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# Associations of dietary choline, lutein and zeaxanthin with cognitive function in the Framingham Offspring Study

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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**ASSOCIATIONS OF DIETARY CHOLINE, LUTEIN AND ZEAXANTHIN WITH  
COGNITIVE FUNCTION IN THE FRAMINGHAM OFFSPRING STUDY**

by

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2025



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**ASSOCIATIONS OF DIETARY CHOLINE, LUTEIN AND ZEAXANTHIN WITH  
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**YIFEI CAI**

**ABSTRACT**

**Background:** With the rapid aging of populations worldwide, cognitive impairment—including mild cognitive impairment (MCI) and dementia—has emerged as a significant public health concern. Nutritional factors such as choline, lutein, and zeaxanthin have been suggested to play a role in cognitive aging, but their long-term effects remain underexplored.

**Objectives:** This study aimed to investigate the prospective associations between dietary intake of choline, lutein, and zeaxanthin and cognitive functioning, including measures of verbal and visual memory, verbal learning, attention and concentration, executive function, abstract reasoning, language, reading, visual-perceptual skills, and global cognitive function among women and men in the Framingham Offspring Study (FOS). In secondary analyses, we also evaluated prospective associations of dietary choline, lutein and zeaxanthin with repeated measures of cognitive function in the FOS. Lastly, we evaluated prospective associations of dietary choline, lutein and zeaxanthin with risk of incident dementia in the FOS.

**Methods:** In this prospective cohort study, I analyzed dietary intake data obtained from three-day diet records and neuropsychological test scores in the FOS. Multivariable analysis of covariance models was employed to evaluate the associations between categories of nutrient intake and cognitive performance while adjusting for confounders. The median score value within three categories of choline and lutein and zeaxanthin was used in linear regression models to test for linear trend. We also use linear mixed models to test repeated measures of cognitive outcomes associated with choline, lutein and zeaxanthin. Lastly, logistic regression models were used to estimate relative odds of developing dementia. All models were adjusted for age and sex. Potential confounders were evaluated based on their impact on the age- and sex- adjusted parameter estimates. We retained those variables in the model that changed the parameter estimates by approximately 10% or more.

**Results:** Higher dietary intake of choline, lutein, and zeaxanthin were associated with better overall cognitive performance using a global cognitive function score. In addition, choline, lutein and zeaxanthin were positively associated with better visual memory and abstract reasoning. Lutein and zeaxanthin intake were more modestly associated with better attention and concentration, language, visuo-perceptual organization and premorbid educational achievement. Participants with moderate or higher intakes of choline, lutein and zeaxanthin (vs. lower) retained better global cognitive function over time. Lastly, higher dietary (vs. lower) choline intake was associated with lower odds of incident dementia.

**Conclusion:** The findings support the hypothesis that higher dietary intake of choline, lutein, and zeaxanthin may contribute to better cognitive function in aging populations. These results highlight the importance of dietary interventions in mitigating cognitive decline and suggest the need for further randomized trials to confirm these associations.

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## LIST OF ABBREVIATIONS

BU.....	Boston University
AD .....	Alzheimer’s Dementia
AI .....	Adequate Intake
APOE.....	Encoding Apolipoprotein E
BNT.....	Boston Naming Test
cm.....	Centimeters
DHA.....	Docosahexaenoic Acid
FFQ .....	Food Frequency Questionnaire
FHS.....	Framingham Heart Study
FOS .....	Framingham Offspring Study
HDL .....	High-Density Lipoprotein Cholesterol
HEI .....	Healthy Eating Index
HVOT .....	Hooper Visual Organization Test
JNC-7 .....	Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
Kg.....	Kilograms
kg/m <sup>2</sup> .....	Kilograms per Meter Squared
LDL.....	Low-Density Lipoprotein Cholesterol
LMD.....	Logical Memory–Delayed Recall
LMI.....	Logical Memory–Immediate Recall
LMR.....	Logical Memory–Recognition Recall

MCI.....	Mild Cognitive Impairment
Mod.....	Moderate
N .....	Sample Size
NDSR.....	Nutrition Data System for Research
NP.....	Neuropsychological
OR.....	Odds Ratio
PA.....	Physical Activity
PAI.....	Physical Activity Index
PAL.....	Physical Activity Level
PASD.....	Paired Associates–Delayed Recall
PASI.....	Paired Associates–Immediate Recall
SIM.....	Wechsler Memory Scale Similarities Subtest
TEE.....	Total Energy Expenditure
TG.....	Triglycerides
TrA.....	Trail-Making Test Part A
TrB.....	Trail-Making Test Part B
TrBA.....	Trail-Making Test B–A Difference
UL.....	Upper Limit
VRD.....	Visual Memory–Delayed Recall
VRI.....	Visual Memory–Immediate Recall
WHO.....	World Health Organization
WHtR.....	Waist-to-Height Ratio

WRAT..... Wide Range Achievement Test (Reading Subtest)

## INTRODUCTION

### **Spectrum of cognitive deterioration**

With rapid aging of populations worldwide, cognitive impairment, especially Mild Cognitive Impairment (MCI) and dementia, have emerged as major public health<sup>1</sup>. In the United States, Alzheimer's dementia (AD) affects around 6.5 million Americans aged 65 and older. Globally, based on the World Health Organization (WHO, 2021)<sup>2</sup> by the year 2050, dementia is projected to impact approximately 152 million individuals worldwide, creating significant socioeconomic and healthcare challenges. In addition to the high prevalence and public health impact of dementia, approximately 12% to 18% of people age 60 or older are living with MCI<sup>3</sup>. Combined, these conditions represent a growing public health concern as the U.S. population continues to age.

### **Types of cognitive decline**

Aging-related cognitive decline, MCI, dementia, and AD are interconnected in a continuum of cognitive health. While aging-related cognitive decline represents the baseline changes expected with aging<sup>4</sup>, MCI is a preclinical, transitional stage between healthy aging and dementia<sup>5</sup>. Dementia signifies a later stage of cognitive decline, with AD being the most common type.

Some cognitive functions, such as certain numerical skills and general knowledge, experience little decline with age. However, other mental abilities begin to deteriorate from middle age or even earlier<sup>6</sup>. These include memory, executive functions, processing speed, and reasoning—abilities collectively referred to as 'fluid' cognitive skills. Fluid

intelligence refers to the capacity for abstract thinking, rapid reasoning, and problem-solving without relying on prior knowledge<sup>7</sup>. These fluid abilities are crucial for performing daily tasks, maintaining independence, and leading a fulfilling life.

### *Mild cognitive impairment*

The National Institute on Aging describes MCI as a condition in which individuals experience greater memory or thinking problems than is typical for their age, but these issues do not substantially interfere with daily life. Symptoms may include losing things often, missing appointments, or struggling to recall words. The National Institute on Aging emphasizes that MCI can increase the risk of developing AD or other forms of dementia, though it does not always lead to these conditions<sup>8</sup>. About 15% of MCI cases may proceed to dementia after two years<sup>9</sup>, while one-third of cases proceed to dementia after five years<sup>10</sup>. As a result, not all individuals with MCI develop dementia; some may even regain normal cognitive function. For example, one study found that 15% of MCI patients recovered normal cognition after a follow-up of 11 years<sup>11</sup>.

### *Dementia*

Dementia is a neurological disorder that leads to deterioration across various cognitive domains, accompanied by functional impairments. Over time, individuals affected by dementia gradually lose their independence and become unable to conduct routine activities of daily living. The WHO describes dementia as a syndrome marked by cognitive decline exceeding the typical effects of aging. This decline affects memory,

reasoning, orientation, comprehension, calculation, learning ability, language, and judgment, while consciousness remains unaffected<sup>12</sup>.

Adopting a healthy lifestyle can help slow the progression of MCI to dementia. Aerobic exercise is particularly beneficial, as it enhances neuroplasticity and improves vascular health, thereby supporting cognitive function<sup>13</sup>. Consuming a nutrient-rich diet, for example, Mediterranean or DASH diet, which emphasizes whole grains, fruits, vegetables, and healthy fats, is also linked to a reduced rate of cognitive decline<sup>14,15</sup>. Additionally, engaging in intellectually stimulating pursuits like solving puzzles, reading, or acquiring new skills fosters cognitive resilience<sup>16</sup>, a concept that refers to the brain's ability to resist the adverse effects of cognitive decline<sup>17</sup>. Those individuals with higher cognitive resilience may not progress to dementia despite the presence of cognitive impairment. Social interaction contributes significantly to cognitive resilience, as active participation in community activities promotes emotional stability and mental well-being, highlighting the significance of comprehensive lifestyle changes in preserving cognitive health<sup>18,19</sup>. Nutrition has also been shown to benefit cognition and it is possible that it may play a role in promoting resilience<sup>20</sup>.

#### *Alzheimer's disease (AD)*

AD is the most common form of dementia linked to aging and is classified as a degenerative disorder<sup>21</sup>. The key pathological features of AD involve the accumulation of amyloid in certain regions of the brain, forming amyloid plaques, along with the presence of extracellular amyloid plaques and intracellular misfolded tau proteins, which lead to

the formation of neurofibrillary tangles<sup>22,23</sup>. As the lesions expand to other parts of the brain, the symptoms progress beyond simple forgetfulness to impair additional cognitive domains.

The National Institute on Aging and the Alzheimer's Association in 2024 defined the condition through its biological markers, emphasizing the use of blood-based biomarkers, imaging techniques like PET scans, and cerebrospinal fluid analysis to identify amyloid beta and tau proteins. The framework adopts a comprehensive staging system that combines biomarker findings with clinical evaluations and accounts for factors such as comorbidities, cognitive resilience, and individual variability, offering a thorough approach to diagnosing and managing AD<sup>24</sup>.

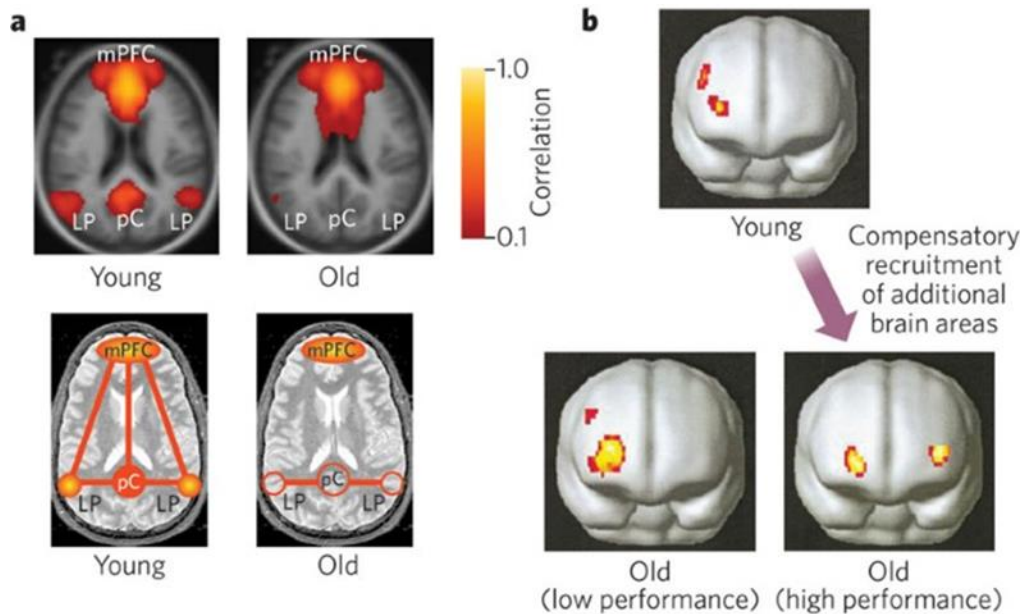
### **Aging-related cognitive function decline**

#### *Aging process in the human brain*

In humans, the aging process is characterized by distinct structural and neurophysiological modifications in the brain, coupled with differing degrees of cognitive decline<sup>25</sup>. Advances in functional brain imaging have offered a detailed perspective on neural activity and its transformation over time (Figure1). These studies have demonstrated that the interaction between distinct brain regions involved in higher-order cognitive functions becomes less coordinated as people age, leading to a global decline in integrative functioning<sup>26</sup>. Notably, this decreased synchronization of brain activity is linked to poorer performance across multiple cognitive domains<sup>26</sup>. Moreover,

neural activity in certain brain areas, particularly the prefrontal cortex, becomes less localized in older adults when engaging in tasks that require executive functioning<sup>6,27</sup>. In contrast, younger adults tend to engage more specific brain parts for the identical tasks and show stronger integration between these regions. Interestingly, older adults who exhibit more diffuse brain activity tend to perform better cognitively compared to those with more localized activity, suggesting that this delocalization may serve as a compensatory mechanism<sup>28</sup>. These findings indicate that the brain's systems-level functioning undergoes significant changes during normal aging, even without disease.

The deterioration of higher-order brain systems with age may be partially attributed to the breakdown of myelinated fibers, which play a crucial role in enabling communication between neurons across various cortical regions<sup>26</sup>. While neuronal loss remains minimal in most cortical regions of a typically aging brain<sup>29</sup>, alterations in synaptic physiology may contribute to impaired connectivity and reduced integrative function. Research on the prefrontal cortex of both humans and rhesus macaques shows a marked age-related downregulation of genes involved in GABA ( $\gamma$ -aminobutyric acid)-mediated inhibitory neurotransmission<sup>30</sup>. This alteration could disrupt the equilibrium between inhibitory and excitatory signals, potentially leading to heightened neural activity in the prefrontal cortex of older individuals<sup>30</sup>. Although this increase in activity may initially serve as a compensatory mechanism, it could also predispose individuals to excitotoxicity and neurodegenerative diseases over time<sup>4</sup>.



**Figure1:** Altered functional activation of brain systems during brain ageing<sup>26,28</sup>.  
 a Top: Functional magnetic resonance imaging (fMRI) scans illustrate that in young adults, the medial prefrontal cortex (mPFC), posterior cingulate (pC), and lateral parietal cortex (LP) exhibit synchronized activation. However, this temporal coordination is significantly diminished in older individuals<sup>26</sup>. Bottom: Theoretical models suggest that strong connectivity between the mPFC, pC, and LP facilitates coordinated activation in younger brains. In contrast, reduced connectivity between these regions may contribute to the disrupted coordination observed in aging brains. (Images courtesy of C. Koch, California Institute of Technology, Pasadena.) b, Positron emission tomography (PET) scans indicate that younger individuals performing a memory task predominantly show right-lateralized activation in the prefrontal cortex (PFC). Among older adults, those with lower task performance also exhibit right-lateralized activation. However, older individuals with better performance display bilateral activation, suggesting that recruiting additional brain regions may serve as a compensatory mechanism for age-related functional decline in primary cognitive processing areas<sup>28</sup>. (Figure and legend adapted from Roberto, 2002 and Andrews, 2007).

### *Genetic contributions to cognitive aging*

Research on heritability, based on twin studies and data from families with adopted children, suggests that approximately 50% of general cognitive ability is influenced by genetic factors. This heritability appears to increase from childhood through adulthood

and continues into old age. Evidence suggests that genetics contribute not only to overall intelligence across the lifespan but also to cognitive decline associated with aging<sup>31,32</sup>.

Research on candidate genes has explored the links between specific genetic variations and cognitive decline with aging. These genes include those previously linked to cognitive function and longevity<sup>32</sup>. Although numerous associations between candidate genes and cognitive aging have been identified, the gene encoding apolipoprotein E (APOE) stands out as one of the few consistently replicated across multiple studies<sup>33</sup>.

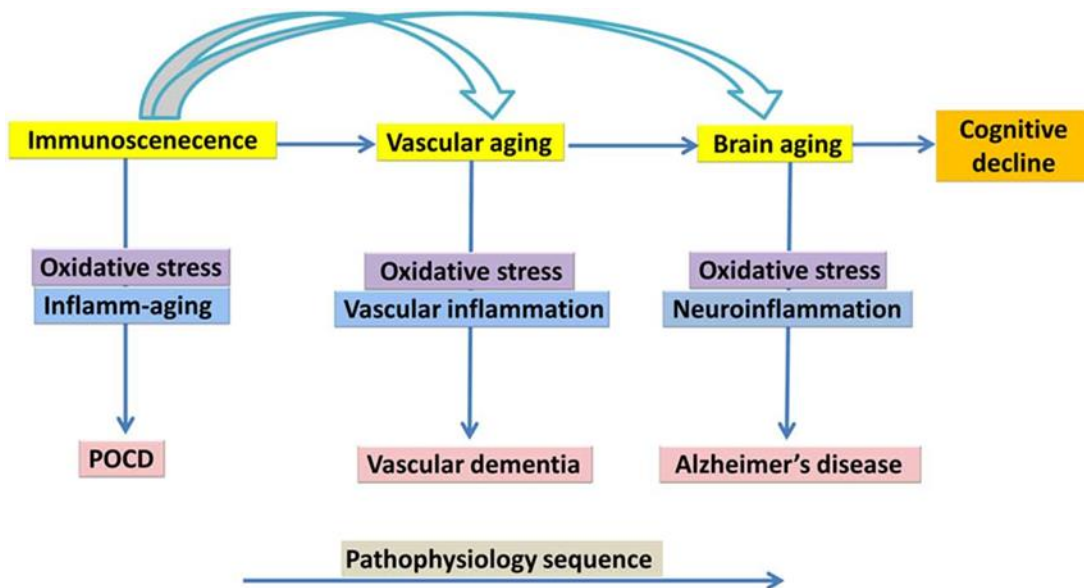
Among individuals experiencing normal cognitive aging, those who carry the E4 allele of the APOE gene tend to exhibit slightly worse performance in overall cognitive ability<sup>34</sup>.

#### *Cardiovascular disease and cognitive decline*

The brain and circulatory system share a delicate relationship that, when disrupted by vascular disease, can impair normal brain function<sup>35</sup>. Various neurological symptoms can arise from either temporary or permanent interruptions in blood flow to the brain. After an acute ischemic stroke, often resulting from large-artery atherosclerosis, long-term sensorimotor and behavioral disabilities are commonly observed<sup>36</sup>. A single large brain infarct, or multiple infarcts, increases the likelihood of cognitive decline, a condition known as multi-infarct dementia. Even without developing dementia, stroke survivors typically perform worse on cognitive tests compared to healthy individuals<sup>37</sup>.

