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Association of Docosahexaenoic Acid Supplementation With Alzheimer Disease Stage in Apolipoprotein E ε4 Carriers
A Review

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IMPORTANCE The apolipoprotein E ε4 (*APOE4*) allele identifies a unique population that is at significant risk for developing Alzheimer disease (AD). Docosahexaenoic acid (DHA) is an essential ω-3 fatty acid that is critical to the formation of neuronal synapses and membrane fluidity. Observational studies have associated ω-3 intake, including DHA, with a reduced risk for incident AD. In contrast, randomized clinical trials of ω-3 fatty acids have yielded mixed and inconsistent results. Interactions among DHA, *APOE* genotype, and stage of AD pathologic changes may explain the mixed results of DHA supplementation reported in the literature.

OBSERVATIONS Although randomized clinical trials of ω-3 in symptomatic AD have had negative findings, several observational and clinical trials of ω-3 in the predementia stage of AD suggest that ω-3 supplementation may slow early memory decline in *APOE4* carriers. Several mechanisms by which the *APOE4* allele could alter the delivery of DHA to the brain may be amenable to DHA supplementation in predementia stages of AD. Evidence of accelerated DHA catabolism (eg, activation of phospholipases and oxidation pathways) could explain the lack of efficacy of ω-3 supplementation in AD dementia. The association of cognitive benefit with DHA supplementation in predementia but not AD dementia suggests that early ω-3 supplementation may reduce the risk for or delay the onset of AD symptoms in *APOE4* carriers. Recent advances in brain imaging may help to identify the optimal timing for future DHA clinical trials.

CONCLUSIONS AND RELEVANCE High-dose DHA supplementation in *APOE4* carriers before the onset of AD dementia can be a promising approach to decrease the incidence of AD. Given the safety profile, availability, and affordability of DHA supplements, refining an ω-3 intervention in *APOE4* carriers is warranted.

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Supplemental content

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Alzheimer disease (AD) is the most common degenerative brain disorder, characterized by cognitive impairment and abnormal deposition of amyloid plaques and neurofibrillary tangles in the brain. The apolipoprotein E ε4 (*APOE4*) allele contributes the greatest attributable genetic risk for AD.[1] Humans express 3 alternative isoforms of APOE—ε2, ε3, and ε4—the most common of which is *APOE* ε3. The ε4 allele frequency in the general population is 15% but is increased to 40% in patients with AD. Individuals with 1 ε4 allele are 3 to 4 times more likely to develop AD as those without an ε4 allele, and people with 2 ε4 alleles have a 12-fold higher risk of developing AD.[2]

Several mechanisms have been proposed by which *APOE4* might lead to an increased risk for AD, including (1) less effective clearance of β-amyloid (Aβ) from brain to blood,[3] (2) reduced neuroplasticity and resilience in the face of brain injury,[4] (3) pronounced inflammatory response,[5] and (4) a direct pathologic effect on the cerebrovascular system.[6] Clinical studies suggest an interaction between *APOE4* and docosahexaenoic acid (DHA). Docosahexaenoic acid is an essential long-chain, ω-3 polyunsaturated fatty acid.[7] Among brain lipids, DHA is of particular importance in AD because the brain requires DHA for production and clearance of Aβ, maintenance of neuronal membranes, modulation of inflammation,[8] and improvement of vascular health.[9] Levels of DHA are reduced in the brains of humans with AD compared with cognitively healthy older adults.[10] Owing to these observations, randomized clinical trials of ω-3 fatty acid supplementation for AD treatment have been undertaken but yielded mixed and inconsistent results.

In this review, we summarize landmark observational and clinical trials that associate DHA with AD risk and focus on the differential interactions between ω-3 and *APOE* genotype during the dementia and predementia stages of AD. We present mechanisms to support inefficient delivery of DHA by APOE4 proteins to brain

Table 1. Randomized Clinical Trials of ω-3 Supplementation and Cognitive Outcomes

