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Discoveries

FORUM REVIEW ARTICLE

The Eicosanoids, Redox-Regulated Lipid Mediators in Immunometabolic Disorders

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Abstract

Significance: The oxidation of arachidonic acid *via* cyclooxygenase (COX) and lipoxygenase (LOX) activity to produce eicosanoids during inflammation is a well-known biosynthetic pathway. These lipid mediators are involved in fever, pain, and thrombosis and are produced from multiple cells as well as cell/cell interactions, for example, immune cells and epithelial/endothelial cells. Metabolic disorders, including hyperlipidemia, hypertension, and diabetes, are linked with chronic low-grade inflammation, impacting the immune system and promoting a variety of chronic diseases.

Recent Advances: Multiple studies have corroborated the important function of eicosanoids and their receptors in (non)-inflammatory cells in immunometabolic disorders (*e.g.*, insulin resistance, obesity, and cardiovascular and nonalcoholic fatty liver diseases). In this context, LOX and COX products are involved in both pro- and anti-inflammatory responses. In addition, recent work has elucidated the potent function of specialized pro-resolving mediators (*i.e.*, lipoxins and resolvins) in resolving inflammation, protecting organs, and stimulating tissue repair and remodeling.

Critical Issues: Inhibiting/stimulating selected eicosanoid pathways may result in anti-inflammatory and pro-resolution responses leading to multiple beneficial effects, including the abrogation of reactive oxygen species production, increased speed of resolution, and overall improvement of diseases related to immunometabolic perturbations.

Future Directions: Despite many achievements, it is crucial to understand the molecular and cellular mechanisms underlying immunological/metabolic cross talk to offer substantial therapeutic promise. *Antioxid. Redox Signal*. 29, 275–296.

Keywords: eicosanoids, innate immunity, inflammation, metabolic diseases, drug development

Introduction

ARACHIDONIC ACID (AA) is the source of eicosanoids, a well-known group of lipid mediators, comprising prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), and lipoxins (LXs) with a variety of physiological and pathogenic functions (31, 55). Although eicosanoids are important regulators of blood pressure (72), renal function (73), reproduction and host defense against microorganisms (161, 172), these molecules when generated in excess can induce pain, fever, and diverse inflammatory reactions. Continued inflammation that fails to resolve may lead to chronic inflammatory diseases, for example, asthma, rhinitis, atherosclerosis, thrombosis, and cancer. Therefore, multiple drugs have been developed that target selected steps of the eicosanoid pathway, including specific enzymes and receptors (53).

Eicosanoid biosynthesis is initiated by controlled oxygen radical reactions, catalyzed by specific oxygenases, which in turn are dependent on the intracellular redox state and peroxide tone. Thus, both cyclooxygenases (COXs) and lipoxygenases (LOXs) are activated by lipid hydroperoxides, which are regulated by glutathione (GSH) peroxidases and therefore indirectly by the levels of GSH (52, 150). Moreover, reactive oxygen species (ROS) can attack the double bonds of polyunsaturated fatty acids, such as AA, and elicit unspecific radical reactions leading to products structurally

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similar, or even identical, to enzymatically formed eicosanoids. One typical example is the family of isoprostanes that on one hand are used as an index of oxidative stress and on the other hand include compounds with significant biological activity (112). Hence, eicosanoid biosynthesis involves both specific and unspecific oxygen radical reactions under control of cellular redox state.

Metabolic diseases and inflammation have been thoroughly studied in the past; however, emerging evidence indicates the presence of complex interactions between inflammatory and metabolic processes, which may underlie what are known as immunometabolic disorders, that is, obesity, insulin resistance, type 2 diabetes, cardiovascular diseases (CVD), and nonalcoholic fatty liver diseases (NAFLD). Although eicosanoids are well-known mediators of innate immune responses and inflammation with important functions in disease, their potential involvement in the pathogenesis of immunometabolic conditions has only recently been recognized and is presently gaining considerable attention.

In this review, we focus on the initial enzymatic oxygenation reactions and subsequent catalytic steps along the COX, LOX, and cytochrome P450 (CYP) pathways leading to cellular release of bioactive lipid mediators. We also discuss mass spectrometry (MS)-based methodologies for metabolic profiling and quantification of eicosanoids in biological matrices. Finally, we summarize recent work on the participation of eicosanoids and related lipid mediators in a number of metabolic diseases and potential opportunities for therapeutic intervention based on insights into the eicosanoid cascades.

AA Metabolism

AA metabolism through COX-1 and COX-2 with associated downstream terminal synthases generates PGs, prostacyclin and TXs. The 5-LOX and 12/15-LOX pathways produce several classes of LTs and LXs. The CYP pathways comprise monooxygenases and epoxygenases to form hydroxy-eicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EpETrEs or EETs), which are processed by soluble epoxide hydrolase (sEH) to generate the corresponding dihydroxy-eicosatrienoic acids (DiHETrEs or DHETs). These pathways for AA metabolism are summarized in Figure 1A.

AA is the main substrate for COX, LOX, and CYP and is primarily released by one isoform of the phospholipase A_2 superfamily (PLA_2), namely cytosolic $PLA_2\alpha$ group IV (13). This enzyme has an N-terminal Ca^{++}-dependent phospholipid-binding domain, which under Ca^{++}- stimuli leads to activation and translocation of cytosolic phopholipase A2 ($cPLA_2$)α to the endoplasmic reticulum and perinuclear membranes with simultaneous phosphorylation of the protein (50, 99). Most cells and tissues constitutively express $cPLA_2\alpha$; however, certain proinflammatory cytokines and growth factors may contribute to increased $cPLA_2\alpha$ expression (52). In ischemia- and inflammation-related diseases, inflammatory cytokines and ROS increase the activity of $cPLA_2$, thus allowing augmented release of AA from membranes (85). Mechanistically, it has been described that increased nicotinamide adenine dinucleotide phosphate, NADPH, oxidase activity increases ROS levels, inactivating protein phosphatases such as protein tyrosine phosphatase, mitogen-activated protein kinase (MAPK) phosphatase 1, as well as protein phosphatase 1 and 2A (PP1 and PP2A, respectively). This results in activation of MAPK cascades such as c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase, responsible for $cPLA_2\alpha$ activation (85).

Biosynthesis of PGs and TXs

The majority of our cells synthesize PGs, which act in an autocrine and paracrine manner. PGs are synthesized *de novo*, in response to mechanical injury or by specific stimuli (*e.g.*, cytokines and growth factors) (149). AA is metabolized by COX, also known as prostaglandin H (PGH) synthase (PGHS), to an intermediate endoperoxide, PGH_2, which is further metabolized into PGE_2, PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2), and TXA_2 (67, 149). The first phase of the COX reaction is abstraction of the 13-pro S hydrogen from AA by a tyrosyl radical (Tyr385) to form an arachidonyl radical, which then reacts with O_2 in a regio- and stereoselective manner (Fig. 2A). After antarafacial oxygen insertion at C-11, the resulting 11(R)-hydroperoxyl radical reacts at C-9 to generate an endoperoxide group and a C-8 radical. Then, C-8 and C-12 are connected through cyclization to form a five-membered ring and a carbon-centered radical on C-15. In the final step, a second O_2 reacts at C-15 to form a 15-hydroperoxyl radical, which extracts a hydrogen from Tyr385 to form PGG_2. Peroxidases such as GST peroxidase may contribute to the reduction of PGG_2 to PGH_2 (149, 150) (Fig. 2B).

COX is present in COX-1 (PGHS-1) and COX-2 (PGHS-2) isoforms (149). COX-1 is constitutively expressed in most tissues, while COX-2 expression is transiently and rapidly induced in response to a variety of cell stimuli such as proinflammatory agents, cytokines, and growth factors. In addition, COX-2, unlike other oxygenases in the eicosanoid cascades, is upregulated by hypoxia *via* an HIF-1-dependent mechanism (81).

The action of PGs and TXA_2 is mediated through binding to a series of G protein-coupled receptors (GPCRs), the PGE receptors: EP1 (E prostanoid receptor 1), EP2, EP3, and EP4; PGD receptors: DP1 and DP2; PGF receptor: FP; PGI receptor: IP; and TXA_2 receptor: TP; on a variety of cell types exerting a multitude of physiological functions (Fig. 1B).

Biosynthesis of Leukotrienes

The canonical reaction was catalyzed by LOX isoxygenation of polyunsaturated fatty acids containing a 1,4-*cis*-pentadiene structure. In the case of 5-LOX, oxygenation of AA starts with stereospecific abstraction of the pro-S hydrogen at C-7 followed by radical migration to C-5 and formation of a Δ6-*trans* double bond. The radical at C-5 reacts with O_2 to form 5(S)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HpETE). In a following step, chemically similar to the first oxygenation, 5-HpETE is converted into LTA_4. This conversion involves an initial abstraction of the pro-R hydrogen at C-10 of 5-HpETE, radical migration to C-6, and rearrangements of the double bonds to Δ7,9-*trans* yielding a conjugated triene system, which is the physicochemical basis for the typical leukotriene chromophore. Finally, the radical combines with the 5(S)-hydroperoxy group, resulting in dehydration to form the epoxide moiety of LTA_4 (52) (Fig. 2C).

