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Harris et al. Blood n-3 fatty acid levels and total and cause- specific mortality from 17 prospective studies. Nat Comm. 2021;12:2329.

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

The research question that never dies in nutrition. While so much of the debate and supposed 'controversies' in nutrition relate to foods with negative associations for human health - red meat, saturated fat, sugar - there is also debate over *positive* associations, and whether certain foods/nutrients are in fact *beneficial* for health outcomes.

The marine omega-3 fatty acids eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA] have become one such battleground; are they beneficial or not? This debate has been heightened by the current 'plant vs. animal' dichotomised perspective on the health effects of foods, which assumes that food source dictates health effects and a bias against nutrients derived primarily from animal sources.

The reality is that the evidence, as it always does in nutrition, has multiple factors that are important in conceptualising the findings, including:

- Is the exposure of interest is fish, supplements, or both?
- What is the outcome of interest? [e.g., total cardiovascular disease, myocardial infarction, stroke, etc.];
- If biomarkers* are being investigated, what tissue compartment are they measuring? [e.g., plasma, adipose tissue, etc.].

Because of the challenges of dietary assessment and distinguishing the potential benefits of fish overall from EPA and DHA specifically [as constituents of fish, but not the only nutrients fish provides], biomarker studies have become more attractive to study the essential fatty acids. This is because these fatty acids cannot be produced in adequate amounts in the body, therefore the levels of these nutrients in tissues tend to better reflect the levels of dietary intake.

The present study investigated the relationship between blood levels of EPA and DHA and mortality outcomes.

*Geek Box: Dietary Biomarkers

The term "biomarker" means use of a specific biochemical measure that provides an indication of nutrient intakes. This isn't always as straightforward as "nutrient in = nutrient measured", because nutritional status is influenced by variations in the digestion, absorption, metabolism, distribution, and excretion of a nutrient, which differs from nutrients to nutrient. For example, when measuring fatty acids, whether it is the phospholipid content of cell membranes, lipoproteins, or adipose tissue measured, each will provide different indications of dietary intake. Biomarkers may be classified according to what measurement they allow for. A biomarker for which there is a quantitative relationship between dietary intake and the value of the biomarker, such that absolute intake over a 24hr period can be measured accurately, is known as a "recovery biomarker". "Recovery" reflects the fact that all intake over a 24hr period is excreted, usually through urine, with minimal losses through other excretory pathways. These are very rare in nutrition science: only 24hr urinary sodium, 24hr urinary potassium, 24hr urinary nitrogen, and total energy measured by doubly-labeled water, are considered recovery biomarkers. The most commonly used biomarkers, when measuring the concentration of a nutrient in a plasma, red blood cells, or adipose tissue, are known as "concentration biomarkers", as they are measuring the concentration of that particular nutrient in the circulation or tissue. The use of biomarkers is very attractive for nutritional epidemiology, as it allows for an objective assessment of the validity of dietary questionnaires, and quantification of dietary intake that is independent of measurement error. However, there remain limitations to their application. First, there is not a reliable biomarker for every nutrient of interest to nutrition science. Secondly, many non-dietary factors may influence the status of a biochemical indicator, thus introducing a potential measurement error that is unrelated to actual dietary intake. Nonetheless, for exposures of interest like sodium, potassium, fatty acids, or total energy expenditure, biomarkers are reliable, and provide a means of quantifying accurate dietary intake.

The Study

The Fatty Acid and Outcome Research Consortium [FORCE] of studies comprises a large number of prospective cohorts in which measurements of circulating blood levels fatty acids have been conducted, together with confirmed mortality outcomes. The present study included data from 17 cohorts across 10 countries.

'Blood' levels included measures of plasma phospholipids, cholesterol esters, red blood cells, and whole plasma. Levels were reported as a percentage of total fatty acids in a particular compartment.

Omega-3 fatty acids included the plant-sourced alpha linolenic acid [ALA], docosapentanoic acid [DPA, which is a long-chain fatty acid between EPA and DHA], and EPA and DHA [both individually and combined].

The primary outcome was total mortality [i.e., death from any cause]. Secondary outcomes included cardiovascular disease [CVD] mortality and cancer mortality.

Results: The final analysis included 42,466 individuals, of which 15,720 died during an average follow-up period of 16yrs. Participants average age was 65yrs at baseline, and 55% of the participants were female.