Source by Study Condition	Mean Age, y	Intervention	Duration	Outcomes	No. of Participants	Comments
Alzheimer disease						
Quinn et al,[13] 2010	76	2 g/d of DHA vs placebo	18 mo	Cognitive tests	384	No overall effect
Freund-Levi et al,[14] 2006	73	1.7 g/d of DHA and 0.6 g/d of EPA vs placebo	12 mo	Cognitive tests	204	No overall effect
Mild cognitive impairment						
van de Rest et al,[15] 2008	70	1800 mg/d of EPA-DHA and 400 mg/d of EPA-DHA vs placebo	6 mo	Cognitive tests	302	No overall effect
Chiu et al,[16] 2008	75	1080 mg/d of EPA and 720 mg/d of DHA vs placebo	24 mo	Cognitive tests	30	No overall effect. Among participants with MMSE score >27, improved cognition scores
Lee et al,[17] 2013	65	430 mg/d of DHA and 150 mg/d of EPA vs placebo	12 mo	Cognitive tests	36	Improved short-term and working memory
Sinn et al,[18] 2012	74	EPA (1.67 g/d EPA + 0.16 g/d DHA), DHA (1.55 g/d of DHA + 0.40 g/d of EPA) or linoleic acid (2.2 g/d)	6 mo	GDS and cognitive tests	54	Verbal fluency improved in the DHA group
Cognitively healthy						
Stonehouse et al,[19] 2013	33	1.16 g/d of DHA vs placebo	6 mo	Cognitive tests	176	Improved memory retention times
Külzow et al,[20] 2016	50-75	2200 mg/d of DHA and EPA vs placebo	6 mo	Memory outcomes	44	Cued recall was significantly better after ω-3 supplementation
Benton et al,[21] 2013	22	400 mg/d of DHA vs placebo	50 d	Memory outcomes	285	No effect on cognitive scores
Jackson et al,[22] 2012	22	1 g/d of DHA or 1 g/d of EPA vs placebo	12 wk	Cognitive tests	159	No effect on cognitive scores
Yurko-Mauro et al,[23] 2010	70	900 mg/d of DHA vs placebo	6 mo	Memory outcomes	485	Improved learning and memory function
Johnson et al,[24] 2008	68	800 mg/d of DHA vs placebo	4 mo	Memory outcomes	20	Improved verbal fluency scores
Danghour et al,[25] 2010	75	200 mg/d of EPA plus 500 mg/d of DHA vs placebo	24 mo	Memory outcomes	867	Cognitive function did not decline in either study arm for duration
Geleijnse et al,[26] 2012	69	400 mg/d of EPA-DHA vs placebo	40 mo	Global cognition	1265	No effect of dietary doses of ω-3 fatty acids on global cognitive decline

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GDS, Geriatric Depression scale; MMSE, Mini-Mental State Examination.

regions involved in AD during the predementia stage. In contrast, we present data to support DHA catabolism in the dementia stage. We hypothesize that DHA supplementation in *APOE4* carriers can prevent or delay the onset of AD when the timing precedes the onset of neurodegeneration. We suggest 3 disease stages, delineated by specific biomarkers, to guide the evaluation of the differential efficacy of treatment.

Methods

We searched the PubMed database for original articles, systematic reviews, and meta-analyses of ω-3 studies in AD that were published before August 30, 2016. Our results included preclinical studies, cross-sectional studies, longitudinal cohorts, and randomized clinical trials. For preclinical studies, we discuss findings from a 2012 meta-analysis from Hooijmans et al.[11] For observational studies, we discuss key findings of cross-sectional and longitudinal cohorts and present summaries of these studies in the eTable in the Supplement. The results of a meta-analysis on ω-3 and AD incidence conducted by Zhang et al[12] are then discussed. For randomized clinical trials, we present summaries of key findings of 14 ω-3 trials in **Table 1** and discuss conclusions of a Cochrane systematic review[27] from 2012 and a systematic review by Yurko-Mauro et al[28] from 2015. We then examine mechanistic studies that link *APOE* genotype with DHA metabolism to understand the effect of *APOE4* on DHA brain delivery and discuss the role of recent advances in brain imaging.

Observations

Role of Long-term DHA Supplementation in the Prevention of AD in Animal Studies

Docosahexaenoic acid significantly affects hippocampal neuronal development and synaptic function in the developing hippocampus. In embryonic neuronal cultures, DHA supplementation promotes neurite growth and synaptic protein expression.[29] Long-term deficiency of DHA in the diet led to learning impairment in an animal model.[30] A systematic review that focused on the effects of relatively long-term ω-3 supplementation (minimum period, 10% of an average total lifespan) in AD animal models reported significant reductions in detergent-insoluble amyloid levels and plaque burden and improved cognitive outcomes.[11] The largest beneficial effects were observed when DHA supplementation was started before

