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Review Article

The octadecanoids: an emerging class of lipid mediators

Alessandro Quaranta[1], Johanna Revol-Cavalier[1,3] and Craig E. Wheelock[1,2,4]

[1]Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden; [2]Department of Respiratory Medicine and Allergy, Karolinska University Hospital, 171 76 Stockholm, Sweden; [3]Larodan Research Laboratory, Karolinska Institutet, 171 65 Stockholm, Sweden; [4]Gunma University Initiative for Advanced Research (GIAR), Gunma University, Maebashi, Gunma 371-8511, Japan

Correspondence: Craig E. Wheelock (craig.wheelock@ki.se)

Oxylipins are enzymatic and non-enzymatic metabolites of mono- or polyunsaturated fatty acids that encompass potent lipid mediators including the eicosanoids and docosanoids. Previously considered of low interest and often dismissed as 'just fat', octadecanoid oxylipins have only recently begun to be recognized as lipid mediators in humans. In the last few years, these compounds have been found to be involved in the mediation of multiple biological processes related to nociception, tissue modulation, cell proliferation, metabolic regulation, inflammation, and immune regulation. At the same time, the study of octadecanoids is hampered by a lack of standardization in the field, a paucity of analytical standards, and a lack of domain expertise. These issues have collectively limited the investigation of the biosynthesis and bioactivity of octadecanoids. Here, we present an overview of the primary enzymatic pathways for the oxidative metabolism of 18-carbon fatty acids in humans and of the current knowledge of the major biological activity of the resulting octadecanoids. We also propose a systematic nomenclature system based upon that used for the eicosanoids in order to avoid ambiguities and resolve multiple designations for the same octadecanoid. The aim of this review is to provide an initial framework for the field and to assist in its standardization as well as to increase awareness of this class of compounds in order to stimulate research into this interesting group of lipid mediators.

Introduction

Oxylipins are broadly defined as enzymatic metabolites of mono- or polyunsaturated fatty acids (PUFAs) produced by at least one step of mono- or dioxygenase-catalyzed oxygenation as well as non-enzymatic products [1]. These lipids are involved in multiple biological processes including inflammation and immune activation, tissue modulation and cellular proliferation, ion transport and airway smooth muscle contraction [2–6]. Oxylipins can be classified according to the length of the carbon chain of the parent fatty acid (e.g. docosanoids (C-22), eicosanoids (C-20), and octadecanoids (C-18)). The most well-known oxylipins are the eicosanoids, which are produced from 20-carbon-containing PUFAs (e.g. dihomo-γ-linolenic acid, arachidonic acid, and eicosapentaenoic acid (EPA)) and have been demonstrated to exert profound function in multiple physiological processes [4,6,7]. More recently, the docosanoids have emerged, with a focus on the omega-3 fatty acid-derived specialized pro-resolving mediators (SPMs) (e.g. resolvins, protectins, and maresins) [8]. In contrast, the role of octadecanoids in human physiology has been largely overlooked as opposed to plant biology. In plants, octadecanoids play a pivotal role as the main class of phytohormones and are involved in the regulation of biotic and abiotic stress signaling, as well as in plant growth and development processes, especially via the Jasmonate pathway [9]. In mammals, the 18-carbon fatty acids have to date been primarily considered as building blocks for

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longer-chain PUFAs given that linoleic acid (LA) and α-linolenic acid (ALA), possessing unsaturations on the ω-6 and ω-3 carbon, respectively, are essential fatty acids that cannot be produced by mammalian enzymatic systems (Figure 1) [10].

The 18-carbon fatty acids are by far the most abundant long-chain PUFAs consumed in the western diet, with LA and ALA accounting for 7.2% and 0.7% of the total energy intake, respectively, in the average USA diet [11]. These levels mark a 3-fold and 2-fold increase for LA and ALA, respectively, compared to the early 20th century. At the same time, only a small fraction of these dietary 18-carbon fatty acids is converted to longer-chain PUFAs (e.g. only 10% of ALA is elongated to EPA) [12]. The majority is incorporated into membranes where it has structural functions, but can be oxidized by the same enzymatic systems that form eicosanoids or by non-enzymatic oxidation, both *in situ* or after release by phospholipases [13]. The 18-carbon PUFAs are generally poorer substrates for oxidative enzymes, compared to 20- or 22-carbon PUFAs [14,15]; however, their endogenous levels are normally higher [16,17] which is reflected in the subsequent oxylipin concentrations. For example, human serum levels of selected octadecanoids were reported to be 5 times higher compared to oxylipins from longer-chain PUFAs (the summed concentration of the 21 detected octadecanoids was ∼158 ng/ml, compared to ∼29 ng/ml for the 54 detected eicosanoids/docosanoids [16]). The bioactivity of the majority of these compounds is still largely unexplored [6], and dedicated analytical methods are needed in order to expedite their study [18]. Recent studies have started unveiling the importance of octadecanoids in human physiology, highlighting their involvement in inflammation and immune modulation [19–21], in the mediation of metabolic processes [22–25], in cell proliferation and tissue structure modulation [26], as well as in pain transmission and nociception [27]. Simultaneously, the expansion of knowledge on octadecanoids is hampered by a lack of field harmonization, a paucity of analytical standards, and limited domain expertise.

The scope of this review is to provide a framework on the knowledge of octadecanoids to date, focusing on the primary oxidative metabolism mechanisms and products in humans for the major 18-carbon fatty acids: oleic acid (OA), LA, ALA, and γ-linolenic acid (GLA). The primary known biological effects exerted by these metabolites in humans are also briefly discussed, while metabolic processes exclusive to plants are excluded because many comprehensive reviews already exist [9,28,29]. Additionally, we propose a harmonization of the octadecanoid nomenclature based upon defined rules in use for eicosanoids.

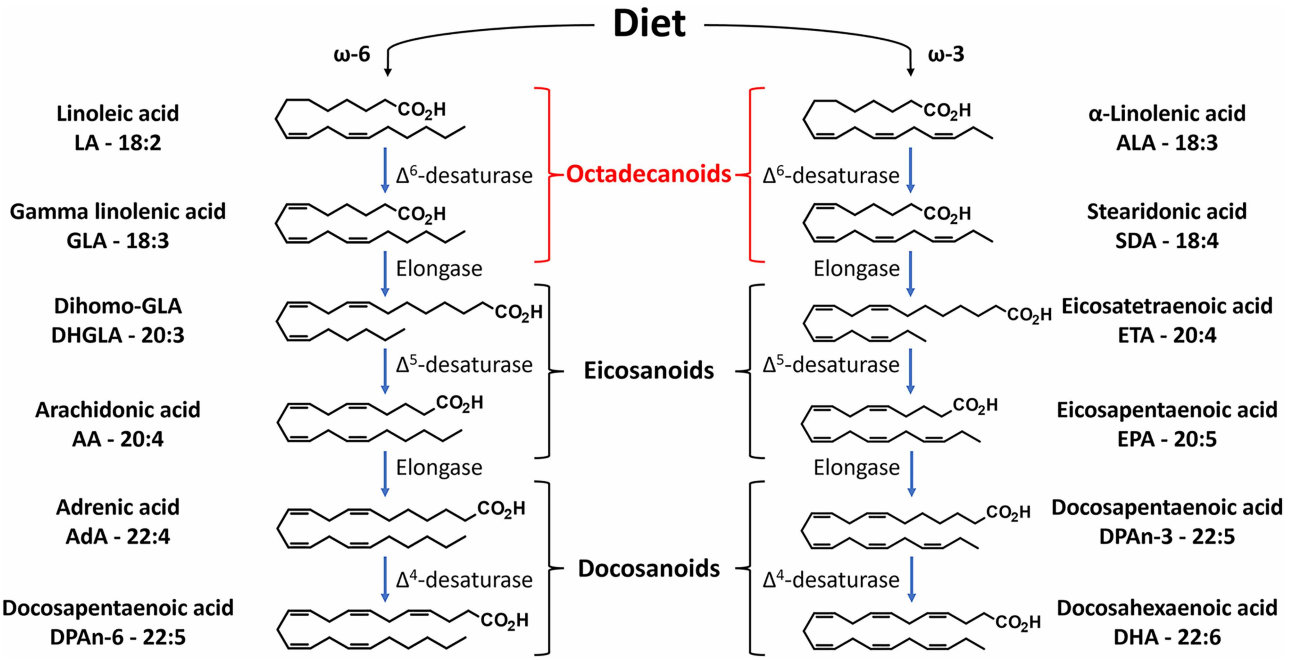

Figure 1. Biosynthesis pathways for ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) and the resulting class of oxylipin.

Linoleic (LA) and α-linolenic acid (ALA) are essential fatty acids that must be obtained via the diet. These essential fatty acids are converted via a series of desaturation and 2-carbon elongation processes to produce the longer-chain PUFAs.

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Octadecanoid nomenclature

The absence of a set nomenclature is a limiting factor for the development of the octadecanoid field, introducing unclarity in the comparison of different studies. Octadecanoids are generally abbreviated following the same criteria used for eicosanoids; however, the abbreviations are sometimes used ambiguously (e.g. 'T' indicating both trienoic and tetraenoic, depending on the user [30]), or replaced by generic or research group-specific abbreviations (e.g. 9-KOTrE defined as 9-oxo-OTA (9-oxo-octadecatrienoic acid) [31], or 10-HOME described as Hydroxide A or HYA [32]). To harmonize octadecanoid nomenclature, we propose the following explicitly defined abbreviation system, considering that the oxidative metabolism of 18-carbon fatty acids results in the introduction of a limited number of oxygenated functions, namely hydroperoxides, epoxides, ketones, monohydroxyls, diols, and triols. The abbreviation of each compound starts with the position of the main oxygenated function (multiple different functions are expressed in order of decreasing polarity), followed by the abbreviation of the function ('Hp' for hydroperoxides, 'H' for mono-hydroxyl, 'DiH' for diols, 'TriH' for triols, 'K' for oxo, and 'Ep' for epoxide), then by a capital 'O' indicating 'octadeca' (similarly to how 'E' is used for 'eicosa'), and then by the number of unsaturations ('M': mono-unsaturated; 'D': di-unsaturated; 'Tr': tri-unsaturated; 'T': tetra-unsaturated). Finally, an 'E' (for 'enoic') indicates that the species is unsaturated. For saturated compounds the 'E' is replaced by 'DA' (for '**D**ec**A**noic'). With this system, **9**-oxo-**o**ctadeca**trie**noic acid is 9-KOTrE, while 10-hydroxyoctadecenoic acid (**10**-**h**ydroxy-**o**ctadeca**m**ono**e**noic acid) is 10-HOME. A scheme showing the application of this nomenclature to LA and ALA metabolites is shown in Figure 2. When known, the chirality and geometry should be indicated in terms of the hydroxy and epoxide moieties and the unsaturation(s) (e.g. *R*/*S*, *cis*/*trans*, *threo*/*erythro*, *E*/*Z*). According to IUPAC nomenclature, a '=O' moiety bonded to the corresponding numbered carbon is indicated by the term 'oxo'(33); however, we propose the use of 'K' ('keto') to

