectivity (Musen et al., 2012). More specifically, the dorsal attention network has shown disruptions in functional connectivity in individuals with metabolic dysfunction (Xia et al., 2015). Importantly, insulin resistance is modifiable and Mediterranean-

style diets with a high proportion of monounsaturated fatty acids have been shown to enhance insulin sensitivity (Esposito et al., 2004; Shai et al., 2008). In fact, dietary guidelines on macronutrient intake to improve glucose-insulin profiles specifically recommend increasing foods rich in monounsaturated fatty acids and reducing saturated fatty acids (Aranceta and Pérez-Rodrigo, 2012; Evert et al., 2013). Thus, our results corroborate the role of monounsaturated fatty acids in metabolic function, and further provide novel evidence for the role of monounsaturated fatty acids in supporting intrinsic connectivity networks that underlie general intelligence.

The strengths of this study include: (i) the use of blood biomarkers to measure physiological status of monounsaturated fatty acids and saturated fatty acids, (ii) the use of functional MRI and graph theory metrics to measure functional connectivity, and (iii) the assessment of a critical cognitive ability that impacts everyday life. Directions for future research include: (i) replication of results in a larger sample, (ii) determination of the optimal intake of monounsaturated fatty acids, (iii) implementation of a longitudinal study to examine how changes in intake or nutritional status of monounsaturated fatty acids relate to changes in general intelligence and functional connectivity, (iv) examination of the mechanisms that support the relationship between monounsaturated fatty acids and functional connectivity within the dorsal attention network, and (v) investigation of the relationship between monounsaturated fatty acids, cognition, and functional connectivity in other model systems, including animal models and clinical populations.

Research at the frontline of nutritional cognitive neuroscience aims to incorporate cutting-edge measures of nutritional intake, cognitive function, and brain health to confirm that cognition is dependent not only on brain structure and function, but also on nutritional status and

dietary intake. In doing so, nutritional cognitive neuroscience endeavors to bridge the gap between largely disparate literatures of aging within the fields of nutritional epidemiology and cognitive neuroscience, and offer a novel perspective on brain health. Accumulating evidence suggests that certain nutrients may influence particular aspects of cognitive function by targeting specific features of brain health (Bowman et al., 2013; Gu et al., 2016; Zamroziewicz et al., 2015, 2016a, 2016b, 2017, 2018; Zamroziewicz and Barbey, 2016). The present finding contributes to this research program and provides novel evidence for the benefits of monounsaturated fatty acids on intrinsic connectivity networks that underlie general intelligence. Ultimately, this line of work can inform personalized and comprehensive nutritional interventions for healthy brain aging.

Tables and Figures

Table 7.1. Characteristics of sample

Demographics	***n* = 99**
Age in years (M ± SD)	69 ± 3
Female (%)	63
Education (%)	
Some high school	1
High school degree	11
Some college	16
College degree	71
Income (%)	
< $15,000	1
$15,000 - $25,000	3
$25,000 - $50,000	15
$50,000 - $75,000	24
$75,000 - $100,000	25
>$100,000	31
BMI (M ± SD)	26 ± 4
Depression indicated (%)	5
Plasma phospholipid nutrients	**(M ± SD, μmol/L)**
Capric acid (10:0)	0.14 ± 0.14
Lauric acid (12:0)	1.32 ± 0.87
Myristic acid (14:0)	12.60 ± 4.49
Pentadecylic acid (15:0)	6.34 ± 1.60
Palmitic acid (16:0)	723.81 ± 139.08
Stearic acid (18:0)	402.28 ± 88.05
Arachidic acid (20:0)	10.27 ± 2.08
Behenic acid (22:0)	30.85 ± 7.24
Lignoceric acid (24:0)	20.52 ± 5.40
Myristoleic acid (14:1)	1.10 ± 0.97
cis-7-Hexadecenoic acid (16:1n-9)	4.07 ± 1.09
Palmitoleic acid (16:1n-7)	15.58 ± 7.20
Oleic acid (18:1n-9)	240.22 ± 58.39
cis-Vaccenic acid (18:1n-7)	34.71 ± 7.79
Gondoic acid (20:1n-9)	3.53 ± 0.91
Erucic acid (22:1n-9)	0.43 ± 0.23
Nervonic acid (24:1n-9)	28.12 ± 7.10
Cognition	**(M ± SD)**
General intelligence	115 ± 13
Small world propensity	**(M ± SD)**
Visual network	0.542 ± 0.116
Motor network	0.515 ± 0.122
Dorsal attention network	0.584 ± 0.104
Ventral attention network	0.518 ± 0.137
Limbic network	0.554 ± 0.147
Frontoparietal network	0.664 ± 0.059
Default network	0.672 ± 0.032

