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Greupner T, Kutzner L, Nolte F, Strangmann A, Kohrs H, Hahn A, Schebb NH, Schuchardt JP. Effects of a 12-week high-α-linolenic acid intervention on EPA and DHA concentrations in red blood cells and plasma oxylipin pattern in subjects with a low EPA and DHA status. Food Funct. 2018 Mar 1;9(3):1587-1600.

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

We know that shifts to a more plant-based diet are moving from the realm of niche dietary paradigm to environmental prerogative. However, such a dramatic shift in population dietary habits warrants attention to the 'unknown unknowns' of removing and/or replacing foods and food groups that currently contribute significantly to nutritional status across the population.

One such debate that, since the release of yet another Netlfix documentary [currently above systematic review and meta-analysis in the public hierarchy of evidence], has become a focal point of the incessant ideological mudslinging between diet tribes, is that of plant vs. animal source omega-3 fatty acids.

Alpha linolenic acid [ALA] is the primary plant-derived omega-3 fatty acid, while eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA] constitute the primary very-long-chain omega-3 fatty acids, which can be synthesised from ALA*. However, the average conversion from ALA is minimal, around 8-12% conversion to EP and ~1% to DHA [(1)]. There are also important sex differences in conversion, with women converting significantly higher levels of ALA to EPA and DHA of, respectively, 21% and 9% [(2)]. Conversely, men only convert around 0.3-8% of ALA to EPA, and <1% to DHA [(2)].

The low conversion of ALA to, in particular, DHA, has generated questions over whether consuming ALA alone is sufficient for health effects associated with DHA. The present study tested the effects of very high dose ALA supplementation on EPA and DHA levels in participants with low levels of EPA/DHA.

