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Apolipoprotein E genotype moderates the association between dietary polyunsaturated fat and brain function: An exploration of cerebral glutamate and cognitive performance

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Abstract

Objective: To investigate the effect of Apolipoprotein E (APOE) genotype on the association between dietary polyunsaturated fat (PUFA), cognitive function, and cerebral glutamate.

Methods: A participant sample of 122 middle-aged adults were grouped according to APOE genotype ($\epsilon 4$ carrier or $\epsilon 4$ non-carrier) and asked to record dietary intake for three consecutive days. All participants also underwent neuropsychological testing and a proton magnetic resonance spectroscopy (^1H MRS) scan to assess glutamate in the posterior cingulate cortex.

Results: Multiple regression analyses revealed a significant interaction between APOE genotype and PUFA intake on memory performance, $F(1,113) = 6.749$, $p = .016$. Greater PUFA intake was associated with better memory performance in healthy middle-aged adults who were APOE $\epsilon 4$ non-carriers, but not for $\epsilon 4$ carriers. Furthermore, there was a significant interaction between APOE genotype and PUFA intake on cerebral glutamate, in that dietary PUFA was associated with greater cerebral glutamate in APOE $\epsilon 4$ carriers, but not for $\epsilon 4$ non-carriers, $F(1,114) = 5.173$, $p = .025$.

Conclusions: The findings suggest that PUFA action on the brain differs according to APOE polymorphism and points towards cerebral glutamate as a potential marker of genetic risk for Alzheimer's disease (AD). Early treatment consisting of PUFA supplementation that is tailored to APOE genotype may be an important intervention for the prevention of cognitive decline.

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Disclosure of Interest

The authors report no conflict of interest.

Keywords

Apolipoprotein E; Polyunsaturated fat; Diet; Proton magnetic resonance spectroscopy; Memory; Aging; Alzheimer's disease

Epidemiological research supports an association between polyunsaturated fat (PUFA) intake and reduced risk of cognitive decline and Alzheimer's disease (AD) (1, 2). In particular, intake of omega-3 (ω -3) PUFAs, which include both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been associated with better cognitive performance (3) and better brain integrity (4). The underlying mechanisms explaining the beneficial effects of PUFA have not been fully elucidated, although researchers have posited vascular, anti-inflammatory, and neuroprotective mechanisms (5–7). Additionally, prior work has suggested that PUFA consumption may be linked with more efficient regulation of glutamatergic synapses, which play a crucial role in memory (7, 8). In particular, ω -3 PUFAs are instrumental in supporting the integrity of astrocytes, which are known for maintaining homeostasis of the glutamatergic synapse and maintaining a safe concentration of Glu in the neuronal environment (9). Given that one physiological mechanism of age-related cognitive decline has been attributed to the deregulation of glutamatergic synapse functioning (10), maintaining adequate intake of dietary PUFA throughout the lifespan may be a valuable strategy for preserving cognitive function.

The apolipoprotein E (APOE) genotype appears to affect the association between PUFA intake and cognition. In particular, some studies have suggested that carriers of the APOE ϵ 4 allele, a genetic risk factor for AD, do not reap the same cognitive and health benefits. Prospective dietary studies have observed that high fish-containing diets may be protective against dementia for APOE ϵ 4 allele non-carriers, but not for APOE ϵ 4-positive individuals (11, 12). In a placebo-controlled DHA supplement trial, only ϵ 4 allele non-carriers with AD showed reduced cognitive decline relative to the placebo group (13). Similar results have been reported whereby APOE ϵ 4 status moderated the inverse relation between ω -3 PUFA concentrations in erythrocyte membranes and cognitive function (14). However, other studies have shown contradictory results. In some studies, the benefit of dietary PUFA appeared to be restricted to APOE ϵ 4 positive individuals (15). Others have reported no effect of APOE genotype with respect to dietary PUFA and cognitive outcomes (16). Therefore, the interaction between APOE ϵ 4 status and dietary PUFA on cognitive function is likely complex, and warrants more research in this area.

