Intake and metabolism of omega-3 and omega-6 polyunsaturated fatty acids: nutritional implications for cardiometabolic diseases

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Prospective observational studies support the use of long-chain omega-3 polyunsaturated fatty acids (PUFAs) in the primary prevention of atherosclerotic cardiovascular disease; however, randomised controlled trials, have often reported neutral findings. There is a long history of debate about the potential harmful effects of a high intake of omega-6 PUFAs, although this idea is not supported by prospective observational studies or randomised controlled trials. Health effects of PUFAs might be influenced by Δ-5 and Δ-6 desaturases, the key enzymes in the metabolism of PUFAs. The activity of these enzymes and modulation by variants in encoding genes (*FADS1-2-3* gene cluster) are linked to several cardiometabolic traits. This Review will further consider non-genetic determinants of desaturase activity, which have the potential to modify the availability of PUFAs to tissues. Finally, we discuss the consequences of altered desaturase activity in the context of PUFA intake, that is, gene–diet interactions and their clinical and public health implications.

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Introduction

Fatty acids present in different lipid molecules are the major components of dietary fats. The physical and chemical characteristics and the nutritional and health effects of dietary fatty acids are influenced greatly by the types and proportions of the component fatty acids.[1] The predominant fatty acids are either saturated fatty acids or fatty acids that contain carbon–carbon double bonds—ie, monounsaturated fatty acids with one double bond, and polyunsaturated fatty acids (PUFAs) with two or more double bonds. Although foods rich in saturated fatty acids (eg, fatty meat and dairy products, coconut and palm oil, confectionary and cakes) and monounsaturated fatty acids (some vegetable oils, meats) contribute to the intake of these fatty acids, they can also be produced endogenously. By contrast, the PUFA linoleic acid and α-linolenic acid are essential fatty acids and mainly derived from vegetable oils, nuts, and seeds.[2] Humans have some capacity to synthesise eicosapentaenoic acid, and to a lesser extent docosahexaenoic acid, from α-linolenic acid.[3] Although eicosapentaenoic acid and docosahexaenoic acid are long-chain omega-3 PUFAs, and therefore not strictly essential, they are consumed together as fish or fish oil capsules (either as over-the-counter dietary supplements or pharmaceutical-grade preparations). Microalgae is a dietary source of long-chain omega-3 PUFAs for vegetarians. Although the intake of PUFAs has been a cornerstone of dietary recommendations, controversy remains about the optimal absolute and relative intakes of the main dietary omega-3 and omega-6 PUFAs.[2,3]

Prospective observational studies support the role of long-chain omega-3 PUFAs, eicosapentaenoic acid and docosahexaenoic acid, in the primary prevention of atherosclerotic cardiovascular disease, with the underlying mechanisms of action widely described.[4,5] However, randomised controlled trials (RCTs) have often reported neutral findings for these PUFAs,[6,7] which called into question the use of fish oil supplements to reduce the risk of heart disease or stroke.[8] There is also debate about potentially harmful effects of a high intake of omega-6 PUFAs, specifically if omega-6 PUFAs far exceeds omega-3 PUFAs.[9] Because omega-6 PUFAs account for the majority of PUFAs in normal diets,[10] it is important to understand the complex epidemiology and metabolism related to PUFA intake, and the clinical and public health implications of omega-3 and omega-6 PUFA intake for cardiometabolic diseases, such as type 2 diabetes and atherosclerotic cardiovascular disease.

This Review starts with a summary of evidence relating intake of omega-3 and omega-6 PUFAs to cardiometabolic diseases. We then address how PUFA metabolism is linked to Δ-5 and Δ-6 desaturases, and review the evidence linking enzymes' activities and their modulation by genetic determinants, specifically genetic variants in the *FADS1-2-3* gene cluster, to several cardiometabolic traits. Finally, we discuss the consequences of altered desaturase activity in the context of PUFA intake, that is, gene–diet interactions and their clinical and public health implications.

Effect of omega-3 PUFA intake on atherosclerotic cardiovascular disease and type 2 diabetes

Prospective cohort studies consistently support the role of eicosapentaenoic acid and docosahexaenoic acid for primary prevention of atherosclerotic cardiovascular disease, with underlying mechanisms, including an effect on concentration of plasma triglycerides, size of lipoproteins, inflammation and plaque stability, vascular function, and arrhythmias.[4,5,11] Existing RCTs on clinical endpoints are largely secondary prevention trials (eg, GISSI[12] and Alpha Omega[13]) or mixed primary and secondary prevention trials (eg, JELIS,[14] REDUCE-IT,[15] and the Risk and Prevention Study;[16] table 1). Only VITAL[7] was a primary prevention study recruiting healthy men and women with no history of cardiovascular disease. Earlier secondary prevention trials reported a 20%–30% reduction in cardiovascular deaths.[12,14,27] Similarly, advice to consume fatty fish lowered total mortality.[28] However, subsequent

	Population	Intervention	Duration	Outcome	Effect size	Comment
Omega-3 PUFA						
GISSI (1999)[12]	Post-myocardial infarction less than 3 months	850–882 mg/day eicosapentaenoic acid plus docosahexaenoic acid	3·5 years	Primary: death, myocardial infarction, stroke; secondary: atherosclerotic cardiovascular disease death	Primary 0·85 (0·74–0·9); secondary 0·70 (0·56–0·87)	Four medications prescribed, 5% of patients on statins
JELIS (2007)[14]	Men with hypercholesterolaemia (aged 40–75 years) and postmenopausal women (aged ≤ 75 years)	1800 mg eicosapentaenoic acid (icosapent ethyl)	4·6 years	Primary: sudden cardiac death, fatal and non-fatal myocardial infarction, unstable angina, angioplasty, stenting, coronary artery bypass graft	0·81 (0·69–0·95)	All patients on statins, 64% on antihypertensive medication, 14% on antiplatelet drugs, 12% on hypoglycaemic agents
Alpha Omega (2010)[13]	Post-myocardial infarction	400 mg/day eicosapentaenoic acid plus docosahexaenoic acid (trigylcerides); 2 g/day α-linolenic acid	3·7 years	Primary: fatal and non-fatal atherosclerotic cardiovascular disease, percutaneous coronary intervention, and coronary artery bypass graft	Eicosapentaenoic acid plus docosahexaenoic acid *vs* α-linolenic acid or placebo 1·01 (0·87–1·17); α-linolenic acid *vs* eicosapentaenoic acid plus docosahexaenoic acid or placebo 0·91 (0·78–1·05)	Median eicosapentaenoic acid plus docosahexaenoic acid intake at baseline 120–130 mg/day, no subgroup difference by baseline intake; suggestive effect of α-linolenic acid in women (HR 0·73 [0·51–1·03])
ORIGIN (2012)[17]	High atherosclerotic cardiovascular disease risk and prediabetes or diabetes	900 mg/day eicosapentaenoic acid plus docosahexaenoic acid (ethyl esters)	6·2 years	Primary: atherosclerotic cardiovascular disease death; secondary: non-fatal myocardial infarction, stroke, or atherosclerotic cardiovascular disease death	Primary 0·98 (0·87–1·10); secondary 1·01 (0·93–1·10)	High prevalence of cardiovascular medication, statin use 54%; median eicosapentaenoic acid plus docosahexaenoic acid intake at baseline 210 mg/day
Risk and Prevention Study (2013)[16]	At least four atherosclerotic cardiovascular disease risk factors or vascular disease, previous myocardial infarction precluded	850 mg/day eicosapentaenoic acid plus docosahexaenoic acid (ethyl esters)	5·0 years	Primary: time to death from atherosclerotic cardiovascular disease or hospital admission for atherosclerotic cardiovascular disease; secondary: atherosclerotic cardiovascular disease death	Primary 0·98 (0·88–1·08); secondary 1·03 (0·82–1·30)	Nine medications were prescribed, statin use 41%; at 1 year the event rate was lower than anticipated and the primary endpoint was revised
ASCEND (2018)[6]	Type 2 diabetes, no evidence of atherosclerotic cardiovascular disease	840 mg/day eicosapentaenoic acid plus docosahexaenoic acid (ethyl esters)	7·4 years	Primary: atherosclerotic cardiovascular disease death, myocardial infarction, stroke, or transient ischaemic attack; secondary: non-fatal myocardial infarction, non-fatal ischaemic stroke, or vascular death	Primary 1·00 (0·91–1·09); secondary: non-fatal myocardial infarction 0·93 (0·76–1·14), non-fatal ischaemic stroke 1·01 (0·84–1·22), vascular death 0·81 (0·67–0·99)	High prevalence of cardiovascular medication, statin use 75%
VITAL (2019)[7]	Healthy men (aged ≥50 years), women (aged ≥55 years)	840 mg/day eicosapentaenoic acid plus docosahexaenoic acid (ethyl esters)	5·3 years	Primary: atherosclerotic cardiovascular disease death, myocardial infarction, or stroke; secondary: atherosclerotic cardiovascular disease death or total myocardial infarction	Primary 0·92 (0·80–1·06); secondary: atherosclerotic cardiovascular disease death 0·96 (0·76–1·21), total myocardial infarction 0·72 (0·59–0·90)	Atherosclerotic cardiovascular disease death, myocardial infarction, and stroke HR 0·81 (0·67–0·98) and total myocardial infarction HR 0·60 (0·45–0·81) with fish intake less than 1·5 servings per week; no effect in patients with high fish intakes of at least 1·5 servings per week (HR 1·08 and 0·94)
REDUCE-IT (2019)[15]	Atherosclerotic cardiovascular disease, or aged ≥ 50 years and had type 2 diabetes and at least one additional atherosclerotic cardiovascular disease risk factor	4 g/day eicosapentaenoic acid (icosapent ethyl)	4·9 years	Primary: atherosclerotic cardiovascular disease death, myocardial infarction, stroke, coronary revascularisation, or unstable angina	0·75 (0·68–0·83)	HR 0·74 (0·65–0·83) for composite atherosclerotic cardiovascular disease death, myocardial infarction, and stroke (key secondary endpoint)
STRENGTH (2018)[18] and (2020)[19]	High atherosclerotic cardiovascular disease risk or previous atherosclerotic cardiovascular disease or type 2 diabetes, high triglycerides and low HDL-cholesterol	2·2 g eicosapentaenoic acid plus 0·8 g docosahexaenoic acid (carboxylic acids)	..	Primary: cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, emergent coronary revascularisation, elective coronary revascularisation, or hospitalisation for unstable angina	Trial stopped	Intervention judged unlikely to show a benefit by the independent data monitoring committee

