ALINEA

NUTRITION

SEPTEMBER 2023

www.alineanutrition.com

TABLE OF CONTENTS

Vauzour D, Scholey A, White DJ, Cohen NJ, Cassidy A, Gillings R, Irvine MA, Kay CD, Kim M, King R, Legido-Quigley C, Potter JF, Schwarb H, Minihane AM. A combined DHA-rich fish oil and cocoa flavanols intervention does not improve cognition or brain structure in older adults with memory complaints: results from the CANN randomized, controlled parallel-design study. Am J Clin Nutr. 2023 Aug;118(2):369-381.

What We Know, Think We Know, or Are Starting to Know

The study of nutrition in brain and cognitive function is challenging. On one hand, we know as a matter of biological fact that specific nutrients play critical roles in brain and cognitive development, including long-chain omega-3 and omega-6 fatty acids, iodine, zinc and iron, folate, and vitamin B12 [(1)]. And current evidence has added non-nutritive but biologically active (poly)phenol compounds to this list of dietary factors that influence cognitive function [(2,3)].

On the other hand, the older ages at which neurodegenerative diseases are diagnosed [i.e., prevalence of dementia increases significantly >80yrs] render long-term intervention trials with "hard" clinical endpoints such as dementia practically impossible. Added to this challenge is the difficulty in deliberately exposing individuals to low levels of any nutrient known to be important for cognitive function, which would be deemed unethical [(4)].

Two dietary factors which have attracted substantial research interest are illustrative of these challenges: the long-chain omega-3 docosahexaenoic acid [DHA], and the (poly) phenols found in cocoa, namely flavonols [(5,6)]. The effects of DHA may require longer durations of intake for brain-related outcomes, while cocoa flavonols may primarily exert acute effects on cognition [(6–8)].

Recently, the potential synergistic effect of nutrients in the brain has gathered interest, with interactions between the omega-3's eicosapentaenoic acid [EPA] and DHA and B-vitamins demonstrated for cognitive outcomes in the elderly [(9)]. However, whether omega-3 may have synergistic effects with (poly)phenols in humans has limited evidence.

The Study

The CANN [Cognitive Ageing, Nutrition and Neurogenesis] trial was a randomised, double-blind, and placebo-controlled study. Participants were randomly assigned to an active treatment or a placebo, thus:

- **Active Omega-3 + Cocoa Flavanols [hereafter "O3-CF"]:** Combined dose of 1,100mg DHA + 400mg EPA per day consumed in capsules, and 508mg total flavan-3-ols per day consumed through chocolate drops, both taken with the main meal of the day.
- **Placebo Cocoa Flavanols + Placebo Omega-3 [hereafter "control"]:** The control in this condition was an 80/20 palm/corn oil mix for the omega-3's, and chocolate drops that were isocaloric to the active cocoa flavanol treatment and matched for macronutrients, only with <50mg total flavonols. Both placebos were also taken with the main meal of the day.

Participants were adults aged ≥55yrs with either subjective cognitive impairment [SCI] or clinically assessed mild cognitive impairment [MCI], but no diagnosis of dementia or major depression. Participants were recruited both in Norwich, UK, and in Melbourne, Australia. Participants were excluded if they had a high daily flavonoid intake and high [>6%] red blood cell EPA/DHA status.

The duration of the intervention was 12-months, and participants underwent cognitive assessments and blood/urine sampling at screening, the baseline of the study period, 3-months, and 12-months. A subgroup of participants also underwent MRI neuroimaging at baseline and 12-months. Diet was assessed at baseline and 12-months using a validated food frequency questionnaire [FFQ].

The primary outcome was hippocampal cognitive function, assessed through the number of false-positives on the Picture Recognition Task which tests episodic memory and memory speed. A second test assessed another domain of hippocampal cognitive function in relational memory, i.e., arbitrary associations between events or objects.

Results: 125 participants were randomised into the O3-CF group, and 121 to the control group, of which 95 from the O3-CF group and 102 from the control group completed the 12-month study. Average age of participants was ~65yrs, and 57% of participants were female. 42% of participants had MCI.

