Review

Intake and metabolism of n-3 and n-6 PUFA: Nutritional implications for cardiometabolic diseases

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SUMMARY

Prospective observational studies support a role of long-chain n-3 PUFA in the primary prevention of atherosclerotic cardiovascular disease, however, RCTs have often reported neutral findings. There has been also a long history of debate about potential harmful effects of high n-6 PUFA intake, although this notion is not supported by prospective observational studies or RCTs. Health effects of dietary PUFA may be influenced by delta-5 and delta-6 desaturases, key enzymes in the metabolism of PUFA. The activity of these enzymes and its modulation by variants in encoding genes (*FADS1-2-3* gene cluster) are linked to several cardiometabolic traits. This review will furthermore consider non-genetic determinants of desaturase activity, which have the potential to modify tissue PUFA availability. Finally, it will discuss the consequences of altered desaturase activity in the context of PUFA intake, i.e. gene-diet interactions, and their clinical and public health implications.

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 kinds and proportions of the component fatty acids.[1] The predominant fatty acids are either saturated fatty acids (SFA) or contain carbon-carbon double bonds: monounsaturated fatty acids (MUFA) with one and polyunsaturated fatty acids (PUFA) with two or more double bounds. While foods rich in SFA (e.g. fatty meat and dairy products, coconut and palm oil, confectionary and cakes) and MUFA (some vegetable oils, meats) contribute to the intake of these fatty acids, they can also be produced endogenously. In contrast, the PUFA linoleic acid (LA) and α-linolenic acid (ALA) are essential fatty acids and mainly derived from vegetable oils, nuts and seeds.[2] Humans have a limited capacity to synthesize eicosapentaenoic acid (EPA), and to a lesser extent docosahexaenoic acid (DHA), from ALA.[3] While EPA and DHA as long chain (LC) n-3 PUFA are therefore not strictly essential, they are consumed together as fish or fish oil capsules (either over the counter (OTC) dietary supplements or pharmaceutical grade preparations). Microalgae is a dietary source for vegetarians. Although the intake of PUFA has been a cornerstone of dietary recommendations, controversy remains about the optimal absolute and relative intakes of the main dietary n-3 and n-6 PUFA.[2,3]

Prospective observational studies support the role of LC n-3 PUFA, EPA and DHA in the primary prevention of atherosclerotic cardiovascular disease (ASCVD), with the underlying mechanisms of action widely described.[4,5] However, recent RCTs have often reported neutral findings,[6,7] which has called into question the use of fish oil supplementation as a strategy to reduce the risk of heart disease or stroke.[8] There has been also a long history of debate about potential harmful effects of high n-6 PUFA intake, specifically if their intakes far exceed those of n-3 PUFA.[9] In light of the fact that n-6 PUFA account for the majority of PUFA in normal diets,[10] it is important to shed light on the complex epidemiology and metabolism related to PUFA intake and to better understand the clinical and public health implications of both n-3 and n-6 PUFA intake for cardiometabolic diseases such as type 2 diabetes (T2DM) and ASCVD.

This review will start with a summary of evidence relating intake of n-3 and n-6 PUFA to cardiometabolic diseases. It will then address how PUFA metabolism is linked to delta-5 and delta-6 desaturases and 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, it will discuss the consequences of altered desaturase activity in the context of PUFA intake. i.e. gene-diet interactions, and their clinical and public health implications.

N-3 PUFA intake and its relation to ASCVD and T2DM

Prospective cohort studies consistently support the role of EPA and DHA in the primary prevention of ASCVD, with underlying mechanisms including an impact on plasma triglycerides and lipoprotein size, inflammation and plaque stability, vascular function, and arrhythmias.[4,5,11] Existing RCTs on clinical endpoints are largely secondary prevention (e.g. GISSI [12] and Alpha Omega [13]) or mixed primary and secondary prevention trials (e.g. JELIS,[14] REDUCE-IT [15] and the Risk and Prevention Study [16]) (**Table 1**). Only VITAL was a primary prevention study recruiting healthy men and women with no history of CVD.[7] Earlier secondary prevention trials reported a 20-30% reduction in cardiovascular deaths.[12,14,17] Similarly, advice to consume fatty fish lowered total mortality.[18] However, subsequent RCTs have often observed no effect on ASCVD incidence or deaths.[6,7,13,15,16,19] These have been summarized by several systematic reviews [17,20] which have questioned a role of supplementation with LC n-3 PUFA in secondary ASCVD prevention. This notion seems generally supported by recent RCTs. For instance, no benefit of EPA+DHA supplementation to prevent ASCVD was observed among patients with T2DM or prediabetes in the ASCEND study [6] or healthy individuals in the VITAL study.[7] However, REDUCE- IT observed significantly lower risk of ASCVD and borderline significant reduction in mortality with high doses of EPA (4g per day) among patients with established ASCVD or risk factors (including high triglycerides).[15] Up to 4g EPA/DHA is approved as an alternative to fibrates as a triglyceride reducing agent.[21] The effect-size in REDUCE-IT (hazard ratio 0.75) is suggestive of benefits beyond triglyceride lowering.[4,5] Due to the chemical form used (i.e. icosapent ethyl, a highly purified and stable EPA ethyl ester) these results cannot be directly extrapolated to OTC formulations. These RCTs have been included in two very recent meta-analysis. One analysis, including 13 RCTs, concluded that LC n- 3 PUFA supplementation lowers risk for MI, coronary heart disease (CHD) death, total CHD, ASCVD death, and total ASCVD, with risk reductions linearly related to dose.[22] According to the latest Cochrane meta-analysis, LC n-3 PUFA supplementation reduces CHD death and CHD events by approximately 10%, although no significant effect is evident for total ASCVD events or for stroke.[23]

Direct comparison between observational and trial evidence has several obstacles. Observational studies are generally prone to confounding, thus other factors associated with LC n-3 PUFA intake might explain observed benefits. Also, observational studies usually capture dietary sources of LC n-3 PUFA (fish intake) where the LC n-3 PUFA are predominantly in the triglyceride form with some phospholipid EPA and DHA also present, rather than supplementation with isolated PUFA. Additionally, when comparing the outcomes from individual RCTs it is important to consider the chemical form and dose of n-3 PUFAs, whether the formulation contains both EPA and DHA and the ratio, and whether the supplement used was OTC or a prescription grade preparation. Supplemental (OTC) or prescription EPA and DHA is available as a free fatty acid, triglyceride, phospholipid or ethyl ester forms. The bioavailability of fatty acids from ethyl esters is known to be substantially lower than from the other forms, particularly when consumed fasting or in a low-fat meal.[24] Although this is unlikely to have been an issue in REDUCE-IT given the high dose of EPA administered,[15] in ORIGIN,[19] the Risk and Prevention Study,[16] ASCEND,[6] and VITAL [7] reduced EPA+DHA bioavailability may have contributed to the lack of efficacy observed (**Table 1**). Furthermore, more aggressive use of cardiovascular medications over the last two decades may lower the therapeutic opportunity since EPA and DHA having overlapping targets with the prescribed drugs. For example, both fibrates and EPA/DHA mediate triglyceride lowering via PPAR-alpha dependent mechanisms.[25] However, subgroup analyses in ORIGIN, VITAL or REDUCE-IT and the positive impact of EPA intervention in statin users in the JELIS trial do not support a general stronger benefit of LC n-3 PUFA supplementation among non-users of cardiovascular medication.[7,14,15,19] Still, recent RCTs may have the limitation of a high habitual EPA and DHA intake in the trial participants at baseline.[17] For example, in VITAL, subgroup analysis indicated an effect of EPA+DHA on major cardiovascular events (HR 0·81) and myocardial infarction (MI) incidence (HR 0·64) in those with lower fish intake at baseline (<1·5 servings per week) but not in those with higher intakes (≥1·5 servings per week).[7] While such a difference was not observed in the ORIGIN trial,[19] a systematic evaluation of effect modification by baseline n-3 PUFA status across existing trials is so far lacking, as is an RCT which specifically tests the effect of supplementation among individuals with low habitual n-3 PUFA intake.