### *Inflammation and cognitive aging*

Neuroinflammation refers to a multifaceted series of cellular and molecular events within the brain, where immune cells play a pivotal role in both its initiation and progression<sup>38,39,40</sup>. Cognitive aging is a complex process, involving several key biological mechanisms. In this context, Tangestani Fard et al. have identified three primary processes that potentially elucidate cognitive aging: immunosenescence, vascular aging, and brain aging<sup>41</sup>. This model highlights how these processes share common pathological features (Figure 2)<sup>41</sup>.



**Figure 2:** Immunosenescence, vascular aging, brain aging in association with cognitive decline, a suggested model of underlying mechanism. (Figure from Tangestani, 2019)<sup>41</sup>.

### *Pharmacotherapy*

There are limited pharmacological options available for reducing amyloid levels and alleviating symptoms in individuals with dementia. For example, Aducanumab has been shown to reduce amyloid plaques and slightly improve symptoms, while Donepezil is used to treat memory and cognitive difficulties in moderate-to-severe dementia<sup>42,43</sup>. However, its clinical benefits remain a topic of debate. Notably, aducanumab has been associated with significant adverse effects, including amyloid-related imaging abnormalities, which encompass brain swelling and microhemorrhages. Patients may also experience headaches and an increased risk of falls<sup>44</sup>. Given these limitations, there has been a growing interest in alternative strategies, particularly lifestyle changes<sup>45,46</sup>. Among these, dietary supplements have gained considerable attention for their potential to not only slow the progression from MCI to dementia but also delay the onset of Alzheimer's disease<sup>47</sup>.

### *Modifiable risk factors for cognitive decline*

The influence of diet and lifestyle factors on brain aging has become an area of increasing interest within scientific research. According to the World Dementia Council, there is enough data to suggest a relationship between many modifiable risk factors and a lower chance of cognitive decline<sup>48</sup>. Specifically, the influence of diet and lifestyle factors on brain aging has become an area of increasing interest within scientific research. Emerging evidence indicates that dietary improvements in older adults may contribute to delaying the onset or mitigating the progression of cognitive decline associated with

aging. For example, a calorie-restricted diet for three months was found to significantly improve memory performance in a prospective interventional study of healthy, normal-to-overweight senior participants<sup>49</sup>. This enhancement in memory may be attributed to increased insulin sensitivity resulting from calorie restriction, which in turn enhances insulin signaling in the brain<sup>50</sup>.

Additionally, current investigations primarily emphasize the impact of specific nutrients and overall dietary patterns in this context. Several studies highlight the significance of a diet rich in B-vitamins, antioxidants, and omega-3 fatty acids in maintaining cognitive health. B-vitamins, especially B12, B6, and folate (B9), are necessary for maintaining normal brain functions and memory. Epidemiological evidence suggests that these vitamins have a protective effect on cognitive performance<sup>51</sup>. Cross-sectional studies have provided evidence of a positive relationship between antioxidant levels and cognitive performance in older adults<sup>52</sup>. Beyond antioxidant vitamins, certain fruits and vegetables, such as berries, are rich in plant polyphenols—bioactive compounds believed to have positive impacts by changing neural transmission and stress signaling, thereby enhancing their functional capacity during the aging process<sup>53,54</sup>. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA), are abundant in the brain. Regular consumption of oily fish rich in omega-3s in older adults has been linked with a reduced risk of cognitive impairment and dementia<sup>55</sup>.

The complexity of nutrient interactions must be acknowledged, as dietary intake is influenced by both macronutrients and micronutrients<sup>34</sup>. Consequently, some research has

shifted toward examining the impact of overall dietary patterns. The Mediterranean diet is distinguished by a high intake of unrefined cereals, fruits, vegetables, legumes, and olive oil, moderate consumption of dairy products and alcohol, and a low intake of meat<sup>56</sup>. Following a Mediterranean-style diet has been linked to a lower risk of developing various chronic diseases<sup>57</sup>. Its protective effects are thought to arise from the combined benefits of monounsaturated fatty acids and polyphenols in olive oil, polyunsaturated fatty acids from fish, and antioxidants from fruits, vegetables, and wine<sup>58</sup>. In contrast, diets high in added-sugar, cholesterol, and trans fats have been associated with worse cognitive performances in aging populations<sup>59</sup>.

In addition to diet, other lifestyle factors such as smoking and alcohol consumption play a role in cognitive aging<sup>59,60</sup>. Evidence increasingly suggests that light-to-moderate alcohol intake in adults is associated with better cognitive outcome compared to both abstinence and heavy drinking, and may provide a protective effect against cognitive decline<sup>61</sup>. Conversely, smoking is a major risk factor for cognitive impairment, primarily through its harmful impact on vascular health. A dose-response relationship has been observed between the amount of cigarettes smoked through a lifetime and the degree of cognitive decline<sup>62</sup>.

### **Choline, lutein and zeaxanthin**

Among dietary factors, another area of interest relates to egg consumption and its possible influence on cognitive outcomes among adults. Several studies found that eating more eggs was related with improved performance on some cognitive tests and a lower

risk of dementia<sup>63-65</sup>. Eggs are the main source of choline, lutein and zeaxanthin, all of which have been linked with alterations in human cognitive performance<sup>66-68</sup>. As the role of these nutrients in cognitive function became evident, researchers began focusing on dietary sources of these compounds, prompting investigations into the impact of egg consumption on cognitive health. This growing body of evidence underscores the significance of further exploring the association between egg intake and cognitive outcomes, with focuses on choline, lutein and zeaxanthin, which are the primary nutrients of interest in this study.

#### *Recommended intake values for choline, lutein and zeaxanthin*

In 1998, the U.S. Institute of Medicine's Food and Nutrition Board set both an Adequate Intake (AI) and a tolerable Upper Limit (UL) for choline . The recommended AI is 425 mg per day for women and 550 mg per day for men, with higher amounts suggested for pregnant and breastfeeding women. For infants, the AI is based on estimated choline intake from breast milk. While there are no official recommended dietary intake levels for lutein and zeaxanthin<sup>70</sup>, a study published in 1994 proposed an intake of 6 mg per day as a potential dietary target to help lower the risk of age-related macular degeneration<sup>71</sup>.

#### *Sources of choline, lutein, and zeaxanthin*

Choline and its esters are prevalent across various food sources, though animal-based products generally provide higher concentrations per unit weight compared to plant-based foods. Excellent dietary sources of choline include eggs, meat, fish, and milk, alongside

certain plant foods like cruciferous vegetables and some legumes, all of which offer at least 10% of the daily recommended intake per serving<sup>72</sup>. Dietary choline intake varies significantly, with national surveys indicating that only 11% of U.S. adults meet the AI level for choline<sup>73</sup>. Additionally, foods often contain the choline metabolite betaine<sup>72</sup>, while not convertible into choline, functions as a methyl donor and can reduce the body's need for choline<sup>72</sup>. Plant-based foods, particularly grains, are rich in betaine. Furthermore, many processed foods include lecithin (phosphatidylcholine), which boosts overall choline consumption<sup>74</sup>. However, most commercially available multivitamin supplements, including those for prenatal use, either lack choline or include it in minimal amounts (25–50 mg)<sup>74</sup>.

Lutein and zeaxanthin are the most abundant xanthophylls found in green leafy vegetables such as kale, spinach, and broccoli, as well as in egg yolks<sup>75</sup>. The lutein-to-zeaxanthin ratio in green vegetables typically ranges from 12 to 63, with kale having the highest ratio, while in yellow-orange fruits and vegetables, this ratio ranges between 0.1 and 1.4<sup>76</sup>. Egg yolks are a superior source of these nutrients compared to fruits and vegetables due to their enhanced bioavailability, attributed to the digestible lipid matrix of the yolk<sup>77</sup>. Egg yolks contain approximately  $292 \pm 117 \mu\text{g}$  of lutein and  $213 \pm 85 \mu\text{g}$  of zeaxanthin in an average yolk weighing 17–19 g<sup>78</sup>. These levels are likely influenced by the chicken's feed and are mainly present in non-esterified form<sup>78</sup>.

*Choline, lutein and zeaxanthin and cognitive function decline*

Accumulating evidence shows that oxidative and inflammatory damage contribute to the progress of AD and age-related cognitive decline<sup>79,80</sup>. In our knowledge, if increases in sensitivity to oxidative stress and inflammation in the aging brain cause cognitive deficits, interventions with antioxidants and anti-inflammatory agents may delay the extent of oxidative damage to neural tissues, potentially slowing cognitive decline and the development of neurologic diseases<sup>68</sup>.

Lutein and zeaxanthin are among the most abundant carotenoids in human brain tissue<sup>81</sup>. Previous studies demonstrate that the xanthophyll carotenoids act as both antioxidants and anti-inflammatory agents<sup>82</sup>, consuming these nutrients may benefit cognitive health. One double-blind, randomized, placebo-controlled trial conducted by Hammona et al.<sup>83</sup> concludes that older adults receiving the active lutein and zeaxanthin supplement had statistically significant improvements in attention and cognitive flexibility domains ( $p < 0.05$ ), relative to participants taking the placebo. Although the molecular basis of lutein and zeaxanthin's neuroprotective effects is unknown, several mechanisms have been discussed, including reduced oxidative stress, activation of anti-inflammatory pathways<sup>84,85</sup>, and modulation of synaptic membrane functional properties, as well as changes in their structural features<sup>86</sup>.

Additionally, choline is a vital nutrient affects brain functions in early life. However, there is limited data supporting its possible neuroprotective effects in later life<sup>66</sup>. Some investigators offer a prospective analysis in middle- and older-aged men with a follow-up

of more than two decades, demonstrating that dietary intake of choline was related with improved function in cognitive tests assessing verbal fluency and memory<sup>87</sup>. One proposed explanation by Blusztajn et al. for the effect of contemporaneous choline intake on adult cognition is that it acts as a precursor to the phospholipid phosphatidylcholine, which is a major component of all biological membranes, including those in neurons cells<sup>88</sup>. However, another more recent study in a sample of older adults 60 years + from the National Health and Nutrition Examination Survey shows neither the total choline intake nor the average daily dietary intake was associated with changes in cognitive test scores<sup>66</sup>.

Data from the Framingham Offspring Cohort (FOS) in 2011 revealed positive cross-sectional associations between dietary choline intake and both visual and verbal memory, as assessed through Food Frequency Questionnaire (FFQ). In this study, We proposed to use food records since food records often provide more accurate nutrient intake data<sup>89</sup> and take advantages of the repeated measures of cognitive function over the follow-up of FOS. Also, in our prospective study, researchers were allowed to observe the temporal sequence between exposure (e.g., nutrient intake) and outcome (e.g., cognitive decline).

### **Purpose and aims of this study**

Since aging-related cognitive decline has been linked with oxidative stress and inflammation, it is possible that dietary choline, lutein, and zeaxanthin may benefit cognitive function during the aging process. Overall, given the gaps in evidence and inconsistent results from previous studies, here, we aimed to prospectively evaluate

associations between intakes of dietary choline as well as lutein and zeaxanthin and cognitive function, including measures of verbal and visual memory, verbal learning, attention and concentration, executive function, abstract reasoning, language, reading, visual-perceptual skills and global cognitive function among women and men in the FOS.

## **METHODS**

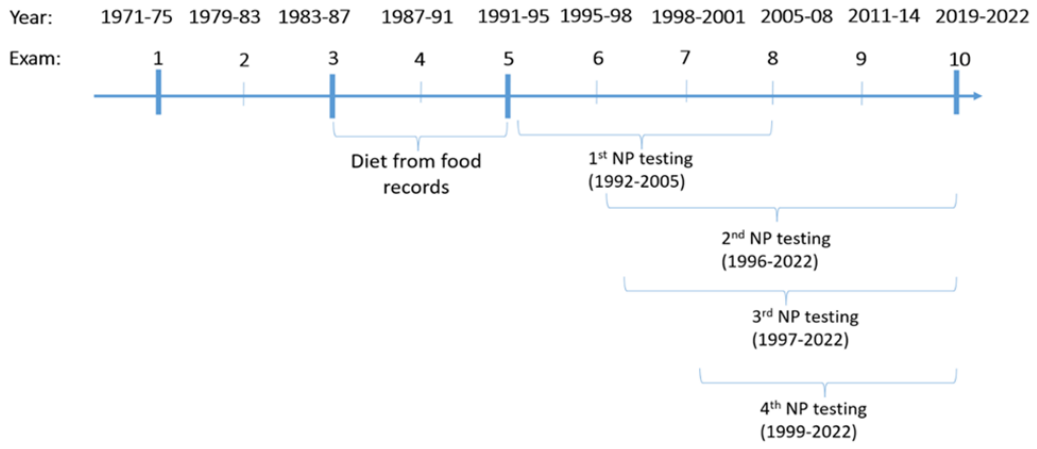
### **Study population and timeframe**

The Framingham Offspring Cohort, established in 1971 with 5,124 participants, has been monitored for over 40 years. Participants attended clinic visits every four years, where data on demographics, lifestyle, physical measurements, blood pressure, and medical history updates were collected. Dietary data was recorded using three-day diet record during exams 3 and 5, setting exam 5 as the baseline for dietary analyses.

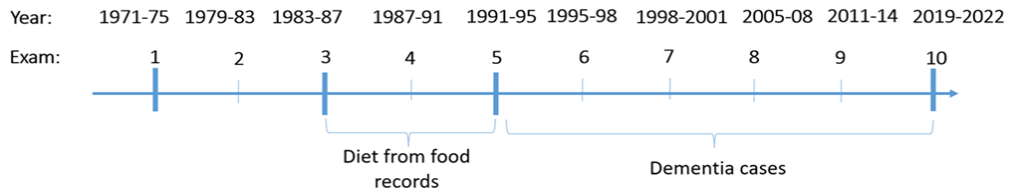
Neuropsychological (NP) testing was introduced in exam 5, with most participants completing their initial tests by exam 7. Testing intervals were generally 4-6 years but individuals with potential cognitive impairment were tested every 1-2 years.

As shown in Figure 3 the NP testing and the cohort examination cycles were not conducted concurrently, therefore, we included individuals who had their first NP testing within 2 SD (1 SD=8.34) years away from the time of the final dietary assessment at exam 5. In this study, we excluded participants with dietary data or extremes of energy intake (<1,000 kcals/day or >3,500 kcals/day for females; <1,200 kcals/day or >4,000 kcals/day for males) as has been previously done in this cohort. We excluded those who

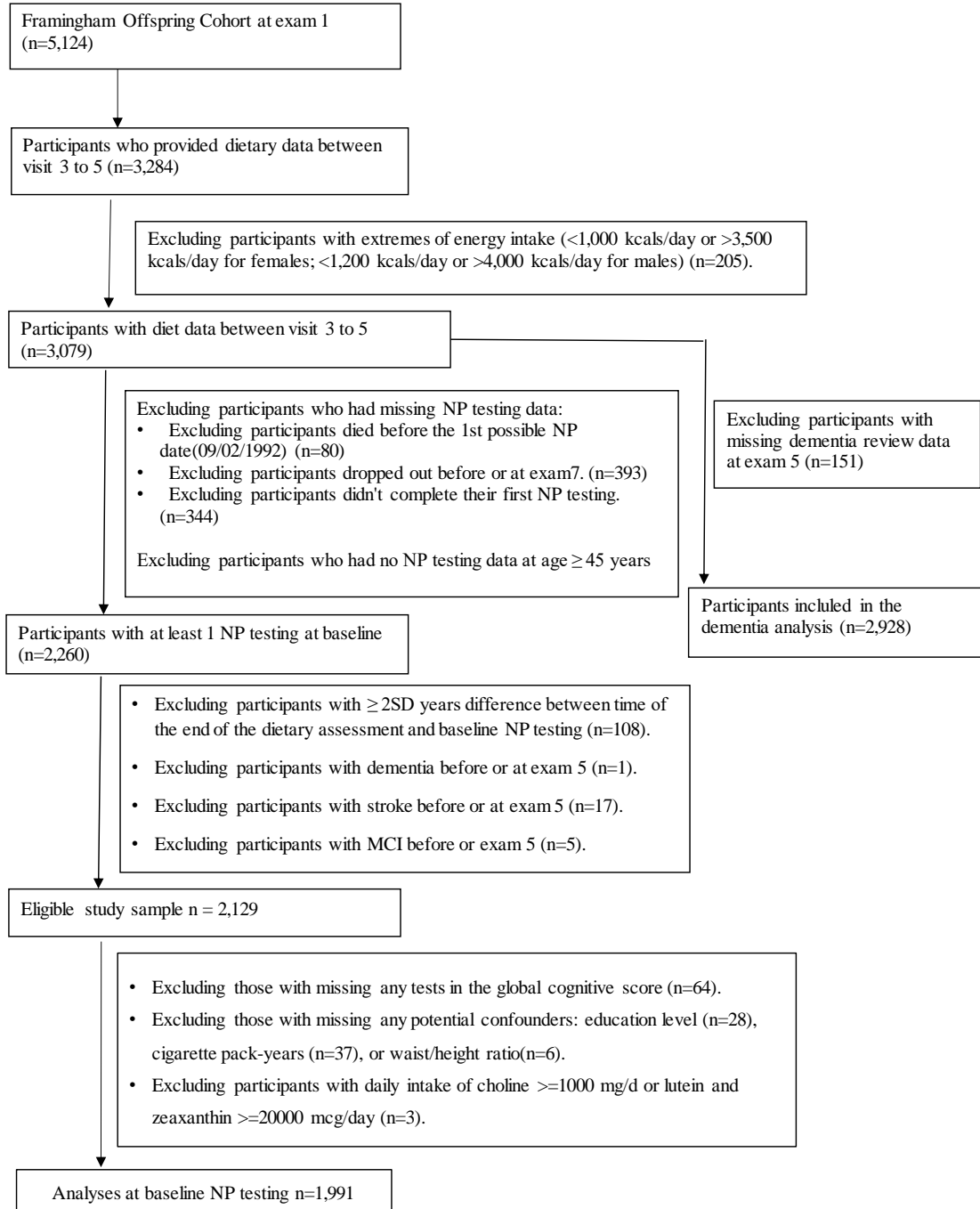
had missing NP testing data, which included participants who died before the first NP testing date (n=80), those who dropped out at or before exam 7 (n=393), and those who did not complete any NP testing (n=344). We also excluded 2 persons who had no NP test data at  $\geq 45$  years of age. Then we excluded those participants with prevalent dementia (n=1), stroke (n=17), or mild cognitive impairment (n=5) before or at exam 5. Dementia was adjudicated throughout the follow-up of the FOS as shown in Figure 4. The criteria used in FHS to diagnose dementia were comparable to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition<sup>90</sup>. Lastly, we also excluded those with missing any tests in the global cognitive score (n=64), those with missing any potential confounders: education level (n=28), cigarette pack-years (n=37), waist/height ratio (n=6), and those with daily intake of choline  $\geq 1000$  mg/d or lutein and zeaxanthin  $\geq 20,000$  mcg/day (n=3). After all exclusions, a total of 1,991 participants remained eligible in this study. Additionally, for dementia analyses, we excluded individuals who had missed dementia review data, yielding a sample size of 2,928. Details and sample sizes for individual exclusion criteria are shown in Figure 5.