The central enzyme 5-LOX, a nonheme dioxygenase, is activated by Ca^{++}. Coactosin-like protein (CLP) binds to 5-LOX, which then translocates from the cytoplasm to the endoplasmic and perinuclear membrane where CLP contributes to 5-LOX protein assembly (121). At this site, 5-LOX interacts

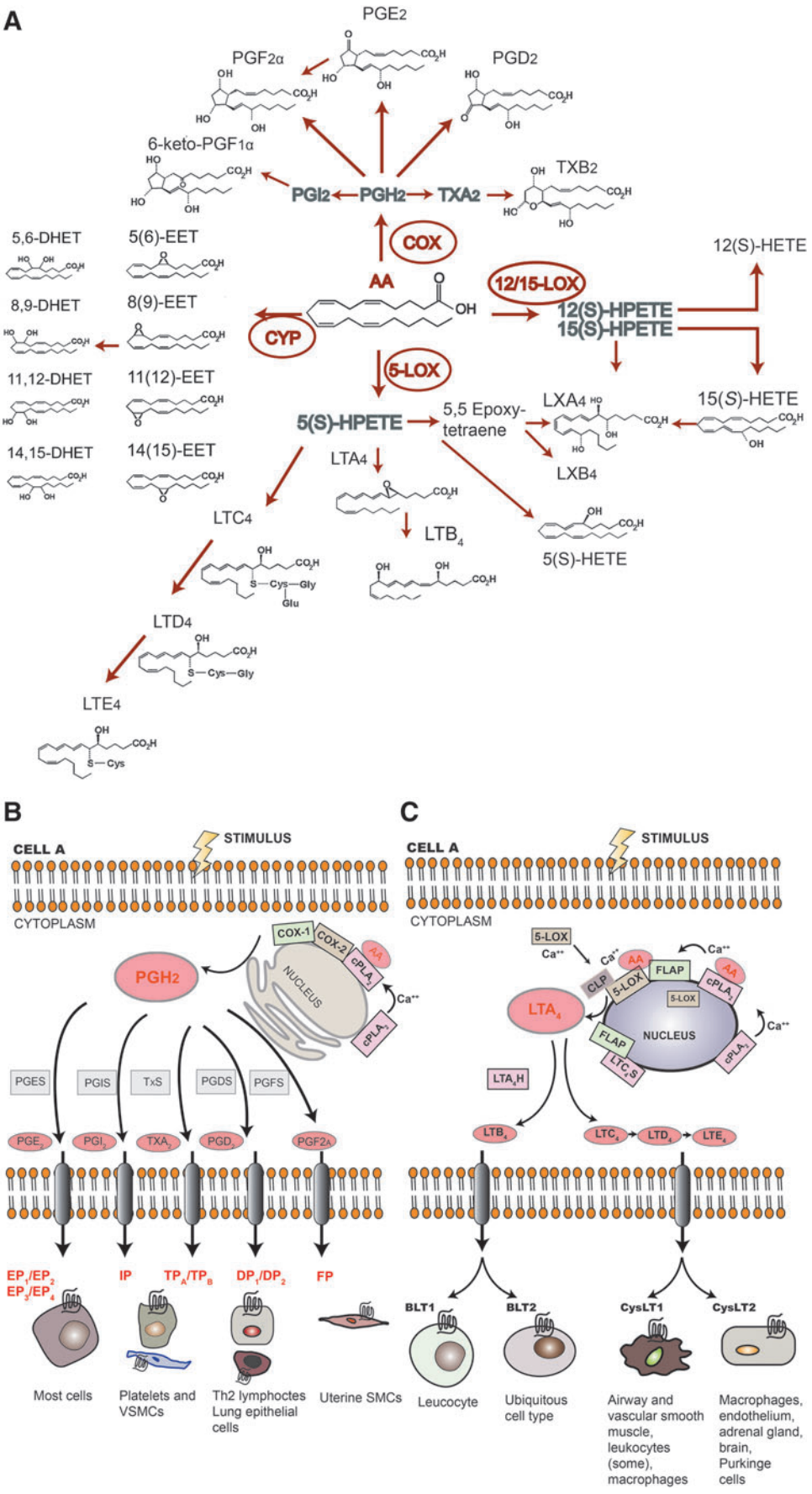

FIG. 1. AA cascade and cellular COX and LOX biosynthesis. (A). This scheme illustrates the major pathways for production of the main eicosanoids discussed. AA is substrate for oxidation by lipoxygenase (LOX; 5-LOX and 12/15-LOX), COX and CYP enzymes, which collectively produce a large spectrum of eicosanoids. **(B).** Cellular COX biosynthesis. COX catalyzes the formation of PGG_2, and then, subsequent peroxidase activity further converts it to PGH_2. From this precursor, downstream enzymes initiate the production of prostaglandins and thromboxanes, whose bioactions are mediated *via* signaling through distinct GPCRs. These COX metabolites are exported through the plasma membrane *via* an export carrier known as PGT, and thus secreted to reach their corresponding receptors on diverse target cells to elicit an array of physiological responses. **(C).** Cellular LT biosynthesis. Under cellular stimulation, intracellular Ca^{2+} is increased, thus activating and translocating cPLA2α and 5-LOX to the nuclear membrane. There, AA is released from membrane and converted to LTA_4 by 5-LOX and FLAP. Subsequently, LTA_4 is metabolized into LTB_4 or LTC_4 by the LTA4H or LTC4S enzymes, respectively. LTs are exported through the plasma membrane *via* an export carrier known as MRP, and secreted LTs then reach their corresponding receptors on diverse target cells. AA, arachidonic acid; COX, cyclooxygenase; CYP, cytochrome P450; DHET, dihydroxyeicosatrienoic acids; EET, epoxyeicosatrienoic acids; FLAP, 5-lipoxygenase activating protein; GPCRs, G protein-coupled receptors; HETEs, hydroxy-eicosatetraenoic acids; HpETEs, hydroperoxyeicosatetraenoic acids; MRP, multidrug resistance protein; LT, leukotriene; LTA_4H, LTA_4 hydrolase; LOX, lipoxygenase; LXs, lipoxins; PGH, prostaglandin H; PGT, prostaglandin transporter; PLA^2, phospholipase A^2 superfamily. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

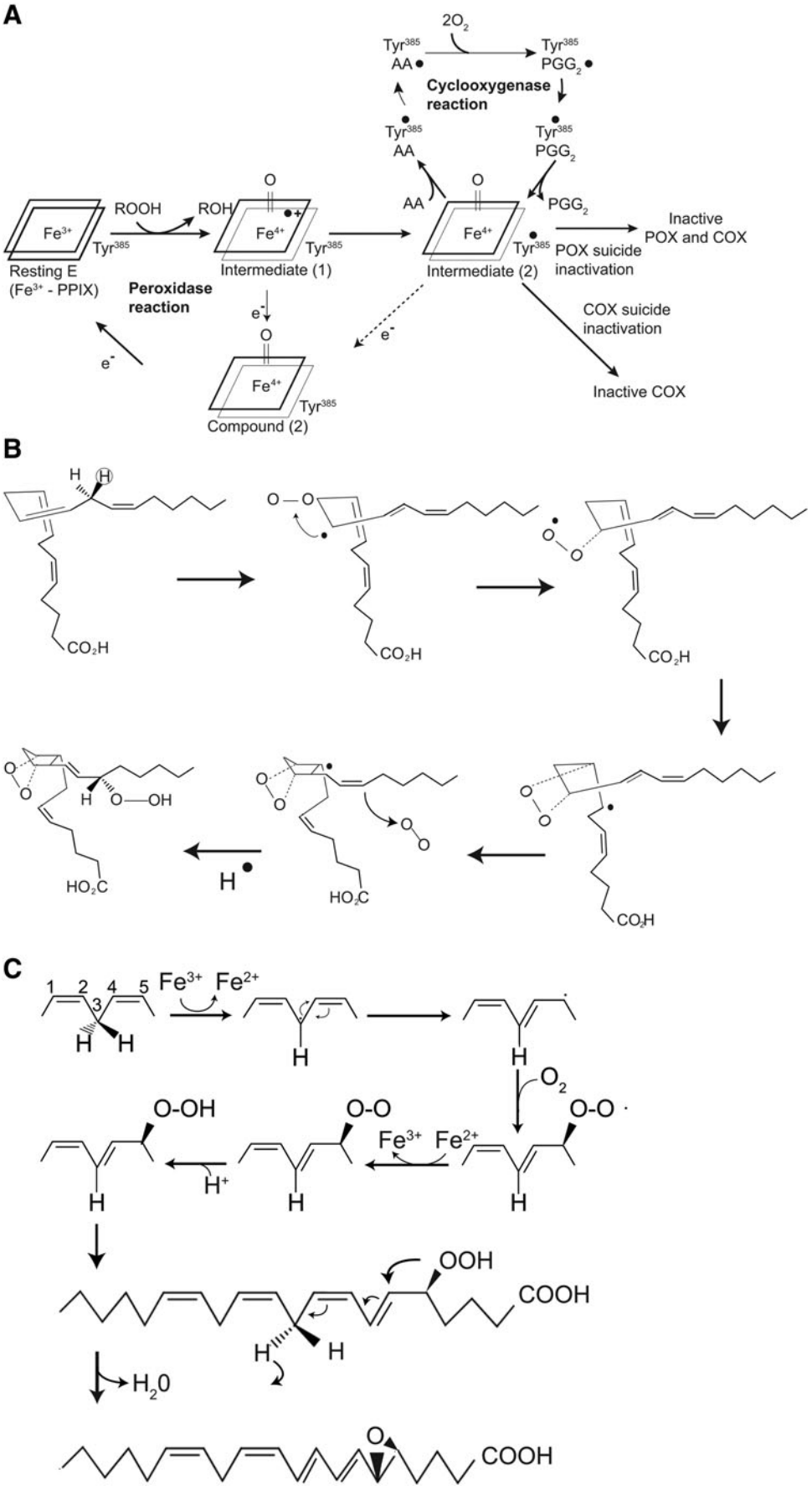

FIG. 2. Biosynthesis of COX- and LOX-derived metabolites. (A, B). Mechanism of COX. Combined cyclooxygenase (dioxygenase) and hydroperoxidase (peroxidase) activity converts free AA to prostaglandin G_2 (PGG_2) and then PGH_2. First, the 13-proS hydrogen atom is removed from AA by a Tyr385 radical forming an arachidonyl radical. The 11-hydroperoxyl radical is then formed by the addition of O_2 to C-11. An endoperoxide is formed by the action of the 11-hydroperoxyl radical at C-8. The substrate then undergoes cyclization (C-8 to C-12 bond formation) to form a five-membered ring and a carbon-centered radical on C-15. An additional O_2 is added at C-15 and readdition of the abstracted hydrogen atom. **(C).** Mechanism of 5-lipoxygenation. According to the common lipoxygenase mechanism, a hydrogen atom is specifically removed at C-3 of the *cis*,*cis*-1,4-pentadiene (pro-S hydrogen at C-7 of AA). Subsequently, this hydrogen reduces the iron to Fe^{2+} (inactive), and unpaired radical electrons combine with one of the electrons of the adjacent double bond, forming a 1-*cis*, 3-*trans*-conjugated diene with relocation of the radical to C-5, where oxygen then forms a peroxy radical. The iron is oxidized and protonated to provide the hydroperoxide and Fe^{3+} (active) enzyme. 5-LOX then catalyzes a second hydrogen abstraction of the pro-R hydrogen at C-10 of 5-HpETE followed by radical migration to C-6, combination with the 5(S)-hydroperoxy group, and dehydration to form the epoxide moiety of LTA_4. 5-HpETE, 5(S)-hydroperoxy-6-trans-8,11, 14-cis-eicosatetraenoic acid.

with 5-lipoxygenase activating protein (FLAP) and together with cPLA$_2\alpha$ constitute a biosynthetic complex that releases and oxidizes AA to form the transient epoxide intermediate LTA_4 (132). In leukotriene biosynthesis, LTA_4 is further converted into the dihydroxy acid LTB_4 by the cytosolic enzyme LTA_4 hydrolase (LTAH), while LTC_4 synthase (LTC_4S; a specific membrane-bound GST) conjugates LTA_4 with GSH to form LTC_4. The lipid/peptide adduct, LTC_4, is the parent compound of the so-called cysteinyl-leukotrienes (cys-LTs; LTC_4, LTD_4, and LTE_4). Thus, leukotrienes comprise two classes of mediators, LTB_4 and the cys-LTs, which act *via* two corresponding sets of GPCRs, BLT1/2 and CysLT1/2, respectively. Leukotrienes are mainly biosynthesized by leukocytes from the myeloblastic (neutrophils, eosinophils, and mast cells) and monoblastic lineages (monocytes/macrophages). These lipid mediators act in a paracrine manner exerting their functions at nanomolar concentrations and causing different responses according to cell type (52) (Fig. 1C).