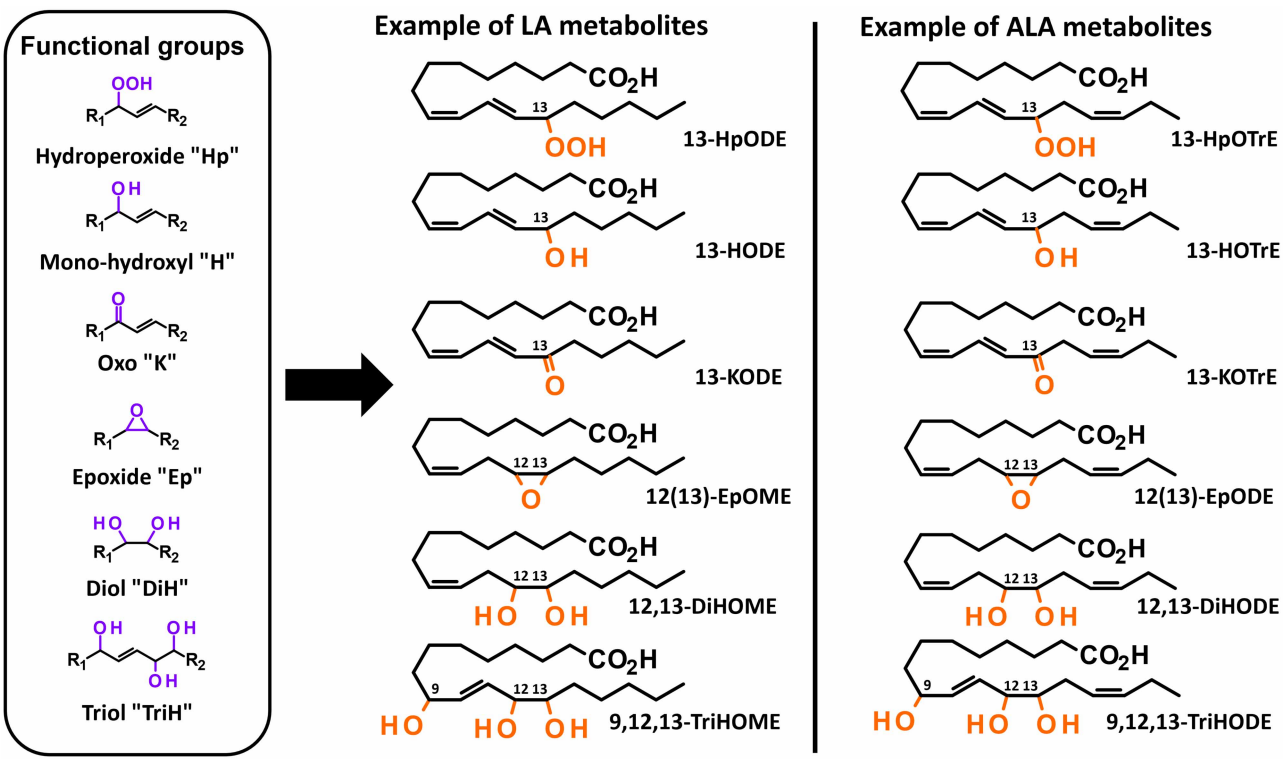

Figure 2. Proposed abbreviated nomenclature system for octadecanoids.

Compound names start with the positioning of the oxygenated moiety on the carbon backbone. The number(s) is followed by the abbreviation of the moiety ('Hp' for hydroperoxides, 'H' for mono-hydroxyl, 'DiH' for diols, 'TriH' for triols, 'K' for oxo, and 'Ep' for epoxide) and by the alkyl chain length (for octadecanoids, a capital 'O') and by the number of unsaturations ('M': mono-unsaturated; 'D': di-unsaturated; 'Tr': tri-unsaturated; 'T': tetra-unsaturated). The last letter indicates if the compound is unsaturated ('E' for enoic, unsaturated; 'DA' for decanoic, saturated). For example, with this system, **9,10**-**ep**oxy **o**cta**d**eca**n**oic acid is 9(10)-EpODA.

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avoid cacophonic and difficult to pronounce names such as 9-Oxo-ODE, and to maintain an identifiable link in series of metabolites (e.g. the series of LOX-derived 9-HpODE — 9-HODE — 9-KODE). For species possessing more than one oxygenated function, the functions are expressed in order of increasing polarity (hydroperoxyls > hydroxyls > ketones > epoxides). The following suffixes are used: 'Hp' for hydroperoxides, 'OH' for hydroxyls, 'DiOH' for diols, 'TriOH' for triols, and 'oxo' for ketones (e.g. 10(*E*)-9-oxo-12(13)-EpOME, 9(*Z*)-11-OH-12(13)-EpOME). The position of the double bonds is always indicated for metabolites presenting multiple functions. A list of the full nomenclature for many of the octadecanoid species discussed in this paper is available in a recent publication [18].

Oxidative metabolism of 18-carbon fatty acids

Oxidative metabolism of 18-carbon fatty acids is primarily performed by enzymes belonging to the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) families. Humans possess two COX isoforms, COX-1 and COX-2, both of which are heme-containing dioxygenases with peroxidase and COX functions. While COX forms cyclic oxylipins from 20- and 22-carbon fatty acids via the COX activity, interaction with 18-carbon fatty acids is limited to the peroxidase activity [14]. The expression of these isoforms is generally different; COX-1 is ubiquitously expressed in tissues while COX-2 is synthesized in response to inflammatory stimuli [33,34]. This distinction, however, is not strict and the inducibility of COX-1 following an external stimulus, as well as constitutive expression of COX-2 in many tissues, have been observed [35]. Both isoforms have strong affinity for arachidonic acid, from which they produce eicosanoids including prostaglandins and thromboxanes, but show variable affinity for 18-carbon PUFAs. While LA is a substrate for both isoforms, ALA is metabolized only by COX-2 [14,36].

LOX are also dioxygenases, but do not contain a heme moiety. Humans possess genes encoding for six LOX isoforms: 5-LOX, 12(*S*)-LOX, 12(*R*)-LOX, 15-LOX-1, 15-LOX-2, and eLOX3 [37]. 5-LOX and 15-LOX-1 are highly expressed in leukocytes, with 15-LOX-1 highly expressed in the lungs [38], while 12(*R*)-LOX and eLOX3 are exclusively expressed in the epidermis [39]. 15-LOX-2 and 12(*S*)-LOX are expressed in a multitude of tissues including skin, prostate, liver, colon, kidneys, and brain [40]. The different isoforms show variable affinity for 18-carbon fatty acids. For example, LA is a good substrate for 15-LOX-1, but has poor affinity for 15-LOX-2, and is not a substrate for 12(*S*)-LOX [41]. The eLOX3 isoform can metabolize 9(*R*)-HpODE, but no information on its affinity for other C-18 species has been reported [42].

Both COX and LOX catalyze the specific abstraction of a hydrogen atom from *bis*-allylic positions in PUFAs possessing a 1,4-*cis* pentadiene moiety. While COX abstracts specifically the *pro*-(*S*) proton [43], abstraction by LOX depends on the enzyme isoform [44]. Oxidation by COX and LOX results in the production of stereochemically defined hydroperoxides, which are subsequently reduced to monohydroxyls by peroxidases. Monohydroxyls are stable LOX metabolites that can also be further oxidized to ketones by fatty acid dehydrogenase [45,46]. The main stable COX metabolites of LA are 9(*R*)- and 13(*S*)-HODEs, with a preference for the 9-isomer (82% and 67% for COX-1 and COX-2, respectively) [47], whereas LOX produces 13(*S*)-HODE. The epidermal isoform 12(*R*)-LOX is able to produce 9(*R*)-HODE as well [48]. Finally, eLOX3 has a hydroperoxide isomerase function that can metabolize 9(*R*)-HpODE and 13(*S*)-HpODE into hydroxy-epoxides (11-OH-9(10)-EpOME and 13-OH-9(10)-EpOME from the former, 9-OH-12(13)-EpOME and 11-OH-12(13)-EpOME from the latter), which can be further hydrolyzed to triols (9,10,13-, 9,10,11-, 11,12,13-, and 9,12,13-TriHOMEs, with stereochemistry depending on the formation route) or oxidized to the respective epoxy-ketones [48]. ALA and GLA are also metabolized by LOX, with 15-LOX-1 transforming ALA to 9(*S*)-HOTrE, which is subsequently oxidized to 9-KOTrE, while 5-LOX produces 13(*S*)-HOTrE (and 13-KOTrE) [31,49], whereas GLA is metabolized to 6(*Z*),9(*Z*),11(*E*)-13-HOTrE (13-HOTrE-γ) and 6(*Z*),8(*E*),12(*Z*)-10-HOTrE by LOX enzymes in human platelets [50]. Additionally, since ALA and GLA possess two pentadiene moieties, they can be targeted by two sequential LOX oxidation, yielding non-vicinal diols. This mechanism has been reported for ALA resulting in the production of 9,16-DiHOTrE [51], but not for GLA. COX oxidation of ALA results in the formation of 12-HOTrE following hydrogen abstraction from the ω-5 position [14]. While GLA is known to be a substrate of COX-2, the resulting products have not been characterized [14]. As opposed to the eicosanoids, the production of cyclic metabolites from COX oxidation has not been described for octadecanoids.