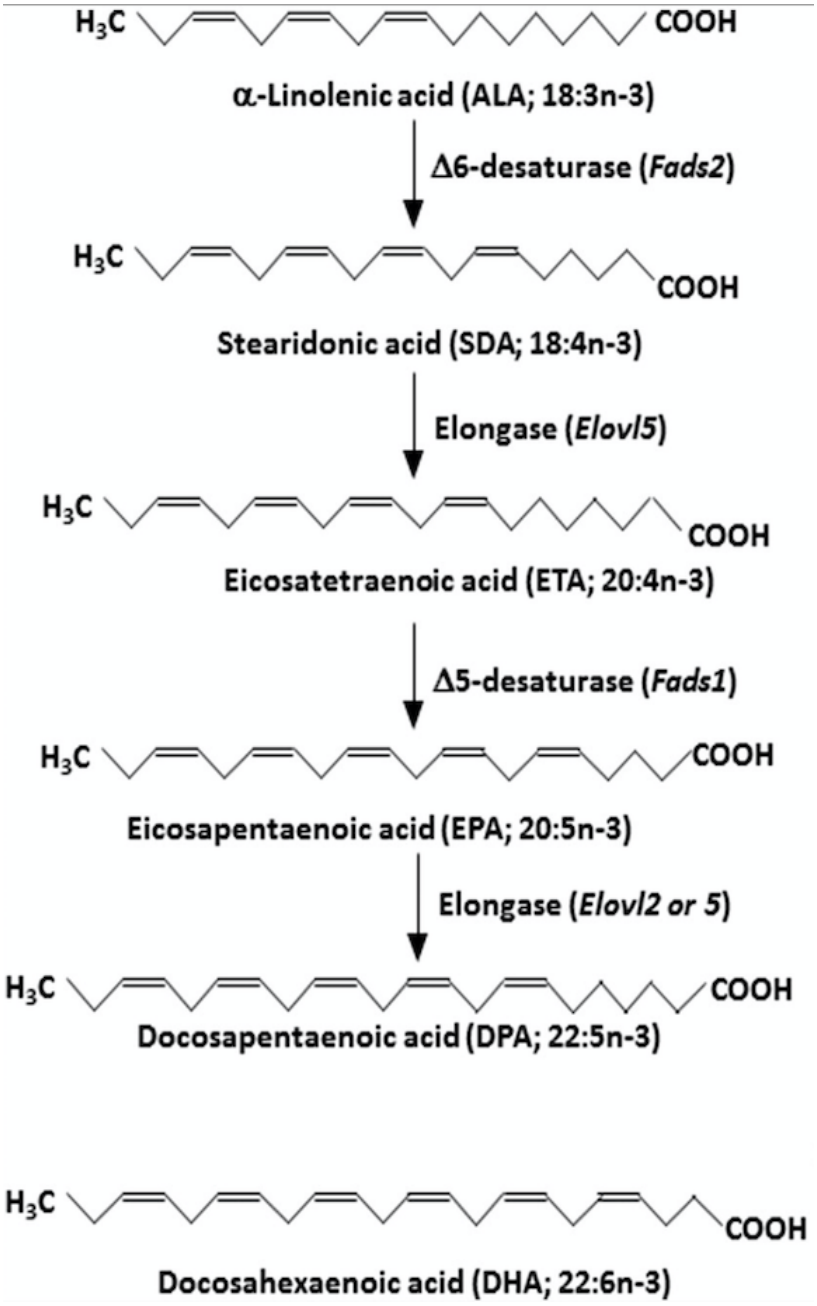

Figure *from [(1)] illustrating the omega-3 fatty acid pathway from ALA to DHA production. As you can see to the right of the arrow from ALA [very top of figure], the delta-6-desaturase enzyme is required to convert ALA to longer-chain omega-3 fatty acids. However, humans lost the full activity of the D6D enzyme during evolution, reflecting the consumption of EPA and DHA from direct dietary pre-formed sources. D6D is therefore considered the "rate-limiting" enzyme, which means that the rate at which conversion may occur is limited by that step in the chain. However, pay attention to stearidonic acid [SDA] above - it is passed the D6D rate-limiting step, and therefore may be a more viable option for plant-based approaches to increasing long-chain omega-3 fatty acid status.*

*Geek Box: ALA and DHA as Essential Fatty Acids

*Up to 60% of the brain's dry weight is comprised of fat, of which up to 30% is comprised of polyunsaturated essential fatty acids [EFAs]. In particular, DHA comprises over 90% of omega-3 fatty acids in the brain, while both EPA and [ALA are low in brain tissue, comprising less than 1% of total brain fatty acid composition. The critical role of DHA in neural growth and development suggests an important evolutionary requirement for this long-chain omega-3. This predominant role of DHA in the brain has generated a "shore-based perspective" of human evolution. Anthropological evidence for the expansion of humans from the African rift valley suggests that migration along coastal and inland watercourses provided access to both marine and freshwater sources of fish, and in particular long-chain omega-3 fatty acids. Stable isotope analysis of bones of early Sapiens have indicated that marine protein sources constituted significant proportions of daily energy in the human diet. The evidence suggests that consistent access to such food sources over multiple generations coincided with the period of exponential encephalisation [the growth of the brain], preceding the rapid development of language, complex reasoning, and problem solving cognitive capacities associated with the prefrontal cortex. Relative to other mammalian species, and indeed our primate cousins, the human brain is disproportionately large compared to body size. A number of lines of nutritional evidence support the anthropological research. First, other mammals with high levels of other polyunsaturated fats in membranes, but without a direct dietary source of preformed DHA, did not develop large brains, indicating a foundational need for preformed DHA in the early modern human diet. Secondly, humans lost the full activity of the delta-6-desaturase enzyme responsible for converting ALA to EPA and ultimately to DHA. While it is highly active in neonatal periods, in adulthood there is very little conversion of ALA through to DHA, which again suggests a direct source of preformed DHA as a foundational dietary characteristic associated with human encephalisation. Anthropological theory also suggests that fish would have been a far less risky prey for humans to hunt, and that the cost of obtaining land mammals as prey may have been quite high. However, only ALA is considered an essential fatty acid, due to the fact that humans lack the necessary enzyme to produce ALA in the body, therefore requiring a direct dietary source. Conversely, technically DHA may be synthesised from ALA, notwithstanding that this conversion is very low. A number of researchers in this area therefore consider DHA to be essential, due to the requirement for a preformed source of DHA to maintain brain concentrations. There remain many gaps in our knowledge, however, and this area is likely to be one of contention as the frankly boring argument about whether nutrients of primarily animal source have a place in the modern human diet continues. If this topic is of interest to you, see references **3-5**.*

The Study

Healthy participants aged 20-40yrs were recruited to undergo a 12-week intervention, followed by an 8-week follow-up period, investigating the effects of supplementing linseed [flaxseed] oil. The fatty acid composition of linseed oil is 58% ALA, and participants were instructed to consumed 22.3g of linseed oil per day, resulting in a daily ALA intake of 12.9g per day.

During the intervention, participants underwent 5 examinations at the investigators research centre, which included blood sampling and questionnaires about diet, medication, and lifestyle. These examinations were conducted at baseline [week 0], followed by week 1, week 3, week 6, and week 12. Two additional examinations were conducted during an 8-week follow-up period, at week 14 and week 20.

For four weeks prior to the study, participants were instructed to refrain from eating fatty fish [salmon, herring, tuna and mackerel] and ALA-rich vegetable foodstuffs such as linseeds, chia seeds, or walnuts [including related oils].

