REVIEW

Quantitative metabolic profiling of lipid mediators

David Balgoma, Antonio Checa, Daniel García Sar, Stuart Snowden and Craig E. Wheelock

Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II, Karolinska Institutet, Stockholm, Sweden

Received: December 19, 2012
Revised: May 7, 2013
Accepted: May 8, 2013

Lipids are heterogeneous biological molecules that possess multiple physiological roles including cell structure, homeostasis, and restoration of tissue functionality during and after inflammation. Lipid metabolism constitutes a network of pathways that are related at multiple biosynthetic hubs. Disregulation of lipid metabolism can lead to pathophysiological effects and multiple lipid mediators have been described to be involved in physiological processes, (e.g. inflammation). Accordingly, a thorough description of these pathways may shed light on putative relations in multiple complex diseases, including chronic obstructive pulmonary disease, asthma, Alzheimer's disease, multiple sclerosis, obesity, and cancer. Due to the structural complexity of lipids and the low abundance of many lipid mediators, mass spectrometry is the most commonly employed method for analysis. However, multiple challenges remain in the efforts to analyze every lipid subfamily. In this review, the biological role of sphingolipids, glycerolipids, oxylipins (e.g. eicosanoids), endocannabinoids, and *N*-acylethanolamines in relation to health and disease and the state-of-the-art analyses are summarized. The characterization and understanding of these pathways will increase our ability to examine for interrelations among lipid pathways and improve the knowledge of biological mechanisms in health and disease.

Keywords:
Disease / Inflammation / Lipidomics / Lipids / MS

Correspondence: Dr. Craig Wheelock, Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II, Karolinska Institutet, Scheeles väg 2, SE – 171 77 Stockholm, Sweden
E-mail: Craig.Wheelock@ki.se
Fax: +46-8-736-04-39

Abbreviations: AD, Alzheimer's disease; **AEA**, anandamide; **2-AG**, 2-arachidonoylglycerol; **C1P**, ceramides-1-phosphate; **Cer**, ceramides; **CNS**, central nervous system; **CoA**, coenzyme A; **COX**, cyclooxygenase; **CYP450**, cytochrome P450; **cys-LT**, cysteinyl-leukotriene; **dhCer**, dihydroceramides; **EC**, endocannabinoid; **FAAH**, fatty acid amide hydrolase; **GL**, glycerolipid; **GlcCer**, glucosylceramide; **GP**, glycerophospholipid; **GSLs**, glycosphingolipids; **HETE**, hydroxyeicosatetraenoic acid; **HODE**, hydroxyoctadecadienoic acid; **LacCer**, lactosylceramide; **LOX**, lipoxygenase; **LT**, leukotriene; **LTB_4**, leukotriene B_4; **LTC_4**, leukotriene C_4; **LTD_4**, leukotriene D_4; **LTE_4**, leukotriene E_4; **LXA_4**, lipoxin A_4; **MRM**, multiple reaction monitoring; **MSD**, multiple sclerosis disease; **NAE**, *N*-acylethanolamine; **NL**, neutral loss; **NP**, normal phase; **OEA**, *N*-oleoylethanolamine; **PAF**, platelet-activating factor; **PEA**, *N*-palmitoylethanolamine; **PG**, prostaglandin; **PGI_2**, prostacyclin; **PPAR**, peroxisome proliferator-activator receptor; **S1P**, sphingosine-1-phosphate; **SEA**, *N*-stearoylethanolamine; **SL**, sphingolipids; **SM**, sphingomyelins; **Spa**, sphinganine; **Sph**, sphingosines; **TQ-MS/MS**, triple quadrupole mass spectrometer; **TriHOME**, trihydroxyoctadecenoic acids; **UHPLC**, ultra-high performance liquid chromatography

1 Introduction

Once considered as mere structural blocks and energy stores, lipids have been thoroughly described to play key roles in cell signaling, homeostasis, and disease [1]. In particular, lipids play a distinct role in inflammatory processes, are part of the innate immunity defense and take part in the remodeling of tissues with the aim of restoring homeostasis and functionality. During inflammation, a plethora of protein and metabolite mediators, orchestrated by changes in gene expression, work in concert to eliminate the challenges to the organism that can compromise function [1, 2]. However, uncontrolled inflammation can have pathophysiological consequences and lead to further damage. Multiple diseases evidence distinct inflammatory components including asthma [3], chronic obstructive pulmonary disease [4], obesity [5], cancer [6], and Alzheimer's disease (AD) [7]. Accordingly, the study of disease mechanism requires suitable analytical tools to characterize the suite of molecular mediators involved in the inflammatory process.

The literature has seen an emphasis on development of analytical methods capable of assessing an increasing number of genes, proteins, and metabolites in a single analysis. These techniques have been coined with the suffix omics. While proteomics and genomics methods are complex, the total number of genes and proteins are constituted by a limited

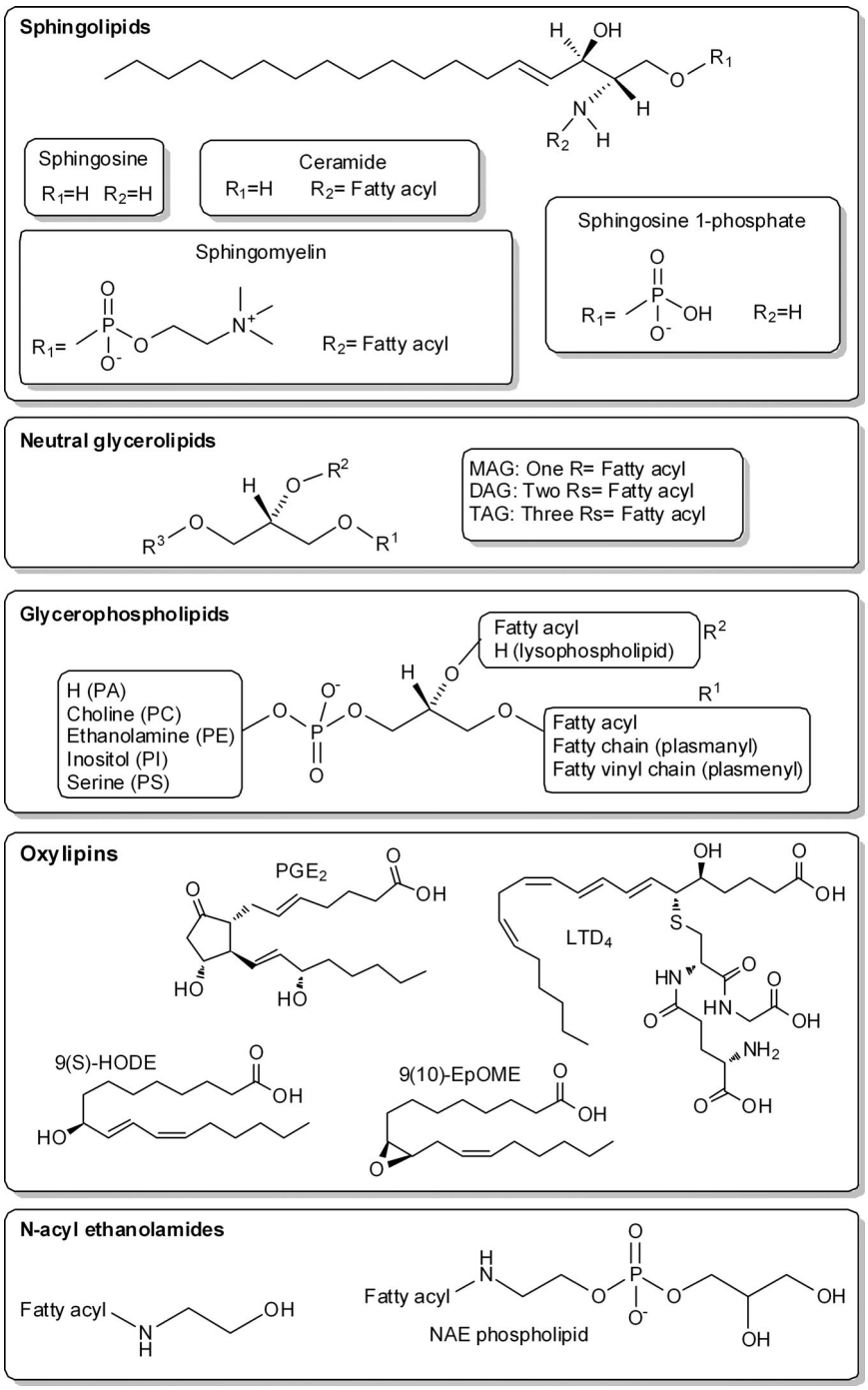

Figure 1. Chemical structure of major lipid mediator classes, including sphingolipids, GLs, oxylipins, and *N*-acyl ethanolamides.

number of structural units and these disciplines can therefore be systematized. On the other hand, the metabolome possesses a wealth of structural units, which often require further subdivision for analysis (e.g. lipidomics and glycomics). This high degree of complexity has led to a substantial degree of analysis compartmentalization. Specifically, lipids constitute a complex family that possesses a range of physicochemical properties (it has been estimated that there are 180 000 different potential species of molecular lipids [8]), necessitating separate analyses of different lipid subfamilies. Many of these lipids, such as sphingolipids (SLs), glycerolipids (GLs), oxylipins (oxidized free fatty acids, including eicosanoids), endocannabinoids (ECs), and *N*-acylethanolamines (NAEs), constitute an intricate network and exert a key role in the mechanisms of physiological and pathophysiological inflammation [9–13]. This structural diversity is shown in Fig. 1.

Although there are multiple lipidomics methods published that enable the large-scale profiling of lipid species [14],

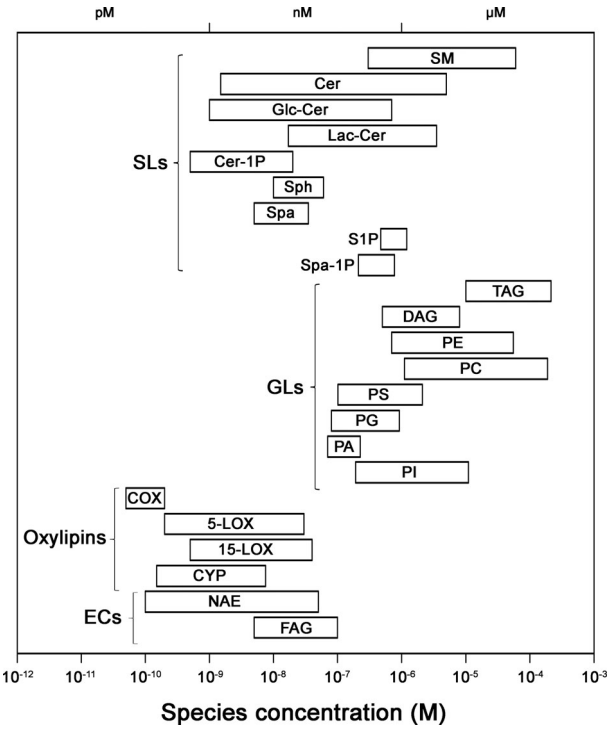

Figure 2. Concentration range of sphingolipids (SLs), GLs, oxylipins, and ECs in plasma or serum. The range of endogenous concentrations from the most to least abundant member of each lipid mediator family is shown (on a logarithmic scale). Levels for SLs (plasma), oxylipins (serum), and ECs (plasma) are from laboratory data, while GLs (plasma) are from [14].

these methods often lack specificity and are usually only semi-quantitative. Many of these lipid species are present at very low levels (Fig. 2) in endogenous systems and therefore require targeted methods for quantification.