In addition, 5-HpETE can be metabolized to 5-HETE, 5-oxo-ETE (5-KETE), and the LX family of lipid mediators (145). The enzymes described as 12/15-LOX include the 12-LOX and 15-LOX originally found in porcine leukocytes and afterward in murine tissues, and in reticulocytes, respectively. In humans, the 12/15-LOX is expressed by eosinophils, reticulocytes, and airway respiratory epithelium, while in mice it is mostly found in peritoneal macrophages (43). 12/15-LOX oxidizes AA at C-12 and C-15 in an isoform- and species-dependent manner (32). In humans, the 12-LOX and 15-LOX isoforms produce 12- and 15-hydroperoxy-eicosatetraenoic acid (12-HpETE) and (15-HpETE), respectively, and are often referred to as 12/15-LOX. The resulting stereochemistry is generally only the S-isomer (12(S)-HETE and 15(S)-HETE) (52); however, R-lipoxygenases have also been reported (142).

Cythochrome P450 Monooxygenase Pathway of Eicosanoid Biosynthesis

The CYP monooxygenase family of enzymes catalyzes the redox-coupled activation of molecular oxygen and the transport of one oxygen atom to ground state carbons (Fig. 3A). In the synthesis of eicosanoids, CYP oxidizes AA either *via* bisallylic oxidation to form the HETEs or *via* the epoxidation of an alkene to generate EETs (22). This broad enzyme family consists of many isoforms that possess overlapping biosynthetic activity. The CYP4F and CYP4A families are able to oxidize AA to the HETEs (11); however, the most common pathway is the ω hydroxylation of AA to produce 20-HETE. In contrast to LOX activity, the CYP-catalyzed formation of HETEs generally produces the R isomer (113). The CYP epoxygenase enzyme adds an oxygen atom to one of the four olefines of AA, which can produce four different epoxide regioisomers, 5(6)-, 8(9)-, 11(12)-, and 14(15)-EET. These epoxides can subsequently serve as substrates for the sEH enzyme, which converts the epoxides to the corresponding vicinal diols, 5,6-, 8,9-, 11,12-, and 14,15- DHET (Fig. 3B).

A range of biological activities have been reported for the CYP-derived AA products. The EETs have been extensively studied and reported to possess a wide range of biological activity, including suppression of peptide hormone secretion, vascular and bronchial smooth muscle tone and vasorelaxation, hemostasis, cell proliferation, and controlling inflammation (154, 191). The HETEs can play an important role in angiogenesis stimulation, apoptosis inhibition, and enhancement of proliferation, as well as migration of cancer cells (21). The chirality of the HETE hydroxyl moiety can play an important role in the observed biological activity. It is common that the HETEs and EETs exert opposite biological functions (*i.e.*, promote *vs.* suppress inflammation, vasoconstriction *vs.* vasodilatation) (100). For example, it has been reported that the CYP-mediated reactions of AA underlie mechanisms leading to both vasodilation (EET) and vasoconstriction (20-HETE) of cerebral arteries (54). Generally, the EETs are considered to be hypotensive and the DHETs to possess hypertensive properties (113). The sEH has been suggested as a drug target for cardiovascular (75) and renal diseases (71). It is also of interest that the CYP pathway cross talks with other pathways. For instance, the EETs can serve as substrates for COX (134). The signal transduction of HETEs, EETs, and DHETs is not fully understood and, only recently, gpr75 was deorphanized as the first receptor for a CYP generated eicosanoid, namely 20-HETE (46).

Biosynthesis of Proresolving Lipid Mediators

LXs were the first eicosanoids reported to have both anti-inflammatory and proresolving actions (145). Their biosynthesis occurs *via* 12- or 15-LOX-mediated conversion of AA to 15-hydroxyeicosatetraenoic acid [15(S)-HETE], followed by further transformation *via* the 5-LOX pathway into LXA_4 and LXB_4 through cell/cell interactions. LXs can also be produced in the vasculature during interactions between platelets and leukocytes (157). LXs are known to promote efferocytosis, that is, macrophage clearance of apoptotic polymorphonuclear leukocytes (155). *In vivo* studies have demonstrated that LX can reduce airway inflammation in lipopolysaccharide-induced pulmonary injury in mice (79), decrease systemic inflammation and augment survival rates in a model of sepsis in rat (171), inhibit inflammation-induced mechanical hypersensitivity in rats (1), and suppress pulmonary and renal fibrosis in mice (137). The suggested mechanism involves inhibition of nuclear factor κB (NF-κB) activation, decreased levels of proinflammatory cytokines, and inhibition of apoptosis in hepatic cells (78). Of note, aspirin acetylates COX-2, which in turn converts AA into 15(R)-HETE, the substrate for 15-epi-lipoxin A_4 (15-epi-LXA_4) production (26). This metabolite is known as aspirin-triggered lipoxin (ATL). It has since been discovered that there are multiple aspirin-triggered variants of the specialized proresolving lipid mediators. These compounds have been reported to collectively exert profound effects on the resolution of inflammation, providing insight into the temporal relationship of the inflammatory cascade (144).

Metabolic Diseases

Metabolic diseases occur when normal metabolism and energy homeostasis are deranged with perturbed hormone balance, blood glucose levels, and/or serum lipoprotein and lipid compositions (dyslipidemia).

Obesity and Diabetes

Obesity has become a worldwide epidemic and is associated with several comorbidities (*i.e.*, hypertension, type 2 diabetes (T2D), certain types of cancer, heart, liver, kidney, and CVD (35).

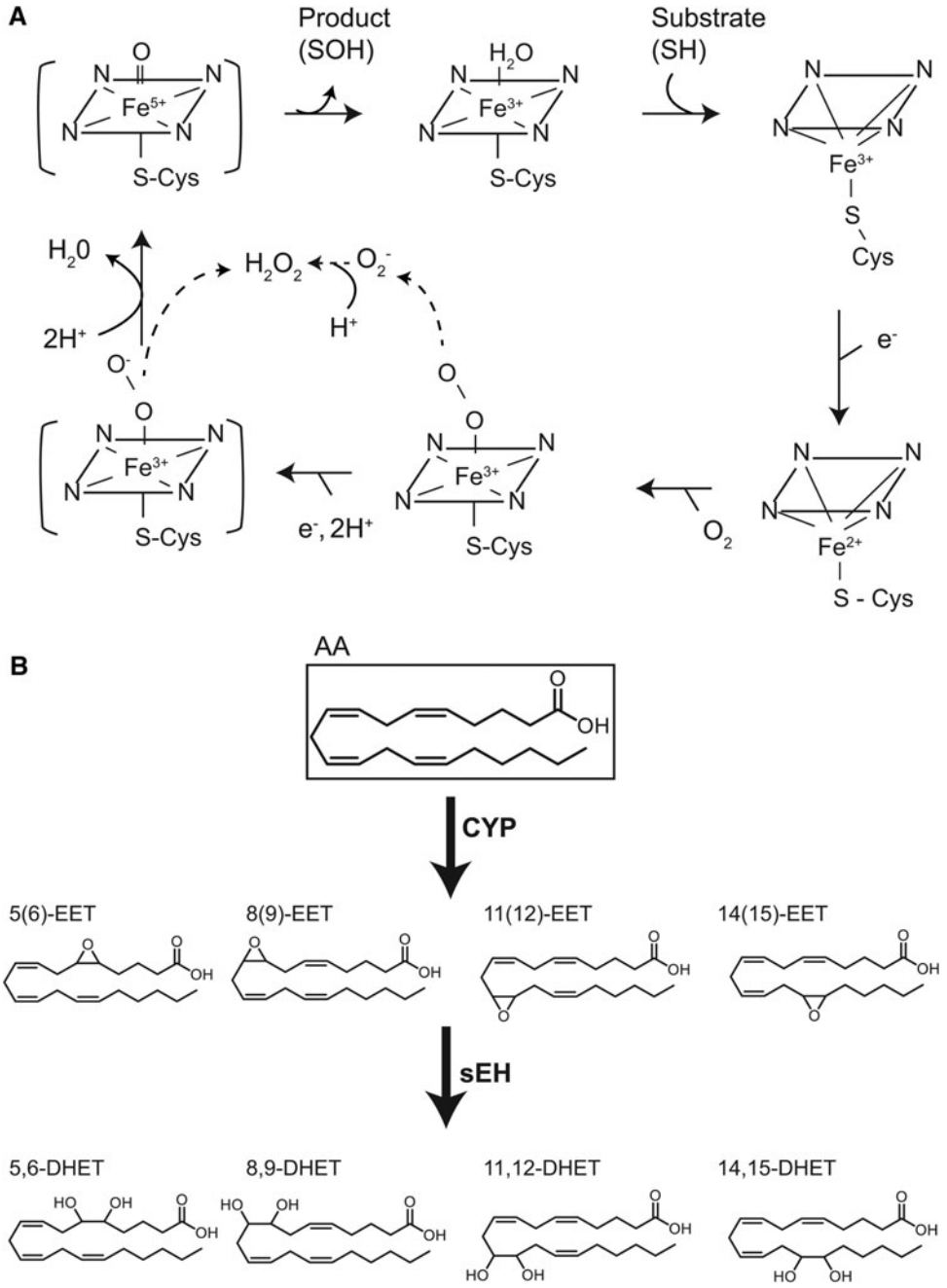

FIG. 3. Cytochrome P450 oxidation of AA. (A). The most common reaction catalyzed by CYP is a monooxygenase reaction, involving insertion of one atom of oxygen into an organic substrate, while the other oxygen atom is reduced to water. This mechanism forms the AA-derived HETEs. For example, 20-HETE is formed by CYP omega hydroxylase. **(B).** AA is also converted by CYP epoxygenases to four different regioisomers of EETs, which are further metabolized by the sEH to the corresponding vicinal diols, DHETs. sEH, soluble epoxide hydrolase.