The role of APOE genotype as a potential moderator of the association between dietary PUFA and cognition may be further clarified through the study of biomarkers of brain function. Glutamate (Glu), a neurometabolite critical for learning and memory (17), but neurotoxic at high concentrations (18), may be a sensitive marker to conditions of brain vulnerability and is implicated in the pathogenesis of neurodegenerative disorders. We have previously demonstrated that greater PUFA intake is related to lower cerebral Glu in a small sample of physically fit and healthy adults (8), adding support to the role of dietary PUFA in maintaining efficient regulation of glutamatergic synapses (7).

Whereas it appears that dietary PUFA, cognitive function, and cerebral Glu are related, it is currently unclear to what extent these links may differ as a function of APOE ϵ 4 allele status. Several studies have indicated that the beneficial cognitive effects of dietary PUFA may be attenuated for APOE ϵ 4 carriers (11–14). However, it is not known if positive correlations between PUFA and cognitive performance differ according to APOE ϵ 4 allele status as early as midlife. Furthermore, no studies to date have explored APOE genotype as a moderator of the association between PUFA and cerebral Glu. Therefore, the purpose of the present study was to investigate the effect of APOE ϵ 4 allele status on the links between dietary PUFA, cognitive function, and cerebral Glu in a sample of middle-aged adults. Specifically, it was hypothesized that greater dietary PUFA would be associated with better cognitive performance in APOE ϵ 4 non-carriers, but not in APOE ϵ 4 carriers. Second, given prior work from our team indicating potentially beneficial effects of dietary PUFA on cerebral Glu, it was hypothesized that a similar association between dietary PUFA and cerebral Glu would be observed in APOE ϵ 4 non-carriers, in that dietary PUFA would be inversely related to Glu, with Glu concentrations at the lower end of the normative range potentially indicative of lower risk for neurotoxicity, but that this association would not be present for APOE ϵ 4 carriers.

Method

Participants

Participants were comprised of a subsample of individuals recruited through flyers, newspaper, and online advertisements in the community as part of a larger study investigating cardiometabolic health and brain function. Exclusionary criteria included history of overt cardiovascular disease (e.g., coronary artery disease, angina pectoris, transient ischemic attack, myocardial infarction, heart failure, or cardiac surgery), neurological disease (e.g., stroke, Parkinson's disease, or clinically significant traumatic brain injury), major psychiatric illness (e.g., schizophrenia, bipolar disorder), substance abuse, or contraindications to magnetic resonance imaging (MRI). Individuals that completed the health assessment, dietary assessment, cognitive evaluation, and neuroimaging session portions of the larger study, comprised the participant sample for the current study. Dietary data were also checked for unusual or implausible caloric values via linear regression of caloric intake versus body mass index (BMI). Five participants were excluded from analysis due to having total energy intake residuals greater than 3 standard deviations from the mean, resulting in a total of 122 participants.

Procedure

The study was approved by the university Institutional Review Board and was conducted in accordance with the guidelines of the Helsinki Declaration of 1975. All volunteers provided written informed consent prior to participation. Participants completed an initial medical history questionnaire to screen for exclusionary medical conditions and subsequently underwent a health assessment. Within two months of the initial assessment, all eligible participants completed additional neuropsychological assessment, brain imaging, and dietary assessment. Participants did not report any major lifestyle or dietary changes during the data collection period.

APOE Genotyping

Saliva samples were collected using the Oragene Discover (OGR-500) kit, and stored at room temperature prior to analysis. DNA extraction using 500 uL of saliva was performed using the prepIT-L2P kit from DNAgenotek, and samples were stored at -40 degrees Celsius prior to genotyping. PCR was conducted using APOE-Fwd4 (GCT GAT GGA CGA GAC CAT GAA GGA GTT) and APOE-snapR (GCC CCG GCC TGG TAG ACT GCC A) primers. Polymerase chain reaction (PCR) amplification was performed with 10 ng of DNA and 10 pMol primer, with the following amplification protocol: 95 °C for 15 min, 35 cycles of (95 °C 30 sec., 65 °C 30 sec., 72 °C 30 sec.) and hold at 4°C.

APOE genotyping was conducted using PCR amplification and Sanger sequencing(19) with Variant Reporter Software from Life Technologies (Thermo Fisher Scientific). Sequence data was obtained with KB basecaller and chromatograms were analyzed via visual inspection for the rs429358C>T and rs7412C>T SNPs. Participants were categorized according to allele type, either ε4 negative (ε2/ε2, ε2/ε3, ε3/ε3) or ε4 positive (ε2/ε4, ε3/ε4, ε4/ε4). Due to sample size, APOE ε4 hetero- and homozygous individuals were combined together (n = 37), and compared to all APOE ε4 non-carriers (n = 85).