Abbreviations: mean (M), standard deviation (SD), body mass index (BMI)

Table 7.2. Nutrient biomarker pattern construction: Pattern structure and variance explained[1]

Plasma phospholipid fatty acid	Nutrient biomarker pattern[2]			
	1	**2**	**3**	**4**
Palmitoleic acid (16:1n-7)	0.815*			
Pentadecanoic acid (15:0)	0.769*			
Myristic acid (14:0)	0.742*			
Palmitic acid (16:0)	0.535*	-0.321		-0.416
Arachidic acid (20:0)		-0.914*		
Lignoceric acid (24:0)		-0.914*		
Behenic acid (22:0)		-0.873*		
Nervonic acid (24:1n-9)		-0.758*		
Stearic acid (18:0)		-0.393		-0.391
Capric acid (10:0)	-0.412		0.725*	
Erucic acid (22:1n-9)			0.674*	
Myristoleic acid (14:1)			0.513*	
Lauric acid (12:0)	0.308		0.492	
Gondoic acid (20:1n-9)	-0.326			-0.928*
Oleic acid (18:1n-9)				-0.769*
cis-Vaccenic acid (18:1n-7)				-0.701*
cis-7-Hexadecenoic acid (16:1n-9)	0.408			-0.578*
Percent variance explained by each NBP	43.040	13.452	8.263	6.679
Cumulative percent variance explained with each extraction	43.040	56.493	64.756	71.435

Abbreviations: saturated fatty acid (SFA), monounsaturated fatty acid (MUFA)

[1] Extraction method: principal component analysis; rotation method: oblimin

[2] NBP interpretation based on strongest loading coefficients within each pattern; only loadings with an absolute value $\geq$ 0.3 are shown in the table

* Nutrients with absolute loadings $\geq$ 0.5 that are considered as dominant nutrients contributing to the particular nutrient pattern

Table 7.3. Linear regression models: Small world propensity of intrinsic connectivity networks associated with general intelligence

Network small world propensity		**General intelligence**	
		Model 1[a]	**Model 2**[b]
Visual	**β**	12.252	9.353
	SE	9.995	10.248
Somatomotor	**β**	-10.242	-7.686
	SE	10.853	10.778
Dorsal attention	**β**	32.222*	25.451*
	SE	11.844	12.065
Ventral attention	**β**	-2.333	1.356
	SE	8.587	8.644
Limbic	**β**	-0.415	-2.277
	SE	7.966	8.065
Frontoparietal	**β**	42.555*	40.313*
	SE	19.357	19.125
Default	**β**	9.994	1.024
	SE	36.201	36.506
Model	**R^2**	0.347*	0.392*

[a] Model 1: general intelligence = small world propensity of 7 networks + age + gender + education
[b] Model 2: general intelligence = model 1 + income + body mass index + depression status
* $p<0.05$

Table 7.4. Linear regression models: Nutrient biomarker patterns associated with small world propensity of intrinsic connectivity networks

NBP		**Dorsal attention**		**Frontoparietal**	
		Model 1[a]	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**
SFA	**β**	-0.025	-0.028	-0.001	-0.002
	SE	0.012	0.012	0.007	0.007
SEED OILS	**β**	0.012	0.015	0.002	0.002
	SE	0.012	0.012	0.007	0.007
MIXED	**β**	-0.007	-0.008	-0.005	-0.005
	SE	0.011	0.011	0.007	0.007
MUFA	**β**	-0.044*	-0.044*	-0.001	-0.001
	SE	0.012	0.012	0.008	0.008
Model	**R^2**	0.158*	0.219*	0.049	0.054

Abbreviations: nutrient biomarker pattern (NBP), nutrient biomarker pattern 1 (SFA), nutrient biomarker pattern 2 (SEED OILS), nutrient biomarker pattern 3 (MIXED), nutrient biomarker pattern 4 (MUFA)

[a] Model 1: small world propensity of network = 4 NBPs + age + gender + education

[b] Model 2: small world propensity of network = model 1 + income + body mass index + depression status

* $p<0.05$, FDR-corrected

Table 7.5. Linear regression models: Nutrient biomarker patterns associated with general intelligence