2 Analysis

The complete characterization of lipid mediator pathways requires analytical approaches that combine (i) selectivity for the characterization of structurally similar compounds and (ii) sensitivity for quantification of biologically relevant concentrations. The state-of-the-art technique that enables quantification of large numbers of lipid mediators is MS [15]. Depending on the structure and abundance of the compounds to be analyzed, there are multiple MS approaches that can be applied. A common approach is to focus metabolic profiling efforts on distinct groups of structurally related compounds with common physicochemical properties (e.g. oxylipins [16]). Lipids can be analyzed by MS with two general strategies: (i) direct infusion or (ii) previous separation by LC [17, 18]. The present review briefly summarizes the role of these lipid mediators in inflammation and discusses the

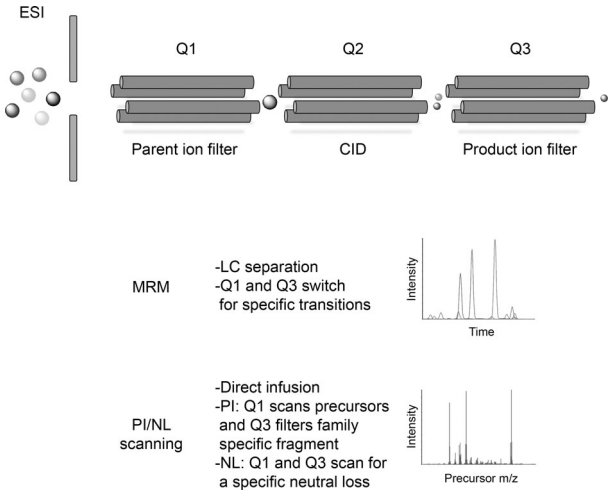

Figure 3. Scheme of triple quadrupole mass analyzer and modalities of analysis (Q = quadrupole; MRM = multiple reaction monitoring; PI = product ion; NL = neutral loss).

state-of-the-art methods and challenges in their analysis by MS.

2.1 Sample preparation

Depending on the characteristics of the sample and the analytes to be quantified, different sample preparation modalities can be addressed. General extraction procedures apply liquid–liquid extraction for cellular, tissue, and aqueous matrices. These methods generally employ chloroform-based extractions (Bligh-Dyer [19] or Folch [20] methods) or methyl *tert*-butyl ether extractions [21]. For aqueous matrices, SPE is extensively used [22, 23]; with a protein precipitation step often employed prior to loading onto the cartridge [24].

2.2 MS analysis

The most commonly used MS detector in quantitative analysis of lipid mediators is the triple quadrupole mass spectrometer (TQ-MS/MS, Fig. 3). Ionization is commonly carried out by ESI [18, 25]. In TQ-MS/MS, depending on the physicochemical characteristics of the analytes, the analysis can be performed by direct infusion and precursor ion/neutral loss (NL) scan or with previous separation by LC and multiple reaction monitoring (MRM) [26]. On the other hand, essentially all lipids can be analyzed via separation by LC and subsequent detection by TQ-MS/MS using MRM. The MRM process is shown in Fig. 3. The analyzer filters an ion in the first quadrupole in order to fragment it by collision induced dissociation in a second cell. The last quadrupole filters selective products ions to be detected (Fig. 3). This technique is necessary for

Table 1. Class specific fragments of glycerophospholipids and sphingolipids

Class	Precursor	Class fragmentation
SM	$[M+H]^+$	PIn 184
Cer	$[M+H]^+$ /$[M+H\text{-}H_2O]^+$	PIn 264
PA	$[M\text{-}H]^-$	PIn 153
PIn	$[M\text{-}H]^-$	PIn 241
PS	$[M\text{-}H]^-$	NL 87
PE	$[M\text{-}H]^-$	PIn 196
PC	$[M+H]^+$	PIn 184

Cer, ceramide; NL, neutral loss; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PIn, phosphatidylinositol; PI, product ion; PS, phosphatidylserine; SM, sphingomyelin

complex matrices with high matrix effects [27] and for analytes in low concentration (e.g. prostaglandins (PGs), ECs). When the analyte is grouped in abundant families with a common structural unit (e.g. glycerophospholipids (GPs)), the chromatographic step can be skipped and analytes can be infused directly and quantified by precursor or NL scanning [28]. The specific transitions for major GPs and SLs are provided in Table 1.

2.3 Internal standards

The selection of appropriate internal standards is crucial in order to achieve accuracy and precision. In the targeted strategy, isotope enriched analogs of the analytes are the preferred internal standards. However, when small molecules are highly deuterated, the behavior of the internal standard and the analyte may diverge, compromising the accuracy of the analytical method [29]. In the case of SLs and GLs (in both targeted- and direct-infusion methodologies), surrogates of short fatty acid chains or with an odd number of carbons have been used. In these cases, the differences in the length of the chain and the number of the unsaturations between the internal standard and the analyte may halve the accuracy and the precision [30].

3 Sphingolipids (SLs)

The SL family, including ceramides (Cer), sphingomyelins (SM), sphingosines (Sph), sphingosine-1-phosphate (S1P), and ceramide-1-phosphate (C1P), constitutes a remarkably diverse group of lipids found in many different types of cells. SLs were long considered to play primarily structural roles as components of membranes, lipoproteins, skin, and other biomaterials. However, recent research on SL metabolism and function has revealed the members of the SL family to be bioactive molecules with profound roles as mediators in intracellular and extracellular signaling pathways [31, 32]. All eukaryotic cells are able to produce SLs via de novo pathways in the ER [33].

3.1 SL metabolism and biological roles

The SL metabolic pathway displays a complex network of reactions resulting in the formation of a multitude of SLs, where Cer and dihydroceramides (dhCer) form the hub of SL biosynthesis, catabolism, and precursors of more complex SLs such as SM and glycosphingolipids (GSLs). This de novo biosynthesis begins with condensation of the amino acid *L*-serine and palmitoyl coenzyme A (CoA) by serine palmitoyltransferase to form 3-ketosphinganines. In the next steps, 3-ketosphinganine is reduced to sphinganine (Spa) by 3-ketosphinganine reductase followed by *N*-acylation to dhCer by a family of Cer synthases. The subsequent dhCer are converted to Cer, the key intermediate, via insertion of a 4,5-trans-double bond into the sphingoid base backbone by dihydroceramide desaturases [32]. Sph are not directly synthesized in this pathway; rather, Cer must be hydrolyzed to the free sphingoid base and fatty acids by ceramidase. Furthermore, Sph can also undergo phosphorylation to yield S1P. This phosphorylated SL is an important lipid mediator and an intermediate of SL turnover. In the following SL biosynthetic reactions, Cer are primarily used for the synthesis of more complex SLs including SMs and GSLs. SMs are present in the plasma membranes of animal cells and are especially abundant in myelin. GSLs are ceramides with one or more sugar residues joined in a β-glycosidic linkage at the 1-hydroxyl position [34]. The first step in GSL synthesis is the glucosylation of Cer-forming glucosylceramide (GlcCer). This cerebroside can be converted to lactosylceramide (LacCer), which can then serve as the substrate for gangliosides [35, 36]. Moreover, Cer can also be phosphorylated to C1P. There is increasing evidence suggesting that C1P can regulate cell proliferation and apoptosis and Chalfant and co-workers have demonstrated that C1P is a potent proinflammatory agent [37].

Furthermore, activation of the cytokine system as well as mobilization of immune cells to the tissues during inflammatory events is regulated by several SLs [37–39]. In this sense, investigations of the role of S1P have provided more direct evidence for its importance in inflammation due to the fact that there is a better characterization of the mode of action compared with ceramide and C1P [40]. However, the effects of S1P may vary dependent on the environment. Intracellular sphingosine kinase activation can produce S1P in response to specific stimuli. Regarding the role of S1P in inflammation, there is also a degree of cell-type specificity. A deficiency in the enzyme SK2 results in a decreased production of eicosanoids and cytokines highlighting the importance of S1P production [41]. Mast cells express S1P1 and S1P2 receptors and these mediate important effects of mast-cell activation [42]. In other cell types, S1P is also involved as part of the inflammatory process, particularly in response to the cytokine TNF-α [43].

3.2 SLs in disease

SLs are increasingly recognized as critical mediators playing myriad and profound roles in a variety of human diseases [37], including diabetes [38], cardiovascular disease [44, 45], AD, and multiple sclerosis disease (MSD) [46–49]. Some recent in vivo studies highlighted the importance of S1P and S1P receptor signaling in pathological inflammation. For example, activation of the S1P2 receptor on corneal endothelial cells is critical for upregulation of COX-2-induced inflammation [50]. In addition, it is known that acute lung injury is characterized by an inflammation resulting in damage to the endothelial and epithelial cell barriers. The possible role of S1P in disruption of this barrier integrity has also been reported [51].

