Impact of Circulating Esterified Eicosanoids and Other Oxylipins on Endothelial Function

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Eicosanoids, including epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, and other oxylipins derived from polyunsaturated fatty acids, have emerging roles in endothelial inflammation and subsequent atherosclerosis. Unlike eicosanoids in the prostanoid series, they are known to be esterified in cell lipids such as phospholipids and triglycerides; however, our understanding of these reservoirs is in its infancy. This review focuses on recent work identifying circulating oxylipins, primarily esterified with lipoprotein lipids, and their effects on markers of endothelial dysfunction. These oxylipins are known to be released by at least one lipase (lipoprotein lipase) and to mediate increased expression of inflammatory markers in endothelial cells, which coincides with the known roles of lipoproteins in endothelial dysfunction. The implications of the lipolytic release of lipoprotein-bound oxylipins for the inflammatory response, challenges to analysis of this oxylipin compartment, and the potential importance of non–arachidonate-derived oxylipins are discussed.

Introduction

Eicosanoids, or oxygenated 20-carbon polyunsaturated fatty acids (PUFAs), are thought to play key roles in the progression of atherosclerosis. They can be derived from cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome p450 (CYP), or through the interaction of reactive oxygen species with arachidonic acid (AA), as produced by myeloperoxidase within atherosclerotic plaques [1]. Collectively, over 50 species of oxygenated fatty acids (FAs) derived from AA are known. They have been summarized by Buczynski et al. [2••], and available techniques for their detection and quantification have been reviewed recently [3]. In addition to well-known AA metabolites, other PUFAs can also be oxygenated to produce the octadecanoid products of linoleic acid (LA), α-linolenic acid, and γ-linolenic acid; the eicosanoid products of eicosapentaenoic acid (EPA) and dihomo-γ-linolenic acid; and the docosanoid products of docosapentaenoic acid (n-6 DPA) and docosahexaenoic acid (DHA) (Table 1). These oxygenated PUFAs add to AA-derived eicosanoids and are collectively organized into a superclass termed *oxylipins*, which better describes the in vivo oxygenated lipid matrix. Oxylipins act locally in autocrine or paracrine modes, yet other roles for these highly bioactive molecules remain largely unexplored. This review addresses the evidence for and implications of oxylipins esterified in circulating lipid pools, particularly as they affect endothelial function.

Oxylipin Sources

Oxygenation of PUFAs is dependent on the activation and transfer of molecular oxygen to an unsaturated carbon. These events can occur within the active site of multiple enzymes or proceed through the direct interaction of the lipid with free reactive oxygen species, which can be derived deliberately from enzymatic sources (eg, myeloperoxidase, NADPH oxidase) or inadvertently through inefficiencies in either enzymatic reactions or electron transport. In the context of atherogenic inflammation, the myeloperoxidase-dependent formation of reactive oxygen is likely a dominant source of lipid peroxidation contributing significantly to the circulating esterified lipid pools [1,4]. Although the COX-derived prostanes (eg, prostaglandins and thromboxanes) are not known to be incorporated into glycerol phospholipids, both of the LOX-derived and CYP-derived metabolites can be incorporated into membranes within vascular tissues; however, their function within this pool is debated [5]. It is not known whether lipoproteins have the ability to upload these oxygenated lipids from tissues; therefore, the source of LOX metabolites and CYP metabolites in the circulating esterified lipid pool is not known at this time. The rearrangement and metabolism of lipid hydroperoxides yields a structurally diverse array of bioactive lipids

Table 1. Formal names and abbreviations* of major oxylipins esterified in plasma and their analogs

