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Jernerén F, Elshorbagy AK, Oulhaj A, Smith SM, Refsum H, Smith AD. Brain atrophy in cognitively impaired elderly: the importance of long-chain v-3 fatty acids and B vitamin status in a randomized controlled trial. Am J Clin Nutr. 2015;102:215–21.

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

We know that in the epidemiology of diet and dementia/Alzheimer' s Disease, the long-chain marine omega-3 fatty acids, eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], exhibit the strongest associations with reduced risk of these neurodegenerative diseases [1-3].

We also know that supplemental interventions of these fatty acids, either alone or in combination, have been overall underwhelming, leading to some to suggest that perhaps they are not as brain-boosting as the epidemiology would imply. However, we think that a major reason why these trials have largely returned ' null' results relates to low doses, insufficient study duration, consumption of fish in the intervention and control groups, and potentially the modifying effects of the stage of disease relative to the timing of interventions [4].

We also know that, from a mechanistic perspective, B-vitamins have specific neurological functions. Specific interest in vitamins B6 (pyridoxine), B9 (folate), and B12 (cobalamin) is based on their roles in neurotransmitter function and the methylation cycle [5,6]. However, the evidence supporting a beneficial effect of these B-vitamins is the inverse of what we see with EPA/DHA; the epidemiology of B-vitamins is inconsistent [7], while a number of recent intervention studies - namely the Folic Acid and Carotid Intima-media Thickness [FACIT] and Homocysteine and B Vitamins in Cognitive Impairment [VITACOG] trials - have demonstrated significant benefits for cognitive performance and protection against brain atrophy from supplementation with either folic acid alone [FACIT] or a combination B6/B9/B12 supplement [VITACOG] [8,9].

In both the FACIT and VITACOG trials, the effect of B-vitamin supplementation was attributable to reductions in homocysteine, which provided biological plausibility to the benefit of B-vitamins given that elevated homocysteine levels are associated with a significant increase in risk for dementia/Alzheimer' s [10,11]. However, research in humans has also indicated a relationship between polyunsaturated fats, methylation and homocysteine, and B-vitamins [12]. Specifically, folate deficiency impairs lipid metabolism in the brain, while high levels of omega-3 fatty acids have also been shown to decrease homocysteine [13,14].

This begs the question: could there be a relationship between omega-3 status and the effects of B-vitamin supplementation on cognitive health? The present study examined this question.

The Study

The Homocysteine and B Vitamins in Cognitive Impairment [VITACOG] trial was an randomised controlled trial investigating the effects of a combination 0.8mg B9, 0.5mg B12, and 20mg B6 supplement taken daily compared to a placebo on brain health. The duration of the study was 2yrs, and the primary outcome measures were brain atrophy [shrinkage] assessed by MRI, and cognitive performance. The results of the brain atrophy outcome and cognitive performance outcomes were published separately [9,15].

In the MRI study, B-vitamin supplementation resulted in 31.1% less brain atrophy over 2yrs compared to the placebo group, an effect which was significantly associated with the 22.5% reduction in homocysteine levels in the intervention group [9]. In the cognitive function testing, B-vitamin supplementation result in significant attenuation of cognitive decline, and the magnitude of effect on the Clinical Dementia Rating scale scores was most pronounced in participants with the highest homocysteine levels [15].

The present study was a retrospective analysis*of the VITACOG trial data, based on plasma fatty acid measures taken at baseline and at the end of the 2yr intervention.