CYP450 is a superfamily of ubiquitous monooxygenases participating in reductive, oxidative, and peroxidative metabolism of a variety of endogenous chemicals and xenobiotics, including fatty acids [52]. Humans possess 57 genes coding for CYP enzymes, widely distributed in many tissues and highly expressed in brain, liver, lungs, and kidneys [53]. CYP enzymes are deeply involved in lipid metabolism, for which they catalyze

four main reactions: epoxidation, *bis*-allylic hydroxylation, hydroxylation with double-bond migration (allylic hydroxylation), and ω-side chain hydroxylation [54]. All 18-carbon fatty acids are CYP450 substrates and are mainly metabolized to *cis*-epoxides, which can subsequently be converted to vicinal *erythro*-diols by the soluble epoxide hydrolase (sEH) [55]. Stable epoxides and related vicinal diols have been reported for LA (9(10)-EpOME and 12(13)-EpOME and the respective diols 9,10-DiHOME and 12,13-DiHOME [55]), ALA (9(10)-EpODE, 12(13)-EpODE, 15(16)-EpODE and the respective diols 9,10-DiHODE, 12,13-DiHODE, and 15,16-DiHODE [56]), and OA (9(10)-EpODA and 9,10-DiHODA [57]), whereas only epoxides have been reported for GLA (6(7)-EpODE-γ, 9(10)-EpODE-γ, and 12(13)-EpODE-γ [56]). Numerous mono-hydroxides produced by the other described mechanisms have been reported for LA [55,58], while ω-side chain hydroxyls have been also reported for ALA and OA [59,60]. An overview of the metabolic mechanisms applied to the oxidation of LA by the discussed enzymatic systems is shown in Figure 3.

In addition to human enzymatic systems, octadecanoids can also be produced by symbiotic microbes and fungi, such as the gut microflora, and from pathogenic microorganisms. These microorganisms can contribute to the octadecanoid pool of the host by metabolizing both 18-carbon fatty acids and oxylipins (e.g. by hydrolyzing EpOMEs to DiHOMEs via bacterial EH [19]). Moreover, gut bacteria possess specific sets of enzymes responsible for the saturation of 1,4-*cis* pentadienes, which are toxic [61,62], resulting in the production of specific octadecanoids that can be transferred to the host. The identified metabolites from LA and ALA and the enzymes responsible for their formation in *Lactobacillus plantarum* were first described by Kishino et al. [61].

Together with endogenous metabolites, a large variety of plant and fungal octadecanoids can also be introduced via the diet. Plant and fungi contain high concentrations of octadecanoids, which can have structures specifically derived by plant enzymatic systems or share the same structure of human metabolites. It is, therefore, often challenging to understand the origin of a specific octadecanoid because different sources can produce compounds with the same structure. While metabolites exclusive to plants and fungi are not described in this review, the biological functions of common metabolites are discussed with no distinction related to their source. The main human enzymatic metabolites deriving from LA are displayed in Figure 4, while Figure 5 shows the primary metabolites from OA and ALA.

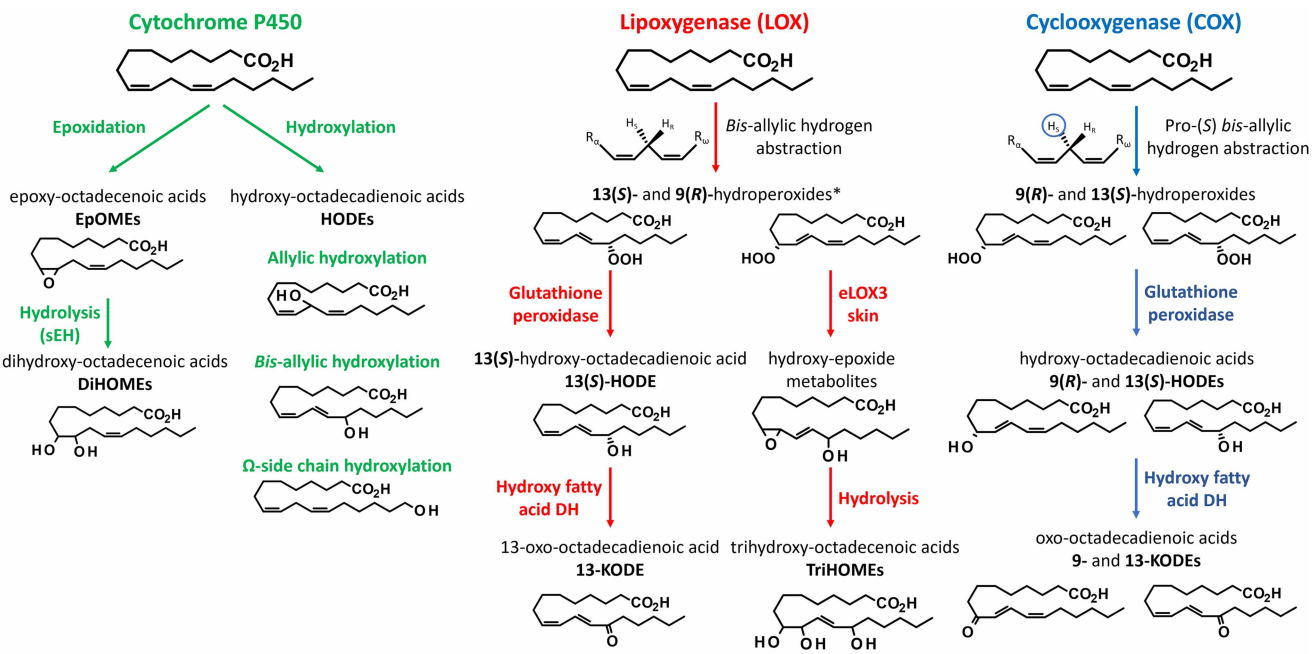

Figure 3. Principal mechanisms for the main enzymatic pathways of LA metabolism in humans.

*(*R*)-hydroperoxides in humans are exclusively formed by 12(*R*)-LOX in the epidermis and can be further metabolized to 9(*R*)-HODE or to hydroxy–epoxy metabolites by eLOX3 [48]. Mice have been shown to produce 9(*S*)-hydroperoxide from LA by 15-LOX-2, which is then metabolized to 9(*S*)-HODE [40]. sEH, soluble epoxide hydrolase; DH, dehydrogenase.

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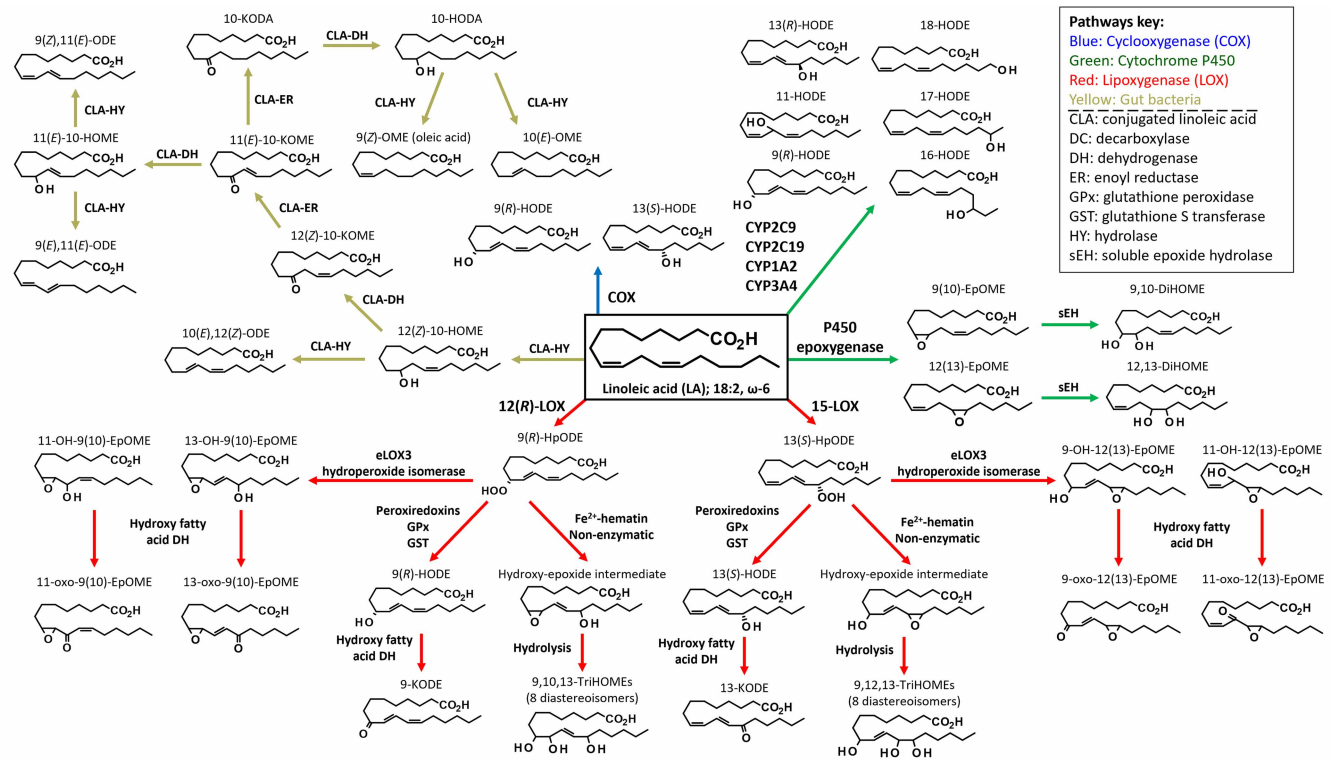

Figure 4. Primary enzymatic pathways for linoleic acid (LA)-derived octadecanoids in humans.
The yellow pathways indicate metabolites that are exclusively formed from symbiotic gut bacteria, as described by Kishino et al. [61].

