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Mechanistic insights into cardiovascular protection for omega-3 fatty acids and their bioactive lipid metabolites

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Patients with well-controlled low-density lipoprotein cholesterol levels, but persistent high triglycerides, remain at increased risk for cardiovascular events as evidenced by multiple genetic and epidemiologic studies, as well as recent clinical outcome trials. While many trials of low-dose ω3-polyunsaturated fatty acids (ω3-PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have shown mixed results to reduce cardiovascular events, recent trials with high-dose ω3-PUFAs have reignited interest in ω3-PUFAs, particularly EPA, in cardiovascular disease (CVD). REDUCE-IT demonstrated that high-dose EPA (4 g/day icosapent-ethyl) reduced a composite of clinical events by 25% in statin-treated patients with established CVD or diabetes and other cardiovascular risk factors. Outcome trials in similar statin-treated patients using DHA-containing high-dose ω3 formulations have not yet shown the benefits of EPA alone. However, there are data to show that high-dose ω3-PUFAs in patients with acute myocardial infarction had reduced left ventricular remodelling, non-infarct myocardial fibrosis, and systemic inflammation. ω3-polyunsaturated fatty acids, along with their metabolites, such as oxylipins and other lipid mediators, have complex effects on the cardiovascular system. Together they target free fatty acid receptors and peroxisome proliferator-activated receptors in various tissues to modulate inflammation and lipid metabolism. Here, we review these multifactorial mechanisms of ω3-PUFAs in view of recent clinical findings. These findings indicate physico-chemical and biological diversity among ω3-PUFAs that influence tissue distributions as well as disparate effects on membrane organization, rates of lipid oxidation, as well as various receptor-mediated signal transduction pathways and effects on gene expression.

Introduction

The recent success of clinical studies with high-dose omega-3 polyunsaturated fatty acids (ω3-PUFAs),

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eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and specifically EPA, has reignited interest in the potential mechanism of action of ω3-PUFA therapy in cardiovascular disease (CVD). Reporting in 2018, the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) was the largest randomized trial to date to employ high-dose EPA (4 g/day). Results from REDUCE-IT indicated that EPA significantly reduced risk of ischaemic events, including death from cardiovascular causes, in patients with high triglycerides (TG) despite statin use.[1] In addition, OMEGA-REMODEL, a smaller randomized trial that examined high-dose ω3-PUFAs (EPA + DHA, 4 g/day) on remodelling for 6 months post-myocardial infarction (MI), reported significant reductions in ventricular remodelling and inflammation.[2] In stark contrast, the Vitamin D and Omega-3 Trial (VITAL), also reporting in 2018, found no improvement in primary cardiovascular outcomes with low-dose ω3-therapy (1 g/day).[3] However, despite the positive outcomes from REDUCE-IT and OMEGA-REMODEL, there remains controversy regarding the efficacy of ω3-PUFAs in CVD. This controversy stems from a history of clinical trials with low-dose ω3-PUFAs that have failed to show efficacy.[3-9] More recently, the STRENGTH trial using high-dose mixed ω3-PUFAs was stopped for futility.[10] Additionally, there is ongoing debate regarding the mechanistic basis for the cardioprotective effects of ω3-PUFAs, which has also hampered their wider use in CVD. While there has long been interest in how the effects of ω3-PUFAs differ from ω6-PUFAs, recent results have highlighted the potential that EPA has unique benefits. Therefore, the goals of this review are to outline the mechanistic basis for EPA cardioprotective effects in CVD, and finally to suggest new directions in understanding EPA in CVD. ω3-polyunsaturated fatty acids have multiple biological activities including: (i) distinct physiochemical properties; (ii) as a precursor to oxylipins and other lipid mediators; and as direct receptor agonists at (iii) free fatty acid receptors (Ffars) or (iv) peroxisome proliferator-activated receptors (PPARs).

Distinct effects of eicosapentaenoic acid on membrane structure, lipid oxidation, biomarkers, plaque composition, and endothelial function

ω3-polyunsaturated fatty acids modify lipid composition of caveolae and cellular localization of nitric oxide synthase

Rather than diffusing in an unrestricted fashion, membrane constituents are organized into a complex 'mosaic' of specific lipids that create distinct environments or microdomains for various proteins involved in signal transduction and receptor binding.[11,12] Such microdomains or 'lipid rafts' are typically detergent-resistant and enriched with unesterified cholesterol and sphingolipids. These sphingolipids contain saturated fatty acids (SFAs) that provide a highly ordered structure and sequester specific proteins.[13] Proteins that preferentially localize to these domains have a certain tertiary structure that supports their function as an integral membrane protein. Lipid rafts move or 'float' as a coherent structural unit within the surrounding liquid-disordered lipid bilayer and can also cluster with other rafts to form larger platforms. The basis for differences in fluidity between rafts and the surrounding membrane is the differences in the degree of hydrocarbon chain saturation of constituent sphingolipids that associate strongly with cholesterol.[11] Treatment of cells with ω3-PUFAs like EPA modifies the acyl chain composition of membrane phospholipids and thereby alters the properties and protein function of various lipid domains.[14]

Caveolae are a lipid raft subtype that appear as microscopic, flask-shaped invaginations along the membrane surface and are commonly found in endothelial cells, adipocytes, and smooth muscle cells. The principal protein component of caveolae is caveolin, a scaffolding protein that binds cholesterol efficiently and interacts with various signalling macromolecules, including G proteins and calcium regulating proteins.[15] Caveolin modulates endothelial nitric oxide synthase (eNOS) activity as it binds directly to the enzyme, thereby blocking access of the co-factor, calcium/calmodulin.[16] The expression of caveolin is markedly elevated under conditions of hypercholesterolaemia because of enrichment of plasma membrane cholesterol levels, resulting in reduced eNOS activation in a manner that can be reversed with statin treatment.[17] In cultured human endothelial cells, EPA treatment modifies the subcellular distribution of eNOS and dramatically changes the lipid composition of caveolae.[14] As a result of its incorporation into phospholipid acyl chains, EPA changes the fluid dynamics of the caveolae by increasing acyl chain unsaturation. Eicosapentaenoic acid treatment also displaces caveolin-1 from caveolae and causes the translocation of eNOS from caveolae fractions to soluble fractions where it can be activated. Finally, EPA changes the overall distribution of caveolin-1 and eNOS in plasma membranes.