*Geek Box: Retrospective Study

If you've heard of a retrospective study, most likely it is in the context of a retrospective cohort study, in which the study is conducted after participants have developed a particular outcome of interest. Such a study tends to look back at records or other data to try to identify any relationship between an exposure and an outcome. For example, let's say the investigators think there may be a relationship between exposure to lead in paint factories and cancer incidence. They could look at back records of people who died from cancer, that also worked in paint factories, and compare them to an unexposed control group. They could identify a higher prevalence of cancer incidence. As you can probably see, as data is not collected at baseline, and as the study participants are all identified and compared after the outcomes have occurred, retrospective studies are more prone to bias than other observational research. But what about a randomised controlled trial, can there be a 'retrospective RCT'? Well, yes: all this means is that a retrospective analysis is conducted with data from an RCT. This means that it is not part of the direct hypothesis tested in the initial intervention, and the analysis is conducted to explore further relationships in the data. The difference may also be defined as explanatory vs. exploratory. In an explanatory RCT, the intervention is designed with the hypothesis of testing whether Exposure A results in Outcome B, and 'explaining' any such cause-effect relationship. Exploratory study means an initial exploration of a theory, from which a hypothesis could be generated. In the present study, the analysis was retrospective: the initial study was completed, but the data was available to explore whether there was any relationship between the primary intervention [B-vitamins] and plasma omega-3 fatty acid levels. Finding a relationship in turn generates further hypotheses to be tested directly in further study. The key distinction in interpreting this retrospective study is that it is not demonstrated any cause-effect relationships, and is in effect an observation.

Results: 168 participants completed both baseline and final MRI scans [85 in the intervention group, 83 in the placebo group]. In the intervention group, adherence was confirmed by the plasma analysis and adherence to taking the supplement or placebo was high in each respective group.

Average baseline levels and follow-up levels of combined EPA+DHA, EPA alone, and DHA alone, were similar in both the intervention group and placebo group.

- ***Overall omega-3 fats acid status and brain atrophy rate:*** Absolute levels (i.e., rather than the division into tertiles) of omega-3 fatty acid status were associated with significant reductions in rate of brain atrophy in the B-vitamin intervention group, but not the placebo group.
- ***Effect of B-vitamins relative to omega-3 status:*** Compared to the placebo group, participants in the highest tertile of combined EPA+DHA, EPA alone, and DHA alone, in the B-vitamin group exhibited the following results:
    - Combined EPA+DHA: 40% slowed rate of brain atrophy
    - EPA: 45.8% slowed rate of brain atrophy
    - DHA: 43.4% slowed rate of brain atrophy

There was a significant effect of increasing omega-3 fatty acid levels, i..e, brain atrophy rate decreased as levels of omega-3 fatty acids increased. However, the placebo group showed no significant reduction in brain atrophy rate at any level of omega-3 fatty acid status.