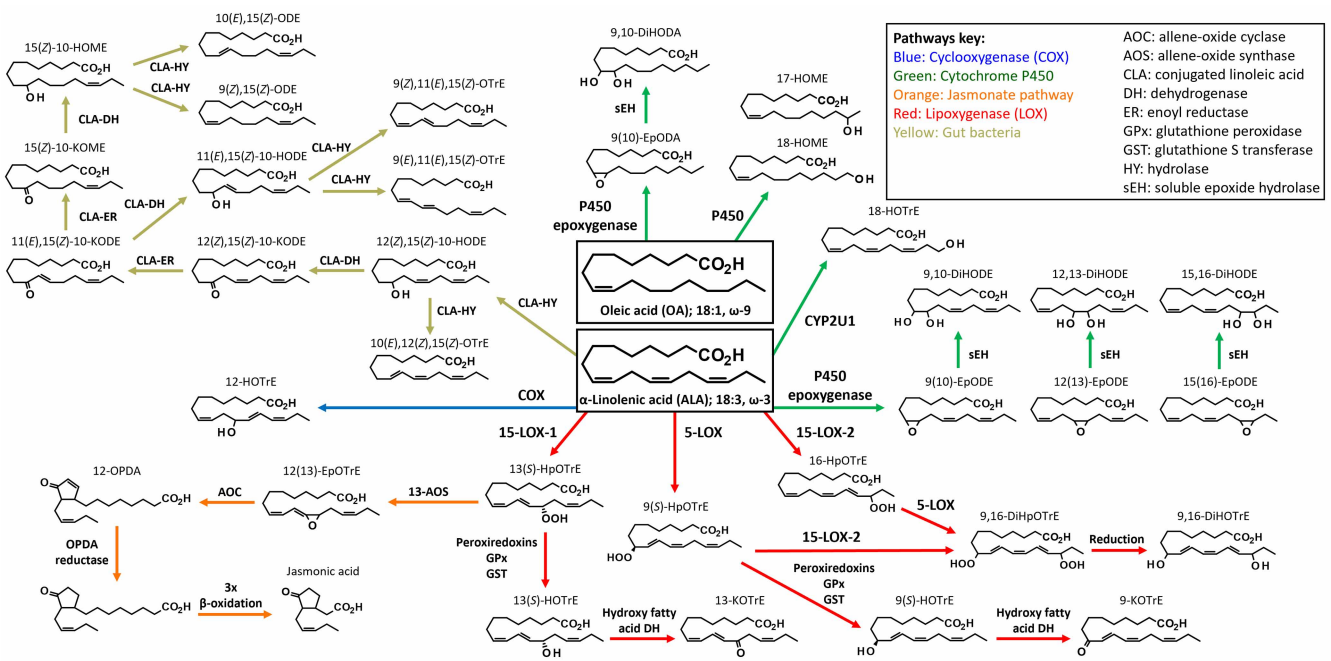

Figure 5. Primary enzymatic pathways for oleic acid (OA)- and α-linolenic acid (ALA)-derived octadecanoids in humans.
The yellow pathways indicate metabolites which are exclusively formed from symbiotic gut bacteria, as described by Kishino et al. [61]. The orange pathway is the 'Jasmonate pathway', a pathway present in plants that leads to the biosynthesis of the major class of phytohormones, the jasmonates. This pathway was historically considered the only biologically relevant pathway of octadecanoids and was named the 'Octadecanoid pathway' and is, therefore, shown in this figure for historical context.

Enzymatic biosynthesis is not the only source of octadecanoids from 18-carbon PUFAs, because 1,4-*cis* pentadienes are prone to oxidation through free radicals or to photosensitized oxidation, producing a high diversity of octadecanoid structures [63]. Linear octadecanoids produced by non-enzymatic oxidation may possess the same chemical structure of enzymatic octadecanoids but are synthesized with undefined stereochemistry (racemic mixtures). In addition, cyclic structures such as endoperoxides can be formed exclusively from autoxidation, while PUFAs possessing three or more unsaturations can produce two additional families of cyclic compounds: phytoprostanes (PhytoPs) and phytofurans (PhytoFs). The mechanisms of formation of non-enzymatic octadecanoids are outside the scope of this review and have been broadly reviewed elsewhere [64].

Biological activity

Historically, study of the biological effects of octadecanoids has focused on a limited number of LA metabolites, such as the CYP450 epoxides (EpOMEs) and diols (DiHOMEs), as well as the HODEs. In particular, the potent toxic effects exerted by 9(10)-EpOME were first identified by Ozawa et al. [65], who detected high concentrations of this compound in lung lavages of rats exposed to hypoxia and demonstrated its capacity to uncouple mitochondrial respiration, ultimately causing cell death and tissue damage, resulting in pulmonary edema. Because 9(10)-EpOME is synthesized by leukocytes, it was named 'leukotoxin' (and its regioisomer 12(13)-EpOME 'isoleukotoxin'). The toxic effects of leukotoxins were further explored and these compounds were found to induce heart failure when injected intravenously in dogs [66], possess vasoconstrictor effects in cat carotid arteries [67], and induce dysfunction in isolated rabbit renal cortical mitochondria [68]. Most remarkably, intravenous injection of both epoxides in mice caused acute pulmonary edema with dispersed bleeding spots, with a 100% mortality rate [69]. Further studies linked the toxic effect to the corresponding diols: 9,10-DiHOME and 12,13-DiHOME (leukotoxin-diol and isoleukotoxin-diol, respectively) [70]. Diols are produced from the epoxides by the sEH, which is ubiquitously expressed in human tissues and possess high conversion efficiency [71]. It was demonstrated that the diols and not the epoxides were responsible for mitochondrial dysfunction, leading to cell death in rabbit renal proximal tubular cells [72] and for the pulmonary edema causing acute respiratory distress syndrome (ARDS) in mice [73]. Additionally, mice administered with a sEH inhibitor to block the conversion of epoxides to diols had a significantly decreased mortality rate [73]. Recent studies have highlighted a potential role of the DiHOMEs in the pathophysiology of severe COVID-19 [74,75]. The biological role of 12,13-DiHOME has expanded beyond its cytotoxic effects as demonstrated by multiple recent papers that review different aspects of its biological activity [76–79]. In addition, our knowledge on the impact of octadecanoids other than EpOMEs and DiHOMEs on human pathophysiology has been amplified by the numerous studies discussed below.

Epidermal barrier formation

LA is the most abundant fatty acid in mammal skin [80]. Brash and colleagues have extensively investigated the role of LA-derived octadecanoids in epidermal barrier formation and found that LA is almost exclusively esterified in epidermis-specific ceramides [81]. Two of the six human LOX enzymes, eLOX3 and 12(*R*)-LOX, are highly expressed in the epidermis [39]. eLOX3 can metabolize 9(*R*)-HpODE to form hydroxy–epoxy metabolites, while 12(*R*)-LOX can oxidize LA to the 13(*R*)-HpODE. Oxidation of esterified LA plays a fundamental role for correct functioning of the skin. Esterified LA is oxidized to hydroxy-epoxides and the subsequent hydrolysis to triols facilitates its cleavage from skin ceramides and enables the formation of the corneocyte lipid envelope, fundamental to prevent transepidermal water loss [42]. There is also evidence that octadecanoids derived from other LA regioisomers are involved in the formation of the epidermis (e.g. sebaleic acid, 5(*Z*),8(*Z*)-octadecadienoic acid) [82]. *In vitro* experiments demonstrated that human sEH (EPHX2) as well as human and murine EPHX3 hydrolyzed LA-derived hydroxy-epoxides to the corresponding triols. The resulting stereochemistry of the triol formation matched that of the major triol isomers in human, murine, and porcine epidermis [42,83]. While microsomal epoxide hydrolase (mEH, EPHX1) was also tested, it was found to be inactive [83]. Follow-up *in vivo* experiments demonstrated the ability of EPHX3 to hydrolyze 11(*E*)-13(*R*)-OH-*trans*-9(*R*)(10(*R*))-EpOME, esterified in the epidermal ceramide in the outer layer of the murine epidermis, to the corresponding 9(*R*),10(*S*),13(*R*)-TriHOME [84]. Taken together, these collective findings highlight the role that esterified linoleates play in the formation of the mammalian epidermal water permeability barrier, while additional studies suggest that they may be a source of bioactive lipids formation [85].

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Nociception

The involvement of LA octadecanoids in nociception was first proposed by Ramsden and colleagues when they identified increased concentrations of hydroxy–epoxy and hydroxy-oxo octadecanoids in psoriatic skin lesions and observed increased scratching behavior after injection of these compounds in rodents. In particular, 9(*Z*)-11-OH-12(13)-EpOME and 12(*Z*)-11-OH-9(10)-EpOME sensitized primary afferent dorsal root ganglion (DRG) neurons in *ex vivo* calcitonin gene-related peptide (CGRP) release assays, and induced C-fiber-mediated pain-related hypersensitivity in rats; because these compounds share a 3-hydroxy-(*Z*)-pentenyl-*trans*-epoxide moiety, this was proposed as the pharmacophore mediating nociception [48]. Both these compounds were found to stimulate trigeminal neurons by eliciting Ca^{2+} responses, suggesting involvement in chronic headaches and craniofacial pain syndromes [86]. High levels of 9(*Z*)-11-OH-12(13)-EpOME and 11(*E*)-13-OH-9(10)-EpOME were detected for the first time in animal tissues in the brain of chronic pain model rats [87]. The former was capable of sensitizing nociceptors in the dorsal root ganglia and eliciting pain-related behavior *in vivo* [87], and its plasma concentrations inversely correlated with pain reduction in a small clinical trial manipulating LA and omega-3 PUFAs in the diet of people suffering from chronic daily headaches [48].

Among the other LA-derived octadecanoids, 9- and 13-HODEs and KODEs were found to be involved in nociception via interaction with the capsaicin receptor TRPV1 (transient receptor potential cation channel subfamily V member 1). These compounds were formed in rodent biopsies after exposure to noxious heat and were released in large amounts following cell injuries [88]. Blocking the production or the action of these octadecanoids substantially decreased the heat sensitivity of TRPV1 in rats and mice and reduced nociception. 9-KODE and its precursor 9-HODE were found to be the most abundant oxylipins in human skin, confirming the high activity of 12(*R*)-LOX [27], and systemic levels of both 9- and 13-HODE directly correlated with increased pain scores in women suffering from chronic neck pain [89]. TRPV1 was also activated by 12,13-DiHOME resulting in thermal pain hypersensitivity [90]. A systematic investigation of the molecular mechanisms behind nociception and inflammation integrating transcriptomics and lipidomics measurements was published by Domenichiello et al. [27]. The results highlighted the unique position of octadecanoids in pain modulation due to their high abundance in skin and their possibility to interact with receptors involved in the pain circuit.

Tissue modulation and cell proliferation

The monohydroxyls produced by oxidation of LA, 9- and 13-HODEs, are PPAR-γ agonists and were found to increase the uptake of 18-carbon fatty acids in monocyte cell lines. Additionally, 9-HODE alone increased the uptake of 16-carbon fatty acids and induced apoptosis in the same cell lines [91]. Activation of PPAR-γ by the HODEs induced monocyte maturation to macrophages [92] and resulted in antiproliferative and pro-apoptotic effect in Caco2 cell lines [93]. Chiral characterization determined the opposite effect of the (*S*)- and (*R*)-enantiomers in Caco2 cell lines, with only the (*S*)-enantiomer being responsible for the antiproliferative effect. Conversely, the (*R*)-enantiomer activated the BLT-1 and BLT-2 receptors and induced cell proliferation through COX activation. 13(*S*)-HODE was also found to downregulate and deactivate PPAR-δ, restoring apoptosis in colorectal cancer cells [94] and inhibiting cell growth in a time- and dose-dependent fashion in MCF-7 and MDA-MB231 breast cancer cell lines [95]. Moreover, 13(*S*)-HODE specifically blocked the pro-metastatic effect of the eicosanoid 12(*S*)-HETE, which enhanced surface expression of integrin α11bβ3 in lung tumor cells, both in low and high metastatic B16 cell lines [96]. Other than cell proliferation, 13-HODE is also the major chemorepellent in blood vessels endothelium, to which it imparts flexibility and thromboresistance [97]. A comprehensive review describing the effects of the HODEs in the regulation of inflammation in metabolic syndrome, cell adhesion, apoptosis, and mitogenesis in cancer has been published by Vangaveti *et al.* in 2016 [26].