Also noteworthy, individual RCTs point towards beneficial effects on specific cardiovascular endpoints as secondary outcome measures. For instance, EPA+DHA intervention reduced the

risk of vascular deaths among patients with prevalent CHD according to a meta-analysis of RCTs [26] and in patients with diabetes in the ASCEND study.[6] Supplementation with LC n-3 PUFA reduced risk of MI in VITAL.[7] Furthermore, there is no direct comparison of EPA vs DHA in primary or secondary prevention trials. Such evidence is needed to inform policy, if individual EPA and DHA recommendations are to be developed. Although DHA has emerged as being more effective than EPA in reducing plasma triglycerides and improving vascular function,[27] high dose EPA reduced incident ASCVD in the REDUCE-IT trial.[15] The STRENGTH trial,[28] with a study population comparable to REDUCE-IT,[15] has recently been stopped due to its low likelihood of demonstrating a benefit.[29] Relative to REDUCE-IT (4g EPA as ethyl ester), STRENGTH supplemented with slightly lower dose of and different form of LC n-3 PUFA (2·2g EPA and 0·8g DHA as free fatty acids). These recent findings suggest that the formulation of the LC n-3 PUFA tested may be very important.

Similar to LC n-3 PUFA, prospective cohorts indicate an approximate 10-15% reduced risk of cardiovascular events associated with higher ALA intake.[30,31] These observational data are supported by RCTs: a recent Cochrane review concluded that 'increased ALA may slightly reduce risk of cardiovascular events and probably reduces risk of CHD mortality and arrhythmia' with modest effect sizes.[32] However, large-scale long-term trials on ALA supplementation are rather scarce (**Table 1**). In the only trial meeting this criteria - the Alpha Omega trial - providing an ALA-rich margarine to persons with previous MI did not significantly reduce major ASCVD events compared to a placebo margarine, although the effect size points towards benefits.[13] Any impact of ALA might be due to both an indirect effect on EPA status and a direct effect on other cardiometabolic pathways or risk factors.

Furthermore, there is little indication that EPA and DHA improve indices of glycaemia and insulin sensitivity or reduce incident T2DM risk.[33] In a pooled analyses of cohort studies, sea food and fish derived LC n-3 PUFA had no clear association with T2DM risk.[34] That LC n-3 PUFA might be less important with regard to diabetes compared to ASCVD risk is supported by Mendelian randomization studies where triglyceride lowering reduced ASCVD risk but not diabetes risk.[35,36] In contrast, ALA was modestly inversely linked to diabetes risk.[34] An inverse association between ALA plasma levels and T2DM risk was also seen in the EPIC-InterAct study.[37] However, evidence from RCTs on ALA supplementation with T2DM as endpoint is largely missing so far.[33]

N-6 PUFA intake and its relation to ASCVD and T2DM

Several older trials on the effect of cholesterol-lowering PUFA-rich diets replacing SFA from dairy and meat on risk of ASCVD and death have been conducted (**Table 1**).[38-44] In these trials, mostly LA as n-6 PUFA was used or a mixture of n-6 (LA) and n-3 (ALA) from vegetable oils. PUFA was associated with a moderate reduction of total CHD and fatal deaths relative to SFA in one meta-analyses.[45] The risk reduction (10% for each 5% energy from SFA substituted with PUFA) accorded well with the lowering of serum cholesterol and cholesterol/HDL ratio in these trials. Similarly, a recent Cochrane review supports a risk reduction for MI if n-6 PUFA replace SFA,[46] although no clear benefit was observed for overall ASCVD. No evidence was found for dose-response, but there was a suggestion of greater ASCVD protection in participants with lower baseline n-6 PUFA intake across outcomes. The trial findings are fairly consistent with a pooled analysis of 11 prospective cohort studies which specifically evaluated a substitution of PUFA (mostly LA) for SFA [47] and a meta-analysis of 13 cohort studies on LA versus SFA.[48] Notably, in prospective cohort studies with n-6 PUFA biomarkers (circulating or tissue LA levels), higher LA levels were associated with lower risk of all ASCVD outcomes, including ASCVD mortality, even after taking n-3 PUFA levels into account.[49] According to a recent meta-analysis of cohort studies, higher LA intake and tissue levels are related not only to lower ASCVD mortality, but also total and cancer mortality.[50] However, secondary analyses of older trials suggest that high intake of LA in combination with ALA is more favorable than high n-6 PUFA alone, which may have unwanted effects at higher doses.[51-53] These older trials are, however, 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 PUFA-rich margarines used (**Table 1**). Still, meta-analyses of trials show somewhat inconsistent results for n-6 PUFA, depending on study inclusions.[45,46,53-56] Taken together, evidence from RCTs and prospective cohort studies suggests that plant oils rich in LA seem to be moderately protective against CHD, especially MI.

Regarding n-6 PUFA intake and T2DM risk, there are no available data from trials designed to investigate diabetes incidence as an outcome.[33] However, when taking into account short-term feeding trials and prospective cohort studies using n-6 biomarkers or food questionnaires, a previous review suggests inverse associations between n-6 PUFA (LA in particular) and incident T2DM.[57] This has been confirmed in several more recent cohort studies, including the pan-European EPIC-InterAct study,[37] and the pooled meta-analyses

from the FORCE consortium.[58] In the latter which measured LA and arachidonic acid (AA) in circulating lipids in prospective cohorts, LA, but not AA, was inversely and linearly associated with incident T2DM, and this robust association was not modified by n-3 PUFA status.[58] In support, a quite large number of small and mostly shorter-term randomized feeding trials indicate that PUFA, when isocalorically compared with SFA or carbohydrates, improve markers of insulin sensitivity and glycemic control.[59] Still, more definitive RCTs of longer duration are lacking.[33]

Role of n-6/n-3 ratio

Concern that consuming a relatively high proportion of dietary n-6 fats compared with n-3 fats has detrimental effects, particularly on inflammatory status, have generally not been supported by study evidence. Indeed, several feeding trials showed that increasing n-6 PUFA (e.g. LA) while keeping n-3 intake unchanged, thus resulting in several-fold higher n-6/n-3 ratio, has no adverse effects on either multiple markers of inflammation or oxidative stress,[60] not even under energy excess conditions at very high LA intake.[61] In line with these findings, a recent meta-analysis did not find evidence to suggest an important role of the n-6/n-3 ratio on glucose metabolism.[33] While longer term RCTs on n-6 PUFA are insufficient to conclude on the relevance of the n:6/n-3 ratio,[46] prospective cohort studies do not indicate any adverse role of a high n-6/n-3 ratio.[62] The reasons for the lack of apparent importance of the n-6/n-3 ratio to predict cardiometabolic disease or inflammation probably include the fact that this ratio is partly based on a number of incorrect and simplified assumptions,[63] e.g. n-6 PUFA overall is proinflammatory, n-6 PUFA and LA in particular have adverse effects on CVD risk (while in fact both n-6 and n-3 PUFA are related to lower risk), and lowering intake of LA will lower AA levels (in contrast, supplementing LA does not increase AA plasma or adipose tissue levels [61,64]). In addition, a clear problem arises when combining different n-6 (and n-3) PUFA despite distinct inflammatory and cardiovascular effects of individual PUFA as well as their different metabolites.[63] It would be important to standardize the calculation of this ratio, as different studies have used somewhat different ratios (e.g. considering all n-3 PUFA or EPA and DHA only). Also, this ratio calculated from dietary intake data cannot be compared with a ratio calculated from plasma or tissue PUFA levels.