NBP		General intelligence	
		Model 1[a]	**Model 2[b]**
SFA	**β**	0.129	-0.075
	SE	1.308	1.301
SEED OILS	**β**	0.596	0.801
	SE	1.322	1.295
MIXED	**β**	-1.014	-1.233
	SE	1.262	1.229
MUFA	**β**	-3.832*	-3.696*
	SE	1.399	1.375
Model	**R^2**	0.302*	0.364*

Abbreviations: nutrient biomarker pattern (NBP), nutrient biomarker pattern 1 (SFA), nutrient biomarker pattern 2 (SEED OILS), nutrient biomarker pattern 3 (MIXED), nutrient biomarker pattern 4 (MUFA)

[a] Model 1: general intelligence = 4 NBPs + age + gender + education

[b] Model 2: general intelligence = model 1 + income + body mass index + depression status

* $p<0.05$, FDR-corrected

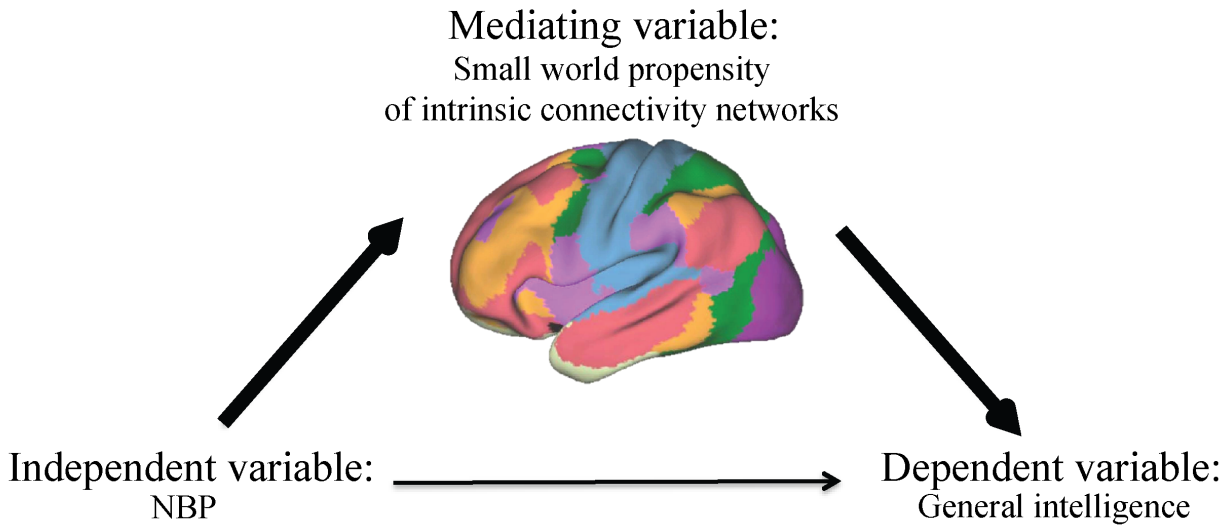

Figure 7.1. Proposed mediation model: The primary requirement for mediation is a significant indirect mediation effect, defined as the effect of the independent variable (nutrient biomarker pattern, NBP) through the mediator (small world propensity of intrinsic connectivity networks) on the dependent variable (general intelligence).