***Primary Outcome – Picture Recognition*:** There was no significant differences between groups, with both groups averaging ~85% accuracy in recognition at baseline and 12-months, respectively. The outcomes did not differ according to baseline cognitive status, sex, or *ApoE-4* carrier* status [see ***Geek Box**, below, for further detail].

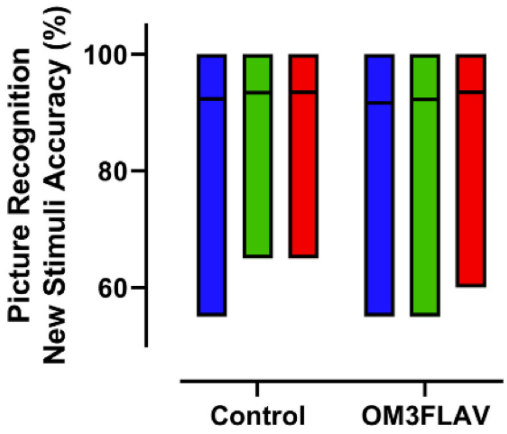

***Figure** from the paper illustrating the changes in accuracy of picture recognition cognitive tests, as a measure of hippocampal cognitive function, at baseline [**blue bars**], 3-months [**green bars**], and 12-months [**red bars**], in the control group and intervention groups. There is nothing doing in this data.*

***Secondary Outcomes – Cognitive Tests and MRI*:** There were no significant effects of the intervention on secondary outcomes, except for the individual outcomes of reaction time variability, executive function, and alertness, each of which showed modestly lower performance in the O3-CF group. For reaction time and executive function, the improvements were only significant in participants with SCI, not MCI.

The MRI scans did not reveal any significant effect of the intervention on brain volume, with the exception of cortical volume which showed a greater decrease in the O3-CF group compared to the control group.

