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Park S, Lee S, Kim Y, et al. Causal Effects of Serum Levels of n-3 or n-6 Polyunsaturated Fatty Acids on Coronary Artery Disease: Mendelian Randomization Study. *Nutrients*. 2021;13(5):1490.

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

If we anthropomorphised polyunsaturated fats [PUFA], you would definitely feel like they are being bullied. And being the good person that you are, you'd want to stop the bullying and set the world to rights. Except, in the seemingly conflicting world of science and evidence we don't have bullies, but we do have bad research and, as Richard Feynman said, the easiest person to fool is ourselves.

For omega-6 PUFA, there is an entire community in nutrition who have fooled themselves into thinking that these fats are harmful, when the evidence from prospective cohort studies [1,2], tissue biomarker studies [1,3], randomised controlled trials [4,5], and controlled feeding studies [6–8], all demonstrate benefits to these fats for cardiovascular health.

For omega-3 PUFA, the irony is that these are the subtypes of PUFA where almost everyone in the popular nutrition conversation is convinced of a range of benefits, yet the evidence requires much more dissection to come to any conclusion, and that conclusion could be debated [9,10]. The inconsistency in the evidence likely relates to the fact that both EPA and DHA have distinct mechanisms in cardiovascular health, and the form of supplement, ratio of EPA to DHA, and total dose, are all moderating factors influencing the outcomes in RCTs to date [11,12].

The challenges of teasing out cause-effect relationships in science, particularly in nutrition science, has led to an explosion in the popularity of genetic studies known as Mendelian randomisation [MR] as a research design [***see Geek Box**, below, for further detail]. The present study conducted an MR analysis of the effects of omega-6 and omega-3 on coronary artery disease [CAD].

The Study

The study was conducted as a "two-sample MR", which is a type of MR analysis where the exposure is measured in one genetic sample and the outcome is measured from another genetic sample. Thus, the present two-sample set up in this study was:

- **Exposure:** European ancestry genetic study [known as "genome-wide association study" or GWAS] on serum omega-3 and omega-6 levels
- **Outcome:** UK Biobank using individual-level data [*n* = 337,129] and the CARDIoGRAMplusC4D database using summary-level data [*n* = 184,305].

"Individual-level data" is where information on genetic variants and outcomes are available for each participant in the study, and the ultimate results reflect the average across all individuals. "Summary-level data" is where the estimated effect of a genetic variant on an outcome has already been summarised from previous genetic analysis.

The present study thus conducted two separate two-sample MR analyses:

1. **Individual-Level**: GWAS omega-3/omega-6 data & UK Biobank data on risk of myocardial infarction [MI].
2. **Summary-Level**: GWAS omega-3/omega-6 data & CARDIoGRAMplusC4D data on risk of MI and CAD.

The "exposure" was genetic variants associated with higher levels of omega-3 and omega-6 in the serum [the fluid portion of blood remaining after blood has clotted].