Adipose tissue (AT) is composed of adipocytes and several different cell types. AT is divided into white (WAT) and brown (BAT) with slightly different functions. Thus, WAT primarily stores nutrients, while BAT both stores nutrients and releases their inherent energy (7, 190). More recently, a third type of AT called browning or beige has been defined, for a review see Ref. (8). Obesity can also trigger the renin–angiotensin–aldosterone system and the sympathetic nervous system, promoting inflammation, insulin resistance, hypertension, oxidative stress, and sodium retention (152). Obesity is typified by a general increase in subcutaneous WAT, but ectopic accumulation around and within some organs is also observed. The enlarged white adipocytes release proinflammatory adipokines (*i.e.*, tumor necrosis factor α [TNFα], interleukin [IL]-6, IL18, leptin, C-C motif chemokine [CCl]2, and C-X-C chemokine [CXCL]5), increase the levels of nonesterified fatty acids, and promote lipotoxicity, as well as infiltration of proinflammatory cells and eicosanoid release (70) (Fig. 4).

As referred in the AA Metabolism section, an excess of lipid mediators is related to modifications of the microenvironment such as accumulation and activation of immune cells and consequences of cell/cell interaction, which in turn promote development of disease. It has been shown that pancreatic islets can also produce COX (PGE_2), LOX (12(S)-HETE) metabolites and, although less explored, CYP metabolites (EETs). These mediators seem to have an important function as modulators of islet function [for review (103, 162)]. Interestingly, it was demonstrated that COX (PGE_2) and LOX

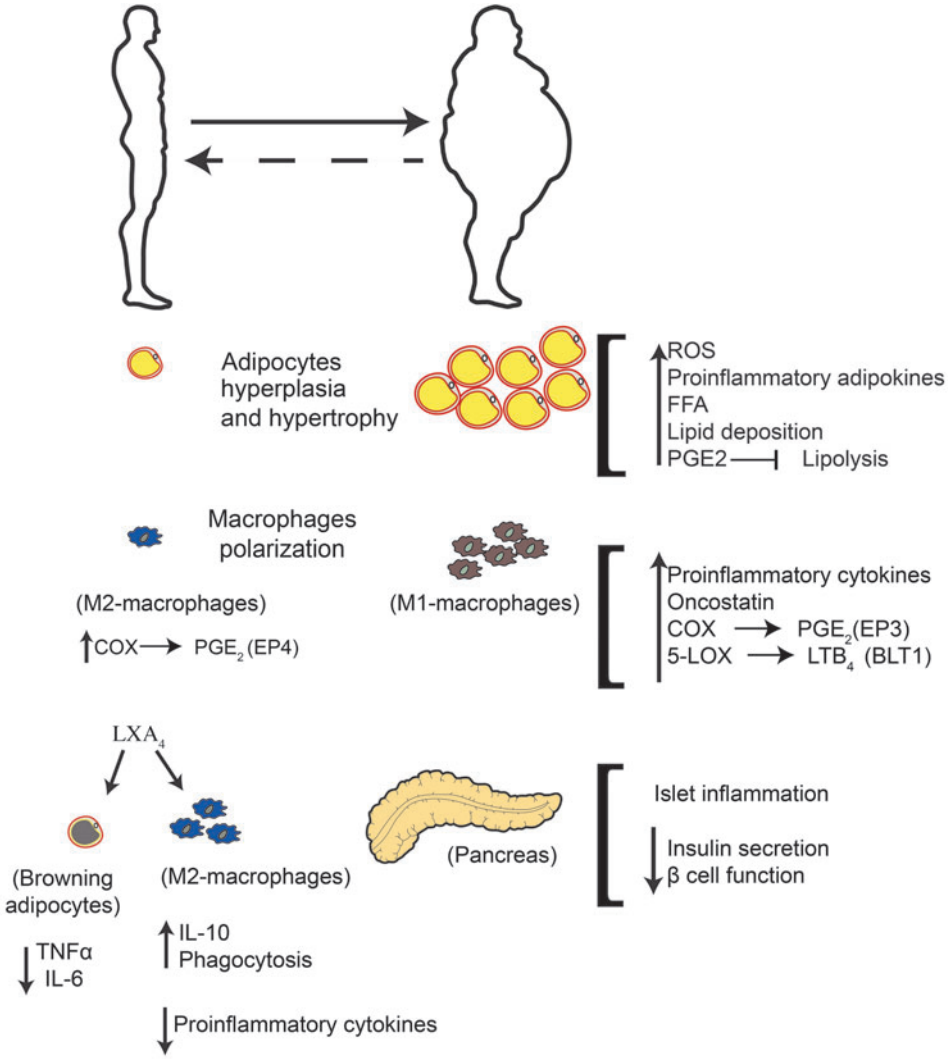

FIG. 4. Eicosanoids in obesity and diabetes. Excess of caloric intake stimulates adipocyte hypertrophy and hyperplastic dysfunction mainly in adipose tissue, with concomitant increase of proinflammatory adipokines, ROS, and lipid accumulation (mainly FFA). This stress recruits inflammatory cells stimulating the release of proinflammatory factors (eicosanoids and cytokines). As a consequence of cross talk between WAT and immune cells (M1), proinflammatory cytokines are released. Pancreatic β cells are overloaded with lipids and become dysfunctional, while insulin has reduced action. In healthy individuals or weight loss, eicosanoids can stimulate anti-inflammatory reactions and browning adipocytes. FFA, free fatty acids; ROS, reactive oxygen species; TNFα, tumor necrosis factor α; WAT, white adipose tissue. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

(LTB_4, LTC_4, and 15-HETE) metabolites, released by pancreatic islets, decrease cyclic adenosine monophosphate (cAMP) levels in β cells with a resulting decrease in glucose-stimulated insulin secretion (103, 120).

Since metabolic diseases stimulate low grade of inflammation, infiltration of inflammatory cells and imbalanced production of eicosanoids in the kidneys, eyes, nerves, and cardiovascular system contribute to the development of diabetes pathogenesis and its complications (Fig. 4). Therefore, nonsteroidal anti-inflammatory drugs (NSAIDs), which interfere broadly with prostanoid biosynthesis, have been tested for their ability to control diabetes nephropathy and retinopathy with some beneficial effects (162). COX-2 inhibitors appear attractive but since they are accompanied by an increased cardiovascular risk (see in use of NSAIDs and risks for patients with CVD section), their use in (cardio)metabolic disease would be hazardous. Thus, there is a need to develop novel safe drugs that selectively block certain eicosanoids relevant to the pathogenic context as potential therapeutics for metabolic diseases and the major component T2D.

COX-derived metabolites in obesity and T2D

Prostanoids, a collective name for PGs and TXs, are implicated in the progression of obesity-associated AT inflammation resulting in insulin resistance. Breyer and colleagues (23) demonstrated that through the deletion of one of the PGE_2 receptors, EP3, mice under high-fat diet (HFD) displayed an obesity-related phenotype (*i.e.*, overweight, ectopic lipid accumulation, and insulin resistance) when compared to wild-type (WT) controls. Therefore, it has been proposed that activation of COX-2 and subsequent PGE_2-EP3 signaling has a crucial function for recruitment of adipose immune cells contributing to the onset and progression of inflammatory responses in AT. Moreover, *in vivo* and *in vitro* treatment with a selective COX-2 inhibitor and EP3 antagonist (celecoxib and L798106, respectively) showed efficacy in reverting inflammation in AT and insulin resistance (24). In contrast to previous publications, it was shown that the PGE_2-EP4 axis exerts a proinflammatory function on obesity-related inflammation (185). Administration of an EP4 agonist (ONO-AE-329) in *db/db* mice receiving chow diet revealed that EP4 has a potent regulatory effect on adipose tissue macrophages (ATMs), since the transcription of genes encoding proinflammatory adipokines (*i.e.*, TNFα, IL-6, and monocyte chemotactic protein 1 [MCP-1]) was reduced in stromal vascular fractions of epididymal WAT, a region rich in ATM. Consequently, the infiltration of proinflammatory macrophages (M1) was reduced in contrast to the augmented differentiation of macrophages into the anti-inflammatory type

(M2). In line with that finding, García-Alonso *et al.* (47) demonstrated that PGE_2 has an anti-inflammatory action in obese AT, by inhibition of inflammatory gene expression in *ex vivo* experiments with human WAT and by decreasing the inflammatory response in differentiated adipocytes stimulated by the bacterial product (lipopolysaccharide). In addition, PGE_2 decreased the expression of certain profibrotic genes such as *Collagen alpha-1* (*I*) *chain* (*Col1a1*) and *Col1a2* considered as a hallmark of metabolically dysfunctional AT. In this context, one may also note that hyperglycemia induces COX-2 expression in monocytes (147) and increases the production of prostanoids in streptozotocin-induced diabetes in rats (141). Recently, it has been proposed that targeting PGE_2 receptor EP3 might contribute to the control of obesity and T2D (115). Altogether, these studies indicate that regulation of the COX signaling might be important for pathogenesis of obesity and diabetes type 2 (related) diseases.

LOX-derived metabolites in obesity and T2D

Proinflammatory leukotrienes have also been strongly implicated in the pathogenesis of immunometabolic diseases. In both obese humans and mice, macrophage accumulation occurs within insulin-sensitive tissues (*i.e.*, WAT). It was demonstrated that enzymes and receptors of the 5-LOX pathway were upregulated in AT (65). Importantly, a key end product of this pathway, namely LTB_4, appears to play a pivotal role in the development of AT inflammation accompanied with NF-κB activation and increased production of proinflammatory adipokines, MCP-1, IL-6, and TNFα (65). In agreement with this, pharmacological FLAP inhibition (Bay-X-1005) had a protective effect in mice with dietary obesity in reducing the subsequent 5-LO products and the number of macrophages in AT (65). Mothe-Satney *et al.* (114) reported that both mouse and human adipocytes secrete LTs, suggesting that these eicosanoids may contribute to macrophage and T cell recruitment to AT and insulin resistance. In addition, pharmacological or genetic ablation of the 5-LOX pathway in HFD-fed WT mice resulted in reduction of ATM and insulin resistance. Similar results have also been obtained with mice deficient in BLT1, the cognate receptor for LTB_4, which exhibited reduced ATM infiltration and increase of insulin sensitivity in a diet-induced obesity model (156).