**Figure 3.** Timeframe of diet and cognition assessment in Offspring Cohort.



**Figure 4.** Timeframe of diet and dementia assessment in the Offspring Cohort.



**Figure 5:** Flowchart of study participants for the analyses related the NP testing in the Offspring Cohort.

Abbreviations: n, sample size; NP, Neuropsychology; MCI, Moderate Cognitive Impairment"

### **Dietary exposure**

In the FOS, diet was assessed using three-day diet records, collected during the exam 3 and exam 5 cycles. Approximately 70% of the participants were compliant with the dietary data collection, thereby yielding approximately 16,000 days of diet records that were entered into the Nutrition Data System for Research (NDSR) to calculate individual's daily intake of nutrients. Food intake in each of the USDA (United States Department of Agriculture) food groups and subgroups were calculated by the applicants (since the NDSR software did not provide any food group data at that time). In addition, at the time the dietary records were collected in the FOS, the NDSR did not include data on dietary choline or lutein and zeaxanthin. Therefore, the estimation of daily intakes for dietary choline (mg), lutein (mcg), and zeaxanthin (mcg) involved matching food and ingredient codes obtained from their corresponding USDA food codes. This approach facilitates precise calculation of intakes of dietary choline, lutein and zeaxanthin per day.

### **Neuropsychological (NP) testing**

The neuropsychological test battery used in Framingham is shown in table 1. In this way, cognitive performance was evaluated using a formal NP test battery by a trained technician starting in 1999. The test battery consisted of several individual NP tests that were combined to determine different cognitive domains, such as memory (verbal and visual), attention and concentration, abstract reasoning, language, reading, visual-perceptual skills, and executive function. Verbal memory (i.e., Logical Memory-Immediate Recall, LMI and Logical Memory-Delayed Recall, LMD) was assessed by

asking the participant to recall the details of a story presented to them (immediately and after a delay) as part of the Wechsler Memory Scale, Logical Memory Test. Visual memory was assessed by asking the participant to look at a geometric shape and then draw it from memory. Once again, there were two components (i.e., Visual Memory-Immediate Recall, VRI, and Visual Memory-Delayed Recall, VRD] as part of the Wechsler Memory Scale-Visual Reproduction Test. A common test of verbal memory was used in Framingham — the Wechsler Memory Scale-Paired Associates test. In this, the individual is asked to recall pairs of words that had been presented to them [Paired Associates—Immediate Recall, PASI, and Paired Associates—Delayed Recall, PASD]. To assess cognitive performance on attention and concentration, we used the Trail-making test (parts A [TrA] and B [TrB]). For this test, participants were asked to connect a series of numbered circles in order (part A) and then to alternate between numbers and letters in sequence (part B) as quickly as possible. For the assessment of abstract reasoning, Framingham investigators used the Wechsler Memory Scale Similarities subtest [SIM]. In this test, participants are given pairs of words and asked to identify the similarity between them. The Boston Naming Test [BNT] was used to measure language abilities, in which an individual's ability to retrieve and articulate the names of objects was assessed. To test visual-perceptual organization skills (which relate to the ability to organize visual stimuli), the Hooper Visual Organization Test [HVOT] was used. In this, the individual is required to evaluate fragments of an image (some of which may be rotated) and put them together to identify the object in the picture. Lastly, to assess premorbid educational achievement, the Wide Range Achievement Test (reading subtest)

[WRAT] was used. In this, participants were asked to read aloud a list of words of increasing difficulty.

Higher scores on all domains indicate superior performance, apart from the Trail-making Test, where higher scores indicate slower task completion. To normalize skewed distributions, performance on the Trail-making Test (A and B), Wechsler Memory Scale Similarities subtest, Boston naming test, Hooper Visual Organization Test and Wide Range Achievement Test were normalized using natural logarithms. The directionality of Trail-making Test scores was reversed, so that higher scores on all tests indicated better performance. The Trail-making Test B-A difference [TrBA] score was calculated to isolate the executive functions required to complete Trail-making Test B. This score is derived by subtracting the time taken for Trail Making Test A, which primarily assesses simple sequencing and psychomotor speed, from the time taken for Trail-making Test B, which includes additional demands on executive abilities such as cognitive flexibility and task switching. By removing the shared components of sequencing and psychomotor demands, the difference score provides a more focused measure of the executive processes unique to Trail-making Test<sup>91</sup>. To address any missing or extreme NP subtest score at baseline, we substitute it with its first possible follow-up score, at the same time we follow the principle that individuals who had their first possible NP test score within 2 SD (1 SD=8.34) years away from the time of the final dietary assessment at exam 5 can be substituted.

**Table 1.** Neuropsychological test battery

Cognitive domain	Description	Primary cognitive domain	NP test	Score range (min-max)	Higher score indicates
Verbal memory	Participants are presented with a story and are immediately asked to recall as many details as possible and then asked to recall again after a time delay.	This test assesses verbal episodic memory, language processing, and narrative recall, relying on the left-hemisphere brain regions, particularly the hippocampus and temporal lobes.	WMS-III logical memory-immediate recall	0-23	Better performance
			WMS-III logical memory-delayed recall	0-23	Better performance
			WMS-III logical memory-recognition recall	0-11	Better performance
Visual memory	Participants are shown a drawing for 10s and are then asked to draw the items and then asked to draw them again after a time delay.	This test assesses visual-spatial memory, perceptual organization, and constructional ability, relying on right-hemisphere brain regions, particularly the occipital and parietal lobes.	WMS-III visual reproductions-immediate recall	0-14	Better performance
			WMS-III visual reproductions-delayed recall	0-14	Better performance
			WMS-III visual memory-recognition recall	0-4	Better performance
Verbal learning	Participants are presented with a list of words and asked to recall the word pairs and then asked to recall again after a time delay	This test assesses verbal associative memory, learning efficiency, and retrieval strategies, relying on the hippocampus, medial temporal lobes, and prefrontal cortex.	WMS-III paired associates-immediate recall	0-21	Better performance
			WMS-III paired associates-delayed recall	0-10	Better performance
Attention and concentration	Participants are asked to connect a series of numbered circles in order (Part A) and alternate between numbers and letters in sequence (Part B) as quickly as possible	Tests relies on the prefrontal cortex, parietal lobes, and motor pathways and is sensitive to executive dysfunction and cognitive decline.	Trail-making test A	0.10-7.0	Poorer performance
			Trail-making test B	0.32-10.0	Poorer performance
Executive function	/	This test reflects a purer measure of the executive abilities required to complete the Trail Making Test B by subtracting the simple sequencing and psychomotor demands common to both Trail Making Test A and B	Trail-making B – Trail-making A	/	Poorer performance
Abstract reasoning	Participants are given pairs of words and are asked to identify the similarity between them	This test assesses abstract reasoning, semantic memory, and verbal intelligence, relying on the prefrontal cortex and left temporal lobe.	WAIS-III similarities subset	0-26	Better performance
Language	Participants are asked to name a series of line-drawn images of objects	The test assesses language processing, lexical access, and semantic memory, relying on the left temporal lobe, particularly the inferior temporal gyrus.	Boston naming test	0-30	Better performance
Visual-perceptual organization	Participants are shown fragmented line drawings of objects and are asked to identify what each object is	This test measures visual-spatial processing, perceptual integration, and object recognition, relying on the right parietal and occipital lobes.	Hooper visual organization test	0-30	Better performance
Premorbid educational achievement	Participants are asked to read aloud a list of words of increasing difficulty	The WRAT Reading subtest measures basic reading ability, word recognition, and phonological processing, relying on the left temporal and parietal lobes.	Wide Range Achievement Test-reading (WRAT)	15-57	Better performance

Abbreviations : NP, Neuropsychology.

**Table 2.** Description of neuropsychological tests

Cognitive domain	NP test measure	Raw NP test	Raw NP test mean (min, max)	Transformed code manipulation	Transformed NP test mean (min, max)	Z_score (min, max)
<u>Verbal memory</u>	WMS-III logical memory-immediate recall	LMI	11.27 (0, 22)	\	\	-0.081 (-3.387, 3.065)
	WMS-III logical memory-delayed recall	LMD	10.34 (0, 22)	\	\	-0.085 (-3.023, 3.227)
	WMS-III logical memory-recognition recall	LMR	9.45 (3, 11)	\	\	-0.061 (-5.344, 1.213)
	Average Z_score of verbal memory	LM	\	\	\	-0.076 (-3.820, 2.502)
<u>Visual memory</u>	WMS-III visual reproductions-immediate recall	VRI	8.86 (0, 14)	\	\	-0.113 (-2.952, 1.535)
	WMS-III visual reproductions-delayed recall	VRD	7.97 (0, 14)	\	\	-0.115 (-2.530, 1.712)
	WMS-III visual memory-recognition recall	VRR	3.00 (0, 4)	\	\	-0.087 (-3.121, 0.919)
	Average Z_score of visual memory	VR	\	\	\	-0.105 (-2.868, 1.389)
<u>Verbal learning</u>	WMS-III paired associates-immediate recall	PASI	13.71 (0, 21)	\	\	-0.083 (-4.252, 2.131)
	WMS-III paired associates-delayed recall	PASD	8.21 (0, 10)	\	\	-0.100 (-5.839, 1.154)
	Average Z_score of verbal learning	PAS	\	\	\	-0.091 (-5.046, 1.642)
<u>Attention and concentration</u> <i>[median (min-max)]</i>	Trail-making test A (sec)	TrA	30.0 (12, 346)	LTrAc= -LOG (TrA)	-3.40 (-5.84, -2.48)	-0.145 (-7.334, 2.726)
	Trail-making test B (sec)	TrB	73.0 (25, 600)	LTrB= -LOG (TrB)	-4.29 (-6.40, -3.22)	-0.193 (-4.923, 2.468)
<u>Executive function</u> <i>[median (min-max)]</i>	Trail-making B – Trail-making A	TrBA	42 (-67, 582)	TrBA=TrB-TrA LTrBA=LOG(2+TrBA)	-4.70 (-6.47, 0)	-0.156 (-5.912, 15.858)
<u>Abstract reasoning</u>	WAIS-III similarities subset	SIM	16.66 (0, 25)	\	17.01 (3, 25)	-0.089 (-4.794, 2.268)
<u>Language [median (min-max)]</u>	Boston naming test	BNT	28 (3, 30)	LBNT= -LOG(31-BNT)	-1.10 (-3.43, 0)	-0.094 (-3.500, 1.422)
<u>Visual-perceptual organization [median (min-max)]</u>	Hooper visual organization test	HVOT	25.5 (3, 30)	LHVOT= -LOG(31-HVOT)	-1.70 (-3.33, 0)	-0.092 (-3.338, 3.196)
<u>Premorbid educational achievement [median (min-max)]</u>	Wide Range Achievement Test-reading (WRAT)	WRAT	49 (15, 57)	LWRAT= -LOG(58-WRAT)	-2.20 (-3.76, 0)	-0.052 (-2.636, 3.060)
<u>Global cognitive function score</u>		\	\	\	\	-0.102 (-3.526, 1.460)

Abbreviations: NP, Neuropsychology.

## **Global Cognitive Function Scores**

The composite subtests summary score was developed to standardize and aggregate cognitive performance across multiple neuropsychological tests. This score was calculated using Z-scores by subtracting the sample mean ( $\bar{\mu}$ ) from a participant's mean score ( $x$ ) and then dividing by the overall SD( $\sigma$ ): [Z-score =  $(x - \bar{\mu}) / \sigma$ ]. Once a Z-score was calculated for each subtest, the average of the Z-scores was calculated to obtain the composite subtests summary score. Individual test's raw score and Z-score range are also shown in table 2<sup>92</sup>.

## **Assessment of potential confounding**

We evaluated a broad range of potential confounders measured at baseline (exam 5) including demographic and lifestyle factors such as age, physical activity, smoking status, alcohol consumption, total energy intake, dietary components, cardiometabolic risk factors, and the use of lipid lowering, blood pressure lowering, or glucose-lowering medications. The dietary variables encompassed food groups (whole grains, eggs, fruits, vegetables, dairy, legumes, nuts and seeds, meats, poultry, fish, and sweets), as well as nutrient intakes (carbohydrates, protein, animal and plant protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, omega-3 fat, fiber, betaine, vitamin B6, vitamin B12, folate, and alcohol). Additionally, we considered total energy expenditure (TEE) as a confounder and explore the use of the Healthy Eating Index (HEI) as a marker of diet quality.

Education level was self-reported during the first NP examination in the Offspring Cohort. Self-reported education was used to classify participants into one of two educational groups: (1) high school or less, and (2) some college or more. Cigarette smoking was evaluated using questionnaires at exam 5. The questionnaire inquired about participants' smoking history, as well as the frequency and quantity of current smoking. The amount smoked was then characterized. The calculation of pack-years of cigarette smoking was derived from an individual's smoking history recorded up to and at the time of dietary assessment. Cigarette pack-years of smoking was defined as a continuous variable. In these cohorts, most individuals were non-smokers at the time, reporting no daily cigarette consumption and a pack-year smoking history of zero.

Physical activity in Framingham was also measured using questionnaires that ask for the number of hours spent each day sleeping or engaging in sedentary, light, moderate, or strenuous activity (including occupationally linked activity)<sup>93</sup>. A moderate/vigorous index of physical activity was calculated by multiplying the number of hours spent in each type of activity by the anticipated energy expenditure for that activity<sup>94</sup>. TEE was determined as a mean from exams 3 and 5 and was based on each participant's sex, age, weight, and physical activity level, using the following equations: (a) for males:  $864 - (9.72 \times \text{AGE}) + \text{PA} \times (14.2 \times \text{Weight} + 503 \times \text{Height})$ ; (b) for females:  $387 - (7.31 \times \text{AGE}) + \text{PA} \times (10.9 \times \text{Weight} + 660.7 \times \text{Height})$ . In these equations, PA represents the physical activity coefficient corresponding to the participant's physical activity index (PAI) category. The PAI cutoffs and their respective PA coefficients were defined as

follows: (a) Sedentary ( $0 \leq \text{PAI} < 32.3$ ) with  $\text{PA} = 1.0$ ; (b) Low activity ( $32.3 \leq \text{PAI} < 35.7$ ) with  $\text{PA} = 1.12$ ; (c) Active ( $35.7 \leq \text{PAI} < 41.6$ ) with  $\text{PA} = 1.27$ ; and (d) Very Active ( $\text{PAI} > 41.6$ ) with  $\text{PA} = 1.45$ . Typically, physical activity level (PAL) cutoffs are established using the doubly labeled water technique, which derives PAL from the sum of activity time multiplied by the basal metabolic rate for each activity. However, because the Framingham Heart Study (FHS) does not have basal metabolic rate data, this study instead used quartiles of the physical activity index from Exam 3 and 5 to define PAL cutoffs.

For weight and height measurements, participants were requested to don a hospital gown, take off their shoes, and stand on a standard beam balance. Average height before age 60 was used to address possible measurement errors or any height loss that may occur when aging. BMI was determined as weight (kg) divided by average height squared ( $\text{m}^2$ ). A participant's waist circumference was measured using tape put at the level of the umbilicus. Waist circumference (cm) was measured in inches and converted to centimeters. The waist-to-height ratio (WHtR) was calculated by dividing waist circumference by average adult height.

Participants were asked to fast for 12 hours before undergoing laboratory assessments. At the time of testing, they reported the last instance of food consumption, allowing for the calculation of total fasting duration. Only individuals who fasted for at least eight hours were included in the analysis, while those with shorter fasting periods were excluded. Blood samples were drawn from the antecubital vein and stored in aliquots at  $-80^\circ\text{C}$  to

maintain sample integrity. Measurements included high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and blood glucose, with quality control procedures implemented to ensure precise and reliable data.

Various laboratory values were assessed for blood pressure and lipid levels. Blood pressure, including systolic and diastolic readings, was measured at each examination visit using a standard mercury sphygmomanometer (Baumanometer). Two measurements were taken on the participant's left arm while seated, ensuring that the cuff's midpoint was aligned with heart level. A minimum rest period of 30 seconds was observed between measurements, and the average of the two readings was recorded. Hypertension was classified according to modified criteria from the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure<sup>95</sup> (JNC 7) as follows: (1) a single visit with an average systolic blood pressure of at least 160 mm Hg or a diastolic pressure of 95 mm Hg or higher; (2) an initial visit with a systolic pressure between 140–159 mm Hg or a diastolic pressure of 90–94 mm Hg, followed by a subsequent visit where systolic pressure was at least 140 mm Hg or diastolic pressure was at least 90 mm Hg; or (3) the first visit in which the participant reported newly starting antihypertensive medication.

### **Statistical analysis**

Statistical analyses were done using SAS statistical software (version 9.4; SAS). The overall goal of this thesis was to examine the prospective association between intakes of dietary choline as well as lutein and zeaxanthin and cognitive function in the FOS. The

dietary choline intake (grams per day) was adjusted for the individuals' body weight at exam 5 (during the dietary assessment) by adding the residuals from linear regression models to the overall mean intake values. In the main analyses, choline intake and lutein and zeaxanthin intake (micrograms per day) were initially divided into quintiles. To reduce variability, a three-category grouping was created: the 1st quintile remained as a separate category, the 2nd and 3rd quintiles were combined into a second category, and the 4th and 5th quintiles were grouped into a third category. To assess global cognitive function, we standardized each cognitive domain score using z-scores, as these measures are on different scales. The overall cognitive function score was derived by computing the mean of the standardized cognitive domain scores.

To evaluate cognitive decline at baseline NP testing, we employed analysis of covariance (ANCOVA) to estimate adjusted mean values both global cognitive function and individual cognitive domain scores according to categories of choline and lutein and zeaxanthin intake. The median score value within each score category for choline and lutein and zeaxanthin was used in linear regression models to test p-values for linear trends. All models were adjusted for age and sex. Additional potential confounders were evaluated based on their impact on the age- and sex- adjusted parameter estimates; confounding factors that were included in the final models were those that changed the parameter estimates by approximately 10% or more. Multivariable logistic regression model was also to assess associations between choline, L&Z intake and odds of dementia.