Health assessment

Participants were instructed to fast overnight for at least 8 hours and to abstain from physical exercise, alcohol consumption, and caffeine for at least 24 hours prior to attending the health assessment. Height and body weight were measured using a stadiometer and digital scale to calculate body mass index (BMI) kg/m². Waist circumference was measured with a non-elastic measuring tape placed around the trunk at the top of the iliac crest (20). A blood sample was taken from the antecubital vein through venipuncture by a certified phlebotomist to assess blood concentrations of triglycerides, HDL-cholesterol, and glucose. Arterial blood pressure was assessed in the supine position after resting for 15 minutes using a standard oscillometric blood pressure monitor.

Diet assessment

Characterization of nutrient intake was determined through 3-day food records in which participants were instructed to record all dietary intake for three consecutive days of their choice. Dietary intake entries were clarified by a trained research assistant and subsequently analyzed by a Registered Dietitian through the use of Nutritionist Pro Software (Axxya Systems, Stafford, TX, USA). Average energy and PUFA intake per day in grams was calculated and provided by the software. Due to previous work from our lab indicating similar associations between ω-3 and ω-6 PUFA intake and cerebral metabolism (8) as well as to reduce the number of statistical tests, the effects of overall PUFA (including both ω-3 and ω-6 PUFA) were examined in the present study.

¹H-magnetic resonance Spectroscopy

¹H-MRS data were acquired from a single session using a 3T Siemens Skyra scanner (Siemens Medical Solutions, Malvern, PA) with a 32-channel head coil. Anatomical scans of the brain were collected using high-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequences (256 × 256 matrix, flip angle = 7°, field of view

(FOV) = $24 \times 24 \text{ cm}^2$, 1 mm slice thickness, 0 gap, voxel size = $1.0 \times 1.0 \times 1.0 \text{ mm}^3$, TR = 2530.0 ms). Point-RESolved Spectroscopy (PRESS) sequence (svs_se_30) was used to obtain cerebral metabolite ratios using the following parameters: TE/TR = 30/3000 ms, 80 excitations, 2000 Hz spectral width, volume $\sim 6 \text{ cm}^3$ in the occipitoparietal grey matter including the posterior cingulate gyrus. The posterior cingulate gyrus was chosen given its close relation to cognition(21) and that metabolic changes in that region have been implicated in early stages of dementia(22). To ensure consistency of voxel placement, a digital archive was saved and reviewed (Figure 1). The concentration of Glu was reported as a ratio relative to creatine (Cr), a marker of energy metabolism, which is considered to be a stable metabolite for use as an internal reference (23). Glu was quantified at a frequency of 2.34 ppm, and the commercially available software, LCModel, was used to separate the metabolite resonance from the macromolecule background (24).

Neuropsychological Assessment

A battery of standard neuropsychological assessment instruments with established reliability and validity was administered to participants (25). The measures were grouped according to two cognitive domains: memory and executive function. Raw scores for each assessment were converted to z-scores and averaged with the other tests in each respective domain. Timed-test scores were multiplied by -1 so that higher scores indicated superior performance. The California Verbal Learning Test-II immediate and delayed recall, a measure of rote verbal learning and memory, comprised the *memory* domain (26). The Trail Making Test B (27) time to completion (a task of speeded mental flexibility), WAIS-III Digit Span Subtest(28) (a task of attention and working memory), and Controlled Oral Word Association Test (29) (a task of verbal fluency) made up the *attention-executive* domain. Global cognitive functioning was assessed by the Mini Mental Status Exam (MMSE) (30), a 30-point, brief assessment to determine cognitive impairment, and estimated intelligence was derived from scores on the Wechsler Test of Adult Reading (31), a measure that assesses vocabulary level as an indicator of premorbid intellectual function. A trained research assistant administered and scored the test battery using standard administration and scoring criteria.