(Table 1 continues on next page)

	Population	Intervention	Duration	Outcome	Effect size	Comment
(Continued from previous page)						
Omega-6 PUFA						
LA veterans (1969)[20]	Men with or without coronary heart disease	Corn and soybean oil	At least 8 years	Myocardial infarction, sudden death, and cerebral infarction	0·74 (0·53–1·03)	Confirmation of compliance by adipose tissue fatty acid analyses. Secondary outcome for total atherosclerotic cardiovascular disease showed approximately 30% reduced risk, RR 0·68 (0·52–0·91)
MRC Soy (1968)[21]	Men post-myocardial infarction	Soybean oil 80 g approximately	5 years	Myocardial infarction, sudden death, and all-cause mortality	0·86 (0·61–1·22)	Confirmation of compliance by weighted food records and adipose tissue fatty acid analyses; intake at least 43 g soybean oil unheated (often drunk with fruit juice)
Oslo diet-heart study (1970)[22]	Men post-myocardial infarction	Soybean oil, cod liver oil	5 years	Myocardial infarction and sudden cardiac death	0·75 (0·57–0·99)	Multifactorial intervention with modification of dietary composition other than fat
Finnish mental hospital study (1979)[23]	Men with myocardial infarction	Soybean oil	6 years	Myocardial infarction (ECG change) and coronary heart disease mortality	0·55 (0·34–0·88)	Institutionalised population; assignment by hospital, not individually randomised; confirmation of compliance with a large increase of linoleic acid in adipose tissue
Finnish mental hospital study (1983)[24]	Women with myocardial infarction	Soybean oil	6 years	Myocardial infarction (ECG change) and coronary heart disease mortality	0·64 (0·41–1·00)	Institutionalised population; assignment by hospital, not individually randomised
Minnesota Coronary Survey (1989)[25]	Men and women	Corn oil	4·5 years or less	Atherosclerotic cardiovascular disease events, atherosclerotic cardiovascular disease, and total mortality	1·08 (0·84–1·37)	Institutionalised population; mean follow-up only 1 year; dropout rate approximately 75%; high dose of corn oil (13% *vs* 3% energy from linoleic acid)
Sydney Diet Heart Study (1978)[26]	Men post-myocardial infarction or with coronary heart disease	Safflower oil	2–7 years	Myocardial infarction and cardiac death	1·86 (0·63–5·44)	Short study duration (median follow-up 39 months), with potential confounding by trans fatty acids; high dose of omega-6 PUFAs, without any increase in omega-3 PUFAs

Data are HR (95% CI) or RR (95% CI). The table does not provide an exhaustive description of all PUFA intervention studies done to date, but rather gives illustrative examples of earlier and later trials, and those that are discussed in the text. PUFAs=polyunsaturated fatty acids. RCT=randomised controlled trial.

Table 1: **Key RCTs examining the effect of PUFA supplementation on incident cardiovascular disease**

RCTs often observed no effect on atherosclerotic cardiovascular disease incidence or deaths.[6,7,13,15–17] These RCTs were summarised by several systematic reviews that questioned the likely benefit of supplementation with long-chain omega-3 PUFAs in the secondary prevention of atherosclerotic cardiovascular disease.[27,29] This notion seems to be generally supported by subsequent RCTs. For instance, no benefit was seen from eicosapentaenoic acid plus docosahexaenoic acid supplementation to prevent atherosclerotic cardiovascular disease in patients with type 2 diabetes or prediabetes in the ASCEND study,[6] or in healthy individuals in the VITAL study.[7] However, REDUCE-IT[15] saw a significantly lowered risk of atherosclerotic cardiovascular disease and a non-significant reduction in mortality (hazard ratio [HR] 0·87, 95% CI 0·74–1·02) with high doses (4 g/day) of icosapent ethyl (a highly purified and stable eicosapentaenoic acid ethyl ester) among patients with established atherosclerotic cardiovascular disease or risk factors (including a high concentration of triglycerides). Up to 4 g eicosapentaenoic acid plus docosahexaenoic acid is approved as an alternative to fibrates as a triglyceride-reducing agent.[30] The effect size in REDUCE-IT (HR 0·75) suggests benefits beyond triglyceride lowering.[4,5] Because of the chemical form used (ie, icosapent ethyl) these results cannot be directly extrapolated to over-the-counter formulations. These RCTs were included in two meta-analyses published in 2019 and 2020.[31,32] One analysis (13 RCTs) concluded that long-chain omega-3 PUFA supplementation reduced the risk of myocardial infarction, coronary heart disease death, total coronary heart disease, atherosclerotic cardiovascular disease death, and total atherosclerotic cardiovascular disease, with risk reductions linearly related to dose.[31] According to the latest Cochrane meta-analysis, supplementation with long-chain omega-3 PUFAs reduce coronary heart disease death and coronary heart disease

events by approximately 10%, although no significant effect was shown for total atherosclerotic cardiovascular disease events or stroke.[32]

Making direct comparisons between observational and trial evidence has several limitations. Observational studies are generally prone to confounding, thus other factors associated with intake of long-chain omega-3 PUFAs might explain the observed benefits. Also, observational studies usually capture dietary sources of long-chain omega-3 PUFAs (fish intake), in which the long-chain omega-3 PUFAs are predominantly in the triglyceride form with some phospholipid eicosapentaenoic acid and docosahexaenoic acid also present, rather than supplementation with isolated PUFAs alone. Also, when comparing outcomes from individual RCTs, it is important to consider various factors, such as the chemical form and dose of omega-3 PUFAs, whether the formulation contains eicosapentaenoic acid and docosahexaenoic acid, what their ratio to each other is, and whether the supplement used was an over-the-counter or a prescription-grade preparation. Supplemental (over-the-counter) or prescription eicosapentaenoic acid and docosahexaenoic acid are available in free fatty acid, triglyceride, phospholipid, or ethyl ester forms. The bioavailability of fatty acids from ethyl esters is known to be substantially lower than that from the other forms, particularly if consumed when fasting or as part of a low-fat meal.[33] Although bioavailability is unlikely to have been an issue in REDUCE-IT[15] because of the high dose of eicosapentaenoic acid used, reduced eicosapentaenoic acid and docosahexaenoic acid bioavailability might have contributed to the absence of efficacy seen in ORIGIN,[17] Risk and Prevention Study,[16] ASCEND,[6] and VITAL[7] (table 1). Furthermore, increasingly intensive use of cardiovascular medications over the past 2 decades might reduce the therapeutic opportunity, because eicosapentaenoic acid and docosahexaenoic acid have overlapping targets with the prescribed drugs. For example, both fibrates and long-chain omega-3 PUFAs mediate triglyceride lowering via PPAR-α-dependent mechanisms.[34] However, subgroup analyses in ORIGIN, VITAL, or REDUCE-IT trials, and the positive effect of eicosapentaenoic acid in statin users in the JELIS trial, do not support a generally increased benefit of long-chain omega-3 PUFA supplementation among non-users of cardiovascular medication.[7,14,15,17] However, RCTs might have the limitation of a high habitual intake of eicosapentaenoic acid and docosahexaenoic acid in trial participants at baseline.[27] For example, in VITAL, subgroup analysis indicated an effect of eicosapentaenoic acid plus docosahexaenoic acid on major cardiovascular events (HR 0·81) and myocardial infarction incidence (HR 0·64) in people with low intakes of fish at baseline (<1·5 servings per week) but not in people with high intakes (≥1·5 servings per week).[7] Although such a difference was not observed in the ORIGIN trial,[17] there is no systematic evaluation of effect modification by baseline omega-3 PUFA status across existing trials, or an RCT that specifically tests the effect of supplements for individuals with a low habitual intake of omega-3 PUFAs.