To evaluate cognitive function at baseline for each NP test, we substituted for all the missing values by substituting the value for the first non-missing NP test score from subsequent NP testing. To evaluate cognitive changes between baseline and 1st follow-up NP test, we substituted for missing data by taking the mean of the adjacent non-missing NP test scores. In these analyses, missing data for body weight, waist, hip, BMI, moderate to vigorous physical activity index, pack-years of cigarette smoking, fasting blood glucose, systolic blood pressure, diastolic blood pressure, fasting triglycerides, total cholesterol, and HDL cholesterol were imputed using available information from adjacent examination cycles. For instance, if data were unavailable at exam 5 in the FOS, the non-missing mean value from exams 4 and 6 was used as a substitute. In cases where physical activity data were missing at exam 5, values from exams 4 and 7 were averaged, as this measure was not collected during exam 6. We adjusted for WHtR instead of BMI in the models for choline, as it showed lower collinearity with body weight-adjusted choline intakes.

## **RESULTS**

### **Baseline characteristics**

Table 3 and table 4 show the sex-specific baseline characteristics of Offspring cohort participants stratified by body weight-adjusted choline intake categories. Participants in the highest choline intake group (315.2–691.8mg/day) were, on average, slightly younger ( $62.2 \pm 0.32$  years) than those in the lowest category (57.7–271.7 mg/day;  $63.1 \pm 0.46$  years). In addition, higher choline intake was associated with a more favorable

anthropometric profile, including lower BMI ( $26.2 \pm 0.16 \text{ kg/m}^2$  vs.  $29.4 \pm 0.23 \text{ kg/m}^2$ ), lower waist-to-height ratios, as well as lower systolic blood pressure levels. Lipid profiles also improved with increasing choline intake, with HDL cholesterol rising and triglyceride levels falling. There were fewer comorbidities associated with higher choline intakes (e.g., lower prevalence of hypertension, diabetes, and dyslipidemia). Dietary assessments revealed that self-reported energy intake but not TEE increased across choline categories, with higher consumption of whole grains, fruits and vegetables, dairy, red meats, poultry, fish, and eggs also increasing (Table 4).

Table 5 and table 6 present the sex-specific baseline characteristics of participants across categories of lutein and zeaxanthin intake in the Offspring cohort. Participants in the highest category of lutein and zeaxanthin intake were slightly older and had a lower BMI than those in the lowest category. Participants with higher intakes of these nutrients also had better lipid profiles (i.e., higher HDL cholesterol and lower triglyceride levels) compared to those in the lowest intake category. Smoking status varied by intake level, with the lowest prevalence of current smokers (10.9%) in the highest category of lutein and zeaxanthin intake compared with the lowest category (23.3%) of intake. Higher intakes were also associated with fewer pack-years of cigarette smoking ( $13.5 \pm 0.69$  pack-years) compared with the lowest category ( $17.8 \pm 0.99$  pack-years). The highest category also had a greater proportion of individuals with a college degree or higher, indicating a better education level. Food-based dietary intakes differed substantially across lutein and zeaxanthin intake categories, with those in the highest category

consuming more whole grains, fruits and vegetables, poultry, fish, and eggs, while also having higher self-reported energy intake from food diaries compared with the lowest category. As expected, higher lutein and zeaxanthin intakes were linked to better diet quality (HEI) and increased consumption of plant-based foods (Table 6).

**Table 3.** Sex-specific baseline characteristics of the participants included in all analyses across body weight adjusted choline intake categories in the Offspring cohort.

	<b>Choline (mg/day)</b>		
	<b>Low (57.7-271.7) (n=397)</b>	<b>Mod. (232.7-379.6) (n=797)</b>	<b>High (315.2-691.8) (n=797)</b>
	<i>Mean (SE)*</i>		
Age (years)	63.1 (0.46)	63.3 (0.32)	62.2 (0.32)
BMI (kg/m <sup>2</sup> )	29.4 (0.23)	26.9 (0.16)	26.2 (0.16)
Waist to hip ratio	0.912 (0.003)	0.896 (0.002)	0.891 (0.002)
Waist to height ratio	0.577 (0.004)	0.543 (0.003)	0.532 (0.003)
Systolic blood pressure (mmHg)	128.5 (0.83)	124.5 (0.58)	124.2 (0.58)
Fasting blood glucose (mg/dL)	103.7 (1.17)	98.0 (0.83)	98.1 (0.83)
Total cholesterol (mg/dL)	206.5 (1.7)	205.2 (1.2)	201.3 (1.2)
HDL cholesterol (mg/dL)	46.6 (0.67)	49.7 (0.48)	52.0 (0.48)
Triglycerides (mg/Dl)	170.3 (5.1)	149.0 (3.6)	134.7 (3.7)
Physical activity index-moderate to high	9.9 (0.44)	10.9 (0.31)	10.8 (0.31)
Pack-years of cigarette smoking	14.6 (0.98)	12.5 (0.69)	16.4 (0.69)
Energy intake (kcal/day)	1587.0 (19.3)	1844.6 (13.7)	2241.0 (13.7)
Total Energy Expenditure (TEE)	2328.3 (21.6)	2253.3 (15.2)	2242.8 (15.2)
	<b>Column %</b>		
Females	53.4	53.3	53.3
Current smoker	15.2	11.4	16.5
High school or less	38.0	35.4	35.1
>=College	62.0	64.6	64.9
Employment: full time	38.3	38.2	38.9
Employment: part-time	14.9	14.2	14.4
Retired	3.5	2.3	3.8
Unemployed	43.3	45.2	42.7
Volunteer	0.0	0.1	0.1
Hypertension	35.0	27.4	23.3
Treated hypertension	20.5	15.7	13.8
Treated diabetes	5.1	1.9	1.9
Treated dyslipidemia	12.1	6.8	4.7

\*Adjusted for age and sex, except age adjusted for sex only, and TEE & age not adjusted at all.

**Table 4.** Sex-specific baseline dietary characteristics of the participants included in all analyses across body weight adjusted choline intake categories in the Offspring cohort.

	<b>Choline (mg/day)</b>		
	<b>Low (57.7-271.7) (n=397)</b>	<b>Mod. (232.7-379.6) (n=797)</b>	<b>High (315.2-691.8) (n=797)</b>
	<i>Mean (SE)*</i>		
<b>Daily foods intake, servings per day</b>			
Whole grains	0.50 (0.04)	0.67 (0.03)	0.65 (0.03)
Fruits	1.1 (0.05)	1.3 (0.04)	1.4 (0.04)
Vegetables	1.4 (0.04)	1.8 (0.03)	2.2 (0.03)
Total fruits and vegetables	2.5 (0.07)	3.1 (0.05)	3.5 (0.05)
Dairy	1.1 (0.04)	1.3 (0.03)	1.7 (0.03)
Legumes, nuts and seeds	0.04 (0.005)	0.04 (0.004)	0.04 (0.004)
Red Meat	1.7 (0.08)	2.1 (0.06)	3.1 (0.06)
Poultry	1.1 (0.06)	1.3 (0.04)	1.6 (0.04)
Fish	1.0 (0.06)	1.2 (0.05)	1.5 (0.05)
Red Meat + poultry +fish	3.9 (0.09)	4.8 (0.07)	6.4 (0.07)
Egg (oz/wk)	1.2 (0.11)	2.3 (0.08)	4.0 (0.08)
<b>Daily body weight adjusted nutrients intake</b>			
Choline(mg)	214.0 (2.40)	300.9 (1.69)	422.5 (1.69)
Lutein + zeaxanthin (mcg)	1304.5 (73.4)	1813.3 (51.8)	2101.7 (51.8)
Carbohydrates(g)	185.1 (3.2)	216.4 (2.2)	247.7 (2.24)
Protein(g/kg/day)	0.76 (0.01)	0.99 (0.01)	1.29 (0.01)
Protein(g)	59.4 (0.74)	75.4 (0.52)	96.2 (0.52)
Animal protein(g)	40.0 (0.68)	52.6 (0.48)	70.0 (0.48)
Plant protein(g)	18.7 (0.37)	21.9 (0.26)	24.8 (0.26)
Total fat (g)	57.9 (1.10)	71.3 (0.78)	90.5 (0.78)
Saturated fat (g)	19.7 (0.45)	24.3 (0.32)	31.4 (0.32)
Poly fat(g)	12.5 (0.29)	15.0 (0.21)	17.8 (0.21)
Mono fat (g)	21.4 (0.44)	26.5 (0.31)	34.0 (0.31)
Omega3 fat (g)	1.2 (0.03)	1.5 (0.02)	1.8 (0.02)
Dietary cholesterol (mg)	158.0 (4.1)	231.9 (2.9)	347.1 (2.9)
Added sugars(g)	11.2 (0.39)	12.4 (0.27)	13.4 (0.27)
Total Fiber (g)	13.4 (0.30)	16.1 (0.21)	18.3 (0.21)
Vitamin B-6 (mg)	1.3 (0.03)	1.8 (0.02)	2.2 (0.02)

Vitamin B-12 (mcg)	4.4 (0.48)	6.0 (0.34)	9.3 (0.34)
Folate (mcg)	209.9 (5.8)	267.0 (4.1)	322.5 (4.1)
Methionine (gm)	1.4 (0.02)	1.7 (0.01)	2.2 (0.01)
Alcohol (g)	6.8 (0.72)	9.0 (0.51)	14.1 (0.51)
Caffeine (mg)	238.0 (11.0)	254.2 (7.8)	301.8 (7.8)
<b>TOTAL HEI-2015 SCORE</b>	<b>53.7 (0.55)</b>	<b>57.3 (0.39)</b>	<b>57.2 (0.39)</b>

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\*Adjusted for age and sex.

**Table 5.** Sex-specific baseline characteristics of the participants included in all analyses across lutein & zeaxanthin intake categories in the Offspring cohort.

	<b>Lutein + Zeaxanthin (mcg/day)</b>		
	<b>Low (51.8-759.6) (n=397)</b>	<b>Mod. (759.4-1696.4) (n=797)</b>	<b>High (1637.8-8833.5) (n=797)</b>
	<b>Mean (SE)*</b>		
Age (years)	61.1 (0.46)	63.1 (0.32)	63.5 (0.32)
BMI (kg/m <sup>2</sup> )	27.8 (0.24)	27.1 (0.17)	26.7 (0.17)
Waist to hip ratio	0.903 (0.003)	0.895 (0.002)	0.896 (0.002)
Waist to height ratio	0.558 (0.004)	0.545 (0.003)	0.540 (0.003)
Systolic blood pressure (mmHg)	126.7 (0.83)	124.2 (0.59)	125.4 (0.59)
Fasting (>= 8 hours) blood glucose (mg/dL)	101.3 (1.18)	99.2 (0.83)	98.2 (0.83)
Total cholesterol (mg/dL)	205.8 (1.74)	204.1 (1.22)	202.8 (1.22)
HDL cholesterol (mg/dL)	49.5 (0.68)	49.4 (0.48)	50.9 (0.48)
Triglycerides (mg/Dl)	158.1 (5.23)	147.5 (3.66)	142.6 (3.67)
Physical activity index-moderate to high	10.7 (0.44)	10.8 (0.31)	10.5 (0.31)
Pack-years of cigarette smoking	17.8 (0.99)	13.8 (0.69)	13.5 (0.69)
Energy intake (kcal/day)	1787.1 (22.78)	1947.3 (16.03)	2038.7 (16.04)
Total Energy Expenditure (TEE)	2303.7 (21.59)	2255.5 (15.23)	2252.9 (15.23)
	<b>Column %</b>		
Females	53.4	53.3	53.3
Current smoker	23.3	13.0	10.9
High school or less	42.8	37.3	30.9
>=College	57.9	62.7	69.1
Employment: full time	42.2	39.0	36.1
Employment: part-time	12.9	14.4	15.2
Retired	2.3	2.9	3.8
Unemployed	42.7	43.7	44.5
Volunteer	0	0	0.25
Hypertension	27.1	26.5	28.1
Treated hypertension	13.4	15.5	17.6
Treated diabetes	3.1	2.5	2.3
Treated dyslipidemia	5.4	7.8	7.1

\*Adjusted for age and sex, except age adjusted for sex only, and TEE & age not adjusted at all.

**Table 6.** Sex-specific baseline dietary characteristics of the participants included in all analyses across lutein & zeaxanthin intake categories in the Offspring cohort.

	<b>Lutein + Zeaxanthin (mcg/day)</b>		
	<b>Low (51.8-759.6)</b> (n=397)	<b>Mod. (759.4-1696.4)</b> (n=797)	<b>High (1637.8-8833.5)</b> (n=797)
	<b>Mean (SE)*</b>		
<b>Daily foods intake, servings per day</b>			
Whole grains	0.42 (0.04)	0.57 (0.02)	0.79 (0.02)
Fruits	0.81 (0.05)	1.28 (0.03)	1.56 (0.03)
Vegetables	1.2 (0.04)	1.7 (0.03)	2.3 (0.03)
Total fruits and vegetables	2.0 (0.06)	3.0 (0.04)	3.9 (0.04)
Dairy	1.3 (0.05)	1.4 (0.03)	1.5 (0.03)
Legumes nuts and seeds	0.05 (0.005)	0.04 (0.004)	0.04 (0.004)
Red Meat	2.5 (0.08)	2.4 (0.06)	2.3 (0.06)
Poultry	1.0 (0.06)	1.4 (0.04)	1.5 (0.04)
Fish	1.2 (0.07)	1.2 (0.05)	1.4 (0.05)
Red Meat + poultry +fish	4.9 (0.11)	5.2 (0.07)	5.4 (0.07)
Egg (oz/wk)	2.3 (0.12)	3.0 (0.09)	2.8 (0.09)
<b>Daily body weight adjusted nutrients intake</b>			
Choline(mg)	288.1 (4.5)	330.0 (3.2)	356.4 (3.2)
Lutein + zeaxanthin (mcg)	517.5 (51.5)	1186.2 (36.3)	3121.4 (36.3)
Carbohydrates(g)	195.5 (3.3)	220.6 (2.3)	238.4 (2.3)
Protein(g/kg/day)	0.94 (0.01)	1.06 (0.01)	1.13 (0.01)
Protein(g)	72.0 (0.99)	80.0 (0.70)	85.4 (0.70)
Animal protein(g)	52.5 (0.89)	57.0 (0.62)	59.3 (0.63)
Plant protein(g)	18.4 (0.37)	21.9 (0.26)	24.9 (0.26)
Total fat (g)	71.2 (1.26)	77.4 (0.89)	77.7 (0.89)
Saturated fat (g)	25.4 (0.50)	26.5 (0.35)	26.4 (0.35)
Poly fat(g)	13.7 (0.31)	15.8 (0.22)	16.4 (0.22)
Mono fat (g)	26.6 (0.50)	29.2 (0.35)	28.8 (0.35)
Omega3 fat (g)	1.4 (0.03)	1.6 (0.02)	1.7 (0.02)
Dietary cholesterol (mg)	237.3 (5.5)	270.2 (3.9)	269.4 (3.9)
Added sugars(g)	12.8 (0.39)	12.6 (0.28)	12.4 (0.28)
Total Fiber (g)	12.3 (0.28)	15.6 (0.20)	19.4 (0.20)
Vitamin B-6 (mg)	1.4 (0.03)	1.8 (0.02)	2.1 (0.02)

Vitamin B-12 (mcg)	7.4 (0.49)	6.6 (0.35)	7.2 (0.35)
Folate (mcg)	197.7 (5.7)	262.6 (4.0)	333.1 (4.0)
Methionine (gm)	1.7 (0.02)	1.9 (0.02)	2.0 (0.02)
Alcohol (g)	10.2 (0.74)	10.2 (0.52)	11.2 (0.52)
Caffeine (mg)	296.3 (11.1)	261.9 (7.8)	265.0 (7.8)
<b>TOTAL HEI-2015 SCORE</b>	49.6 (0.52)	55.7 (0.37)	60.8 (0.37)

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\*Adjusted for age and sex.

### **Association between daily weight-adjusted choline intakes and NP test results**

The association between daily weight-adjusted choline and mean age- and sex-adjusted scores on various NP tests are shown in Table 7. Overall, higher choline intake was positively associated with global cognitive function and several cognitive domains, particularly visual memory and abstract reasoning, when adjusted only for age and sex. However, these associations were attenuated and no longer statistically significant after additional adjustments for education level, cigarette pack-years of smoking, waist-to-height ratio, and alcohol intake (Table 8).

In these cross-sectional analyses, no statistically significant associations were found between dietary choline intake and several other cognitive domains, such as the verbal memory (immediate, delayed, and recognition recall) tests, attention and concentration (assessed by the Trail-Making Test A and B), executive functioning (measured by Trail-Making Test B-A), language skills, and visuo-perceptual organization).

After further adjustments for education level, cigarette pack-years, waist-to-height ratio, and alcohol intake (Table 8), previously significant associations for visual memory (recognition recall) and abstract reasoning were weakened and no longer statistically significant in the fully adjusted model. Similarly, the association between choline intake and global cognitive function, which was significant in the age- and sex-adjusted model, was attenuated and no longer significant ( $P\text{-trend} = 0.228$ ) after full adjustment.