Recent data demonstrate a similar role for LTB_4 in promoting liver steatosis and insulin resistance in muscle and AT, reinforcing the role of the LTB_4–BLT1 signaling axis as a main driver for the inflammation–insulin resistance syndrome in obesity (98). Moreover, a report published this year demonstrated that in $BLT1^{-/-}$ mice or mice treated with a BLT1 antagonist (CP-105696), recruitment of B2 lymphocytes into visceral fat depots is reduced (188). In as much as B cells can also regulate AT function in obesity (30), these results further corroborate the notion that inhibition of LTB_4 signaling can be a valuable therapeutic strategy in diseases related to insulin resistance.

Specialized proresolving mediatorsin obesity and T2D

The COX and LOX pathways are also involved in the generation of anti-inflammatory, proresolving, lipid mediators, typified by LX and ATL, which can attenuate low-grade inflammation in immunometabolic disorders. LX controls leukocyte infiltration into tissues and promotes resolution. An elegant study demonstrated that mice overexpressing ALOX5AP in AT, displayed increased levels of LXA_4 rather than the expected LTB_4 (37). This shift in equilibrium between proinflammatory and anti-inflammatory eicosanoids promoted browning of WAT and led to augmented energy expenditure, diminished adiposity, and control of obesity and T2D development.

Similarly to obesity, aging is associated with continuous low-grade inflammation. Börgeson *et al.* (15) demonstrated that AT explants from perigonadal fat pads of aging WT female (12 months old) mice treated with LXA_4 (1 n*M*) attenuated IL-6 release, while the mRNA levels of important components of the insulin-sensitizing cascade, glucose transporter (GLUT)-4 and insulin receptor substrate (IRS)-1, were found to be upregulated. Later, the same group showed that the LXA_4 synthetic analog (15[R]-Benzo-LXA_4) in obese mice diminished AT inflammation by stimulation of M1 to M2 polarization in WAT as well as control of adipose autophagy, which is usually increased in the HFD model, as judged by restoration of protein levels of the adipose autophagy markers, microtubule-associated protein-1 light chain (LC) 3 (conjugated form) LC3II and p62 (Sequestosome-1) (14). Furthermore, obesity-associated diseases such as liver and chronic kidney diseases were also assessed. In these settings, LXs reduced liver weight and serum alanine aminotransferase (ALT) and triglyceride levels, while causing a reduction of albuminuria, urine hydrogen peroxide/creatinine ratios, and profibrotic signaling in the kidney (14), suggesting that LXA_4 and LX analogs protect from aging- and obesity-induced diseases.

CYP-derived metabolites in obesity and T2D

Insulin resistance is often linked to hypertension and T2D. The epoxygenases (CYP2C, CYP2J) and their EET products decrease blood pressure and may improve glucose homeostasis. CYP2C-derived EETs have been shown to contribute to insulin sensitivity in both mice (192) and humans (45), suggesting that increasing the levels of circulating EETs could be a novel approach to improve insulin sensitivity and treat hypertension. Inhibition of the sEH enzyme, which is the primary route for metabolism of the EETs, provides renal protection in the initial phase of diabetic nephropathy (138) and beneficial effects on glucose homeostasis (25) in murine models. Accordingly, reduction of EET bioavailability in AT appears to be a consequence of obesity, and approaches to stimulate the protective effects of EETs in AT may offer therapeutic opportunities for medical management of obesity and obesity-linked pathologies.

Cardiovascular Diseases

The risk of developing CVD, that is, atherosclerosis, coronary heart disease, stroke, and heart failure (90, 135), is increased in patients with metabolic disorders. Atherosclerosis is regarded as a chronic inflammatory disease characterized by subendothelial formation of plaque composed of cholesterol, foam cell formation, influx of immune cells, in particular monocytes and T cells, smooth muscle cells, and fibroblasts, and thickening of the intimal part of the arterial wall (Fig. 5). Atherothrombosis occurs when a plaque ruptures, causing thrombus formation, narrowing or occlusion of vessel lumen leading to local or peripheral ischemic complications, in particular myocardial infarction and ischemic stroke (57).

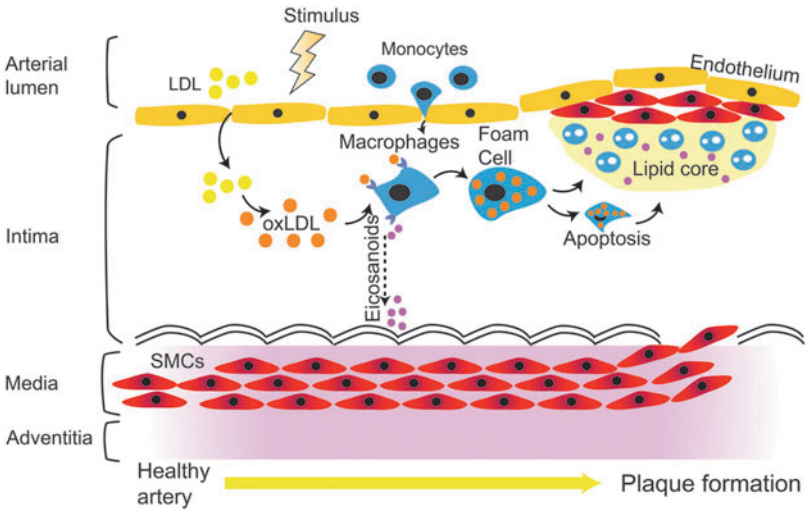

FIG. 5. Eicosanoids in atherosclerosis. Chronic endothelial injury (*i.e.*, hyperlipidemia) stimulates monocyte adhesion and migration from the lumen to the intima. There, lipoproteins (*i.e.*, LDL) accumulate and are subsequently oxidized and taken up by macrophages, initiating foam cell formation. M1-macrophages release proinflammatory lipid mediators, disturbing the VSMCs. Later, these cells proliferate and migrate to the subendothelium and together with other immune cells contribute to plaque formation. VSMC, vascular smooth muscle cell. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

COX-derived metabolites and atherosclerosis

Given the key role of COX in inflammation, many studies have attempted to define the function of COX isoforms and prostanoids in atherosclerosis. Surprisingly, genetic deletion or pharmacological inhibition of COX-2, the proinflammatory isoform, may retard, accelerate, or leave unchanged atherogenesis in mouse models of atherosclerosis (164). In contrast, COX-1 deletion significantly attenuated lesion development in the apolipoprotein E $(ApoE)^{-/-}$ mouse; a similar effect was found when COX-1 and COX-2 were inhibited together in low-density lipoprotein receptor $(LDLr)^{-/-}$ mice (108, 126). Thus, COX-1 products, such as TXA_2, promote atherogenesis, whereas the role of COX-2 is more uncertain. On the contrary, deletion of the downstream microsomal prostaglandin E synthase 1 (mPGES-1), responsible for PGE_2 synthesis during inflammation, leads to retarded atherogenesis and reduced plaque burden in $LDLr^{-/-}$ mice (173) and diversion of the substrate PGH_2 into PGI_2 synthesis promotes an antithrombotic phenotype (160). PGI_2 may also have an atheroprotective role since deletion of the IP receptor induces an early development of atherosclerosis in hyperlipidemic mice (36). It has been shown that PGE_2 is produced in atherosclerotic plaque of $ApoE^{-/-}$ mice under HFD, and its receptor (EP3) is then activated (51). Therefore, by blocking *in vivo* the receptor EP3 with the antagonist DG-041, atherothombosis was inhibited (165). Interestingly, the PGE_2-EP3 signaling seems specific for thrombosis without affecting hemostasis.

Use of NSAIDs and risks for patients with CVD

Aspirin and several other well-known NSAIDs share the same mechanism of action, namely, inhibition of COX. This enzyme inhibition reduces PG synthesis and accounts for the analgesic, anti-inflammatory, and antipyretic effects of these drugs. Low-dose aspirin is one of the most frequently prescribed, mild antithrombotic medications, whose mechanisms of action is related to promotion of endothelial synthesis of vasodilatory and antithrombotic PGI_2 with simultaneous inhibition of proaggregatory platelet TXA_2. In the early 1990s, several laboratories independently discovered a second COX isoform that turned out to be specifically linked to inflammatory responses and cancer. Unlike the classical COX, this new COX was expressed at low levels in the gastric mucosa (149). The pharmaceutical industry quickly developed potent and selective COX-2 inhibitors, which were marketed as ''safe'' NSAIDs carrying less gastric side effects (ulcerations and bleeding). These drugs, nowadays known as coxibs, proved very efficacious for treatment of inflammatory pain, especially in patients suffering from rheumatoid arthritis and osteoarthritis.

However, concerns were raised regarding the cardiovascular safety of COX-2 inhibitors. When the results of a clinical trial of rofecoxib for treatment of colon cancer, the so-called APPROVe (Adenomatous Polyp Prevention on Vioxx) study, were disclosed, the data indicated a significant risk of thrombotic events, mainly myocardial infarction and stroke among patients taking rofecoxib (Vioxx), which in turn led to an immediate withdrawal of the drug from the market (18). There are still uncertainties regarding the cellular and molecular mechanism(s) underlying these serious side effects. However, a large body of evidence indicates that coxibs inhibit COX-2-dependent PGI_2 synthesis, particularly in the endothelium. Reduced levels of PGI_2 in the vessel surface would then increase the sensitivity to thrombotic and hypertensive signals and could also promote atherogenesis (41). Later meta-analyses of randomized trials have shown that not only COX-2 inhibitors but also several frequently prescribed traditional NSAIDs are associated with a significant cardiovascular risk, with naproxen exhibiting the lowest risk (82). In this context, it is worth noticing that certain COX-2 inhibitors, for example, Celebrex® (celecoxib), are still widely prescribed and clinical trials (*i.e.*, PRECISION) have concluded that the risks in developing side effects by the use of certain COX-2 inhibitors may be related to dose and dosing interval, offering treatment regimens with reduced cardiovascular risk (10, 20, 116).