MSD is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that, due to the composition of myelin, has been associated with SLs. Among others, Jana et al. [48] provide a comprehensive review of the roles for dysfunctional SL metabolism in MSD. It is well known that S1P receptors are expressed in a variety of cell types including immune and CNS cells, and are thought to play a critical role in MSD indicating a possible link between S1P signaling and MSD [52]. The trafficking of immune cells is regulated by S1P signaling via the S1P receptor-1 (S1PR-1 or S1P1) [53]. Regulatory T cells (Treg), which are important to decrease inflammatory and autoimmune responses, are regulated by the S1P1 signaling system [54]. The immunomodulating drug Fingolimod (FTY720) binds the S1P receptor (S1PR) and has been recently approved for the treatment of MSD. This structural analog of Sph prevents the migration of immune cells into the circulation, keeping them within the lymph nodes of the immune tissue and stopping their movement into the CNS, where they would normally attack and damage neurons in patients with MSD [47, 55, 56]. In MSD patients who were administered Fingolimod, levels of circulating TH17 cells (that stimulate inflammatory reactions) were reduced, whereas Treg concentrations increased [57]. Focused studies identified an upregulation of specific Cer species during demyelination, revealing an associated increase of Sph and suppression of S1P. These findings highlight that disruptions in SL pathways may indirectly contribute to brain cell injury in different cerebral disorders [58]. The potential for modulating the Cer/S1P pathway to promote myelin repair has also been reported [59]. Interest in GSLs has increased recently, in part due to the recognition that they modulate immune responses [60]. For instance, in contrast to healthy individuals, T cells from MSD patients were activated upon stimulation with α-galactosyl ceramide [61]. GlcCer and its catabolism to ceramide is believed to have an immunomodulatory effect [62]. Saadatt et al. [63] reported that mice lacking GlcCer synthesis do not show myelin abnormalities.

Multiple lipidomics, metabolomics, and targeted approaches have identified pathways and products of SL metabolism that are altered early in the course of AD and contribute to the neuropathological alterations associated with disease. Mielke et al. [64–67] reported that elevated levels of serum long-chain ceramides predicted memory impairment in a population-based study of community-dwelling, cognitively normal women. Elevated plasma ceramide levels predicted faster rates of cognitive decline among AD patients. Accordingly, decreasing ceramide levels via sphingomyelinase inhibitors may slow the rate of progression among AD patients. Initial investigations in humans showed that ceramide and SM levels in brain tissue and cerebrospinal fluid, as well as gene expression patterns of enzymes participating in the SL metabolism pathway, varied with AD severity.

It is expected that interest in SL metabolism will continue to increase substantially and that this area will be productive in the search for new therapeutic targets (e.g. psoriasis, systemic lupus erythematosus). For instance, many drugs that are currently in clinical use have been identified as SL metabolism blockers such as the COX-2 inhibitor celecoxib, which inhibits dihydroceramide desaturase and increases SL biosynthesis [68].

3.3 MS analysis specificities

Recent advances in MS analysis of lipids are expected to enhance our understanding of metabolic diseases and its correlation between genetic determinants and SL levels [69]. Whereas SLs can be analyzed by direct infusion MS modality [70, 71], they have been systematically analyzed with chromatographic separation by LC coupled to TQ-MS/MS over a decade in order to identify, quantify, and elucidate their structures [35, 72, 73]. Typically, chromatographic separation of SLs has been done by both reversed (RP) and normal phase (NP) modes. In RP chromatography, separations are based on the length and unsaturation level of the sphingoid base and/or *N*-acyl fatty acid, for example, to separate Sph and Spa [35, 72, 74]. On the other hand, NP separations are based on the polarity of the head group (i.e. separation of Cer, GlcCer, LacCer, and SM) [75–77]. LC-TQ-MS/MS-based analysis in MRM mode is currently the most suitable technique. These approaches have a number of advantages over other analytical methodologies (1) improved accuracy (2) powerful tools for structural annotation (3) allows rapid simultaneous quantification of a large number of lipids. To date, one of the major challenges in the analysis of SLs by LC-TQ-MS/MS is to obtain standards both for methods development and to be used as internal standards in order to control variability in extraction recovery and ionization. The ideal internal standard would be a stable isotope labeled version of each analyte. However, the number of potential target SLs screened in a typical sphingolipidomic study is quite large, rendering this approach unfeasible. To date, a more practical approach has been introduced in order to obtain internal standards that are similar in structure and physical properties, as well as ionization and fragmentation features of the corresponding family of compounds under investigation. Normally, these internal standards are commercially available and contain uncommon chain-length sphingoid bases (C17) for Sph, Spa, S1P, Cer, SM, and LacCer and

on the other hand, short fatty acid analogs of dhCer, Cer1P, and GlcCer (C6:0, C8:0 and C12:0). Another challenge is the optimization of the extraction conditions where the analytes of interest and adequate internal standards are recovered in high yield. For instance, highly polar (S1P) or nonpolar (Cer) SLs differ in chain length and other biophysical features.

4 Glycerolipids (GLs)

Traditionally considered a mere barrier or energy source, GLs are also involved in the regulation of biological processes [1, 78]. Specifically, GPs are the precursors of the PUFAs, which are transformed to eicosanoids by means of four pathways (see section oxylipins). PUFAs are mainly found esterified in the *sn*-2 position of GPs that possess in the *sn*-1 position a hydrophobic chain linked by ester, ether, or vinylether bonds. A phosphate-linked polar group in the *sn*-3 position characterizes the GP family (no group, choline, ethanolamine, serine, glycerol, and inositol GPs as well as cardiolipins).

4.1 Glycerolipid metabolism and biological roles

Liberation of the PUFAs is catalyzed by phospholipase A_2 enzymes [79], and reincorporation by association of the free fatty acid to CoA and esterification to the glycerol backbone by acyl-CoA transferases [80]. In addition, the traffic of PUFAs among GPs is fatty acid dependent [81] and arachidonic acid shows an extra component, the CoA-independent transacylation from choline GPs to ethanolamine GPs [82, 83].

By means of this trafficking, the amount of free PUFAs (especially arachidonic acid) is controlled by the inflammatory cell as the rate-limiting step in early eicosanoid production. Nevertheless, other lipid mediators containing a glycerol backbone are involved in biological processes. For example, platelet-activating factor (PAF) [84] is controlled by cleavage of the ester bond in the *sn*-2 position of *sn*-1 alkyl choline GPs by PLA_2 activity and then regulated by the balance between lysoPAF acetyl-transferases and PAF acetylhydrolases. On the other hand, inositol GPs can be phosphorylated in the inositol head group to yield phosphoinositides. Although phosphoinositides mediate in different intracellular processes [13, 85], they can be hydrolyzed by phospholipases C and yield diacylglycerols (protein kinase C agonists [86]) and inositolphosphates (that provoke cytosol calcium increase via regulation of channels in ER [87]). The pathway crosstalk for different lipid mediator families is demonstrated by the fact that diacylglycerol is the precursor of the EC 2-arachidonoylglycerol.

GPs show compartmentalization at different levels of organization. At the cellular level, the inner leaflet of eukaryotic membranes shows enrichment in serine and ethanolamine GPs, while choline GPs and SM are mainly in the outer leaflet [88]. Serine GPs membrane leaflet gradient have been the most studied [89]. This spatial gradient control is involved in several biological processes. For instance, the externalization of serine GPs has been described in apoptosis [90, 91]. In a supracellular level of organization, a unique composition of GPs in lung surfactant has been observed, which lowers the surface tension at the air–water interface in the airways. The surfactant is 90% lipids, and 80% of the GPs possess a choline headgroup, where the dipalmitoyl species is the most abundant [92].

Lipid mediators have been involved in receptor-involved signal transduction. PAF exerts its biological activity by interaction with the PAF receptor, a G-protein-coupled membrane receptor. Activation of PAF receptors provokes the release of other lipid and related mediators, such as arachidonic acid liberation, activation of mitogen-activated protein kinases, cyclic AMP synthesis, and intracellular Ca^{2+} mobilization [93]. The effects of PAF receptor activation is cell dependent [94]. LPA triggers varied cellular responses via G-protein-coupled receptors [95, 96]. It induces monocyte migration, protects T cells from apoptosis and is a macrophage recruiter in wound healing. On the other hand, it has been reported that LPA is peroxisome-proliferator activator receptors γ agonist (PPARγ) [97].

4.2 GLs in disease

While the fine regulation of lipid trafficking plays a key role in physiological functions, it can be altered in pathophysiological conditions. In Barth syndrome, when the TAZ gene is mutated, cardiolipins, which are involved in mitochondrial function, show lower levels and different distribution of fatty acids. This might halve mitochondrial respiratory function and lead to cardiomyopathy [98]. Patients with Scott syndrome, an autosomal recessive bleeding disorder, show lower calcium-induced scramblase activity, diminishing the levels of serine GPs in the outer leaflet of platelets and impairing their function [99]. In other cases, the relation with disease is more complex. In the case of asthma, apart from the involvement of oxylipins, the composition of GPs in the airway surfactant has been found to be different: the proportion of dipalmitoyl phosphatidylcholine is decreased in sputum, but not in bronchoalveolar lavage fluid [100]. Moreover, elevated levels of phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$) have been described in several cancer types. For example, HER2 characterizes a subtype of breast tumors with worse prognosis and plays a role in two pathways of GL signaling. This receptor activates phosphoinositide-3-kinase [101] and thus levels of $PI(3,4,5)P_3$ and hence modulating not only AKT pathway, but also phospholipase C-γ1 that provokes calcium signaling via inositoltrisphosphate and proteinkinase C via diacyglycerol. These pathways play a key role in breast cancer metastatic processes [13]. LPA plays a role in ovarian cancer cell [102]. LPA synthesis is increased in ovarian cancerous cells and shows increased levels in ascitic fluid and plasma from ovarian cancer donors [96].

4.3 MS analysis specificities

As described, most GLs are relatively abundant and can be analyzed by either direct infusion or LC-based approaches [26]. Direct infusion GL analyses take into account that the members of a GL class possess a common polar head group that fragments similarly in the mass analyzer. Therefore, the extract (the Bligh-Dyer liquid–liquid extraction is commonly used for sample preparation [19, 83]) is analyzed by direct infusion and TQ-MS/MS. In this case, the first quadrupole scans in the mass range of the subfamily and the second quadrupole filters those masses that render the fragment specific for the polar head group (*m/z* 184 in the case of choline GPs ionized in positive mode, Table 1). When the specific fragment of a group of lipids is an NL, both quadrupoles scan in coordination with this difference of *m/z* (e.g. all serine GPs show an NL of 87 *m/z* when ionized in negative mode, Table 1). This strategy is straightforward and rapid. Nevertheless, the information that it yields is only the sum of the carbons and unsaturations of the acyl chains. In addition, highly abundant species in the sample may quench the detection of minor species. When minor species or the discrimination of the acyl chains linked to the glycerol are to be characterized, chromatographic separation of the analytes prior to MS analysis is necessary [26]. The behavior of the GLs in NP and RP chromatography is similar to SLs, in NP they separate primarily by the polar head group and in RP by the length and number of unsaturations of the acyl chains attached to the glycerol backbone. Whereas GLs automatic analyses by direct infusion or with previous chromatographic separation have experienced a breakthrough [103, 104], fully quantitative and automatic strategies are still to be developed.