Statistical Analyses

The interaction between APOE $\epsilon 4$ allele status and PUFA intake on cognitive function and cerebral Glu was tested using three multiple regression models defined in the PROCESS macro tool for SPSS (32). The PROCESS macro is a conditional process modeling program that uses an ordinary least squares-based path analytical approach to test for both direct and indirect effects. The simple moderation model (Model 1) was used, which is appropriate for testing and probing interactions by a single, hypothesized moderator variable. When assessing cognitive function, the composite cognitive performance scores (either overall executive function or overall memory performance) were entered as dependent variables. Clinically relevant covariates (age, gender, waist circumference, education, and total energy intake) were included in the models based on published associations with cognitive function (33–37). Similarly, when assessing neurochemistry, cerebral Glu/Cr was entered as the dependent variable, and covariates included age, gender, waist circumference, and total energy intake. In both models, APOE $\epsilon 4$ allele status was entered as the moderator variable,

and PUFA intake was entered as a continuous independent variable. All models included bias corrected nonparametric bootstrapping of 5000 samples and mean centering of the interaction term. All statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The criterion level for significance was set at an alpha level of 0.05.

The assumptions required by multiple regression (linearity, normality, multicollinearity, and homoscedasticity) were met. Linearity between the outcome and independent variable was assessed through visual examination of scatterplots. Shapiro-Wilk tests revealed that residuals for all three models were normally distributed ($p > 0.05$). Multicollinearity diagnostics were found to be acceptable (variance inflation factors for each model < 10 and correlation coefficients among all independent variables $< .80$). Finally, examining the plots of standardized residuals versus predicted values for each of the models suggested that points tended to be equally distributed across all values of the independent variables.

Results

Descriptive Statistics

Demographic data for participants ($n = 122$) are presented in Table 1. Participants did not significantly differ in total energy intake, total PUFA, carbohydrate, or protein intake ($p > .10$). Participants also did not statistically differ for physiological health and demographic variables according to APOE $\epsilon 4$ allele status.

PUFA, cognition, and APOE genotype

As evidenced in Table 2, individuals did not significantly differ in overall memory performance, executive function, or estimated IQ based on APOE $\epsilon 4$ allele status. When controlling for age, sex, education, and waist circumference, there was a significant effect of PUFA intake on memory performance ($p < .001$). Additionally, there was a significant interaction between PUFA intake and APOE genotype on memory performance, $F(1,113) = 6.749$, $p = .016$, $R^2 = .044$; Figure 2). For $\epsilon 4$ non-carriers, there was a significant, positive association between PUFA intake and the composite memory score ($t = 3.108$, $p < .001$, 95% CI .027 to .091) with higher PUFA intake resulting in better memory performance. PUFA intake was not significantly related to memory performance in $\epsilon 4$ carriers ($t = -.242$, $p = .809$, 95% CI $-.042$ to $.033$). Age was negatively associated with overall memory performance ($p = .004$), and females exhibited better overall memory performance relative to males ($p = .003$). A post hoc power analysis conducted with G*Power using the observed effect size ($R^2 = .044$) showed a post hoc power of 0.65.

A significant main effect of PUFA intake was also observed on performance in the executive-attention domain ($p = .013$), with greater PUFA intake being related to better overall executive function. Greater educational attainment was also positively associated with executive function performance ($p = .021$).

PUFA, cerebral glutamate, and APOE genotype

There was a significant interaction between PUFA intake and APOE $\epsilon 4$ allele status on Glu/Cr, $F(1,114) = 5.173$, $p = .025$, $R^2 = .037$ (Figure 3) such that greater PUFA intake was

significantly associated with greater cerebral Glu for $\epsilon 4$ carriers ($t = 2.7963$, $p = .006$, 95% CI .002 to .013) but no association was detected in the $\epsilon 4$ non-carriers ($t = -.115$, $p = .908$, 95% CI $-.005$ to $.004$). Age was also negatively associated with Glu/Cr ($p = .002$), and males showed greater Glu concentrations relative to females ($p = .039$). Post hoc power analysis conducted with G*Power using the observed effect size ($R^2 = .047$) indicated a post hoc power of 0.57.