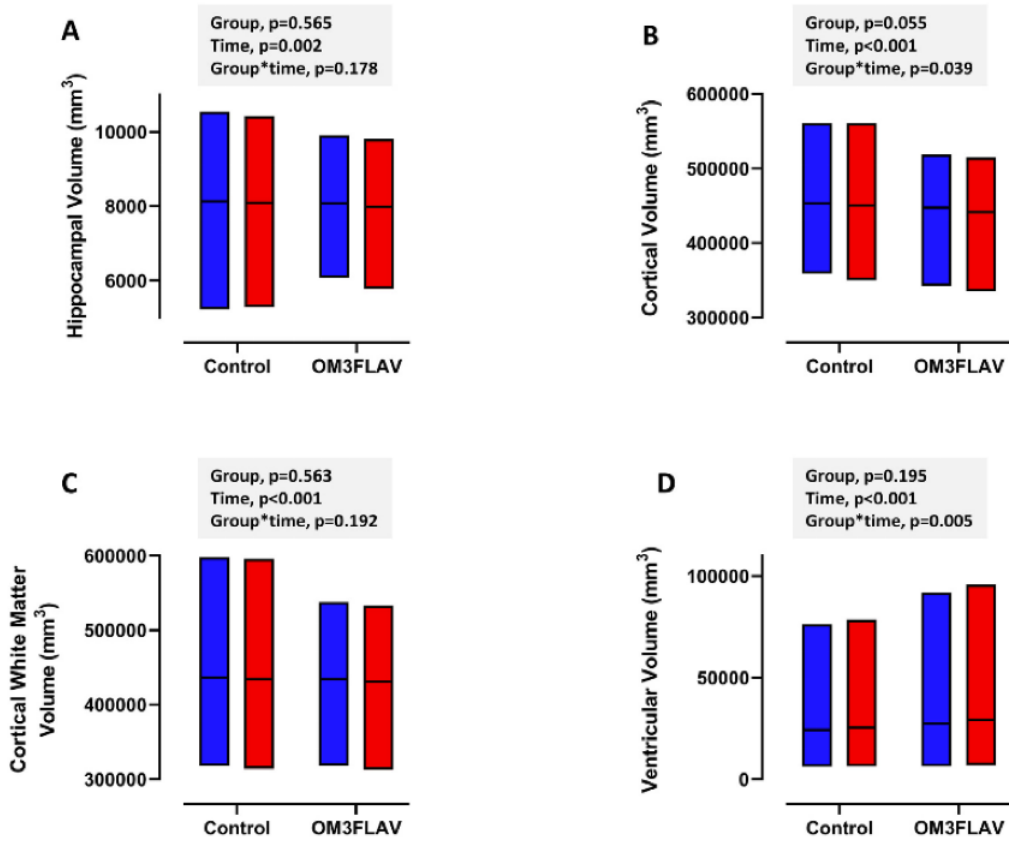

*Figure from the paper illustrating the changes in MRI measures between baseline [**blue bars**] and 12-months [**red bars**] in the control group and intervention group. The bars represent the minimum to maximum values from participants' measures, with the horizontal line in the bars representing the mean. The only significant difference was for changes in cortical volume [**top right**], which decreased modestly in the intervention group.*

*Geek Box: ApoE-4

Apolipoprotein E [ApoE] is a protein expressed in tissues throughout the body, but with particularly high expression in the liver and in the brain. The e4 variant of the ApoE gene [ApoE-4] is strongly associated with Alzheimer's Disease. The mechanisms identified to date include the influence of this gene on metabolism of amyloid-beta protein in the brain. The high metabolic activity, and therefore waste generation, by the brain results in the production of amyloid-B precursor protein [APP]. APP can be metabolised through a number of pathways: the gamma-secretase pathway is strongly implicated in the build-up of amyloid-beta and plaque formation in the brain. Mechanistically, high cholesterol levels contribute to this abnormal processing of amyloid-beta protein. The ApoE-4 genetic variant may increase risk for Alzheimer's by resulted in disordered amyloid-beta metabolism in the brain, either through impaired clearance of amyloid-beta or through influencing disordered cholesterol metabolism [the two may interact]. While the mechanistic processes remain to be fully elucidated, there is compelling evidence supporting a significantly increased Alzheimer's risk with the ApoE-4 genetic variant. Those with this variant are generally advised to follow a diet very low in saturated fat, lower in total fat, and low in dietary cholesterol.

The Critical Breakdown

Pros: Overall, the study had a strong design. The study hypothesis was clearly stated. Investigators and participants were blinded to the group allocation, which was randomly assigned. The placebos for both omega-3 and cocoa flavonols were well-designed to prevent unblinding. The groups were balanced for sex and there were no differences between groups on any baseline measures, confirming the effectiveness of randomisation. Compliance was very high at >95% in both groups, confirmed both through supplement container returns and validated against plasma and urinary biomarkers of omega-3 and flavanol status, respectively. Diet was assessed using the validated European Prospective Investigation into Cancer and Nutrition [EPIC] cohort FFQ. The statistical analysis included only adherent participants, but conducted a sensitivity analysis using intention-to-treat [including any participant with post-randomisation data]. The analysis was also stratified for relevant covariates known to influence cognition, in particular ApoE-4 status, cognitive status, and sex. The analysis also sought to include only participants with low intakes of the intervention nutrients of interest, who may be expected to benefit from deliberately increasing intakes.

Cons: While the study was well-designed and thought through in its methodology, recall the issue of temporality in any effects of nutrients; it is possible that the duration of intervention was too short to detect longer-term effects of DHA in the brain [average half-life of 2.5yrs], while effects of cocoa flavonols to date appear to be mostly acute. Thus, the interactive effects of the respective components of the intervention may exhibit different temporal associations with cognition. While the study was adequately powered initially [the sample size estimate was for *n* = 108/group], by 12-months there was a loss of power with higher-than-expected dropouts from the study. The inclusion of both participants with SCI and MCI may also have lowered the power to detect any intervention effects, particularly where effects of DHA may be more evident in MCI [10,11]. Finally, there was no detail on changes in bodyweight during the intervention.