Metabolic and hormone regulation

Octadecanoids have been shown to affect hormone modulation and lipid metabolism. At low concentration, 12,13-DiHOME acts as a lipokine by promoting insulin sensitivity and glucose tolerance and by stimulating brown adipose tissue (BAT) activity [25]. Levels of 12,13-DiHOME were found to negatively correlate with body-mass index and insulin resistance, and injections of 12,13-DiHOME activated BAT fuel uptake and enhanced cold tolerance, which resulted in decreased levels of serum triglycerides [25]. Acute 12,13-DiHOME treatment of mice *in vivo* increased skeletal muscle fatty acid uptake and oxidation, but not glucose uptake [24]. The LA derivative 10(*E*)-9-oxo-12(13)-EpOME (commonly known as EKODE) is known to regulate

aldosterone production *in vitro*, and can favor the onset of hypertension [98]. Lipid metabolism can be altered by octadecanoids such as 13-HODE and ketones oxidized on C-9. It was shown that 13-HODE can interfere with the assembly and composition of triacylglycerol-rich lipoproteins secreted by intestinal cells and decrease the secretion of triacylglycerol in Caco-2 cells [99]. The ALA-derived ketone 9-KOTrE was found to promote fatty acid uptake and oxidation via activation of PPAR-α and by inducing the mRNA expression of PPAR-α target genes [31]. The same effect was caused by 10(*E*),12(*E*)-9-KODE, a LA-derived ketone found in tomato fruits, which was also reported to inhibit cellular triglyceride accumulation in hepatocytes [100]. These effects were confirmed *in vivo* on KK-Ay obese mice fed a high-fat diet, for which treatment with 10(*E*),12(*E*)-9-KODE decreased the levels of plasma and hepatic triglycerides, suggesting a possibility to improve obesity-induced dyslipidemia and hepatic steatosis [101].

Inflammation and immune modulation

Various octadecanoids can regulate inflammation and possess anti-inflammatory activity. The LA metabolite 13-KODE induces transcriptional repression of pro-inflammatory factors followed by amelioration of colitis in animal models of inflammatory bowel disease (IBD), via the activation of the PPAR-γ receptor [102]. Activation of this receptor by 13(*S*)-HpOTrE or 13(*S*)-HOTrE inactivates the NLRP3 inflammasome complex leading to the downregulation of LPS-induced pro-inflammatory markers in LPS-challenged RAW 264.7 cells and mouse peritoneal macrophages. Additionally, the anti-inflammatory effects of these two metabolites were mediated by the induction of apoptosis and inhibition of autophagy in the LPS-challenged macrophages [20]. The generation of epoxy metabolites from LA during inflammation resolution constitutes an important control point limiting the accumulation of pro-inflammatory Ly6chi monocytes [103]. The diol 9,16-DiHOTrE significantly decreased the levels of prostaglandins synthesized by recombinant COX-1. Both the 9(*R*)/16(*S*)- and the 9(*S*)/16(*S*)-stereoisomers exhibited an inhibitory effect on human recombinant COX-2, which is a major mode of action to reduce inflammation. Additionally, the 9(*R*)/16(*S*)-isomer caused a decrease in pro-inflammatory LTB_4 and 5(*S*)-HETE formation from arachidonic acid, indicating the additional inhibition of the 5-LOX pathway [51].

The DiHOMEs have been suggested to function as key drivers of immune cell dysfunction in severe burn injury through hyperinflammatory neutrophilic and impaired monocytic actions [104], further suggesting that inhibition of the sEH might be a useful therapeutic target for treating burn patients. Lynch and co-workers [21] found that levels of 12,13-DiHOME produced by gut bacteria in neonates correlated with the propensity to develop atopic asthma. A follow-up study provided a mechanistic link showing that perturbation of the gut microbiome resulted in the overexpression of bacterial epoxide hydrolase genes, which produced increased levels of 12,13-DiHOME. This, in turn, impeded immune tolerance and promoted allergic inflammation by altering the cell immune metabolism [19].

Microbiome-derived octadecanoids

The deep connection between host and symbiotic microflora is attracting increasing attention, showing important biological actions of microbial metabolites on the host health status. In particular, octadecanoids produced by gut bacteria enzymatic oxidation on C-10 of LA and ALA have been shown to modulate a variety of metabolic and hormonal processes via interaction with various PPARs, TRPV1, and GPCR40. The LA metabolites 12(*Z*)-10-HOME and 11(*E*)-10-HOME are both PPAR-α agonists [105]. The 12(*Z*)-isomer was recognized by GPCR40 with higher affinity than LA, leading to amelioration of intestinal inflammation in dextran sulfate sodium (DSS)-induced colitis in mice [32] and TNFR2, through which it was found to regulate intracellular levels of glutathione and trigger antioxidant/detoxifying effects, which in turn are responsible for its immunomodulatory ability [106].

The production of 12(*Z*)-10-HOME from gut bacteria diverts excess of LA from the arachidonic acid cascade reducing the production of pro-inflammatory mediators [22]. Acute administration of 12(*Z*)-10-HOME promoted intestinal peristalsis (via activation of the EP3 receptor in the gut) and increased the secretion of GLP-1, which caused appetite suppression, improved insulin resistance and glucose homeostasis, and conferred protection against fat-induced obesity in mice [22].

Ketones produced from the hydroxides via conjugated linoleic acid dehydrogenase (CLA-DH) were also found to interact with the same receptor systems. Both 12(*Z*)-10-KOME and 11(*E*)-10-KOME are PPAR-α agonists, whereas only the 12(*Z*)- isomer shows significant PPAR-γ activation, resulting in increased insulin-stimulated glucose uptake and induction of adipocytes differentiation in 3T3-L1 cells [105]. 12(*Z*)-10-KOME was also found to be a potent activator of TRPV1 and enhanced noradrenalin turnover in adipose tissues,

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resulting in the improvement in the conditions of glucose intolerance, insulin resistance, and increased adiposity in obese and diabetic-model mice [23]. The 11(*E*) isomer, on the other hand, stimulated the secretion of gut hormones such as cholecystokinin through interaction with GPCR40 [107]. The same effect was observed upon administration of the ALA metabolite 9(*Z*),15(*Z*)-13-KODE, which in addition lowered the gastric emptying rate in rats upon oral administration [107] and, together with 9(*Z*),15(*Z*)-13-HODE, induced differentiation of anti-inflammatory M2 macrophages [108].

Perspectives

- 18-carbon fatty acids are the most abundant long-chain PUFAs consumed in the Western diet. These compounds can be converted to downstream metabolic products collectively called octadecanoids, which are increasing being established as functional lipid mediators. Given the high consumption of 18-carbon fatty acids, the octadecanoid lipid mediators have a commensurate elevated concentration relative to the traditional eicosanoid and docosanoid lipid mediators, increasing the potential for these compounds to exert physiological effects.

- Octadecanoids have been demonstrated to exert functional roles in multiple biological functions including epidermal barrier formation, inflammation and immune modulation, metabolic processes, cell proliferation and tissue structure modulation, as well as pain transmission and nociception. However, there remains a lack of knowledge regarding the biological activity of known octadecanoids as well as a need for the discovery and characterization of new species, particularly in relation to the microbiome.

- The majority of octadecanoid studies to date lack mechanistic insight into the functional role of these lipid mediators. Future directions in this nascent field should strive to fully characterize the diverse structures that can be formed from both enzymatic and non-enzymatic processes (including stereochemistry), determine the production of these putative mediators in known systems including the triggers of biosynthesis and identification of factors that change the setting and fidelity of their formation. The investigation of specific octadecanoid receptors should also be a priority. Increased understanding of the biological activity of octadecanoids represents an opportunity to understand the relationship between alterations in dietary fat composition and the physiology of multiple disease processes.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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A.Q., J.R.-C., and C.E.W. co-wrote the manuscript.

Abbreviations

ALA, α-linolenic acid; BAT, brown adipose tissue; COX, cyclooxygenase; CYP450, cytochrome P450; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; LOX, lipoxygenase; OA, oleic acid; PUFAs, polyunsaturated fatty acids; sEH, soluble epoxide hydrolase.

References

1 Gerwick, H., Moghaddam, M. and Hamberg, M. (1991) Oxylipin metabolism in the red alga gracilariopsis lemaneiformis: mechanism of formation of vicinal dihydroxy fatty acids. *Arch. Biochem. Biophys.* **290**, 436–444 https://doi.org/10.1016/0003-9861(91)90563-X

2 Ruan, Y.C., Zhou, W. and Chan, H.C. (2011) Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. *Physiology (Bethesda)* **26**, 156–170 https://doi.org/10.1152/physiol.00036.2010

3 Samuelsson, B., Dahlén, S.E., Lindgren, J.A., Rouzer, C.A. and Serhan, C.N. (1987) Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* **237**, 1171–1176 https://doi.org/10.1126/science.2820055

4 Dennis, E.A. and Norris, P.C. (2015) Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* **15**, 511–523 https://doi.org/10.1038/nri3859

5 Dyall, S.C., Balas, L., Bazan, N.G., Brenna, J.T., Chiang, N., da Costa Souza, F. et al. (2022) Polyunsaturated fatty acids and fatty acid-derived lipid mediators: recent advances in the understanding of their biosynthesis, structures, and functions. *Prog. Lipid Res.* **86**, 101165 https://doi.org/10.1016/j.plipres.2022.101165

6 Gabbs, M., Leng, S., Devassy, J.G., Monirujjaman, M. and Aukema, H.M. (2015) Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv. Nutr.* **6**, 513–540 https://doi.org/10.3945/an.114.007732

7 Hajeyah, A.A., Griffiths, W.J., Wang, Y., Finch, A.J. and O'Donnell, V.B. (2020) The biosynthesis of enzymatically oxidized lipids. *Front. Endocrinol.* **11**, 591819 https://doi.org/10.3389/fendo.2020.591819