Exploratory analyses

Due to differences in participant sample composition in that of Oleson et al. (2017), which included relatively healthy and physically fit adults, and that of the current study (participants included those with various vascular health risk factors and components of Metabolic Syndrome), we conducted an exploratory analysis examining the relation between ω -3 dietary PUFA and cerebral Glu in a sample that more closely resembled that of the previously done study in our lab (8). The participant sample was limited to APOE $\epsilon 4$ non-carriers that were also free of major health factors known to influence lipid-lipoprotein metabolism (central adiposity and dyslipidemia) (38). In this healthy sample of $\epsilon 4$ non-carriers, there was a significant association between ω -3 dietary PUFA and cerebral Glu, in that greater ω -3 dietary PUFA was related to lower cerebral Glu ($r = -.434$, $n = 26$, $p = .027$; Figure 4), similar to that observed in Oleson et al. (2017). However, the association was attenuated after controlling for age and gender, possibly due to lack of power ($\beta = -0.299$, $t = -1.335$, $p = .195$). The associations between total PUFA and Glu ($p = .195$) and ω -6 PUFA and Glu ($p = .550$) were non-significant.

Additionally, given growing evidence suggesting the importance of ω -3 intake relative to ω -6 intake for supporting brain function (39), the ratio of ω -3 to ω -6 was computed and examined in relation to Glu concentrations. For analyses, the ratio was computed by dividing ω -3 by ω -6 intake, so that greater values were indicative of more balanced intake. First, the interaction between the ω -3/ ω -6 ratio and APOE status was tested on Glu concentrations in the total sample. The interaction was not statistically significant ($p = .919$). When the sample was constricted to healthy $\epsilon 4$ non-carriers, there was a marginal trend toward more balanced intake of ω -3 and ω -6 PUFA being associated with lower cerebral Glu ($r = -.360$, $n = 26$, $p = .071$). However, this association was attenuated after controlling for age and gender, ($\beta = -0.243$, $t = -1.174$, $p = .253$).

Discussion

The major aim of the present study was to investigate the effect of APOE $\epsilon 4$ allele status on the links between PUFA intake, cognition, and cerebral Glu in a sample of middle-aged adults. Key findings from the analysis indicated an interaction between APOE genotype and PUFA intake on cognitive function, in that greater intake was associated with better overall memory performance specifically for $\epsilon 4$ non-carriers, but not for $\epsilon 4$ carriers. Additionally, greater Glu concentrations were observed in the posterior cingulate cortex for APOE $\epsilon 4$ carriers relative to non-carriers, potentially indicating altered cerebral Glu metabolism as one source of vulnerability to neurodegenerative disease in $\epsilon 4$ carriers. A significant interaction between APOE genotype and PUFA intake on cerebral Glu was also detected

such that greater dietary PUFA intake was related to higher Glu concentrations in $\epsilon 4$ carriers, but not in $\epsilon 4$ non-carriers. The current study builds on previous work connecting dietary fat, cerebral metabolism, and cognition (8), and to our knowledge, is the first study to explore the interaction between genetic and environmental factors (APOE genotype and dietary fat intake) on cognitive function and cerebral Glu.

A significant interaction between APOE genotype and dietary PUFA on cognition was observed in the present study such that there was a positive association between dietary PUFA intake and memory performance for $\epsilon 4$ non-carriers, but not for $\epsilon 4$ carriers. Whereas previous studies have examined dietary PUFA and cognition in middle age (40, 41), few have stratified results by APOE genotype. However, a growing area of research suggests that PUFA-rich diets do not appear to confer the same risk protection against dementia for $\epsilon 4$ carriers as they do non-carriers (11, 12). Interestingly, clinical trials involving PUFA supplementation have yielded mixed results (42, 43). One explanation for this may be differential effects of PUFA supplementation according to APOE $\epsilon 4$ allele status. Furthermore, evidence from clinical trials also suggests that cognitive benefits conferred by dietary PUFA are greatest before the onset of dementia symptoms or related neuropathology (44). These findings, along with the present study results, suggest a need for early dietary intervention studies that take APOE $\epsilon 4$ status into consideration.