The aim of the study was to investigate the effects of 14g/d ALA supplemented through linseed oil, on EPA and DHA concentrations in participants with low EPA and DHA status. The study also investigated the effect of ALA on oxylipins, the term for a group of bioactive fats ['lipids'] produced from polyunsaturated fats [PUFA] in the diet through 'COX' [cyclooxygenase] and 'LOX' [lipoxygenase] enzymes, which are implicated in the health benefits of PUFA. The oxylipins produced through COX and LOX enzymes in turn act on a number of pathways related to cardiovascular and metabolic function, and the type of oxylipins produced reflects the composition of PUFA in the diet.

Results: Of 20 participants enrolled in the study, 19 completed the intervention and attended all 5 in-person test days. Mean age was 26yrs, and BMI of 24kgm2. Subjects had low fish intake and low measured blood EPA/DHA levels, and consumed a general omnivorous 'Western' diet with low vegetable and fruit intake. Analysis of 3-day dietary questionnaires indicated that dietary linoleic acid [LA], ALA [i.e., background ALA not due to the intervention], EPA, and DHA, were all constant throughout the intervention.

The supplemental ALA resulted in average ALA intake going from 1.39g/d at week 0, to 13.9g/d at week 6 and 14g/d at week 12.

- ***Effect of ALA on Red Blood Cells [RBCs] Fatty Acids:*** During the intervention, the concentrations of ALA and EPA increased significantly in RBCs. ALA concentrations increased by 238% and peaked at week 6, while EPA levels doubled by week 12. However, the concentration of DHA in RBCs decreased significantly in response to the high ALA intake.