Table 2. Effect of *APOE* Genotype on Cognitive Outcomes in ω-3 Studies

Source by Study Type	Mean Age, y	Participant Condition	Association Between ω-3 and Cognitive Outcomes	Beneficial Response
Randomized clinical trial				
Quinn et al,[13] 2010	76	Alzheimer disease	ω-3 Improved 2 cognitive scores (ADAS-cog and MMSE)	*APOE4* noncarriers
Stonehouse et al,[19] 2013	33	Cognitively healthy	ω-3 Improved memory retention time	*APOE4* carriers
Vellas et al,[39] 2015	75	Cognitively healthy	ω-3 Improved composite score	*APOE4* carriers
van de Rest et al,[15] 2008	70	Cognitively healthy	ω-3 Improved attention domain	*APOE4* carriers
Observational studies				
Daiello et al,[35] 2015	75	Alzheimer disease	Association of ω-3 intake with cognitive scores and brain volumes	*APOE4* noncarriers
Huang et al,[32] 2005	72	Cognitively healthy	Fatty fish consumption associated with reduced risk for dementia	*APOE4* noncarriers
Barberger-Gateau et al,[33] 2007	>65	Cognitively healthy	Association of fish consumption with reduced risk of dementia	*APOE4* noncarriers
Whalley et al,[34] 2008	64	Cognitively healthy	Association of cognitive scores with RBC DHA	*APOE4* noncarriers
Laitinen et al,[36] 2006	50	Cognitively healthy	Fatty fish consumption associated with reduced risk for dementia	*APOE4* carriers
Samieri et al,[38] 2011	74	Cognitively healthy	Plasma DHA associated with slower decline on Benton Visual Retention Test	*APOE4* carriers
Morris et al,[37] 2016	83	Cognitively healthy	Association of seafood consumption with brain autopsy AD neuropathologic changes was stronger	*APOE4* carriers

Abbreviations: ADAS-cog, cognitive subscale of the Alzheimer's Disease Assessment Scale; *APOE4*, apolipoprotein E ε4 allele; DHA, docosahexaenoic acid; MMSE, Mini-Mental State Examination; RBC, red blood cell.

initiating a toxic experimental model of AD (such as infusing Aβ peptide in the rat cerebral ventricle). Supplementation with DHA attenuated AD pathologic changes in *APOE4* transgenic mouse models of AD[31] by restoring memory and learning and synaptic functions and reducing hippocampal Aβ42 levels to *APOE3* levels. This finding underscores a role of long-term DHA supplementation in the prevention rather than in the treatment of AD, and that the AD phenotype in *APOE4* transgenic mouse model can be prevented with DHA supplementation.[11]

Observational Studies Linking ω-3 Intake and Levels With Cognitive Outcomes

Most observational studies associate higher levels of seafood, ω-3 consumption, or ω-3 blood levels with decreased incidence of AD, better cognitive measures, or preserved brain volume in AD-vulnerable regions (eTable in the Supplement). In general, observational studies investigated participants without evidence of cognitive impairment at baseline for a follow-up duration ranging from 2 to 20 years and evaluated outcomes such as AD incidence or cognitive decline. Zhang et al[12] summarized 21 of these studies in a meta-analysis of 181 580 participants, with 4438 dementia cases identified during follow-up ranging from 2.1 to 21 years. The authors concluded that a 1-serving/wk increment of dietary fish was associated with significantly lower risk for AD dementia (relative risk, 0.93; 95% CI, 0.90-0.95; $P = .003$).

Randomized Clinical Trials of ω-3s in AD Dementia and Predementia

The effects of ω-3 supplementation on cognitive outcomes in randomized clinical trials[13-26] have been mixed (Table 1). Supplementation was not effective in the treatment of symptomatic AD.[13,14] In studies that included participants with mild or no cognitive impairment, the results were mixed. A Cochrane review[27] in 2012 found no evidence to support ω-3 supplementation in cognitively healthy older individuals. In contrast, a 2015 meta-analysis that examined additional studies[28] concluded that ω-3 supplementation significantly improves episodic memory. In cognitively healthy individuals, 6 randomized clinical trials demonstrated better memory functions with ω-3 supplementation compared with placebo,[17-20,23,24] whereas 6 trials reported no benefit.[15,16,21,22,25,26] Some of these randomized clinical trials had limitations. For example, in 2 trials of cognitively healthy younger adults, the duration of follow up was less than 6 months.[21,22] Some trials used low doses of ω-3.[21,26] In 1 trial, no cognitive decline in the placebo and the ω-3 intervention arms was observed.[25]

We suggest the following 4 factors that might explain these variable results: (1) limitations or differences in study design (eg, age, dose, or duration); (2) selection of participants who will not progress to AD dementia; (3) stage of disease at the time of ω-3 supplementation; and (4) baseline ω-3 consumption. Given the safety profile, availability, and affordability of ω-3 supplements, refining ω-3 interventions and trial designs to identify a population that might have a beneficial response is worthwhile.