The examination of cerebral Glu in the present study allows for one useful method to explore brain biomarkers in the context of dietary effects on cognition, and may also improve our understanding of early neuropathological changes in individuals at risk for AD. Whereas Glu serves as the major mediator of excitatory signals in the central nervous system, and plays an essential role in neuronal growth, synaptic plasticity, and regulation of neurotransmission, the excessive production of Glu becomes neurotoxic. Importantly, Glu levels are found to be higher in neurodegenerative disease states such as AD in comparison to normal aging. The finding that APOE $\epsilon 4$ carriers showed higher Glu concentrations relative to $\epsilon 4$ allele non-carriers is consistent with previous work indicating APOE-related differences in glutamatergic metabolism and transmission. Previous research utilizing rodent models has demonstrated altered Glu metabolism in that of APOE $\epsilon 4$ targeted replacement mice (45, 46). Specifically, APOE $\epsilon 4$ targeted replacement mice showed greater levels of vesicular glutamate transporter (VGLUT1), which is believed to be correlated with the amount of Glu that is stored and released (47). Prior work has also demonstrated impairments in synaptic plasticity and N-methyl-D-aspartate (NMDA) receptor phosphorylation associated with APOE $\epsilon 4$ (48). Taken together, these findings have led some researchers to propose that altered glutamatergic activity associated with APOE $\epsilon 4$ serves as one mechanism through which the $\epsilon 4$ isoform confers risk for or accelerates onset of dementia and neuronal degeneration.

The interaction between APOE genotype and dietary PUFA on cerebral Glu concentrations in the present study also provides insight into the mechanistic effects of dietary PUFA on the brain that may differ as a function of genetic risk for AD. Specifically, greater intake of dietary PUFA was correlated with higher Glu concentrations in $\epsilon 4$ carriers, but this link was not observed in $\epsilon 4$ non-carriers. One interpretation of this finding may be that greater PUFA intake in $\epsilon 4$ carriers is associated with elevated risk for neurotoxicity or cognitive decline.

Whereas PUFA has been linked to health and cognitive benefits generally, there is evidence suggesting that the APOE $\epsilon 4$ allele is related to alterations in lipid metabolism that leads to inefficient delivery of PUFA to neurons as well as altered lipid homeostasis in the brain (49). Furthermore, $\epsilon 4$ positive individuals have been observed to show greater sensitivity to dietary patterns such as those high in dietary fat (50), and have even shown increased total cholesterol and LDL levels following ω -3 PUFA supplementation relative to $\epsilon 4$ non-carriers (51). Accordingly, modulating dietary fat intake or determining appropriate fat intake levels for $\epsilon 4$ individuals may be especially critical for the prevention of cognitive decline in this genetically at-risk population.

An alternative interpretation of the current findings may be that the present study demonstrates an association between PUFA and Glu for $\epsilon 4$ carriers that could be attenuated, or even reversed, at greater or varied levels of PUFA intake. Regarding ω -3 PUFA supplement use, research has suggested that $\epsilon 4$ carriers may require greater doses of ω -3 PUFA relative to $\epsilon 4$ non-carriers to obtain comparable cognitive and health benefits (52). Similarly, the American Heart Association recommends at least 1 gram of daily ω -3 PUFA intake for healthy individuals, but has also suggested increasing dosage for individuals with existing cardiovascular risk factors (53). Although not a statistically significant difference in the present study, the average ω -3 PUFA intake for $\epsilon 4$ carriers ($M = 1.11$ grams) was on the lower end of recommended daily intake, relative to $\epsilon 4$ non-carriers ($M = 1.42$ grams). However, the APOE groups did not differ in overall ratio of ω -3 to ω -6 PUFA intake. Despite this, one possibility may be that the optimal ratio of these two fatty acids for supporting brain function may differ according to APOE genotype. Based on our exploratory analyses, there was no statistically significant association between the ratio of ω -3 to ω -6 dietary PUFA and Glu concentrations in $\epsilon 4$ carriers. Interestingly, there was a marginal trend suggesting that more balanced intake of ω -3 and ω -6 PUFA may be predictive of Glu concentrations in healthy $\epsilon 4$ non-carriers when the sample was restricted to individuals without central adiposity or dyslipidemia. Therefore, this may suggest PUFA composition and cardiovascular health status are important factors to consider for improving our understanding APOE-PUFA effects on brain function. Future studies with greater sample size to assess these complex associations, and that also allow for manipulation of dietary PUFA intake composition will likely be important for clarifying optimal levels of either absolute or relative PUFA to support brain function according to APOE status.