Individual RCTs suggest beneficial effects of long-chain omega-3 PUFA supplementation on specific cardiovascular endpoints as secondary outcome measures. For instance, eicosapentaenoic acid plus docosahexaenoic acid reduced the risk of vascular death among patients with prevalent coronary heart disease, according to a meta-analysis of RCTs,[35] and in patients with diabetes in the ASCEND study.[6] Long-chain omega-3 PUFA supplements reduced risk of myocardial infarction in VITAL.[7] There is no direct comparison of eicosapentaenoic acid versus docosahexaenoic acid in primary or secondary prevention trials, though such evidence is needed to inform policy, if individual eicosapentaenoic acid and docosahexaenoic acid recommendations are to be developed. Although docosahexaenoic acid is more effective than is eicosapentaenoic acid in reducing plasma triglycerides and improving vascular function, high-dose eicosapentaenoic acid reduced incidence of incident atherosclerotic cardiovascular disease in the REDUCE-IT trial.[15,36] The STRENGTH trial,[18] with a similar study population to REDUCE-IT,[15] was stopped because of its low likelihood of showing a benefit.[19] STRENGTH used a lower dose and different form of long-chain omega-3 PUFAs (2·2 g eicosapentaenoic acid and 0·8 g docosahexaenoic acid as free fatty acids) than did REDUCE-IT (4 g eicosapentaenoic acid as ethyl ester). These findings suggest that the formulation of the long-chain omega-3 PUFAs tested might be highly important.

Similar to long-chain omega-3 PUFAs, prospective cohorts indicate an approximate 10%–15% reduced risk of cardiovascular events associated with high α-linolenic acid intake.[37,38] These observational data are supported by RCTs: a 2018 Cochrane review concluded that increased α-linolenic acid intake might slightly reduce the risk of cardiovascular events and probably reduces the risk of coronary heart disease mortality and arrhythmia with modest effect sizes.[39] However, large-scale, long-term trials on α-linolenic acid supplementation are rather scarce (table 1). In the only trial meeting these criteria, the Alpha Omega trial,[13] giving margarine rich in α-linolenic acid to patients with previous myocardial infarction did not significantly reduce major atherosclerotic cardiovascular disease events compared with a placebo margarine, although the effect size suggests such benefits exist. Any effect due to α-linolenic acid might be caused by an indirect effect on eicosapentaenoic acid status and a direct effect on other cardiometabolic pathways or risk factors.

There is little indication that eicosapentaenoic acid and docosahexaenoic acid improve indices of glycaemia and insulin sensitivity, or reduce risk of incident type 2 diabetes.[40] In a pooled analyses of cohort studies, long-chain omega-3 PUFAs derived from seafood and fish had no clear association with type 2 diabetes risk.[41] Intake of long-chain omega-3 PUFAs might be less important for diabetes risk than for atherosclerotic cardiovascular

disease risk. This idea is supported by Mendelian randomisation studies, which found that lowering triglyceride concentrations reduced atherosclerotic cardiovascular disease risk, but did not reduce type 2 diabetes risk.[42,43] By contrast, α-linolenic acid intake was inversely linked to type 2 diabetes risk, although modestly.[41] An inverse association between plasma concentrations of α-linolenic acid and type 2 diabetes risk was also seen in the EPIC-InterAct study.[44] However, evidence from RCTs on α-linolenic acid supplementation with type 2 diabetes as an endpoint is needed.[40]

Effect of omega-6 PUFA intake on atherosclerotic cardiovascular disease and type 2 diabetes

Several trials assessed the effect of replacing saturated fatty acids from dairy and meat with a diet rich in cholesterol-lowering PUFAs on the risk of atherosclerotic cardiovascular disease and death (table 1).[20–26] These trials mostly used linoleic acid as omega-6 PUFA, or a mixture of omega-6 (linoleic acid) and omega-3 (α-linolenic acid) PUFAs from vegetable oils. In one meta-analysis, PUFAs were associated with a moderate reduction of total coronary heart disease and fatal deaths compared with saturated fatty acids.[45] The risk reduction (10% for each 5% of energy from saturated fatty acids substituted with PUFAs) accorded well with the lowering of serum cholesterol and the cholesterol to HDL ratio in these trials. Similarly, a 2018 Cochrane review supports a risk reduction for myocardial infarction if omega-6 PUFAs replace saturated fatty acids, although no clear benefit was seen for overall atherosclerotic cardiovascular disease.[46] No evidence was found for dose–response, but there was a suggestion of increased protection for atherosclerotic cardiovascular disease across outcomes in participants with low omega-6 PUFA intake at baseline. The protective effects found in trials are consistent with a pooled analysis of 11 prospective cohort studies that evaluated replacing saturated fatty acids with PUFAs (mostly linoleic acid), and a meta-analysis of 13 cohort studies comparing linoleic acid and saturated fatty acids.[47,48] In prospective cohort studies with omega-6 PUFA biomarkers (circulating or tissue linoleic acid concentrations), higher linoleic acid concentrations were associated with a lower risk of all atherosclerotic cardiovascular disease outcomes, including atherosclerotic cardiovascular disease mortality, even after taking omega-3 PUFA concentrations into account.[49] According to a 2020 meta-analysis of cohort studies, high linoleic acid intake and tissue concentrations are related not only to lower atherosclerotic cardiovascular disease mortality, but also to lower total and cancer mortality.[50] However, secondary analyses of recaptured data from two trials done during the 1970s–1980s suggest that a high intake of linoleic acid plus α-linolenic acid is more favourable than a high intake of omega-6 PUFA alone, which might have unwanted side-effects at high doses.[51–53] However, these trials are difficult to interpret, because of the short duration of some trials, small numbers of events, high drop-out rates, and confounding by trans fats that were commonly abundant in the PUFA-rich margarines used (table 1). Meta-analyses of trials show inconsistent results for omega-6 PUFAs, depending on study inclusions.[45,46,53–56] Taken together, evidence from RCTs and prospective cohort studies suggests that plant oils rich in linoleic acid seem to be moderately protective against coronary heart disease, especially myocardial infarction.

Regarding intake of omega-6 PUFAs and type 2 diabetes risk, there are no available data from trials designed to investigate diabetes incidence as an outcome.[40] However, after considering short-term feeding trials and prospective cohort studies using omega-6 biomarkers or food questionnaires, a previous review suggests inverse associations between omega-6 PUFA (linoleic acid in particular) and incident type 2 diabetes.[57] This suggestion is supported by several subsequent cohort studies, including the pan-European EPIC-InterAct study,[44] and the pooled meta-analyses from the FORCE consortium.[58] FORCE considered linoleic acid and arachidonic acid concentrations in circulating lipids in prospective cohorts and found that linoleic acid, but not arachidonic acid, was inversely and linearly associated with incident type 2 diabetes, and this robust association was not modified by omega-3 PUFA status.[58] In support, numerous small and mostly short-term, randomised feeding trials indicated that PUFA, when isocalorically compared with saturated fatty acids or carbohydrates, improved markers of insulin sensitivity and glycaemic control,[59] although additional definitive RCTs of a longer duration are needed.[40]

Role of the omega-6 to omega-3 PUFA ratio

Concerns that consuming a high proportion of dietary omega-6 fats compared with omega-3 fats can have detrimental effects, particularly on inflammatory status, are not generally supported by study evidence. Indeed, several feeding trials showed that increasing omega-6 PUFA intake (eg, linoleic acid) while keeping omega-3 PUFA intake unchanged (increasing several times the omega-6 to omega-3 ratio), has no adverse effects on either multiple markers of inflammation or oxidative stress,[60] even under energy excess conditions at high linoleic acid intake.[61] In line with these findings, a meta-analysis did not find evidence to suggest an important role of the omega-6 to omega-3 PUFA ratio on glucose metabolism.[40] Although long-term RCTs on omega-6 PUFAs are insufficient to conclude on the relevance of the omega-6 to omega-3 PUFA ratio,[46] prospective cohort studies do not indicate any adverse role of a high omega-6 to omega-3 PUFA ratio.[62] The reason that the omega-6 to omega-3 PUFA ratio seems unimportant in predicting cardiometabolic disease or inflammation is probably because concerns about this ratio are partly formed on the basis of a number of incorrect and simplified assumptions—for example, that omega-6 PUFAs overall are proinflammatory, that omega-6 PUFAs (and linoleic acid in particular) have adverse effects on cardiovascular disease risk (although in

fact omega-6 and omega-3 PUFAs are related to lower risk), and that reducing linoleic acid intake will lower arachidonic acid concentrations (by contrast, linoleic acid supplements do not increase arachidonic acid concentrations in plasma or adipose tissue).[61,63,64] In addition, a clear problem arises when combining different omega-6 (and omega-3) PUFAs despite the distinct inflammatory and cardiovascular effects and different metabolites of individual PUFAs.[63] It is important to standardise the calculation of this ratio, because different studies have used different ratios (eg, some considered all omega-3 PUFAs or eicosapentaenoic acid and docosahexaenoic acid only). Also, a ratio calculated from dietary intake data cannot be compared with a ratio calculated from plasma or tissue PUFA concentrations.