In the aftermath of the Vioxx scandal, new strategies to develop safe NSAIDS have emerged. For instance, enzymes downstream of COX-2, such as mPGES-1 responsible for proinflammatory PGE production (77), have attracted considerable attention (20, 84, 89). Another ongoing approach is to antagonize the prostanoid receptors to achieve a more targeted interference with discrete parts of the eicosanoid cascade, thereby avoiding side effects (16, 80, 122, 178).

LOX-derived metabolites in atherosclerosis

A large body of evidence has implicated the 5-LOX pathway, in particular LTB_4, in the pathogenesis of atherosclerosis

(52), although the role of 5-LOX metabolites is not fully understood in this disease (58, 59). Thus, increased expression of 5-LOX, FLAP, and LTA_4H was observed in human atherosclerotic plaque (128), and molecular genetic studies showed that certain 5-LOX, FLAP, and LTA_4H genotypes were associated with increased risk of atherosclerosis and myocardial infarction (2, 44, 128, 153). The pharmaceutical industry has made several efforts to develop drugs targeting the 5-LOX pathway, including inhibitors of 5-LOX and LTA_4H as well as BLT1 antagonists, as new medicines against atherosclerosis but has not yet produced a small molecule that has reached the clinic.

Studies have revealed opposing roles of 12/15-LOX and the products in atherosclerosis where this pathway can stimulate atherosclerotic lesions (48, 69, 139, 194) or exert a protective function (109). Proatherogenic effects are usually attributed to the ability of 12/15-LOX to oxidize LDL, a key villain in atherogenesis. Protective effects, on the contrary, may be related to synthesis of proresolving mediators (146). Despite these opposing data, signaling along this pathway has been identified as a regulator of IL-12 and Th1 responses, which may contribute to vascular effects of 12/15-LOX expression and enzyme activity (111). There is 40% homology between 15-LOX-1 and 15-LOX-2 at the amino acid level, but these enzymes differ in their catalytic activities (17). 15-LOX-2 was observed in macrophages and hypoxia was found to increase its expression (140). Later, 15-LOX-2 was found highly expressed in human carotid plaques (48). Moreover, this enzyme activity can be modulated by hypoxia inducible factor 1-α (HIF-1α), suggesting that 15-LOX-2 contributes to atherogenesis by augmenting the inflammatory response and recruitment of inflammatory cells (32).

Specialized pro-resolving mediators and atherosclerosis

Specialized pro-resolving mediators (SPM) the LXs, resolvins, protectins, and maresins, counteract and temper the effects of eicosanoids and other proinflammatory mediators, diminishing recruitment of neutrophils and promoting efferocytosis. Early work by Brezinski *et al.* (19) showed that angioplasty increases intracoronary levels of LXs. Furthermore, levels of ATL were lower in the plasma of patients with both peripheral and coronary atherosclerosis when compared to healthy volunteers, indicating that ATL is part of a protective response against peripheral atherosclerosis. In addition, the ALX (receptor for LXA_4 and ATL) was expressed in vascular smooth muscle cells (VSMCs) and the migration of the PDGF-stimulated VSMCs pretreated with ATL was reduced (63), suggesting that ATL could be used to control abnormal SMC responses and as a biomarker for progressive vascular inflammation. More recent studies have focused on the therapeutic use of SPMs in various forms of CVD with positive effects in a number of experimental models of atherosclerosis (40).

CYP-derived metabolites in atherosclerosis

The EETs have been reported to have beneficial effects on CVD, including the ability to dilate coronary arteries, hyperpolarize VSMCs, and suppress adhesion molecules (88, 123). In addition, a number of atherosclerosis models have suggested that sEH inhibition, with a concomitant decrease in the degradation of EETs, may attenuate both the development of atherosclerosis and aneurysm formation (19, 63, 68). The antiatherosclerotic effects of inhibiting the sEH correlate with increased circulating levels of the EETs and are associated with elevated HDL and reduced LDL. For example, the genetic ablation or pharmacological inhibition of sEH increased the ratio of EETs/DHETs (a metric for inferred sEH activity) and diminished the expression of P-selectin glycoprotein ligand 1 (PSGL-1) in murine peripheral blood mononuclear cells (97). The inhibition of sEH was also shown to increase EET levels in the plasma of Sprague-Dawley rats, resulting in reduced neointima formation in the femoral artery cuff model (174). Dysregulation in CYP, and its major enzymatic products 20-HETE and EETs, contributes to endothelial dysfunction and CVD, including hypertension, ischemic injury, and atherosclerosis, suggesting that there is potential to target these pathways for treating atherosclerosis (74).

Nonalcoholic Fatty Liver Diseases

NAFLD is one of the most common hepatic diseases in Western countries. NAFLD is defined by augmented lipid accumulation in hepatocytes (>5%), absence of hepatic viral infections plus no significant alcohol intake (>20 g of alcohol/d), and the use of drugs with high potential to develop hepatotoxic reactions (117, 189). This disease can progress to steatosis with inflammation (NAFL), nonalcoholic steatohepatitis (NASH), NASH with fibrosis, cirrhosis, and hepatocellular carcinomas (27). For the progression from NAFL to NASH, a variety of metabolic inflammatory events are involved, one of them is insulin resistance, leading to free fatty acids and lipid accumulation in peripheral blood and hepatocytes, followed by a sequence of innate immune responses from kupffer cells (KCs) (9, 163) (Fig. 6).

COX-derived metabolites in NAFLD/NASH

There are controversial data about the role of PGs in hepatocyte metabolism. PGE_2 produced by KCs acts on receptors (EP3 and EP4) present on hepatocytes increasing triglyceride accumulation *via* mechanisms involving cAMP, which might contribute to the fat accumulation in hepatocytes and development of steatosis (38). PGE_2 also enhances the production of oncostatin M, a mediator of insulin resistance, in KCs contributing to hepatic insulin resistance (62). On the contrary, it was reported that PGE_2 acts in a paracrine manner suppressing the transcription of certain lipogenic genes in hepatocytes (*i.e.*, fatty acid synthase [*Fasn*], l-pyruvate kinase [*L-pk*] and thyroid hormone responsive [*Sport14*]) and the *de novo* lipogenesis (107); and attenuation of triglyceride incorporation into very low-density protein (VLDL) in primary cultures of rat hepatocytes (12, 61).

LOX-derived metabolites in NAFLD/NASH

Although the role of LOX has not been well investigated in the context of hepatic inflammation, Titos *et al.* (166) demonstrated that attenuation of 5-LOX signaling by administration of Bay-X-1005 (FLAP inhibitor) in carbon tetrachloride (CCl_4)-treated rats caused reduced cys-LT and LTB_4 levels along with increased levels of LXA_4 in the first 6 weeks of treatment. These shifts correlated with reduction of metalloproteinase (MMP) activity and tissue MMP2 (*TIMP-2*) mRNA level in the liver. Using the same hepatotoxic agent, CCl4, which is frequently used to induce

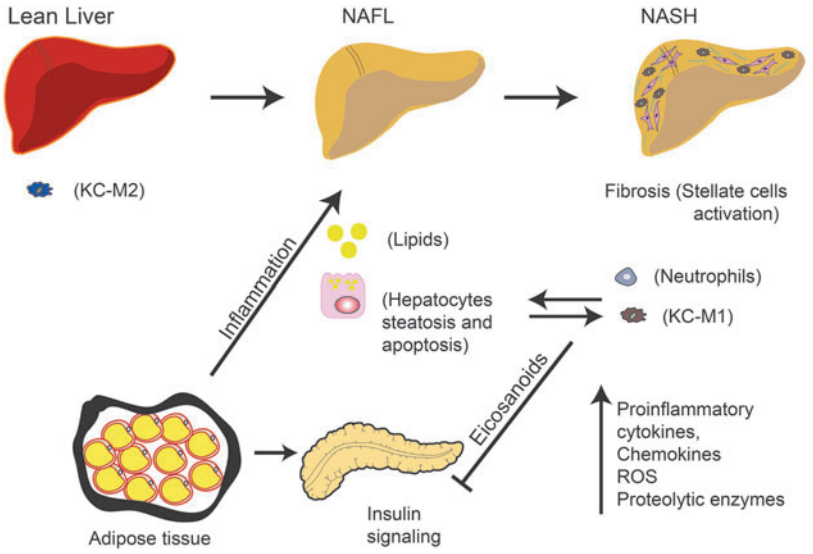

FIG. 6. Eicosanoids in nonalcoholic fatty liver disease. Accumulation of fatty acids in hepatocytes is characteristic of NAFL. Obesity exerts a chronic low-grade inflammation, activating the infiltration and polarization of proinflammatory KC (KC-M1), neutrophils, and other immune cells, and thus stimulating the progression of disease to NASH. Lipid mediators produced by KC-M1 (5-LOX and COX metabolites) contribute to fat accumulation in the hepatocytes as well as insulin resistance. Fibrosis may also be present during this phase. KC, kupffer cells; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

inflammation, steatosis, fibrosis, and liver cancer in mice (33), it was later demonstrated that deletion of *Alox5* in mice exerts a protective effect in liver injury, reducing the effects of hepatic necroinflammation, hepatocyte ballooning, and reducing the serum ALT levels (167). The effect of treatment with the 5-LOX inhibitor CJ-13,610 for 14 days in *db/db* mice also revealed a protection against hepatic steatosis (101). Interestingly, the combination of a selective COX-2 (SC-236) and 5-LOX (CJ-13,610) inhibitor reduced both necroinflammation and fibrosis in CCl4 models (66). It has been revealed that 5-LOX signaling has a potential effect on the progression from steatosis to NASH in hyperlipidemic models and its metabolite, LTB_4, might have a crucial function for the development of disease. Therefore, the deletion of BLT1 in obese mice protects the animals from developing hepatic steatosis, mostly by reduction of proinflammatory M1-like and increase of anti-inflammatory M2-like macrophages in AT, thus preventing lipolysis (156). Although the mechanisms by which 5-LOX signaling triggers liver dysfunction and damage are not fully understood, both genetic and pharmacological abrogation of 5-LOX in $ApoE^{-/-}$ mice under HFD led to reduced infiltration of macrophages, insulin resistance, and hepatic inflammation (decrease of proinflammatory cytokines, *Tnfα*, *Mcp-1,* and *Il-1b*) through mechanisms related to the suppression of NF-kB activity (104). Until now, no studies on the role of the second LT class, cys-LT, have been reported. Altogether, these reports suggest that controlling the 5-LOX pathway might result in an interesting therapeutic approach against the progression of liver steatosis into NAFLD.