8 Serhan, C.N. (2014) Novel pro-resolving lipid mediators in inflammation are leads for resolution physiology. *Nature* **510**, 92–101 https://doi.org/10.1038/nature13479

9 Wasternack, C. (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot.* **100**, 681–697 https://doi.org/10.1093/aob/mcm079

10 Spector, A.A. and Kim, H.-Y. (2015) Discovery of essential fatty acids. *J. Lipid Res.* **56**, 11–21 https://doi.org/10.1194/jlr.R055095

11 Blasbalg, T.L., Hibbeln, J.R., Ramsden, C.E., Majchrzak, S.F. and Rawlings, R.R. (2011) Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am. J. Clin. Nutr.* **93**, 950–962 https://doi.org/10.3945/ajcn.110.006643

12 Brenna, J.T. (2002) Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr. Opin. Clin. Nutr. Metab. Care* **5**, 127–132 https://doi.org/10.1097/00075197-200203000-00002

13 Liu, G.-Y., Moon, S.H., Jenkins, C.M., Sims, H.F., Guan, S. and Gross, R.W. (2019) Synthesis of oxidized phospholipids by Sn-1 acyltransferase using 2–15-HETE lysophospholipids. *J. Biol. Chem.* **294**, 10146–10159 https://doi.org/10.1074/jbc.RA119.008766

14 Laneuville, O., Breuer, D.K., Xu, N., Huang, Z.H., Gage, D.A., Watson, J.T. et al. (1995) Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and −2. *J. Biol. Chem.* **270**, 19330–19336 https://doi.org/10.1074/jbc.270.33.19330

15 Kutzner, L., Goloshchapova, K., Heydeck, D., Stehling, S., Kuhn, H. and Schebb, N.H. (2017) Mammalian ALOX15 orthologs exhibit pronounced dual positional specificity with docosahexaenoic acid. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1862**, 666–675 https://doi.org/10.1016/j.bbalip.2017.04.001

16 Psychogios, N., Hau, D.D., Peng, J., Guo, A.C., Mandal, R., Bouatra, S. et al. (2011) The human serum metabolome. *PLoS One* **6**, e16957 https://doi.org/10.1371/journal.pone.0016957

17 Schuchardt, J.P., Schmidt, S., Kressel, G., Dong, H., Willenberg, I., Hammock, B.D. et al. (2013) Comparison of free serum oxylipin concentrations in hyper- vs. normolipidemic men. *Prostaglandins Leukot. Essent. Fat. Acids* **89**, 19–29 https://doi.org/10.1016/j.plefa.2013.04.001

18 Quaranta, A., Oger, C., Keyes, G.S., Wheelock, Å.M, Durand, T., Galano, J.-M. et al. Development of a chiral supercritical fluid chromatography −tandem mass spectrometry and reversed-phase liquid chromatography−tandem mass spectrometry platform for the quantitative metabolic profiling of octadecanoid oxylipins. *Anal. Chem.* **94**, 14618–14626 https://doi.org/10.1021/acs.analchem.2c02601

19 Levan, S.R., Stamnes, K.A., Lin, D.L., Panzer, A.R., Fukui, E., McCauley, K. et al. (2019) Elevated faecal 12,13-DiHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance. *Nat. Microbiol.* **4**, 1851–1861 https://doi.org/10.1038/s41564-019-0498-2

20 Kumar, N., Gupta, G., Anilkumar, K., Fatima, N., Karnati, R., Reddy, G.V. et al. (2016) 15-Lipoxygenase metabolites of α-linolenic acid, [13-(S)-HPOTrE and 13-(S)-HOTrE], mediate anti-inflammatory effects by inactivating NLRP3 inflammasome. *Sci. Rep.* **6**, 31649 https://doi.org/10.1038/srep31649

21 Fujimura, K.E., Sitarik, A.R., Havstad, S., Lin, D.L., Levan, S., Fadrosh, D. et al. (2016) Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat. Med.* **22**, 1187–1191 https://doi.org/10.1038/nm.4176

22 Miyamoto, J., Igarashi, M., Watanabe, K., Karaki, S., Mukouyama, H., Kishino, S. et al. (2019) Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids. *Nat. Commun.* **10**, 4007 https://doi.org/10.1038/s41467-019-11978-0

23 Kim, M., Furuzono, T., Yamakuni, K., Li, Y., Kim, Y.-I., Takahashi, H. et al. (2017) 10-Oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, enhances energy metabolism by activation of TRPV1. *FASEB J.* **31**, 5036–5048 https://doi.org/10.1096/fj.201700151R

24 Stanford, K.I., Lynes, M.D., Takahashi, H., Baer, L.A., Arts, P.J., May, F.J. et al. (2018) 12,13-DiHOME: an exercise-induced lipokine that increases skeletal muscle fatty acid uptake. *Cell Metab.* **27**, 1111–1120.e3 https://doi.org/10.1016/j.cmet.2018.03.020

25 Lynes, M.D., Leiria, L.O., Lundh, M., Bartelt, A., Shamsi, F., Huang, T.L. et al. (2017) The cold-induced lipokine 12,13-DiHOME promotes fatty acid transport into brown adipose tissue. *Nat. Med.* **23**, 631–637 https://doi.org/10.1038/nm.4297

26 Vangaveti, V.N., Jansen, H., Kennedy, R.L. and Malabu, U.H. (2016) Hydroxyoctadecadienoic acids: oxidised derivatives of linoleic acid and their role in inflammation associated with metabolic syndrome and cancer. *Eur. J. Pharmacol.* **785**, 70–76 https://doi.org/10.1016/j.ejphar.2015.03.096

27 Domenichiello, A.F., Sapio, M.R., Loydpierson, A.J., Maric, D., Goto, T., Horowitz, M.S. et al. (2021) Molecular pathways linking oxylipins to nociception in rats. *J. Pain* **22**, 275–299 https://doi.org/10.1016/j.jpain.2020.09.001

28 Feussner, I. and Wasternack, C. (2002) The lipoxygenase pathway. *Annu. Rev. Plant Biol.* **53**, 275–297 https://doi.org/10.1146/annurev.arplant.53.100301.135248

29 Mosblech, A., Feussner, I. and Heilmann, I. (2009) Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiol. Biochem.* **47**, 511–517 https://doi.org/10.1016/j.plaphy.2008.12.011

30 Petta, T., Secatto, A., Faccioli, L.H. and Moraes, L.A.B. (2014) Inhibition of inflammatory response in LPS induced macrophages by 9-KOTE and 13-KOTE produced by biotransformation. *Enzyme Microb. Technol.* 58–59, 36–43 https://doi.org/10.1016/j.enzmictec.2014.02.011