5 Oxylipins

Oxylipins are endogenous signaling molecules that are highly diverse in terms of their structure, metabolism, and biological function. They play both detrimental and beneficial roles in a number of important pathological processes, including fever [105], pain [106], and as both pro (e.g. prostaglandins(PGs)) [9] and antiinflammatory/proresolving agents (e.g. resolvins (RVs) and lipoxins) [107, 108]. While all oxylipins that contain 20 carbons are technically eicosanoids (eicosa meaning 20 in Greek), the term is more commonly used to refer specifically to products of arachidonic acid. A full understanding of how these compounds affect human health requires insight into their biosynthetic pathways (see [1, 109] for comprehensive reviews).

5.1 Metabolism and biological roles

As discussed above, these mediators are synthesized from PUFAs including AA [110], linoleic acid [111], and other omega-3 and 6 fatty acids [112]. Some oxylipins, such as the hydroxyeicosatetraenoic acids (HETEs) can also be formed by auto-oxidation; however, the majority are formed via three major enzymatic pathways, cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) [113–115]. COX enzymes incorporate two oxygen molecules into AA to form PG peroxides, which are subsequently converted into a variety of PGs by a range of terminal PG synthases [116]. COX exists in two isoforms, a constitutive form (COX-1) and an inducible form (COX-2); both isoforms are susceptible to inhibition by nonsteroidal antiinflammatory drugs such as aspirin [117, 118]. Mammals possess up to six different LOX isoforms, which are categorized by the carbon position at which they incorporate an oxygen molecule [119, 120], 5-LOX, 8-LOX, 12(S)-LOX, 12(R)-LOX, and two forms of 15-LOX [1], although the difference between the 12(S)-LOX, 12(R)-LOX is unclear; however, only four of these isoforms 5-LOX, 12-LOX, 15-LOX-1, and 15-LOX-2 are found in humans [121]. Of these isoforms, 5-LOX is the most well studied, and catalyzes an oxidation and subsequent dehydration of AA to produce leukotriene A_4 [114, 122]. This can be further converted into leukotriene B_4 (LTB_4) by leukotriene A_4 hydrolase [123], or into the cysteinyl-leukotrienes (cys-LT) by leukotriene C_4 (LTC_4) synthase [124, 125]. CYP450 enzymes are monooxygenases that incorporate an oxygen atom into their substrates. These include AA, which is converted to form epoxyeicosatrienoic acids and subsequently into the corresponding diols via the activity of soluble epoxide hydrolase [126–128], and linoleic acid, which is converted to epoxyoctadecenoic acids and subsequently into the corresponding dihydroxyoctadecenoic acids. The role of CYP450-derived oxylipins in pathophysiology is less known than those derived from the COX and LOX. However, a growing body of work has highlighted their role in cardiovascular disease, hypertension, and respiratory disease [129–132].

All known mammalian systems possess enzymes that catalyze the metabolism of arachidonic and linoleic acid, with work to date focusing on the traditional S-isoforms of these enzymes. However, a number of enzymes have been shown to produce the R-isomer, including CYP450 [133] and 12R-LOX [134], with the R enantiomer measured in human umbilical cells [135] and bovine aorta [136]. These R enantiomers have been shown to have significant effects in mammalian systems with properties sometimes differing from the S isoform. For example, 5(R)-HETE has been shown to be a more powerful chemotactic agent than 5(S)-HETE [137]. Accordingly, in order to fully understand the role of oxylipins in disease, it is necessary to perform chiral-based analyses and whenever possible, the chirality of any quantified species should be reported.

5.2 Prostaglandins (PGs)

PGs are eicosanoids that contain a 5-carbon ring, are present in the majority of tissues and organs, and are produced in most nucleated cells. PGs were first discovered and isolated

from human semen in the 1930s by Ulf von Euler, who initially thought that PGs were produced by the prostate gland. PGs act as local autocrine and paracrine signaling molecules [138,139] and bind a range of G-protein-coupled receptors FP, DP_{1-2}, IP_{1-2}, TP [140], EP_{1-4} [141], and CRTH2 [142]. This diversity of receptors means that PGs act on a wide range of cells to produce a variety of effects. The TP receptor was the first eicosanoid receptor to be cloned in humans [143]. Thromboxane A_2 (TxA_2), an endogenous TP ligand, is a powerful bronchoconstrictor [144], and has also been shown to exacerbate hyperresponsiveness. PGD_2, a ligand of DP and CRTH2 receptors, has long been linked to inflammation, and has been shown to produce airway bronchoconstriction [145,146]. The inflammatory effects of PGD_2 appear to be mediated by both DP and CRTH2, with both receptors binding PGD_2 with equal affinity [142]; however, the CRTH2 receptor shows little structural similarity with the DP or any of the other PG receptors. PGE_2 acts as a ligand of four receptors EP_1, EP_2, EP_3, and EP_4, this wide range of receptors provides PGE_2 a wide range of often contradictory biological effects, for example, eliciting dilation and constriction in guinea pig ileum [147]. EP_1 and EP_3 interactions with PGE_2 have both been shown to elicit pain in inflammatory hyperalgesia [148], with EP_4 mediating antiinflammatory effects of PGE_2 in allergic airways [149]. Prostacyclin (PGI_2) is produced by the majority of cell types in response to inflammation [150]. PGI_2 acts peripherally by binding the PGI_2 receptor (IP), and has been reported to mediate both acute inflammation [151], and inflammatory pain transduction by producing hyperalgesia [150]. The biological properties of PGs include sensitizing peripheral nerve terminals to pain [152], regulating smooth muscle contraction [153], inflammatory mediation [12,154], and influencing cell growth [155]. PGE_2 and $PGF_{2\alpha}$ are both used to induce child birth in pregnancies that reach full term [156], and PGE_1 is used to assess and treat numerous conditions including congenital heart disease [157], risk of heart failure [158], pulmonary hypertension [157], and erectile dysfunction [159].

In the recent literature, the paradigm of the roles of COX-1 and 2 in health and disease has shifted. Whereas COX-1 was considered to be involved in homeostasis and inducible COX-2 in inflammation, the development and clinical trials of selective inhibitors of COX-2 have shown a more complex role of the two enzymes. COX-2 selective inhibitors were developed to avoid gastrointestinal side effects associated with the inhibition of COX-1 by nonselective drugs. In the cardiovascular system, prothrombotic and proatherogenic thromboxane A_2 are synthesized *via* COX-1 mainly in platelets, antithrombotic, and antiatherogenic PGI_2 is mainly derived *via* COX-2 from endothelium and smooth muscle [160]. Selective inhibition of COX-2 provokes an imbalance between TxA_2 and PGI_2 and an increase in cardiovascular events [160]. It might be considered that the best option to analyze this imbalance is to assess the biosynthesis of these two antagonistic mediators in blood. However, platelets are activated in the process of blood extraction and can produce TxA_2, confounding the analysis of basal levels. One approach is to measure these mediators as their urinary metabolites, with the caveat that urine reflects systemic production [161,162]. Mediators acting as autocrine and paracrine signaling molecules are rapidly metabolized via multiple pathways. PGD_2 and PGE_2 undergo a 15-hydroxy dehydrogenation, Δ^{13} unsaturation reduction, and β- and ω-oxidation to yield tetranor metabolites [163]. PGI_2, TxA_2, and PGD_2 yield nonenzymatic metabolites in a first step (6-keto$PGF_{1\alpha}$, TxB_2, and 11-β-$PGF_{2\alpha}$, respectively) that undergo β-oxidation to yield the excreted 2,3-dinor metabolites [164].

5.3 Leukotrienes

As the name suggests, leukotrienes (LT) were first discovered in leukocytes and contain three conjugated double bonds [165]. Similar to PGs, LTs contain 20 carbons, but lack the 5-carbon ring structure. LTs act as autocrine and paracrine signaling molecules acting via G-protein-coupled receptors [166], although they can bind other receptors as well (e.g. peroxisome proliferator-activated receptors (PPARs)) [167]. The glutathione conjugate forms are termed cys-LTs and include LTC_4, leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4). LTC_4 and LTD_4 are metabolized to LTE_4, which is often used as a marker for this pathway [168] due to its in vivo stability. Cys-LTs are potent bronchoconstrictors that have been shown to possess specific effects on peripheral airways [169]. They are also powerful vasoconstrictors [170] that affect the contraction and proliferation of smooth muscle [171]. Parachuri et al. 2009 showed the role of LTE_4 in pulmonary inflammation is mediated by $P2Y_{12}$, an ADP-reactive purigenic receptor [172]. With regard to pulmonary disease, the analysis of these metabolites has been proven useful in elucidating disease mechanism. For example, higher levels of LTE_4 as well as PGD_2 metabolites have been reported in urine from atopic asthmatic patients following allergen provocation [173], this is potentially due to mast-cell activation during the allergic process [174]. LTB_4 has been shown to be involved in numerous inflammatory diseases, including bronchial asthma [175] and inflammatory bowel disease [176]. LTB_4 binds to two receptors, BLT_1 and BLT_2, which has led to antagonists, such as the BLT_2 agonist Ly-2552837 [177] being suggested as a potential treatment for inflammatory diseases.

5.4 Antiinflammatory oxylipins

Although numerous oxylipins have been shown to possess proinflammatory properties, several classes, including the lipoxins [178,179], RVs [178,180], protectins (PDs) [181], and maresins [182,183], have been shown to possess powerful antiinflammatory and proresolving effects. Lipoxins are trihydroxyeicosatetraeonic acids derived from AA that are generally formed via interactions with LOX enzymes, with the two main lipoxins, lipoxin A_4 (LXA_4), and lipoxin B_4 (LXB_4), formed via 12- and 15-LOX activity. The antiinflammatory

effect of LXA_4 is mediated via the alpha lipoxin receptor [184, 185], which is a G-protein-coupled receptor, and has been shown to inhibit the production of the cys-LTs providing protection from inflammation. LXA_4 possesses a number of properties, including inhibition of neutrophil adherence and chemotaxis, reducing neutrophil activation and increased monocyte removal by neutrophils, potentially aiding in the resolution of inflammation [178]. The protective effects of LXA_4 could also be the result of the inhibition of NF-κβ signaling pathway reducing the abundance of a range of proinflammatory cytokines including TNF-α, IL1, and IL6 [186].

