ALINEA

NUTRITION

MAY 2021

www.alineanutrition.com

TABLE OF

CONTENTS

Li J, Guasch-Ferré M, Li Y, Hu FB. Dietary intake and biomarkers of linoleic acid and mortality: systematic review and meta-analysis of prospective cohort studies. Am J Clin Nutr. 2020 Jul 1;112(1):150-167.

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

Throughout the early research into the Diet-Heart Hypothesis, two findings became clear:

1. Polyunsaturated fats had the greatest blood cholesterol-lowering effect;
2. Hydrogenation of polyunsaturated fats resulted in the opposite effect.

In 1957, Beveridge et al. had published the results of feeding polyunsaturated and monounsaturated rich oils, either in natural form or in hydrogenated form, and showed opposite effects: the natural oil significantly lowered blood cholesterol, while the hydrogenated form increased blood cholesterol levels [(1)].

These early metabolic ward studies also clearly demonstrated that saturated fats had twice the effect on raising blood cholesterol levels as polyunsaturated fats had on lowering blood cholesterol [(2)]. Thus, the most beneficial dietary adjustment to lower blood cholesterol levels, which at the time were already implicated in heart disease risk, was to replace saturated with polyunsaturated fats.

In the 1960' s and 1970' s, a number of randomised controlled trials were conducted on this basis: the intervention group would replace saturated fat sources, like butter and lard, with polyunsaturated fats from margarines and/or vegetable oils. The control group would continue with habitual saturated fat intake. One such study was the Sydney Diet Heart Study [SDHS], in which men who had existing coronary heart disease [CHD] were randomised to either continue with SFA intake of 16% or increase PUFA to 15%. The intervention group were provided with a commercially available safflower-based margarine, known as *Marrickville Margarine*. The problem is that commercial margarines at the time often contained anywhere from 25-40% trans fatty acids, due to hydrogenation [(3)]. Over ~3yrs of follow-up in the study, it was actually participants in the intervention group who experienced a statistically insignificant increased risk for a further coronary event.

In 2013, a group obtained old data from the original SDHS researchers and re-analysed the data, purporting to now show a statistically significant risk of cardiovascular disease [CVD] and CHD [(4)]. However, none of the fundamental limitations of the original study had changed. The SHDS was also included in a meta-analysis of other, massively flawed trials from the same period, all pointing in the direction of omega-6 linoleic acid increasing risk for CVD/CHD [(5)].

Needless to say, this was all music to the ears of Diet-Heart revisionists. The present study examined the long-term effects of omega-6 linoleic acid [LA] intake from diet, and circulating biomarkers of LA on mortality risk, in prospective cohort studies.

The Study

A systematic review and meta-analysis of prospective cohort studies or nested case-control/ case-cohort studies* was conducted with all-cause mortality, CVD mortality, and cancer mortality, as endpoints. Studies had to have reported a risk estimate [i.e., relative risk or hazard ratios] that was adjusted for multiple variables, for the association between LA and at least one mortality outcome.

Studies on dietary intake and biomarkers were analysed separately. A dose-response analysis was conducted to examine the relationship between dose of LA and mortality. The heterogeneity between studies was assessed [25% = low; >75% = high], and the included studies were examined for potential sources of heterogeneity [i.e., baseline age, sex, follow-up duration].