Distinct membrane interactions of eicosapentaenoic acid and docosahexaenoic acid

ω3-polyunsaturated fatty acids and their bioactive lipid metabolites incorporate into cellular membranes and lipoproteins associated with vascular tissues, along with the atherosclerotic plaque, where they interfere with inflammation, oxidative stress, and endothelial dysfunction.[18-22] Treatment with EPA, at pharmacologic doses, affects lipid oxidation rates, membrane organization, and signal transduction.[18-21] Eicosapentaenoic acid and DHA have disparate effects on membrane lipid dynamics due to differences in their carbon length and number of double bonds. Eicosapentaenoic acid inserts into lipoprotein particles and cellular membranes where it scavenges free radicals through electron stabilization mechanisms associated with its multiple double bonds (*Figure 1*). The antioxidant effects of EPA were not reproduced with DHA over time or other FDA-approved TG-lowering agents such as gemfibrozil, as well as fenofibric and nicotinic acids.[18,23,24] The antioxidant effects of EPA are also observed in membranes under conditions of hyperglycaemia.[23] Unlike EPA, other TG-lowering agents like the fibrates are relatively hydrophilic and do not possess chemical moieties capable of stabilizing unpaired free electrons in the membrane hydrocarbon core, while EPA has an extended and stable

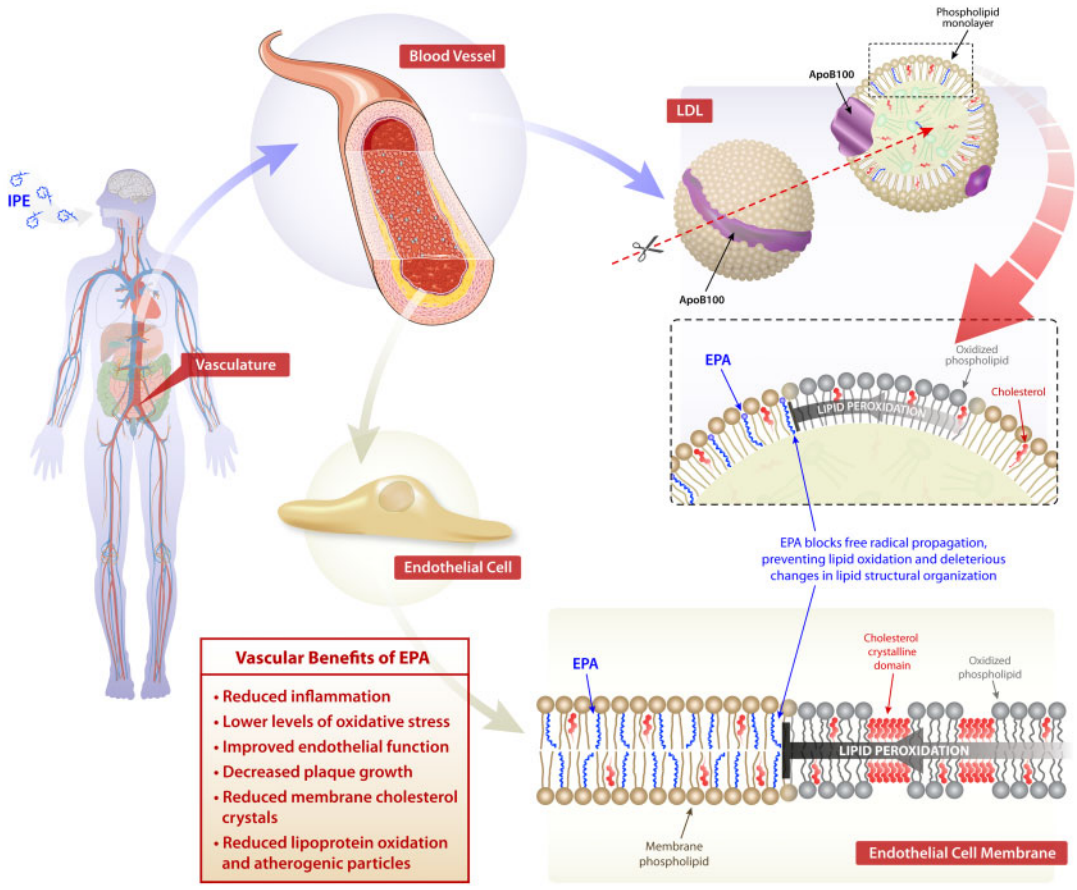

Figure 1 Molecular lipid interactions of eicosapentaenoic acid. Schematic illustration of the proposed location of phospholipid-linked eicosapentaenoic acid in cellular membranes and lipoproteins. Eicosapentaenoic acid is rapidly esterified and incorporated into lipoproteins and membrane phospholipids. Biophysical studies indicate eicosapentaenoic acid has an extended orientation that preserves membrane fluidity and inhibition of lipid oxidation and membrane cholesterol domain formation.

orientation in the membrane as determined by small-angle X-ray diffraction approaches.[25] In contrast, DHA interacts with the phospholipid head group region with concomitant changes in hydrocarbon core electron density consistent with increased membrane disorder. These differences in membrane interactions with DHA are consistent with the rapid isomerization or conformational changes over a nanoscale time frame.[18,19,21,24,26] In a more recent study, the antioxidant effects of EPA in small dense low density lipoprotein (sdLDL) and membranes were compared to other long-chain fatty acids. The results showed that hydrocarbon length and number of double bonds for fatty acids are important predictors of antioxidant activity in lipoproteins and membranes, along with membrane cholesterol domain formation.[27] Eicosapentaenoic acid appears to have the optimal combination of chain length and unsaturation for these antioxidant properties, followed by other ω3-PUFAs. Fatty acids with two or fewer double bonds and the ω6-PUFA arachidonic acid (AA) failed to exhibit antioxidant activity as compared to ω3-PUFAs.

Effects of ω3-polyunsaturated fatty acids on membrane cholesterol crystal formation

A characteristic feature of an atherosclerotic plaque is the formation of cholesterol crystals that originate from cholesterol-enriched membranes of various cells, including macrophages and smooth muscle cells.[28,29] Over time, these toxic cholesterol crystals destabilize the plaque as they continue to form or precipitate from membrane domains.[30] Using various imaging approaches,[31] cholesterol crystals have been observed in vascular tissue where they associated with the plaque's fibrous cap.[32] In patients that have experienced myocardial infarction, there is abundant evidence of cholesterol crystals in the diseased plaque in a proportional manner.[33] In addition to excess cholesterol accumulation, membrane cholesterol domains are also stimulated by oxidative stress and high glucose.[34] Along with oxidized LDL, cholesterol crystals activate nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasomes, which regulates the processing of pro-interleukin 1 beta (IL-1β) into an active cytokine.[35]

Due to its potent antioxidant activity and lipophilicity, EPA significantly inhibits glucose-induced cholesterol crystalline domain formation in membrane lipid vesicles at pharmacologically relevant concentrations.[23,36] The inhibition of cholesterol domain formation by EPA is not reproduced with other TG-lowering agents or vitamin E due to differences in membrane distribution and antioxidant activities.[23] These findings suggest that EPA preferentially

intercalates into the hydrocarbon core of the membrane bilayer where it can trap free radicals with its multiple double bonds, leading to reduced cholesterol domain formation. The effects of EPA on cholesterol domain formation was not observed with DHA as it has different interactions with the highly planar steroid ring structure of cholesterol that is oriented parallel to surrounding phospholipid acyl chains.[18,37,38]

Effects of ω3-polyunsaturated fatty acids on cardiovascular biomarkers, plaque biology, and high density lipoprotein (HDL) function