PUFA metabolism

PUFA fulfill various functions within the human body, besides being a source of energy. They are central structural components of the phospholipid layer of cell membranes,

influencing membrane fluidity and selective permeability. Furthermore, PUFA can directly influence several metabolic pathways, being ligands for transcription factors like sterol regulatory element binding protein 1 (SREBP-1), nuclear factor κB (NF-κB), hepatocyte nuclear factor 4α (HNF-4α), and peroxisome proliferator-activated receptors (PPARs), which play a central role in lipid metabolism. PUFA are furthermore substrates for the formation of various lipid-related metabolites, e.g. eicosenoids, leukotrienes, prostaglandins, thromboxanes, lipoxins, endocannabinoids, or resolvins, which themselves are highly bioactive. In this context, the sphingolipids ceramides are an additional interesting group of lipid molecules. Prospective studies have linked ceramides to cardiometabolic risk [65,66] and experimental data suggest causal links to insulin resistance, and potentially also ASCVD.[67] Interestingly, n-6 PUFA (LA) decrease several plasma ceramide species as compared with SFA.[61]

Importantly, although the essential precursor PUFA LA and ALA are the main PUFA sources in the diet, other PUFA can be produced endogenously, although the bioconversion efficiency to DHA is limited.[3] The bioconversion of LA and ALA to the longer chain PUFA (γ-linolenic acid [GLA], dihomo-γ-linolenic acid [DGLA], AA, EPA, DHA) is catalyzed by elongases and desaturases – with the delta-6 and delta-5-desaturases being the key enzymes in this process (**Figure 1**). Specifically, delta-6-desaturase is considered the rate limiting step of conversion of LA and ALA to downstream metabolites. Noteworthy also, both n-6 and n-3 PUFA compete for the same enzymes in this processes, although a preferential affinity to n-3 PUFA exists.[68] PUFA are the main dietary component that regulate the activity of these desaturases. In a rodent model, both desaturases seem to be suppressed by dietary PUFA.[69] A stable isotope study showed that increased LC n-3 PUFA intakes inhibits ALA bioconversion.[3]

PUFA often exert their physiological effects through a host of oxidative bioactive products (**Figure 1**), collectively named ‘oxylipins‘, produced by the action of cycloxygenases (encoded by *COXs*), lipoxygenases (encoded by *ALOXs*), and some members of *CYP450* superfamily (*CYP1-4* families). Oxylipins have long been known to have a pro-inflammatory effect as thromboxanes (TXs), prostaglandins (PGs) and leukotrienes (LTs). TXs, 2 series-PGs and the 4 series-LTs are AA-derived while EPA metabolism produces 3 series-PGs and 5 series-LTs. EPA products are generally less pro-inflammatory than their AA counterparts.[70] However, the inflammatory impact of AA or EPA derivates is nuanced both producing pro- or

anti-inflammatory products. For example, AA-derived PGI2 is traditionally known to inhibit platelet aggregation.[71]

In addition to competing with AA, recent studies show that EPA and DHA play a direct anti-inflammatory and resolving role through the production of signalling molecules, named Specialised Pro-resolving Mediators (SPM).[72] The SPM Resolvins, Protectins and Maresins, together with AA-produced lipoxins, contribute to the active process of resolution of inflammation by inhibiting the influx of neutrophils into the site of inflammation and enhancing phagocytosis. Evidence to date is largely derived from rodent studies, with confirmation of the role of SPMs in human homeostasis and pathophysiology needed.

Prostaglandins and thromboxanes are produced mainly by the action of COX enzymes on AA and EPA. COX-1 is involved in basic physiological functions while COX-2 products are mainly produced in response to inflammation and in malignant conditions such as colo-rectal 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] In this context, it would be interesting to better understand how the differences between nonsteroidal anti-inflammatory drugs (including aspirin), n-3 PUFA, and n-6 PUFA on prostaglandin metabolism may relate to their different effects on ASCVD risk (e.g. some adverse effects of selective COX-2 inhibitors vs. aspirin).[75]

Lipoxygenases (encoded by *ALOX5*, *ALOX5AP*, *ALOX12* and *ALOX15*) modulate the production of the pro-inflammatory LTs and the anti-inflammatory SPMs.[76] In obese patients with T2DM, increased *ALOX5*, *ALOX12* and *ALOX15* expression in adipose tissue has been reported compared to obese-nondiabetic patients.[77] The ALOX5 enzymatic pathway is activated in cardiovascular diseases and suggests an important role of LTs in atherosclerosis and in its ischemic complications such as MI and stroke.[78] CYP1-4 families produce epoxyeicosatrienoic acid (EET) and hydroxyeicosatetraenoic (HETE) from AA, EPA and DHA, with EETs being further catalyzed to their respective regioisomers (DiHETE) through epoxide hydrolase (sEH). Pre-clinical and clinical studies show the potential role of these metabolites as vasodilators and subsequent regulators of blood pressure, with some preliminary evidence of their anti-arrhythmic and cardio-protective functions.[79]

Relationship of desaturase activity to cardiometabolic health

The relevance of endogenous formation of more unsaturated FAs from dietary precursor FAs (LA and ALA) for cardiometabolic diseases 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 based on product-to-precursor ratios of FAs measured in blood fractions. Several studies observed that a higher estimated delta-6 activity (ratio of GLA/LA) is related to an increased T2DM risk, while the contrary was the case for estimated delta-5 activity (AA/DGLA).[37,58,80] A similar, although less clear picture emerges for ASCVD, where a pooled analysis of 30 cohort studies revealed inverse associations for both LA and AA. However, studies on ASCVD considering intermediate n-6 PUFA or specific FA ratios to reflect their bioconversion are scarce. DGLA was related to higher CHD risk,[81] but less convincing stroke risk in the ARIC study,[82] but GLA and/or DGLA were not clearly associated with ASCVD risk in other studies.[83-85] Still, these studies suggest overall that higher delta-6 activity but lower delta-5 activity, which would both lead to the accumulation of intermediate FAs (GLA and DGLA) increase cardiometabolic risk, not a higher accumulation of AA.