*Geek Box: Nested Case-Control & Case-Cohort Studies

Prospective cohort studies take a cohort of people from the population, and follow them prospectively, i.e., over time. The advantage to this design is that exposures - like diet, smoking, etc. - can be assessed before a disease develops in the participants, and this reduces the potential for recall bias. Another advantage of prospective cohort studies is the potential sample size, which can range into the thousands to hundreds of thousands of participants. However, this comes with a potential disadvantage in terms of gathering more granular detail regarding exposures and participant characteristics, which would be prohibitively expensive to do with a cohort of, for example, >100,000 people. To navigate this potential hurdle, some additional study designs have been used, in particular nested case-control and case-cohort designs. In a nested case-control design, a number of healthy participants are selected as controls for each case of an outcome, e.g., each case of coronary heart disease that is diagnosed. The design is termed 'nested', because it is within a larger defined cohort. In this design, the researchers identify cases of their outcome of interest - we'll use CHD for example - that have already occurred, or as they occur if the parent study is a prospective cohort, and then select up to 4 to 5 healthy controls to match with a case of CHD. The nested case-control study would then compare, for example, blood cholesterol levels and blood pressure in the healthy controls vs. the CHD cases. While nested case-control studies have some advantages, they can face some logistical hurdles, in particular if more than one disease outcome is of interest - which is common in prospective cohort studies - then it can be highly inefficient, as each case of each different disease would require its own set of healthy controls to be matched with. As a result, the case-cohort design was proposed as an alternative to the nested case-control. In a case-cohort design, rather than select 4-5 healthy controls for each case, a random sample [known as a 'subcohort'] is selected along with all identified cases of the disease outcome of interest. Thus, the case-cohort consists of the cases and the subcohort. The main advantage of the case-cohort design is that, by the selection of a random sample, multiple disease outcomes can be examined from the same subcohort. This is because the subcohort is not matched to specific cases, as occurs in a nested case-control design. Both study designs share the advantage of lower cost and more efficiency in execution, and where the outcome of interest is a single disease endpoint, the difference between the two designs is small. However, where multiple disease endpoints are of interest, the case-cohort design is more efficient due to the random sampling of the subcohort.

Results: A total of 21 prospective cohorts were included, totalling 811,069 participants, 170,076 total deaths, 50,786 CVD deaths, and 59,684 cancer deaths. The follow-up duration ranged from 5-30yrs. The following reported findings are for statistically significant outcomes.

Dietary LA Intake:

- **All-cause Mortality:** Comparing high vs. low levels of LA intake, there was a 13% lower risk [RR 0.87, 95% CI 0.81 - 0.94]
- **CVD Mortality:** Comparing high vs. low levels of LA intake, there was a 13% lower risk [RR 0.87, 95% CI 0.82 - 0.92]
- **Cancer Mortality:** Comparing high vs. low levels of LA intake, there was a 11% lower risk [RR 0.89, 95% CI 0.85 - 0.93]

Dose-Response Analysis:

- **All-cause Mortality:** Each 10% of energy from LA was associated with a 19% lower risk [RR 0.81, 95% CI 0.69 - 0.96]
- **CVD Mortality:** Each 5% of energy from LA was associated with a 7% lower risk [RR 0.93, 95% CI 0.91 - 0.95]
- **Cancer Mortality:** Each 10% of energy from LA was associated with a 17% lower risk [RR 0.83, 95% CI 0.78 - 0.89]

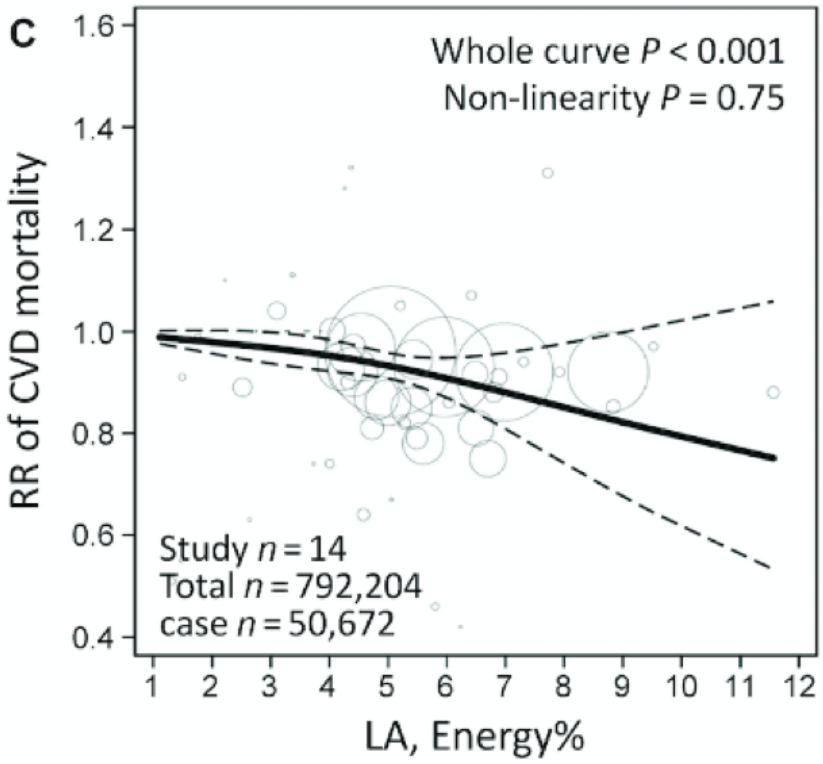


Figure *rom paper illustrating the linear relationship in the dose-response analysis between increase LA as a percentage of energy, and lower CVD risk. The relationships for all-cause mortality and cancer were non-linear, but similar to the CVD mortality outcome showed a significant reduction in risk >8% of total energy from omega-6 LA.*