**Table 7.** Age- and sex-adjusted mean NP test scores associated with three categories of dietary choline intake.

	<b>Choline (mg/day)</b>			<i>P trend*</i>
	<b>Low (57.7-271.7) (n=397)</b>	<b>Mod. (232.7-379.6) (n=797)</b>	<b>High (315.2-691.8) (n=797)</b>	
	<i>Mean (95%CI)</i>			
<b><u>Verbal memory</u></b>				
Immediate recall	11.1 (10.8, 11.4)	11.2 (11.0, 11.5)	11.4 (11.1, 11.6)	0.185
Delayed recall	10.1 (9.7, 10.5)	10.3 (10.1, 10.6)	10.4 (10.2, 10.7)	0.161
Recognition recall	9.4 (9.3, 9.5)	9.4 (9.4, 9.5)	9.5 (9.4, 9.5)	0.466
Verbal memory (overall Z-score)	-0.13 (-0.22, -0.04)	-0.08 (-0.15, -0.02)	-0.06 (-0.12, 0.00)	0.187
<b><u>Visual memory</u></b>				
Immediate recall	8.6 (8.3, 8.9)	9.0 (8.8, 9.2)	8.9 (8.7, 9.1)	0.228
Delayed recall	7.6 (7.3, 7.9)	8.1 (7.9, 8.4)	8.0 (7.8, 8.2)	0.251
Recognition recall	2.9 (2.8, 3.0)	3.0 (2.9, 3.1)	3.1 (3.0, 3.1)	0.040
Visual memory (overall Z-score)	-0.19 (-0.27, -0.11)	-0.08 (-0.14, -0.02)	-0.08 (-0.14, -0.03)	0.085
<b><u>Verbal learning</u></b>				
Immediate recall	13.5 (13.2, 13.8)	13.6 (13.4, 13.8)	13.8 (13.6, 14.0)	0.062
Delayed recall	8.1 (7.9, 8.2)	8.2 (8.1, 8.3)	8.2 (8.1, 8.3)	0.232
Verbal learning (overall Z-score)	-4.78 (-4.81, -4.74)	-4.77 (-4.79, -4.74)	-4.76 (-4.78, -4.74)	0.102
<b><u>Attention and concentration<sup>†</sup></u></b>				
Trail-making test A	-3.47 (-3.50, -3.44)	-3.44 (-3.46, -3.42)	-3.44 (-3.46, -3.42)	0.196
Trail-making test B	4.39 (-4.43, -4.34)	4.36 (-4.39, -4.32)	4.36 (-4.39, -4.33)	0.582
<b><u>Executive function<sup>†</sup></u></b>				
Trail-making B – A	-4.78 (-4.81, -4.74)	-4.77 (-4.79, -4.74)	-4.76 (-4.78, -4.74)	0.633
<b><u>Abstract reasoning</u></b>				
WAIS-III similarities subset	16.2 (15.9, 16.6)	16.8 (16.5, 17.0)	16.8 (16.6, 17.0)	0.023
<b><u>Language<sup>†</sup></u></b>				
Boston naming test	-1.09 (-1.15, -1.02)	-1.06 (-1.11, -1.02)	-1.02 (-1.07, -0.98)	0.101
<b><u>Visuo-perceptual organization<sup>†</sup></u></b>				

Hooper visual organization test	-1.67 (-1.72, -1.63)	-1.68 (-1.72, -1.65)	-1.68 (-1.71, -1.64)	0.958
<b><u>Premorbid educational achievement<sup>†</sup></u></b>				
Wide Range Achievement Test-reading (WRAT)	-2.13 (-2.20, -2.07)	-2.04 (-2.08, -1.99)	-2.04 (-2.08, -1.99)	0.052
<b><u>Global cognitive function score<sup>‡</sup></u></b>	-0.17 (-0.22, -0.11)	-0.10 (-0.14, -0.06)	-0.08 (-0.12, -0.04)	0.038

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\* Model 1 adjusted for age and sex.

<sup>†</sup> Based on log-transformed data.

<sup>‡</sup> Based on Z-score data.

**Table 8.** Fully adjusted mean NP scores associated with three categories of dietary choline intake.

	<b>Choline (mg/day)</b>			<i>P trend*</i>
	<b>Low (57.7-271.7) (n=397)</b>	<b>Mod. (232.7-379.6) (n=797)</b>	<b>High (315.2-691.8) (n=797)</b>	
	<i>Mean (95%CI)</i>			
<b><u>Verbal memory</u></b>				
Immediate recall	10.8 (10.5, 11.2)	10.9 (10.7, 11.2)	11.1 (10.8, 11.3)	0.231
Delayed recall	9.9 (9.5, 10.2)	10.0 (9.8, 10.3)	10.1 (9.9, 10.4)	0.281
Recognition recall	9.3 (9.2, 9.5)	9.4 (9.3, 9.5)	9.4 (9.3, 9.5)	0.691
Verbal memory (overall Z-score)	-0.12 (-0.21, -0.04)	-0.08 (-0.14, -0.03)	-0.06 (-0.13, 0.00)	0.305
<b><u>Visual memory</u></b>				
Immediate recall	8.5 (8.2, 8.8)	8.8 (8.6, 9.0)	8.7 (8.5, 8.9)	0.668
Delayed recall	7.5 (7.2, 7.8)	7.9 (7.7, 8.1)	7.7 (7.5, 8.0)	0.629
Recognition recall	2.9 (2.8, 3.0)	2.9 (2.9, 3.0)	3.0 (2.9, 3.1)	0.188
Visual memory (overall Z-score)	-0.16 (-0.24, -0.08)	-0.08 (-0.14, -0.03)	-0.10 (-0.15, -0.04)	0.376
<b><u>Verbal learning</u></b>				
Immediate recall	13.4 (13.0, 13.7)	13.5 (13.2, 13.7)	13.6 (13.4, 13.8)	0.146
Delayed recall	8.0 (7.9, 8.2)	8.2 (8.1, 8.3)	8.1 (8.0, 8.2)	0.486
Verbal learning (overall Z-score)	-0.16 (-0.25, -0.07)	-0.10 (-0.16, -0.04)	-0.08 (-0.15, -0.02)	0.252
<b><u>Attention and concentration</u><sup>†</sup></b>				
Trail-making test A	-3.47 (-3.50, -3.43)	-3.45 (-3.47, -3.42)	-3.45 (-3.48, -3.43)	0.489
Trail-making test B	-4.39 (-4.44, -4.35)	-4.38 (-4.41, -4.35)	-4.39 (-4.43, -4.36)	0.794
<b><u>Executive function</u><sup>†</sup></b>				
Trail-making B – A	-4.78 (-4.81, -4.75)	-4.78 (-4.81, -4.76)	-4.78 (-4.81, -4.76)	0.824
<b><u>Abstract reasoning</u></b>				
WAIS-III similarities subset	16.0 (15.6, 16.3)	16.4 (16.1, 16.6)	16.4 (16.2, 16.7)	0.076
<b><u>Language</u><sup>†</sup></b>				
Boston naming test	-1.12 (-1.19, -1.05)	-1.12 (-1.17, -1.08)	-1.09 (-1.14, -1.05)	0.419
<b><u>Visuo-perceptual organization</u><sup>†</sup></b>				
Hooper visual organization test	-1.68 (-1.73, -1.63)	-1.70 (-1.73, -1.66)	-1.69 (-1.73, -1.66)	0.761
<b><u>Premorbid educational achievement</u><sup>†</sup></b>				

Wide Range Achievement Test-reading (WRAT)	-2.17 (-2.22, -2.11)	-2.13 (-2.17, -2.09)	-2.14 (-2.18, -2.10)	0.680
<b><u>Global cognitive function score</u></b> <sup>‡</sup>	-0.20 (-0.26, -0.15)	-0.16 (-0.20, -0.12)	-0.15 (-0.19, -0.11)	0.228

\* Model 2 adjusted for age, sex, education level, cigarette pack-years, waist/height ratio and alcohol intake.

† Based on log-transformed data.

‡ Based on Z-score data.

### **Association between daily intakes of lutein and zeaxanthin and NP test results**

Table 9 presents the age- and sex-adjusted mean neuropsychological test scores across categories of lutein and zeaxanthin intake in the FOS. Overall, higher lutein and zeaxanthin intake was positively associated with global cognitive function and several cognitive domains, including visual memory, attention and concentration, abstract reasoning, language, visuo-perceptual organization, and premorbid educational achievement.

Individuals in the highest category of lutein and zeaxanthin intake had significantly higher scores in visual memory immediate recall (P-trend = 0.0003), delayed recall (P-trend = 0.002), and recall recognition (P-trend = 0.004) compared to those in the lowest intake category. Notably, those with the highest lutein and zeaxanthin intake exhibited significantly better overall visual memory recall ability (P-trend = 0.0002). Additionally, higher lutein and zeaxanthin intake was positively associated with premorbid educational achievement.

Unlike dietary choline intake, lutein and zeaxanthin intake was statistically significantly associated with improved attention and concentration (Trail-Making Test b, P-trend = 0.012), language (P-trend < 0.001), and visuo-perceptual organization (P-trend = 0.004). Global cognitive function scores were also statistically significantly associated with lutein and zeaxanthin intake (P-trend < 0.0001), indicating a potential overall cognitive benefit.

After additional adjustments for education level, cigarette pack-years, and waist-to-height ratio (Table 10), associations remained for global cognitive function (P-trend = 0.01), overall visual memory ability (P-trend = 0.017), visual memory immediate recall (P-trend = 0.016), visual memory recognition recall (P-trend = 0.05), abstract reasoning (P-trend = 0.002), visuo-perceptual organization (P-trend = 0.018), and premorbid educational achievement (P-trend = 0.001). However, some other associations (i.e., attention and concentration, and executive functioning) were attenuated in the fully adjusted model.

**Table 9.** Age- and sex-adjusted means association between NP test scores and three categories of lutein & zeaxanthin intake

	<b>Lutein + Zeaxanthin (mcg/day)</b>			<i>P trend*</i>
	<b>Low (51.8-759.6) (n=397)</b>	<b>Mod. (759.4-1696.4) (n=797)</b>	<b>High (1637.8-8833.5) (n=797)</b>	
	<i>Mean (95%CI)</i>			
<b><u>Verbal memory</u></b>				
Immediate recall	10.9 (10.6, 11.3)	11.3 (11.0, 11.5)	11.4 (11.1, 11.6)	0.069
Delayed recall	9.9 (9.6, 10.3)	10.4 (10.2, 10.6)	10.4 (10.2, 10.7)	0.071
Recognition recall	9.4 (9.3, 9.5)	9.4 (9.3, 9.5)	9.5 (9.4, 9.6)	0.191
Verbal memory (overall Z-score)	-0.17 (-0.25, -0.08)	-0.08 (-0.14, -0.02)	-0.05 (-0.11, 0.02)	0.059
<b><u>Visual memory</u></b>				
Immediate recall	8.2 (7.9, 8.5)	9.0 (8.8, 9.2)	9.1 (8.9, 9.3)	0.0003
Delayed recall	7.4 (7.1, 7.7)	8.1 (7.9, 8.3)	8.2 (7.9, 8.4)	0.002
Recognition recall	2.9 (2.8, 3.0)	3.0 (2.9, 3.1)	3.1 (3.0, 3.1)	0.004
Visual memory (overall Z-score)	-0.28 (-0.36, -0.19)	-0.08 (-0.14, -0.02)	-0.04 (-0.10, 0.02)	0.0002
<b><u>Verbal learning</u></b>				
Immediate recall	13.35 (13.0, 13.7)	13.7 (13.5, 14.0)	13.8 (13.5, 14.0)	0.108
Delayed recall	8.1 (7.9, 8.2)	8.2 (8.1, 8.3)	8.2 (8.1, 8.3)	0.077
Verbal learning (overall Z-score)	-0.19 (-0.28, -0.11)	-0.09 (-0.16, -0.03)	-0.07 (-0.13, -0.01)	0.069
<b><u>Attention and concentration<sup>†</sup></u></b>				
Trail-making test A	-3.49 (-3.52, -3.46)	-3.43 (-3.46, -3.41)	-3.44 (-3.46, -3.41)	0.105
Trail-making test B	-4.43 (-4.47, -4.39)	-4.35 (-4.38, -4.32)	-4.34 (-4.37, -4.31)	0.012
<b><u>Executive function<sup>†</sup></u></b>				
Trail-making B – A	-4.79 (-4.83, -4.76)	-4.76 (-4.79, -4.74)	-4.75 (-4.78, -4.73)	0.079
<b><u>Abstract reasoning</u></b>				
WAIS-III similarities subset	15.6 (15.3, 16.0)	16.9 (16.6, 17.1)	17.0 (16.7, 17.2)	<.0001
<b><u>Language<sup>†</sup></u></b>				
Boston naming test	-1.20 (-1.27, -1.14)	-1.03 (-1.07, -0.98)	-1.00 (-1.04, -0.95)	<.0001
<b><u>Visuo-perceptual organization<sup>†</sup></u></b>				
Hoopper visual organization test	-1.73 (-1.78, -1.69)	-1.68 (-1.72, -1.65)	-1.65 (-1.68, -1.61)	0.004

**Premorbid educational achievement<sup>†</sup>**

Wide Range

Achievement	-2.21 (-2.27, -2.15)	-2.06 (-2.11, -2.02)	-1.97 (-2.02, -1.93)	<.0001
Test-reading (WRAT)				

**Global cognitive  
function score<sup>‡</sup>**

	-0.24 (-0.30, -0.19)	-0.09 (-0.13, -0.05)	-0.05 (-0.09, -0.01)	<.0001
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\* Model 1 adjusted for age and sex.

† Based on log-transformed data.

‡ Based on Z-score data.

**Table 10.** Fully adjusted mean NP scores associated with three categories of dietary choline intake.

	<b>Lutein + Zeaxanthin (mcg/day)</b>			<i>P trend*</i>
	<b>Low (51.8-759.6) (n=397)</b>	<b>Mod. (759.4-1696.4) (n=797)</b>	<b>High (1637.8-8833.5) (n=797)</b>	
	<i>Mean (95%CI)</i>			
<b><u>Verbal memory</u></b>				
Immediate recall	10.80 (10.50, 11.10)	11.00 (10.80, 11.30)	11.00 (10.80, 11.30)	0.474
Delayed recall	9.80 (9.40, 10.10)	10.10 (9.90, 10.40)	10.10 (9.80, 10.30)	0.487
Recognition recall	9.30 (9.20, 9.50)	9.40 (9.30, 9.40)	9.40 (9.30, 9.50)	0.592
Verbal memory (overall Z-score)	-0.20 (-0.29, -0.12)	-0.14 (-0.20, -0.08)	-0.15 (-0.21, -0.08)	0.453
<b><u>Visual memory</u></b>				
Immediate recall	8.20 (7.90, 8.50)	8.80 (8.60, 9.00)	8.80 (8.60, 9.00)	0.016
Delayed recall	7.30 (7.00, 7.60)	7.90 (7.70, 8.10)	7.80 (7.60, 8.10)	0.083
Recognition recall	2.90 (2.80, 3.00)	3.00 (2.90, 3.00)	3.00 (2.90, 3.10)	0.05
Visual memory (overall Z-score)	-0.28 (-0.36, -0.20)	-0.13 (-0.19, -0.07)	-0.12 (-0.18, -0.06)	0.017
<b><u>Verbal learning</u></b>				
Immediate recall	13.30 (13.00, 13.60)	13.60 (13.40, 13.80)	13.50 (13.30, 13.80)	0.438
Delayed recall	8.10 (7.90, 8.20)	8.10 (8.00, 8.20)	8.20 (8.10, 8.30)	0.316
Verbal learning (overall Z-score)	-0.21 (-0.30, -0.12)	-0.14 (-0.20, -0.07)	-0.14 (-0.20, -0.07)	0.337
<b><u>Attention and concentration</u><sup>†</sup></b>				
Trail-making test A	-3.49 (-3.52, -3.46)	-3.44 (-3.47, -3.42)	-3.45 (-3.47, -3.42)	0.320
Trail-making test B	-4.43 (-4.48, -4.39)	-4.38 (-4.41, -4.35)	-4.38 (-4.41, -4.35)	0.206
<b><u>Executive function</u><sup>†</sup></b>				
Trail-making B –A	-4.80 (-4.83, -4.77)	-4.78 (-4.80, -4.76)	-4.78 (-4.80, -4.75)	0.508
<b><u>Abstract reasoning</u></b>				
WAIS-III similarities subset	15.50 (15.20, 15.80)	16.60 (16.30, 16.80)	16.50 (16.30, 16.70)	0.002
<b><u>Language</u><sup>†</sup></b>				
Boston naming test	-1.227 (-1.29, -1.16)	-1.081 (-1.13, -1.04)	-1.079 (-1.13, -1.03)	0.009
<b><u>Visuo-perceptual organization</u><sup>†</sup></b>				
Hooper visual organization test	-1.74 (-1.78, -1.69)	-1.70 (-1.73, -1.66)	-1.66 (-1.70, -1.63)	0.018
<b><u>Premorbid educational achievement</u><sup>†</sup></b>				

Wide Range Achievement Test- reading (WRAT)	-2.23 (-2.29, -2.17)	-2.14 (-2.18, -2.10)	-2.09 (-2.13, -2.05)	0.001
<b><u>Global cognitive function score</u></b>	-0.260 (-0.31, -0.21)	-0.141 (-0.18, -0.10)	-0.137 (-0.18, -0.10)	0.01

\* Model 2 adjusted for age, sex, education level, cigarette pack-years and waist/height ratio.

† Based on log-transformed data.

‡ Based on Z-score data.

**The effect of daily choline and lutein & zeaxanthin intake categories on all  
immediate NP subtests and delayed NP subtests**

Table 11 presents the adjusted Z-score means for immediate and delayed recall in verbal memory, visual memory, and verbal learning across categories of choline (body weight-adjusted) and lutein & zeaxanthin intake in the FOS. Overall, higher choline intake was only significantly associated with better immediate Z-score of verbal memory, visual memory and verbal learning recall, when just adjusting for age and sex. However, higher intake of lutein and zeaxanthin was significantly associated with better performance on both immediate and delayed recall of verbal memory, visual memory and verbal learning tests, when just adjusting for age and sex.

For choline exposure, participants in the highest intake group had better immediate recall (Z-score = -0.21, 95% CI: -0.35, -0.06) and better delayed recall (Z-score = -0.43, 95% CI: -0.57, -0.28) compared to lower intake group when adjusting for age and sex. But these trends disappeared in the fully adjusted model, and only immediate recall association was statistically significant in model 1 (P-trend = 0.04).

In contrast, higher lutein and zeaxanthin intake was significantly associated with better immediate and delayed recall performance in the age- and sex-adjusted model.

Participants in the highest intake category had significantly better immediate recall (Z-score = -0.16, P-trend = 0.001) and delayed recall (Z-score = -0.19, P-trend = 0.003) compared to those in the lowest intake group (Z-score = -0.68 for immediate recall, Z-

score = -0.70 for delayed recall). However, in the fully adjusted model (Model 2), these trends disappeared in the fully adjusted model, and none of these associations were statistically significant in model 2.

**Table 11.** Adjusted Z-score means for the association between all immediate and delayed NP tests and choline (body weight adjusted) and Lutein & zeaxanthin intake categories in the Offspring Cohort.