Genetic determinants of PUFA metabolism

Genetic factors have been clearly linked to the fatty acid composition of biosamples, specifically blood fractions. Variations in *FADS1* and *FADS2*, the genes encoding the delta-5- and delta-6-desaturases, have been related to PUFA blood levels by candidate gene approaches.[86] Furthermore, GWAS have identified this region to have the strongest genetic link to PUFA blood levels. For example, variant alleles at single nucleotide polymorphisms (SNPs) in the *FADS1-2-3* gene cluster were associated with higher levels of ALA and lower levels of EPA and DPA in the CHARGE consortium.[87] The strongest associated SNPs explained ~4%, 2% and 9% of the variance of ALA, EPA and DPA, respectively. Similarly, variants in the *FADS1-2-3* gene cluster were strongly associated with n-6 PUFA levels (LA, GLA, DGLA, AA) in CHARGE, with the top SNP (rs174547) explaining ~10% variation in DGLA and >20% in AA.[88] Similarly, GWAS considering FA ratios as estimates of delta-5- or delta-6 desaturase have identified the *FADS1-2-3* gene cluster as prominent locus.[89,90]

In addition to their relationship to tissue FA composition, variants in the *FADS1-2-3* gene cluster are among the strongest genetic variants linked to TG levels [91,92] and are associated with other lipids, e.g. cholesterol.[92] Furthermore, GWAS support that variants in this gene cluster are among the strongest signals related to specific lipids, particularly phospholipids

(phosphatidylcholines and phosphatidyletholamines).[93,94] Several of these lipids were related to risk of diabetes and ASCVD in prospective studies.[95,96]

Variants in the *FADS1-2-3* gene cluster have not been identified to relate to diabetes and ASCVD risk on a genome-wide level,[97,98] despite their association with multiple other cardiometabolic traits, including inflammatory markers,[91] and fasting glucose.[99] However, they have recently been used in Mendelian randomization analyses to support causal roles of desaturases, PUFA and specific phospholipids for cardiometabolic diseases.[100-102] However, investigating genetic variation in the *FADS1-2-3* gene cluster to disentangle the potentially different role of PUFA is hampered by strong linkage disequilibrium in this region.[80] Common variants in *FADS1* are strongly correlated with variants in *FADS2* and minor alleles of variants in *FADS1* are not only related to higher DGLA (substrate of delta-5-desaturase) and lower AA concentrations (product), but also higher LA and lower GLA concentrations (substrate and product of delta-6-desaturase) in European populations.[88] Importantly in this context, prospective studies using PUFA biomarkers support that both desaturases have opposing associations with cardiometabolic risk and confounding by linkage disequilibrium in genetic studies may mask true associations.[103]

Also noteworthy, 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 lower ability to convert plant based PUFA into longer-chain and more unsaturated FAs, is largely absent in African, relatively common in European and dominating in American populations (**Figure 2**).[104] Similarly, the *FADS2* variant rs174570, related to lower desaturase activity, is much more frequent in Greenlandic Inuit (allele frequency 99%) than in Chinese (34%) or European populations (16%).[105] This highlights a potential human adaptation to varying dietary PUFA sources. Variants in the *FADS1-2-3* gene cluster which increase LC-PUFA synthesis from plant-based PUFA might have been of advantage in geographic regions with limited access to marine sources for LC-PUFA. The other extreme can be found in native Inuit who traditionally consume extremely high levels of LC n-3 PUFA from fish and marine mammals and for whom the *FADS1-2-3* gene cluster was found to be the strongest outlier region based on patterns of allele frequency differentiation compared to other populations.[105] There is little evidence that the *FADS1-2-3* gene cluster relates to systems and biologic processes related to food preferences, e.g. 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 a number of experimental models and human investigations. In *ALOX5* KO mice, a reduction in LTB4 and reverse cholesterol transport suggests a new mechanism through which lipoxygenase pathway likely influences atherogenesis.[108] Large-scale GWAS reported associations between *ALOX5* SNPs and HDL-cholesterol levels in humans.[92,108] Variation in the tandem repeats of the *ALOX5* promoter and in *ALOX5* activating protein (*ALOXAP*) was associated with incident MI in cohort studies.[109,110] However, *ALOX* genotypes were not significantly associated with T2DM or coronary artery disease in recent GWAS.[71,111]

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**).[112-118] Unfortunately, most studies were cross-sectional in nature or have investigated intake of LC n-3 PUFA intake,[112,114,116,118] which are, however, not the substrate of desaturases. Only two trials investigated supplementation with plant-derived PUFA. Gillingham et al. investigated the effect of a ALA-rich flaxseed oil intervention in comparison to a Western diet and an oleic acid rich diet. Minor allele carriers of 4 different variants in the *FADS1-2-3* gene cluster had substantially lower increase in EPA plasma concentrations after the ALA intervention compared to wild-type: EPA levels in individuals homozygote for the major *FADS1* rs174561 allele were 2·2% (ALA intervention) versus 0·6% (Western diet) and 0·7% (oleic acid diet) after the intervention, but 0·9% versus 0·3 and 0·4% in individuals being homozygote for the minor allele (P for interaction <0·001).[115] In the FADSDIET trial, AA levels in plasma phospholipids decreased in participants with the rare CC genotype of *FADS1* rs174550 in response to a LA-rich sunflower oil, while AA levels remained unchanged in individuals with the TT genotype.[117] These findings are supported by animal studies. Knockout of *FADS2* in mice fed a LA-rich diet is related to lower availability of AA [119] and incorporation of AA into phospholipids.[120] Similarly, knockout of *FADS2* depletes EPA and DHA in mice fed an ALA-rich diet.[121] *FADS2* variants alter *FADS2* gene expression and tissue AA concentration in pigs.[122] Similarly, *FADS1* knockdown results in reorganization of both n-6 and n-3 PUFA levels and their associated proinflammatory and proresolving lipid mediators.[123]

Similar to modifying effects on blood PUFA levels, modification of effects of dietary PUFA on blood lipid levels by variants in the *FADS1-2-3* gene cluster can be hypothesized. Studies addressing this question have found mixed results, but are mostly observational and cross-sectional in nature (**Table 2**).[113,124,125] Only one trial, involving 208 participants, has specifically focused on the triglyceride response to dietary LC n-3 PUFA intervention, but did not observe an effect modification.[126] However, as mentioned above, LC n-3 PUFA are not the main substrate of desaturases. Given the current lack of interventional studies which directly tested response to the intake of plant derived LA and ALA rather than LC n-3 PUFA, this hypothesis remains unconfirmed so far. Still, modifying effects of the *FADS1-2-3* gene cluster have been described for other cardiometabolic risk factors. For example, the FADSDIET trial observed modification of a LA-rich diet response by *FADS1* rs174550 in terms of inflammation (hsCRP).[118] Influences of inhibited delta-5- and delta-6-desaturase activities on glycaemic traits and atherosclerosis have been described from knock-out animal studies.[127]

Whether variants in the *FADS1-2-3* gene cluster modify cardiometabolic effects of dietary PUFA in terms of risk for clinical endpoints has been investigated in two case control and several cohort studies (**Table 2**).[49,58,128-130] In a Swedish cohort study, higher ALA intake appeared to be more beneficial with regard to ASCVD risk among carriers of *FADS1* rs174546 genotype related to lower desaturase activities.[129] While FORCE, a consortium of several prospective cohort studies, did not find that the *FADS1* rs174547 variant modulated the relationship between LA and AA biomarkers and T2DM risk,[58] but effect modification was observed for ASCVD endpoints, specifically stroke.[49] The latter finding implies that a protective effect of higher LA is restricted to individuals homozygous for the common rs174547 allele, thus a higher genetically determined ability to convert LA to AA and subsequent products. Still, the *FADS1* variant considered has already strong influence on LA tissue concentrations and interpretation of this PUFA biomarker as a proxy of dietary intake is problematic. Clearly, prospective studies investigating associations between intake of plant-derived LA and ALA and subsequent risk of diabetes and ASCVD and its modification by variants in the *FADS1-2-3* gene cluster would be informative to substantiate the findings of FORCE.