Eicosapentaenoic acid and other ω3-PUFAs have been shown to favourably influence circulating levels of biomarkers associated with cardiovascular risk in patients with dyslipidaemia. Prescription EPA (2–4 g/day) in patients with high or very high triglyceride (TG) levels reduces high-sensitivity C-reactive protein (hsCRP), lipoprotein-associated phospholipase A_2 (Lp-PLA_2), and oxidized LDL-cholesterol (oxLDL) levels, as well as the arachidonic acid (AA)-to-EPA ratio, as compared to placebo.[39–44] The ability of EPA to reduce hsCRP is actually enhanced in combination with a statin in proportion to its intensity.[40] Similar effects on hsCRP were not observed with DHA in a similar patient population even with 4 g/day but, like EPA, treatment was associated with reduced Apolipoprotein CIII.[45] Eicosapentaenoic acid treatment also down-regulates cAMP responsive element protein 1 (CREB1) and hypoxia inducible factor 1 (HIF1) gene expression.[46] In cellular studies, EPA reduces production of inflammatory mediators in macrophages, including TNF-α and IL-1β, compared to DHA.[47]

Eicosapentaenoic acid also has direct vascular effects that impact the progression of atherosclerosis as compared to other ω3-PUFAs. In mice that lack apolipoprotein E ($Apoe^{-/-}$) fed a Western diet supplemented with EPA (1%, w/w) or DHA (1%, w/w) for 3 weeks, EPA treatment reduces plaque volume as compared to DHA.[48] Eicosapentaenoic acid and its metabolites, especially 12-hydroxyeicospentaenoic acid (12-HEPE), preferentially associate with thin-cap plaques and accumulation of anti-inflammatory M2 macrophages, while DHA associates with plaques of various sizes. In the aortic root, total EPA and 12-HEPE levels follow a concentration gradient from the vascular endothelium to the media. In patients with coronary artery disease (CAD), treatment with EPA improves high-density lipoprotein mediated cholesterol efflux along with antioxidant and anti-inflammatory effects.[49] In human endothelial cells, EPA-enriched HDL increases resolvin E3 production while reducing cytokine-stimulated vascular cell adhesion molecule 1 (VCAM-1) expression.[50] As observed in Apo-B containing particles, the lipophilic properties and molecular dimensions of EPA allow it to insert more efficiently into the HDL particle, with improved antioxidant function, as compared to DHA.[51]

Effects of ω3-polyunsaturated fatty acids and statins on endothelial function

Atherosclerosis is causally related to endothelial cell (EC) dysfunction characterized by a loss of normal nitric oxide (NO) bioavailability that results in abnormal vasodilation and plaque development.[52–54] In isolated tissue, EPA reverses endothelial dysfunction triggered by either oxLDL or high glucose in a manner that is enhanced in combination with a statin.[55] This improvement in function is evidenced by pronounced increases in the release ratio of NO to peroxynitrite ($ONOO^-$) in the endothelial cells. The combined benefit of EPA with a statin on endothelial function and NO synthase activity was not reproduced with DHA under identical conditions. By reducing LDL oxidation, EPA also attenuated the effects of oxLDL on endothelial function unlike DHA over the same time course. Similar effects of EPA vs. DHA were seen *ex vivo* in arterial segments from a rodent model.[55]

The endothelial benefits of EPA are not caused by differences in expression of endothelial nitric oxide synthase (eNOS) but are likely due to improved eNOS coupling.[55] In the absence of eNOS coupling, excessive O_2^- rather than NO is generated by eNOS, which leads to increased levels of highly reactive $ONOO^-$ molecules that cause cellular and lipoprotein oxidation (oxLDL). EPA treatment of human ECs, along with a statin, reduces the effects of oxLDL on endothelial dysfunction and preserves NO bioavailability. The favourable effects of EPA and the statin may be due to shared antioxidant properties and a common molecular distribution in cellular membranes.[23] There are also benefits of EPA in an *ex vivo* rodent system.[55] The combined treatment of EPA and a statin produces an additional improvement with respect to NO bioavailability following exposure to hyperglycaemia.

Summary

Based on its unique molecular structure, EPA has several significant effects on cellular and lipoprotein membrane structure and dynamics, including:

(1) Eicosapentaenoic acid incorporated in phospholipid acyl chains modifies membrane dynamics of caveolae (lipid rafts), displaces caveolin-1, the structural backbone of caveolae, and displaces signalling proteins localized to caveolae, like eNOS, allowing for increased activation.
(2) Eicosapentaenoic acid inserts into lipoprotein particles and cellular membranes where it scavenges free radicals through electron stabilization due to its multiple double bonds. Eicosapentaenoic acid, as opposed to DHA, appears to have the optimal combination of chain length and unsaturation for these antioxidant properties.
(3) Eicosapentaenoic acid, as opposed to DHA, significantly inhibits glucose-induced cholesterol crystalline domain formation in membrane lipid vesicles, which is not reproduced with other TG-lowering agents.
(4) Eicosapentaenoic acid and other ω3-PUFAs reduce circulating levels of biomarkers associated with cardiovascular risk in patients with dyslipidaemia.
(5) Eicosapentaenoic acid, in combination with statins, reverses endothelial dysfunction as evidenced by increased nitric oxide bioavailability due to shared

antioxidant properties and a common molecular distribution in cellular membranes.

Oxylipins as signalling metabolites derived from eicosapentaenoic acid

Oxylipins are the subclass of lipid mediators derived by enzymatic oxygenation of PUFAs that have specific biological activities.[56] At the molecular level, PUFAs serve as a reservoir of precursors from which a broad array of lipid mediators are synthesized through oxygenation,[57] amidation,[58] nitrosylation,[59] and more. Oxylipins are produced by enzymes in multiple signalling pathways, including cyclooxygenase (COX) and lipoxygenase families (5-LOX, 12-LOX, and 15-LOX), as well as a broad cross section of cytochrome p450s with epoxygenase (CYP_{epox}), hydroxylase (CYP_{hx}), or ω/ω-1 hydroxylase ($CYP_{\omega\text{-}hx}$) activities. In many cases, CYPs exhibit these activities simultaneously. While COX enzymes are selective for arachidonic acid (AA), LOX, and CYP have activity on multiple PUFAs including AA and EPA and the pleiotropic activity forms complex webs of overlapping signalling pathways. *Table 1* summarizes the most common AA and EPA oxylipins in LOX and CYP signalling. The summary exemplifies the complexity of signalling matrices generated because: (i) the same signalling molecule can be produced from distinct enzymes—e.g. 12-HEPE is produced by 12-LOX and by CYP_{hx}; and (ii) the same enzymes can produce oxylipins from multiple PUFAs—e.g. 12-LOX produces 12-hydroxyeicotetraenoic (12-HETE) from AA and 12-HEPE from EPA.