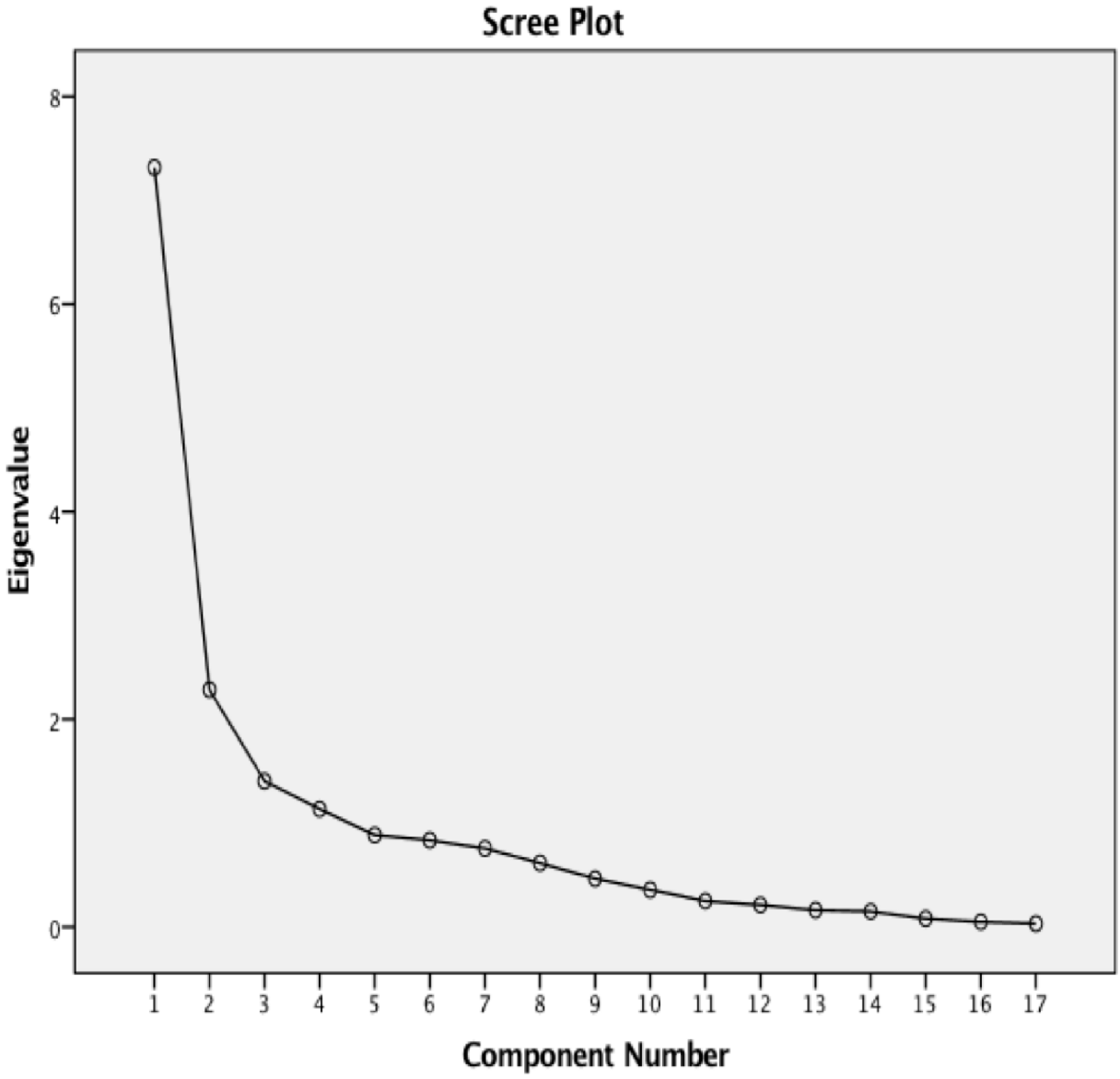

Figure 7.2. Scree plot: Inspection of the scree plot indicated that the inflection point occurred after the fourth component, or nutrient biomarker pattern, was extracted.