Oxylipin	Octadecanoid (18 carbon)		Eicosanoid (20 carbon)		Docosanoid (22 carbon)
	Linoleic acid (LA; n-6)	α-Linolenic acid (aLA; n-3)	Arachidonic acid (AA; n-6)	Eicosapentaenoic acid (EPA; n-3)	Docosahexaenoic acid (DHA; n-3)
Alcohols	HODE	HOTE	HETE	HEPE	HDoHE†
	Hydroxyoctadecadienoic acid	Hydroxyoctadecatrienoic acid	Hydroxyeicosatetraenoic acid	Hydroxyeicosapentaenoic acid	Hydroxydocosahexaenoic acid
Ketones	KODE (ie, oxo-ODE)	KOTE (ie, oxo-OTE)†	KETE (ie, oxo-ETE)	KEPE (ie, oxo-ETE)†	KDoHE†
	Keto-octadecadienoic acid	Keto-octadecatrienoic acid	Ketoeicosatetraenoic acid	Ketoeicosapentaenoic acid	Ketodocosahexaenoic acid
Epoxides	EpOME	EpODE	EpETrE (ie, EET)	EpETE (ie, EEQ)†	EpDoPE (ie, EDP)†
	Epoxyoctadecamonoenoic acid	Epoxyoctadecadienoic acid†	Epoxyeicosatrienoic acid	Epoxyeicosatetraenoic acid	Epoxyedocosapentaenoic acid
Diols	DiHOME	DiHODE	DiHETrE (ie, DHET)	DiHETE†	DiHDoPE†
	Dihydroxyoctadeca(mono)-enoic acid	Dihydroxyoctadeca(di)enoic acid	Dihydroxyeicosatrienoic acid	Dihydroxyeicosatetraenoic acid	Dihydroxydocosapentaenoic acid

*Alternate abbreviations, which are more commonly used, are in parentheses.
†Not yet observed in human plasma.

that includes oxygenated products of all PUFAs. Of these species, the mid-chain alcohols [4,6] and isoprostanes [7] appear to be the most abundant circulating esterified species; however epoxides and their hydrolysis products, the vicinal diols, are also routinely observed.

Oxylipins in Atherosclerosis

Epoxides and vicinal diols

The epoxyeicosatrienoic acids (EETs) derived from AA have anti-inflammatory and cardioprotective actions [5,8], and polymorphisms in the genes regulating their formation and metabolism have been associated with atherosclerosis and cardiovascular health risks [9–13]. The epoxides of other long-chain PUFAs are also bioactive [14,15], but their impact on atherogenesis has not been investigated. Acting through nuclear factor-κB, vanilloid receptors, peroxisome proliferator–activated receptors (PPAR), and cyclic adenosine monophosphate–mediated mechanisms, EETs have antiatherogenic effects. Increasing endogenous epoxide levels may have antiatherogenic affects. For example, reducing the rate of epoxy fatty acid hydrolysis via 4 weeks of treatment with a soluble epoxide hydrolase (sEH) inhibitor reduced lesion size by 50% in an apolipoprotein E $(apoE)^{-/-}$ model of atherosclerosis [16••]. Inhibitor treatment increased plasma concentrations of some, but not all, EET isomers as well as linoleate-derived epoxides (epoxyoctadecamonoenoic acids [EpOMEs]), and plasma epoxide concentrations were inversely correlated to atherosclerotic plaque area. Whereas hydrolysis of the EET epoxide is often considered a deactivation step, evidence for diol-dependent effects on PPARα activation [17], cellular hypoxic responses process [18], and cytotoxicity [19] have been reported. Thus, a reduction in diol formation and an increase in epoxide concentration could both contribute to the sEH inhibitor mechanism of action.