Our understanding of the initiation of oxylipin synthesis and generation of signalling activity is guided by the common model depicted in *Figure 2*. Precursor PUFAs stored in membrane phospholipids are made available to enzymes by the activity of phospholipase A_2 (PLA_2), which releases PUFAs from the sn-2 position into the cytosol where COX, LOX, and CYP divert them into each pathway.[60–63] Over 70 metabolites of AA are known to exist, and since EPA has an additional double bond (five compared to four in AA), even more metabolites of EPA are likely to exist, and almost 50 have been described to date.[64]

Eicosapentaenoic acid administration changes the amount and types of oxylipins in plasma

Pharmaceutical EPA as icosapent ethyl at 4 g/day increases membrane EPA content by nearly 8-fold but reduces membrane AA content by 27%.[94] 1.86 g/day EPA as omega-3 acid ethyl esters (in conjunction with 1.5 g/day DHA) not only induces a 540% increase in %EPA but also induces an 11% decrease in %AA.[95] This shift in membrane substrate availability is reflected in treatment-dependent changes in tissue oxylipins, with a general pattern of increasing EPA membrane content, at the expense of AA. Like fatty acids, oxylipins are distributed through plasma and the majority circulate esterified in the glycerolipids of lipoproteins (VLDL, LDL, and HDL). A small portion (<5%) circulate unesterified and bound to albumin in a manner similar to non-esterified fatty acids (often termed 'free' oxylipins, which are most often reported). As a general rule, treatments that increase tissue EPA result in concurrent increases in total (esterified + unesterified) plasma alcohols and epoxides derived from EPA: 4 weeks of 3.4 g/day omega-3 acid ethyl esters induced an 8.4-fold increase in plasma %EPA and concurrently increased plasma esterified HEPEs by 5.7-fold, epoxytetraenoic acids (EpETEs) by 4.7-fold, and dihydroxyeicostetraenoic acids (DiHETEs) by 4.1-fold.[96] Treatment reduced %AA to 0.88-fold of baseline and similarly reduced oxylipins derived from AA: HETEs to 0.8-fold of baseline and dihydroxytrienoic acids (DiHETrEs) to 0.81-fold of baseline; epoxyeicosatrienoic acids (EpETrEs) were not significantly reduced, but the data were most consistent with a reduction to 0.9-fold of baseline. For both EPA- and AA-oxylipins, changes in plasma concentration were reflective of the abundance their PUFA precursors. Eicosapentaenoic acid treatment is not only effective at increasing steady-state abundance of EPA oxylipins, but it also decreases the abundance of AA oxylipins in proportion to decreases in AA availability. However, a more detailed examination in the randomized clinical trial (RCT) setting reveals pool-specific changes. In subjects with metabolic syndrome responding to oral EPA (combined with DHA) at 3.4 g/day ω3-acid-ethyl esters, the reduction in AA-oxylipins was restricted to two HETEs, oxoeicosatetraenoic acids (KETEs), and leukotriene B4 (LTB4) derivatives in LDL and HDL respectively, but the same VLDL oxylipins were unchanged. In addition, AA-oxylipins including EpETrEs and DiHETrEs were unchanged for all lipoproteins,[95] indicating that after treatment CYP_{epox} signalling is sustained but LOX and CYP_{hx} signalling are limited by AA.

Eicosapentaenoic acid oxylipins share ligand activity with oxylipins from other polyunsaturated fatty acids

In addition to enzymes that can produce oxylipins from multiple PUFAs, oxylipins also have overlapping activities as receptor ligands, and the classic model of a unique ligand-receptor pair does not hold. This expectation follows from Burr's original findings that linoleic acid[97] and alpha-linolenic acid[98] are both effective in rescuing rats with essential fatty acid deficiency,[99] which was explained in 1930 as complementary PUFA activities. Unfortunately, few studies systematically compare the bioactivity of ω6 and ω3 homologs. AA and EPA are both effective substrates for CYP_{epox} activity, producing EpETrEs and EpETEs from AA and EPA, respectively. Both series potently induce dilation of rat coronary microvessels by activation of BK_{Ca} channels.[88] EpETEs appear to be moderately more potent than their EpETrE homologs, but all homologs bind and activate the channel. Hence, the most likely benefit of EPA is to add the benefit of EpETEs to that of EpETrEs. Additive instances are common. Among 12-LOX pathway metabolites, 12-hydroperoxides (12-HpETE for AA, 12-HpEPE for EPA) equivalently suppress collagen-mediated platelet aggregation and serotonin release.[65]

Instances in which the effect of EPA-oxylipins are different from the AA-homolog are also reported, with examples both where the EPA-oxylipin is more potent than the AA-homolog, and also where it is less potent. Among 5-LOX pathway metabolites, the EPA-derived 5-KEPE is 10-fold less potent than the AA-derived 5-KETE in mobilizing

Table 1 Examples of arachidonic acid vs. eicosapentaenoic acid oxylipin activities[a]

Family LOX	Specific enzyme	PUFA AA	Activities	EPA	Activities	Direct comparisons
	5-LOX	5-HpETE	**Inflammatory:** Inhibits platelet aggregation[65]	5-HpEPE	**Inflammatory:** Inhibits platelet aggregation[65]	5-HpETE < 12-HpETE 5-HpEPE < 12-HpEPE
		5-HETE	**Inflammatory:** Chemotaxis, immune cell activation[66-68] **Cardiovascular:** Inhibits PGI_2 synthesis	5-HEPE	**Inflammatory:** Chemotaxis[67] **Metabolic:** Glucose-dependent insulin secretion[69]	**Inflammatory:** AA > EPA
		5-KETE	**Inflammatory:** Eosinophil and neutrophil migration[70]	5-KEPE	**Inflammatory:** Eosinophil and neutrophil migration[70]	**Inflammatory:** AA ≫ EPA
		LtB_4	**Inflammatory:** Neutrophil aggregation[71] Polymorphonuclear cell activation[72]	LtB_5	**Inflammatory:** Neutrophil aggregation[71]	**Inflammatory:** EPA < AA
		LtC_4	**Inflammatory:** Block coronary anaphylaxis-induced vascoconstriction[73]	LtC_5	**Inflammatory:** Block coronary anaphylaxis-induced vascoconstriction[73]	**Inflammatory:** EPA < AA
		LxB_4	**Inflammatory:** Immune cell migration and activation[74] **Cardiovascular:** Aortic vasodilation[74]	LxB_5	**Cardiovascular:** No activity[74]	**Inflammatory:** AA > EPA
	12-LOX	12-HpETE	**Inflammatory:** Inhibits platelet aggregation[65]	12-HpEPE	**Inflammatory:** Inhibits platelet aggregation[65]	**Inflammatory:** 12-HpETE > 5/15-HpETE 12-HpEPE > 5/15-HpEPE
		12-HETE	**Inflammatory:** Inhibits platelet aggregation[65] Increased monocyte adhesion to fatty streaks[75,76] **Cardiovascular:** Increased mitochondrial Ca^{2+}/nitric oxide[77]	12-HEPE	**Inflammatory:** Inhibits platelet aggregation[65]	**Inflammatory:** AA = EPA HETEs < HpETEs HEPEs < HpEPEs
	15-LOX	15-HpETE	**Inflammatory:** Inhibits platelet aggregation[65] **Cardiovascular:** Loss of cardiomyocyte membrane integrity[78] Species/context dependent vasodilation/vaso-constriction[79]	15-HpEPE	**Inflammatory:** Inhibits platelet aggregation[65]	**Inflammatory:** AA = EPA Peroxides > alcohols 12-LOX > 15-LOX

(continued)