With regard to potential modification of dietary PUFA effects by *ALOX5*, evidence from studies is more limited. AA intake was related to enhanced influence of the variant

rs59439148, related to the number of tandem repeats of the Sp1 binding site in the *ALOX5* promoter (the rare allele having 1-2 fewer tandem repeats), on arterial thickness in a cross-sectional study [131] and with the incidence of MI in a cohort study.[109] However, in the Danish Diet, Cancer and Health study, the same tandem repeat variant in *ALOX5* did not interact with adipose tissue AA and EPA in relation to risk of MI.[132] In an intervention, individuals homozygote for the rare variant (deletion of tandem repeats) showed less increase in erythrocyte EPA and DHA levels in response to a fish oil supplementation compared to subjects with the common allele.[133] Similarly, concentrations of EPA derived metabolites showed a more marked increase after the fish oil supplementation in individuals homozygote for the common allele compared to carriers of the rare allele.[134] Still, results of this small-scale trial have not been replicated so far.

Implications

Intake of n-6 PUFA varies largely across different global regions, ranging from 2·5% to 8·5% of daily energy according to the Global Burden of Disease Study (GBDS).[10] In Western Europe and the USA, mean intake is estimated to be 5·2 and 6·7% of daily energy. Even more pronounced differences in intake levels of n-3 PUFA are evident, where, for example, with LC n-3 PUFA from seafood ranging within Western Europe between ~100 mg/d (Ireland) to ~1200 mg (Denmark and Iceland), with a mean USA consumption of ~140mg/d. The intake of plant n-3 PUFA (ALA) ranges 10-fold across global regions from ~300-3200 mg/day, which standardized to a 2000 kcal per day represents <1 to 14% of daily energy intake.

Reference values for adults according to EFSA are 4% of energy from LA and 0·5% from ALA.[135] These values are based on the lowest estimated mean intakes of various populations across Europe, where overt deficiency symptoms are not present – they do not reflect optimal intake levels for the prevention of cardiometabolic diseases. Interestingly, reference values for n-3 and n-6 PUFA vary between EFSA and several European countries.[2] Ranges of intake for LA as an energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients, has been specified by the US Institute of Medicine (5-10%) [136] and the FAO/WHO (2·5–9·0%).[137] This implies that LA intakes exceeding these ranges are considered sub-optimal and potentially harmful, which is in contrast to EFSA recommendations where no upper limit for n-6 PUFA was set.[135] With regard to recommendations for LC n-3 PUFA intake, adequate intake levels have been more comparably set at 250-500mg/d EPA+DHA by most organizations.[135-138]

Guidelines and position statements of the American Heart Association [17] and the European Society of Cardiology [139] regarding the role of PUFA in the prevention of ASCVD highlight the importance of substituting energy from SFA by PUFA, but are less optimistic for LC n-3 PUFA. Specifically, the intake of fish oil supplements with dosages of EPA and DHA substantially higher than the adequate intake level is not routinely recommended at a population level. The American Heart Association, however, evaluates fish oil supplementation to be a reasonable (but not recommended) treatment for secondary prevention among patients with pre-existing CHD.[17] Beneficial effects of fish oils observed in subgroups of low-fish consumers in some trials [7] also indicates cardio-protection in those with low EPA+DHA status, an area which needs closer attention in further research. Both societies recommend a usual intake of 1-2 portions of fish per week, mainly based on their role as dietary sources of LC n-3 PUFA.[139,140] The inconsistent literature on fish intake (beneficial, but largely based on observational studies) and individual fish oil supplementation trials demonstrates the complexity of comparing food bioactives in isolation to their complex food sources. Still, given the observed ASCVD risk reduction when evidence from recent trials is included in meta-analysis [22] implies that the more conservative recommendations regarding EPA and DHA supplementation may need to be revised.

The strong role of PUFA metabolism, specifically of desaturases, highlights that fatty acid intakes and availability along with genetic variants which influence PUFA metabolism, might be considered in the future when setting ALA, EPA and DHA recommendations (**Figure 3**). In regions with limited access to seafood, intake of the plant-derived ALA might need to be considerably higher, specifically if genetic variants in the *FADS1-2-3* gene cluster are present which limit bioconversion of ALA to LC n-3 PUFA. Alternatively, supplementation with fish-oil would be an option in such a setting. Similarly, if plenty of plant oils rich in LA and ALA are consumed and the genetic make-up supports bioconversion to longer-chain, more unsaturated PUFA, there could be a lower need for intake of LC n-3 PUFA from seafood or supplements.

Contributors

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

Conflicts of interest

MBS reports grants from European Commission and German Federal Ministry of Education and Research, outside the submitted work. RNMS reports grants from EU JPI-BBSRC (UK Government), during the conduct of the study. AMM reports grants from EU JPI- BBSRC (UK Government), outside the submitted work. UR declares to have no conflicts of interest.

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Search strategy and selection criteria

PubMed database was searched for systematic review articles written in English from January 1990 up to August 2019, to identify reports about associations of PUFA intake and cardiometabolic outcomes. The search terms used were "PUFA", "Polyunsaturated fatty acids", "linoleic acid", alpha-linolenic acid", "eicosapentaenoic acid", "docosahexaenoic acids", "n-3 fatty acids", "n-6 fatty acids" together with terms for cardiovascular outcomes and T2DM. The reference lists of the identified papers were used to identify individual papers of interest. Furthermore, we search PubMed database for studies on interaction of *FADS* gene variants and PUFA intake using search terms "*FADS1*", "*FADS2*", "fatty acid desaturase", "D5D", "Delta-5-Desaturase", "D6D", "Delta-6-Desaturase", "*FADS* polymorphisms", "*FADS* gene variants" in combination with "PUFA", "Polyunsaturated fatty acids", "linoleic acid", alpha-linolenic acid", "eicosapentaenoic acid", "docosahexaenoic acids", "n-3 fatty acids", "n-6 fatty acids". The final reference list was selected on the basis of relevance to the subject of this review.

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Figure 1 Key enzymes involved in the metabolism of PUFA

Conversion of linoleic acid and α-linolenic acid to longer-chain n-6 and n-3 polyunsaturated fatty acids is catalized by the action of delta-6 desaturase, delta-5 desaturase, and elongases. Arachidonic acid (AA), eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) often exert their physiological effects through 'oxylipins' produced by the action of cycloxygenases (COXs), lipoxygenases (ALOXs), and some members of CYP450 superfamily.