Genetic variance in epoxide metabolism also appears to be associated with atherosclerotic risk. In the Atherosclerosis Risk in Communities (ARIC) study [9], a 3.5-fold increase in hazard rate ratio for coronary heart disease was observed for an sEH gene (*EPHX2*) polymorphism that was associated with reduced sEH function among white participants in that study. Importantly, the linoleate epoxide to diol (ie, substrate/product) ratio was found to be a phenotypic marker of the functional atheroprotective state in the white individuals in ARIC. In the Coronary Artery Risk Development in Young Adults (CARDIA) study, a different sEH polymorphism was related to calcified plaque size [10]. Additionally, polymorphic variants in the primary human CYP epoxygenases in the 2C and 2J families have been linked to ethnicity-specific and gender-specific differences in cardiovascular disease risk [11–13,20], with higher epoxygenase activities being protective. These studies support an antiatherogenic role in atherosclerosis that is consistent with the mechanisms inferred from animal and cell culture models.

Mid-chain alcohols and ketones

The direct role of PUFA alcohols in susceptibility to atherogenesis and progression is unclear. In a case-control study (n = 104) of coronary artery disease, only 9-hydroxyeicosatetraenoic acid (9-HETE; an AA-derived alcohol) and F2-isoprostanes were positively associated with the prevalence of disease [21], supporting a role for oxidative stress but not necessarily generalized HETE production. The 9-HETE and F2-isoprostanes appear to be markers of autooxidation because they are not known to be enzymatically generated. Thus, 9-HETE is unlike other HETE isomers, which can be enzymatically generated. These findings provide some evidence that other HETEs are not simply markers of oxidative stress; however, this should be confirmed in a larger study.

The proinflammatory metabolic cascade initiated by the formation of 5-hydroperoxyecosatetraenoic acid (5-HpETE) is commonly associated with 5-LOX–dependent proinflammatory effects (as summarized by Whatling et al. [22•]) and promotes immune cell activation and recruitment. The glutathione peroxidase–dependent 5-HpETE reduction product 5-HETE and its dehydrogenase product 5-oxo-eicosatetraenoic acid (ETE) mediate immune cell activation via the oxoeicosanoid receptor (OXE) [23]. As opposed to the proinflammatory effects of the 5-LOX AA metabolites, there is a spectrum of activity (from pro- to anti-inflammatory) of oxylipin regioisomers derived from other LOX isoforms [24,25•]. In addition to eicosanoid and docosanoid LOX metabolites, the mid-chain octadecanoid alcohols have bioactivities, again both pro- and anti-inflammatory. The linoleate-derived 13-hydroxyoctadecadienoate (13-HODE) is the most abundant alcohol in oxidized low-density lipoprotein (LDL) and has been reported to have numerous physiologic effects, including macrophage accumulation in atherosclerotic plaques [26].

Esterified Oxylipins

The oxylipin classes discussed are known to be esterified in cellular phospholipids and cholesterol esters. For example, early reports demonstrated that stimulated leukocytes rapidly esterified HETEs largely into neutral lipids (eg, triglycerides) and to a lesser extent into phospholipids [27]. In contrast, coronary endothelial cells incorporate more than 80% of EETs into phospholipids and only 15% into neutral lipids [28]. Whether these differences are cell-type or substrate specific is not known, but it is clear that biochemical pathways exist that incorporate oxygenated lipids into esterified pools.

The consequences of esterification have been discussed [8,29,30], but many studies are not designed to detect a role for oxylipins in the absence of direct synthesis. The occurrence of oxylipins in the sn-2 position of phospholipids implies that phospholipase A_2 (PLA_2) could act by releasing active oxylipin into the cytosol even in the absence of the active enzymes (eg, COX, LOX, or CYP), provided oxylipins are also high-affinity substrates

of PLA_2. Supporting this, esterified oxylipins are rapidly released from cellular phospholipids via Ca^{2+}-stimulated mechanisms, and coronary artery relaxation is potentiated by EET loading into cellular phospholipids, mirroring the activation of PLA_2 [31]. Still, the importance of esterified oxylipins is unclear because their actual enrichment, when expressed as whole tissue oxylipin-to-arachidonate ratio, may not be sufficient to elicit a response [8]. Thus, for esterified oxylipins to be important in eliciting intracellular responses, unique subcellular localization or preferential enzymatic release would seem to be required. Regardless, esterification may also be important for clearing oxylipin signals rather than producing them, a mechanism that is usually more important than synthesis rates in determining the duration of a stimulus.