RVs are low level (endogenous concentrations ranging from the nano- to picomolar level) lipid mediators that are involved in the resolution of inflammation. These compounds are produced from the omega-3 fatty acids eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The RVs are categorized into two groups based on their biosynthetic origin, E series RVs are formed from EPA, while D series RVs are produced from DHA [187]. Chemoattractant receptor 23 (ChemR23), which was the first RV receptor identified, is a G-protein coupled receptor exhibiting a 36% homology to the alpha lipoxin receptor [188]. RvE_1 has also been shown to interact with the LTB_4 receptor BLT_1, but not with the BLT_2 receptor [187]. This interaction with BLT_1 has been shown to attenuate LTB_4-induced NF-κβ activation [187], suggesting a potential mechanism for the antiinflammatory activity.

PDs and maresins are, like RVs, produced from the omega-3 fatty acids, EPA and DHA [178]. Originally termed "neuroprotectins", the name was shortened to PDs after they were identified in tissues outside the brain. PDs and lipoxins share similarities in their protective properties, both have been shown to act to block the production of the proinflammaotry IL-13 and cys-LTs in bronchoalveolar lavage fluid, however, PD_1 exhibits its protective effects at lower concentrations than LXA_4 [189–191]. The administration of PD1 has been shown to reduce the levels of LXA_4. In vivo administration is also associated with reductions in PGD_2, which induces 15-LOX subsequently leading to lipoxin synthesis [189, 190], suggesting that PDs possess lipoxins-independent effects.

Maresins are newly described lipid mediators involved in the resolution of inflammation; their name is derived from Macrophage mediator in resolving inflammation [183]. Owing to the recent nature of their discovery, there is little work describing their biological function; however, they have been shown to reduce polymorphonuclear neutrophils migration and increased macrophage phagocytosis in peritonitis [182].

5.5 Other oxylipins

There are numerous, less well-studied groups of oxylipins whose biology is often unclear. Enzymatically produced HETEs are primarily LOX derived, with the exception of 19-HETE and 20-HETE, which are hydroxylated by CYP450. However, HETEs may also be formed via auto-oxidation [192]. HETEs have been shown to possess a variety of biological functions, 15-HETE can inhibit 5-LOX activity in neutrophils, thereby potentially reducing leukotriene biosynthesis [193, 194]. Reducing the production of these powerful inflammatory agents suggests a protective role of 15-HETE against inflammation. 20-HETE also potentially possesses proactive activity and has been shown to relax human bronchi by the activation of BKCa ion channels [195]. The biological function of epoxyeicosatrienoic acids (EETs) is poorly understood; however, they primarily act as local signaling molecules in the kidney and cardiovascular systems and regulate ion transport to produce vasorelaxation and constriction [196–199], influence bronchomotor tone [200], and act in pulmonary inflammation [201].

Hydroxyoctadecadienoic acids (HODEs) are LOX products derived from linoleic acid. These lipids can be further oxidized to form the corresponding oxooctadecadienoic acid (KODEs). These lipids interact with PPAR-γ, 9-HODE, 9- and 13-KODEs have been shown to bind directly to PPAR, and while 13-HODE does not bind directly, it upregulates with mitogen-activated protein kinase and PPAR phosphorylation, which in turn reduces the activity of PPAR-γ [202]. The HODEs can also be metabolized to form trihydroxyoctadecenoic acids (TriHOMEs) via epoxyhydroxyoctadecenoic acid [203]. As in the previous cases, there is a dearth of information about the functions played by the TriHOMEs; however, treatment of spontaneously hypersensitive rats with a soluble expoxide hydrolase inhibitor has been shown to lower both blood pressure and the levels of 9,12,13-TriHOME [204].

5.6 MS analysis specificities

Oxylipins possess a wide range of chemical structures, as well as numerous structural isomers, making it necessary to apply multidimensional separation techniques such as LC-TQ-MS/MS in MRM mode. However, the application of these approaches to oxylipin analysis is not trivial, with many of the species susceptible to degradation or auto-oxidation. This is especially problematic in the analysis of the cys-LTs, which have been shown to be unstable in some biological matrices, with recoveries as low as 25% reported [205]. In addition, specific transitions should be chosen in order to achieve selectivity (oxylipins are carboxylic acids and undergo an NL of 44 atomic mass units, which is not selective given that it is the loss of CO_2). Some isomers exhibit the same mass and fragmentation pattern (e.g. PGD_2 and PGE_2). In these cases, targeted analysis requires a good separation by LC. On the other hand, in TQ-MS, several transitions can be analyzed simultaneously by MRM allowing the possibility of analyzing coeluting compounds. The introduction of ultra-high performance LC has enabled the achievement of good separation of these structural isomers, decreasing the required chromatographic time and allowing the determination of an increasing number of analytes [16, 206]. Nevertheless, several analytical challenges remain. While many oxylipins

are produced enzymatically, some of them, which are associated to oxidative stress, are produced in radical-driven reactions that yield diastereoisomers and enantiomers (e.g. isoprostanes) [207]. In addition, biological activity of enantiomers can be different and can indicate the synthetic source of the compound (e.g. LOX-derived compounds). Therefore, chiral separation of compounds is necessary to analyze both enantiomers [208]. To date, commercial chiral ultra-high performance LC columns are not available.

6 Endocannabinoids (ECs) and *N*-acylethanolamines (NAEs)

ECs, a group of endogenous cannabinoid receptor ligands, mainly belong to two families of compounds, NAEs and glycerolacylesters. NAEs are a group of lipid mediators that contain a long-chain fatty acid linked to an ethanolamine moiety via an amide group.

6.1 Metabolism and biological roles

First reported as an EC in 1992 [209], *N*-arachidonoylethanolamine (AEA, anandamide) is so far the most studied NAE. The affinity of two other NAEs, *N*-docosatetraenoylethanolamine and *N*-dihomo-γ-linoleoylethanoamine, for the cannabinoid receptors was proven in 1993 [210]. Even though they do not interact with the cannabinoid receptors, other NAEs derived from long-chain fatty acids are present in a range of tissues [211]. For example, *N*-oleoylethanolamine (OEA), *N*-stearoylethanolamine (SEA), *N*-palmitoylethanolamine (PEA), and *N*-linoleoylethanolamine usually exhibit higher concentrations in tissues than the ECs. NAEs are synthesized from membrane phospholipids and released on demand as a response to physiological and pathological stimuli, such as neural depolarization and bacterial lipopolysaccharides [212]. At present, there are three known principal enzymatic pathways for the biosynthesis of NAEs in mammals and all of them have *N*-acylated ethanolamine phospholipids as precursors [213]. *N*-acylated ethanolamine phospholipids are formed by transfer of the *sn*-1 fatty acid from a donor phospholipid to the amino group of ethanolamide, a reaction catalyzed by a ubiquitous, but poorly characterized calcium-activated *N*-acyltransferase [214]. To date, three enzymes have been identified as being responsible for the hydrolysis of NAEs into ethanolamine and their corresponding fatty acid in mammals, of which the most important is the membrane protein fatty acid amide hydrolase (FAAH-1) [215]. The other enzymes are FAAH-2, an isozyme of FAAH-1 present in humans, but not in rodents, and the NAE-hydrolyzing acid amidase, a lysosomal enzyme, whose role is poorly understood [216]. Another route of degradation of NAEs is the oxidation of the fatty moiety by COX-2, LOX, and CYP450, though the biological activity of these compounds remains to be determined [217].

The other major EC found in tissues is 2-arachidonoylglycerol (2-AG) [218, 219]. The main source of 2-AG is the hydrolysis of diacylglycerols containing an arachidonate moiety in the 2 position via a diacylglycerol lipase selective for the *sn*-1 position [220]. 2-AG degradation is mainly performed by the cytosolic enzymes monoacylglycerol lipases, though other enzymes have been found to contribute to this EC degradation [221]. Other ECs discovered to date include 2-arachidonoylglycerol ether (noladin ether) [222], *O*-arachidonoyl ethanolamine (*O*-AEA, virodhamine) [223] and *N*-arachidonoyl dopamine [224], but their presence in tissue has been questioned and is still a matter of debate [225, 226].

The two types of endogenous cannabinoid receptors identified to date are the transmembrane G-protein-coupled receptors, CB1 and CB2. Though they are mainly expressed in the nervous and immune system respectively, both have been found at much lower concentrations in other tissues and cells [227, 228].

As an EC, AEA presents cannabis-like effects such as hypoalgesia, catalepsy, and nociception. It was the first endogenous ligand found for transient potential vanilloid receptor type 1 [229]. Increased levels of NAEs during inflammation have been described in several in vitro and animal models [230]. The activity of PEA against inflammation was reported 30 years ago [231]. PEA levels are enhanced in peripheral blood or tissues affected by neuropathic and inflammatory pain, both in animals and humans, suggesting its properties against these inflammatory disorders [232]. PEA is the NAE secreted in largest concentrations, and food intake is directly related with its plasma levels, with decreases of around 40% after meals [233]. Though it does not present CB1 activity, PEA is also a substrate of the FAAH and enhances its effects by competitively inhibiting its inactivation.

6.2 ECs and NAEs in disease

An increase of plasma levels of AEA, PEA, and OEA has been shown in patients with MSD, though it has been suggested that this increase may depend on the disease subtype [234]. In the same study, however, no changes in 2-AG levels were found. Another study showed that AEA levels in bronchoalveolar lavage are increased more than fourfold in patients with allergic asthma after allergen provocation, confirming the implication of ECs in this disease [235]. Elevated levels of AEA and 2-AG have also been reported in several types of tumors [236].

6.3 MS analysis specificities

ECs and NAEs have mainly been analyzed using targeted chromatographic methods, either GC or LC, coupled to MS or TQ-MS/MS. As in the case of the different lipid

mediator families previously described, low abundance presence of these compounds has made tandem TQ-MS/MS the current method of choice. In this case, MRM is based on the monitoring of the transition from the protonated form $[M+H]^+$ to the ethanolamine moiety (m/z 62) in the case of NAEs, and the transition $[M+H]^+$ to the protonated fatty acid backbone in the case of *N*-acylglycerols. Traditionally, methodologies for the determination of NAEs in tissues and biofluids included only the selected compounds of interest [237]. Nowadays, the improvement in MS instrumentation allows the development of targeted methods for the simultaneous quantification of all the known compounds that may contribute to an improved understanding of their implications in inflammatory diseases. Moreover, 2-AG and the related acylglycerols exhibit similar behavior to the NAEs both in the extraction protocols and chromatography, and thus they can also be easily included in the methodologies. For instance, Balvers et al. have developed a method that includes the simultaneous quantification of six NAEs together with 2-AG and related ECs, which has been used to profile NAEs in several matrices during induced inflammation in mice [230,238]. The inclusion of all these related compounds in a single analysis is useful for understanding biological pathways. However, multiple factors should be taken into account when quantifying these compounds, which are reflected by the discrepancies reported for their levels in the literature. First, it is important to know that some NAEs are used in industrial processes and may be found in products such as detergent liquids [213]. Moreover, the presence of PEA and SEA has been reported in commercial chloroforms [239]. In addition, we have observed PEA, OEA, SEA, and *N*-linoleoylethanolamine in plastics commonly used for sample preparation (e.g. collecting tubes and SPE cartridges). Thus, it is advised that a rigorous check of all laboratory solvents and materials is performed during method development. Another issue is the well-characterized isomerization of 2-AG into 1-AG. 1-AG does not activate the cannabinoids receptors and has not yet been proven to be present endogenously. Therefore, it is recommended that chromatographic baseline separation is achieved to enable reporting of both isomers. In order to reduce compound isomerization, protic solvents should be avoided during sample extraction [211, 240]. Moreover, samples must be stored at −80°C to avoid compound degradation. With regard to internal standards, as for the rest of lipid mediators described, the use of deuterium-labeled compounds, which are commercially available for several NAEs and 2-AG, represent an advantage in terms of precision and accuracy in the quantification. Quantification accuracy will increase for NAEs and AG if enzymatic activity is inactivated after fluid or tissue sampling to prevent ex vivo metabolism.