	Immediate Recall Variables (Verbal memory, visual memory and verbal learning)		Delayed Recall Variables (Verbal memory, visual memory and verbal learning)		
	Model 1	Model 2	Model 1	Model 2	
	<i>Z-score Mean (95% CI) †</i>				
<b>Choline (mg/day)</b>	Low	-0.49 (-0.70, -0.28)	-0.63 (-0.84, -0.43)	-0.56 (-0.78, -0.35)	-0.69 (-0.90, -0.47)
	Mod.	-0.28 (-0.43, -0.14)	-0.48 (-0.62, -0.34)	-0.24 (-0.39, -0.09)	-0.44 (-0.58, -0.29)
	High	-0.21 (-0.35, -0.06)	-0.43 (-0.57, -0.28)	-0.27 (-0.42, -0.12)	-0.49 (-0.64, -0.33)
	<i>P trend</i>	0.04	0.15	0.09	0.29
<b>Lutein &amp; zeaxanthin (mcg/day)</b>	Low	-0.68 (-0.89, -0.48)	-0.76 (-0.96, -0.56)	-0.70 (-0.91, -0.49)	-0.76 (-0.97, -0.55)
	Mod.	-0.24 (-0.38, -0.09)	-0.41 (-0.55, -0.27)	-0.26 (-0.41, -0.11)	-0.43 (-0.57, -0.28)
	High	-0.16 (-0.30, -0.01)	-0.42 (-0.57, -0.28)	-0.19 (-0.34, -0.04)	-0.45 (-0.60, -0.30)
	<i>P trend</i>	0.001	0.07	0.003	0.12

Model 1 adjusted for age and sex.

Model 2 adjusted for age, sex, education level, cigarette pack-years, waist/height ratio.

Analyses in choline model were additionally adjusted for alcohol.

† Based on Z-score data.

## **Associations of dietary choline, lutein, and zeaxanthin with incidence of dementia**

Table 12 presents the adjusted odds ratios for the association between choline, lutein, and zeaxanthin intake categories and the odds of developing dementia. Participants were grouped into three intake categories, with the first category representing the lowest intake and serving as the reference group.

In the fully adjusted model (Model 4), which accounted for age, sex, TEE, education, and pack-years of cigarette smoking, participants in the highest choline intake category had significantly lower odds of developing dementia compared to those in the lowest category (OR = 0.67, 95% CI: 0.43–0.98). This inverse association was consistent across all models, with ORs ranging from 0.67 to 0.71, although statistical significance was only observed in the fully adjusted model. The middle intake category was not significantly associated with dementia odds in any model.

In contrast, no significant associations were observed between lutein and zeaxanthin intake categories and odds of dementia. Across all four models, ORs for the middle and highest intake categories were close to 1.00, and all corresponding confidence intervals included the null value. For example, in Model 4, the OR for the highest lutein and zeaxanthin intake category was 0.88 (95% CI: 0.57–1.36).

**Table 12.** Adjusted ORs for association between choline, lutein and zeaxanthin intake categories and dementia odds.

		<b>New cases of dementia</b>	<b>Cumulative incidence%</b>	<b>OR (95% CI)<sup>1</sup></b>	<b>OR (95% CI)<sup>2</sup></b>	<b>OR (95% CI)<sup>3</sup></b>	<b>OR (95% CI)<sup>4</sup></b>
<b>Choline categories</b>	Low (n=585)	62	10.6	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	Moderate (n=1172)	121	10.32	0.94 (0.64-1.38)	0.89 (0.60-1.31)	0.89 (0.61-1.33)	0.89 (0.61-1.33)
	High (n=1171)	95	8.11	0.71 (0.48-1.07)	0.67 (0.45-1.00)	0.67 (0.45-1.02)	0.67 (0.43-0.98)
<b>Lutein &amp; zeaxanthin categories</b>	Low (n=585)	47	8.03	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
	Moderate (n=1172)	116	9.98	0.98 (0.65-1.50)	0.98 (0.64-1.49)	0.997 (0.65-1.52)	0.98 (0.64-1.50)
	High (n=1171)	115	9.74	0.89 (0.58-1.35)	0.88 (0.58-1.35)	0.91 (0.59-1.39)	0.88 (0.57-1.36)

Model 1 adjusted for age and sex.

Model 2: Model 1 + TEE

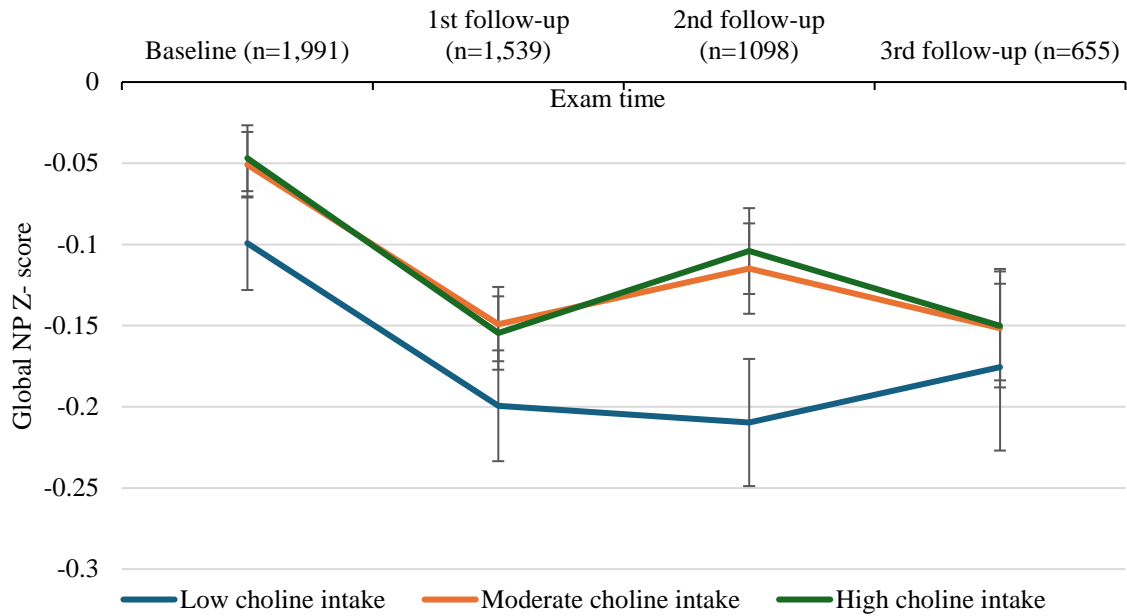
Model 3: Model 2 + education

Model 4: Model 3 + pack-years of cigarette smoking

**The effect of daily choline and lutein & zeaxanthin intake categories on repeated measures of global cognitive function (Z-score) over time.**

Figure 6 shows repeated measures of adjusted Z-score means for global cognition function across categories of choline (body weight-adjusted) intake in the FOS. Overall, participants who had moderate to high choline intake (232.7 – 691.8 mg/day) had continuous better global cognition function compared to those who had low choline intake (57.7 – 271.7 mg/day) over time.

Figure 7 shows repeated measures of adjusted Z-score means for global cognition function across categories of lutein and zeaxanthin intake in the FOS. Overall, participants who had moderate to high lutein and zeaxanthin intake (759.4 – 1696.4 mg/day) also had continuous better global cognition function compared to those who had low intake (1637.8 – 8833.5 mg/day) over time. Notably, greater category Z-score difference was observed in lutein and zeaxanthin model than in choline model.

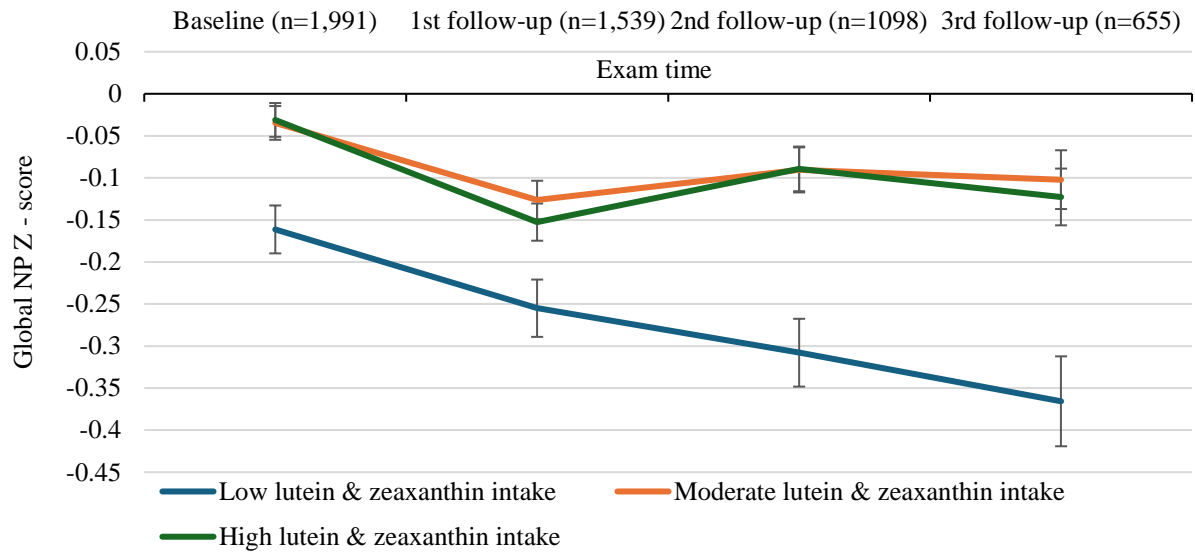


**Figure 6.** Categories of daily choline intake and repeated measures of global cognitive function over time.

Low choline intake: 57.7 - 271.7 mg/day.

Moderate choline intake: 232.7 - 379.6 mg/day.

High choline intake: 315.2 - 691.8 mg/day.



**Figure 7.** Categories of daily lutein & zeaxanthin intake and repeated measures of global cognitive function over time

Low lutein & zeaxanthin intake: 51.8 - 759.6 mcg/day.

Moderate lutein & zeaxanthin intake: 759.4 - 1696.4 mcg/day.

High lutein & zeaxanthin intake: 1637.8 – 8833.5 mcg/day.

## DISCUSSION

In this study, we investigated the prospective associations between dietary choline, lutein, and zeaxanthin intake and cognitive function in the FOS. Our findings suggest that higher choline intake is positively associated with global cognitive function, visual memory and, abstract reasoning domains when adjusted for age and sex, but most of these associations were attenuated when education, pack-years of cigarette smoking, waist-to-height ratio, and alcohol were added to the model. Additionally, we found that dietary lutein and zeaxanthin intakes were positively associated with global cognitive function and several cognitive domains, including visual memory, attention and concentration, abstract reasoning, language, visuo-perceptual organization, and premorbid educational achievement domain when adjusting for age, sex. In the fully-adjusted models, we still found statistically significant relationships between lutein and zeaxanthin intake and global cognitive function, including some individual cognitive domains such as visual memory, language, abstract reasoning, visuo-perceptual organization, and premorbid educational achievement. These results contribute to the growing body of evidence linking dietary nutrients with cognitive health in aging populations.

Our findings partly align with previous research, such as a 2011 analysis that demonstrated positive associations between dietary choline intake and visual and verbal memory individuals in FOS without dementia<sup>96</sup>. However, we found no association between choline intake and verbal memory. Few studies have evaluated dietary choline intake during middle-aged to old adults with various cognitive domains in the

Framingham Heart Study. In other similar studies, however, Ylilauri et al. found statistically significant associations between both total choline intake and phosphatidylcholine and verbal memory function in men in eastern Finland<sup>87</sup>. In addition, a recent cross-sectional study using NHANES data demonstrated that compared with those consuming <187.6 mg/day, a total choline intake of 187.06-399.50 mg/day was associated with a protective effect on cognitive function, including learning ability, verbal fluency, processing speed, sustained attention, and working memory<sup>97</sup>. Other investigations have assessed choline status using blood biomarkers while others examined choline intake primarily from supplements, or focused on maternal choline consumption<sup>98-99</sup>. These studies have generally found that higher choline levels are associated with improved cognitive function. However, several methodological challenges complicate direct comparisons. First, dietary choline intake does not strongly correlate with plasma choline concentrations<sup>100</sup>, indicating that circulating choline is an unreliable biomarker for dietary intake. Second, variations in choline supplementation, including differences in dosage and molecular form, influence bioavailability and may contribute to the discrepancies observed across studies<sup>99</sup>. Thus, findings on the relationship between choline compounds and cognitive performance have been inconsistent across studies.

The potential mechanisms through which choline may influence cognitive function include its role as a precursor to acetylcholine, a neurotransmitter involved in memory and learning, as well as its involvement in methylation processes that affect brain

function<sup>101</sup>. Research in animal models has demonstrated the neuroprotective properties of choline supplementation. Studies indicate that providing choline to rats during prenatal development enhances memory function, with benefits extending into adulthood<sup>102,103</sup>. In another study, Teather and Wurtman explored the impact of cytidine (5')-diphosphocholine, a choline precursor, on memory decline in older rats<sup>104</sup>. Their findings suggest that supplementation mitigates age-associated cognitive impairments.

The metabolites derived from choline contribute significantly to maintaining the structural integrity of cell membranes and play a crucial role in cholinergic signaling during neuronal development<sup>105</sup>. Several investigations propose that administering choline and its derivatives in pharmacologic doses may be beneficial for older adults experiencing cognitive difficulties, impaired memory, or early-stage Alzheimer's disease<sup>106,107</sup>. Magil et al. demonstrated that dietary choline, particularly in the form of lecithin, markedly increased concentrations of choline and acetylcholine in both the bloodstream and brain tissue<sup>108</sup>.

Neuropathological examinations of postmortem brains have identified notable reductions in cortical cholinergic markers, which exhibit a strong correlation with AD pathology<sup>109,110</sup>. Analyses of brain samples from individuals who succumbed to AD reveal an accelerated breakdown of membrane phosphatidylcholine and a diminished supply of choline throughout neural structures. Some researchers hypothesize that cholinergic neurons degrade their own choline-containing membranes, ultimately leading to cellular degeneration<sup>111</sup>. On the other hand, sufficient levels of acetylcholine in the

brain are thought to confer protective effects against certain forms of dementia, including AD<sup>112</sup>.

Regarding lutein and zeaxanthin, although fewer studies within the Framingham Heart Study have explored the association between lutein and zeaxanthin and cognitive function, our findings align with previous research demonstrating the neuroprotective properties of these carotenoids. In a combined cross-sectional and longitudinal study involving 442 individuals aged 65 to 94 years, Perrig et al found that elevated plasma concentrations of  $\beta$ -carotene correlated with superior performance in memory-related tasks, including free recall, recognition, and vocabulary assessments<sup>113</sup>. Similarly, findings from the Rotterdam Study, which examined 5,182 community-dwelling participants between the ages of 55 and 95 years, revealed that lower dietary intake of  $\beta$ -carotene was linked to diminished cognitive ability, as evaluated using the Mini-Mental State Examination (MMSE)<sup>114</sup>.

More research has examined the relationship between serum and brain concentrations of dietary carotenoids and cognitive function, underscoring the need for further investigation into biomarkers of lutein and zeaxanthin in both serum and brain tissue. For example, in one cross-sectional study of individuals aged 80 years and older, including centenarians, they identified significant associations between dietary carotenoid levels in both serum and brain tissue and multiple aspects of cognitive function<sup>115</sup>. Rather than a single cognitive domain demonstrating the strongest correlation with carotenoid

concentrations, significant relationships were observed across several areas, including memory, executive function, and language.

The precise mechanisms by which carotenoids influence brain function remain an area of active investigation, though several hypotheses have been proposed. One possibility is that their neuroprotective effects stem from mitigating oxidative and inflammatory stress, processes known to contribute to neurodegenerative conditions<sup>116</sup>. This aligns with findings linking lower macular pigment optical density to cognitive function<sup>117</sup>, reinforcing the idea that carotenoids may serve a protective role in cognitive aging.

Beyond this broad protective function, a more direct and mechanistically distinct pathway has been suggested, particularly in the context of younger individuals or palliative interventions. The "neural efficiency hypothesis" posits that carotenoids enhance cognitive function by directly interacting with neural cells to optimize processing efficiency<sup>118,119</sup>. This idea is based on three key observations. Firstly, lutein and zeaxanthin are present in brain regions involved in vision and cognition, like the occipital and frontal lobes, and their levels vary among individuals<sup>81</sup>. Secondly, lab studies show that lutein and zeaxanthin enhance cell-to-cell communication, such as improving gap junctions<sup>120</sup>. Lastly, research links higher macular pigment levels to faster processing speed and better cognitive function, indicating their role in optimizing neural efficiency<sup>121</sup>.

Lastly, regarding the NP test, we noticed that previous studies assessing global cognitive function have notably excluded the Wide Range Achievement Test (WRAT) as a subtest

in composite cognitive function scores. The WRAT, particularly its Reading subtest, is widely utilized as a measure of literacy levels and premorbid intelligence<sup>122</sup>, as reading ability is thought to remain relatively stable across the lifespan, even in the presence of cognitive decline<sup>123</sup>. The test–retest reliability of WRAT scores has been well-documented in aging populations, including individuals with MCI and AD, supporting its use as a consistent measure in longitudinal studies of cognitive decline. One study<sup>124</sup> specifically examined the stability of the WRAT-3 Reading subtest over a one-year period among older adults with varying cognitive statuses: cognitively normal (n = 200), MCI (n = 137), and possible or probable AD (n = 41). Results showed that raw WRAT-3 Reading scores exhibited high stability across all groups (test–retest reliability: controls = 0.81, MCI = 0.92, AD = 0.90). However, categorical descriptive classifications (e.g., "average", "high average") were inconsistent, with 26% of participants changing classifications over time, and the greatest variability observed in the AD group (36%). These findings suggest that while the WRAT-3 Reading subtest is reliable for tracking reading performance over time, it is not suitable for categorically estimating premorbid ability or literacy levels, as small score fluctuations can lead to changes in classification even when actual ability remains stable. Moreover, the WRAT-3 Reading subtest exclusively assesses the ability to read irregularly spelled words and does not evaluate memory, reasoning, executive function, attention, or concentration—all of which are critical components of global cognitive function. Consequently, an individual with cognitive impairment may still perform well on WRAT-3 if their reading ability remains intact, potentially leading to a misleading impression of preserved cognitive function.

These limitations likely explain why the WRAT-3 Reading subtest is not typically included in global cognitive function assessments.

A key strength of this study is the use of prospective data with repeated dietary and cognitive assessments. The use of three-day diet records enhances the accuracy of nutrient intake estimates compared to food frequency questionnaires. Additionally, the inclusion of multiple cognitive domains, allowing for a comprehensive assessment of nutrients' potential cognitive effects.