Previous work utilizing rodent models has also demonstrated interactions between APOE and dietary nutrients on glutamatergic function. One study observed reduced levels of hippocampal Glu in mice after supplementation of a multi-nutrient diet rich in ω -3 fatty acids in APOE knockout, but not in APOE $\epsilon 4$ positive mice (54). However, another study demonstrated benefits of ω -3 PUFA in $\epsilon 4$ positive rats, with fish oil supplementation resulting in attenuation of APOE-related alterations in presynaptic Glu function (46). These findings, as well as that of the present study, indicate that diet interacts with APOE genotype to affect Glu metabolism; however, results appear to be mixed regarding the precise direction of these findings. Previous work from our team has demonstrated that in middle-aged adults, lower dietary PUFA was associated with greater, potentially more neurotoxic cerebral Glu (8). Whereas a similar negative correlation between dietary PUFA and cerebral Glu was observed for $\epsilon 4$ non-carriers in the present study, this association was not

statistically significant. One potential explanation for the seemingly discrepant findings between our two studies is the difference in composition of our participant samples. Our previous study sample was mainly composed of “supercontrols” – middle aged adults with very few health conditions and relatively high level of fitness – but the current study participants were composed of individuals with varying cardiometabolic health, many of whom had pre-clinical and clinical hypertension, hyperglycemia, obesity, and dyslipidemia. This hypothesis is supported by an exploratory analysis in the present study that demonstrated a statistically significant negative association between ω -3 PUFA and Glu (similar to previous work from our lab (8)) when the ϵ 4 non-carrier sample was restricted to individuals without dyslipidemia or abdominal obesity. Therefore, it is possible that cardiovascular risk factors that influence lipid metabolism may also impact glutamatergic functioning.

Although the present study has several strengths including detailed characterization of physiological health factors and brain function in a sample of middle-aged adults with varying genetic risk for AD, some important limitations must also be acknowledged. First, conclusions drawn regarding the link between dietary PUFA, cerebral Glu, and cognition are limited by the cross-sectional nature of the study. Additionally, given the results of post hoc power analyses conducted for the present study (power ranged from 0.57 to 0.65), it will be important for future studies that are amply powered with greater sample size to replicate these results. Future intervention studies and those that employ longitudinal methods with greater sample size are needed to fully clarify the differential effects of dietary PUFA according to APOE genotype. Furthermore, whereas validated 3-day dietary records used to characterize nutritional intake are considered an ideal method for assessing nutritional intake (55), this method still heavily relies on participant self-report and may not represent long-term dietary habits. Additionally, it is possible that differences in lipid metabolism based on APOE ϵ 4 status **or other factors** may underlie the effects observed in the present study. Prior studies have also indicated that endogenous sex hormones appear to influence lipid metabolism (56), which raises the possibility of sex differences or menopausal status potentially confounding the current results. Of note, our participant sample included more women than men, and menopausal status was not controlled for in the current study. Although sex was included as a covariate in all analyses, it is still possible that the influence of hormonal status on lipid metabolism may influence PUFA-brain associations. Future studies will likely benefit from comparing dietary record data and measures of PUFA uptake in erythrocytes or plasma in order to account for variability in lipid metabolism.

Overall, the current study demonstrated an interaction between APOE ϵ 4 allele status and dietary PUFA on cerebral Glu and cognition during midlife. Specifically, higher PUFA intake was related to higher cerebral Glu concentrations in the posterior cingulate cortex for APOE ϵ 4 carriers, but this was not the case for APOE ϵ 4 non-carriers. At the same time, the finding that higher PUFA intake was associated with better memory performance was isolated to APOE ϵ 4 non-carriers. The present results add to an accumulating body of research suggesting APOE-related differences in cognitive outcomes are connected with dietary PUFA, and propose dietary PUFA effects on glutamatergic function as one possible mechanism underlying this relation. Effective treatments for dementia are currently lacking

and therefore, the study of modifiable lifestyle factors such as diet in conjunction with identifying useful biomarkers of brain health is a critical strategy for early intervention. Dietary interventions that are tailored to APOE genotype may be beneficial for the preservation of cognitive functioning.

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Figure 1.
Anatomical image with superimposed voxel outline of ¹H MRS volume in the occipitoparietal gray matter.

Figure 2.

Interaction between APOE genotype and PUFA intake on overall memory performance ($p = .031$) indicated by multiple regression analysis. PUFA intake was positively associated with memory performance in $\epsilon 4$ non-carriers ($p = .002$), but not for $\epsilon 4$ carriers ($p = .657$).

Figure 3.

