

COMMENTARY

What was lost in translation in the DHA trial is whom you should intend to treat

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Abstract

The results of a randomized double-blind placebo-controlled trial with docosahexaenoic acid (DHA) supplementation in mild to moderate Alzheimer's disease (AD) published by Quinn and colleagues in *JAMA* argues against overall efficacy of DHA in slowing progression. However, certain caveats in the results caution against discarding DHA altogether, raising questions about oxidation, dosage, pharmacogenomics and stage of intervention. One potential misconception is that what works for prevention will slow progression in AD subjects. Preclinical studies with DHA supported the rationale for early stage intervention; and three epidemiological studies indicated DHA intake was associated with reduced risk in non-apolipoprotein E4 (ApoE4) carriers. Putative drugs are initially tested for impact on progression because prevention approaches are problematic. However, should a drug be discarded for prevention if it fails to modify progression? Consistent with epidemiology, DHA significantly benefited two measures of cognition in mild to moderate non-ApoE4 carriers. Although the results of this trial were overall negative, failing to modify other outcomes, this commentary discusses important questions raised by them. Should future trials pursue DHA in non-ApoE4 carriers for slowing progression? Since *in vivo* oxidation of DHA may have adverse effects, particularly in ApoE4 patients, should preclinical and clinical studies be performed to optimize dose and mitigate oxidation before pursuing intervention or prevention trials with DHA? And finally, should DHA be tested now for mild cognitive impairment or prevention?

Subjects with baseline mini-mental state examination (MMSE) scores of mild to moderate Alzheimer's disease (AD) were treated with algal docosahexaenoic acid (DHA, 2,000 mg/day) for 18 months in a randomized double-blind placebo-controlled trial to determine the impact on AD progression. The rationale for testing DHA was strong. It is enriched in neuronal membranes but depleted in AD. Multiple epidemiological studies report diets rich in fish or DHA reduce AD risk, most clearly in non-apolipoprotein E4 (ApoE4) carriers [1]. Preclinical studies with DHA have not yet modeled ApoE isoform pharmacogenomics, but mice transgenic for familial dominant AD mutations that elevate β -amyloid ($A\beta$) production are vulnerable to dietary DHA depletion. DHA and its metabolites pleiotropically impact $A\beta$ production, insulin/neurotrophic signaling, tau kinase activation and synaptic plasticity [1]. Although DHA had no impact on cognitive or functional decline based on intent to treat AD, in non-ApoE4 carriers, DHA supplementation appeared to reduce declines in MMSE and Alzheimer Disease Assessment Scale-Cognitive (ADAS-Cog). This commentary discusses the trial's results and important questions raised, including the need for optimization of dose and antioxidant combinations and whether there should be further investigation of the impact of DHA on slowing cognitive decline in non-ApoE4 carriers. Perhaps a larger issue is whether agents directed at amyloid or tau pathology or associated with reduced AD risk should be translated with an intent-to-treat earlier disease stages rather than mild to moderate AD.

The randomized trial of DHA for AD provides evidence that DHA supplementation provides no general benefit to AD patients, including no overall impact on Clinical Dementia Rating (CDR) scale, ADAS-Cog, MMSE, Neuropsychiatric Inventory (NPI; $P = 0.11$) and Activities of Daily Living (ADL) [2]. The authors argue that DHA may still have potential for prevention. Thus, a fundamental question arising out of this study is the stage at which we should treat. First, three smaller studies showed an apparent benefit from fish oil treatment in mild cognitive impairment but not in mild to moderate AD subjects [3-5]. With age-associated memory

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impairment, one small trial [6] and the larger 485-subject MIDAS (Memory Improvement with DHA Study) trial [7] found significant cognitive benefits with DHA. While there have been two fish oil trials in unimpaired elderly in which no cognitive benefits were observed [8,9], subjects in both were cognitively normal at baseline, and the latter failed to show significant cognitive decline in the placebo group. This study argues that fish oil is not a cognitive enhancer, but does not examine disease modification in subjects with pathology-driven memory deficits. Second, animal studies report DHA/fish oil act on two pathological endpoints that plateau by mild to moderate stages: A β accumulation [10] and loss of superior cortical drebrin, an excitatory synaptic marker [11] (reviewed in [1]). Dramatic medial and superior temporal drebrin loss plateaus early with mild cognitive impairment by MMSE 26 [12], so loss has already occurred in trial subjects. While DHA reduced both A β and tau pathology in 3xTg AD mice [13], that intervention was early (pre-pathology). In contrast, with late post-pathology intervention in human tau transgenic mice with significant neuron loss, we find DHA treatment is insufficient to produce significant cognitive and synaptic improvements (GMC and SAF, Society for Neuroscience presentations, 2010). Finally, epidemiological risk factors may be relevant to prevention, but not necessarily to treatment. Animal model data with DHA support early intervention for primary prevention or mild cognitive impairment and suggest a failure to impact tangle and neuron loss driven deficits at later stages.

Although the data demonstrate that DHA has no general benefit for AD, a concern remains as to whether the key negative effect may be driven by the failure of ApoE4 subjects to respond. Since non-ApoE4 carriers comprise a large segment of the US (approximately 75%) and AD (approximately 50%) populations, whether DHA may slow progression in non-ApoE4 carriers is important. Figure 3 in [2] indicates that 40% of non-ApoE4 carriers showed significant ($P = 0.03$) stabilization of both ADAS-Cog and MMSE, but not with correction for multiple comparisons. The authors point out that three epidemiological studies showed reduced risk with fish consumption only in the ApoE4 non-carriers, but add that pharmacogenomic interaction was not seen with CDR, ADL or NPI. For example, NPI showed a trend independent of genotype, worsening less (2.93 points) in the DHA group than in the placebo group (5.09 points, $P = 0.11$). Are pathogenic mechanisms impacting NPI, ADL, CDR and MMSE/ADAS-Cog the same? Thus, any pharmacogenomic potential of DHA requires clarification.