However, several limitations must be acknowledged. First, while the use of food records improves dietary assessment accuracy, measurement error remains a possibility due to self-reported intake. Second, we lack a biomarker of choline, such as serum choline concentrations, which could serve as an exposure in place of dietary choline. Third, the observational nature of this study prevents us from establishing causality. For example, it is possible that some participants with very healthy diets (rich in choline, lutein, and zeaxanthin) may have improved their diet quality because of prevalent diseases; randomized controlled trials would be needed to confirm the potential benefits of these nutrients in such a situation. Lastly, since our study sample is only from the Framingham Heart Study cohort and these individuals are predominantly of western European descent, this may limit generalizability to more diverse populations.

A growing body of evidence suggests that promoting dietary patterns rich in choline, lutein, and zeaxanthin could be a viable strategy for supporting cognitive health in aging populations. Many adults do not meet the recommended adequate intake (AI) for choline

and many others have low intakes of lutein and zeaxanthin. Thus, a balanced diet rich in plant-based foods as well as eggs, dairy, and other protein-source foods may aid in the prevention of aging-related cognitive decline. Our findings indicate that the associations between these nutrients and cognitive performance were not uniform across all cognitive domains. While some measures showed statistically significant associations, others did not, suggesting potential specificity in how these nutrients influence different aspects of cognition. This discrepancy may reflect differences in the underlying neurobiological mechanisms supporting various cognitive functions or could be influenced by the sensitivity of different tests in capturing diet-related cognitive changes. Future studies should aim to clarify whether these nutrients predominantly benefit specific domains, such as verbal memory, executive function, or visuospatial abilities, and investigate potential moderating factors such as sex, obesity, or overall diet quality.

Future research should also explore the long-term impact of dietary choline, lutein, and zeaxanthin intake on cognitive trajectories, particularly through large-scale longitudinal studies that assess whether midlife choline intake protects against cognitive decline in later years. Additionally, incorporating neuroimaging techniques such as MRI and PET scans could provide insight into whether higher choline, lutein, and zeaxanthin intakes are associated with structural brain differences, including greater gray matter volume, reduced hippocampal atrophy, or enhanced neural connectivity. Given the observational nature of most existing studies, randomized controlled trials are essential to ascertain the causal relationship of these supplements on cognitive performance in at-risk populations.

These trials should also assess optimal dosage, duration, and potential interactions with other neuroprotective nutrients. Addressing these research gaps will help refine dietary recommendations and evaluate the potential of these nutrients as a preventive strategy against age-related cognitive decline.

## CONCLUSION

In summary, our findings suggest that higher dietary intakes of choline, lutein, and zeaxanthin are associated with better performance in memory-related cognitive tasks and global cognitive function in middle-aged and older adults. These findings support the potential neuroprotective role of these nutrients, particularly in maintaining cognitive function during aging. However, given the observational nature of our study, future research is needed to confirm these associations and establish causal relationships.

**ABBREVIATIONS**

<i>Ageing Res Rev</i> .....	<i>Ageing Research Reviews</i>
<i>Alzheimers Res Ther</i> .....	<i>Alzheimer's Research &amp; Therapy</i>
<i>Altern Med Rev</i> .....	<i>Alternative Medicine Review</i>
<i>Am J Clin Nutr</i> .....	<i>The American Journal of Clinical Nutrition</i>
<i>Am J Epidemiol</i> .....	<i>The American Journal of Epidemiology</i>
<i>Annu Rev Nutr</i> .....	<i>The Annual Review of Nutrition</i>
<i>Annu Rev Psychol</i> .....	<i>The Annual Review of Psychology</i>
<i>Arch Intern Med</i> .....	<i>Archives of Internal Medicine</i>
<i>Behav Neurol</i> .....	<i>Behavioral Neurology</i>
<i>Br Med Bull</i> .....	<i>British Medical Bulletin</i>
<i>Clin Interv Aging</i> .....	<i>Clinical Interventions in Aging</i>
<i>Clin Ophthalmol Auckl NZ</i> .....	<i>Clinical Ophthalmology (Auckland, N.Z.)</i>
<i>Clin Ther</i> .....	<i>Clinical Therapeutics</i>
<i>Curr Nutr Rep</i> .....	<i>Current Nutrition Reports</i>
<i>Dev Brain Res</i> .....	<i>Developmental Brain Research</i>
<i>Eur J Nutr</i> .....	<i>The European Journal of Nutrition</i>
<i>Free Radic Biol Med</i> .....	<i>Free Radical Biology and Medicine</i>
<i>Front Aging Neurosci</i> .....	<i>Frontiers in Aging Neuroscience</i>
<i>J Agric Food Chem</i> .....	<i>The Journal of Agricultural and Food Chemistry</i>
<i>J Aging Health</i> .....	<i>The Journal of Aging and Health</i>
<i>J Am Diet Assoc</i> .....	<i>The Journal of the Academy of Nutrition and Dietetics</i>
<i>J Clin Exp Neuropsychol</i> .....	<i>Journal of Clinical and Experimental Neuropsychology</i>
<i>J Gerontol Ser A</i> .....	<i>The Journals of Gerontology: Series A</i>
<i>J Geriatr Psychiatry Neurol</i> .....	<i>Journal of Geriatric Psychiatry and Neurology</i>

<i>J Neurol Neurosurg Psychiatry</i> .....	<i>The Journal of Neurology, Neurosurgery and Psychiatry</i>
<i>J Nutr Gerontol Geriatr</i> .....	<i>Journal of Nutrition in Gerontology and Geriatrics</i>
<i>J Nutr Health Aging</i> .....	<i>The Journal of Nutrition, Health and Aging</i>
<i>Mol Aspects Med</i> .....	<i>Molecular Aspects of Medicine</i>
<i>Neurobiol Aging</i> .....	<i>Neurobiology of Aging</i>
<i>Neurochem J</i> .....	<i>Journal of Neurochemistry</i>
<i>Neurochem Res</i> .....	<i>Neurochemical Research</i>
<i>Neurodegener Dis Manag</i> .....	<i>Neurodegenerative Disease Management</i>
<i>Neurosci Biobehav Rev</i> .....	<i>Neuroscience &amp; Biobehavioral Reviews</i>
<i>Nutr Neurosci</i> .....	<i>Nutritional Neuroscience</i>
<i>Nutr Rev</i> .....	<i>Nutrition Reviews</i>
<i>Proc Natl Acad Sci</i> .....	<i>Proceedings of the National Academy of Sciences of the United States of America</i>
<i>Prog Neuropsychopharmacol Biol Psychiatry</i> .....	<i>Progress in Neuropsychopharmacology &amp; Biological Psychiatry</i>

## REFERENCES

1. Assessment of Cognition Using Surveys and Neuropsychological Assessment: The Health and Retirement Study and the Aging, Demographics, and Memory Study | The Journals of Gerontology: Series B | Oxford Academic. Accessed March 14, 2025.  
[https://academic.oup.com/psychsocgerontology/article/66B/suppl\\_1/i162/556770](https://academic.oup.com/psychsocgerontology/article/66B/suppl_1/i162/556770)
2. Dementia. Accessed March 14, 2025. <https://www.who.int/news-room/fact-sheets/detail/dementia>
3. Mild Cognitive Impairment (MCI) | Symptoms & Treatments | alz.org. Alzheimer's Association. Accessed March 14, 2025. [https://www.alz.org/alzheimers-dementia/what-is-dementia/related\\_conditions/mild-cognitive-impairment](https://www.alz.org/alzheimers-dementia/what-is-dementia/related_conditions/mild-cognitive-impairment)
4. Bishop NA, Lu T, Yankner BA. Neural mechanisms of ageing and cognitive decline. *Nature*. 2010;464(7288):529-535. doi:10.1038/nature08983
5. Anderson ND. State of the science on mild cognitive impairment (MCI). *CNS Spectr*. 2019;24(1):78-87. doi:10.1017/S1092852918001347
6. Park DC, Reuter-Lorenz P. The Adaptive Brain: Aging and Neurocognitive Scaffolding. *Annu Rev Psychol*. 2009;60(Volume 60, 2009):173-196. doi:10.1146/annurev.psych.59.103006.093656
7. Fluid Intelligence - an overview | ScienceDirect Topics. Accessed November 2, 2024. <https://www.sciencedirect.com/topics/psychology/fluid-intelligence>
8. What Is Mild Cognitive Impairment? National Institute on Aging. April 12, 2021. Accessed January 2, 2025. <https://www.nia.nih.gov/health/memory-loss-and-forgetfulness/what-mild-cognitive-impairment>
9. Practice guideline update summary: Mild cognitive impairment [RETIRED]: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. *Neurology*. 2018;90(3):126-135. doi:10.1212/WNL.0000000000004826
10. Rate of Conversion from Prodromal Alzheimer's Disease to Alzheimer's Dementia: A Systematic Review of the Literature | Dementia and Geriatric Cognitive Disorders Extra | Karger Publishers. Accessed January 2, 2025.  
<https://karger.com/dee/article/3/1/320/103150/Rate-of-Conversion-from-Prodromal-Alzheimer-s>

11. Tyas SL, Salazar JC, Snowdon DA, et al. Transitions to Mild Cognitive Impairments, Dementia, and Death: Findings from the Nun Study. *Am J Epidemiol.* 2007;165(11):1231-1238. doi:10.1093/aje/kwm085
12. Dementia. Accessed January 2, 2025. <https://www.who.int/news-room/fact-sheets/detail/dementia>
13. Chapman SB, Aslan S, Spence JS, et al. Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging. *Front Aging Neurosci.* 2013;5. doi:10.3389/fnagi.2013.00075
14. Effects of the Dietary Approaches to Stop Hypertension Diet, Exercise, and Caloric Restriction on Neurocognition in Overweight Adults With High Blood Pressure | Hypertension. Accessed March 12, 2025. <https://www.ahajournals.org/doi/full/10.1161/HYPERTENSIONAHA.109.146795>
15. Mediterranean Diet and Age-Related Cognitive Decline: A Randomized Clinical Trial | Lifestyle Behaviors | JAMA Internal Medicine | JAMA Network. Accessed March 12, 2025. <https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2293082>
16. Effects of Cognitive Training Interventions With Older Adults: A Randomized Controlled Trial | Dementia and Cognitive Impairment | JAMA | JAMA Network. Accessed March 12, 2025. <https://jamanetwork.com/journals/jama/fullarticle/195506>
17. Cognitive Resilience in Brain Health and Dementia Research - Mahesh S. Joshi, James E. Galvin, 2022. Accessed March 14, 2025. <https://journals.sagepub.com/doi/full/10.3233/JAD-220755>
18. Dodds L, Brayne C, Siette J. Associations between social networks, cognitive function, and quality of life among older adults in long-term care. *BMC Geriatr.* 2024;24(1):221. doi:10.1186/s12877-024-04794-9
19. Risk reduction of cognitive decline and dementia: WHO guidelines. Accessed January 2, 2025. <https://www.who.int/publications/i/item/9789241550543>
20. Lieberman HR. Nutrition, brain function and cognitive performance☆. *Appetite.* 2003;40(3):245-254. doi:10.1016/S0195-6663(03)00010-2
21. Alzheimer Disease in the US Population: Prevalence Estimates Using the 2000 Census | Dementia and Cognitive Impairment | JAMA Neurology | JAMA Network. Accessed October 13, 2024. <https://jamanetwork.com/journals/jamaneurology/article-abstract/784558>

22. Neurofibrillary Tangles Mediate the Association of Amyloid Load With Clinical Alzheimer Disease and Level of Cognitive Function | Dementia and Cognitive Impairment | JAMA Neurology | JAMA Network. Accessed October 13, 2024. <https://jamanetwork.com/journals/jamaneurology/article-abstract/785529>
23. Classification and basic pathology of Alzheimer disease | Acta Neuropathologica. Accessed October 13, 2024. <https://link.springer.com/article/10.1007/s00401-009-0532-1>
24. Alzheimer's & Dementia. Alzheimer's Association. Accessed January 2, 2025. <https://alz-journals.onlinelibrary.wiley.com/journal/15525279>
25. Neural mechanisms of ageing and cognitive decline - PMC. Accessed March 14, 2025. <https://pmc.ncbi.nlm.nih.gov/articles/PMC2927852/>
26. Andrews-Hanna JR, Snyder AZ, Vincent JL, et al. Disruption of Large-Scale Brain Systems in Advanced Aging. *Neuron*. 2007;56(5):924-935. doi:10.1016/j.neuron.2007.10.038
27. Cabeza R. Hemispheric asymmetry reduction in older adults: The HAROLD model. *Psychol Aging*. 2002;17(1):85-100. doi:10.1037/0882-7974.17.1.85
28. Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging Gracefully: Compensatory Brain Activity in High-Performing Older Adults. *NeuroImage*. 2002;17(3):1394-1402. doi:10.1006/nimg.2002.1280
29. The Aging Brain | Annual Reviews. Accessed October 14, 2024. <https://www.annualreviews.org/content/journals/10.1146/annurev.pathmechdis.2.010506.092044>
30. Loerch PM, Lu T, Dakin KA, et al. Evolution of the Aging Brain Transcriptome and Synaptic Regulation. *PLOS ONE*. 2008;3(10):e3329. doi:10.1371/journal.pone.0003329
31. Genetic foundations of human intelligence | Human Genetics. Accessed October 15, 2024. <https://link.springer.com/article/10.1007/s00439-009-0655-4>
32. Deary IJ, Wright AF, Harris SE, Whalley LJ, Starr JM. Searching for genetic influences on normal cognitive ageing. *Trends Cogn Sci*. 2004;8(4):178-184. doi:10.1016/j.tics.2004.02.008
33. Small BJ, Rosnick CB, Fratiglioni L, Bäckman L. Apolipoprotein E and Cognitive Performance: A Meta-Analysis. *Psychol Aging*. 2004;19(4):592-600. doi:10.1037/0882-7974.19.4.592

34. Deary IJ, Corley J, Gow AJ, et al. Age-associated cognitive decline. *Br Med Bull.* 2009;92(1):135-152. doi:10.1093/bmb/ldp033
35. Waldstein SR, Elias MF, Elias MF, eds. *Neuropsychology of Cardiovascular Disease.* Psychology Press; 2000. doi:10.4324/9781410600981
36. Brown GG, Zorrilla LTE. Neuropsychological Aspects of Stroke. In: *Neuropsychology of Cardiovascular Disease.* Psychology Press; 2001.
37. Hochstenbach J, Mulder T, van Limbeek J, Donders R, Schoonderwaldt H. Cognitive Decline Following Stroke: A Comprehensive Study of Cognitive Decline Following Stroke\*. *J Clin Exp Neuropsychol.* 1998;20(4):503-517. doi:10.1076/jcen.20.4.503.1471
38. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms Underlying Inflammation in Neurodegeneration. *Cell.* 2010;140(6):918-934. doi:10.1016/j.cell.2010.02.016
39. Role of Antioxidants and Natural Products in Inflammation - Arulselvan - 2016 - Oxidative Medicine and Cellular Longevity - Wiley Online Library. Accessed October 15, 2024. <https://onlinelibrary.wiley.com/doi/full/10.1155/2016/5276130>
40. Rizzo MR, Barbieri M, Boccardi V, Angellotti E, Marfella R, Paolisso G. Dipeptidyl Peptidase-4 Inhibitors Have Protective Effect on Cognitive Impairment in Aged Diabetic Patients With Mild Cognitive Impairment. *J Gerontol Ser A.* 2014;69(9):1122-1131. doi:10.1093/gerona/glu032
41. Tangestani Fard M, Stough C. A Review and Hypothesized Model of the Mechanisms That Underpin the Relationship Between Inflammation and Cognition in the Elderly. *Front Aging Neurosci.* 2019;11. doi:10.3389/fnagi.2019.00056
42. Dietary choline intake is necessary to prevent systems-wide organ pathology and reduce Alzheimer's disease hallmarks - Dave - 2023 - Aging Cell - Wiley Online Library. Accessed October 18, 2024. <https://onlinelibrary.wiley.com/doi/full/10.1111/accel.13775>
43. Cummings J, Aisen P, Lemere C, Atri A, Sabbagh M, Salloway S. Aducanumab produced a clinically meaningful benefit in association with amyloid lowering. *Alzheimers Res Ther.* 2021;13(1):98. doi:10.1186/s13195-021-00838-z
44. Li HC, Luo KX, Wang JS, Wang QX. Extrapyrarnidal side effect of donepezil hydrochloride in an elderly patient: A case report. *Medicine (Baltimore).* 2020;99(11):e19443. doi:10.1097/MD.00000000000019443

45. Martins LB, Malheiros Silveira AL, Teixeira AL. The Link Between Nutrition and Alzheimer's Disease: From Prevention to Treatment. *Neurodegener Dis Manag.* 2021;11(2):155-166. doi:10.2217/nmt-2020-0023
46. 2013 Alzheimer's disease facts and figures - - 2013 - Alzheimer's & Dementia - Wiley Online Library. Accessed October 18, 2024. <https://alz-journals.onlinelibrary.wiley.com/doi/full/10.1016/j.jalz.2013.02.003>
47. Mosconi L, McHugh PF. Let Food Be Thy Medicine: Diet, Nutrition, and Biomarkers' Risk of Alzheimer's Disease. *Curr Nutr Rep.* 2015;4(2):126-135. doi:10.1007/s13668-014-0111-5
48. Baumgart M, Snyder HM, Carrillo MC, Fazio S, Kim H, Johns H. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. *Alzheimers Dement.* 2015;11(6):718-726. doi:10.1016/j.jalz.2015.05.016
49. Witte AV, Fobker M, Gellner R, Knecht S, Flöel A. Caloric restriction improves memory in elderly humans. *Proc Natl Acad Sci.* 2009;106(4):1255-1260. doi:10.1073/pnas.0808587106
50. Martin B, Mattson MP, Maudsley S. Caloric restriction and intermittent fasting: Two potential diets for successful brain aging. *Ageing Res Rev.* 2006;5(3):332-353. doi:10.1016/j.arr.2006.04.002
51. Zhang DM, Ye JX, Mu JS, Cui XP. Efficacy of Vitamin B Supplementation on Cognition in Elderly Patients With Cognitive-Related Diseases: A Systematic Review and Meta-Analysis. *J Geriatr Psychiatry Neurol.* 2017;30(1):50-59. doi:10.1177/0891988716673466
52. Van Dyk K, Sano M. The Impact of Nutrition on Cognition in the Elderly. *Neurochem Res.* 2007;32(4):893-904. doi:10.1007/s11064-006-9241-5
53. Fruit Polyphenols and Their Effects on Neuronal Signaling and Behavior in Senescence - JOSEPH - 2007 - Annals of the New York Academy of Sciences - Wiley Online Library. Accessed March 12, 2025. <https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1196/annals.1395.052>
54. Brain foods: the effects of nutrients on brain function | Nature Reviews Neuroscience. Accessed October 16, 2024. <https://www.nature.com/articles/nrn2421>
55. Dietary Influences on Cognitive Function with Aging - PARROTT - 2007 - Annals of the New York Academy of Sciences - Wiley Online Library. Accessed October 16, 2024.