Biomarker Analysis:

- **All-cause Mortality:** Combined analysis of LA in all measured tissue compartments [i.e., adipose tissue, plasma] was associated with an 9% lower risk [RR 0.91, 95% CI 0.87 - 0.95]
- **CVD Mortality:** Combined analysis of LA in all measured tissue compartments [i.e., adipose tissue, plasma] was associated with an 11% lower risk [RR 0.89, 95% CI 0.85 - 0.94]
- **Cancer Mortality:** Combined analysis of LA in all measured tissue compartments [i.e., adipose tissue, plasma] was associated with an 9% lower risk [RR 0.91, 95% CI 0.84 - 0.98]

The Critical Breakdown

Pros: The included studies provided an enormous total sample size of >800,000 participants, in which a substantial number of mortality events occurred. The study combined an analysis of dietary intake, including a dose-response analysis [more under ***Key Characteristic***, below], and analysis of tissue biomarkers of LA. For CVD and cancer mortality, there was very low heterogeneity between studies in the meta-analysis. Study quality was assessed using appropriate guidelines for reporting systematic reviews and observational meta-analysis.

Cons: Notwithstanding the low heterogeneity in specific outcomes, there was high heterogeneity in the all-cause mortality outcomes. No substitution analysis was conducted, so it is unclear to what extent the effect of LA would be mediated by what nutrient it replaces in the diet, i.e., carbohydrate or saturated fat. There is always the caveat with meta-analysis of prospective cohort studies that residual confounding may influence the outcomes. There is also the caveat with meta-analysis of prospective cohort studies that the 'high' vs. 'low' comparison may not be uniform, and the paper did not specify what these levels were. Were it not for the dose-response analysis, this would be more of a limitation.

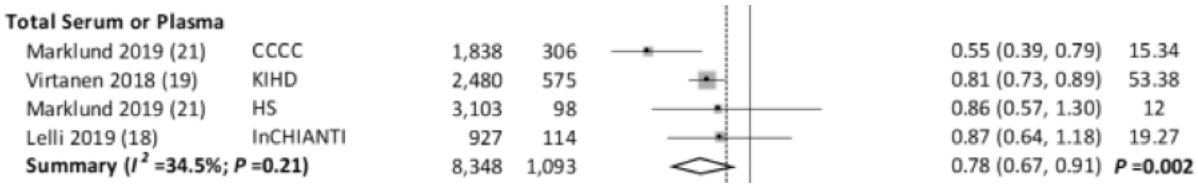
Key Characteristic

The dose response analysis provided important additional insight into levels of omega-6 LA associated with reduce risk of disease. This is useful where the 'high vs. low' comparison levels were not defined in the main analysis of dietary intake. However, we know from the dose-response analysis that LA intake ranged from 1.1% to 11.6% of total energy intake. This is important because dose-response relationships for nutrients are most likely to be non-linear, due to the bell-curve shape of nutrient action from insufficient intakes, to adequate, to excess. Thus, dose-response relationships tend to depend on the starting point of intake on a range of potential exposure to the nutrient [(6)]. And if nutrient intake is already sufficient, then the curve may be flat or non-linear [(6)]. PUFA also generally do not constitute more than 11% of dietary energy, and an analysis of data from 40 countries indicated a range of average PUFA intakes of 2.8 to 11.3% [(7)]. The dose-response analysis in the present study thus reflects habitual PUFA intakes, and from very low levels of 1.1% demonstrated that 5% to 10% increases in LA were associated with significant reductions in mortality risk.

Interesting Finding

Although the main biomarker analysis in the paper presented the relative risks for all tissue compartments, in the supplementary data the effects of each specific tissue compartment were analysed separately. And interestingly, plasma levels of LA were associated with the largest effect size reducing risk for CVD mortality, followed by cholesterol esters. Plasma fatty acids tend to reflect very recent dietary intake, over a matter of preceding 3-4 days, while cholesterol esters reflect the previous 2-3 weeks [8]. In fact, LA levels appear to be greatest in cholesterol esters [8].