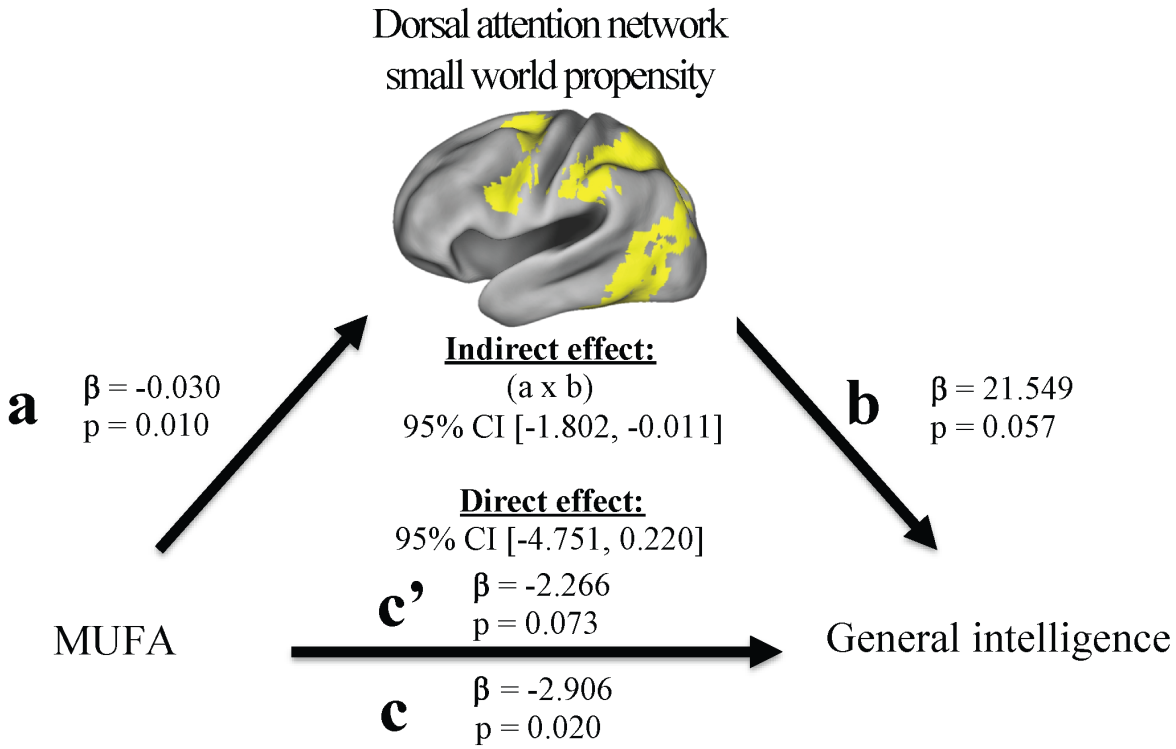

Figure 7.3. Mediation model statistics: Higher levels of nutrient biomarker pattern 4 (MUFA) associated with higher small world propensity of the dorsal attention network (**path a**). Higher MUFA also associated with better general intelligence (**path c**). The indirect pathway of mediation (i.e., the effect of MUFA through dorsal attention network small world propensity on general intelligence; **path a-b**) was statistically significant. The direct pathway of mediation (i.e., the effect of MUFA on general intelligence, accounting for small world propensity of the dorsal attention network; **path c'**) was not significant. Therefore, small world propensity of the dorsal attention network fully mediated the relationship between MUFA and general intelligence.

CHAPTER 8: CONCLUSION

This series of experiments substantiates the role of nutrition in healthy cognitive and brain aging, thus furthering the field of nutritional cognitive neuroscience. By integrating cutting-edge techniques from nutritional epidemiology and cognitive neuroscience, this work demonstrates that particular nutrients and nutrient patterns influence specific aspects of brain health to support facets of cognition. The main conclusions of each chapter and their contributions to these efforts are summarized below.

Although evidence suggests that diet has a substantial influence on the aging brain, the question of how individual nutrients might relate to specific aspects of cognition remains unanswered. Thus, the first three empirical chapters explore the relationship between particular nutrients and specific features of cognition and brain health in healthy older adults. Chapter two begins to define specific and sensitive mediatory relationships between nutrition, cognition, and brain health by demonstrating a novel structural mediation between plasma omega-3 polyunsaturated fatty acid levels and cognitive flexibility (Zamroziewicz et al., 2015). Chapter three provides further evidence that specific nutrients may slow or prevent decline in executive function by supporting brain structure in detailing a novel structural mediation between plasma phosphatidylcholine levels and cognitive flexibility (Zamroziewicz et al., 2016b). Chapter four demonstrates that these mediatory relationships are not limited to executive function by reporting a novel structural mediation between serum lutein and crystallized intelligence (Zamroziewicz et al., 2016a). These results demonstrate the importance of measuring domain-specific cognitive abilities as well as subtle variability in brain structure. The implementation of such techniques allowed for the detection of relationships between nutrition, cognition, and brain health that appear to be specific to particular domains of cognition and underlying brain structures.

Accumulating evidence indicates that the effects of nutrition on brain health are complex and multifactorial, reflecting the influence of particular nutrient combinations on specific aspects of brain aging. In light of this evidence, the last three empirical chapters examine the role of nutrient patterns in cognition and integrity of underlying structural and functional circuits across the brain. Chapter five demonstrates novel evidence for particular omega-3 polyunsaturated fatty acid patterns in supporting fluid intelligence and its underlying structural gray matter circuit (Zamroziewicz et al., 2017). Chapter six builds upon prior work to depict the relationship between patterns of omega-3 and omega-6 polyunsaturated fatty acids, memory, and underlying structural connectivity, as measured by white matter integrity (Zamroziewicz et al., 2018). Finally, chapter seven shows that patterns of monounsaturated fatty acids play a role in promoting general intelligence, and for the first time, that functional connectivity within a critical brain network may support the relationship between nutrition and cognition. These results demonstrate the predictive power of nutrient patterns, which capture the interactive effects and metabolic processing of constituent nutrients. These nutrient patterns were shown to influence cognitive domains highly sensitive to age- and disease-related decline, along with their underlying brain circuits.