2-PGs: 2 series prostaglandins, 3-PGs: 3 series prostaglandins 4-LTs: 4 series leukotrienes, 5-LTs: 5 series leukotrienes, ALOXs: Arachidonate Lipoxygenases, ALOX5AP: 5-Lipoxygenase Activating Protein, COX-2: cylcloxygenase-2, CYP-450: cytochrome-P450, DHEQs: dihydroxyeicosatetraenoic acids, DHET: dihydroxyeicosatrienoic acid, DiHDPA: dihydroxydocosapentaenoic acid, EDP: epoxydocosapentaenoic acid, EEQ: epoxyeicosatetraenoic acid, EETs: epoxyeicosatrienoic acid, HDHA: hydroxydocosahexaenoic acid, HEPE: hydroxyeicosapentaenoic acid, HETE: hydroxy-eicosatetraenoic acid, HpDHA: hydroperoxide intermediate of DHA, sEH: serum epoxide hydrolase enzyme, TX: thromboxanes

Figure 2. Allele frequencies (percent) for *FADS1* rs174547 across populations

Genetic variation in *FADS1* is in strong linkeage disequilibrium with variation in *FADS2*. *FADS1* rs174547, one of the lead SNPs related to circulating PUFA levels identified in large-scale GWAS,[88] shows strong differences across different populations worldwide. Data from 1000 Genomes Project Phase 3.[104]

Figure 3. Conceptual framework for hypothesized cardiometabolic benefits of n-6 and n-3 PUFA intake depending on genetic variation in PUFA metabolism

Genetic variation in the *FADS1-2-3* gene cluster relates to the activity of d-5 and d-6-desaturases, key enzymes in the formation of longer-chain FA from precursor n-3 α-linolenic acid (ALA) and n-6 linoleic acid (LA). Mutations related to lower desaturase activity are frequent in Native Inuit (e.g. rs174570: 99% [105]), relatively common in European populations (16%) and largely absent in African populations (1%).[104] Higher d5-desaturase activity and thus higher ability to metabolize LA and intermediate n-6 PUFA (GLA and DGLA) to AA is related to lower cardiometabolic risk.[49] In the presence of high intake of LC n-3 PUFA from fish and seafood, a lower genetically determined desaturase activity may compensate for a decreased intake of plant-based PUFAs. However, there is uncertainty whether higher ALA intake is more beneficial in the context of higher ability to convert ALA to LC n-3 PUFA.[128]

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

Study/ Publication year	Population	Intervention	Duration	Outcome	Effect size HR or RR (95% CI)	Comment
GISSI [12]	Post-MI < 3m	850-882 mg/d EPA+DHA	3·5y	P: Death, MI stroke S: ASCVD death	0·85 (0·74-0·9) 0·70 (0·56-0·87)	4 medications prescribed, statins use 5%
JELIS [14]	Hypercholesterolemic men (40-75y) and ostmenopausal women	1800mg EPA (ethyl esters)	4·6y	P: sudden cardiac death, fatal and non-fatal MI, unstable angina, angioplasty, stenting, CABG	0·81 (0·69-0·95)	All on statins, 64% on antihypertensive medication, 14% on antiplatelets, 12% on hypoglycemic agents
Alpha Omega [13]	Post-MI	1: 400 mg/d EPA+DHA (trigylcerides) 2: 2 g/d ALA	3·7y	P: fatal and nonfatal ASCVD, PCI+CABG	EPA+DHA vs. ALA/placebo: 1·01 (0·87-1·17) ALA vs. EPA+DHA/placebo: 0·91 (0·78-1·05)	Median EPA+DHA intake at baseline: 120-130 mg/d, no subgroup difference by baseline intake; Suggestive effect of ALA in women (HR: 0·73 (0·51–1·03))
ORIGIN [19]	High ASCVD risk and prediabetes/diabetes	900 mg/d EPA+DHA (ethyl esters)	6·2 y	P: ASCVD death S: nonfatal MI, stroke or ASCVD death	0·98 (0·87-1·10) 1·01 (0·93-1·10	High prevalence of cardiovascular medication, statin use ~54%; Median EPA+DHA intake at baseline: 210 mg/d
Risk and Prevention Study [16]	ASCVD risk factors ≥4 or vascular disease, previous MI precluded	850 mg/d EPA+DHA (ethyl esters)	5·0y	P: Time to death from ASCVD or hospital admission for ASCVD S: ASCVD death	0·98 (0·88-1·08) 1·03 (0·82-1·30)	9 medications prescribed, statin use 41% At 1y, event rate lower than anticipated, the primary end point was revised

ASCEND [6]	Type 2 diabetes, no evidence of ASCVD	840 mg/d EPA+DHA (ethyl esters)	7·4y	P: ASCVD death, MI, stroke, transient ischemic attack S: nonfatal MI S: nonfatal ischemic stroke S: vascular death	1·00 (0·91-1·09) 0·93 (0·76-1·14) 1·01 (0·84-1·22) 0·81 (0·67-0·99)	High prevalence of cardiovascular medication, statin use ~75%
VITAL [7]	Healthy, men (≥50y), women (≥55y)	840 mg/d EPA+DHA (ethyl esters)	5·3y	P: ASCVD death, MI and stroke S: ASCVD death S: Total MI	0·92 (0·80-1·06) 0·96 (0·76-1·21) 0·72 (0·59-0·90)	HR of ASCVD death, MI and stroke of 0·81 (0·67-0·98) and total MI of 0·60 (0·45-0·81) with fish intake of <1·5 servings per week. No effect in those with higher fish intakes of ≥1·5 servings per week (HR 1·08 and 0·94)
REDUCE-IT [15]	ASCVD or T2DM ≥ 1 ASCVD risk factor, high TG	4 g/d Ethyl-EPA (icosapent ethyl)	4·9y	P: ASCVD death, MI, stroke, coronary revascularization or unstable angina	0·75 (0·68-0·83)	HR of 0·74 (0·65–0·83) in composite ASCVD death, MI and stroke secondary end point
STRENGTH [28,29]	high ASCVD risk or previous ASCVD or DM, high TG and low HDL-c	4g oil providing 2·2 EPA + 0·8g DHA (carboxylic acids)	-	P: cardiovascular death, nonfatal MI, nonfatal stroke, emergent/elective coronary revascularization, or hospitalization for unstable angina	stopped	Intervention judged unlikely to demonstrate a benefit by the independent data monitoring committee
LA veterans [38]	Men with or without CHD	Corn and soybean oil	≤8y	MI, sudden death + cerebral infarction	0·74 (0·53-1·03)	Confirmation of compliance by adipose tissue fatty acid analyses. Secondary outcome for total

						ASCVD showed ~ 30% reduced risk, RR of 0.68 (0.52–0.91).
MRC Soy [39]	Post MI, men	Soybean oil ~80g	5y	MI, 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. ≥43 g soybean oil unheated (often drunk with fruit juice)
Oslo diet-heart study [40]	Post MI, men	Soybean oil, Cod liver oil	5y	MI + Sudden cardiac death	0·75 (0·57-0·99)	Multifactorial intervention with modification of dietary composition other than fat
Finnish mental hospital study [41]	Men with MI	Soybean oil	6y	MI (ECG change) + CHD mortality	0·55 (0·34-0·88)	Institutionalized population; assignment by hospital, not individually randomized; confirmation of compliance with a large increase of LA in adipose tissue.
Finnish mental hospital study [42]	Women with MI	Soybean oil	6y	MI (ECG change) + CHD mortality	0·64 (0·41-1·00)	Institutionalized population; assignment by hospital, not individually randomized
Minnesota Coronary Survey [43]	Men and women	Corn oil	≤4·5y	ASCVD events, ASCVD and total mortality	1·08 (0·84-1·37)	Institutionalized population; mean follow-up only 1 year; drop-out rate ~75%; very high dose of corn oil (13 vs 3 energy % from LA)
Sydney Diet Heart Study [44]	Post MI or with CHD, men	Safflower oil	2-7y	MI + cardiac death	1·86 (0·63-5·44)	Relatively short study duration (median follow-up 39 months), with potential confounding by *trans*-fatty acids; very high dose of n-6 PUFA, without any increase of n-3