Table 1 Continued

Family LOX	Specific enzyme	PUFA AA	Activities	EPA	Activities	Direct comparisons
		15-HETE	**Inflammatory:** Inhibit platelet aggregation[65] **Cardiovascular:** Species/context dependent vaso-dilation and vasoconstriction[79]	15-HEPE	**Inflammatory:** Inhibition of 5-LOX[80]	**Inflammatory:** AA = EPA 15-HpETE < 12-HpETE 15-HpEPE < 12-HpEPE
CYP		AA	Activities	EPA	Activities	Direct comparisons
	CYP_{hx} $CYP_{\omega/\omega\text{-}1\ hx}$	HETEs[b] *18-HETE*[c]		HEPEs[a] 18-HEPE	**Inflammatory:** Decreased TNFα secretion by macrophages[81] **Cardiovascular:** Protects heart from pressure Overload fibrosis; blocks cardiac Fibroblast inflammation[82]	
	CYP_{epox}	5(6)-EpETrE	**Cardiovascular:** Vasodilatory[83-85]	5(6)-EpETE		
		8(9)-EpETrE	**Cardiovascular:** Vasodilatory[83,84] Protects against reperfusion injury[86]	8(9)-EpETE	**Inflammatory:** Inhibit platelet aggregation[87] **Cardiovascular:** Vasodilatory[88]	EpETE > EpETrE[87] EpETrE = EpETE[88]
		11(12)-EpETrE	**Inflammatory:** Inhibition of NF-κB[89] **Cardiovascular:** Vasodilatory[85,88] Protection against reperfusion injury[86]	11(12)-EpETE	**Inflammatory:** Inhibit platelet aggregation[87] **Cardiovascular:** Vasodilatory[88]	EpETE > EpETrE[87] EpETrE = EpETE[88]
		14(15)-EpETrE	**Cardiovascular:** Vasodilatory, >14,15-DiHETrE[90] Vasodilatory <14,15-DiHETrE[83,84] Protects against reperfusion injury[86]	14(15)-EpETE	**Inflammatory:** Inhibit platelet aggregation[87] **Cardiovascular:** Vasodilatory[88]	EpETE > EpETrE[87] EpETrE = EpETE[88]
				17(18)-EpETE	**Inflammatory:** Inhibit platelet aggregation EpETE[87] Anti-inflammation[91] **Cardiovascular:** Vasodilatory[92]	

(continued)

Table 1 Continued

Family LOX	Specific enzyme	PUFA AA	Activities	EPA	Activities	Direct comparisons
		5,6-DiHETrE	**Cardiovascular:** Vasodilatory; >5(6)-EpETrE[83] Coronary artery hyperpolarizing (BKc)[93]	5,6-DiHETE		
		8,9-DiHETrE	**Cardiovascular:** Vasodilatory; >8(9)-EpETrE[83,84] Coronary artery hyperpolarizing (BKc)[93]	8,9-DiHETE	**Inflammatory:** Inhibit platelet aggregation ≪ EpETE[87] **Cardiovascular:**	
		11,12-DiHETrE	**Cardiovascular:** Vasodilatory; >8(9)-EpETrE[83,84] Coronary artery hyperpolarizing (BKc)[93]	11,12-DiETE		
		14,15-DiHETrE	**Cardiovascular:** Vasodilatory; >8(9)-EpETrE[3,84] Coronary artery hyperpolarizing (BKc)[93]	14,15-DiETE		
				17,18-DiHETE	NA	

[a]As summarized from Gabbs *et al.*[64] with modifications and emphasis on CVD-outcomes.
[b]CYPs have generalized oxygenation activity, both at double bonds (similar to LOX) and at saturated positions. For simplicity, only a few unique activities are shown.
[c]18-HEPE is produced distinctly from 18-HETE. The former is oxygenated at the ω3-double bond (Δ18) of EPA, the latter is produced at the saturated Δ18 position of AA.

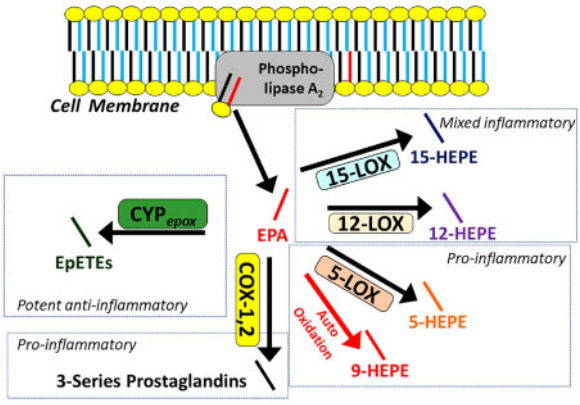

Figure 2 The common model of oxylipin production by oxylipin producing enzymes, including various lipoxygenase, cyclooxygenases, and cytochrome p450 epoxygenases. Polyunsaturated fatty acids are released by phospholipase A_2 from the membrane, making them available to enzymes for downstream signalling by oxylipins. Eicosapentaenoic acid is depicted in red, other polyunsaturated fatty acids are depicted in blue. The relative production of eicosapentaenoic acid-derived oxylipins is a function of the relative provision of eicosapentaenoic acid to enzymes by phospholipase A_2, which in turn, reflects their relative abundance in the phospholipid membrane. Generalized pathway activities are summarized. For details, see *Table 1*. COX, cyclooxygenase; CYPepox, cytochrome P450 expoxygenases; LOX, lipoxygenase.

cytosolic Ca^{2+} in human neutrophils, and it is less potent in stimulating neutrophil or eosinophil migration.[70] Another EPA-oxylipin derived from 5-LOX (5-HEPE) acts on GPR119 to produce an insulinogenic effect but the AA homolog (5-HETE) has only half the effect.[69] Hence, different GPR119-dependent insulinogenic responses among individuals could potentially be explained by availability of cell membrane AA and EPA, independent of genetic polymorphisms. By considering the interactions of PUFAs with each other, a better assessment of how the bioactivities of mixtures are established can be derived.

Finally, ω3-PUFAs can produce distinct oxylipins with no direct homolog. With EPA, this occurs because ω3-PUFAs have one more double bond compared to AA, and hence an additional point for oxygenation not available on AA. For mid-chain alcohols, this metabolite is 18-HEPE, which is a target of much investigative interest, in part because of its role as a precursor to specialized pro-resolving mediators such as Resolvin E1. Interestingly, 18-HEPE protects against pressure overload-induced heart failure.[82] For fatty acid epoxides, the unique metabolite is 17(18)-EpETE, and it is thought to have potent anti-inflammatory activity and to direct macrophage polarization towards the M2 phenotype.[91]

Finally, pathway diversion is a potential mechanism. The phenomenon has been demonstrated in the context of heart failure, where activation of mitochondrial $iPLA_{2\gamma}$ diverts oxylipin production away from epoxides (EpETrEs) towards mid-chain alcohols (HETEs) which promotes pathology by inducing necrosis and apoptosis,[100] and such a shift could amplify cases where EPA is a neutral antagonist to an oxylipin producing enzyme as is the case with COX.[101]

Summary

There are at least four ways increasing EPA availability can alter the oxylipin signalling pathways and affect downstream physiologic function:

(1) Eicosapentaenoic acid-oxylipins can add to the activity of AA-oxylipins.
(2) Eicosapentaenoic acid-oxylipins can have effects distinct from their AA-homologs.
(3) Eicosapentaenoic acid-oxylipins can neutralize the activity of their AA-homologs.
(4) Eicosapentaenoic acid could divert activity from one oxylipin pathway to another.

As the results from STRENGTH are clarified, if evidence for differences between EPA and DHA emerges, the same molecular rationale that accounts for differences between AA and EPA can similarly explain differences between EPA and DHA oxylipins.