- ***Total Mortality:*** Combined blood levels of EPA+DHA were associated with a 13% [HR 0.87, 95% CI 0.83–0.90] lower risk for total mortality. DPA levels were also associated with a 13% [HR 0.87, 95% CI 0.84–0.91] lower risk.
- ***CVD Mortality:*** Combined blood levels of EPA+DHA were associated with a 15% [HR 0.85, 95% CI 0.79–0.91] lower risk for total mortality. DPA levels were also associated with a 9% [HR 0.91, 95% CI 0.84–0.99] lower risk.
- ***Cancer Mortality:*** Combined blood levels of EPA+DHA were associated with a 11% [HR 0.89, 95% CI 0.83–0.96] lower risk for total mortality. DPA levels were also associated with a 13% [HR 0.87, 95% CI 0.81–0.95] lower risk.

ALA levels were not associated with any effect on any mortality outcome [more under ***Interesting Finding*** and ***Relevance***, below].

- ***Specific Tissue Compartments & EPA+DHA:*** Combined levels of EPA+DHA in phospholipids were associated with a 14% [HR 0.86, 95% CI 0.83–0.91] lower risk for total mortality. Levels in cholesterol esters and in whole plasma were each associated with a 6% lower risk, which was not significant.

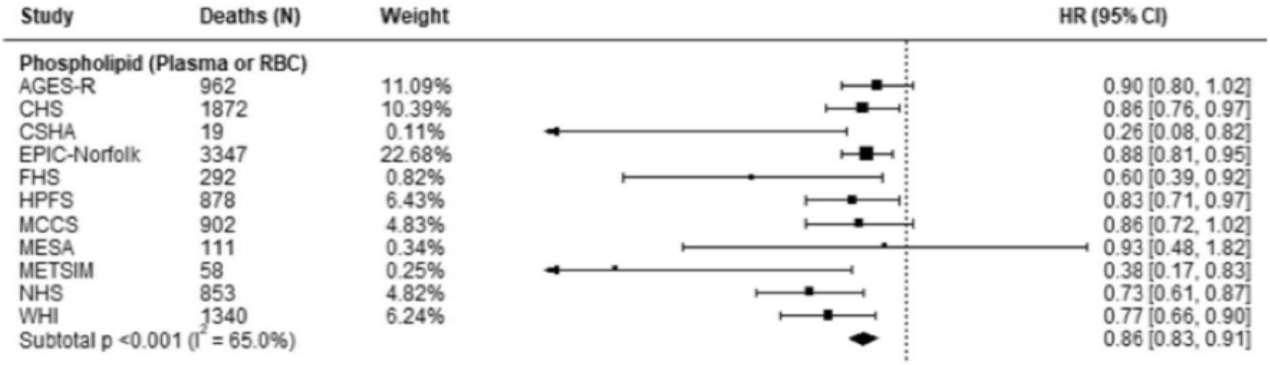

Figure *from the paper illustrating the analysis of EPA+DHA in phospholipids, which included both plasma phospholipids and red blood cell phospholipids. The forest plot shows a consistent direction of effect; each square represents the point estimate for that study, all of which are left of the dotted line [which represents 1.0, or the 'null']. The bars on either side represent the confidence intervals for that study. You can see that only a single study is spreadeagled across the 1.0 line, i.e., across the null. And you can see that while other studies may cross over it just about, the direction of effect of those studies is all toward lower risk.*

- ***Linear Analysis:*** Analysis of linear effects for EPA, DHA, DPA, and EPA+DHA revealed significant linear associations for DPA, DHA, and EPA+DHA. The relationship for EPA was significant, but non-linear; the effect was greatest between the lowest intakes to the 50th percentile, before plateauing. Each were associated with 15-18% lower risk of total mortality.