[https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1196/annals.1396.028?casa\\_token=cpOBAbAfyvoAAAAA%3Aqz1qb4dPVLx4w359IEUSmVXCeJ0S07ZCQNJ3jSTkNgIXiRVEggmX88X\\_thfHQ4pHyv1JjbeZLA15bC8](https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1196/annals.1396.028?casa_token=cpOBAbAfyvoAAAAA%3Aqz1qb4dPVLx4w359IEUSmVXCeJ0S07ZCQNJ3jSTkNgIXiRVEggmX88X_thfHQ4pHyv1JjbeZLA15bC8)

56. Mediterranean diet pyramid today. Science and cultural updates | Public Health Nutrition | Cambridge Core. Accessed October 16, 2024.  
<https://www.cambridge.org/core/journals/public-health-nutrition/article/mediterranean-diet-pyramid-today-science-and-cultural-updates/70359644D12A038AC003B935AA04E120>
57. Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies - Schwingshackl - 2014 - International Journal of Cancer - Wiley Online Library. Accessed October 16, 2024.  
[https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.28824?casa\\_token=JIlmo3lvSnUAAAAA:nSjF3gT6osIHY0eF6hDwNu8hs8NnfBu1fTnvPVraLVPV6Wh7PwQ\\_qN-wgbqeVBrVM\\_k0-ATPFXePadw](https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.28824?casa_token=JIlmo3lvSnUAAAAA:nSjF3gT6osIHY0eF6hDwNu8hs8NnfBu1fTnvPVraLVPV6Wh7PwQ_qN-wgbqeVBrVM_k0-ATPFXePadw)
58. Adherence to a Mediterranean Diet and Survival in a Greek Population | New England Journal of Medicine. Accessed October 16, 2024.  
[https://www.nejm.org/doi/full/10.1056/NEJMoa025039?casa\\_token=dy0wbiboZSkAAAAA%3AVeKNF40S8YbbSrPneuGjT61mwSdZ8EwQSA\\_bKxBOHHory\\_-qb8XP1wm0k5UWhOY0ZJovDGr7dfFjNqI](https://www.nejm.org/doi/full/10.1056/NEJMoa025039?casa_token=dy0wbiboZSkAAAAA%3AVeKNF40S8YbbSrPneuGjT61mwSdZ8EwQSA_bKxBOHHory_-qb8XP1wm0k5UWhOY0ZJovDGr7dfFjNqI)
59. Cooper C, Goswami U, Sahakian BJ. *Mental Capital and Wellbeing*. John Wiley & Sons; 2009.
60. Salthouse TA. When does age-related cognitive decline begin? *Neurobiol Aging*. 2009;30(4):507-514. doi:10.1016/j.neurobiolaging.2008.09.023
61. Zarezadeh M, Mahmoudinezhad M, Faghfour AH, et al. Alcohol consumption in relation to cognitive dysfunction and dementia: A systematic review and dose-response meta-analysis of comparative longitudinal studies. *Ageing Res Rev*. 2024;100:102419. doi:10.1016/j.arr.2024.102419
62. Smoking and Cognitive Decline Among Middle-Aged Men and Women: The Doetinchem Cohort Study | AJP | Vol. 98 Issue 12. Accessed October 16, 2024.  
<https://ajph.aphapublications.org/doi/full/10.2105/AJPH.2007.130294>
63. Association of dietary intake and lifestyle pattern with mild cognitive impairment in the elderly | The journal of nutrition, health & aging. Accessed June 24, 2024.  
<https://link.springer.com/article/10.1007/s12603-014-0524-2>

64. Iannotti LL, Lutter CK, Waters WF, et al. Eggs early in complementary feeding increase choline pathway biomarkers and DHA: a randomized controlled trial in Ecuador†. *Am J Clin Nutr*. 2017;106(6):1482-1489. doi:10.3945/ajcn.117.160515
65. Aparicio Vizuete A, Robles F, Rodríguez-Rodríguez E, López-Sobaler AM, Ortega RM. Association between food and nutrient intakes and cognitive capacity in a group of institutionalized elderly people. *Eur J Nutr*. 2010;49(5):293-300. doi:10.1007/s00394-009-0086-y
66. An R, Li D, Xiang X. Choline Intake and Cognitive Function Among U.S. Older Adults. *J Nutr Gerontol Geriatr*. 2023;42(1):30-45. doi:10.1080/21551197.2023.2179565
67. Edwards CG, Walk AM, Thompson SV, et al. Dietary lutein plus zeaxanthin and choline intake is interactively associated with cognitive flexibility in middle-adulthood in adults with overweight and obesity. *Nutr Neurosci*. Published online July 3, 2022. Accessed June 23, 2024. <https://www.tandfonline.com/doi/abs/10.1080/1028415X.2020.1866867>
68. Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly12345. *Am J Clin Nutr*. 2012;96(5):1161S-1165S. doi:10.3945/ajcn.112.034611
69. Medicine I of, Board F and N, Nutrients S on URL of, Choline SC on the SE of DRI and its P on F Other B Vitamins, and. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press; 2000.
70. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary Carotenoids, Vitamins A, C, and E, and Advanced Age-Related Macular Degeneration. *JAMA*. 1994;272(18):1413-1420. doi:10.1001/jama.1994.03520180037032
71. Rasmussen HM, Johnson EJ. Nutrients for the aging eye. *Clin Interv Aging*. 2013;8:741-748. doi:10.2147/CIA.S45399
72. Concentrations of Choline-Containing Compounds and Betaine in Common Foods - ScienceDirect. Accessed October 16, 2024. <https://www.sciencedirect.com/science/article/pii/S0022316622158554>
73. Assessment of Total Choline Intakes in the United States: Journal of the American College of Nutrition: Vol 35, No 2. Accessed October 16, 2024. <https://www.tandfonline.com/doi/abs/10.1080/07315724.2015.1080127>

74. Zeisel SH, Klatt KC, Caudill MA. Choline. *Adv Nutr*. 2018;9(1):58-60. doi:10.1093/advances/nmx004
75. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products - ScienceDirect. Accessed October 16, 2024. [https://www.sciencedirect.com/science/article/pii/S0889157508001336?casa\\_token=EVWJcugUy1IAAAAA:UdRkt7fq9Jml0oLfyg61ZeNnEt0d8qPQnjRhc\\_LB7qrc8IF7jJE\\_LH3ZuV6y9soEu7KkccXPBtk](https://www.sciencedirect.com/science/article/pii/S0889157508001336?casa_token=EVWJcugUy1IAAAAA:UdRkt7fq9Jml0oLfyg61ZeNnEt0d8qPQnjRhc_LB7qrc8IF7jJE_LH3ZuV6y9soEu7KkccXPBtk)
76. Humphries JM, Khachik F. Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Fruit, Vegetables, Wheat, and Pasta Products. *J Agric Food Chem*. 2003;51(5):1322-1327. doi:10.1021/jf026073e
77. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. *J Am Diet Assoc*. 1993;93(3):284-296. doi:10.1016/0002-8223(93)91553-3
78. Handelman GJ, Nightingale ZD, Lichtenstein AH, Schaefer EJ, Blumberg JB. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk2. *Am J Clin Nutr*. 1999;70(2):247-251. doi:10.1093/ajcn.70.2.247
79. Pappolla MA, Smith MA, Bryant-Thomas T, et al. Cholesterol, oxidative stress, and Alzheimer's disease: expanding the horizons of pathogenesis1. *Free Radic Biol Med*. 2002;33(2):173-181. doi:10.1016/S0891-5849(02)00841-9
80. Oxidants, oxidative stress and the biology of ageing | Nature. Accessed June 24, 2024. <https://www.nature.com/articles/35041687>
81. Craft N, Haitema T, Garnett K, Fitch K, Dorey C. Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *J Nutr Health Aging*. 2004;8:156-162.
82. Krinsky NI. Possible Biologic Mechanisms for a Protective Role of Xanthophylls. *J Nutr*. 2002;132(3):540S-542S. doi:10.1093/jn/132.3.540S
83. Hammond BR, Miller LS, Bello MO, Lindbergh CA, Mewborn C, Renzi-Hammond LM. Effects of Lutein/Zeaxanthin Supplementation on the Cognitive Function of Community Dwelling Older Adults: A Randomized, Double-Masked, Placebo-Controlled Trial. *Front Aging Neurosci*. 2017;9. doi:10.3389/fnagi.2017.00254
84. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med*. 2005;26(6):459-516. doi:10.1016/j.mam.2005.10.001
85. Johnson EJ. Obesity, Lutein Metabolism, and Age-Related Macular Degeneration: a Web of Connections. *Nutr Rev*. 2005;63(1):9-15. doi:10.1111/j.1753-4887.2005.tb00105.x

86. Krinsky NI, Mayne ST, Sies H. *Carotenoids in Health and Disease*. CRC Press; 2004.
87. Ylilauri MP, Voutilainen S, Lönnroos E, et al. Associations of dietary choline intake with risk of incident dementia and with cognitive performance: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr*. 2019;110(6):1416-1423.
88. Blusztajn JK, Slack BE, Mellott TJ. Neuroprotective actions of dietary choline. *Nutrients*. 2017;9(8):815.
89. McKeown NM, Day NE, Welch AA, et al. Use of biological markers to validate self-reported dietary intake in a random sample of the European Prospective Investigation into Cancer United Kingdom Norfolk cohort. *Am J Clin Nutr*. 2001;74(2):188-196. doi:10.1093/ajcn/74.2.188
90. Frances A, First MB, Pincus HA. *DSM-IV Guidebook*. American Psychiatric Association; 1995:x, 501.
91. Nishtala A, Piers RJ, Himali JJ, et al. Atrial fibrillation and cognitive decline in the Framingham Heart Study. *Heart Rhythm*. 2018;15(2):166-172. doi:10.1016/j.hrthm.2017.09.036
92. Downer B, Fardo DW, Schmitt FA. A Summary Score for the Framingham Heart Study Neuropsychological Battery. *J Aging Health*. 2015;27(7):1199-1222. doi:10.1177/0898264315577590
93. Kannel WB, Sorlie P. Some Health Benefits of Physical Activity: The Framingham Study. *Arch Intern Med*. 1979;139(8):857-861. doi:10.1001/archinte.1979.03630450011006
94. Physical activity and physical demand on the job and risk of cardiovascular disease and death: The Framingham Study - ScienceDirect. Accessed January 30, 2025. <https://www.sciencedirect.com/science/article/pii/S0002870386904801>
95. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and Evidence From New Hypertension Trials | Hypertension. Accessed February 18, 2025. <https://www.ahajournals.org/doi/full/10.1161/01.HYP.0000110061.06674.ca>
96. Poly C, Massaro JM, Seshadri S, et al. The relation of dietary choline to cognitive performance and white-matter hyperintensity in the Framingham Offspring Cohort. *Am J Clin Nutr*. 2011;94(6):1584-1591.

97. Liu L, Qiao S, Zhuang L, et al. Choline Intake Correlates with Cognitive Performance among Elder Adults in the United States. Lin MS, ed. *Behav Neurol*. 2021;2021:1-11. doi:10.1155/2021/2962245
98. Plasma free choline, betaine and cognitive performance: the Hordaland Health Study | *British Journal of Nutrition* | Cambridge Core. Accessed March 12, 2025. <https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/plasma-free-choline-betaine-and-cognitive-performance-the-hordaland-health-study/A07F06DC93C7678B188229FB072272E2>
99. A Comprehensive Review of Eggs, Choline, and Lutein on Cognition Across the Life-span: *Journal of the American College of Nutrition*: Vol 37, No 4. Accessed March 8, 2025. <https://www.tandfonline.com/doi/abs/10.1080/07315724.2017.1423248>
100. The Relationship Between Dietary Intake of Choline, Choline Serum Levels, and Cognitive Function in Healthy Elderly Persons - SANCHEZ - 1984 - *Journal of the American Geriatrics Society* - Wiley Online Library. Accessed March 8, 2025. <https://agsjournals.onlinelibrary.wiley.com/doi/abs/10.1111/j.1532-5415.1984.tb02004.x>
101. ZEISEL S. Choline and human nutrition. *Annu Rev Nutr*. 1994;14:269-296.
102. Meck WH, Williams CL. Choline supplementation during prenatal development reduces proactive interference in spatial memory. *Dev Brain Res*. 1999;118(1):51-59. doi:10.1016/S0165-3806(99)00105-4
103. Meck WH, Williams CL. Metabolic imprinting of choline by its availability during gestation: implications for memory and attentional processing across the lifespan. *Neurosci Biobehav Rev*. 2003;27(4):385-399. doi:10.1016/S0149-7634(03)00069-1
104. Teather LA, Wurtman RJ. Dietary cytidine (5')-diphosphocholine supplementation protects against development of memory deficits in aging rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003;27(4):711-717. doi:10.1016/S0278-5846(03)00086-1
105. Wurtman RJ. Cholinemetabolism as a basisfor the selective vulnerabil#y of cholinergic neurons. 1992;15(4).
106. Conant R, Schauss AG. Therapeutic Applications of Citicoline for Stroke and Cognitive Dysfunction in the Elderly: A Review of the Literature. *Altern Med Rev*. 2004;9(1).

107. De Jesus Moreno Moreno M. Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: A multicenter, double-blind, randomized, placebo-controlled trial. *Clin Ther.* 2003;25(1):178-193. doi:10.1016/S0149-2918(03)90023-3
108. Magil SG, Zeisel SH, Wurtman RJ. Effects of Ingesting Soy or Egg Lecithins on Serum Choline, Brain Choline and Brain Acetylcholine. *J Nutr.* 1981;111(1):166-170. doi:10.1093/jn/111.1.166
109. Davis KL, Mohs RC, Marin D, et al. Cholinergic Markers in Elderly Patients With Early Signs of Alzheimer Disease. *JAMA.* 1999;281(15):1401-1406. doi:10.1001/jama.281.15.1401
110. Geula C, Mesulam MM. Systematic Regional Variations in the Loss of Cortical Cholinergic Fibers in Alzheimer's Disease. *Cereb Cortex.* 1996;6(2):165-177. doi:10.1093/cercor/6.2.165
111. Davies P. Challenging the Cholinergic Hypothesis in Alzheimer Disease. *JAMA.* 1999;281(15):1433-1434. doi:10.1001/jama.281.15.1433
112. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatry.* 1999;66(2):137-147. doi:10.1136/jnnp.66.2.137
113. The Relation Between Antioxidants and Memory Performance in the Old and Very Old - Perrig - 1997 - Journal of the American Geriatrics Society - Wiley Online Library. Accessed March 8, 2025. <https://agsjournals.onlinelibrary.wiley.com/doi/abs/10.1111/j.1532-5415.1997.tb01476.x>
114. Jama JW, Launer LJ, Witteman JC, et al. Dietary antioxidants and cognitive function in a population-based sample of older persons. The Rotterdam Study. *Am J Epidemiol.* 1996;144(3):275-280. doi:10.1093/oxfordjournals.aje.a008922
115. Johnson EJ, Vishwanathan R, Johnson MA, et al. Relationship between Serum and Brain Carotenoids,  $\alpha$ -Tocopherol, and Retinol Concentrations and Cognitive Performance in the Oldest Old from the Georgia Centenarian Study. *J Aging Res.* 2013;2013(1):951786. doi:10.1155/2013/951786
116. Fatani AJ, Parmar MY, Abuohashish HM, Ahmed MM, Al-Rejaie SS. Protective effect of lutein supplementation on oxidative stress and inflammatory progression in cerebral cortex of streptozotocin-induced diabetes in rats. *Neurochem J.* 2016;10(1):69-76. doi:10.1134/S1819712416010074

117. Renzi LM, Dengler MJ, Puente A, Miller LS, Hammond BR. Relationships between macular pigment optical density and cognitive function in unimpaired and mildly cognitively impaired older adults. *Neurobiol Aging*. 2014;35(7):1695-1699. doi:10.1016/j.neurobiolaging.2013.12.024
118. Zimmer JP, Hammond BR. Possible influences of lutein and zeaxanthin on the developing retina. *Clin Ophthalmol Auckl NZ*. 2007;1(1):25-35.
119. The relation between the macular carotenoids, lutein and zeaxanthin, and temporal vision - Renzi - 2010 - *Ophthalmic and Physiological Optics* - Wiley Online Library. Accessed March 13, 2025. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1475-1313.2010.00720.x>
120. Biological activities of natural and synthetic carotenoids: induction of gap junctional communication and singlet oxygen quenching. | *Carcinogenesis* | Oxford Academic. Accessed March 13, 2025. <https://academic.oup.com/carcin/article-abstract/18/1/89/2364958>
121. Johnson EJ, McDonald K, Caldarella SM, Chung H yun, Troen AM, Snodderly DM. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutr Neurosci*. 2008;11(2):75-83. doi:10.1179/147683008X301450
122. Lezak MD. *Neuropsychological Assessment*. Oxford University Press; 2004.
123. Practitioner's Guide to Evaluating Change with Neuropsychological Assessment ... - Google Books. Accessed March 5, 2025. [https://books.google.com/books?hl=en&lr=&id=f07aBwAAQBAJ&oi=fnd&pg=PA157&ots=aDbgsEAqbp&sig=IASzQoFmfQKwdR3H0CsefQV\\_1Rw#v=onepage&q&f=false](https://books.google.com/books?hl=en&lr=&id=f07aBwAAQBAJ&oi=fnd&pg=PA157&ots=aDbgsEAqbp&sig=IASzQoFmfQKwdR3H0CsefQV_1Rw#v=onepage&q&f=false)
124. Ashendorf L, Jefferson AL, Green RC, Stern RA. Test–retest stability on the WRAT-3 reading subtest in geriatric cognitive evaluations. *J Clin Exp Neuropsychol*. 2009;31(5):605-610. doi:10.1080/13803390802375557

**CURRICULUM VITAE**