A possible mechanism linking 12/15-LOX to the promotion of obesity, hepatic steatosis, insulin resistance, and inflammation has been described through experimental liver disease model and HFD consumption. To understand the 12/15-LOX activity in this context, Marcos Martinez-Clemente *et al.* (105) demonstrated that double-knockout $ApoE^{-/-} \times 12/15\text{-}LO^{-/-}$ mice had a reduction of NAFLD parameters such as ALT, steatosis, and inflammation due to the decrease of macrophage infiltration as well as expression of inflammatory cytokines (*Mcp-1*, *Il-6*, and *Il-18*). 12/15-LOX catalyzes the oxidation of *ω*-6 PUFAs and its metabolites have been described as proinflammatory. Double knockout $LDLr^{-/-} \times 12/15\text{-}LOX^{-/-}$ fed with PUFA-enriched diet displayed a reduction of total plasma cholesterol (VLDL and LDL), combined with a decrease in triglyceride levels and hepatic cholesterol content improving hepatic steatosis when compared with single knockout $LDLr^{-/-}$ mice (139). Another study revealed that a diet with controlled PUFA *ω*6/*ω*3 ratio exerted a protective effect in WT mice against development of NASH and reduced plasma concentration of oxidized AA metabolites (91, 139). Hence, it has been suggested that inhibition of 12/15-LOX could be an interesting strategy for prevention of obesity associated with metabolic disorders such as liver disease.

CYP-derived metabolites in NAFLD/NASH

CYP enzymes are highly expressed in the liver, catalyzing the oxidative biotransformation of xenobiotics (143); however, the functional relevance of the CYP metabolism pathway in the pathogenesis of NASH remains poorly understood. The omega hydroxylase CYP4A14 has been shown to play an important role in the pathogenesis of both hepatic steatosis and NASH, and suggested as a potential therapeutic target for treatment of NAFLD (192). Wells *et al.* (177) suggested that dysregulation of the CYP epoxy-eicosanoid pathway is a key pathological consequence of NASH, and that promoting the anti-inflammatory and protective effects of EETs warrants further investigation as a therapeutic strategy for NASH. Induction of NAFLD/NASH in mice suppressed hepatic CYP epoxygenase expression and activity, as well as both hepatic and circulating levels of EETs. Interestingly, disrupting soluble epoxide hydrolase restored hepatic and systemic EET levels as well as attenuated NAFLD/NASH-evoked hepatic inflammation and injury. Collectively, these findings suggest that suppression of hepatic EET biosynthesis is a key pathological consequence of NAFLD/NASH, and therapeutic restoration of EET levels is an anti-inflammatory strategy with potential utility for the treatment of fatty liver disease-associated inflammation and injury (143).

Mass Spectrometry Profiling of Lipid Mediators

There is an extensive body of literature on the analysis of eicosanoids by mass spectrometry, which can only be

partially described herein. The quantification of eicosanoids has primarily been conducted by high-performance liquid chromatography (HPLC) or gas chromatography coupled to mass spectrometry (*e.g.*, LC-MS, GC-MS), as well as the generally more sensitive enzyme- and radioimmunoassay techniques (EIA and RIA) (87, 125). While commonly used, EIA and RIA methods have two limitations: (1) chemical cross reactivity can result in high variability in analyte quantification and (2) generally it is only possible to detect a single analyte in a given assay. The primary advantage of GC-MS and LC-MS methods is that they offer increased selectivity for the simultaneous analysis of multiple eicosanoids (6, 168, 170). GC-MS has been used extensively to quantify eicosanoids; however, compounds must be both thermally stable and volatile, which necessitates the use of chemical derivatization for the analysis of most eicosanoids (130, 169). LC-MS does not have these limitations and has therefore become the method of choice for the majority of eicosanoid analyses.

One of the current trends in the biomedical sciences is the development of analytical methods that are able to simultaneously quantify a broad panel of lipids in a single analysis (*e.g.*, lipidomics). While these omics approaches can provide a wealth of biological information, the high degree of lipid structural complexity is a significant analytical challenge. Lipids are a complex family of compounds, possessing a range of physicochemical properties (131). *In silico* analyses estimate the potential for $\sim$180,000 different species of molecular lipids (186), which can require the development of separate analyses for each of the different subfamilies. However, recent technical developments have made it possible to measure thousands of lipid species simultaneously. Many of these lipids, including signaling lipids (*e.g.*, eicosanoids, endocannabinoids) and structural lipids (*e.g.*, glycerolipids, triglycerides, and cholesterol species), constitute a complex physiological network and exercise important roles in the pathobiological processes involved in the inflammatory cascade (42, 60, 102, 136, 182). The structural diversity in combination with the biological importance has resulted in the development of a plethora of analytical methods and approaches to quantify lipid mediators in biological matrices. The continual development of mass spectrometers enables the development of new methods that include more compounds while simultaneously increasing specificity. Multiple methods for eicosanoid profiling have focused on different analytical goals, including maximizing the number of quantified lipid mediators (34, 175), reducing analytical cycle time (151), improving injection (183), increasing specificity for SPMs (28), or being optimized for clinical phenotyping (181). One reason for this success has been the technical improvement of column particle chemistry in combination with enhanced mass spectrometer sensitivity. Together this has led to the ability to monitor hundreds of lipid mediators simultaneously while separating compounds with high structural similarity (5, 49, 92, 106, 133, 158, 181, 183, 184, 193) as reviewed (3, 4, 86, 169, 179). The ability to collect in parallel product ion scans provides structural and confirmatory data, which is important for elucidating structural analogs.

Eicosanoids and their structural analogs (sometimes referred to as oxylipins or more generally lipid mediators) possess a diversity of chemical structures, as well as multiple structural isomers. It is accordingly necessary to apply multidimensional separation techniques to quantify these compounds such as LC-MS/MS in MRM mode. However, the application of these methods to eicosanoid analysis can be challenging, with many of the lipids susceptible to autooxidation or degradation. This degradation can be particularly problematic for the analysis of the cys-LTs. These compounds are unstable in some biological matrices, with reported recoveries as low as 25% (29). To achieve selectivity in compound identification, specific transitions should be chosen (*e.g.*, eicosanoids and their structural analogs are all carboxylic acids that undergo a neutral loss of 44 atomic mass units, which is the loss of CO_2 and is accordingly not selective). Some structural isomers exhibit an identical fragmentation pattern (*e.g.*, PGE_2 and PGD_2). In this instance, LC separation is required to perform targeted analysis. However, LC-MS/MS methods can simultaneously analyze several transitions by MRM, enabling the selective analysis of coeluting compounds. The advent of ultrahigh-performance liquid chromatography (UHPLC) has resulted in greatly improved chromatographic separation of these structural isomers, simultaneously enabling the determination of more analytes while decreasing the chromatographic runtime (148, 184). Nevertheless, there are still a number of analytical challenges. While many eicosanoids and their analogs are produced *via* enzyme activity, some of them are formed *via* oxidative stress-associated processes. For example, free radical-driven reactions can produce the isoprostanes as well as mono-hydroxylated enantiomers (*e.g.*, HETEs, HODEs) (76). The stereochemistry is important because it provides the synthetic source of the species (*e.g.*, LOX-derived lipids are generally the S enantiomer) and the biological activity of enantiomers can differ. Accordingly, chiral-based separation of molecules is recommended when possible (86, 94, 110).

Chiral chromatography is able to separate enantiomers of monohydroxy fatty acids and the SPMs (39, 64). This provides the ability to distinguish between nonenzymatically produced lipids, as during a neutrophilic-driven oxidative burst, and enzymatically produced lipids (3, 129, 133). Many eicosanoids and related compounds, including hydroperoxyoctadecadienoic acids, hydroxyoctadecadienoic acids (HODEs), hydroperoxyeicosatetraenoic acids (HpETEs), HETEs, and EETs, are formed *via* nonenzymatic reactions to produce racemic mixtures (124, 187). Conversely, when these lipids are formed enzymatically, the products are predominantly enantiopure (180). The developing field of SPM analysis focusing on the resolution of inflammation has used chiral LC-MS/MS-based methods to identify novel compounds, including the EPA-derived 18(*R*) and 18(*S*) series of resolvins (118, 119) as well as the docosapentaenoic acid-derived 13 series resolvins (*e.g.*, RvT1, RvT2) (127).

To improve the limit of detection (LOD), derivatizing agents can be used. For example, the carboxyl groups of eicosanoids can be derivatized to pentafluorobenzyl esters (PFB) (56) and analyzed by chiral phase liquid chromatography electron capture atmospheric pressure chemical ionization mass spectrometry (chiral LC-EC/APCI/MS). Enantiomers of regioisomeric HETEs and HODEs have been measured using this approach (96). These methods have been reviewed elsewhere (93, 95). Although the LOD of chiral eicosanoids has improved with these methods, they have some drawbacks, including the need for sample clean up and

derivatization and often give low yields for conversion to the PFB-esters. Due to these limitations, the field is moving toward the analysis of these compounds as the free acid. For example, chiral LC-MS/MS identified 12(*S*)-HETE as the major enantiomer in the urine of diabetes mellitus patients (159). The effects of phenobarbital on the synthesis of enantiomers of CYP-derived AA metabolites (HETEs and EETs) in rat liver were determined by chiral LC-APCI/MS (176). One of the primary limitations of chiral LC-MS is the availability of chiral columns. However, a number of advances have been made and chiral columns, which used to be predominantly normal phase, are being manufactured for reversed-phase applications. The current main obstacle is the preparation of chiral columns with small particle size. The