Effect of *APOE* Genotype on the Association of ω-3 With Cognitive Outcomes

The *APOE* genotype often appears to affect the response to ω-3 supplementation, and, conversely, ω-3 supplementation can affect the influence of *APOE* genotype on AD symptoms,[13,15,19,32-39] although these results are inconsistent (**Table 2**). Some observational studies do not reveal an effect of *APOE* status on the

association of ω-3 with cognitive outcomes.[40-42] An inverse association between low serum DHA levels and cerebral amyloidosis was reported in older nondemented participants independent of *APOE* genotype.[43]

In some observational studies, the benefit of increased seafood or ω-3 consumption on cognition was restricted to *APOE4* noncarriers[32-35] and, in particular, those with limited seafood intake (<1 serving/wk).[32,33] The Alzheimer's Disease Cooperative Study (ADCS)–sponsored DHA trial reported a null effect on cognitive outcomes, but a preplanned analysis revealed a cognitive benefit (using the cognitive subscale of the Alzheimer Disease Assessment Scale) in the DHA treatment arm in *APOE4* noncarriers.[13]

In other studies, benefit was restricted to *APOE4* carriers.[15,19,36,37] The beneficial response in *APOE4* carriers was observed in younger participants in the randomized clinical trial by Stonehouse et al[19] (mean age, 33 years) and the observational cohort of Laitenen et al[36] (mean age, 50 years, with 20-year follow-up). In a cross-sectional study of deceased participants from the Rush Memory and Aging Project,[37] participants were free of dementia at study entry and underwent annual clinical neurologic evaluations and brain autopsy at death with a mean follow-up duration of 8 years. Individuals who were *APOE4* carriers and consumed at least 1 meal of seafood per week or had higher intakes of long-chain ω-3 fatty acids had less AD neuropathologic changes compared with those who consumed lower amounts.

A recent report in the ADCS-sponsored DHA clinical trial found that baseline cerebrospinal fluid (CSF) DHA levels were lower in *APOE4* carriers compared with *APOE2* carriers.[44] After treatment, lower DHA levels were observed in persons with more advanced brain disease as determined by the lowest tertile of CSF Aβ42 levels.[44] These findings agree with those of preclinical studies in 13-month-old, *APOE*–targeted replacement (TR) mice, where brain DHA levels were lower in *APOE4*-TR mice compared with *APOE2*-TR mice.[45] Accordingly, we propose a complex interaction between *APOE4* status and disease stage such that the response to ω-3 supplementation depends on whether supplementation precedes the onset of neurodegeneration. These studies indicate that *APOE4* is a modifiable AD risk factor, and that the effect of *APOE4* on AD pathologic changes can be attenuated with DHA supplementation.

Mechanisms Underlying the Association of *APOE4* With DHA Brain Uptake

Isoforms of APOE differ in structure from each other only at amino acids 112 and 158. The *APOE2* allele has cysteine at both sites, the *APOE4* allele has arginine at both positions, and the *APOE3* allele contains cysteine at amino acid 112 and arginine at amino acid 158. Such minor differences give rise to variability in domain interactions with multiple molecules, including the low-density lipoprotein receptor, cell-surface heparin sulfate proteoglycans, adenosine triphosphate–binding cassette protein 1 (ABCA1), and low-density lipoprotein–related proteins, as well as with protein stability and protein folding.[46] Differences in the metabolism of APOE particles are likely directly related to APOE conformation and receptor binding. APOE2 exhibits a more stable conformation and has lower affinity to the low-density lipoprotein receptor, which may lead to decreased catabolism. In contrast, APOE4 has a less stable conformation, increased affinity to the low-density lipoprotein receptor, and increased catabolism.