*Geek Box: Mendelian Randomisation Research Design

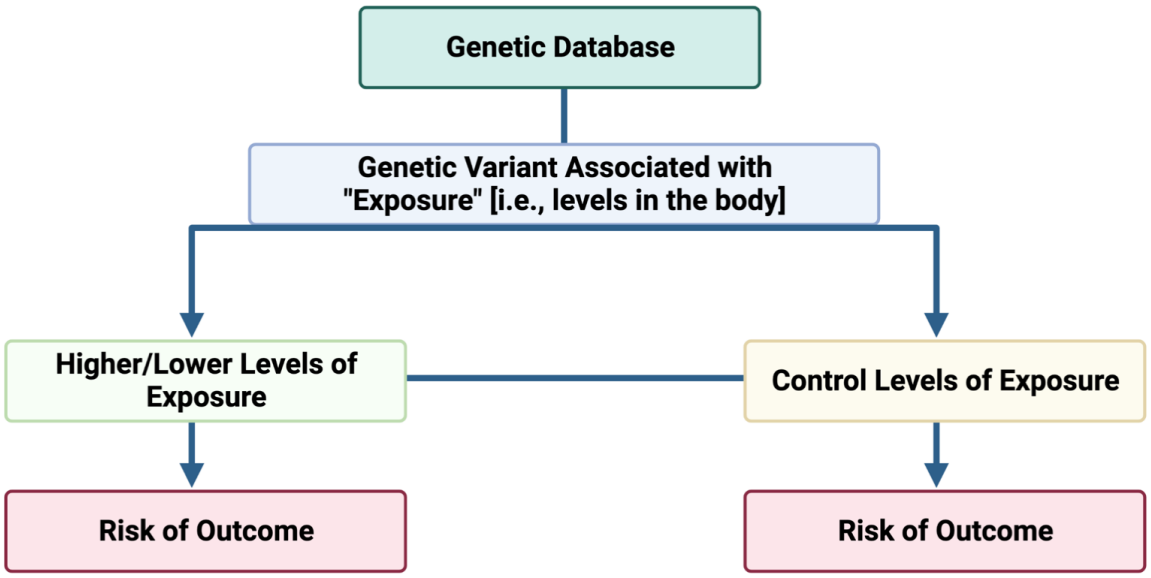
Mendelian randomisation [MR] is a principle of using genetics to mimic a long-term randomised controlled trial [see figure, below], particularly where a long-term intervention study may be unethical or practically infeasible. Because an individuals' genes are 'assigned' when they are conceived, this in effect it is the purest form of randomisation, i.e., the genetic lottery from Mom and Pops.

Well-conducted MR can provide an unconfounded estimate of the relationship between an exposure and an outcome. It is unconfounded because the genetic variant results in a certain physiological response that is independent of other considerations. Thus, to be properly conducted, a MR study must satisfy three criteria:

1. *The genetic variant must be associated with the specific mediating exposure', e.g., LDL-C;*
2. *The genetic variant must not be associated with any potential confounders that could influence the outcome, and;*
3. *The genetic variant must only influence the disease outcome through the specific exposure pathway, not through other mechanisms.*

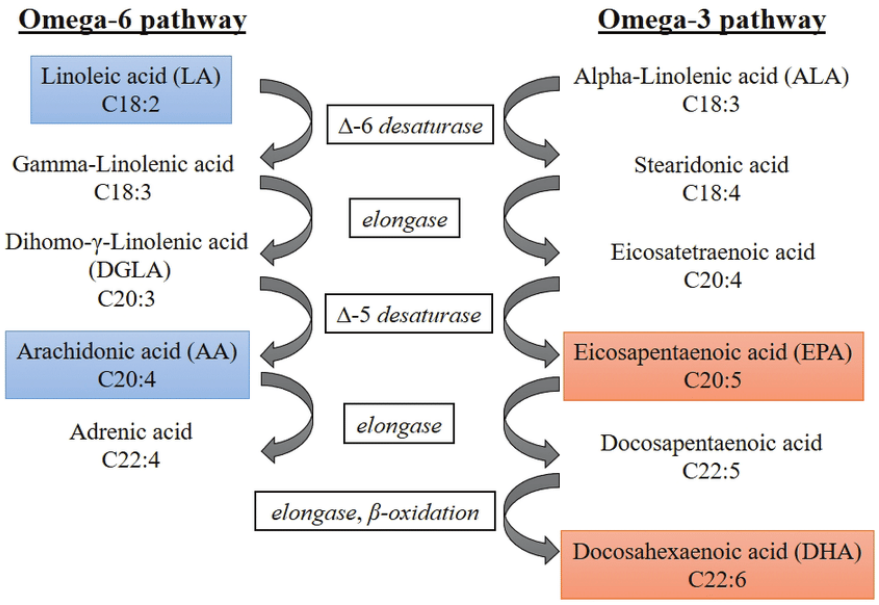
An IV is only valid where the 3 assumptions above hold. This is crucial, because it means that claims of "causality" can only be made where these assumptions are met. Where there may be factors that undermine these assumptions, then an MR study should be considered genetic associations, not necessarily a cause-effect relationship.

When long-term randomised studies are not possible, Mendelian randomisation is a powerful tool to examine potential cause-effect relationships. But we should temper our enthusiasm for thinking anything genetic solves all methodological challenges in our field, as MR of nutritional exposures faces several methodological challenges itself that need to be considered.