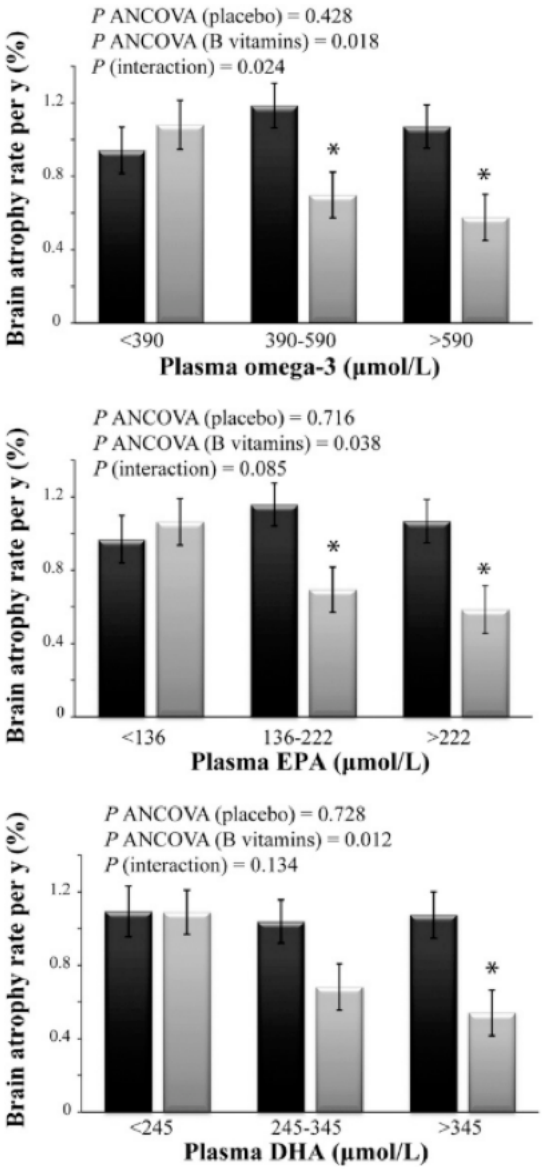


***Figure** from paper demonstrating the relationship between rate of brain atrophy relative to tertiles of omega-3 fatty acid levels in the placebo group (**black bars**) vs. the B-vitamin intervention group (**grey bars**). **Top** displays combined EPA+DHA; **middle** displays EPA alone; **bottom** displays DHA. It is clear in each that the highest tertiles of omega-3 fatty acid levels mediated the effect of the B-vitamin supplemental intervention, and that at low plasma levels of omega-3 fatty acids there was no difference between the intervention group and placebo group.*

- ***Effect of B-vitamins relative to homocysteine (and omega-3) status:*** Homocysteine levels were stratified as <11.3umol/L or >11.3umol/L, with the latter levels associated with increased risk in previous research.

In participants with homocysteine levels <11.3umol/L, there was no significant effect of B-vitamin supplementation on rate of brain atrophy, independent of omega-3 fatty acid levels.

However, in participants with homocysteine levels >11.3umol/L, there was a significant effect of B-vitamin supplementation on rate of brain atrophy that was greater as plasma omega-3 fatty acids increased. This was significant for combined EPA+DHA, EPA alone, and DHA alone. There was no significant difference between placebo and control group in the lowest tertile of omega-3 fatty acid status.