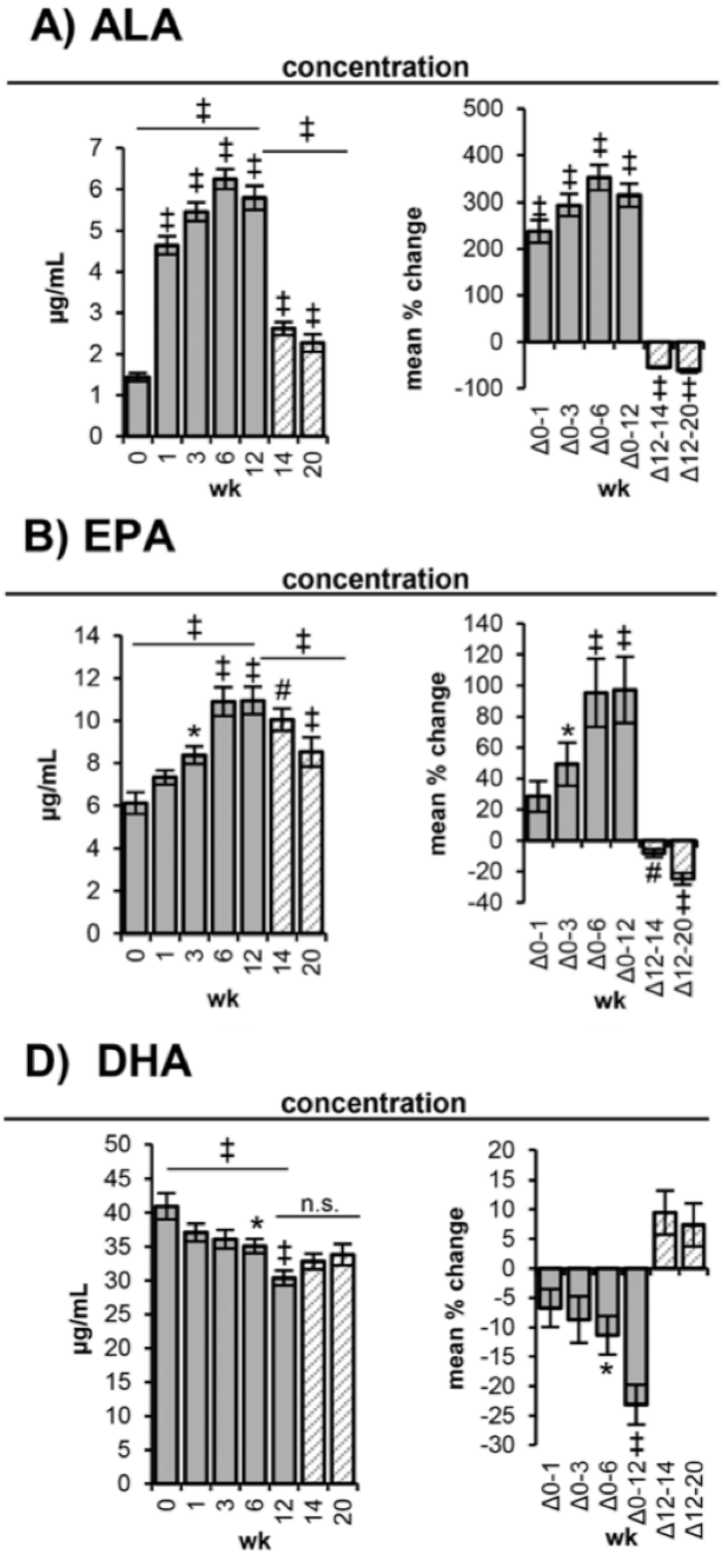

Figure *from the paper illustrating the increase in the concentrations of ALA [top] and EPA [middle] in RBCs, over 12-weeks of the intervention and followed by the 8-week follow-up period after the end of the intervention, where levels decline again. Conversely, DHA [bottom] decreased linearly over the course of the intervention with high dose ALA intake, before increasing again after the intervention had ended.*

- ***Effect of ALA on Oxylipins:*** Oxylipin concentrations in RBCs increased for ALA and EPA-derived oxylipins, while there was no significant change in DHA-derived oxylipins.

The Critical Breakdown

Pros: The research question was clearly focused on a relevant issue, i.e., the effect of a high ALA on EPA and DHA concentrations. The overall study group was very similar in characteristics. Participants were excluded if they consumed fish over 2 times per week, and if they had high blood levels of EPA + DHA. EPA + DHA in RBCs were low, and within a very narrow range. Fatty acid concentrations and oxylipins were analysed using gas chromatography and liquid chromatography-mass spectrometry, respectively, both highly sensitive analytical methods capable of detecting effects with accuracy. The analysis measured red blood cell fatty acids, which is a more robust biomarker of longer term fatty acid status than plasma.

Cons: The study was a non-randomised trial and lacked a control group. Confidence intervals were not reported, which would have provided more detail regarding the precision of the effect. As a caveat of all free-living interventions, it is possible that non-compliance with the background diet requirements, particularly restricting EPA/DHA and ALA-rich foods, occurred [although evaluation of the dietary questionnaires indicates that this did not occur]. The study was confined to men only, which given sex differences in ALA metabolism would have been useful to have included female participants.

Key Characteristic

The deliberate enrolment of individuals with low levels of EPA and DHA was important, given that this population would be expected to show higher conversion of ALA to EPA and DHA. It may be argued that in individuals with adequate EPA and DHA intake, or with regular oily fish consumption, there may be some reverse-inhibition of EPA and DHA synthesis. For example, a study in which participants consumed 6.5g/d DHA showed that the supplemental DHA group had 76% lower EPA conversion from ALA, and 88% lower DHA conversion [6]. A number of studies suggest that high intakes of EPA and DHA may downregulate the conversion of these fatty acids from ALA [6,7]. Thus, the very low EPA+DHA levels of 4.15% in participants in the current study would be expected to negate any potential reverse inhibition of higher direct, preformed EPA and DHA intake. The study therefore was a more direct test of the effects of ALA, and is therefore relevant for the potential application in populations with low/no fish consumption, including people transitioning to either plant-exclusive or plant-predominant dietary patterns [more under ***Relevance***, below].

Interesting Finding

The decrease in DHA is interesting and potentially concerning, although it is a finding that will need further research. The overwhelming majority of the research indicates that either low or high ALA intakes of a range of 2-15g/d do not have any effect on DHA [1,8]. However, the slight decrease in DHA observed in the present study is not an entirely novel finding. The **Figure** below is taken from Baker et al. [1], using data from previous research on the effects of ALA supplementation on the long-chain omega-3 fatty acids, EPA, DPA, and DHA. Supplementation of 0.75-1.5g/d ALA increased EPA levels in plasma and RBCs by 23% and 15%, respectively. However, DHA declined by around 7% in both plasma and RBCs.