31 Takahashi, H., Kamakari, K., Goto, T., Hara, H., Mohri, S., Suzuki, H. et al. (2015) 9-Oxo-10(E),12(Z),15(Z)-octadecatrienoic acid activates peroxisome proliferator-activated receptor α in hepatocytes. *Lipids* **50**, 1083–1091 https://doi.org/10.1007/s11745-015-4071-3
32 Miyamoto, J., Mizukure, T., Park, S.-B., Kishino, S., Kimura, I., Hirano, K. et al. (2015) A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, ameliorates intestinal epithelial barrier impairment partially via GPR40-MEK-ERK pathway. *J. Biol. Chem.* **290**, 2902–2918 https://doi.org/10.1074/jbc.M114.610733
33 Hla, T. and Neilson, K. (1992) Human cyclooxygenase-2 CDNA. *Proc. Natl Acad. Sci. U.S.A.* **89**, 7384–7388 https://doi.org/10.1073/pnas.89.16.7384
34 O'Neill, G.P. and Ford-Hutchinson, A.W. (1993) Expression of MRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett.* **330**, 156–160 https://doi.org/10.1016/0014-5793(93)80263-t
35 Zidar, N., Odar, K., Glavac, D., Jerse, M., Zupanc, T. and Stajer, D. (2009) Cyclooxygenase in Normal human tissues – is COX-1 really a constitutive isoform, and COX-2 an inducible isoform? *J. Cell. Mol. Med.* **13**, 3753–3763 https://doi.org/10.1111/j.1582-4934.2008.00430.x
36 Ringbom, T., Huss, U., Stenholm, Å., Flock, S., Skattebøl, L., Perera, P. et al. (2001) COX-2 inhibitory effects of naturally occurring and modified fatty acids. *J. Nat. Prod.* **64**, 745–749 https://doi.org/10.1021/np000620d
37 Singh, N.K. and Rao, G.N. (2019) Emerging role of 12/15-lipoxygenase (ALOX15) in human pathologies. *Prog. Lipid Res.* **73**, 28–45 https://doi.org/10.1016/j.plipres.2018.11.001
38 Nagasaki, T., Schuyler, A.J., Zhao, J., Samovich, S.N., Yamada, K., Deng, Y. et al. (2022) 15LO1 dictates glutathione redox changes in asthmatic airway epithelium to worsen type 2 inflammation. *J. Clin. Invest.* **132**, e151685 https://doi.org/10.1172/JCI151685
39 Yu, Z., Schneider, C., Boeglin, W.E. and Brash, A.R. (2006) Human and mouse ELOX3 have distinct substrate specificities: implications for their linkage with lipoxygenases in skin. *Arch. Biochem. Biophys.* **455**, 188–196 https://doi.org/10.1016/j.abb.2006.09.002
40 Kuhn, H., Banthiya, S. and van Leyen, K. (2015) Mammalian lipoxygenases and their biological relevance. *Biochim. Biophys. Acta* **1851**, 308–330 https://doi.org/10.1016/j.bbalip.2014.10.002
41 Brash, A.R., Boeglin, W.E. and Chang, M.S. (1997) Discovery of a second 15S-lipoxygenase in humans. *Proc. Natl Acad. Sci. U.S.A.* **94**, 6148–6152 https://doi.org/10.1073/pnas.94.12.6148
42 Chiba, T., Thomas, C.P., Calcutt, M.W., Boeglin, W.E., O'Donnell, V.B. and Brash, A.R. (2016) The precise structures and stereochemistry of trihydroxy-linoleates esterified in human and porcine epidermis and their significance in skin barrier function. *J. Biol. Chem.* **291**, 14540–14554 https://doi.org/10.1074/jbc.M115.711267
43 Marnett, L. J. (2000) Cyclooxygenase mechanisms. *Curr. Opin. Chem. Biol.* 2000 **4**, 545–552 https://doi.org/10.1016/S1367-5931(00)00130-7
44 Coffa, G. and Brash, A.R. (2004) A single active site residue directs oxygenation stereospecificity in lipoxygenases: stereocontrol is linked to the position of oxygenation. *Proc. Natl Acad. Sci. U.S.A.* **101**, 15579–15584 https://doi.org/10.1073/pnas.0406727101
45 Reinaud, O., Delaforge, M., Boucher, J.L., Rocchiccioli, F. and Mansuy, D. (1989) Oxidative metabolism of linoleic acid by human leukocytes. *Biochem. Biophys. Res. Commun.* **161**, 883–891 https://doi.org/10.1016/0006-291X(89)92682-X
46 Kühn, H., Belkner, J., Wiesner, R. and Alder, L. (1990) Occurrence of 9- and 13-keto-octadecadienoic acid in biological membranes oxygenated by the reticulocyte lipoxygenase. *Arch. Biochem. Biophys.* **279**, 218–224 https://doi.org/10.1016/0003-9861(90)90484-G
47 Hamberg, M. (1998) Stereochemistry of oxygenation of linoleic acid catalyzed by prostaglandin–endoperoxide H synthase-2. *Arch. Biochem. Biophys.* **349**, 376–380 https://doi.org/10.1006/abbi.1997.0443
48 Ramsden, C.E., Domenichiello, A.F., Yuan, Z.-X., Sapio, M.R., Keyes, G.S., Mishra, S.K. et al. (2017) A systems approach for discovering linoleic acid derivatives that potentially mediate pain and itch. *Sci. Signal.* **10**, eaal5241 https://doi.org/10.1126/scisignal.aal5241
49 Caligiuri, S.P.B., Love, K., Winter, T., Gauthier, J., Taylor, C.G., Blydt-Hansen, T. et al. (2013) Dietary linoleic acid and α-linolenic acid differentially affect renal oxylipins and phospholipid fatty acids in diet-induced obese rats. *J. Nutr.* **143**, 1421–1431 https://doi.org/10.3945/jn.113.177360
50 Hamberg, M. (1983) Omega 6-oxygenation of 6, 9, 12-octadecatrienoic acid in human platelets. *Biochem. Biophys. Res. Commun.* **117**, 593–600 https://doi.org/10.1016/0006-291x(83)91242-1
51 Liu, M., Chen, P., Véricel, E., Lelli, M., Béguin, L., Lagarde, M. et al. (2013) Characterization and biological effects of di-hydroxylated compounds deriving from the lipoxygenation of ALA. *J. Lipid Res.* **54**, 2083–2094 https://doi.org/10.1194/jlr.M035139
52 Nebert, D.W., Wikvall, K. and Miller, W.L. (2013) Human cytochromes P450 in health and disease. *Philos. Trans. R. Soc. B: Biol. Sci.* **368**, 20120431 https://doi.org/10.1098/rstb.2012.0431
53 Nishimura, M., Yaguti, H., Yoshitsugu, H., Naito, S. and Satoh, T. (2003) Tissue distribution of MRNA expression of human cytochrome P450 isoforms assessedby high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi* **123**, 369–375 https://doi.org/10.1248/yakushi.123.369
54 Oliw, E.H., Bylund, J. and Herman, C. (1996) Bisallylic hydroxylation and epoxidation of polyunsaturated fatty acids by cytochrome P450. *Lipids* **31**, 1003–1021 https://doi.org/10.1007/BF02522457
55 Bylund, J., Ericsson, J. and Oliw, E.H. (1998) Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography–mass spectrometry with ion trap MS2. *Anal. Biochem.* **265**, 55–68 https://doi.org/10.1006/abio.1998.2897
56 Laethem, R.M., Balazy, M. and Koop, D.R. (1996) Epoxidation of C18 unsaturated fatty acids by cytochromes P4502C2 and P4502CAA. *Drug Metab. Dispos.* **24**, 664–668
57 Tsikas, D., Sawa, M., Brunner, G., Gutzki, F., Meyer, H. and Frolich, J. (2003) Gas chromatography–mass spectrometry of Cis-9,10-epoxyoctadecanoic acid (Cis-EODA)I. direct evidence for Cis-EODA formation from oleic acid oxidation by liver microsomes and isolated hepatocytes. *J. Chromatogr. B* **784**, 351–365 https://doi.org/10.1016/S1570-0232(02)00821-8
58 Bylund, J., Kunz, T., Valmsen, K. and Oliw, E.H. (1998) Cytochromes P450 with bisallylic hydroxylation activity on arachidonic and linoleic acids studied with human recombinant enzymes and with human and rat liver microsomes. *J. Pharmacol. Exp. Ther.* **284**, 51–60
59 Chuang, S.S., Helvig, C., Taimi, M., Ramshaw, H.A., Collop, A.H., Amad, M. et al. (2004) CYP2U1, a novel human thymus- and brain-specific cytochrome P450, catalyzes ω- and (ω-1)-hydroxylation of fatty acids. *J. Biol. Chem.* **279**, 6305–6314 https://doi.org/10.1074/jbc.M311830200
60 Adas, F., Salaün, J.P., Berthou, F., Picart, D., Simon, B. and Amet, Y. (1999) Requirement for ω and (ω–1)-hydroxylations of fatty acids by human cytochromes P450 2E1 and 4A11. *J. Lipid Res.* **40**, 1990–1997 https://doi.org/10.1016/S0022-2275(20)32422-6
61 Kishino, S., Takeuchi, M., Park, S.-B., Hirata, A., Kitamura, N., Kunisawa, J. et al. (2013) Polyunsaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition. *Proc. Natl Acad. Sci. U.S.A.* **110**, 17808–17813 https://doi.org/10.1073/pnas.1312937110

62 Polan, C.E., McNeill, J.J. and Tove, S.B. (1964) Biohydrogenation of unsaturated fatty acids by rumen bacteria. *J. Bacteriol.* **88**, 1056–1064 https://doi.org/10.1128/jb.88.4.1056-1064.1964
63 Yin, H., Xu, L. and Porter, N.A. (2011) Free radical lipid peroxidation: mechanisms and analysis. *Chem. Rev.* **111**, 5944–5972 https://doi.org/10.1021/cr200084z
64 Jahn, U., Galano, J.-M. and Durand, T. (2008) Beyond prostaglandins—chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty acids. *Angew. Chem. Int. Ed.* **47**, 5894–5955 https://doi.org/10.1002/anie.200705122
65 Ozawa, T., Hayakawa, M., Takamura, T., Sugiyama, S., Suzuki, K., Iwata, M. et al. (1986) Biosynthesis of leukotoxin, 9, 10-epoxy-12 octadecenoate, by leukocytes in lung lavages of rat after exposure to hyperoxia. *Biochem. Biophys. Res. Commun.* **134**, 1071–1078 https://doi.org/10.1016/0006-291X(86)90360-8
66 Sugiyama, S., Hayakawa, M., Nagai, S., Ajioka, M. and Ozawa, T. (1987) Leukotoxin, 9, 10-epoxy-12-octadecenoate, causes cardiac failure in dogs. *Life Sci.* **40**, 225–231 https://doi.org/10.1016/0024-3205(87)90336-5
67 Siegfried, M.R., Aoki, N., Lefer, A.M., Elisseou, E.M. and Zipkin, R.E. (1990) Direct cardiovascular action of two metabolites of linoleic acid. *Life Sci.* **46**, 7 https://doi.org/10.1016/0024-3205(90)90086-7
68 Moran, J.H., Mitchell, L.A., Bradbury, J.A., Qu, W., Zeldin, D.C., Schnellmann, R.G. et al. (2000) Analysis of the cytotoxic properties of linoleic acid metabolites produced by renal and hepatic P450s. *Toxicol. Appl. Pharmacol.* **168**, 268–279 https://doi.org/10.1006/taap.2000.9053
69 Ozawa, T., Sugiyama, S., Hayakawa, M., Satake, T., Taki, F., Iwata, M. et al. (1988) Existence of leukotoxin 9,10-epoxy-12-octadecenoate in lung lavages from rats breathing pure oxygen and from patients with the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* **137**, 535–540 https://doi.org/10.1164/ajrccm/137.3.535
70 Moghaddam, M.F., Grant, D.F., Cheek, J.M., Greene, J.F., Williamson, K.C. and Hammock, B.D. (1997) Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat. Med.* **3**, 562–566 https://doi.org/10.1038/nm0597-562
71 Newman, J.W., Morisseau, C. and Hammock, B.D. (2005) Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog. Lipid Res.* **44**, 1–51 https://doi.org/10.1016/j.plipres.2004.10.001
72 Moran, J.H., Weise, R., Schnellmann, R.G., Freeman, J.P. and Grant, D.F. (1997) Cytotoxicity of linoleic acid diols to renal proximal tubular cells. *Toxicol. Appl. Pharmacol.* **146**, 53–59 https://doi.org/10.1006/taap.1997.8197
73 Zheng, J., Plopper, C.G., Lakritz, J., Storms, D.H. and Hammock, B.D. (2001) Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. *Am. J. Respir. Cell Mol. Biol.* **25**, 434–438 https://doi.org/10.1165/ajrcmb.25.4.4104
74 Arnardottir, H., Pawelzik, S.-C., Sarajlic, P., Quaranta, A., Kolmert, J., Religa, D. et al. (2022) Immunomodulation by intravenous omega-3 fatty acid treatment in older subjects hospitalized for COVID-19: a single-blind randomized controlled trial. *Clin. Transl. Med.* **12**, e895 https://doi.org/10.1002/ctm2.895
75 McReynolds, C.B., Cortes-Puch, I., Ravindran, R., Khan, I.H., Hammock, B.G., Shih, P.B. et al. (2021) Plasma linoleate diols are potential biomarkers for severe COVID-19 infections. *Front. Physiol.* **12**. https://doi.org/10.3389/fphys.2021.663869
76 Stewart, C.J. (2019) Homing in on 12,13-DiHOME in asthma. *Nat. Microbiol.* **4**, 1774–1775 https://doi.org/10.1038/s41564-019-0599-y
77 Macêdo, A.P.A., Muñoz, V.R., Cintra, D.E. and Pauli, J.R. (2022) 12,13-DiHOME as a new therapeutic target for metabolic diseases. *Life Sci.* **290**, 120229 https://doi.org/10.1016/j.lfs.2021.120229
78 Hildreth, K., Kodani, S.D., Hammock, B.D. and Zhao, L. (2020) Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: a review of recent studies. *J. Nutr. Biochem.* **86**, 108484 https://doi.org/10.1016/j.jnutbio.2020.108484
79 Pinckard, K.M. and Stanford, K.I. (2021) The heartwarming effect of brown adipose tissue. *Mol. Pharmacol.* **102** https://doi.org/10.1124/molpharm.121.000328
80 Hansen, H.S. (1986) The essential nature of linoleic acid in mammals. *Trends Biochem. Sci.* **11**, 263–265 https://doi.org/10.1016/0968-0004(86)90191-X
81 Uchida, Y. and Holleran, W.M. (2008) Omega-O-acylceramide, a lipid essential for mammalian survival. *J. Dermatol. Sci.* **51**, 77–87 https://doi.org/10.1016/j.jdermsci.2008.01.002
82 Stewart, M.E., Grahek, M.O., Cambier, L.S., Wertz, P.W. and Downing, D.T. (1986) Dilutional effect of increased sebaceous gland activity on the proportion of linoleic acid in sebaceous wax esters and in epidermal acylceramides. *J. Investig. Dermatol.* **87**, 733–736 https://doi.org/10.1111/1523-1747.ep12456856
83 Yamanashi, H., Boeglin, W.E., Morisseau, C., Davis, R.W., Sulikowski, G.A., Hammock, B.D. et al. (2018) Catalytic activities of mammalian epoxide hydrolases with cis and trans fatty acid epoxides relevant to skin barrier function. *J. Lipid Res.* **59**, 684–695 https://doi.org/10.1194/jlr.M082701
84 Edin, M.L., Yamanashi, H., Boeglin, W.E., Graves, J.P., DeGraff, L.M., Lih, F.B. et al. (2021) Epoxide hydrolase 3 (Ephx3) gene disruption reduces ceramide linoleate epoxide hydrolysis and impairs skin barrier function. *J. Biol. Chem.* **296**, 100198 https://doi.org/10.1074/jbc.RA120.016570
85 Cossette, C., Patel, P., Anumolu, J.R., Sivendran, S., Lee, G.J., Gravel, S. et al. (2008) Human neutrophils convert the sebum-derived polyunsaturated fatty acid sebaleic acid to a potent granulocyte chemoattractant. *J. Biol. Chem.* **283**, 11234–11243 https://doi.org/10.1074/jbc.M709531200
86 Doolen, S., Keyes, G.S. and Ramsden, C.E. (2020) Hydroxy-epoxide and keto-epoxide derivatives of linoleic acid activate trigeminal neurons. *Neurobiol. Pain* **7**, 100046 https://doi.org/10.1016/j.ynpai.2020.100046
87 Jensen, J.R., Pitcher, M.H., Yuan, Z.X., Ramsden, C.E. and Domenichiello, A.F. (2018) Concentrations of oxidized linoleic acid derived lipid mediators in the amygdala and periaqueductal grey are reduced in a mouse model of chronic inflammatory pain. *Prostaglandins Leukot. Essent. Fat. Acid* **135**, 128–136 https://doi.org/10.1016/j.plefa.2018.07.015
88 Patwardhan, A.M., Akopian, A.N., Ruparel, N.B., Diogenes, A., Weintraub, S.T., Uhlson, C. et al. (2010) Heat generates oxidized linoleic acid metabolites that activate TRPV1 and produce pain in rodents. *J. Clin. Invest.* **120**, 1617–1626 https://doi.org/10.1172/JCI41678
89 Hellström, F., Gouveia-Figueira, S., Nording, M.L., Björklund, M. and Fowler, C.J. (2016) Association between plasma concentrations of linoleic acid-derived oxylipins and the perceived pain scores in an exploratory study in women with chronic neck pain. *BMC Musculoskelet. Disord.* **17**, 103 https://doi.org/10.1186/s12891-016-0951-9
90 Zimmer, B., Angioni, C., Osthues, T., Toewe, A., Thomas, D., Pierre, S.C. et al. (2018) The oxidized linoleic acid metabolite 12,13-DiHOME mediates thermal hyperalgesia during inflammatory pain. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1863**, 669–678 https://doi.org/10.1016/j.bbalip.2018.03.012