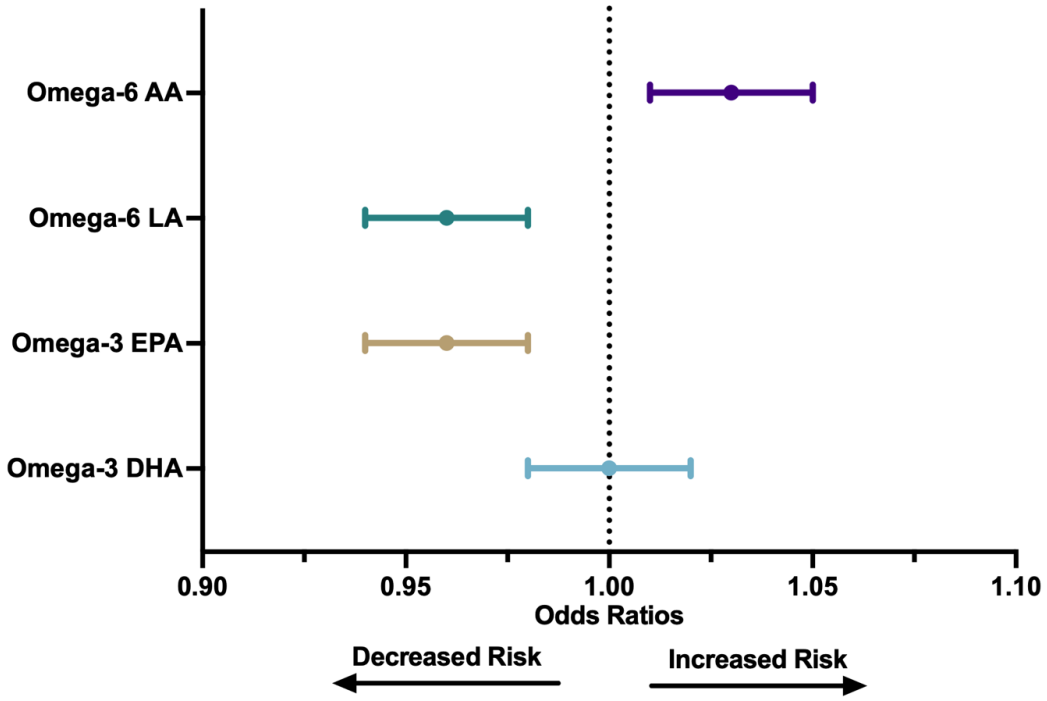


Results: For the individual-level data, the characteristics of the UK Biobank cohort included in this study included an average age of 58yrs, 46% female, and 24% with obesity. Of the 337,129 participants included in the analysis, an MI occurred in 4% [*n* = 12,812]. The results are presented as odds ratio [OR] with lower and upper 95% confidence intervals [95% CI].

The analysis included the omega-3 eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA; an intermediate between EPA and DHA], and docosahexaenoic acid [DHA], and the omega-6 linoleic acid [LA], gamma-linolenic acid [GLA], dihomo- gamma-linolenic acid [DGLA], and arachidonic acid [AA]. To get a bearing on the position of these fatty acids the respective omega-3 and omega-6 pathways, see this ***figure***, below.



- ***Biobank Outcomes – Myocardial Infarction [MI]*:**
  - **Omega-3**:
    - **EPA**: Higher serum EPA was associated with 4% lower odds for MI [OR 0.96, 95% CI 0.94 to 0.98]
    - **DPA**: Higher serum EPA was associated with 2% higher odds for MI [OR 1.02, 95% CI 1.00 to 1.05]
    - **DHA**: Higher serum DHA was not associated with MI [OR 1.00, 95% CI 0.98 to 1.02]
  - **Omega-6**:
    - **LA**: Higher serum LA was associated with 4% lower odds for MI [OR 0.96, 95% CI 0.94 to 0.98]
    - **GLA**: Higher serum GLA was associated with 2% higher odds for MI [OR 1.02, 95% CI 1.00 to 1.04]
    - **DGLA**: Higher serum DGLA was associated with 4% lower odds for MI [OR 0.96, 95% CI 0.95 to 0.98]
    - **AA**: Higher serum AA was associated with 3% higher odds for MI [OR 1.03, 95% CI 1.01 to 1.05]