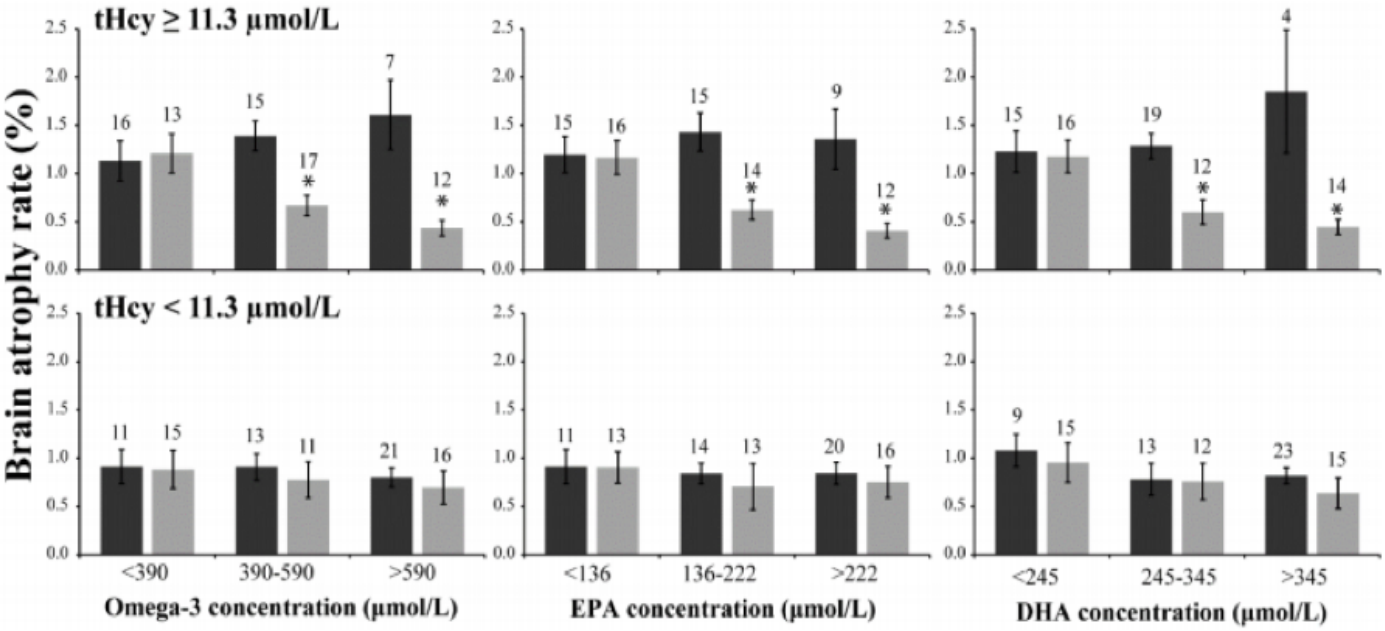


Figure *from paper (supplemental data) displaying the relationship between omega-3 fatty acids and B-vitamin supplementation vs. placebo in participants with homocysteine levels >11.3umol/L (**top**) and <11.3umol/L (**bottom**). A clear mediating effect of homocysteine levels is evident, such that the interaction between omega-3 fatty acid levels and B-vitamin supplementation was only evidence in participants with elevated homocysteine.*

The Critical Breakdown

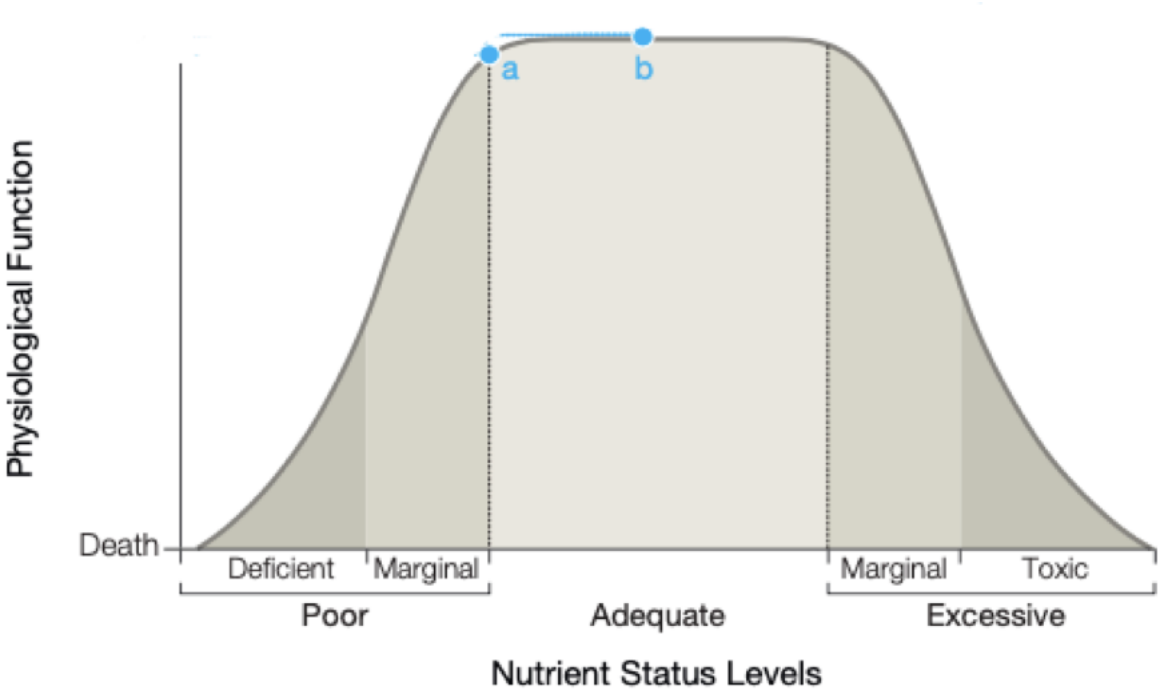
Pros: The VITACOG intervention was well-designed, placebo-controlled, and randomised, in a clinically relevant population of >70yrs with mild cognitive impairment. The maintenance of blinding was achieved by providing tablets labelled with the trial name, which meant the placebo group remained constant. The trial was 2yrs duration and achieved>75% compliance in the intervention and placebo groups. Finally, the use of MRI to quantify brain atrophy, and the measurement of biomarkers of B-vitamin intake, provided strong mechanistic insight into the effects of the intervention.

Cons: The study is not a direct test of omega-3 fatty acids, and this was a retrospective analysis so it is correlational. In certain analyses, like the homocysteine and brain atrophy, there were some very small sample sizes for different levels of B-vitamin and omega-3 fatty acid status. Plasma measures of omega-3 fatty acids were used, which generally are a reflection of very short-term - 3-4 days - dietary intake, and may not reflect habitual longer-term EPA/DHA intake.