7 Concluding remarks

Complex multifactorial inflammatory diseases can manifest patient-specific dysregulation of different inflammatory pathways, warranting the necessity of characterizing these metabolic routes in well-phenotyped patients. Whereas an increasing number of lipids are involved in diseases with an inflammatory component, different MS techniques offer the possibility of analyzing lower quantities of these metabolites in medical samples. Unfortunately, to date, it has not been possible to develop a systematic methodology capable of analyzing all these lipids simultaneously. Hence, different analytical strategies have been used in order to analyze a given subfamily of lipids with common physicochemical properties. Commonly, these strategies are based on targeted LC-MS/MS methods working in MRM mode. Future advances in technical aspects will help achieve the in-depth characterization of the entire lipidome profile, including: (i) improving the method detection limits and dynamic range; (ii) increasing the number of commercially available standards (both external and internal isotopically labeled standards); (iii) optimizing the extraction protocols to allow simultaneous determination of the different lipid families; and (iv) further automating the identification and quantification of lipids.

Quantifying the regulatory lipid and other metabolic pathways in health and disease will result in improved characterization of the pathophysiology and provides mechanistic insight. Lipids constitute an intricate metabolic network (Fig. 1), which precisely regulates many different biological processes, such as energy storage, membrane function, and the production of lipid mediators, involved in physiological and pathophysiologial inflammation. Consequently, the analysis of SLs, GLs, oxylipins, ECs, and NAEs enables the possibility of describing putative cross-talk among the lipid pathways involved in inflammatory diseases and the discovery of combined therapies targeting related lipid pathways.

In the nutritional sciences, health has long been studied within the context of the quantity and type of the fat (lipid) consumed. A particular area of interest includes the balance of ω-3 and ω-6 fatty acids and effects of a fish oil rich diet. However, potential health benefits have been shown to be associated with other types of oil intake, with for example the benefits of virgin olive oil and walnuts reported to reduce the incidence of cardiovascular events [241]. Accordingly, to fully understand the effects and metabolic consequences of nutritional interventions, it is important to understand the concomitant shifts in lipid balance and production of downstream lipid mediators. The pathways highlighted in this review illustrate some of the important biological processes that can be impacted by dietary parameters. The deep quantitative characterization of lipids in individuals will help to unravel and potentially establish beneficial nutrition habits.

This work was supported by the Swedish Heart Lung Foundation, Åke Wiberg Foundation, and the Karolinska Institutet. D.B. has received support from U-BIOPRED, the Innovative Medicines Initiative Joint Undertaking under grant agreement number 115010, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind

contribution. A.C. was supported by the Vinnova research consortium Chronic Inflammation–Diagnosis and Therapy (CiDaT), D.G.S. was supported by Thematic Center for Inflammatory Disease (TCI), S.S. was supported by the Åke Wiberg Foundation, and C.E.W. was supported by the Center for Allergy Research and the Karolinska Institutet.

The authors have declared no conflict of interest.

8 References

[1] Shimizu, T., Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annu. Rev. Pharmacol. Toxicol.* 2009, *49*, 123–150.

[2] Charo, I. F., Ransohoff, R. M., The many roles of chemokines and chemokine receptors in inflammation. *N. Engl. J. Med.* 2006, *354*, 610–621.

[3] Lemanske, R. F., Busse, W. W., Asthma: clinical expression and molecular mechanisms. *J. Allergy Clin. Immunol.* 2010, *125*, S95–S102.

[4] Chung, K. F., Adcock, I. M., Multifaceted mechanisms in COPD: inflammation, immunity, and tissue repair and destruction. *Eur. Resp. J.* 2008, *31*, 1334–1356.

[5] Gregor, M. F., Hotamisligil, G. S., Inflammatory Mechanisms in Obesity. *Annu. Rev. Immunol*, 2011, *29*, 415–445.

[6] Mantovani, A., Allavena, P., Sica, A., Balkwill, F., Cancer-related inflammation. *Nature* 2008, *454*, 436–444.

[7] Holmes, C., Cunningham, C., Zotova, E., Woolford, J. et al., Systemic inflammation and disease progression in Alzheimer disease. *Neurology* 2009, *73*, 768–774.

[8] Yetukuri, L., Ekroos, K., Vidal-Puig, A., Oresic, M., Informatics and computational strategies for the study of lipids. *Mol. Biosyst.* 2008, *4*, 121–127.

[9] Funk, C. D., Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001, *294*, 1871–1875.

[10] Heller, A., Koch, T., Schmeck, J., van Ackern, K., Lipid mediators in inflammatory disorders. *Drugs* 1998, *55*, 487–496.

[11] Lundström, S. L., Yang, J., Källberg, H. J., Thunberg, S. et al., Allergic asthmatics show divergent lipid mediator profiles from healthy controls both at baseline and following birch pollen provocation. *Plos One* 2012, *7*, e33780.

[12] Ricciotti, E., FitzGerald, G. A., Prostaglandins and inflammation. *Arterioscler. Thromb. Vasc. Biol.* 2011, *31*, 986–1000.

[13] Wymann, M. P., Schneiter, R., Lipid signalling in disease. *Nat. Rev. Mol. Cell Biol.* 2008, *9*, 162–176.

[14] Quehenberger, O., Armando, A. M., Brown, A. H., Milne, S. B. et al., Lipidomics reveals a remarkable diversity of lipids in human plasma. *J. Lipid Res.* 2010, *51*, 3299–3305.

[15] Wenk, M. R., The emerging field of lipidomics. *Nat. Rev. Drug. Discov.* 2005, *4*, 594–610.

[16] Yang, J., Schmelzer, K., Georgi, K., Hammock, B. D., Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. *Anal. Chem.* 2009, *81*, 8085–8093.

[17] Murphy, R. C., Axelsen, P. H., Mass spectrometric analysis of long-chain lipids. *Mass Spectrom. Rev.* 2011, *30*, 579–599.

[18] Pulfer, M., Murphy, R. C., Electrospray mass spectrometry of phospholipids. *Mass Spectrom. Rev.* 2003, *22*, 332–364.

[19] Bligh, E. G., Dyer, W. J., A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 1959, *37*, 911–917.

[20] Folch, J., Lees, M., Stanley, G. H. S., A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 1957, *226*, 497–509.

[21] Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A. et al., Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J. Lipid Res.* 2008, *49*, 1137–1146.

[22] Kim, H. Y., Salem, N., Separation of lipid classes by solid phase extraction. *J. Lipid Res.* 1990, *31*, 2285–2289.

[23] Newman, J. W., Watanabe, T., Hammock, B. D., The simultaneous quantification of cytochrome P450 dependent linoleate and arachidonate metabolites in urine by HPLC-MS/MS. *J. Lipid Res.* 2002, *43*, 1563–1578.

[24] Lundström, S. L., Saluja, R., Adner, M., Haeggström, J. Z. et al., Lipid mediator metabolic profiling demonstrates differences in eicosanoid patterns in two phenotypically distinct mast cell populations. *J. Lipid Res.* 2013, *54*, 116–126.

[25] Murphy, R. C., Barkley, R. M., Berry, K. Z., Hankin, J. et al., Electrospray ionization and tandem mass spectrometry of eicosanoids. *Anal. Biochem.* 2005, *346*, 1–42.

[26] Balgoma, D., Montero, O., Balboa, M. A., Balsinde, J., Lipidomic approaches to the study of phospholipase A_2-regulated phospholipid fatty acid incorporation and remodeling. *Biochimie* 2010, *92*, 645–650.

[27] Matuszewski, B. K., Constanzer, M. L., Chavez-Eng, C. M., Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* 2003, *75*, 3019–3030.

[28] Postle, A. D., Wilton, D. C., Hunt, A. N., Attard, G. S., Probing phospholipid dynamics by electrospray ionisation mass spectrometry. *Prog. Lipid Res.* 2007, *46*, 200–224.

[29] Balgoma, D., Larsson, J., Rokach, J., Lawson, J. A. et al., Quantification of lipid mediator metabolites in human urine from asthma patients by electrospray ionization mass spectrometry: controlling matrix effects. *Submitted*.

[30] Koivusalo, M., Haimi, P., Heikinheimo, L., Kostiainen, R. et al., Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response. *J. Lipid Res.* 2001, *42*, 663–672.

[31] Simons, K., Ikonen, E., Functional rafts in cellmembranes. *Nature* 1997, *387*, 569–572.

[32] Bartke, N., Hannun, Y. A., Bioactive sphingolipids: metabolism and function. *J. Lipid. Res.* 2009, *50*(Suppl.), S91–S96.

[33] Michel, C., van Echten-Deckert, G., Conversion of dihydroceramide to ceramide occurs at the cytosolic face

of the endoplasmic reticulum. *FEBS Lett.* 1997, *416*, 153–155.

[34] Merrill, A. H., Jr., Wang, M. D., Park, M., Sullards, M. C., (Glyco)sphingolipidology: an amazing challenge and opportunity for systems biology. *Trends Biochem. Sci.* 2007, *32*, 457–468.

[35] Merrill, A. H., Sullards, M. C., Liu, Y., Chen, Y., Analysis of mammalian sphingolipids by liquid chromatography tandem mass spectrometry (LC-MS/MS) and tissue imaging mass spectrometry (TIMS). *Biochim. Biophys. Acta* 2011, *1811*, 838–853.