The table does not provide an exhaustive description of all PUFA interventions conducted to date, but rather gives illustrative examples of earlier and more recent trials, and those which are discussed in the text

ASCVD- atherosclerotic cardiovascular disease, CABG- coronary artery bypass graft, CHD- coronary heart disease, CI- confidence interval, DHA- docosahexanoic acid, EPA- eicosapentanoic acid, HR- hazard ratio, MI- myocardial infarction, P-primary end-point, PCI- percutaneous coronary intervention, S- secondary end-point, TG- triglycerides

Table 2. Studies investigating interactions between PUFA intake or status and variants in the *FADS1-2-3* gene cluster and FA levels or cardiometabolic outcomes

Author/ Publication year	Design, Population	Intervention/Exposure	Genetic variants	Outcome	Interaction
Fatty acid composition of blood fractions or breast milk					
Molto´-Puigmartı´ et al. 2010 [112]	Cross-sectional	Fatty fish intake	rs174575	Plasma and milk FA	Higher EPA or DHA content in human milk with higher fatty fish intake only in major allele carriers; No difference in plasma phospholipid EPA or DHA by genotype
Dumont et al. 2011 [113]	Cross-sectional	Dietary LA and ALA	rs174546	Serum phospholipid FAs	No interaction
Al-Hilal et al. 2013 [114]	RCT, healthy subjects	EPA+DHA (0·45, 0·9, and 1·8 g/day),	rs174537, rs174561, rs3834458	Plasma and RBC FA	Increase in D5D activity (AA:DGLA) among T-allele carries of rs174537 with higher supplementation
Gillingham et al. 2013 [115]	RCT, hyperlipidemic subjects	ALA rich diet (20·6 g ALA/d)	rs174545, rs174583, rs174561, rs174537	Plasma FA	Substantially smaller absolute EPA in minor allele carriers after ALA intervention
Smith et al. 2015 [118]	Cross-sectional (consortium)	Dietary LA and ALA	rs174538, rs174548	Plasma or RBC LC n-3 PUFA	Interaction between ALA intake and *FADS1* variants on DPA and DHA
Takkunen e al. 2016 [116]	Cross-sectional	LC n-3 PUFA from fish		Plasma and RBC FA	Stronger association between LC n-3 PUFA intake and EPA in minor allele carriers
Juan et al. 2018 [141]	Cross-sectional	Dietary LA, ALA, EPA, DHA	rs174546	Plasma FA	Stronger positive associations between EPA and DHA intake and EPA concentrations with intake with minor T allele; no interactions for other dietary PUFA

Lankinen et al. 2019 [117]	Single group trial	LA rich sunflower oil (17-28 g LA/d)	rs174550	Plasma phospholipid and cholesterol ester FA	Decrease in AA in homozygote for minor allele, no effect in homozygote for major allele
Blood lipids					
Lu et al. 2010 [124]	Cross-sectional	Dietary n-6 and n-3 PUFA	rs174546, rs482548, rs174570	Total, HDL-, and non-HDL-cholesterol	No interactions for total dietary n-3 intake and all outcomes; No interactions for dietary n-6 and most outcomes (only significant interaction for HDL-cholesterol and rs174546)
Dumont et al. 2011 [113]	Cross-sectional	Dietary LA and ALA	rs174546	Serum TG, cholesterol, and lipoproteins	Lower total and non-HDL cholesterol in minor allele carriers with high ALA intake only
Cormier et al. 2012 [126]	Single group trial	1·9 g EPA and 1·1 g DHA per day	Selected SNPs of the *FADS1-2-3* gene cluster	Plasma TG	No interaction observed
Standl et al. 2012 [125]	Cross-sectional	Dietary n-3 PUFA	*FADS1-2-3* gene cluster	Total cholesterol, HDL-cholesterol, LDL-cholesterol, TG	No interaction observed
Dumont et al. 2018 [142]	Cross-sectional	Dietary LA and ALA	rs174547	HDL-cholesterol	Lower HDL-cholesterol with minor allele only with high LA intake; no interaction with ALA
ASCVD or T2DM risk					
Baylin et al. 2007 [128]	Case-control	Adipose tissue ALA	common *FADS1* deletion [T/-]	Non-fatal MI	No interaction observed

Hellstrand et al. 2014 [129]	Cohort	Dietary LA and ALA	rs174546	ASCVD	Inverse association of ALA:LA ratio or ALA with ASCVD/stroke only in minor allele carriers
Liu et al. 2015 [143]	Case-control	Dietary EPA and DHA	rs174547	Coronary artery disease	Common T-allele associated with higher risk only among individuals with lower dietary EPA/DHA intake
Wu et al. 2017 [58]	12 Cohorts	LA and AA biomarker	rs174547	Type 2 diabetes	No interaction
Marklund et al. 2019 [49]	13 Cohorts	LA and AA biomarker	rs174547	ASCVD	Inverse association of LA with total ASCVD and stroke in homozygote common allele carriers, not in minor allele carriers; no interactions for ASCVD mortality or total CHD or for AA

ALA- alpha-linolenic acid, ASCVD- atherosclerotic cardiovascular disease, CHD- coronary heart disease, CI- confidence interval, DGLA- dihomo-gamma linolenic acid, DHA- docosahexanoic acid, EPA- eicosapentanoic acid, HR- hazard ratio, LA- linoleic acid, MI- myocardial infarction, P-primary end-point, s-secondary end-point, TG- triglycerides