Forest plot *indicating the point estimate and 95% confidence intervals for the findings of the main omega-3 and omega-6 polyunsaturated fat analysed in the UK Biobank genetic on risk of MI.*

- ***CARDIoGRAMplusC4D Outcomes – Coronary Artery Disease [CAD] & MI*:**
  - **Omega-3**:
    - **EPA**: Higher serum EPA was associated with 22% lower odds for CAD [OR 0.79, 95% CI 0.62 to 0.97] and 21% lower odds of MI [OR 0.79, 95% CI 0.53 to 1.17]
    - **DPA**: Higher serum EPA was associated with 21% higher odds for CAD [OR 1.22, 95% CI 0.97 to 1.52] and 22% higher odds for MI [OR 1.22, 95% CI 0.95 to 1.57]
    - **DHA**: Higher serum DHA was not associated with CAD [OR 1.00, 95% CI 0.85 to 1.17] or MI [OR 1.05, 95% CI 0.88 to 1.26]
  - **Omega-6**:
    - **LA**: Higher serum LA was associated with 3% lower odds for CAD [OR 0.97, 95% CI 0.95 to 1.00] and 3% lower odds for MI [OR 0.97, 95% CI 0.95 to 0.99]
    - **DGLA**: Higher serum DGLA was associated with 6% lower odds for CAD [OR 0.94, 95% CI 0.89 to 0.98] and 7% lower odds for MI [OR 0.93, 95% CI 0.88 to 0.98]
    - **AA**: Higher serum AA was associated with 1% higher odds for CAD and MI [OR 1.01, 95% CI 1.00 to 1.02 for both findings]

The Critical Breakdown

Pros: Both analyses were based on enormous sample sizes in both genetic cohorts, which was larger in the UK Biobank cohort compared to CARDIoGRAMplusC4D. The study thus had a high level of statistical power. By conducting two separate two-sample MR' s, the study attempted to replicate the findings from the individual-level Biobank analysis with the summary-level CARDIoGRAMplusC4D analysis. The summary-level analysis included 43,676 cases of MI and 60,801 cases of CAD. The individual-level analysis included statistical adjustments for a range of relevant covariates, e.g., LDL-C levels, hypertension, smoking history, cholesterol medications.

Cons: For the UK Biobank data, only 4% of the total cohort experienced an MI, which may reduce some of the statistical power to detect associations, and the effect sizes derived from this analysis were miniscule. A potential imitation is the use of serum long-chain fatty acids as the "exposure" [more under ***Key Characteristic***, below]. The difference in the number of cases between the Biobank and CARDIoGRAMplusC4D data may have influenced the different strengths of associations shown in the respective analyses. The analysis used genetic data only from individuals of White/European ancestry, and thus the findings may not generalise to other ethnic/ancestry population groups.

Key Characteristic

The fact that this analysis focused on *serum* levels of PUFA is the critical design factor to be considered in interpreting the findings. This is because, in an MR analysis, the genetic variant(s) analysed may only be associated with a specific, isolated tissue compartment, e.g., serum, which may not be the sole pathway through which a given nutrient may be associated with an outcome.

It is important to note that what "genetic predisposition to higher serum PUFA" or "genetically higher serum PUFA" means, from the perspective of MR studies; this is intended to mean that these higher levels serve as a *proxy for dietary intake*. Simply put, "genetically higher serum PUFA" translates, in concept, to "higher levels from diet/supplements".

This becomes crucial to interpreting the findings, because we know from biomarker studies – where the *actual* levels of fatty acids are measured in different tissue compartments, e.g., serum, red blood cells, adipose tissue, etc. – that different tissue biomarkers correlate with different reflections of dietary intake.

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.

Within serum, fatty acids may be present in triglycerides, phospholipids, and cholesterol esters [(13)]. These each reflect different time-courses of dietary intake; triglycerides reflect recent hours and immediate days, while phospholipids and cholesterol esters reflect ~2-4 days prior [(13)]. Moreover, red blood cell fatty acids are more stable in response to recent dietary intake, while serum and plasma can be highly variable in response to recent meals [(13,14)].

Thus, as serum PUFA have been analysed in the present study, as a proxy for diet this would only reflect the most immediate intakes of PUFA from diet. This could be too short-term and variable a tissue compartment to make causal claims of long-term dietary intake. The important implication for these findings is that the conclusions should be confined to the specific tissue compartment, not stated as the effect of PUFA more generally in relation to MI/CAD.