Key Characteristic

There is a lot to like about the methodology of the present study, which clearly considered some methodological challenges for nutrition research in intervention trials. In particular, the study sought to recruit participants with low baseline intakes and levels of the respective exposures, by excluding participants with an Omega-3 Index [O3I] of >6% or intake of 15 flavonoid-rich foods per day.

But it is the O3I that provides a puzzling missing piece to this otherwise well-designed study. The O3I is calculated as the sum of DHA + EPA in red blood cells [RBC], expressed as a percentage of the total fatty acids in the RBC measure, and is a more reliable marker of omega-3 status than other biomarkers [12]. And the present study screened participants according to the O3I, and presented their baseline levels: 4.7%.

However, change in the O3I during the study is not reported. This is frustrating because the baseline levels of the O3I were under the 5% level which has been associated with cognitive decline in previous research [13]. However, the most protective effects against cognitive decline were observed at O3I levels of >6.8% [13]. Thus, the lack of outcome data on changes in the O3I omits important and potentially informative data on the lack of omega-3 intervention effects, particularly if participants did not reach a higher O3I status associated with cognitive benefits.

Interesting Finding

It is interesting that there appears to have been a complete absence of interaction effects in the present study. Previous nutritional interventions for cognitive function in older adults have demonstrated some interesting interaction effects. For example, as we covered in a previous Deepdive, the VITACOG trial showed a significant protective effect of a B-vitamin supplement against brain atrophy, however, a secondary analysis of the data showed that this protective effect was only observed in participants with high baseline omega-3 status.

This was not an isolated finding. A meta-analysis from Fairbairn *et al*. [9] of intervention trials using combined omega-3 and B-vitamin supplementation in elderly adults showed small-moderate effect sizes of combined nutrients on episodic memory and other measures of cognition. However, a recent 2018 study found no interactive effects of fish oil supplementation and flavonoid-rich concentrated blueberry powder on cognitive testing outcomes in adults with SCI [14]. Thus, it is possible that flavonoids and omega-3 fatty acids have no discernible interaction effects of note.

Relevance

The evidence for cognitive effects of cocoa flavonols got off to a promising start in trials of acute effects on mental fatigue, visual acuity, and working memory [we covered the research in this area in this previous Research Lecture]. However, the recent COSMOS-Mind trial, which we covered in this Deepdive, which assessed the effects of 500mg cocoa flavonols with or without a multivitamin, found no significant effect of cocoa flavonols on cognitive or memory scores over 3-years follow-up.

The present study adds a further indication that cocoa flavonols may not be protective against cognitive decline over the longer-term, although the COSMOS trial only did singular annual cognitive assessments, which may miss any acute day-to-day effects. In the Cocoa, Cognition, and Aging (CoCoA) Study, ~520mg/d cocoa flavonols resulted in significant improvements in cognitive testing over 8-weeks in elderly adults with MCI, which appeared to relate to reduced insulin resistance from cocoa flavonol supplementation [15].

Another important potential consideration is that while any benefits to DHA may primarily be observed in adults already with some age-related cognitive decline [but not dementia] [11], flavonoids are hypothesised to support benefits earlier, as "lifespan essential" compounds [16]. This presents the conundrum highlighted under ***What We Know...***, above, as studying initially healthy individuals with a high vs. low flavonoid intake for long enough to see differences in cognitive decline is an almost insurmountable research challenge.

For DHA, cognitive status may be important to determining any effects. Progression of cognitive decline may not be attenuated by DHA supplementation where participants already have Alzheimer's Disease [17]. Conversely, in elderly adults with age-related cognitive decline, but free from dementia, 900mg/d DHA improved cognitive function over 24-weeks [11].

Overall, the evidence for both DHA and cocoa flavonols is relatively weak, but contains some potential clues to refine future research, i.e., cocoa flavonols with more repeated-measures designs and DHA in adults with MCI with low O3I levels.