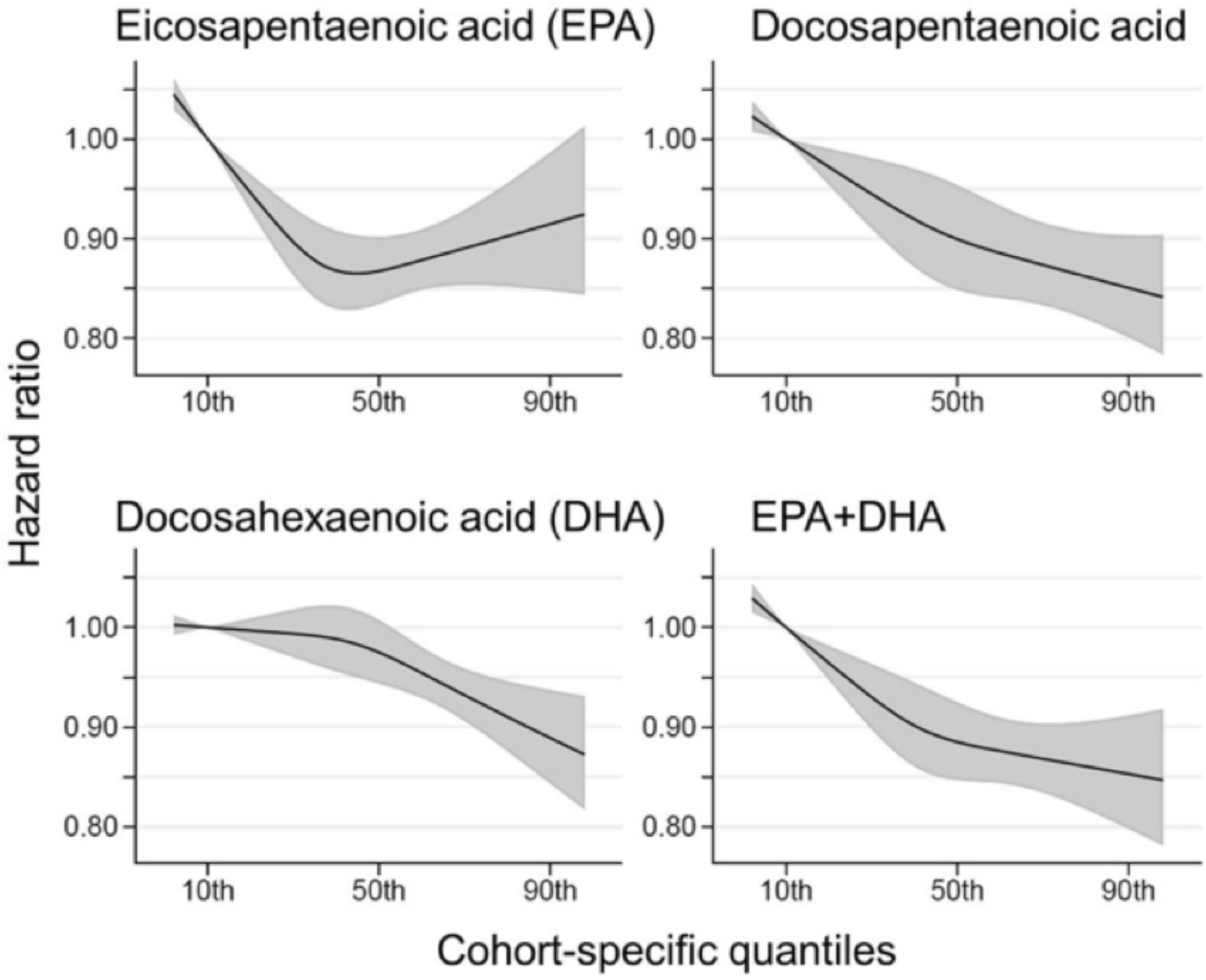

Figure *from the paper illustrating the associations going from the 10th to the 90th percentile of measured omega-3 fatty acids.*

The Critical Breakdown

Pros: In FORCE, all participating studies followed the same standardised analysis protocol, limiting variation from study to study. The included studies encompassed populations in Asia [Japan, Taiwan], Europe [Sweden, Finland, Iceland, France, the UK], Australia and North America [the US and Canada]. Importantly, participants in the included studies were not taking fish oil supplements. The analysis was adjusted for circulating omega-6 levels, which are also associated with lower mortality risk. The study had a large sample size and especially large number of deaths, provided substantial statistical power to detect effects.