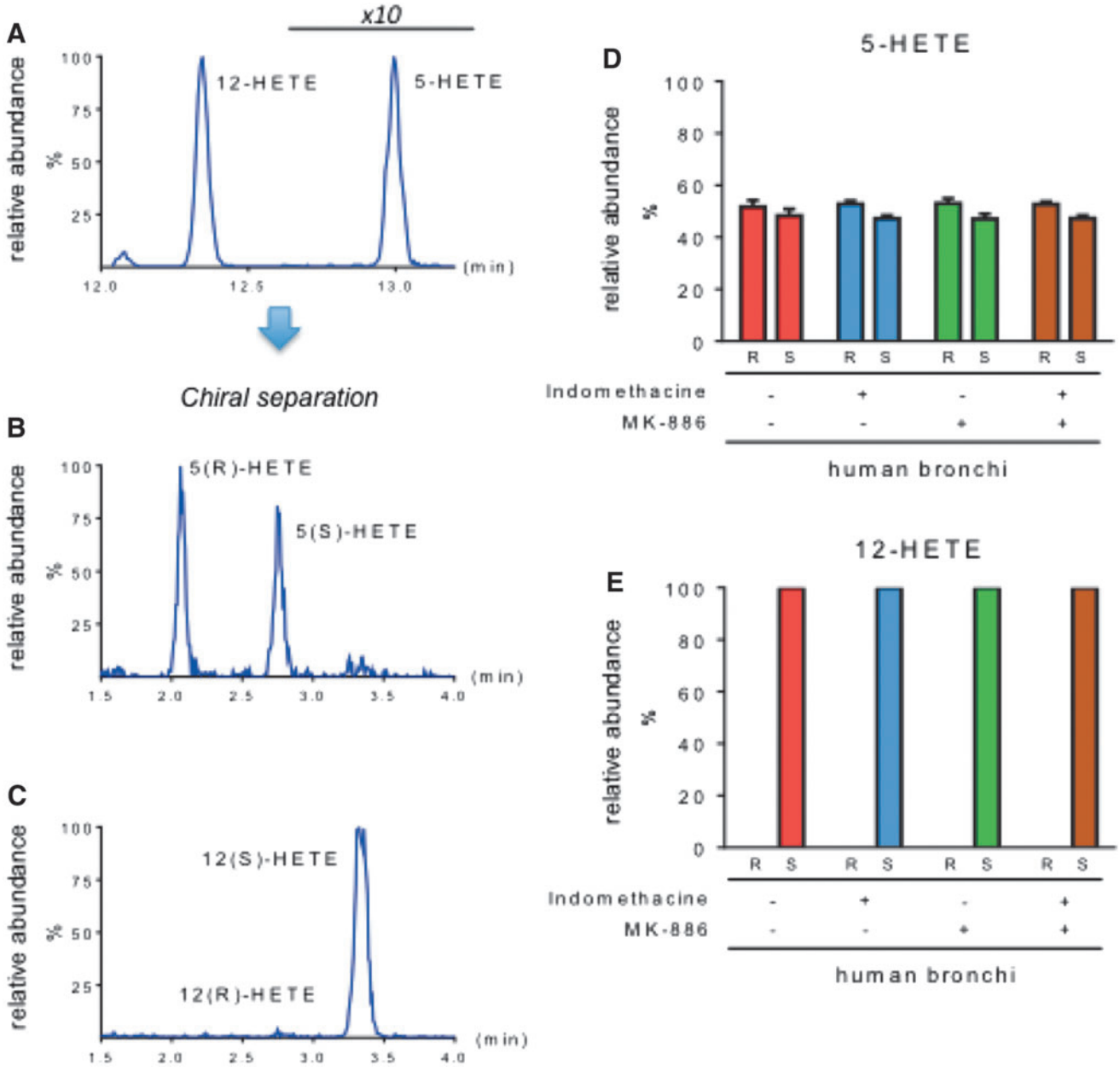

FIG. 7. Utility of chiral chromatography for eicosanoid metabolic profiling. Isolated bronchial rings from human lung were dissected and placed in culture plate wells as previously described (142). After 60-min incubation with indomethacin or the FLAP inhibitor MK866, 3.5 mL of Krebs–Ringer PSS buffer was subjected to eicosanoid profiling by LC-MS/MS as previously described (5). **(A)**. Nonchiral analysis of buffer solution identified both 12-HETE and 5-HETE. **(B)** Chiral analysis of the buffer solution showed that the 5-HETE peak from panel A consisted of an essentially equal mixture of 5(S)-HETE and 5(R)-HETE. **(C)** Chiral analysis of the buffer solution showed that the 12-HETE peak from panel A consisted of primarily 12(S)-HETE. **(D)**. The addition of MK866 had no effect on the ratio of 5(S)-HETE/5(R)-HETE, further indicating that 5-HETE was not formed *via* 5-LOX activity. **(E)** The quantified 12-HETE was >99% 12(S)-HETE, demonstrating that the route of formation was due to lipoxygenation activity. MS, mass spectrometry. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Metabolic diseases:

	COX	LOX	CYP
Obesity/T2D	↑PGE_2 (insulin dysfunction)	↑12-HETE, 15-HETE (stimulation of immune cells, insulin dysfunction)	20-HETE (unbalance)
CVD	↑PGE_2(arterial dilation, immune cells) PGE_2 - TXA_2 (unbalance)	↑ Cys-LTs, LTB_4 (advanced atherosclerosis)	20-HETE, EETs (unbalance)
NAFLD	↑PGD_2, LXA_2 (NAFL resolution) ↑PGE_2 (NASH)	↑ 5-HETE, 15-HETE (NAFL)	↑8,9-DHET, 11,12-DHET (NASH)

FIG. 8. Schematic representation of COX, LOX, and CYP pathway in metabolic diseases. Here we summarize the main eicosanoids involved in obesity/diabetes, CVD, and NAFLD. *Arrows* indicate increased levels of a specific metabolite. CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease.

current limit is around 3 μm, compared with UHPLC columns that offer 1.8 μm particle size. However, even these limitations are being addressed as demonstrated by the development of an LC-MS/MS method for the analysis of EET enantiomers (83). This method is capable of simultaneously quantifying all eight EET enantiomers with an LOQ in the low pg range (Fig. 7).

The field of eicosanoid and lipid mediator profiling will continue to expand with the development of new instrumentation and increased availability of analytical standards. For example, the range of SPM standards available for purchase has recently greatly expanded to include many of the resolvins, protectins, and maresins, as well as the glutathione conjugates (*e.g.*, MCTR1). However, a continued issue will be the need to balance specificity with speed of analysis. In particular, for the SPMs, it is strongly suggested that the published protocols requiring a minimum of 6 diagnostic ions are used (28). Given the structural complexity of these compounds in combination with stereospecificity, a combination of specific targeted profiling methods with chiral chromatography is required to ensure unambiguous identification. In addition, given the low endogenous levels of these compounds, targeted LC-MS/MS-based methods will continue to be necessary to study them in most biological systems. It is not feasible to detect most of these compounds *via* more global metabolomics-based approaches.

Conclusions and Future Directions

Eicosanoids generated through the COX, LOX, or CYP pathways are implicated in low-grade inflammation associated with immunometabolic disturbances such as obesity, insulin resistance, and T2D, as well as atherosclerosis and NASH (Fig. 8). Activation of these pathways occurs *via* multiple immunologic and nonimmunologic cell stimuli. Oxidative stress generates ROS that will trigger radical initiated lipid peroxidation and attenuation of the intracellular hydroperoxide tone. Lipid hydroperoxides are important activators of COX and LOX, thus turning on AA metabolism and eicosanoid production. The AA cascade has previously been successfully targeted for development of anti-inflammatory, antithrombotic, analgesic, and antipyretic drugs, as well as medications against asthma and glaucoma. These existing medications, along with next-generation drugs with different modes of actions, for instance. PG receptor antagonists, or selected enzyme inhibitors and receptor antagonists in the 5-LOX pathway, are exciting candidates for preventive and/or therapeutic use in immunometabolic diseases. The emerging family of SPMs derived from ω3 polyunsaturated fatty acids offers molecular explanations and a rationale for life-style and diet regimens as a means to manage immunometabolic diseases. Moreover, these lipid mediators may be used as templates for development of analogs that can act as agonists at receptors transducing proresolving signaling pathways. It is clear that further research is needed to unlock the full potential of eicosanoids and related lipid mediators in design of therapeutic strategies against immunometabolic diseases.

Acknowledgments

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Abbreviations Used

5-HpETE = 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid
AA = arachidonic acid
ALT = alanine aminotransferase
ApoE = apolipoprotein E
AT = adipose tissue
ATL = aspirin-triggered lipoxins
ATM = adipose tissue macrophage
BAT = brown adipose tissue
cAMP = cyclic adenosine monophosphate
CCl4 = carbon tetrachloride
CLP = coactosin-like protein
COX = cyclooxygenase
$cPLA_2$ = cytosolic phospholipase A2
CVD = cardiovascular disease
CYP = cytochrome P450
Cys-LTs = cysteinyl-leukotrienes
DHET = dihydroxy-eicosatrienoic acids
EETs = epoxyeicosatrienoic acids
FLAP = 5-lipoxygenase activating protein
GC/MS = gas chromatography coupled to mass spectrometry
GPCRs = G protein-coupled receptors
GSH = glutathione
HETEs = hydroxy-eicosatetraenoic acids
HFD = high-fat diet
HODEs = hydroxyoctadecadienoic acids
HpETEs = hydroperoxyeicosatetraenoic acids
HPLC = high-performance liquid chromatography
IL = interleukin
JNK = Jun N-terminal kinase
KCs = kupffer cells
LC/EC/APCI/MS = liquid chromatography electron capture atmospheric pressure chemical ionization mass spectrometry
LDLr = low-density lipoprotein receptor
LOD = limit of detection
LOX = lipoxygenase
LT = leukotriene
LTA_4H = LTA_4 hydrolase

Abbreviations Used (Cont.)

LTC_4S = LTC_4 synthase
LXs = lipoxins
MAPK = mitogen-activated protein kinase
MCP-1 = monocyte chemotactic protein 1
MMP = metalloproteinase
mPEGS-1 = microsomal prostaglandin E synthase 1
MS = mass spectrometry
NADPH = nicotinamide adenine dinucleotide phosphate
NAFLD = nonalcoholic fatty liver disease
NASH = nonalcoholic steatohepatitis
NFκB = nuclear factor κB
NSAID = nonsteroidal anti-inflammatory drug
PFB = pentafluorobenzyl esters
PGHS = prostaglandin H synthase
PGI_2 = prostacyclin
PGs = prostaglandins
PLA_2 = phospholipase A_2 superfamily
PP = protein phosphatase
ROS = reactive oxygen species
sEH = soluble epoxide hydrolase
SPM = specialized pro-resolving mediators
T2D = type 2 diabetes
TNFα = tumor necrosis factor α
TX = thromboxanes
UHPLC = ultrahigh-performance liquid chromatography
VLDL = very low-density protein
VSMC = vascular smooth muscle cell
WAT = white adipose tissue
WT = wild type