These biomarkers are known as "concentration biomarkers", because they are measuring the concentration of that particular nutrient in the circulation or tissue. 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, the half-life of DHA in plasma is only a matter of minutes, and plasma DHA is not a reliable marker for intake of dietary DHA compared to other compartments [8]. The biomarker analysis in the present study thus point to regular intakes of LA.



Forest plot *from supplementary data on plasma biomarkers of LA intake, which were associated with a 22% lower risk of CVD.*

Relevance

It is still possible to find papers, for example from James DiNicolantonio in his very own journal, BMJ Open Heart, pointing the finger at omega-6 vegetable oils as the primary driver of CVD/CHD [9]. This tends to come wrapped up concomitantly with assertions that saturated fats were wrongly implicated. If you haven't yet read the Video Lectures and long-form articles on the topic of saturated fat, I'll refer you to Part 1 & Part 2.

The issue is that this argument is tired, predictable, and played out. It is based on the Sydney Diet Heart Study and a couple of others like it, and the recent meta-analyses of those studies. The argument is divorced from the context of the total literature, which clearly demonstrates that at every level of evidence, polyunsaturated fats are associated with beneficial outcomes. In 2009, Jakobsen et al. published an analysis of data pooled from 11 cohorts, with a range of from 1.7% to 10.6% PUFA intake and 9.4-21.3% SFA intake [10]. Replacing 5% of energy from SFA with PUFA was associated with a 13% relative risk reduction for coronary events and reduced risk for coronary deaths by 26%.

In a meta-analysis by Farvid et al. including 12 cohorts in the US, Europe, and Israel, comparing high vs. low intakes [average 6.4% to 1.5%, respectively] of LA was associated with a 15% lower risk for CHD events, and 21% lower risk for CHD death [11]. This paper also conducted a dose-response analysis, and also found that the relationship between increasing LA as a percentage of energy and lower risk of CHD events and deaths was linear: each 5% increase in LA was associated with a 10% lower risk for events and 13% lower risk for CHD mortality.