Effect of *APOE4* on DHA Transport Before the Onset of Neurodegeneration

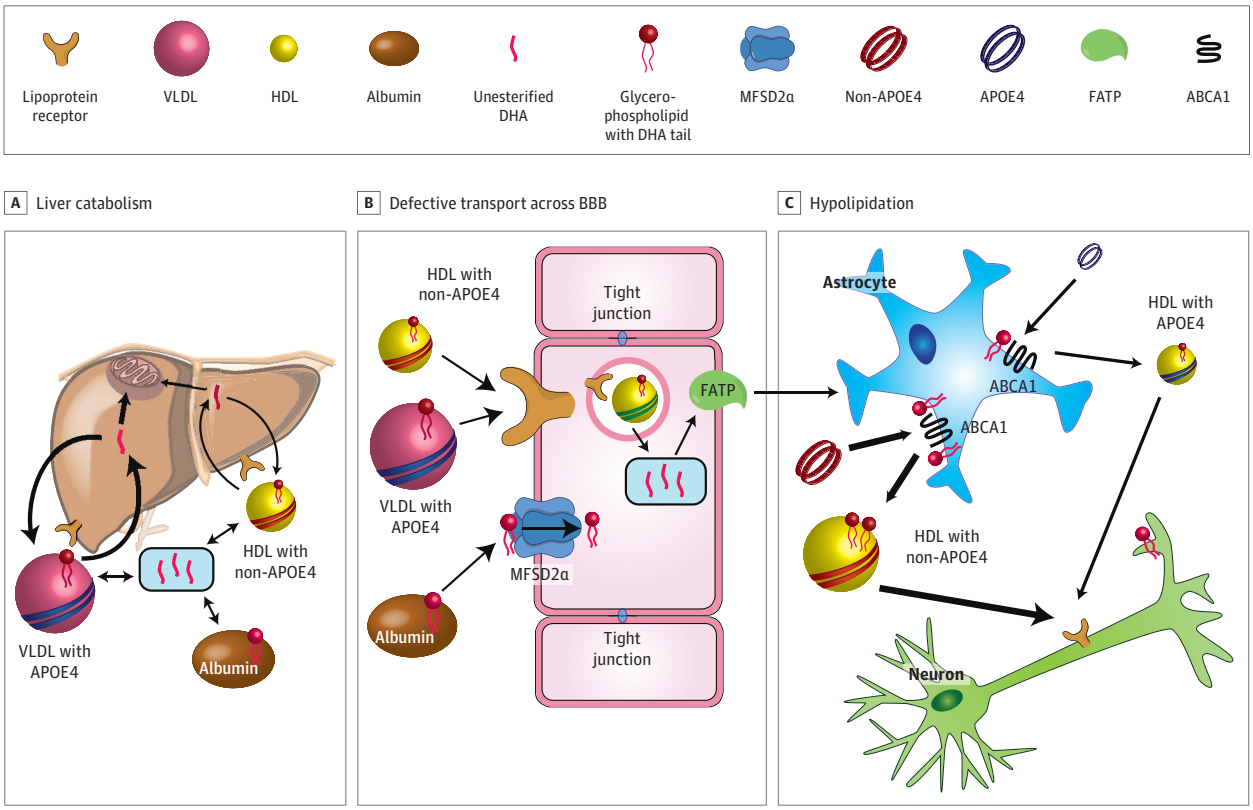
Several mechanisms link *APOE4* with reduced brain DHA metabolism. These mechanisms include (A) accelerated liver catabolism of DHA, (B) defective DHA transfer across the blood-brain barrier, and (C) hypolipidated or decreased APOE particle numbers, resulting in less efficient DHA transport (**Figure 1**). Evidence supports the contention that these *APOE4* mechanisms operate before the onset of neurodegeneration.

Accelerated Catabolism of APOE4 Lipoproteins and Its Relevance to DHA Bioavailability | Lower DHA availability in the brain in *APOE4* carriers may occur in part because of increased liver catabolism of DHA. Docosahexaenoic acid is a fatty acid that is transported on lipoproteins after consumption.[47] Its catabolism depends on its delivery to the liver. Very-low-density lipoproteins (VLDL) are catabolized faster than high-density lipoproteins (HDL). APOE4 apolipoproteins, which preferentially bind to VLDL, are catabolized faster than APOE3 apolipoproteins,[48] which preferentially bind to the longer-lived HDL. One direct example of faster DHA catabolism in *APOE4* carriers comes from a study conducted by Chouinard-Watkins et al.[49] Forty cognitively healthy older participants received a single dose of 40 mg of DHA labeled with carbon 13 (^{13}C). In *APOE4* carriers, ^{13}C-DHA levels in plasma total lipids from 1 hour to 28 days after the dose were 31% lower compared with levels in *APOE4* noncarriers. These findings suggest increased peripheral DHA catabolism, potentially limiting DHA availability to the brain in *APOE4* carriers.

Blood-Brain Barrier Integrity Coupled With Transport of DHA to the Brain | A decrease in the ratio of CSF to plasma DHA was reported in patients with dementia at the lowest tertile of CSF Aβ42 levels.[44] Transgenic mouse models prone to brain amyloid deposition (3xTg-AD mice) demonstrated less delivery of carbon 14–labeled DHA across the blood-brain barrier compared with littermate controls.[50] The *APOE4* allele is associated with breakdown in blood-brain barrier integrity.[51] Delivery of DHA to the brain and blood-brain barrier integrity are both regulated by the major facilitator superfamily domain–containing 2A transporter (MFSD2a).[52] The *APOE4* allele may compromise the integrity of lipoprotein and MFSD2A transporter functions, providing a common mechanism for reduced DHA uptake and loss of blood-brain barrier integrity that associate with early stages of AD pathologic changes.