Esterified Oxylipins in Circulation

Esterified oxylipins are common in the circulation, but their role there is not clear. They could represent the removal of oxylipins from cellular pools or a passive oxidant sink. They could also serve as a source of extracellular oxylipins to be delivered to specific tissues through lipolytic release from lipoproteins or by receptor-mediated particle uptake.

Formed elements

Leukocyte, platelet, and erythrocyte membranes all contain esterified oxylipins. Human platelets contain a broad range of AA-derived oxylipins acylated within phospholipids, including 12-HETE, 20-HETE, and numerous EET isomers. Activated platelets acylate endogenous 12-HETE into phosphatidyl ethanolamine via 12-LOX [32]. Because phosphatidyl ethanolamine is located in the inner leaflet of the plasma membrane, this pool is available for release intracellularly (eg, via PLA_2), possibly participating in signal transduction in activated platelets. Leukocytes [27] and erythrocytes [33] also contain esterified oxylipins, which could potentiate intracellular activities or induce paracrine signaling to the vascular endothelium.

Lipoproteins

Early reports of epoxides demonstrated the presence of EETs in human lipoprotein phospholipids [34] but little work followed except for their identification and function in oxidized LDL [26]. More recently, it has been shown that over 90% of plasma oxylipins are esterified in lipoprotein lipids [35••]. Although the relative contribution of auto-oxidation and enzymatic sources is not known, a broad range of LA and AA oxylipins have been found and include products that can be derived by either hydroperoxide rearrangements or by CYP-dependent and LOX-dependent metabolism (eg, mid-chain alcohols, ketones, epoxides, diols, and triols) [6]. Notably, in this study of lipoproteins from dyslipidemic rats, very low-density lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL) each had unique oxylipin profiles, and those of VLDL and HDL in particular showed increased concentrations of proinflammatory oxylipins. Because the mechanisms and rates of lipid delivery to tissues vary by lipoprotein and lipid class (eg, VLDL triglycerides or HDL phospholipids), the oxylipin distribution and abundance within these pools may influence tissue uptake and delivery. Whether the oxylipin enrichment of plasma lipids in dyslipidemic rats was of auto-oxidative or enzymatic origin, these compounds have the potential to mediate metabolic pathways once exposed to cells.

Lipolysis of Lipoprotein Oxylipins

The lipolytic release of esterified oxylipins represents one means by which circulating oxylipins may act. Their presence in lipoprotein lipids suggests that like FAs, they may be released into their microenvironment by any lipase that acts on their lipid pool. In this scenario, their effect on tissues would depend on the relative abundances, or profiles, of oxylipins released. This is unlike intracellular oxylipin production, where activation of a rate-limiting enzyme increases synthesis of a specific metabolite. Rather, lipase-dependent release would produce a spectrum of oxylipins, potentially with both proinflammatory and anti-inflammatory properties (Fig. 1).

Lipoprotein lipase

Recent studies of the VLDL/lipoprotein lipase (LpL) axis suggest lipolytic delivery of oxylipins to the endothelium can occur, an event with a potentially profound impact on atherosclerosis. LpL is the primary enzyme clearing triglycerides from triglyceride-rich lipoproteins (TGRLs) such as chylomicrons or VLDL. It can release a subset of VLDL oxylipins, which is itself a subset of the overall plasma profile [35••]. LpL produces a high flux of FAs (and potentially oxylipins) in close proximity to endothelia and could thereby affect cellular function, even if the plasma oxylipin concentration is low. The VLDL/LpL clearance axis also targets triglyceride delivery to the endothelia of heart, skeletal muscle, and adipose tissue (in that order per milligram of tissue) and would, in theory, do the same for oxylipins. Eiselein et al. [36] report that endothelial cells treated with TGRL from healthy volunteers showed LpL lipolysis–dependent increases in membrane permeability and disruption of tight membrane junctions. Further studies by Wang et al. [37] also demonstrated that LpL lipolysis affected endothelial cell lipid raft localization and reactive oxygen species production. They also showed that LpL released the 9-hydroxyoctadecadienoate (HODE) and 13-HODE (LA alcohols), along with other FAs, from TGRL, and that the lipolysate increases tumor necrosis factor-α (TNF-α), intercellular adhesion molecule, and reactive oxygen species [38••]. Although the relative abundance of the HODEs was low and their potency was similar to that of their parent FAs, this study established that LpL lipolysate effects on endothelial cells were dependent on the