Biochemical Society Transactions (2022) **50** 1569–1582
https://doi.org/10.1042/BST20210644

91 Hampel, J.K.A., Brownrigg, L.M., Vignarajah, D., Croft, K.D., Dharmarajan, A.M., Bentel, J.M. et al. (2006) Differential modulation of cell cycle, apoptosis and PPARγ2 gene expression by PPARγ agonists ciglitazone and 9-hydroxyoctadecadienoic acid in monocytic cells. *Prostaglandins Leukot. Essent. Fat. Acid* **74**, 283–293 https://doi.org/10.1016/j.plefa.2006.03.002

92 Nagy, L., Tontonoz, P., Alvarez, J.G.A., Chen, H. and Evans, R.M. (1998) Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell* **93**, 229–240 https://doi.org/10.1016/S0092-8674(00)81574-3

93 Cabral, M., Martín-Venegas, R. and Moreno, J.J. (2014) Differential cell growth/apoptosis behavior of 13-hydroxyoctadecadienoic acid enantiomers in a colorectal cancer cell line. *Am. J. Physiol. Gastrointest. Liver Physiol.* **307**, G664–G671 https://doi.org/10.1152/ajpgi.00064.2014

94 Shureiqi, I., Jiang, W., Zuo, X., Wu, Y., Stimmel, J.B., Leesnitzer, L.M. et al. (2003) The 15-lipoxygenase-1 product 13-S-hydroxyoctadecadienoic acid down-regulates PPAR-δ to induce apoptosis in colorectal cancer cells. *Proc. Natl Acad. Sci. U.S.A.* **100**, 9968–9973 https://doi.org/10.1073/pnas.1631086100

95 Tavakoli Yaraki, M. and Karami Tehrani, F. (2013) Apoptosis induced by 13-S-hydroxyoctadecadienoic acid in the breast cancer cell lines, MCF-7 and MDA-MB-231. *Iran J. Basic Med. Sci.* **16**, 653–659

96 Honn, K.V., Nelson, K.K., Renaud, C., Bazaz, R., Diglio, C.A. and Timar, J. (1992) Fatty acid modulation of tumor cell adhesion to microvessel endothelium and experimental metastasis. *Prostaglandins* **44**, 413–429 https://doi.org/10.1016/0090-6980(92)90137-I

97 Buchanan, M.R., Haas, T.A., Lagarde, M. and Guichardant, M. (1985) 13-Hydroxyoctadecadienoic acid is the vessel wall chemorepellant factor, LOX. *J. Biol. Chem.* **260**, 16056–16059 https://doi.org/10.1016/S0021-9258(17)36198-7

98 Goodfriend, T.L., Ball, D.L., Egan, B.M., Campbell, W.B. and Nithipatikom, K. (2004) Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. *Hypertension* **43**, 358–363 https://doi.org/10.1161/01.HYP.0000113294.06704.64

99 Murthy, S., Born, E., Mathur, S. and Field, F.J. (1998) 13-hydroxy octadecadienoic acid (13-HODE) inhibits triacylglycerol-rich lipoprotein secretion by CaCo-2 cells. *J. Lipid Res.* **39**, 1254–1262 https://doi.org/10.1016/S0022-2275(20)32550-5

100 Kim, Y.-I., Hirai, S., Takahashi, H., Goto, T., Ohyane, C., Tsugane, T. et al. (2011) 9-Oxo-10(E),12(E)-octadecadienoic acid derived from tomato is a potent PPAR α agonist to decrease triglyceride accumulation in mouse primary hepatocytes. *Mol. Nutr. Food Res.* **55**, 585–593 https://doi.org/10.1002/mnfr.201000264

101 Kim, Y., Hirai, S., Goto, T., Ohyane, C., Takahashi, H., Tsugane, T. et al. (2012) Potent PPARα activator derived from tomato juice, 13-Oxo-9,11-octadecadienoic acid, decreases plasma and hepatic triglyceride in obese diabetic mice. *PLoS One* **7**, e31317 https://doi.org/10.1371/journal.pone.0031317

102 Altmann, R., Hausmann, M., Spöttl, T., Gruber, M., Bull, A.W., Menzel, K. et al. (2007) 13-Oxo-ODE is an endogenous ligand for PPARγ in human colonic epithelial cells. *Biochem. Pharmacol.* **74**, 612–622 https://doi.org/10.1016/j.bcp.2007.05.027

103 Gilroy, D.W., Edin, M.L., De Maeyer, R.P.H., Bystrom, J., Newson, J., Lih, F.B. et al. (2016) CYP450-derived oxylipins mediate inflammatory resolution. *Proc. Natl Acad. Sci. U.S.A.* **113**, E3240–E3249 https://doi.org/10.1073/pnas.1521453113

104 Bergmann, C.B., McReynolds, C.B., Wan, D., Singh, N., Goetzman, H., Caldwell, C.C. et al. (2022) SEH-derived metabolites of linoleic acid drive pathologic inflammation while impairing key innate immune cell function in burn injury. *Proc. Natl Acad. Sci. U.S.A.* **119**, e2120691119 https://doi.org/10.1073/pnas.2120691119

105 Goto, T., Kim, Y.-I., Furuzono, T., Takahashi, N., Yamakuni, K., Yang, H.-E. et al. (2015) 10-oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, potently activates PPARγ and stimulates adipogenesis. *Biochem. Biophys. Res. Commun.* **459**, 597–603 https://doi.org/10.1016/j.bbrc.2015.02.154

106 Bergamo, P., Luongo, D., Miyamoto, J., Cocca, E., Kishino, S., Ogawa, J. et al. (2014) Immunomodulatory activity of a gut microbial metabolite of dietary linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, associated with improved antioxidant/detoxifying defences. *J. Funct. Foods* **11**, 192–202 https://doi.org/10.1016/j.jff.2014.10.007

107 Hira, T., Ogasawara, S., Yahagi, A., Kamachi, M., Li, J., Nishimura, S. et al. (2018) Novel mechanism of fatty acid sensing in enteroendocrine cells: specific structures in oxo-fatty acids produced by gut bacteria are responsible for CCK secretion in STC-1 cells via GPR40. *Mol. Nutr. Food Res.* **62**, 1800146 https://doi.org/10.1002/mnfr.201800146

108 Ohue-Kitano, R., Yasuoka, Y., Goto, T., Kitamura, N., Park, S.-B., Kishino, S. et al. (2018) α-Linolenic acid-derived metabolites from gut lactic acid bacteria induce differentiation of anti-inflammatory M2 macrophages through G protein-coupled receptor 40. *FASEB J.* **32**, 304–318 https://doi.org/10.1096/fj.201700273R