PUFA metabolism

PUFAs fulfil various functions within the human body in addition to being a source of energy. They are integral structural components of the phospholipid layer of cell membranes, influencing membrane fluidity and selective permeability. Furthermore, PUFAs can directly influence several metabolic pathways, being ligands for transcription factors, such as sterol regulatory element binding protein 1 (SREBP-1), nuclear factor κB (NF-κB), hepatocyte nuclear factor 4α (HNF-4α), and PPARs, which have an important role in lipid metabolism. PUFAs are also substrates for the formation of various lipid-related metabolites (for example, eicosanoids, leukotrienes, prostaglandins, thromboxanes, lipoxygenase interaction products, endocannabinoids, or resolvins), which are highly bioactive. Prospective studies have also linked other lipid molecules, ceramides, to cardiometabolic risk,[65,66] and experimental data suggest causal links to insulin resistance and potentially also to atherosclerotic cardiovascular disease.[67] Omega-6 PUFA (linoleic acid) decreases several plasma ceramide species compared with saturated fatty acids.[61]

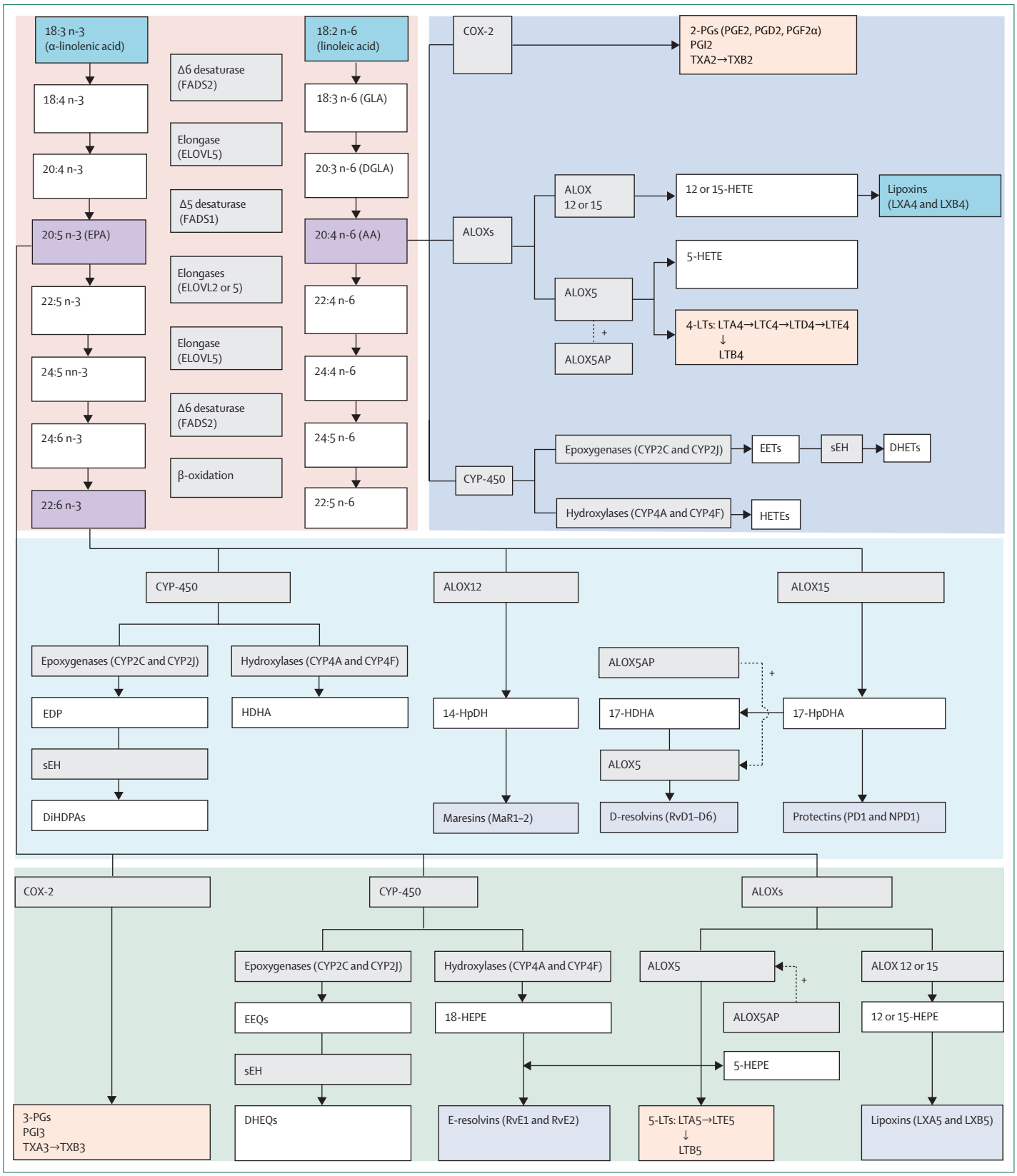
Importantly, although the essential precursor PUFAs, linoleic acid and α-linolenic acid, are the main PUFA sources in the diet, other PUFAs can be produced endogenously, although bioconversion efficiency to docosahexaenoic acid is modest.[3] The bioconversion of linoleic acid and α-linolenic acid to the long-chain PUFAs (γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid) is catalysed by elongases and desaturases, with the Δ-6 and Δ-5-desaturases being the key enzymes in this process (figure 1). Specifically, Δ-6 desaturase is considered the rate-limiting step of conversion of linoleic acid and α-linolenic acid to downstream metabolites. Omega-6 and omega-3 PUFAs compete for the same enzymes in this process, although a preferential affinity to omega-3 PUFAs exists.[68] PUFAs are the main dietary component that regulate the activity of these desaturases. In a rodent model, both desaturases seem to be suppressed by dietary PUFAs.[69] A stable-isotope study showed that increased intake of long-chain omega-3 PUFAs inhibits α-linolenic acid bioconversion.[3]

PUFAs often exert their physiological effects through a number of oxidative bioactive products (figure 1), collectively named oxylipins, which are produced by the action of cycloxygenases (encoded by *COX* genes), lipoxygenases (encoded by *ALOX* genes), and some members of *CYP450* superfamily (*CYP1–4* families). Oxylipins have long been known to have a proinflammatory effect as thromboxanes, prostaglandins, and leukotrienes. Thromboxanes, 2 series-prostaglandins, and the 4 series-leukotrienes are derived from arachidonic acid, and eicosapentaenoic acid metabolism produces 3 series-prostaglandins and 5 series-leukotrienes. Eicosapentaenoic acid products are generally less proinflammatory than their arachidonic acid counterparts.[70] However, the inflammatory effect of arachidonic acid or eicosapentaenoic acid derivates is nuanced, with both producing proinflammatory and anti-inflammatory products. For example, arachidonic acid-derived prostacyclin is known to inhibit platelet aggregation.[71]

In addition to competing with arachidonic acid, studies show that eicosapentaenoic acid and docosahexaenoic acid have a direct anti-inflammatory and resolving role through the production of signalling molecules, named specialised pro-resolving mediators.[72] The specialised pro-resolving mediators resolvins, protectins, and maresins, together with lipoxins produced from arachidonic acid, help to actively resolve inflammation by inhibiting the influx of neutrophils into the site of inflammation and from enhancing phagocytosis. Data are largely derived from rodent studies, and evidence of the role of specialised pro-resolving mediators in human homoeostasis and pathophysiology is needed.