Free fatty acid receptors

While ω3-PUFA incorporation into cellular membranes has profound effects on membrane structure and the organization of membrane-associated signalling complexes, mechanistically, it fails to explain activation of intracellular signalling pathways associated with EPA-mediated cardioprotection. In the early 2000s, a subgroup of orphan receptors, G-protein coupled receptor 40 (GPR40), GPR43, GPR41, and GPR120, were defined as receptors for endogenous free fatty acids and were subsequently renamed free fatty acid receptor 1 (Ffar1), Ffar2, Ffar3, and Ffar4, respectively. Ffar2 and Ffar3 are receptors for short chain FA (≤8 carbons)[102] and will not be discussed further, whereas Ffar1 and Ffar4 are receptors for medium and long-chain FA (≥10 carbons), including, but not limited to, ω3-PUFAs.[102] The discovery of GPRs that bind to and are activated by endogenous free fatty acids establishes and entirely novel paradigm whereby fatty acids function as signalling molecules, not simply as an energy source.

Free fatty acid receptor 1 expression and physiology

In 2003, Ffar1 was the first identified receptor for free FAs and is activated by medium and long-chain FAs.[103-105] It is located at chromosome 19q13.1 in humans and is expressed as a single-gene product. Ffar1 is highly expressed in the pancreas, GI-tract, brain, and lung, with lower levels of expression in skeletal muscle, heart, liver, bone, and brain.[102] In the pancreas, Ffar1 is highly expressed in the pancreatic islets, including β-cells, where long-chain PUFA stimulation enhances glucose-stimulated insulin secretion,[103,104] and α-cells, where it modulates glucagon release. In the gastrointestinal (GI) tract, Ffar1 is expressed in enteroendocrine cells and regulates the release of glucagon like peptide-1 (GLP-1) and peptide hormone YY (PYY) from intestinal L-cells,[106] cholecystokinin (CCK) from intestinal I cells,[107] and gastric inhibitory peptide (GIP) from intestinal K-cells.[108] Additionally, Ffar1 and other Ffars play an important role producing anti-inflammatory signals in the gut to regulate metabolism.[109]

Ffar1 is also expressed in several brain regions including the hypothalamus, hippocampus, medulla oblongata, olfactory bulb, cerebellum, striatum, and cerebral cortex, and might have a role in neurodevelopment, inflammation and pain, and neurodegenerative diseases.[110] Finally, Ffar1 is expressed at low levels in mouse heart, 30-fold lower than Ffar4,[111] although relative expression levels of Ffar1 in human heart might be higher (unpublished observation). However, there are no studies of which we are aware that address Ffar1 function in the heart.

Free fatty acid receptor 4 expression and physiology

In 2005, Ffar4 was identified as another receptor for long-chain FAs.[112] Ffar4, which shows little structural similarity to Ffar1, is located at chromosome 10q23.33 in humans, and two splice variants are expressed in humans, a short isoform (Ffar4S, analogous to rodent Ffar4) and long isoform (Ffar4L) that is unique to humans and differs from Ffar4S in a 16 amino acid insertion in the third intracellular loop.[113,114] Ffar4 expression is detected in several tissues with similar expression patterns in mouse and human,[112] with high levels of expression in lung and GI-tract, and lower levels of expression in other tissues including brain, pancreas, small intestine, adipose, taste buds, muscle, heart, and liver.[112,115,116] Like Ffar1, Ffar4 is expressed in intestinal L-cells and regulates the release of glucagon like peptide-1 (GLP-1) in STC-1 mouse enteroendocrine cells *in vitro*[112] and using α-linolenic acid infusion in rats *in vivo*.[115] However, this was not confirmed in Ffar4 knockout mice, suggesting this effect might be mediated by Ffar1.[117] In the pancreas, Ffar4 is expressed in pancreatic islet α cells where activation induces glucagon release,[118] and ∂ cells where activation induces somatostatin release.[119] Ffar4 might also be expressed in pancreatic, β cells, where Ffar4 was shown to induce insulin release BRINBD11 cells, isolated islets, and *in vivo*,[120] although this was not observed in Ffar4 knockout mice.[118] Ffar4 is also expressed in adipose tissue, and increases surface expression of GLUT4 to attenuate insulin resistance.[121] Ffar4 is expressed in macrophages and induces a more anti-inflammatory phenotype, characterized by increased expression of anti-inflammatory genes including arginase-1, IL-10, MCL1, Ym-1, Clcc7a, and MMR, with decreased expression of pro-inflammatory genes including IL-6, TNFα, MCP-1, IL-1β, iNOS, adiponectin, and CD11c.[121] Furthermore, activation of Ffar4 in macrophages reduced inflammation in adipose tissue and improved insulin sensitivity in mice.[121] The combined insulin-sensitizing and anti-inflammatory effects of Ffar4 in pancreatic islet cells, adipocytes, and macrophages suggest Ffar4 as a potential therapeutic target in the management of type-II diabetes. Finally, Ffar4 is expressed in mouse heart at 30-fold higher levels than Ffar1, while Ffar2/3 were significantly lower levels.[111] Further, Ffar4 is expressed in isolated mouse cardiac myocytes and fibroblasts.[111] Because Ffar4 is the main Ffar expressed in heart, the remainder of the section will focus on Ffar4 in CVD.

Free fatty acid receptor 4 signalling

Although commonly referred to as a receptor for ω3-PUFAs, Ffar4 binds to and is activated by a wide array of saturated, monounsaturated, and polyunsaturated, medium and long-chain FAs with potencies in the low μM range, including ω3-PUFAs, α-linolenic acid (αLA), EPA, and DHA.[122] However, SFAs tend to show lower efficacy for activation of downstream signalling pathways, suggesting a potential functional bias towards PUFAs that likely affects biological activity *in vivo*.[122] Additionally, a novel class of branched chain fatty acids, fatty acid esters of hydroxy fatty acids (FAHFAs), were identified as potential ligands for Ffar4 and have anti-diabetic and anti-inflammatory properties.[123]

Ffar4 signals through both Gαq and β-arrestin-2 (not β-arrestin-1), although there are cell-type specific effects and differences between the Ffar4S and Ffar4L isoforms.[112,114,121] The original description of Ffar4 indicated that activation of the receptor increased intracellular Ca^{2+} and activation of ERK in STC-1 enteroendocrine tumour cells suggesting signalling through $G_{q/11}$,[112] which was confirmed in subsequent studies.[114,124] Alternatively, Ffar4 signals through βarrestin2 to activate TAB1 which inhibits TAK1, preventing NFκB and JNK pro-inflammatory signalling in macrophages.[121] Interestingly, Ffar4 signals through both G_q and βarrestin2 to activate cytoplasmic phospholipase A_2 ($cPLA_2$), resulting in activation of cyclooxygenase 2 (COX2) and production of prostaglandin E_2 (PGE_2) in macrophages.[125] However, in adipocytes, Ffar4 signals through G_q to increase cell surface expression of Glut4.[121] Recent studies also suggest Ffar4 might signal through G_i to induce ghrelin release in the stomach[126] and somatostatin release in pancreatic δ-cells.[119] Adding to the complexity of Ffar4 signalling, the human Ffar4S and Ffar4L isoforms differentially activate Gq and β-arrestin, with the Ffar4S activating both, whereas the Ffar4L only signals through β-arrestin.[114] However, Ffar4L expression appears rather limited, and the physiological significance of different isoform signalling is not entirely clear.[127,128] Collectively, these studies indicate the importance of deciphering Ffar4 cell-specific signalling.