Hypolipidation of APOE4 Lipoproteins in the Brain | APOE4 proteins expressed from astrocytes carry fewer lipids (ie, hypolipidation)[53] at a young age. In vivo, APOE complexes isolated from hippocampal sections of *APOE4* replacement mice[54] are smaller in size, suggesting hypolipidation compared with *APOE3* or *APOE2* replacement mice. These findings can explain why *APOE4* replacement mice have decreased delivery of labeled DHA to the brain compared with *APOE2* replacement mice.[45] Importantly, DHA treatment attenuates the effect of the *APOE4* allele on AD pathologic changes[31] in 4-month-old mice. A recent report[55] found decreased ABCA1-mediated cholesterol efflux capacity in the CSF of *APOE4* homozygous carriers that was associated with decreased CSF lipids compared with *APOE4* noncarriers. Enhancing ABCA1 activity may be a viable strategy to reverse hypolipidation of APOE4 HDL.

Figure 1. Mechanisms Linking Apolipoprotein E ε4 (*APOE4*) Status With Docosahexaenoic Acid (DHA) Delivery to the Brain Before the Onset of Neurodegeneration



Several mechanisms associate the *APOE4* allele with DHA brain delivery, including accelerated liver catabolism of APOE4 lipoproteins, defective transport across the blood-brain barrier (BBB), and hypolipidated APOE particles in the brain. These changes in DHA brain metabolism appear before the onset of neurodegeneration. ABCA1 indicates adenosine triphosphate–binding cassette protein 1; FATP, fatty acid transport protein; HDL, high-density lipoprotein; MFSD2a, major facilitator superfamily domain–containing 2A transporter; and VLDL, very-low-density lipoprotein.

DHA Catabolism With Neurodegeneration

Neurodegeneration in AD dementia is associated with activation of catabolic pathways[56] that oxidize DHA, converting it into F4-neuroprostanes (**Figure 2**). Neuroprostanes accumulate in patients with AD.[57] Phospholipase A_2 (PLA_2) constitutes a complex family of phospholipases that include calcium-independent and calcium-dependent PLA_2. Several lines of evidence suggest that calcium-dependent signaling pathways are dysregulated in the neurons of amyloidosis-prone mice, particularly in the hippocampus.[58] Amyloid pathologic features induce the activity of calcium-dependent PLA_2,[59] which reduces brain DHA consumption through liberation of free DHA from CSF and brain phospholipids. Another investigation[60] demonstrated greater CSF calcium-dependent PLA_2 activity in persons with mild cognitive impairment and in patients with AD compared with age-matched, cognitively healthy older adults. This greater activity was associated with reduced DHA concentrations in CSF phospholipids.

Association of DHA Levels With Cerebral Amyloidosis by Disease Stage

In the ADCS-sponsored DHA trial conducted among persons with mild to moderate AD,[44] plasma DHA levels did not correlate with cerebral amyloidosis as determined by CSF Aβ42 levels. In contrast, Yassine et al[43] demonstrated an association between lower plasma DHA levels and cerebral amyloidosis in the observational Aging Brain Study independent of *APOE* genotype. The Aging Brain Study differed from the ADCS-sponsored DHA trial by recruiting participants without dementia who were enriched for vascular risk factors. **Figure 3** illustrates the association between plasma DHA levels (using scaled DHA and amyloid deposition units to allow for comparison). The association of DHA with amyloid deposition in predementia but not clinical disease led to formulation of a timing hypothesis for a DHA intervention in preclinical *APOE4* carriers. Based on these findings, we hypothesize that an intervention using high-dose DHA supplementation in cognitively healthy *APOE4* carriers can slow the rate of progression to prodromal or clinical AD.