For prevention or treatment, one might expect ApoE genotype-DHA interactions. Because ApoE4 accelerates pathogenesis, age-matched ApoE4 patients may have more intractable AD pathology. Further, one important

target of DHA is insulin resistance [14], but drugs targeting insulin resistance (insulin or peroxisome proliferator-activated receptor (PPAR) γ agonists) appears more effective at reducing cognitive deficits in ApoE3 carriers than ApoE4 carriers [15]. ApoE is a major central nervous system lipid transport protein with isoform-dependent trafficking likely to impact DHA compartmentalization in the brain. Finally, ApoE4 increases oxidative stress, and with six double bonds, DHA is readily oxidized.

This raises other critical issues that need to be addressed before pursuing a future trial: dose and oxidation. The authors discuss the need to investigate potential combinations of DHA with antioxidants in AD patients, given apparent benefits with combinations of fish oil and lutein or lipoate in small trials and with antioxidants in the Souvenaid trial. Oxidation of DHA to neuroprostanes is associated with synaptic loss. Further oxidation produces a toxic end-product, 4-hydroxyhexenal, that contributes to neuron death and defective uptake of glucose by neurons and glutamate by astrocytes. Clinical studies demonstrate that similar dosing with marine n-3 fatty acids (polyunsaturated fatty acids with a double bond at the third carbon), including DHA, can deplete vitamin E and increase some peripheral measures of oxidative damage, particularly with dosing up to 6 months [16]. Because DHA is enriched in the brain where oxidative damage is already increased in AD patients, antioxidant supplements optimized for AD brain appear crucial. Even though marine n-3 fatty acids can deplete vitamin E, high dose vitamin E (900 IU) did not reduce measures of lipid peroxidation in human plasma [17], so vitamin E supplementation is probably not sufficient. In mice the lipophilic phenolic antioxidant food additive butylhydroxytoluene attenuated measures of lipid peroxidation in plasma after high intake of fish oil [18]. The preclinical studies with DHA in AD mouse models require encapsulation of DHA in the chow to minimize oxidation [10,11]. Also, using the US Food and Drug Administration's equation to estimate the human equivalent doses, the clinical trial dose was three-fold higher than the efficacious preclinical dose in mice [10,11], and twice as high as in the MIDAS trial [7], raising questions about whether the dose may have been too high, potentially exacerbating oxidative damage.

Possible cognitive benefits in patient subgroups (pharmacogenomic or otherwise) would be strengthened by evidence of a biomarker response, arguing for the need to validate neuroimaging, cerebrospinal fluid or plasma biomarker responses in preclinical studies going forward. MRI was performed in a small subset of subjects, showing that volumetrics of the left hippocampus in the DHA group showed trends to be smaller than in the placebo group ($P = 0.17$), which may indicate brain shrinkage. In the AN1792 active A β vaccination,

MRI shrinkage was attributed to plaque clearance. Since drugs may only work in a subset of patients, it would be helpful in large studies where neuroimaging or cerebrospinal fluid biomarker analysis are less feasible to identify likely responders with plasma biomarkers. A difficult task at hand is to design future DHA or other trials with earlier intervention to include validated surrogate and/or diagnostic biomarkers that have shown DHA responses in animal models. For tracking adverse effects of DHA, it is important to measure blood vitamin E depletion and lipid peroxides (thiobarbituric acid reactive substances, malondialdehyde, or the specific byproduct of DHA oxidation, 4-hydroxyhexenal). Biomarker validation could track mechanisms and lower trial costs and facilitate choice of efficacious doses before proceeding to longer term, more costly trials to evaluate conversion to AD.

Conclusion

The study by Quinn and colleagues provides additional rationale to test DHA for prevention, with focus on non-ApoE4 carriers, but problems with DHA dosing and oxidation need to be addressed (particularly if an antioxidant could correct a failed ApoE4 response to DHA). Additional preclinical studies of stage-dependent efficacy and ApoE4-DHA interaction may help to clarify whether ApoE genotype affects outcomes and how this can be mitigated, possibly with antioxidants or non-steroidal anti-inflammatory drugs (NSAIDs). Beyond pharmacogenomic roadblocks emerging with DHA and other interventions, all of the epidemiology and most of the animal model data that have been generated are most relevant to early stage interventions, but have been translated in clinical trials in mild to moderate AD, potentially resulting in an intent-to-treat the wrong group. The pre-clinical conclusions may not be wrong, but simply still lost in this translation.

Abbreviations

A β , β -amyloid; AD, Alzheimer's disease; ADAS-Cog, Alzheimer Disease Assessment Scale-Cognitive; ADL, Activities of Daily Living; ApoE, apolipoprotein E; CDR, Clinical Dementia Rating; DHA, docosahexaenoic acid; MIDAS, Memory Improvement with DHA Study; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; NPI, Neuropsychiatric Inventory.

Competing interests

GMC has received reimbursements from Martek Biosciences for travel and lectures that he has presented on DHA and as a member of their expert panel. SAF has no competing financial interests

Authors' contributions

GMC and SAF made equal contributions in writing this commentary.

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