[36] Merrill, A. H., Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem. Rev.* 2011, *111*, 6387–6422.

[37] Pettus, B. J., Chalfant, C. E., Hannun, Y. A., Sphingolipids in Inflammation: roles and implications. *Curr. Mol. Med.* 2004, *4*, 405–418.

[38] Hla, T., Dannenberg, A. J., Sphingolipid signaling in metabolic disorders. *Cell Metab.* 2012, *16*, 420–434.

[39] Nixon, G. F., Sphingolipids in inflammation: pathological implications and potential therapeutic targets. *Br. J. Pharmacol.* 2009, *158*, 982–993.

[40] Fyrst, H., Saba, J. D., An update on sphingosine-1-phosphate and other sphingolipid mediators. *Nat. Chem. Biol.* 2010, *6*, 489–497.

[41] Mitra, P., Oskeritzian, C. A., Payne, S. G., Beaven, M. A. et al., Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 16394–16399.

[42] Jolly, P. S., Bektas, M., Olivera, A., Gonzalez-Espinosa, C. et al., Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. *J. Exp. Med.* 2004, *199*, 959–970.

[43] Pettus, B. J., Bielawski, J., Porcelli, A. M., Reames, D. L. et al., The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-α. *FASEB J.* 2003, *17*, 1411–1421.

[44] Cowart, L. A., Sphingolipids: players in the pathology of metabolic disease. *Trends Endocrinol. Metab.* 2009, *20*, 34–42.

[45] Egom, E. E., Mamas, M. A., Clark, A. L., The potential role of sphingolipid- mediated cell signaling in the interaction between hyperglycemia, acute myocardial infarction and heart failure. *Expert. Opin. Ther. Targets* 2012, *16*, 791–800.

[46] van Echten-Deckert, G., Walter, J., Sphingolipids: critical players in Alzheimer's disease. *Prog. Lipid Res.* 2012, *51*, 378–393.

[47] Podbielska, M., Krotkiewski, H., Hogan, E. L., Signaling and regulatory functions of bioactive sphingolipids as therapeutic targets in multiple sclerosis. *Neurochem. Res.* 2012, *37*, 1154–1169.

[48] Jana, A., Pahan, K., Sphingolipids in multiple sclerosis. *Neuromolecular Med.* 2010, *12*, 351–361.

[49] Haughey, N. J., Sphingolipids in neurodegeneration. *Neuromolecular Med.* 2010, *12*, 301–305.

[50] Skoura, A., Sanchez, T., Claffey, K., Mandala, S. M. et al., Essential role of sphingosine 1–phosphate receptor 2 in pathological angiogenesis of the mouse retina. *J. Clin. Invest.* 2007, *117*, 2506–2516.

[51] Gon, Y., Wood, M. R., Kiosses, W. B., Jo, E. et al., S1P3 receptor-induced reorganization of epithelial tight junctions compromises lung barrier integrity and is potentiated by TNF. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 9270–9275.

[52] Miron, V. E., Schubart, A., Antel, J. P., Central nervous system-directed effects of FTY720 (fingolimod). *J. Neurol. Sci.* 2008, *274*, 13–17.

[53] Cyster, J. G., Schwab, S. R., in: Paul, W. E. (Ed.), *Annual Review of Immunology*, Vol *30*, Annual Reviews, Palo Alto 2012, pp. 69–94.

[54] Guangwei, L., Kai, Y., Samir, B., Sharad, S. et al., The S1P1-mTOR axis directs the reciprocal differentiation of TH1 and Treg cells. *Nat. Immunol.* 2010, *11*, 1047–1056.

[55] Pelletier, D., Hafler, D. A., Fingolimod for multiple sclerosis. *N. Engl. J. Med.* 2012, *366*, 339–347.

[56] Chun, J., Hartung, H. P., Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clin. Neuropharmacol.* 2010, *33*, 91–101.

[57] Mehling, M., Lindberg, R., Raulf, F., Kuhle, J. et al., Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. *Neurology* 2010, *75*, 403–410.

[58] Kim, S., Steelman, A. J., Zhang, Y., Kinney, H. C. et al., Aberrant upregulation of astroglial ceramide potentiates oligodendrocyte injury. *Brain Pathol.* 2012, *22*, 41–57.

[59] Qin, J., Berdyshev, E., Goya, J., Natarajan, V. et al., Neurons and oligodendrocytes recycle sphingosine 1-phosphate to ceramide: significance for apoptosis and multiple sclerosis. *J. Biol. Chem.* 2010, *285*, 14134–14143.

[60] Lahiri, S., Futerman, A., The metabolism and function of sphingolipids and glycosphingolipids. *Cell Mol. Life Sci.* 2007, *64*, 2270–2284.

[61] O'Keeffe, J., Gately, C. M., Counihan, T., Hennessy, M. et al., T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to alpha-galactosylceramide are impaired. *J. Neurol. Sci.* 2008, *275*, 22–28.

[62] Ilan, Y., Elstein, D., Zimran, A., Glucocerebroside: an evolutionary advantage for patients with Gaucher disease and a new immunomodulatory agent. *Immunol. Cell Biol.* 2009, *87*, 514–524.

[63] Saadat, L., Dupree, J. L., Kilkus, J., Han, X. et al., Absence of oligodendroglial glucosylceramide synthesis does not result in CNS myelin abnormalities or alter the dysmyelinating phenotype of CGT-deficient mice. *Glia* 2010, *58*, 391–398.

[64] Mielke, M. M., Haughey, N. J., Bandaru, V. V., Weinberg, D. D. et al., Plasma sphingomyelins are associated with cognitive progression in Alzheimer's disease. *J. Alzheimers Dis.* 2011, *27*, 259–269.

[65] Mielke, M. M., Haughey, N. J., Ratnam Bandaru, V. V., Schech, S. et al., Plasma ceramides are altered in mild

cognitive impairment and predict cognitive decline and hippocampal volume loss. *Alzheimers Dement.* 2010, *6*, 378–385.

[66] Mielke, M. M., Bandaru, V. V., McArthur, J. C., Chu, M. et al., Disturbance in cerebral spinal fluid sphingolipid content is associated with memory impairment in subjects infected with the human immunodeficiency virus. *J. Neurovirol.* 2010, *16*, 445–456.

[67] Mielke, M. M., Bandaru, V. V., Haughey, N. J., Rabins, P. V. et al., Serum sphingomyelins and ceramides are early predictors of memory impairment. *Neurobiol. Aging* 2010, *31*, 17–24.

[68] Schiffmann, S., Sandner, J., Schmidt, R., Birod, K. et al., The selective COX-2 inhibitor celecoxib modulates sphingolipid synthesis. *J. Lipid Res.* 2009, *50*, 32–40.

[69] Hicks, A. A., Pramstaller, P. P., Johansson, A., Vitart, V. et al., Genetic determinants of circulating sphingolipid concentrations in European populations. *PLoS Genet.* 2009, *5*, e1000672.

[70] Han, X. L., Gross, R. W., Shotgun lipidomics: multidimensional MS analysis of cellular lipidomes. *Expert. Rev. Proteomics.* 2005, *2*, 253–264.

[71] Han, X. L., Gross, R. W., Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. *Mass Spectrom. Rev.* 2005, *24*, 367–412.

[72] Merrill, A. H., Shaner, R. L., Allegood, J. C., Park, H. et al., Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers. *J. Lipid Res.* 2009, *50*, 1692–1707.

[73] Merrill, A. H., Sullards, M. C., Allegood, J. C., Kelly, S. et al., Structure-specific, quantitative methods for analysis of sphingolipids by liquid chromatography-tandem mass spectrometry: "inside-out" sphingolipidomics. *Methods Enzymol.* 2007, *432*, 83–115.

[74] Merrill, A. H., Sullards, M. C., Allegood, J. C., Kelly, S. et al., Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. *Methods* 2005, *36*, 207–224.

[75] Farwanah, H., Pierstorff, B., Schmelzer, C. E. H., Raith, K. et al., Separation and mass spectrometric characterization of covalently bound skin ceramides using LC/APCI-MS and Nano-ESI-MS/MS. *J. Chromatogr. B* 2007, *852*, 562–570.

[76] Farwanah, H., Kolter, T., Sandhoff, K., Mass spectrometric analysis of neutral sphingolipids: methods, applications, and limitations. *Biochim. Biophys. Acta* 2011, *1811*, 854–860.

[77] Farwanah, H., Wirtz, J., Kolter, T., Raith, K. et al., Normal phase liquid chromatography coupled to quadrupole time of flight atmospheric pressure chemical ionization mass spectrometry for separation, detection and mass spectrometric profiling of neutral sphingolipids and cholesterol. *J. Chromatogr. B* 2009, *877*, 2976–2982.

[78] Lands, W. E. M., Stories about acyl chains. *Biochim. Biophys. Acta* 2000, *1483*, 1–14.

[79] Schaloske, R. H., Dennis, E. A., The phospholipase A_2 superfamily and its group numbering system. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* 2006, *1761*, 1246–1259.

[80] Pérez-Chacón, G., Astudillo, A. M., Balgoma, D., Balboa, M. A. et al., Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* 2009, *1791*, 1103–1113.

[81] Balsinde, J., Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J.* 2002, *364*, 695–702.

[82] Chilton, F. H., Fonteh, A. N., Surette, M. E., Triggiani, M. et al., Control of arachidonate levels within inflammatory cells. *Biochim. Biophys. Acta Lipid. Lipid. Met.* 1996, *1299*, 1–15.

[83] Balgoma, D., Astudillo, A. M., Pérez-Chacón, G., Montero, O. et al., Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids. *J. Immunol.* 2010, *184*, 3857–3865.

[84] McIntyre, T. M., Prescott, S. M., Stafforini, D. M., The emerging roles of PAF acetylhydrolase. *J. Lipid Res.* 2009, *50*, S255–S259.

[85] Di Paolo, G., De Camilli, P., Phosphoinositides in cell regulation and membrane dynamics. *Nature* 2006, *443*, 651–657.

[86] Gómez-Fernández, J. C., Corbalán-García, S., Diacylglycerols, multivalent membrane modulators. *Chem. Phys. Lipids* 2007, *148*, 1–25.

[87] Foskett, J. K., White, C., Cheung, K. H., Mak, D. O. D., Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol. Rev.* 2007, *87*, 593–658.

[88] Zwaal, R. F. A., Schroit, A. J., Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood* 1997, *89*, 1121–1132.

[89] Kay, J. G., Koivusalo, M., Ma, X. X., Wohland, T. et al., Phosphatidylserine dynamics in cellular membranes. *Mol. Biol. Cell* 2012, *23*, 2198–2212.