Designing an Informative Clinical Trial in *APOE4* Carriers

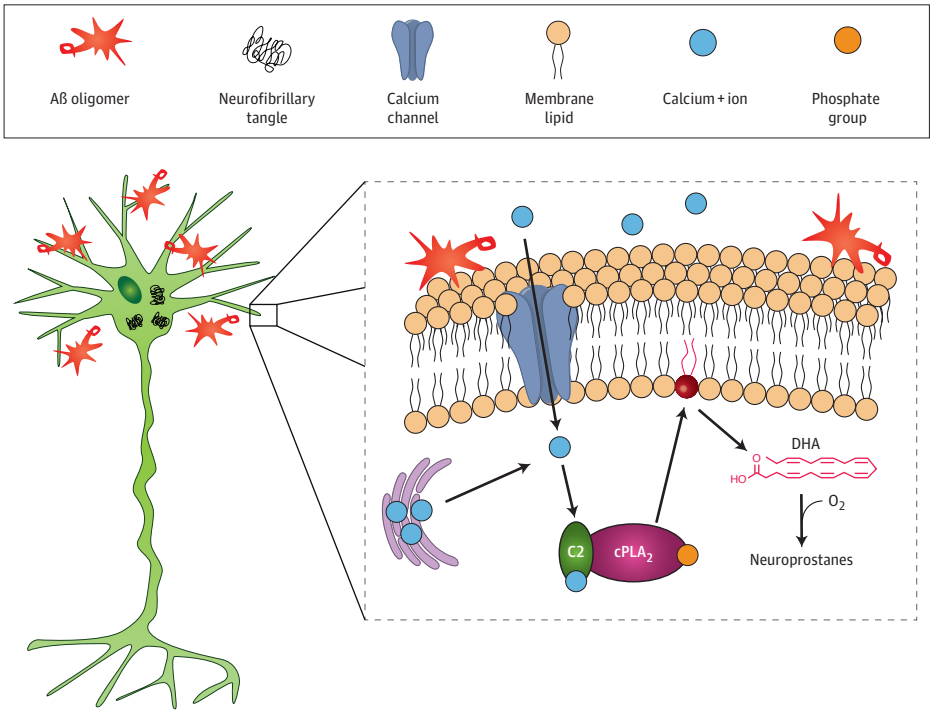
We propose classifying *APOE4* carriers into 3 stages based on disease severity. Stage I represents the earliest predementia phase of the disease with evidence of brain imaging changes in AD-vulnerable areas, but with subtle or no cognitive changes detectable. In this stage, brain DHA metabolism is altered by *APOE4*, and brain imaging or CSF biomarkers can be used to select at-risk individuals and monitor the efficacy of supplementation. Stage II represents an early prodromal stage with evidence of memory and/or executive decline but no significant impairment in activities of daily living. In this stage, long-term, high-dose DHA supplementation

would slow cognitive decline. Stage III represents clinical AD with impairments in multiple cognitive domains, which interfere with usual occupational and social functions (ie, dementia). Intervention with DHA in the dementia stage is not likely to be beneficial.

Cognitive Outcome Measures in *APOE4* Carriers

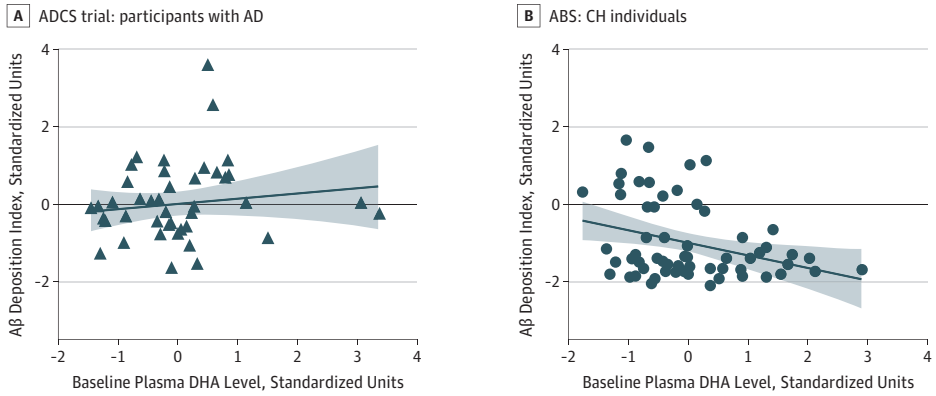
In 2009, Caselli et al[61] reported accelerated memory decline in *APOE4* carriers compared with noncarriers. Eight hundred fifteen participants from 21 to 97 years of age were monitored longitudi-

Figure 2. Mechanisms Linking Apolipoprotein E ε4 (*APOE4*) Status With Docosahexaenoic Acid (DHA) Delivery to the Brain After the Onset of Neurodegeneration



Activation of phospholipase A_2 (PLA_2) associated with neurodegeneration and amyloid toxic effects leads to the liberation of free (unesterified) DHA. Unesterified DHA is oxidized into neuroprostanes. These changes may not be amenable to DHA supplementation. Aβ indicates β-amyloid; C2, calcium-binding domain of PLA_2; $cPLA_2$, calcium-dependent PLA_2; and O_2, oxygen.

Figure 3. Association of Plasma Docosahexaenoic Acid (DHA) Level With Brain Amyloid Deposition by Disease Stage