***Figure 1*: Key enzymes involved in the metabolism of polyunsaturated fatty acids**

Conversion of linoleic acid and α-linolenic acid to longer-chain omega-6 and omega-3 polyunsaturated fatty acids is catalysed by the action of Δ-6 desaturase, Δ-5 desaturase, and elongases. AA, EPA, and DHA often exert their physiological effects through oxylipins produced by the action of cycloxygenases, lipoxygenases, and some members of the CYP450 superfamily. 2-PGs=2-series prostaglandins. 3-PGs=3-series prostaglandins. 4-LTs=4-series leukotrienes. 5-HEPE= 5-hydroxyeicosapentaenoic acid. 5-LTs=5-series leukotrienes. AA=arachidonic acid. ALOX=arachidonate lipoxygenase. ALOX5AP=5-lipoxygenase activating protein. COX-2=cylclooxygenase-2. CYP2C=cytochrome P450 2C. CYP2J=cytochrome P450 2J. CYP4A=cytochrome P450 4A. CYP4F=cytochrome P450 4F. CYP-450=cytochrome P450. DHA=docosahexaenoic acid. DHEQs=dihydroxyeicosatetraenoic acids. DHET=dihydroxyeicosatrienoic acid. DiHDPA=dihydroxydocosapentaenoic acid. EDP=epoxydocosapentaenoic acid. EEQ=epoxyeicosatetraenoic acid. EETs=epoxyeicosatrienoic acids. EPA=eicosapentaenoic acid. HDHA=hydroxydocosahexaenoic acid. HEPE=hydroxyeicosapentaenoic acid. HETE=hydroxyeicosatetraenoic acid. HpDHA=hydroperoxide intermediate of DHA. LTA5=leukotriene A5. LTB5=leukotriene B5. LTE5=leukotriene E5. LXA4=lipoxin A4. LXA5=lipoxin A5. LXB4=lipoxin B4. LXB5=lipoxin B5. n-3=omega-3. n-6=omega-6. PGE2=prostaglandin E2. PGD2=prostaglandin D2. PGF2α=prostaglandin F2α. PG12=prostaglandin 12. PG13=prostaglandin 13. RvE1=Resolvin E1. RvE2=Resolvin E2. sEH=soluble epoxide hydrolase enzyme. TX=thromboxanes. TXA2=thromboxane A2. TXA3=thromboxane A3. TXB2=thromboxane B2. TXB3=thromboxane B3.


18:3 n-3 (α-linolenic acid)
18:4 n-3
20:4 n-3
20:5 n-3 (EPA)
22:5 n-3
24:5 nn-3
24:6 n-3
22:6 n-3
Δ6 desaturase (FADS2)
Elongase (ELOVL5)
Δ5 desaturase (FADS1)
Elongases (ELOVL2 or 5)
Elongase (ELOVL5)
Δ6 desaturase (FADS2)
β-oxidation
18:2 n-6 (linoleic acid)
18:3 n-6 (GLA)
20:3 n-6 (DGLA)
20:4 n-6 (AA)
22:4 n-6
24:4 n-6
24:5 n-6
22:5 n-6
COX-2
2-PGs (PGE2, PGD2, PGF2α)
PGI2
TXA2→TXB2
ALOXs
ALOX 12 or 15
12 or 15-HETE
Lipoxins (LXA4 and LXB4)
ALOX5
5-HETE
4-LTs: LTA4→LTC4→LTD4→LTE4
↓
LTB4
ALOX5AP
CYP-450
Epoxygenases (CYP2C and CYP2J)
EETs
sEH
DHETs
Hydroxylases (CYP4A and CYP4F)
HETEs
CYP-450
Epoxygenases (CYP2C and CYP2J)
Hydroxylases (CYP4A and CYP4F)
EDP
HDHA
sEH
DiHDPAs
ALOX12
14-HpDH
Maresins (MaR1–2)
ALOX15
ALOX5AP
17-HDHA
17-HpDHA
ALOX5
D-resolvins (RvD1–D6)
Protectins (PD1 and NPD1)
COX-2
3-PGs
PGI3
TXA3→TXB3
CYP-450
Epoxygenases (CYP2C and CYP2J)
Hydroxylases (CYP4A and CYP4F)
EEQs
sEH
DHEQs
18-HEPE
E-resolvins (RvE1 and RvE2)
ALOXs
ALOX5
ALOX5AP
5-HEPE
5-LTs: LTA5→LTE5
↓
LTB5
ALOX 12 or 15
12 or 15-HEPE
Lipoxins (LXA5 and LXB5)

Prostaglandins and thromboxanes are produced mainly by the action of COX enzymes on arachidonic acid and eicosapentaenoic acid. COX-1 is involved in basic physiological functions, whereas COX-2 products are mainly produced in response to inflammation and in malignant conditions, such as colorectal cancer.[73] COX-2 oxylipin products and COX inhibitors are important modulators of elements of the cardiometabolic phenotype, including blood pressure, platelet aggregation, and atherogenesis.[74] It would be interesting to understand how the different effects of non-steroidal anti-inflammatory drugs (including aspirin), omega-3 PUFAs, and omega-6 PUFAs on prostaglandin metabolism might relate to their different effects on atherosclerotic cardiovascular disease risk (eg, side-effects of selective COX-2 inhibitors *vs* aspirin).[75]

Lipoxygenases (encoded by *ALOX5*, *ALOX5AP*, *ALOX12*, and *ALOX15*) modulate the production of the proinflammatory leukotrienes and the anti-inflammatory specialised pro-resolving mediators.[76] Greater *ALOX5*, *ALOX12*, and *ALOX15* expression in adipose tissue was reported in people with obesity and type 2 diabetes than in people with obesity but without type 2 diabetes.[77] The ALOX5 enzyme pathway is activated in cardiovascular diseases and suggests leukotrienes have an important role in atherosclerosis and its ischaemic complications, such as myocardial infarction and stroke.[78] *CYP1–4* families produce epoxyeicosatrienoic acid and hydroxyeicosatetraenoic acid from arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, with epoxyeicosatrienoic acids being further catalysed to their respective regioisomers (dihydroxyeicosatetraenoic acid) through soluble epoxide hydrolase. Preclinical and clinical studies show the potential role of these metabolites as vasodilators and subsequent regulators of blood pressure, with some preliminary evidence of their antiarrhythmic and cardioprotective functions.[79]

Relationship between desaturase activity and cardiometabolic health

The relevance to cardiometabolic diseases of endogenous formation of more highly unsaturated fatty acids from dietary precursor fatty acids (linoleic acid and α-linolenic acid) has been investigated across different prospective cohorts. Because direct measurement of liver desaturase activity is not possible, most reports have relied on estimates of activities formed on the basis of product versus precursor ratios of fatty acids measured in blood fractions. Several studies saw that a high estimated Δ-6 activity (ratio of γ-linolenic acid to linoleic acid) is related to an increased risk of type 2 diabetes, although high estimated Δ-5 activity (ratio of arachidonic acid to dihomo-γ-linolenic acid) was associated with reduced risk activity.[44,58,80] A similar, though less clear, picture emerges for atherosclerotic cardiovascular disease, in which a pooled analysis of 30 cohort studies revealed inverse associations with type 2 diabetes risk for linoleic acid and arachidonic acid. However, there are few studies on atherosclerotic cardiovascular disease considering intermediate omega-6 PUFAs or specific fatty acid ratios to reflect their bioconversion. Dihomo-γ-linolenic acid was related to increased coronary heart disease risk, but less convincing to increased stroke risk in the ARIC study,[81,82] but γ-linolenic acid and dihomo-γ-linolenic acid were not clearly associated with atherosclerotic cardiovascular disease risk in other studies.[83–85] Overall, these studies suggest that increased Δ-6 activity and reduced Δ-5 activity lead to the accumulation of intermediate fatty acids (γ-linolenic acid and dihomo-γ-linolenic acid), which increases cardiometabolic risk.

Genetic determinants of PUFA metabolism

Genetic factors are linked to the fatty acid composition of biosamples, specifically blood fractions. Variations in *FADS1* and *FADS2*, the genes encoding the Δ-5 and Δ-6 desaturases, have been related to PUFA blood concentrations by candidate gene approaches.[86] Furthermore, genome-wide association studies identified this region as having the strongest genetic link to PUFA blood concentrations. For example, variant alleles at single nucleotide polymorphisms in the *FADS1-2-3* gene cluster were associated with higher concentrations of α-linolenic acid and lower concentrations of eicosapentaenoic acid and docosapentaenoic acid in the CHARGE consortium meta-analysis.[87] The strongest associated single nucleotide polymorphisms explained approximate variance of 4% in α-linolenic acid, 2% in eicosapentaenoic acid, and 9% in docosapentaenoic acid. Similarly, variants in the *FADS1-2-3* gene cluster were strongly associated with omega-6 PUFA concentrations (linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid) in CHARGE, with the top single nucleotide polymorphism (rs174547) explaining approximately 10% variation in dihomo-γ-linolenic acid and more than 20% in arachidonic acid.[88] Similarly, genome-wide association studies considering fatty acid ratios as estimates of Δ-5 or Δ-6 desaturase identified the *FADS1-2-3* gene cluster as a prominent locus.[89,90]

In addition to their relationship to tissue fatty acid composition, variants in the *FADS1–2-3* gene cluster are among the strongest genetic variants linked to triglyceride concentrations and are associated with other lipids, for example, cholesterol.[91,92] Furthermore, genome-wide association studies support that variants in this gene cluster are among the strongest signals related to specific lipids, particularly phospholipids (phosphatidyl cholines and phosphatidylethanolamines).[93,94] Several of these lipids were related to risk of type 2 diabetes and atherosclerotic cardiovascular disease in prospective studies.[95,96]