Free fatty acid receptor 4 as a mechanistic basis for ω3-polyunsaturated fatty acid-mediated cardioprotection

There are very few studies that directly evaluate Ffar4 as a mechanistic basis for EPA-mediated cardioprotection, but evidence suggests EPA-Ffar4 signalling might explain at least some of the protective effects of EPA in heart failure (HF) and atherosclerosis. In the heart, EPA supplementation prevents interstitial fibrosis and contractile dysfunction in a mouse model of pressure overload heart failure (transverse aortic constriction, TAC).[111,129] In fact, EPA-mediated prevention of fibrosis has now been replicated in many models including: mice,[82,130,131] rats,[132–134] dogs,[135,136] rabbits,[137] and humans.[2] However, dietary supplementation with EPA fails to increase EPA incorporation into cardiac myocytes or fibroblasts, suggesting a mechanism independent of membrane incorporation accounts for EPA-mediated anti-fibrotic and cardioprotective effects.[111,129] Interestingly, Ffar4 is expressed in the mouse

heart, at 30-fold higher levels of expression than Ffar1, and is expressed in both cardiac myocytes and fibroblasts.[111] Ffar4 is also detected in human hearts, although Ffar1 is expressed at proportionally higher levels in human vs. mouse heart (unpublished observation). More importantly, in primary cultures of mouse cardiac fibroblasts, Ffar4 is both sufficient and necessary to prevent TGFβ1-induced fibrosis,[111] but whether Ffar4 mediates EPA-mediated anti-fibrotic and cardioprotective effects *in vivo* remains to be determined. In a model of vascular injury induced by $FeCl_3$, endogenously produced ω3-PUFAs in fat-1 mice prevents neointimal hyperplasia and vascular inflammation in the common carotid artery, which required Ffar4, as protection is lost in fat-1/Ffar4 knockout mice.[138] Interestingly, Ffar4 is anti-inflammatory, reducing macrophage infiltration into adipose tissue in mice fed a high-fat diet and inducing macrophage phenotype switching from pro-inflammatory M1 to anti-inflammatory M2.[121] Given the important role of inflammation in the pathology of CVD, it will be important to understand the interaction between Ffar4-mediated anti-inflammatory signals and direct Ffar4 actions in the cardiovascular system.

Summary

Ffar1 and Ffar4 signalling is the newest mechanism proposed to explain EPA-cardioprotective signalling. In summary:

(1) Ffar1 and Ffar4 are G-protein-coupled receptors for medium and long-chain FAs (C10–C24), which include but are not limited to ω3-PUFAs.
(2) Ffar1 regulates metabolism and is highly expressed in pancreatic islets, regulating insulin release in β cells and glucagon release from α cells, and the GI tract, regulating gut hormone release including, GLP-1, PPY, CCK, and GIP, as well as inflammatory signalling in the gut.
(3) Ffar4 also regulates metabolism and is expressed in the pancreatic islets, regulating glucagon release from α cells and somatostatin release from ∂ cells, and in the GI tract, regulating GLP-1 release.
(4) Ffar4 regulates inflammation, and in macrophages, ω3-PUFAs induce an M2-like anti-inflammatory phenotype. Ffar4 anti-inflammatory signalling in macrophages reduces inflammation in adipose tissue in obesity.
(5) Ffar4 is expressed in the mouse heart, in both cardiac myocytes and fibroblasts, at much higher levels than Ffar1. Ffar4 attenuates pro-fibrotic signalling in cultured fibroblasts, and might mediate at least some of the cardioprotective functions of EPA, but this remains to be tested.

Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptor structure, expression, and pharmacology

The PPARs are a subfamily of ligand-activated nuclear receptors/transcription factors that belong to the superfamily of nuclear receptors.[139,140] Structurally, PPARs contain an N-terminal transactivating domain, a highly conserved DNA-binding domain that recognizes PPAR-specific DNA binding domains, or PPAR-response elements (PPREs), and a C-terminal ligand-binding domain.[141,142] Ligand binding to PPARs facilitates heterodimerization with the retinoid-X-receptor (RXR), binding to PPREs, a six nucleotide dimeric repeat separated by a single nucleotide (AGGTCA-N-AGGTCA), and activation of transcription.[141,142] Of note, the binding pocket of PPARs is relatively large compared to other nuclear receptors, facilitating binding of endogenous FAs and greater overall flexibility in ligand binding.

There are three PPAR isoforms PPARα, PPARβ/∂, and PPARγ that differ in tissue distribution, ligand binding profiles, and regulation of distinct target genes/physiologic functions.[143,144] Peroxisome proliferator-activated receptor α, the first cloned PPAR, is expressed in metabolically active tissues including, liver, heart, skeletal muscle, and kidney, and regulates genes associated with fatty acid uptake, intracellular transport, and β-oxidation.[144–146] Peroxisome proliferator-activated receptor γ is highly expressed in adipocytes and is a master regulator of adipogenesis, energy balance, and lipid biosynthesis; however, PPARγ is also expressed in several other tissues including skeletal and cardiac muscle where it regulates fatty acid oxidation.[144–146] PPARβ/δ is widely expressed, with higher expression in the large and small intestine, heart, brain, and adipose and regulates fatty acid oxidation, improves lipid profiles, and attenuates adiposity.[144–146]

While several synthetic PPAR agonists are in clinical use, including thiazolidinediones (TZDs, PPARγ agonists) and fibrates (PPARα agonists),[143,147] all three PPARs also bind to and are activated by endogenous FAs, typically in the low μM range, although with differing specificity for each PPAR isoform. While all PPARs bind PUFAs with similar affinity, PPARα also shows high affinity for MUFAs and SFAs, PPARβ/δ shows lower affinity for MUFAs and SFAs, and PPARγ is the most selective for PUFAs showing little or no affinity for MUFAs and SFAs.[148] Of note, EPA is one of the most potent PUFAs at all three PPARs.[148] Additionally, PPARs can also bind to certain oxylipins derived from AA,[142,147] and it seems likely oxylipins derived from other FAs would likely also bind to PPARs, although this has not been rigorously examined.