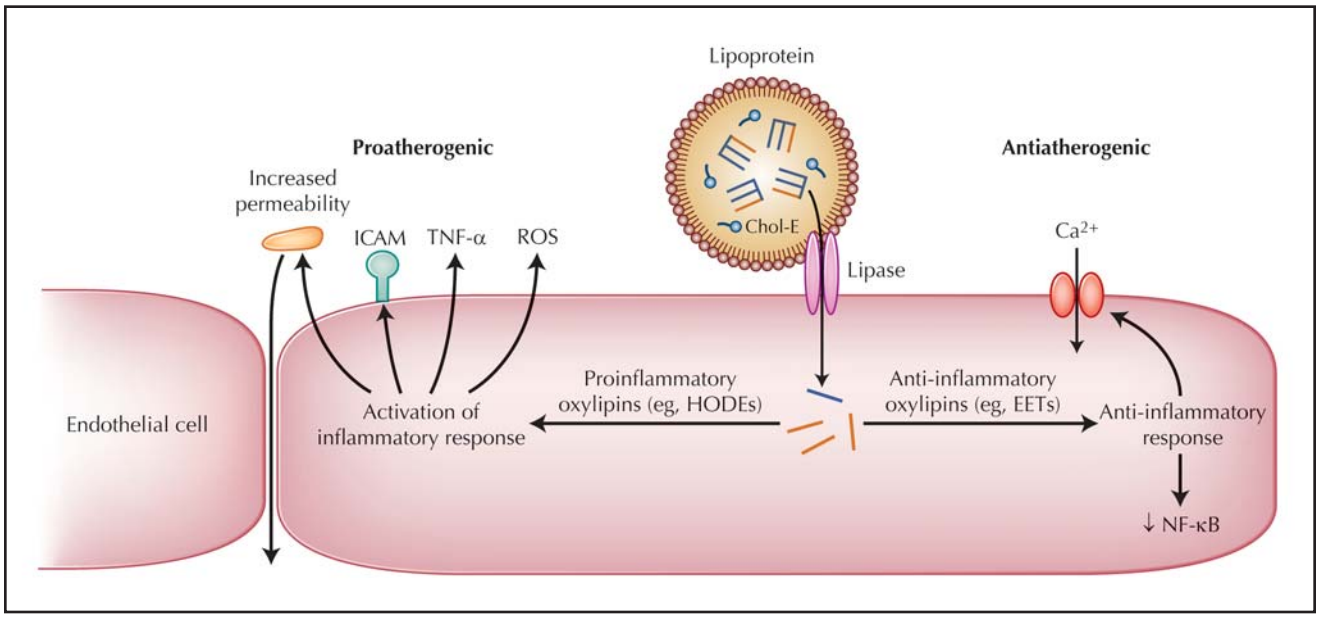

Figure 1. Lipoproteins mediate vasoactive and inflammatory processes on the vascular endothelium via lipolysis of esterified oxylipins. Esterified oxylipins have been demonstrated in each lipoprotein class. Each lipoprotein class has corresponding lipases that anchor to endothelial cells and could act there to release oxylipins. The greatest evidence for lipase-dependent oxylipin effect is for lipoprotein lipase (LpL)–mediated release of hydroxyoctadecadienoates (HODEs), which are linoleic acid–derived mid-chain alcohols. In this case, triglyceride-rich lipoproteins are colocalized to the endothelial surface by LpL and subsequent lipolysis releases the HODEs to the endothelia, where they cause changes in function consistent with endothelial dysfunction. It is expected that lipoproteins containing anti-inflammatory, antiatherogenic oxylipin could likewise exert their effects in the same way. EET—epoxyeicosatrienoic acids; ICAM—intercellular adhesion molecule; NF-κB—nuclear factor–κB; ROS—reactive oxygen species; TNF-α—tumor necrosis factor-α.

released milieu of FAs, which included an array of oxylipins. TNF-α–induced inflammation normally reduces LpL expression on the endothelium; however, when LpL is maintained, increased vascular cell adhesion molecule (VCAM) and E-selectin expression and impaired endothelium-dependent vasodilation are observed [39]. Coleman et al. [40] demonstrated an LpL-dependent role for Cu^{2+}-oxidized VLDL in activating adipocytes via PPARβ/δ, and simultaneously demonstrated the activation of PPARβ/δ by 13-HODE and 15-HETE. In all, these results support a role for oxylipins in the effects of lipolysis on endothelial inflammation.

Endothelial lipase

In the past decade, endothelial lipase (EL) has emerged at the confluence between HDL metabolism and inflammation. In humans, low-dose endotoxin elicits a 2.5-fold increase in circulating EL and is positively correlated with multiple proinflammatory markers [41]. The preference of EL for HDL phospholipids [42] combined with the presence of a large pool of oxylipins within HDL phospholipids suggests that EL could deliver HDL oxylipins to the endothelium. In fact, Ahmed et al. [43] showed that EL mediates a lipolysis-dependent activation of PPARα and the subsequent inhibition of leukocyte adhesion to endothelial cells. However, to date, no studies have specifically demonstrated that EL can hydrolyze acylated oxylipins, and the effects of EL have not been attributed to any specifically released component of the lipolysates.