Notably, this line of work addressed several limitations of previous research (Monti et al., 2014; Sheats et al., 2009). By studying healthy adults in whom nutrition is used to maintain health, rather than treat pathological disease, the limitation of improper sample selection was tackled. In lieu of insensitive nutritional measures, blood biomarkers were assessed to study the effects of single nutrients as well as nutrient patterns. By measuring specific domains of cognition using standardized neuropsychological tests, the insensitivity of coarse cognitive

assessments was avoided. Finally, insensitive measures of brain health were substituted with high-resolution MRI to study the brain structures and functions that underlie cognitive ability.

This research program also paves the way for many future lines of exploration. The results in this dissertation are all derived from one sample of 122 healthy adults aged 65 to 75 years. Future work would benefit from replicating these findings in other samples, such as those that vary in age, ethnicity, socioeconomic status, and geographic location. These findings rely upon measurement of nutrient and nutrient patterns within the blood, and thus do not speak to optimal intake of particular nutrients or dietary components. Additional research will need to determine sufficient and optimal intake of dietary components that support cognition and brain health. The cross-sectional nature of this project does not capture the nature of aging and the relationship between nutrition and decline in cognition and brain health. Thus, future longitudinal studies may serve this purpose. Further, the mediation approach applied in this cross-sectional study does not identify causal relationships between nutrition, cognition, and brain health. Follow-up intervention trials are needed to confirm whether particular nutrients or nutrient patterns cause change in cognition and brain health. In addition, investigating the relationship between nutrition, cognition, and brain health in a human model complicates the examination of underlying mechanisms. Further exploration of these mechanisms in animal models is warranted. Finally, this work is limited to a sample of healthy adults, and the investigation of the identified nutrition-cognition-brain health relationships in clinical populations could serve to determine the limits of nutritional interventions in disease states.

Nutritional cognitive neuroscience aims to integrate cutting-edge measures of nutritional intake, cognitive function, and brain health in an effort to demonstrate that cognition is not only dependent on brain structure and function, but also on nutritional status. Through this effort,

nutritional cognitive neuroscience can begin to bridge the gap between the largely disparate literatures of aging within the fields of nutritional epidemiology and cognitive neuroscience (Zamroziewicz and Barbey, 2016). In light of this goal, the work in this dissertation demonstrated that certain nutrients and nutrient patterns influence particular aspects of cognition by affecting specific features of brain health. The research presented here offers a novel perspective on brain health, and ultimately, aims to inform personalized and comprehensive nutritional interventions for the cognitive and neurological deficits associated with brain aging.

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APPENDIX A: IRB APPROVAL LETTER

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Office of the Vice Chancellor for Research
Institutional Review Board
528 East Green Street
Suite 203
Champaign, IL 61820

December 16, 2012

Aron Barbey
Beckman Institute
Suite 3009 Khan Annex
1206 South 4th Street

RE: *Nutritional Intake, Cognitive Function, and Measures of Brain Aging*
IRB Protocol Number: 12888

Dear Aron:

Your response to required modifications for the project entitled *Nutritional Intake, Cognitive Function, and Measures of Brain Aging* has satisfactorily addressed the concerns of the University of Illinois at Urbana-Champaign Institutional Review Board (IRB) and you are now free to proceed with the human subjects protocol. The UIUC IRB approved the protocol as described in your IRB-1 application with stipulated changes, as part of their monthly review. Certification of approval is available upon request. The expiration date for this protocol, UIUC number 12888, is 12/15/2013. The risk designation applied to your project is *No More than Minimal.*

Copies of the enclosed date-stamped consent form(s) must be used in obtaining informed consent. If there is a need to revise or alter the consent form(s), please submit the revised form(s) for IRB review, approval, and date-stamping prior to use.

Under applicable regulations, no changes to procedures involving human subjects may be made without prior IRB review and approval. The regulations also require that you promptly notify the IRB of any problems involving human subjects, including unanticipated side effects, adverse reactions, and any injuries or complications that arise during the project.

If you have any questions about the IRB process, or if you need assistance at any time, please feel free to contact me or the IRB Office, or visit our Web site at http://www.irb.illinois.edu.

Sincerely,

Anita Balgopal, Director, Institutional Review Board

Enclosure(s)

c: Gene Bowman
Neal Cohen
Kevin Osborne

telephone (217) 333-2670 • *fax* (217) 333-0405 • *email* IRB@illinois.edu