Interesting Finding

The different directions of effect for the two omega-6 markers analysed – linoleic acid [LA] associated with lower risk and arachidonic acid [AA] associated with higher risk [more on AA specifically under ***Relevance***, below] – may be further vindication of LA against the allegations that LA causes inflammation and *increases* risk for adverse health outcomes [15].

When it comes to LA consumed in the diet, a long-standing allegation has been that LA provides a precursor to AA, and AA is used to form pro-inflammatory compounds in the body. This latter statement is correct; AA is used to form what are known as 'eicosanoids', which moderate inflammatory responses in the body, and the precise eicosanoids that AA produces *are* pro-inflammatory [in contrast to the synthesis of anti-inflammatory eicosanoids by EPA] [16].

However, there is actually no experimental evidence for this relationship in humans. A review of 36 human intervention studies highlighted that neither increasing LA levels by up to 551%, or decreasing LA levels by 90%, altered concentrations of AA in plasma, serum, or red blood cells [17].

And a review of 15 human intervention trials investigating the effects of LA on inflammatory biomarkers concluded that *"virtually no data are available from randomised, controlled intervention studies among healthy, non-infant human beings to show that the addition of LA to diets increases markers of inflammation."* [18].

Relevance

The authors continually referred to the findings as "causal effects of PUFA", and it is important to temper this language; it is more appropriate to consider these findings as "genetic associations". Here is why: serum levels act as a proxy for the most short-term dietary intakes, which would themselves be influenced by actual dietary intakes in the "real-world". And the differences in effect estimates, although there were often similar directions of effect between Biobank and the CARDIoGRAMplusC4D cohorts, preclude a confident causal conclusion. Finally, a causal conclusion would require any such relationship to be demonstrated in different populations, while the present analysis is confined to White/European genetic ancestry.

This does not, however, mean that the findings are not a useful addition to the overall evidence. Let's consider how the findings for the main PUFA of interest could be interpreted as it relates specifically to serum measurements. The first is that the lack of effect for serum DHA – a perfect 'null' in both the between Biobank and the CARDIoGRAMplusC4D analyses – DHA is low in any blood compartment and turnover is rapid, thus it may be difficult to detect associations for serum DHA [19,20].

The EPA effect size was a small 4% in the Biobank analysis of MI, while the CARDIoGRAMplusC4D analysis of EPA showed lower 22% lower CAD risk, but for MI although the point estimate was a 21% lower risk the confidence intervals crossed 1.0 up to 1.17, and thus this finding is not reliable. The lack of effect of EPA on MI in the CARDIoGRAMplusC4D data differs to RCTs on this outcome, which are consistent in favour of omega-3 supplementation, both mixed EPA/DHA and EPA in isolation [21]. The effect on CAD, however, is interesting given the evidence that EPA is preferentially incorporated into arterial plaques, improving their morphology, and lowering inflammation [22,23].

What of AA? AA levels in the body are maintained, endogenously, at a relatively constant level [24] Given that the effect size was borderline non-existent – a 3% and 1% higher risk in the Biobank and the CARDIoGRAMplusC4D analyses, respectively, factoring in the relatively constant levels in the body in response to diet, this finding does not likely warrant much weight attached to it.

Application to Practice

Relating the findings from an MR study to actual "real life" dietary intake is always a tad messy. Again, if we bear in mind that "genetically higher levels" of any fatty acid translate, in interpretation, to "higher dietary intakes", then nothing much really turns on this study. In sum:

1. Omega-3 = good
2. Omega-6 = also good

The fact that there were associations for fatty acids like DPA and DGLA doesn't negate that fact, and would require validation in future research. And in practical application, you don't really eat isolated fatty acids, e.g., while oily fish would contain EPA/DHA, it would also contain other omega-3's, and while sunflower oil may contain LA it would also contain other omega-6's.

The argument over the omega-3:omega-6 ratio is likely a reflection of low omega-3's, so ultimately aiming for sufficient alpha-linolenic acid [ALA], EPA and DHA in the diet to retain sufficient fatty acid balance is ideal.

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