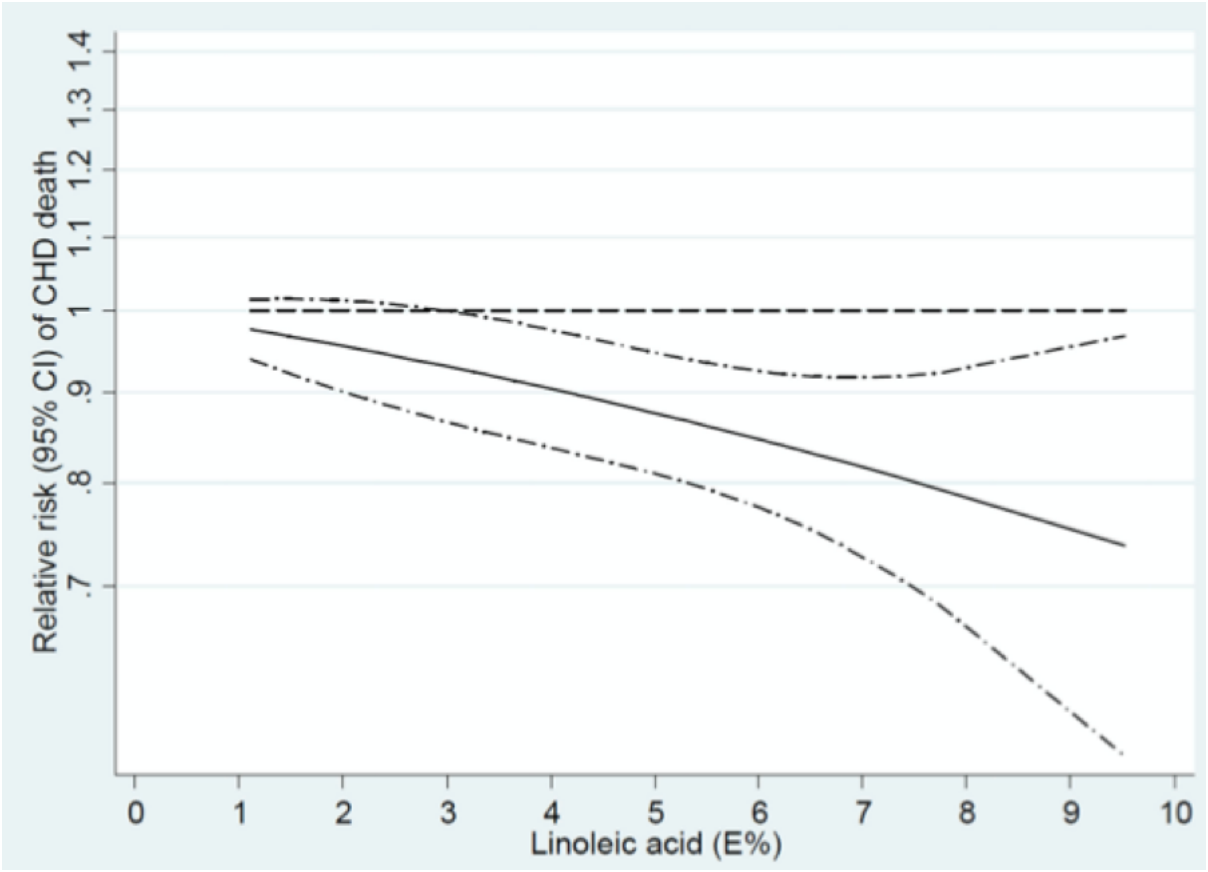


***Figure** from Farvid et al. [11] illustrating linear association between increasing linoleic acid (as a percentage of energy) and reduction in risk for coronary heart disease.*

Although the effect sizes are modest for certain outcomes, this should be considered in the context of the relatively low dose-response levels of intake required for the benefit to be observed.

Application to Practice

When combined with the evidence from tightly controlled interventions examining intermediate risk factors, there is strong corroborating biological plausibility for the reductions in risk observed with higher vs. lower PUFA intake, and omega-6 LA intake. Obviously diet is the sum of its parts, and total PUFA intake also encompasses other beneficial nutrients, the marine omega-3 and plant omega-3 fatty acids included. However, there is no legitimate, science-based reason to avoid certain oils, nuts, or seeds, due to concerns about omega-6 fats. In fact, for people consuming very little, modest increases in daily energy derived from omega-6 appear to be preferable.

References

1. Keys A, Anderson J, Grande F. Prediction of Serum-Cholesterol Responses of Man to Changes in Fats in the Diet. The Lancet. 1957;270(7003):959-966.
2. Beveridge J, Connell W, Mayer G, Firstbrook J, DeWolfe M. The Effects of Certain Vegetable and Animal Fats on the Plasma Lipids of Humans. The Journal of Nutrition. 1955;56(2):311-320.
3. Bernfeld P, Homburger F, Kelley T. Fatty Acid Contents of Margarines and Other Table Fats. The American Journal of Clinical Nutrition. 1962;11(6):554-558.
4. Ramsden C, Zamora D, Faurot K, Majchrzak S, Hibbeln J. The Sydney Diet Heart Study: a randomised controlled trial of linoleic acid for secondary prevention of coronary heart disease and death. FASEB J. 2013;27(S1):127.4-127.4.
5. Ramsden C, Zamora D, Leelarthaepin B, Majchrzak-Hong S, Faurot K, Suchindran C et al. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. BMJ. 2013;346(feb04 3):e8707-e8707.
6. Willett W. Nutritional epidemiology. New York [etc.]: Oxford University Press; 2013.
7. Harika R, Eilander A, Alssema M, Osendarp S, Zock P. Intake of Fatty Acids in General Populations Worldwide Does Not Meet Dietary Recommendations to Prevent Coronary Heart Disease: A Systematic Review of Data from 40 Countries. Annals of Nutrition and Metabolism. 2013;63(3):229-238.
8. Arab L. Biomarkers of Fat and Fatty Acid Intake. The Journal of Nutrition. 2003;133(3):925S-932S.
9. DiNicolantonio J, O' Keefe J. Omega-6 vegetable oils as a driver of coronary heart disease: the oxidized linoleic acid hypothesis. Open Heart. 2018;5(2):e000898.
10. Jakobsen M, O' Reilly E, Heitmann B, Pereira M, Bälter K, Fraser G et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. The American Journal of Clinical Nutrition. 2009;89(5):1425-1432.
11. Farvid M, Ding M, Pan A, Sun Q, Chiuve S, Steffen L et al. Dietary Linoleic Acid and Risk of Coronary Heart Disease: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. Circulation. 2014;130(18):1568-1578.