Figure 1

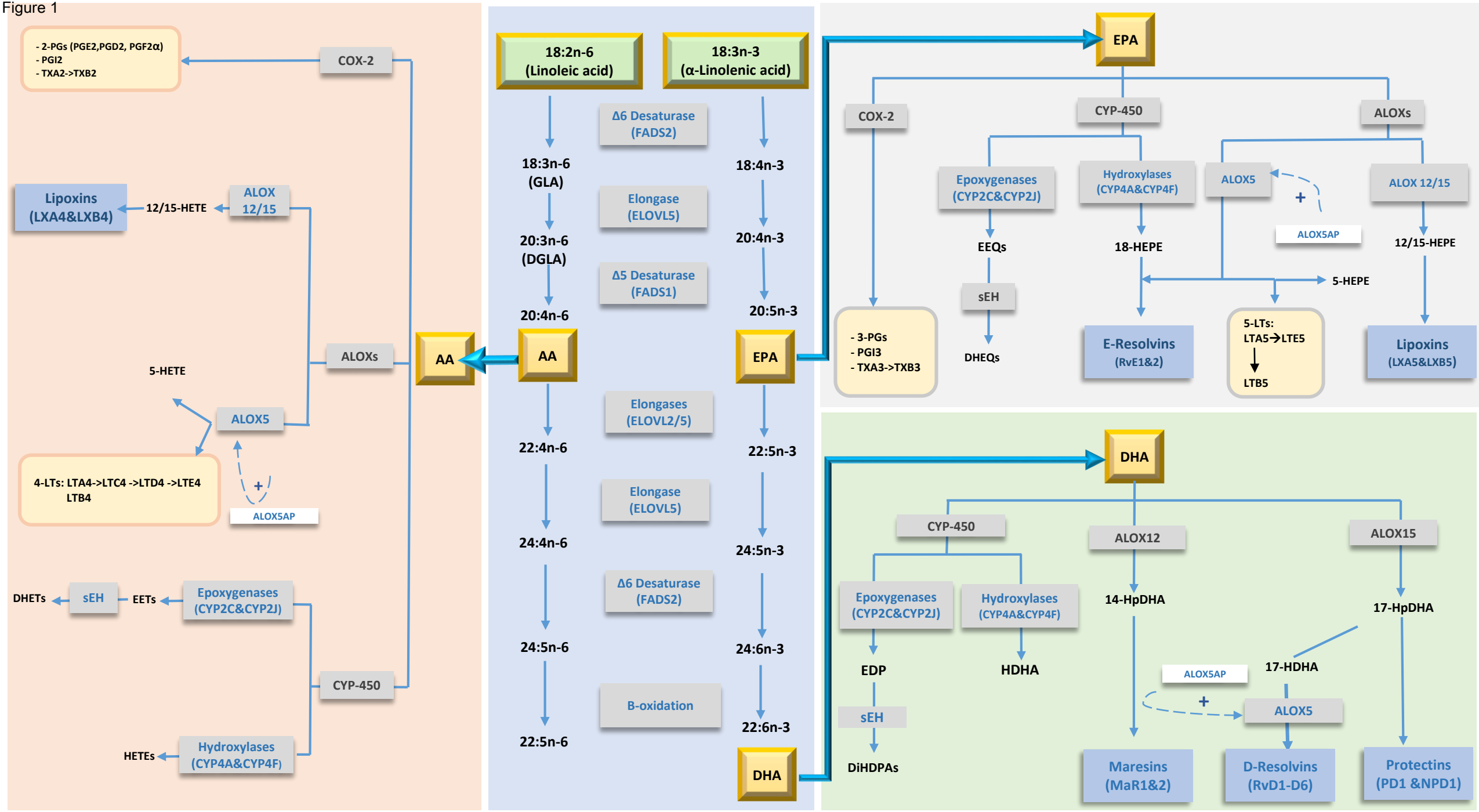
- 2-PGs (PGE2,PGD2, PGF2α)
- PGI2
- TXA2->TXB2
COX-2
Lipoxins (LXA4&LXB4)
12/15-HETE
ALOX 12/15
ALOXs
5-HETE
ALOX5
4-LTs: LTA4->LTC4 ->LTD4 ->LTE4
LTB4
+
ALOX5AP
AA
DHETs
sEH
EETs
Epoxygenases (CYP2C&CYP2J)
CYP-450
HETEs
Hydroxylases (CYP4A&CYP4F)
18:2n-6 (Linoleic acid)
18:3n-3 (α-Linolenic acid)
Δ6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

Figure 2

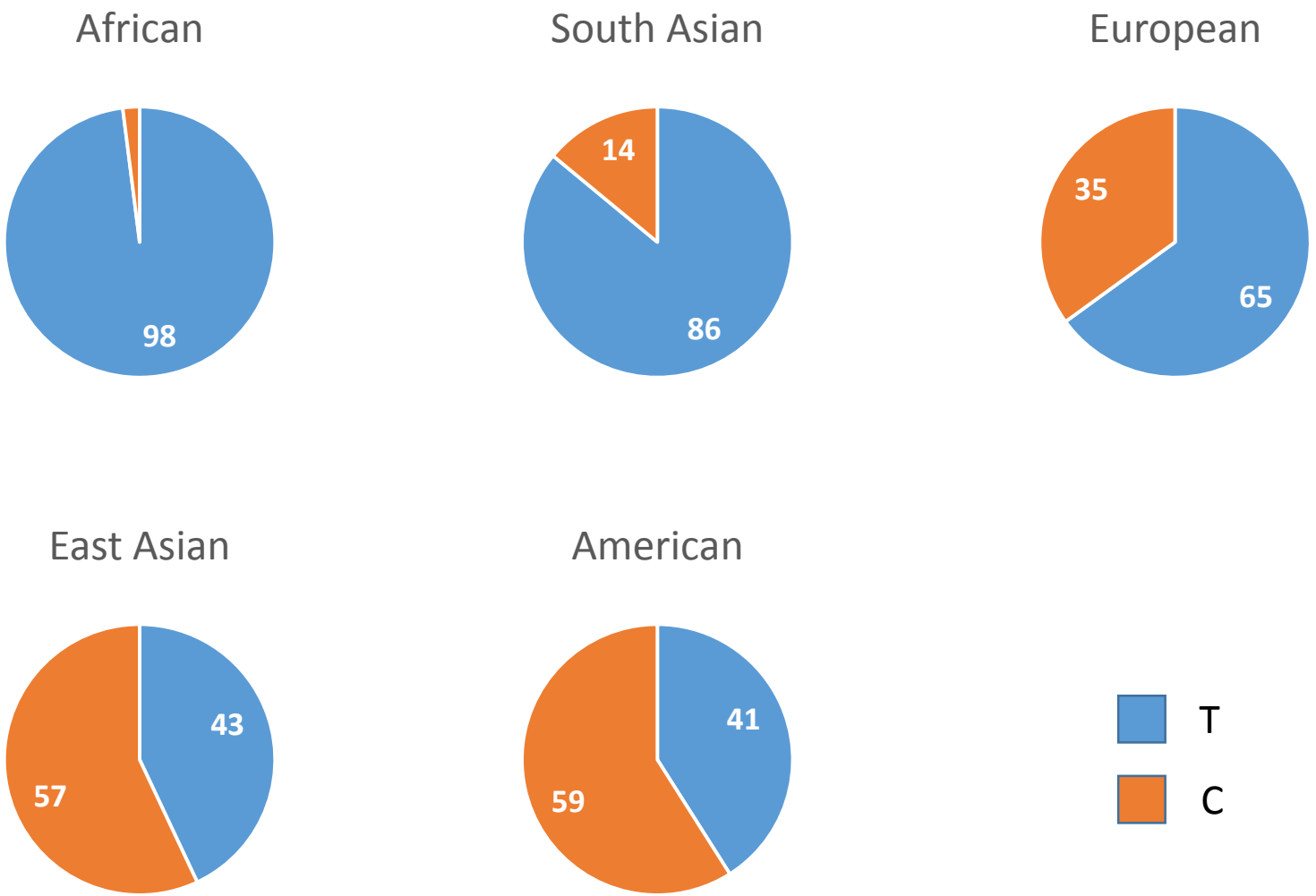
African
98
South Asian
14
86
European
35
65
East Asian
43
57
American
41
59
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Figure 3

Activity of d5- and d6-desaturases

ALA
18:3 n-3

LA
18:2 n-6

GLA
18:3 n-6

DGLA
20:3 n-6

AA
18:2 n-6

EPA
20:5 n-3

DPA
22:5 n-3

DHA
22:6 n-3

ALA
18:3 n-3

LA
18:2 n-6

GLA
18:3 n-6

DGLA
20:3 n-6

AA
18:2 n-6

EPA
20:5 n-3

DPA
22:5 n-3

DHA
22:6 n-3

Cardiometabolic benefit of PUFA intake

ALA-rich plant oils

Fish and fish oil

LA-rich plant oils