Other lipid-trafficking enzymes

Although these works suggest a role for targeted, lipase-mediated delivery of oxylipins to specific tissues, the possibility remains that acylated oxylipins are a substrate for lipid-trafficking enzymes in general. For example, consideration should be given to the role of lecithin cholesteryl acyl transferase or cholesteryl ester transfer protein in mediating oxylipin transport, distribution, and delivery.

Overlap Between Lipoprotein and Oxylipin Actions

There is considerable overlap between the effects of oxylipins and lipoproteins in endothelial dysfunction, hypertension, and vascular disease. The role of LDL in mediating subendothelial lipid deposition and foam cell formation is well known and has been extensively reviewed, but LDL may not be the most important mediator of endothelial inflammation. TGRL lipolysis is central to the inflammatory and proatherogenic effects of postprandial hyperlipidemia [44] and is involved in the acute phase response and/or chronic inflammation [45]. de Goma et al [46•] have summarized HDL's activities, which include inhibition of immune activation, prevention of endothelial dysfunction, and prevention of lipid oxidation. Oxylipins are either active mediators of or a target for each of these activities. The ability of HDL to function as an antiatherogenic particle improves with correction of dyslipidemia in patients with atherosclerosis [47], a condition likely to correspond with a normalized

lipoprotein–oxylipin distribution. Furthermore, apoA-I induces endothelium-dependent vasorelaxation, normalizing it in human patients with endothelial dysfunction and low HDL [48]; HDL also interacts with the endothelium to produce vasoactive effects. Lipid exchange is a common component of this interaction regardless of whether it is ATP binding-cassette A1 (ABCA1)-mediated lipid efflux or EL-mediated lipid delivery. HDL exchanges not only cholesterol via the ABCA1 transporter, but phospholipids as well. It stands to reason that transfer of phospholipids might also entail transfer of esterified oxylipins, as transporters in the ABC family have recently been shown to transport bioactive phospholipids [49].