Variants in the *FADS1-2-3* gene cluster have not been associated with risk of type 2 diabetes and atherosclerotic cardiovascular disease on a genome-wide level,[97,98] despite their association with multiple other cardiometabolic traits, including inflammatory markers, and fasting

glucose.[91,99] However, these variants have been used in Mendelian randomisation analyses to support causal roles of desaturases, PUFAs, and specific phospholipids for cardiometabolic diseases.[100–102] Investigating genetic variation in the *FADS1-2-3* gene cluster to disentangle the potentially different role of individual PUFAs is hampered by strong linkage disequilibrium in this region.[80] Common variants in *FADS1* are strongly associated with variants in *FADS2*, and minor alleles of variants in *FADS1* are not only related to increased dihomo-γ-linolenic acid (substrate of Δ-5 desaturase) and reduced arachidonic acid concentrations (product), but also to increased linoleic acid and reduced γ-linolenic acid concentrations (substrate and product of Δ-6 desaturase) in European populations.[88] Importantly, prospective studies using PUFA biomarkers support the idea that Δ-5 and Δ-6 desaturases have opposing associations with cardiometabolic risk, and confounding by linkage disequilibrium in genetic studies might mask true associations.[103]

Genetic variants in the *FADS1-2-3* gene cluster show strong variability in allele frequency across different populations. For example, the C-allele of *FADS1* rs174547, related to reduced ability to convert plant-based PUFAs into longer-chain and higher unsaturated fatty acids, is largely absent in African, common in European, and dominant in American populations (figure 2).[104] Similarly, the *FADS2* variant rs174570 allele related to lower desaturase activity, is much more frequent in Greenland Inuit (allele frequency 99%) than in Chinese (34%) or European populations (16%).[105] This disparity highlights a potential human adaptation to varying dietary PUFA sources. Variants in the *FADS1-2-3* gene cluster that increase long-chain-PUFA synthesis from plant-based PUFAs might have been of advantage in geographic regions with little access to marine sources for long-chain omega-3 PUFAs. At the other extreme are Inuit, who traditionally consume extremely high amounts of long-chain omega-3 PUFAs from fish and marine mammals, and for whom the *FADS1-2-3* gene cluster was the strongest outlier region, based on patterns of allele frequency differentiation compared with other populations.[105] There is little evidence that the *FADS1-2-3* gene cluster relates to systems and biological processes related to food preferences (eg, fish consumption).[106]

Genetic variations in *ALOX, COX,* and *CYP-450* genes affect oxylipin production.[79,107] *ALOX* genotype was associated with cardiometabolic phenotypes in experimental models and human investigations.[92,108–110] In *ALOX5* knock-out mice, a reduction in LTB4 and reverse cholesterol transport suggests a new mechanism through which the lipoxygenase pathway is likely to influence atherogenesis.[108] Large-scale, genome-wide association studies reported associations between *ALOX5* single nucleotide polymorphisms and HDL cholesterol concentrations in humans.[92,108] Variation in the tandem repeats of the *ALOX5* promoter and in *ALOX5* activating protein *(ALOXAP)* was associated with incident myocardial infarction in cohort studies.[109,110] However, *ALOX* genotypes were not significantly associated with type 2 diabetes or coronary artery disease in genome-wide association studies.[71,111]

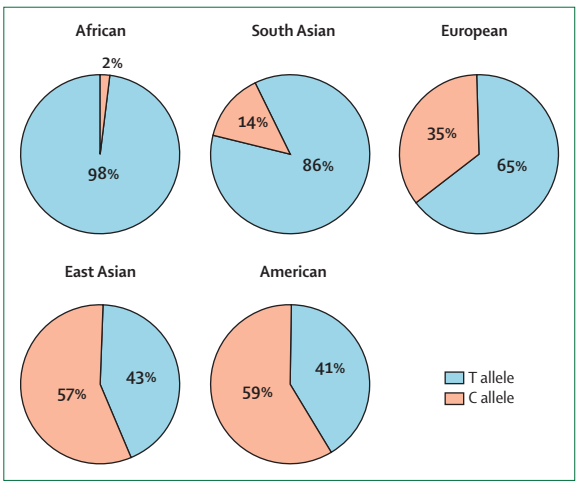


Figure 2: **Allele frequencies (percent) for *FADS1* rs174547 across populations**
Genetic variation in *FADS1* is in strong linkage disequilibrium with variation in *FADS2*. *FADS1* rs174547, one of the lead single nucleotide polymorphisms related to circulating concentrations of polyunsaturated fatty acids identified in large-scale genome-wide association studies,[88] shows strong differences across different populations worldwide. Data are from the 1000 Genomes Project phase 3.[104]

Consequences of altered desaturase and lipoxygenase activity

The strong association of variants in the *FADS1-2-3* gene cluster with tissue PUFA concentrations suggests that response to dietary PUFA intake (in terms of blood PUFA composition) is modified by genetic architecture related to the activity of desaturases. However, this question has been investigated in few studies to date (table 2).[112–119] Unfortunately, most studies were cross-sectional in nature, or investigated intake of long-chain omega-3 PUFAs, which are not the substrate of desaturases.[112,114,116,117] Only two trials investigated supplementation with plant-derived PUFAs. Gillingham and colleagues[115] compared intakes of a flaxseed oil rich in α-linolenic acid, a blend of oils typical of a western diet, and an oil rich in oleic acid. People who were minor allele carriers of four different variants in the *FADS1-2-3* gene cluster had a substantially lower increase in eicosapentaenoic acid plasma concentrations after the α-linolenic acid intervention than did people with the wild type (α-linolenic acid intervention 0·9% *vs* 2·2%, western diet fat 0·3% *vs* 0·6%, oleic acid diet 0·4% *vs* 0·7%; $p_{interaction}<0·001$).[115] In the FADSDIET trial,[119] arachidonic acid concentrations in plasma phospholipids decreased in participants with the rare CC genotype of *FADS1* rs174550 in response to a linoleic acid-rich sunflower oil, but arachidonic acid concentrations remained unchanged in individuals with the TT genotype.[119] These findings are supported by animal studies.

	Design and population	Intervention or exposure	Genetic variants	Outcomes	Interaction
Fatty acid composition of blood fractions or breastmilk					
Moltó-Puigmartí et al (2010)[112]	Cross-sectional	Fatty fish intake	rs174575	Plasma and milk fatty acids	Higher eicosapentaenoic acid or docosahexaenoic acid concentrations in human milk occurred with higher fatty fish intake only in major allele carriers; no difference in plasma phospholipid eicosapentaenoic acid or docosahexaenoic acid by genotype
Dumont et al (2011)[113]	Cross-sectional	Dietary linoleic acid and α-linolenic acid	rs174546	Serum phospholipid fatty acids	No interaction
Al-Hilal et al (2013)[114]	RCT, healthy patients	Eicosapentaenoic acid plus docosahexaenoic acid (0·45 g/day, 0·9 g/day, and 1·8 g/day)	rs174537, rs174561, rs3834458	Fatty acids in plasma and red blood cells	Increase in Δ-5 desaturase activity (arachidonic acid to eicosapentaenoic acid ratio) occurred among T-allele carries of rs174537 with increased supplementation
Gillingham et al (2013)[115]	RCT, patients with hyperlipidaemia	Diet rich in α-linolenic acid (20·6 g/day)	rs174545, rs174583, rs174561, rs174537	Plasma fatty acids	Substantially smaller absolute concentration of eicosapentaenoic acid occurred in minor allele homozygote after α-linolenic acid intervention than in major allele carriers
Smith et al (2015)[116]	Cross-sectional (consortium)	Dietary linoleic acid and α-linolenic acid	rs174538, rs174548	Long-chain omega-3 PUFAs of plasma or red blood cells	Interaction between α-linolenic acid intake and *FADS1* variants on docosapentaenoic acid and docosahexaenoic acid
Takkunen et al (2016)[117]	Cross-sectional	Long-chain omega-3 PUFAs from fish	rs174550	Fatty acids in plasma and red blood cells	Stronger association between long-chain omega-3 PUFA intake and eicosapentaenoic acid in minor allele carriers than in major allele homozygote
Juan et al (2018)[118]	Cross-sectional	Dietary linoleic acid, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid	rs174546	Plasma fatty acids	Stronger positive associations between eicosapentaenoic acid and docosahexaenoic acid intake and eicosapentaenoic acid concentrations in minor allele carriers than in major allele homozygote; no interactions for other dietary PUFAs
Lankinen et al (2019)[119]	Single group trial	Sunflower oil rich in linoleic acid (17–28 g/day)	rs174550	Plasma phospholipid and cholesterol ester fatty acids	Decrease in arachidonic acid concentration in homozygote for minor allele, no effect in homozygote for major allele
Blood lipids					
Lu et al (2010)[120]	Cross-sectional	Dietary omega-6 and omega-3 PUFAs	rs174546, rs482548, rs174570	Total cholesterol, HDL-cholesterol, and non-HDL-cholesterol	No interactions for total dietary omega-3 intake and all outcomes; no interactions for dietary omega-6 PUFAs and most outcomes (only significant interaction was for HDL-cholesterol and rs174546)
Dumont et al (2011)[113]	Cross-sectional	Dietary linoleic acid and α-linolenic acid	rs174546	Serum triglycerides, cholesterol, and lipoproteins	Lower total and non-HDL cholesterol concentration in minor allele carriers occurred with high α-linolenic acid intake only, not with low alpha-linolenic acid intake
Cormier et al (2012)[121]	Single group trial	1·9 g/day eicosapentaenoic acid and 1·1 g/day docosahexaenoic acid	Selected single nucleotide polymorphisms of the *FADS1–2-3* gene cluster	Plasma triglycerides	No interaction observed
Standl et al (2012)[122]	Cross-sectional	Dietary omega-3 PUFAs	*FADS1–2-3* gene cluster	Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides	No interaction observed
Dumont et al (2018)[123]	Cross-sectional	Dietary linoleic acid and α-linolenic acid	rs174547	HDL-cholesterol	Lower HDL-cholesterol concentration with minor allele occurred only with high linoleic acid intake, not with low linolenic acid intake; no interaction with α-linolenic acid
Atherosclerotic cardiovascular disease or type 2 diabetes risk					
Baylin et al (2007)[124]	Case-control	α-linolenic acid from adipose tissue	Common *FADS1* deletion (T/-)	Non-fatal myocardial infarction	No interaction observed
Hellstrand et al (2014)[125]	Cohort	Dietary linoleic acid and α-linolenic acid	rs174546	Atherosclerotic cardiovascular disease	An inverse association of α-linolenic acid to linoleic acid ratio or α-linolenic acid with atherosclerotic cardiovascular disease or stroke occurred only in minor allele carriers
Liu et al (2015)[126]	Case-control	Dietary eicosapentaenoic acid and docosahexaenoic acid	rs174547	Coronary artery disease	Common T-allele associated with higher risk only among individuals with lower dietary eicosapentaenoic acid or docosahexaenoic acid intake, not with higher intake
Wu et al (2017)[58]	12 cohorts	Linoleic acid and arachidonic acid biomarker	rs174547	Type 2 diabetes	No interaction
Marklund et al (2019)[49]	13 cohorts	Linoleic acid and arachidonic acid biomarker	rs174547	Atherosclerotic cardiovascular disease	Inverse association of linoleic acid with total atherosclerotic cardiovascular disease and stroke in homozygote carriers of the common allele, not in minor allele carriers; no interactions for atherosclerotic cardiovascular disease mortality or total coronary heart disease or for arachidonic acid