Application to Practice

Nutrients like omega-3 fatty acids, or bioactive compounds like (poly)phenols, and the foods that provide these nutritional compounds, are broadly consistent with healthy dietary patterns. For cognitive outcomes, that is as general a conclusion we can come to based on current evidence. However, it would be imprudent, given the environmental factors known to increase risk of neurodegenerative disease, to think that diet quality is not a modifiable factor with considering. These are characteristics of diet worthy of inclusion for overall health.

References

1. Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. J Nutr Health Aging. 2006;10(5):377–85.
2. Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. Antioxid Redox Signal. 2013;18(14):1818–92.
3. Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L, et al. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Arch Toxicol. 2014;88(10):1803–53.
4. Frame L, Fischer J, Geller G, Cheskin L. Use of Placebo in Supplementation Studies—Vitamin D Research Illustrates an Ethical Quandary. Nutrients. 2018 Mar 13;10(3):347.
5. Bradbury J. Docosahexaenoic acid (DHA): An ancient nutrient for the modern human brain. Nutrients. 2011;3(5):529–54.
6. Barrera-Reyes PK, de Lara JCF, González-Soto M, Tejero ME. Effects of Cocoa-Derived Polyphenols on Cognitive Function in Humans. Systematic Review and Analysis of Methodological Aspects. Plant Foods for Human Nutrition. 2020 Mar 13;75(1):1–11.
7. Coley N, Raman R, Donohue MC, Aisen PS, Vellas B, Andrieu S. Defining the Optimal Target Population for Trials of Polyunsaturated Fatty Acid Supplementation Using the Erythrocyte Omega-3 Index: A Step Towards Personalized Prevention of Cognitive Decline? J Nutr Health Aging. 2018 Oct 31;22(8):982–8.
8. Cunnane SC, Chouinard-Watkins R, Castellano CA, Barberger-Gateau P. Docosahexaenoic acid homeostasis, brain aging and Alzheimer's disease: Can we reconcile the evidence? Prostaglandins Leukot Essent Fatty Acids. 2013;88(1):61–70.
9. Fairbairn P, Dyall SC, Tsofliou F. The effects of multi-nutrient formulas containing a combination of n -3 PUFA and B vitamins on cognition in the older adult: a systematic review and meta-analysis. British Journal of Nutrition. 2023 Feb 14;129(3):428–41.
10. Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, et al. The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry. 2008 Aug;32(6):1538–44.
11. Yurko-Mauro K, McCarthy D, Rom D, Nelson EB, Ryan AS, Blackwell A, et al. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimer's and Dementia. 2010;6(6):456–64.
12. von Schacky C. Omega-3 Index and Cardiovascular Health. Nutrients. 2014 Feb 21;6(2):799–814.
13. Lukaschek K, von Schacky C, Kruse J, Ladwig KH. Cognitive Impairment Is Associated with a Low Omega-3 Index in the Elderly: Results from the KORA-Age Study. Dement Geriatr Cogn Disord. 2016;42(3–4):236–45.
14. McNamara RK, Kalt W, Shidler MD, McDonald J, Summer SS, Stein AL, et al. Cognitive response to fish oil, blueberry, and combined supplementation in older adults with subjective cognitive impairment. Neurobiol Aging. 2018 Apr;64:147–56.

References

15. Desideri G, Kwik-Uribe C, Grassi D, Necozione S, Ghiadoni L, Mastroiacovo D, et al. Benefits in Cognitive Function, Blood Pressure, and Insulin Resistance Through Cocoa Flavanol Consumption in Elderly Subjects With Mild Cognitive Impairment. Hypertension. 2012 Sep;60(3):794–801.

16. Scalbert A, Williamson G. Dietary Intake and Bioavailability of Polyphenols. J Nutr. 2000 Aug 1;130(8):2073S-2085S.

17. Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, et al. Docosahexaenoic Acid Supplementation and Cognitive Decline in Alzheimer Disease. JAMA. 2010 Nov 3;304(17):1903.