Cons: Given red blood cell phospholipids were a main compartment analysed, it would have been helpful to calculate the 'Omega-3 Index', the amount of EPA/DHA as a percentage of all fatty acids identified in red-blood cell membranes. This is insightful because the fatty acid composition of cell membranes can change rapidly in response to dietary intake; EPA content rises and falls quickly, while DHA remains more constant. The proportion of fatty acids in cell membranes, as a percentage of fatty acids, may be important for the biological activity of these fatty acids.

Key Characteristic

Given the various compartments measured, it was helpful that the associations for different tissue measurements were presented separately. In particular, this allowed for an important finding to be clear; that the strongest magnitude of effect was observed for phospholipid membrane measurements of EPA and DHA, with a 14% lower risk of total mortality.

Depending on whether fatty acids are measured as the composition of fats in circulating blood fats, like triglycerides or lipoproteins, or measured as free fatty acids, or measured from cell membranes, all provide a different indication of the relationship with dietary intake.

For example, measuring fasting blood lipids reflects dietary intake over the previous 3-4 days, while measuring lipoprotein content reflects 2-3 weeks [(1)]. The fatty acid composition of cell membranes is considered a reflection of dietary intake over the previous months, and is therefore a more reliable assessment of consistent intake of EPA/DHA [(1)]. Furthermore, because EPA and DHA are rapidly incorporated into cell membranes, measures of EPA and DHA like whole plasma are not a strong indication of levels of these fatty acids in the body, or of dietary intake. For EPA and DHA, phospholipid membrane levels are a far more robust indicator of EPA/DHA status [(2)].

Interesting Finding

The finding of a linear lower risk of death with increasing circulating levels of DHA warrants comment. The half-life time of DHA in whole plasma is 2 minutes [3]. However, DHA in phospholipids is more stable, and may reflect more medium-term dietary intake [4]. Importantly, ALA levels do not increase plasma DHA levels; in fact, they may drive DHA levels in red blood cell phospholipids down, as we reviewed in a previous Deepdive.

Further, Mendelian randomisation studies of plasma DHA have suggested that serum DHA is "not causally" related to health outcomes [5], although there are questions over whether serum DHA is a relevant proxy for dietary DHA intake or DHA status. This finding would suggest that: a) dietary DHA is the factor driving higher levels of DHA [because ALA does not increase plasma levels], and; b) that some DHA is better than none, and more is preferable to less. This would be consistent with the totality of evidence [2,6,7].

Relevance

Biomarker studies are important parts of the evidential puzzle because, when the biomarker is of a nutrient for which a dietary source of intake is required, it provides a more 'objective' marker than a dietary assessment.

The authors themselves highlight that the hierarchy of effect was red blood cell phospholipid>plasma phospholipid>plasma cholesterol ester>total plasma. This is consistent with red blood cell phospholipids being a more robust reflection of EPA+DHA status, per our ***Key Characteristic***, above.

The lack of association for ALA specifically is also further evidence that the plant-sourced omega-3 fatty acid may not have the same physiological effects as the very-long-chain EPA and DHA. We know that ALA does not increase any plasma levels of DHA, so the finding in relation to DHA is difficult to suggest as merely a reflection of ALA converting to DHA [2]. And although ALA does increase EPA levels, the magnitude of effect for increasing EPA levels is 15-times greater when a direct source of EPA is consumed [2].

However, it should also be noted that a review of 28 studies of both ALA and EPA supplementation demonstrated that neither ALA nor EPA was effective at increasing DHA levels [2]. Thus, DHA levels in the body appear to be only responsive to direct preformed dietary sources of DHA.

Application to Practice

All of this points to direct, preformed EPA+DHA still being important. The evidence is unconvincing that ALA can cover these requirements. More particularly, the fact that red blood cell levels - which reflect dietary intake over the course of the previous ~3-months - show the strongest associations with lower mortality risk indicates that consistent, sustained intakes of EPE+DHA are desirable. We could distill these findings into recommendations which suit all:

- If oily fish is an option, then ~2 servings [120g] per week provides sufficient EPA+DHA;
- If oily fish is not an option, but fish oil supplements are, it may be prudent to supplement a minimum of ~1g/d combined EPA+DHA of a high-quality supplement;
- If neither oily fish or fish oil supplements are an option, ala vegans, it may be prudent to supplement a minimum of ~1g/d combined EPA+DHA from an a high-quality algae-based supplement.

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