Experimental Challenges

Coexistence of oxylipins with complementary and opposing actions

Of the studies reviewed, only a few consider the potential action of oxylipins derived from a source other than AA. Fewer studies consider either the consequences of interaction between oxylipins in the same chemical class but derived from different FAs, or the interactions between oxylipins of different classes. In any oxylipin matrix, the actions of non–AA-derived oxylipins may oppose, enhance, or not interact with the actions of AA oxylipins. For example, in some studies, EET regioisomer effects are ranked, such as the 14(15)- > 11(12)- > 8(9)- >>5(6)-EET in potentiating coronary artery relaxation [31]. Although this is a valuable comparison, extrapolating to an in vivo effect is not simple. It would be a mistake to measure the concentration of 14(15)-EET and to conclude that levels below an effect threshold are unimportant because the combined effects of EETs would contribute to the final effect. Each regioisomer could have similar affinity for the receptor but act differently as agonists, and so different combinations may have unanticipated effects on activity. In order to comprehensively compare the potential oxylipin combinations, heroic experimental designs employing a very large number of mixtures and concentrations (ie, response surfaces) would be needed, and in the end would likely yield results that are difficult to interpret. In addition, it would not end the problem, as non-AA epoxides also occur in vivo. LA-derived EpOMEs occur also and have activity; given the epidemiologic evidence [9,50], these epoxides should not be ignored. Omega-3 variants of EETs derived from EPA and DHA are also likely to exist in vivo because human CYPs efficiently synthesize omega-3 epoxides in vitro [51]. These metabolites are more potent or equipotent compared with EETs in in vitro studies [14,15]. EPA epoxides and DHA epoxides are also likely to be more variable. In a large case control study [50], DHA had an interquartile range that was 65% of the median value and EPA was 80%; AA had an interquartile range that was 25% of its median. This means that individuals, likely as a consequence of dietary patterns, vary much more in their cellular EPA and DHA content than in their AA, and so may also vary more in the EPA and DHA oxylipin content of their plasma lipoproteins. In this case, estimations of epoxide effect size based solely on EETs could be off by as much as 35%, assuming equipotent activities for EPA and DHA epoxides. They would be off by much more if the increased potency of DHA observed in vitro is true in vivo [15]. These assumptions demonstrate that AA-only studies may not extrapolate well to the clinical setting; the entire oxylipin lipidome should be considered when estimating the final consequences in vivo.

A strategy for assessing oxylipin matrices

Regardless of their source or the pathways and receptors on which they act, oxylipins will ultimately exert their effects (complementary or opposing; inflammatory or proinflammatory) at the cell and tissue level; and defining their net effects requires a strategy that takes this into account. One strategy that should be considered is to "phenotype" the action oxylipin profiles on biomarkers of interest rather than measure the effects of individual species. Although an almost unlimited mixture of oxylipins are possible, only a limited number will occur in biology, and combined with dimension reduction techniques such as principal components analysis, simple characteristic markers can be correlated to these complex matrices using approaches such as those recommended by Lemley [52•]. This "oxylipin-omics" approach may prove to be more informative than focusing on one metabolite, one pathway at a time, a situation that does not occur in vivo. Such approaches are proving worthwhile using FAs in similar contexts [50] to the point of outperforming not only individual FA prediction–based models but also combined metrics using traditional risk markers (eg, Framingham scores).

Conclusions

Although the intracellular effects of eicosanoids are normally the only ones considered, evidence for the importance of circulating esterified oxylipins is growing. Lipoproteins can deliver oxylipins via lipase action that affect endothelial inflammation in in vitro models. This could clearly have implications for understanding the molecular basis of endothelial dysfunction and could potentially lead to new treatment strategies based on tissue-targeted oxylipin delivery.

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