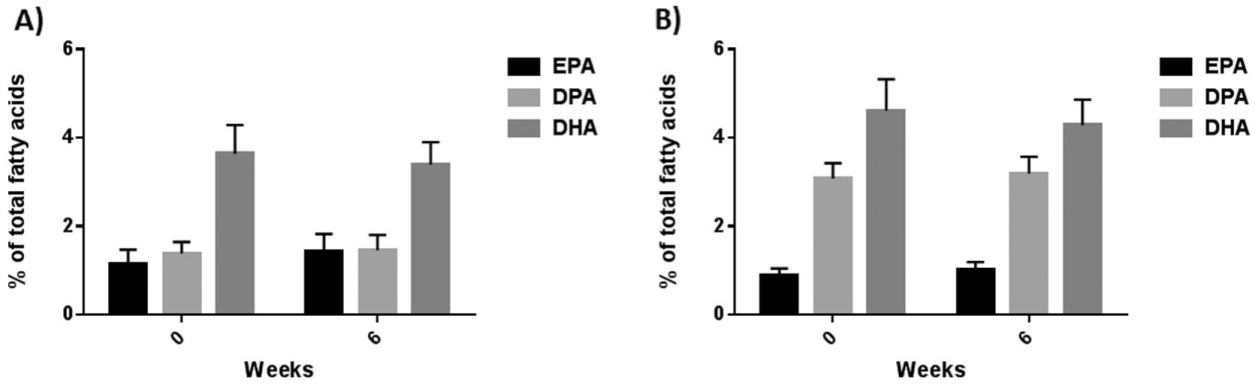

***Figure** from [1] illustrating the effect of 0.75-1.5g/d ALA on long-chain omega-3 fatty acids measured in plasma phospholipids [left graph] and RBCs [right graph].*

However, it should be noted that these findings of decreased DHA levels are the exception, rather than the norm. A review of 28 studies of both ALA and EPA supplementation demonstrated that neither omega-3 was effective at increasing DHA levels [8]. Thus, DHA levels in the body appear to be only responsive to direct preformed DHA sources.

Why might DHA decrease? We do not know exactly. The authors of the current study suggest that reverse-conversion of DHA to EPA may explain, in part, the lower DHA levels. However, it is more likely that the increase in EPA reflected the high dose ALA intake, which has consistently been observed in studies of this kind [1,8]. The physiological relevance and potential implications of decreasing DHA levels in response to high dose ALA remains to be investigated in more detail.

Relevance

This study confirms a consistent finding in the wider literature, which is that supplemental ALA may increase conversion to EPA and EPA levels in certain tissues, but is not sufficient to raise DHA levels. On the contrary, DHA levels were reduced with high dose ALA supplementation.

So, does this mean that ALA is not sufficient for populations consuming diets with no fish? In a small study in Dutch vegans, Fokkema et al. showed that neither 1.17g gamma-linolenic acid or 2.01g ALA alone, or in combination, did not increase EPA and DHA status of multiple compartments to any appreciable extent [9]. The authors hypothesised that DHA levels, even though low, are sufficient and had reached a plateau at low levels, thus any additional DHA would be a "functionally irrelevant surplus" [9]. This is difficult to reconcile with the totality of evidence and overall direction of effect for DHA. A study published in March of this year, for example, compared ALA vs. DHA supplementation on PUFA oxylipin concentrations, which found that while ALA supplementation doubled ALA concentrations, it had no effect on ALA-derived oxylipins [10]. DHA supplementation tripled DHA levels and DHA-derived oxylipins [10]. The totality of evidence for DHA suggests that some is better than none and more is preferable to less [3-5]. However, it could be that low levels of ALA are sufficient to at least preserve DHA functionality: evidence is currently needed to determine whether this is the case.

It should also be noted that although ALA raised EPA levels, the magnitude of effect is up to 15-fold greater when direct preformed EPA is consumed [8]. However, the overall data indicates that each 1g increase in ALA may result in a 10% relative increase in plasma EPA content [1]. In terms of potential related health effects, however, this appears to be a question of dose for ALA. Evidence for beneficial effects on inflammatory and immune responses usually associated with EPA/DHA have only been shown with doses of ALA in the range of 14-18g/d, while studies in a range of 2-10g/d have not shown any effects [11,12]. Bear in mind that average consumption of ALA in Europe, the US, and Australia is 0.5-2.3g/d [1]. Thus, were ALA to be considered an effective alternative for the health effects of direct long-chain omega-3 fatty acids EPA and DHA in the habitual diet, to mimic the health effects would require strategies to increase average consumption by >650% to levels associated with physiological effects.

Thus, the realities of dramatic shifts in population-wide dietary habits without potentially unintended consequences are not as simple, and will require substantial thought.

Application to Practice

It appears that a confident conclusion can be made that increasing ALA levels, even to very high doses, is not an effective strategy to increase DHA levels. While high ALA levels may be sufficient for increasing EPA, the related health effects associated with ALA occur at high doses; this should be born in mind if advising an individual with predominantly plant-based omega-3 sources. From the perspective of DHA, the precautionary principle may apply: in the absence of evidence that long-term exclusion of DHA from the diet does not pose any related health risks, supplementation with a fish or algae-based DHA supplement could be considered a prudent insurance policy while the evidential gaps are filled.

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