Interaction between APOE genotype and PUFA intake on cerebral Glu concentrations ($p = .011$) indicated by multiple regression analysis. Higher PUFA intake was associated with greater Glu concentrations in $\epsilon 4$ carriers ($p = .009$), but PUFA was not significantly related to Glu in $\epsilon 4$ non-carriers ($p = .445$).

Figure 4. correlation between dietary ω -3 PUFA and cerebral Glu in APOE ϵ 4 non-carriers when excluding those with central adiposity and dyslipidemia ($p = .027$). Greater total PUFA was associated with lower cerebral Glu concentrations in healthy APOE ϵ 4 non-carriers.

Table 1.

Selected demographic characteristics and average nutrient intake for study participants.

Total Participants (N = 122)	e4 non-carriers	e4 carriers	
	M (SD)	M (SD)	p value
<i>Demographic and Physiological Characteristics</i>			
Male/Female	39/46	12/25	.162
Age (years)	49.58 (6.31)	48.30 (6.32)	.306
Education (years)	16.45 (2.94)	15.79 (2.27)	.231
Waist circumference (cm)	97.57 (16.65)	99.54 (17.08)	.553
BMI (kg/m ²)	29.84 (6.84)	29.79 (7.05)	.967
Blood glucose (mg/dL)	99.52 (30.47)	95.97 (24.74)	.534
Systolic blood pressure (mm Hg)	121.64 (14.98)	124.78 (16.67)	.312
Diastolic blood pressure (mm Hg)	73.32 (10.45)	74.39 (10.85)	.613
Total cholesterol (mg/dL)	200.51 (40.75)	197.84 (43.90)	.746
Triglycerides (mg/dL)	121.48 (83.76)	133.12 (93.94)	.513
HDL cholesterol (mg/dL)	52.55 (17.59)	49.68 (19.80)	.428
LDL cholesterol (mg/dL)	127.15 (37.29)	119.40 (39.85)	.348
<i>Average Nutrient Intake</i>			
Total caloric intake (kcal/day)	1921.13 (555.68)	1992.40 (662.25)	.541
Carbohydrate (g)	214.13 (76.02)	231.89 (89.62)	.264
Protein (g)	83.28 (28.64)	80.98 (22.91)	.666
Total fat (g)	79.91 (28.66)	78.58 (29.48)	.817
Saturated fat (g)	25.57 (11.97)	24.75 (12.81)	.734
Polyunsaturated fat (PUFA) (g)	13.30 (6.13)	12.09 (6.92)	.337
ω-3 PUFA (g)	1.42 (1.12)	1.11 (0.89)	.105
ω-6 PUFA (g)	10.73 (5.23)	9.68 (6.10)	.337
ω-6/ω-3 ratio	10.43 (8.68)	10.66 (6.36)	.886

HDL = high density lipoprotein; LDL = low density lipoprotein

Bold p-values represent <.05. Values are mean ± standard deviation.

Table 2.

Cerebral Glu and neuropsychological assessment for study participants.

Total Participants (<i>N</i> = 122)	e4 non-carriers <i>M (SD)</i>	e4 carriers <i>M (SD)</i>	<i>p</i> value
<i>Neurochemical concentration</i>			
Glutamate (Glu/Cr)	1.23 (.11)	1.28 (.13)	.018
<i>Estimated Intelligence</i>			
FSIQ	113.53 (14.11)	111.51 (18.48)	.512
<i>Memory (z score)</i>	0.06 (.82)	−0.07 (.97)	.430
CVLT-II immediate recall	11.02 (2.83)	10.30 (3.88)	.249
CVLT-II delayed recall	11.65 (2.59)	10.30 (3.88)	.237
CVLT-II discriminability index	3.01 (.64)	3.04 (.61)	.791
<i>Attention-executive function (z score)</i>	0.01 (.72)	0.01 (.86)	.982
Trail Making Test B time, seconds	65.34 (23.47)	70.46 (49.72)	.443
WAIS-III Digit Span Subtest	19.79 (4.52)	20.89 (4.14)	.206
COWAT	41.45 (12.14)	42.22 (11.38)	.744

FSIQ = Full scale intelligence quotient; CVLT-II = California verbal learning test, second edition; Wechsler adult intelligence scale, third edition;
COWAT = controlled oral association test