PUFAs=polyunsaturated fatty acids. RCT=randomised controlled trial.

Table 2: **Studies investigating interactions between PUFA intake or status and variants in the *FADS1–2-3* gene cluster and fatty acid concentrations or cardiometabolic outcomes**

Knockout of *FADS2* in mice fed a linoleic acid-rich diet is related to reduced availability of arachidonic acid and incorporation of arachidonic acid into phospholipids.[127,128] Similarly, knockout of *FADS2* depletes eicosapentaenoic acid and docosahexaenoic acid in mice fed an α-linolenic acid-rich diet.[129] *FADS2* variants alter *FADS2* gene expression and tissue arachidonic acid concentrations in pigs.[130] Similarly, *FADS1* knockdown results in reorganisation of both omega-6 and omega-3 PUFA concentrations and their associated proinflammatory and pro-resolving lipid mediators.[131]

Similar to modifying effects on blood PUFA concentrations, modification of the effects of dietary PUFAs on blood lipid concentrations by variants in the *FADS1-2-3* gene cluster can be hypothesised. Studies addressing this question have found mixed results, but are mostly

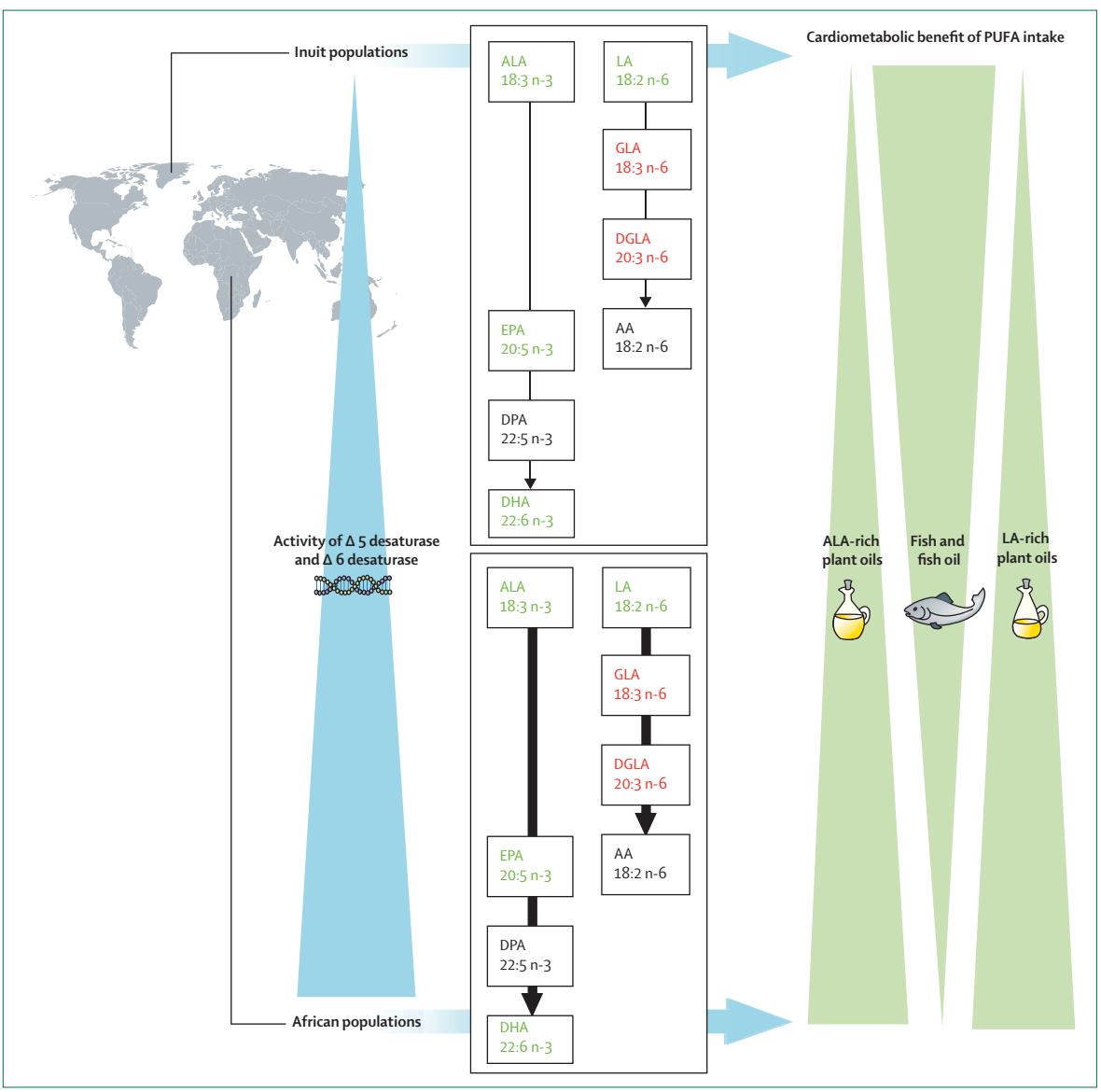


Figure 3: **Conceptual framework for hypothesised cardiometabolic benefits of omega-6 and omega-3 polyunsaturated fatty acids intake depending on genetic variation in polyunsaturated fatty acids metabolism**

Genetic variation in the *FADS1-2-3* gene cluster relates to the activity of Δ-5 and Δ-6-desaturases, key enzymes in the formation of longer-chain higher-unsaturated PUFAs from precursor omega-3 ALA and omega-6 LA. Mutations related to lower desaturase activity are frequent in Greenlandic Inuit populations (eg, rs174570, 99%[105]), common in European populations (16%), and largely absent in African populations (1%).[104] Higher Δ-5-desaturase activity and thus higher ability to metabolise LA and intermediate omega-6 PUFAs (GLA and DGLA) to arachidonic acid is related to lower cardiometabolic risk.[49] In the presence of high intake of long-chain omega-3 PUFAs from fish and seafood, lower genetically determined desaturase activity might compensate for a decreased intake of plant-based PUFAs. However, there is uncertainty about whether higher ALA intake is beneficial in the context of increased ability to convert ALA to long-chain omega-3 PUFAs.[128] ALA=α-linolenic acid. DGLA=dihomo-γ-linolenic acid. DHA=docosahexaenoic acid. DPA=docosapentaenoic acid. GLA= γ-linolenic acid. EPA=eicosapentaenoic acid. LA= linoleic acid. PUFAs=polyunsaturated fatty acids.