Peroxisome proliferator-activated receptors in atherosclerosis

Peroxisome proliferator-activated receptors have received significant attention as potential therapeutic targets for the management of atherosclerosis.[143] As discussed above, clinical trials indicate that EPA, one of the more potent endogenous ligands for PPARs, lowers plasma TG without raising LDL cholesterol and can reduce atherosclerotic plaques.[149] Furthermore, REDUCE-IT demonstrated that EPA (icosapent ethyl) reduced residual atherosclerotic cardiovascular disease (ASCVD) risk beyond statins in patients with ASCVD but also in diabetic patients with multiple risk factors.[150] However, most of the current mechanistic understanding of PPARs in vascular disease is based on studies using high-affinity synthetic PPAR agonists, including fibrates (PPARα) and TZDs (PPARγ).[147]

Functionally, all three PPARs isoforms are expressed in vascular endothelial cells, smooth muscle cells, and multiple inflammatory cells including macrophages and foam cells in atherosclerotic plaques.[143,144] In vascular endothelial cells, PPARα activation inhibits NFκB activation and suppresses inflammation, reducing expression of VCAM-1 and leucocyte adhesion,[151-153] whereas PPARγ and PPARδ may increase nitric oxide (NO) and reduce oxidative stress in diabetes.[154-157] In vascular smooth muscle cells, PPARα attenuates inflammation,[158] whereas PPARγ down-regulates matrix metalloproteinase (MMP) expression, such as MMP9 that might be involved in plaque rupture.[152] In macrophages and foam cells within atherosclerotic lesions, PPARγ generally attenuates inflammation by regulating inflammatory gene expression.[152,159,160]

However, mouse models with global deletion of PPARα (PPARαKO) display slower development of atherosclerosis,[161] conversely PPARα deletion in macrophages increases atherosclerosis,[162] suggesting cell-type specific effects of PPARα signalling are crucial to atherogenesis. Furthermore, endothelial cell-specific deletion of PPARγ induces dyslipidaemia, suggesting that endothelial PPARγ is also a potentially important regulator of atherogenesis.[163] In total, PPARs in the vascular system are important in atherogenesis, and while synthetic PPAR agonists show some promise in therapeutic treatment of atherosclerosis, far fewer studies have examined the benefits of endogenous FAs, such as EPA. However, EPA activation of PPARγ attenuates pro-inflammatory signalling and inhibits endothelial lipase expression in macrophages.[164] Interestingly, DHA signalling through PPARα increases phagocytosis and induces an M2-like phenotype in macrophages,[165] while maresins, a class of oxylipins derived from DHA, signalling through PPARα inhibit inflammation in endothelial cells.[166] In summary, mechanistic studies designed to explain a potential for a clinical benefit of EPA acting through PPARs in atherosclerosis are somewhat lacking.

Peroxisome proliferator-activated receptors in the liver

In the liver, PPARα activation modulates the expression of multiple target genes involved in TG metabolism. Clinically, PPARα agonists (gemfibrozil, clofibrate, fenofibrate, and fenofibric acid) decrease plasma TG levels and increase HDL levels. Despite their ability to effectively lower TGs, clinical trials have failed to show that their use was associated with reduced risk for cardiovascular events when added to statins as compared to statin therapy alone.[167-170] These findings suggest that TG-lowering alone through this mechanism does not lead to reduced cardiovascular risk. As an endogenous ligand for PPARs, EPA may have additional effects through this pathway that limit atherosclerosis and vascular inflammation. Differences between natural and synthetic PPARα ligands are currently being explored.

Peroxisome proliferator-activated receptors in heart failure

Peroxisome proliferator-activated receptor regulation of metabolism in the heart is another potential therapeutic target for the management of HF. Under basal conditions, mitochondrial FA oxidation is the major source of energy production in the myocardium, but in HF, mitochondrial FA oxidation often declines and glycolytic pathways become more predominant, ultimately contributing to contractile dysfunction.[171] Mechanistically, PPARα-RXRα complex formation is decreased in hypertrophic cardiac myocytes,[172] while PPARα in turn might complex with silent information regulator 1 (Sirt1) in HF, contributing to the down-regulation of genes regulating FA oxidation.[173] In PPARαKO mice, chronic pressure overload reduces FA oxidation and worsens pathologic remodelling.[174,175] Conversely, cardiac myocyte-specific overexpression of PPARα induces hypertrophy, cardiac lipotoxicity, and insulin resistance similar to diabetic cardiomyopathy.[176] Cardiac myocyte-specific knockout of PPARγ induces cardiac hypertrophy,[177,178] while cardiac myocyte-specific overexpression of PPARγ and rosiglitazone (PPARγ agonist) also induce hypertrophy.[178,179] Cardiac myocyte-specific deletion of PPARδ disrupts FA oxidation and induces cardiomyopathy.[180] In summary, the data from PPAR knockout and transgenic mice is sometimes conflicting, but suggests that fine regulation of PPAR signalling in cardiac myocytes is essential to maintain cardiac homeostasis.

Eicosapentaenoic acid and mixed ω3-PUFAs demonstrated improved HF outcomes in GISSI-HF and OMEGA-REMODEL.[2,181] However, much less is known about EPA-PPAR signalling in cardiac myocytes. In cultured neonatal cardiac myocytes, EPA mediated activation of PPARα attenuates endothelin-1 induced hypertrophy.[182] In rats fed a standard or high-fat diet, the PPARα agonist WY14643 increased monounsaturated FA and ω3-PUFA replacement with ω6-PUFA.[183] In models of cardiac allografts, EPA reduced allograft rejection, which was abrogated by a PPPAγ antagonist.[184,185] In humans, ω3-PUFA supplementation (3.4 g/day) 2–3 weeks before elective cardiac surgery increased nuclear transactivation of PPARγ, increased FA metabolic, anti-inflammatory, and antioxidant gene expression, and increased mitochondrial respiration rate in atrial biopsies.[186] These studies do suggest that EPA signalling through PPARs have significant biological effects in the heart, but whether EPA signalling through PPARs prevents HF is still an open question.

Summary

Peroxisome proliferator-activated receptors are a second and distinct receptor-mediated mechanism proposed to explain EPA-cardioprotective signalling. In summary:

(1) Peroxisome proliferator-activated receptors are nuclear receptors/transcription factors, and upon ligand binding, PPARs heterodimerize with RXR, bind to PPAR-response elements, and activate transcription.
(2) There are three PPAR isoforms, PPARα, PPARβ/δ, and PPARγ, and synthetic PPAR agonists are in clinical use, including thiazolidinediones (TZDs, PPARγ agonists) and fibrates (PPARα agonists), to regulate diabetes.
(3) Clinical trials suggest that EPA attenuates atherosclerosis. Further, EPA is one of the most potent FA

activators of PPARs, suggesting a potential mechanistic explanation for EPA-mediated protection in atherosclerosis, but this remains to be tested.

(4) PPARs are expressed in the heart and regulate FA oxidation. While EPA and mixed ω3-PUFAs demonstrated improved HF outcomes, whether this is mediated by PPARs remains to be tested.

Final summary and future directions

The recent success of high-dose ω3-PUFAs, particularly EPA administered as icosapent ethyl, in a clinical outcome trial has brought its role in CVD back into the spotlight. However, questions still remain about ω3-PUFAs, as the benefit of mixed and low-dose ω3-PUFAs has been equivocal at best. Undoubtably, understanding the underlying mechanisms of ω3-PUFAs in the cardiovascular system will lead to improved formulations that produce greater efficacy in those patient populations most likely to benefit from such an intervention. Potential mechanisms to explain the cardioprotective benefit of EPA, in particular, include its unique physiochemical interactions in membranes and lipoproteins, the production of EPA-derived cardioprotective oxylipins, activation of Ffar4 signalling, and induction of favourable PPAR-mediated gene expression. A major challenge going forward is how to systematically elucidate the key biological mechanism(s) underpinning the emerging cardioprotective effects of EPA. In light of multiple potential mechanisms to explain such cardioprotection, traditional reductionist experimental approaches might prove inadequate to address what is essentially a systems biological challenge where multiple mechanisms are involved that vary among different cell and tissue types. New experimental paradigms will likely be needed to address this important question.

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