In the Alzheimer Disease Cooperative Study (ADCS)–sponsored DHA trial (A), 43 participants with dementia underwent lumbar puncture before randomization to placebo or DHA supplementation for 18 months. In contrast, the Aging Brain Study (ABS) (B) recruited cognitively healthy (CH) individuals to gain an understanding of risk factors for Alzheimer disease (AD). Sixty-one participants underwent Pittsburgh Compound B positron emission tomography to assess cerebral amyloidosis (including 13 apolipoprotein E ε4 [*APOE4*] carriers). Given the different methods of DHA level measurements and amyloid deposition indices in both studies, the units were scaled from −2 to 4 arbitrary scale units. Cerebrospinal fluid β-amyloid (Aβ) 42 peptide levels were inverted. Plasma DHA levels were not associated with amyloid deposition in the ADCS but were inversely associated with amyloid deposition in carriers and noncarriers of the *APOE4* allele, which supports the timing hypothesis for DHA intervention in *APOE4* carriers. Regression lines illustrate a significant inverse association between plasma DHA levels and brain amyloid deposition in the ABS and a nonsignificant association between baseline plasma DHA levels with brain amyloid deposition in the ADCS-sponsored DHA clinical trial.

nally for a mean of 4.5 years. The greatest difference in cognitive domains by *APOE* status was reflected by the word list memory measure. The divergence in memory outcomes between carriers and noncarriers appeared in their 60s in *APOE4* heterozygotes and in their 50s in *APOE4* homozygotes.

Some evidence links ω-3 levels with some of the cognitive deficits observed in *APOE*4 carriers. Samieri et al[38] reported a significant association between plasma levels of ω-3 fatty acids (DHA or eicosapentaenoic acid) and cognitive decline measured during 7 years on the Benton Visual Retention Test, a test of working memory and attention. Intervention trials with ω-3 supplementation suggested slowing of deterioration in memory and executive cognitive domains (memory reaction times,[19] attention domains,[15] and composite outcome that included cued memory and executive functions[62]) in *APOE4* carriers. The modest effect size of ω-3 supplementation on cognitive outcomes during the predementia stages highlights the challenge for smaller clinical trials to detect significant *APOE* genotype by treatment interactions.

Brain Imaging of *APOE4* Carriers

APOE4 carriers at an increased risk of developing AD demonstrate brain differences in AD-vulnerable regions that are evident on several brain imaging modalities before any evidence of cognitive decline or AD-related brain neuropathologic changes. Of particular interest are *APOE4*-related changes that become more pronounced with age and are observed in regions associated with later cognitive decline. Progressive changes in these brain functions and structures can serve as useful diagnostic targets to guide the efficacy of interventions in the early predementia phase (stage I).

1. Incorporation of DHA into the brain has been quantified using ^{11}C-labeled DHA positron emission tomographic (PET) scans.[63] Umhau et al[64] demonstrated a compensatory increase in brain DHA uptake in the alcohol withdrawal state. The DHA PET scans can be a useful tool to quantify the degree of brain DHA uptake in *APOE4* carriers and inform whether supplementation can enhance brain uptake during the various stages of this disease.
2. Amyloid PET imaging can be used to define a preclinical AD stage in *APOE*4 carriers with amyloid deposition at AD-related brain areas. This amyloid deposition may appear 1 to 2 decades before the onset of symptoms.[65]
3. Fludeoxyglucose F 18–labeled (FDG) PET scans in *APOE4* carriers show abnormally low rates of glucose metabolism bilaterally in the posterior cingulate and parietal, temporal, and prefrontal cortices compared with noncarriers several decades before the onset of AD.[66] Of these regions, hypometabolism in the medial temporal lobe has been shown to predict cognitive decline in cognitively healthy older adults.[67] Docosahexaenoic acid may have a role in the regulation of brain glucose uptake. In 1 nonhuman primate study,[68] DHA supplementation improved glucose brain hypometabolism.
4. Using resting-state functional magnetic resonance imaging, cognitively healthy *APOE4* carriers display disrupted synchronicity of the signal between the hippocampus and the default mode network, a network of regions that are more active at rest than during an effortful task.[69] This disruption of synchronicity may reflect the relative health of the underlying structural connectivity[70] or may be an independent effect related to synaptic or vascular health, all of which may be affected by DHA.[71]
5. Because DHA is enriched in oligodendrocytes as well as neurons, it may play a role in myelin structure[72] and in the brain's connectivity. Such effects may be partially reflected by diffusion tensor imaging measurements. A study by Witte et al[73] found that 26 weeks of ω-3 supplementation resulted in more intact white matter in the inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculus, and corpus callosum compared with placebo.
6. In cognitively healthy older adults, *APOE4* has been associated in some brain imaging studies with smaller hippocampal volume[74] and thinner entorhinal cortex.[75] These brain measures are all vulnerable to changes in patients with AD.

Conclusions

We propose that disease stage–related alterations in DHA transport affecting peripheral and central metabolism in *APOE4* carriers should be considered when designing intervention studies. We hypothesize that DHA supplementation in *APOE4* carriers can result in beneficial outcomes if the timing of the intervention precedes the onset of dementia. Given the safety profile, availability, and affordability of DHA, refining an interventional prevention study in *APOE4* carriers is warranted.

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