observational and cross-sectional in nature (table 2).[113,120–123] Only one trial (208 participants) specifically focused on the triglyceride response to a dietary long-chain omega-3 PUFA intervention, but did not see an effect modification.[121] However, as mentioned earlier, long-chain omega-3 PUFAs are not the main substrate of desaturases. Given the current scarcity of interventional studies that have directly tested response to the intake of plant-derived linoleic acid and α-linolenic acid (rather than long-chain omega-3 PUFAs), this hypothesis remains unconfirmed. However, modifying effects of the *FADS1-2-3* gene cluster have been described for other cardiometabolic risk factors. For example, the FADSDIET trial saw that *FADS1* rs174550 modified the inflammatory response to a diet rich in linoleic acid (as measured by a high-sensitivity C-reactive protein test).[119] The influences of inhibited Δ-5 and Δ-6 desaturase activities on glycaemic traits and atherosclerosis have been described from knockout animal studies.[132]

Whether variants in the *FADS1-2-3* gene cluster modify the cardiometabolic effects of dietary PUFAs on risk of clinical endpoints has been investigated in two case control and several cohort studies (table 2).[49,58,124–126] In a Swedish cohort study, high α-linolenic acid intake appeared to be more effective at reducing atherosclerotic cardiovascular disease risk among carriers of *FADS1* rs174546 genotype related to lower desaturase activity.[125] FORCE, a consortium of several prospective cohort studies, did not find that the *FADS1* rs174547 variant modulated the relationship between linoleic acid and arachidonic acid biomarkers and type 2 diabetes risk.[58] However, effect modification was seen for atherosclerotic cardiovascular disease endpoints, specifically stroke, which implies that a protective effect of high concentrations of linoleic acid is restricted to individuals homozygous for the common rs174547 allele, thus with an enhanced genetically-determined ability to convert linoleic acid to arachidonic acid and subsequent products.[49] However, this *FADS1* variant has a strong influence on linoleic acid concentrations in tissues, and interpretation of this PUFA biomarker as a proxy of dietary intake is problematic. Clearly, prospective studies investi—gating associations between intake of plant-derived linoleic acid and α-linolenic acid, and the subsequent risk of type 2 diabetes and atherosclerotic cardiovascular disease and its modification by variants in the *FADS1-2-3* gene cluster, would help to substantiate the findings of FORCE.

There is scant study evidence for *ALOX5* potentially modifying the effects of dietary PUFAs. Arachidonic acid intake was related to enhanced influence of the variant rs59439148, associated with the number of tandem repeats of the proximal specificity protein 1 binding site in the *ALOX5* promoter (the rare allele having between one and two fewer tandem repeats), on arterial thickness in a cross-sectional study and with the incidence of myocardial infarction in a cohort study.[109,133] However, in the Danish Diet, Cancer and Health study,[134] the same tandem repeat variant in *ALOX5* did not interact with arachidonic acid and eicosapentaenoic acid in adipose tissue in relation to risk of myocardial infarction. In an intervention, an increase in eicosapentaenoic acid and docosahexaenoic acid concentrations in erythrocytes in response to a fish oil supplement was lower in people who were homozygous for the rare variant (deletion of tandem repeats) than in people with the common allele.[135] Similarly, concentrations of eicosapentaenoic acid-derived metabolites showed a more marked increase after the fish oil supplementation in people who were homozygous for the common allele than in people who were carriers of the rare allele.[136] However, results of this small-scale trial have not yet been replicated.

Implications

Intake of omega-6 PUFAs varies largely across different global regions, ranging from 2·5% to 8·5% of daily energy according to the Global Burden of Disease Study.[10] Mean intake as a proportion of daily energy is estimated to be 5·2% in western Europe and 6·7% in the USA.[10] Even greater differences in omega-3 PUFA intake are evident, for example, intake of long-chain omega-3 PUFAs from seafood within western Europe ranges between approximately 100 mg/day in Ireland and approximately 1200 mg/day in Denmark and Iceland, compared with a mean consumption of approximately 140 mg/day in the USA. Approximate intake of plant-based omega-3 PUFA (α-linolenic acid) varies by a factor of 10 across global regions, ranging from 300–3200 mg/day (ie, <1%–14% of a daily energy intake standardised to 2000 kcal per day).

European Food Safety Authority reference values for adults are 4% of energy from linoleic acid and 0·5% from α-linolenic acid.[137] These values are based on the lowest estimated mean intakes for various populations across Europe without overt deficiency symptoms; they do not reflect optimum intake amounts for the prevention of cardiometabolic diseases. Reference values for omega-3 and omega-6 PUFAs vary between the European Food Safety Authority and several European countries.[2] Recommended linoleic acid intake to reduce risk of chronic disease and ensure adequate essential nutrients has been specified as 5%–10% of energy intake by the US Institute of Medicine and 2·5%–9·0% by UN Food and Agriculture Organization and WHO.[138,139] Linoleic acid intakes exceeding these ranges are considered suboptimal and potentially harmful, which is in contrast to European Food Safety Authority recommendations for omega-6 PUFAs, which set no upper limit.[137] Recommended intake for long-chain omega-3 PUFAs is set at 250–500 mg/day by most organisations.[137–140]

Guidelines and position statements of the American Heart Association[27] and the European Society of Cardiology[141] on PUFAs for the prevention of atherosclerotic cardiovascular disease highlight the importance of substituting energy from saturated fatty acids by PUFAs, but are less optimistic for long-chain omega-3 PUFAs.[27,141] Specifically, intake of fish oil supplements with doses of

Search strategy and selection criteria

We searched PubMed for systematic review articles published in English between Jan 1, 1990, and March 31, 2020, to identify reports about associations of PUFA intake and cardiometabolic outcomes. The search terms used were "PUFA" OR "polyunsaturated fatty acids" OR "linoleic acid" OR alpha-linolenic acid" OR "eicosapentaenoic acid" OR "docosahexaenoic acids" OR "n-3 fatty acids" OR "n-6 fatty acids" AND "cardiovascular disease" OR "myocardial infarction" OR "stroke" OR "diabetes mellitus, type 2". The reference lists of the identified papers were used to identify individual papers of interest. Furthermore, we searched PubMed for studies published in English between Jan 1, 1990, and March 31, 2020, on the interaction of *FADS* gene variants and PUFA intake using the search terms "*FADS1*" OR "*FADS2*" OR "fatty acid desaturase" OR "D5D" OR "delta-5-desaturase" OR "D6D" OR "delta-6-desaturase" OR "*FADS* polymorphisms" OR "*FADS* gene variants" AND "PUFA" OR "polyunsaturated fatty acids" OR "linoleic acid" OR "alpha-linolenic acid" OR "eicosapentaenoic acid" OR "docosahexaenoic acids" OR "n-3 fatty acids" OR "n-6 fatty acids". The final references list was selected on the basis of relevance to the subject of this Review.

eicosapentaenoic acid plus docosahexaenoic acid that are substantially higher than the adequate intake level is not routinely recommended at a population level. The American Heart Association, however, evaluates fish oil supplements as a reasonable (but not recommended) treatment for secondary prevention among patients with pre-existing coronary heart disease.[27] Beneficial effects of fish oils in subgroups who are low consumers of fish seen in some trials also indicates cardioprotection in those with low status of eicosapentaenoic acid and docosahexaenoic acid, an area that needs closer attention in further research.[7] The American Heart Association and European Society of Cardiology recommend a usual intake of one to two portions of fish per week, mainly as a dietary source of long-chain omega-3 PUFAs.[141,142] Inconsistent literature on fish intake (deemed beneficial, but largely based on observational studies) and individual trials on fish oil supplements show the complexity of assessing food bioactives in isolation from their complex food sources. However, the risk reduction in atherosclerotic cardiovascular disease seen in meta-analysis of trials implies that conservative recommendations regarding eicosapentaenoic acid and docosahexaenoic acid supplements might need to be revised.[31] The strong role of PUFA metabolism (specifically of desaturases) highlights that fatty acid intake and availability, plus genetic variants that influence PUFA metabolism, might be considered when setting recommendations for α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid intake (figure 3). In global regions with little access to seafood, intake of plant-derived α-linolenic acid might need to be increased considerably, especially if there are genetic variants in the *FADS1-2-3* gene cluster that limit biocon—version of α-linolenic acid to long-chain omega-3 PUFAs. Supplementation with fish oil would be another option in such a setting. Similarly, if plenty of plant oils rich in linoleic acid and α-linolenic acid are consumed, and genetic make-up supports bioconversion to longer-chain higher-unsaturated PUFAs, this could reduce the need for intake of long-chain omega-3 PUFAs from seafood or supplements.

Contributors

All authors contributed to literature search, data interpretation, writing, and critical revision of the manuscript.

Declaration of interests

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