Key Characteristic

Stratifying omega-3 levels into tertiles, which although it is standard practice in epidemiology, is often not undertaken in intervention studies. This often can lead to ' null' findings when only the mean levels of intake of a nutrient are used analysis, whereas if levels of intake had been stratified it may have been possible to detect an effect at a certain threshold - lower or higher - given the bell curve of nutrient action.

This allowed for clear effects of omega-3 fatty acid status to be elucidated. In particular, it clearly demonstrated that in participants in the lowest tertile of omega-3 fatty acids, there was no difference in rate of brain atrophy between the B-vitamin supplement group and placebo group. Conversely, the effect of B-vitamin supplementation on brian atrophy was more pronounced in the middle and highest tertiles of omega-3 fatty acids. The analysis according to tertiles allowed for a much stronger magnitude of effect to be shown than the mean levels alone.



Graph *depicting the bell-curve of nutrient action, illustrating that the effects of a nutrient are not lineal and often dose-dependent, and highlighting the importance of stratifying levels of intake rather than only looking at average levels, which could be within a range of adequacy and therefore generate 'null' findings. Whereas stratification allows for point A above to compared with point B (and even a point C, D, or E as desired).*

Interesting Finding

The relationship between reduced brain atrophy, omega-3 fatty acid levels, and homocysteine levels. Omega-3 fatty acids levels did not mediate the effect of B-vitamin supplementation at any level of omega-3 status, if homocysteine levels were <11.3umol/L.

However, in participants in the B-vitamin intervention group with the highest levels of omega-3 fatty acids, brain atrophy was reduced by 70% compared to the placebo group. The caveat, as the authors highlight, is that there were small group sizes in these comparisons, however the study is exploratory and as such, hypothesis-generating.

And the hypothesis that derives from this finding is interesting, as we typically think of lowering homocysteine as a function in vitamins B9 and B12 - which it is - but this analysis suggests that elevated homocysteine may mediate the biological activity of EPA and DHA. As a corollary, low homocysteine levels may be required for EPA and DHA to have protective effects. And, it may be that EPA and DHA supplementation itself has the capacity to reduce homocysteine levels, as was shown in a meta-analysis of supplemental interventions [(13)]. This will be an interesting any for further research.

Relevance

This study indicated that the effect of B-vitamin supplementation was dependent on levels of the omega-3 fatty acids, EPA and DHA. In the intervention group, there was no effect of B-vitamin supplementation in participants with the lowest omega-3 fatty acids levels. This study is exploratory, and therefore hypothesis-generating; further research will be required to directly test the hypothesis that omega-3 fatty acids and B-vitamins interact to protect against brain atrophy.

Nonetheless, the implication of this research is that the effect of B-vitamin supplementation is mediated by omega-3 fatty acid levels, and any research investigating the effects of B-vitamins on cognitive outcomes should assess omega-3 status in participants. This finding has relevance for both epidemiology and intervention studies.

From a methodological standpoint, and further to the ***Key Characteristic***, above, I want to highlight a line from the paper: "*...our results emphasise the importance of identifying subgroups in clinical trials.*" While I' ll refrain from slow-clapping at my computer, that is the sentiment that arises.

Nutrition interventions, particularly of supplements, often generate null findings, but there are multiple instances where subgroup analysis have revealed effects of an intervention relative to baseline nutrient status.

Because the effects of nutrients are non-linear and likely to be dose-dependent, it is important to dig further into the data to look at potential important subgroup characteristics. Otherwise, potentially important findings - like the present study - may be missed. The assumption then is that an intervention worked for no one, when what we should ask is *did it work for anyone, and if so, what mediated that finding?*

Application to Practice

The effects of this interaction remain to be confirmed in future studies, so this precludes any more specific recommendations that the general advice to consume oily fish (or supplement with an algae-based EPA/DHA formula). Nonetheless, given that this advice is based on sound evidence, the practical application is that, particularly in elderly adults, regular intake of the marine omega-3 fatty acids may be beneficial for the brain.

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