[90] Yeung, T., Gilbert, G. E., Shi, J., Silvius, J. et al., Membrane phosphatidylserine regulates surface charge and protein localization. *Science* 2008, *319*, 210–213.

[91] Leventis, P. A., Grinstein, S., The distribution and function of phosphatidylserine in cellular membranes. *Annu. Rev. Biophys* 2010, *39*, 407–427.

[92] Veldhuizen, R., Nag, K., Orgeig, S., Possmayer, F., The role of lipids in pulmonary surfactant. *Biochim. Biophys. Acta-Mol. Basis Dis.* 1998, *1408*, 90–108.

[93] Stafforini, D. M., McIntyre, T. M., Zimmerman, G. A., Prescott, S. M., Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. *Crit. Rev. Clin. Lab. Sci.* 2003, *40*, 643–672.

[94] Prescott, S. M., Zimmerman, G. A., Stafforini, D. M., McIntyre, T. M., Platelet-activating factor and related lipid mediators. *Annu. Rev. Biochem.* 2000, *69*, 419–445.

[95] Gräler, M. H., Goetzl, E. J., Lysophospholipids and their G protein-coupled receptors in inflammation and immunity. *Biochim. Biophys. Acta* 2002, *1582*, 168–174.

[96] Ishii, I., Fukushima, N., Ye, X. Q., Chun, J., Lysophospholipid receptors: signaling and biology. *Annu. Rev. Biochem.* 2004, *73*, 321–354.

[97] McIntyre, T. M., Pontsler, A. V., Silva, A. R., St Hilaire, A. et al., Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARg agonist. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 131–136.

[98] Chicco, A. J., Sparagna, G. C., Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am. J. Physiol. Cell Physiol.* 2007, *292*, C33–C44.

[99] Zwaal, R. F. A., Comfurius, P., Bevers, E. M., Surface exposure of phosphatidylserine in pathological cells. *Cell. Mol. Life Sci.* 2005, *62*, 971–988.

[100] Wright, S. M., Hockey, P. M., Enhorning, G., Strong, P. et al., Altered airway surfactant phospholipid composition and reduced lung function in asthma. *J. Appl. Physiol.* 2000, *89*, 1283–1292.

[101] Yuan, T. L., Cantley, L. C., PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008, *27*, 5497–5510.

[102] Mills, G. B., Moolenaar, W. H., The emerging role of lysophosphatidic acid in cancer. *Nat. Rev. Cancer* 2003, *3*, 582–591.

[103] Ejsing, C. S., Duchoslav, E., Sampaio, J., Simons, K. et al., Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. *Anal. Chem.* 2006, *78*, 6202–6214.

[104] Ejsing, C. S., Sampaio, J. L., Surendranath, V., Duchoslav, E. et al., Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 2136–2141.

[105] Milton, A. S., Wendlandt, S., Effects on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of unanaesthetized cats and rabbits. *J. Physiol.* 1971, *218*, 325–336.

[106] Xu, Z.-Z., Zhang, L., Liu, T., Park, J. Y. et al., Resolvins RvE_1 and RvD_1 attenuate inflammatory pain via central and peripheral actions. *Nat. Med.* 2010, *16*, 592–597.

[107] Serhan, C. N., Chiang, N., Van Dyke, T. E., Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 2008, *8*, 349–361.

[108] Haworth, O., Cernadas, M., Yang, R., Serhan, C. N. et al., Resolvin E_1 regulates interleukin 23, interferon-[gamma] and lipoxin A_4 to promote the resolution of allergic airway inflammation. *Nat. Immunol.* 2008, *9*, 873–879.

[109] Lundström, S., Balgoma, D., Wheelock, Å. M., Haeggström, J. Z. et al., Lipid mediator profiling in pulmonary disease. *Curr. Pharm. Biotechnol.* 2011, *12*, 1026–1052.

[110] Moghaddam, M. F., Motoba, K., Borhan, B., Pinot, F. et al., Novel metabolic pathways for linoleic and arachidonic acid metabolism. *Biochim. Biophys. Acta* 1996, *1290*, 327–339.

[111] Gundlach, H., Zenk, M. H., Biological activity and biosynthesis of pentacyclic oxylipins: the linoleic acid pathway. *Phytochemistry* 1998, *47*, 527–537.

[112] Serhan, C. N., Gotlinger, K., Hong, S., Arita, M., Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostagl. Other Lipid Mediat.* 2004, *73*, 155–172.

[113] Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A. et al., Cyclooxygenase in biology and disease. *FASEB J.* 1998, *12*, 1063–1073.

[114] Feussner, I., Wasternack, C., The lipoxygenase pathway. *Annu. Rev. Plant Biol.* 2002, *53*, 275–297.

[115] Guengerich, F., in: Ortiz de Montellano, P. R. (Ed.), *Cytochrome P450: Structure, Mechanism, and Biochemistry*, Springer, New York, NY 2005, pp. 377–530.

[116] Herschman, H. R., Hall, W., Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. *Cancer Metastasis Rev.* 1994, *13*, 241–256.

[117] Chenevard, R., Hürlimann, D., Béchir, M., Enseleit, F. et al., Selective COX-2 inhibition improves endothelial function in coronary artery disease. *Circulation* 2003, *107*, 405–409.

[118] Schrör, K., Aspirin and platelets: the antiplatelet action of aspirin and its role in thrombosis treatment and prophylaxis. *Semin. Thromb. Hemost.* 1997, *23*, 349–356.

[119] Ivanov, I., Heydeck, D., Hofheinz, K., Roffeis, J. et al., Molecular enzymology of lipoxygenases. *Arch. Biochem. Biophys.* 2010, *503*, 161–174.

[120] Haeggström, J. Z., Funk, C. D., Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. *Chem. Rev.* 2011, *111*, 5866–5898.

[121] Rai, G., Kenyon, V., Jadhav, A., Schultz, L. et al., Discovery of potent and selective inhibitors of human reticulocyte 15-lipoxygenase-1. *J. Med. Chem.* 2010, *53*, 7392–7404.

[122] Lewis, R. A., Austen, K. F., Soberman, R. J., Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N. Engl. J. Med.* 1990, *323*, 645–655.

[123] Thunnissen, M. M., Norlund, P., Haeggstrom, J. Z., Crystal structure of human leukotriene A_4 hydrolase, a bifunctional enzyme in inflammation. *Nat. Struct. Bio.* 2001, *8*, 131–135.

[124] Sala, A., Folco, G., Neutrophils, endothelial cells, and cysteinyl leukotrienes: a new approach to neutrophil-dependent inflammation? *Biochem. Biophys. Res. Commun.* 2001, *283*, 1003–1006.

[125] Molina, D. M., Wetterholm, A., Kohl, A., McCarthy, A. A. et al., Structural basis for synthesis of inflammatory mediators by human leukotriene C_4 synthase. *Nature* 2007, *448*, 613–616.

[126] Zeldin, D. C., Wei, S. Z., Falck, J. R., Hammock, B. D. et al., Metabolism of epoxyeicosatrienoic acids by cytosolic epoxide hydrolase: substrate structural determinants of asymmetric catalysis. *Arch. Biochem. Biophys.* 1995, *316*, 443–451.

[127] Spector, A. A., Fang, X., Snyder, G. D., Weintraub, N. L., Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog. Lipid Res.* 2004, *43*, 55–90.

[128] Newman, J. W., Morisseau, C., Hammock, B. D., Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog. Lipid Res.* 2005, *44*, 1–51.

[129] Li, N., Liu, J.-Y., Qiu, H., Harris, T. R. et al., Use of metabolomic profiling in the study of arachidonic acid metabolism in cardiovascular disease. *Congest. Heart Fail.* 2011, *17*, 42–46.

[130] Lundström, S. L., Levänen, B., Nording, M., Klepczynska-Nyström, A. et al., Asthmatics exhibit altered oxylipin profiles compared to healthy individuals after subway air exposure. *PLoS One* 2011, *6*, e23864.

[131] Dong, H., Metabolomic profiling of lipids for biomarker discovery. *Biochem. Anal. Biochem.* 2012, *01*, e115.

[132] Podolin, P. L., Bolognese, B. J., Foley, J. F., Long, E. et al., In vitro and in vivo characterization of a novel soluble epoxide hydrolase inhibitor. *Prostaglandins Other Lipid Mediat.* 2013, Doi:10.1016/j.prostaglandins.2013.02.001

[133] Hamman, M. A., Thompson, G. A., Hall, S. D., Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochem. Pharmacol.* 1997, *54*, 33–41.

[134] Boeglin, W. E., Kim, R. B., Brash, A. R., A 12R-lipoxygenase in human skin: mechanistic evidence, molecular cloning, and expression. *Proc. Natl. Acad. Sci. USA* 1998, *95*, 6744–6749.

[135] Camacho, M., Godessart, N., Antón, R., Garca, M. et al. Interleukin-1 enhances the ability of cultured human umbilical vein endothelial cells to oxidize linoleic acid. *J. Biol. Chem.* 1995, *270*, 17279–17286.

[136] Baer, A. N., Costello, P. B., Green, F. A., Stereospecificity of the hydroxyeicosatetraenoic and hydroxyoctadecadienoic acids produced by cultured bovine endothelial cells. *Biochim. Biophys. Acta* 1991, *1085*, 45–52.

[137] Bittleman, D. B., Casale, T. B., 5-Hydroxyeicosatetraenoic acid (HETE)-induced neutrophil transcellular migration is dependent upon enantiomeric structure. *Am. J. Respir. Cell Mol. Biol.* 1995, *12*, 260–267.

[138] Dohadwala, M., Batra, R. K., Luo, J., Lin, Y. et al., Autocrine/paracrine prostaglandin E_2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. *J. Biol. Chem.* 2002, *277*, 50828–50833.

[139] Sales, K. J., Katz, A. A., Howard, B., Soeters, R. P. et al., Cyclooxygenase-1 is up-regulated in cervical carcinomas: autocrine/paracrine regulation of cyclooxygenase-2, prostaglandin e receptors, and angiogenic factors by cyclooxygenase-1. *Cancer Res.* 2002, *62*, 424–432.

[140] Senior, J., Sangha, R., Baxter, G. S., Marshall, K. et al., In vitro characterization of prostanoid FP-, DP-, IP- and TP-receptors on the non-pregnant human myometrium. *Br. J. Pharmacol.* 1992, *107*, 215–221.

[141] Dorsam, R. T., Gutkind, J. S., G-protein-coupled receptors and cancer. *Nat. Rev. Cancer* 2007, *7*, 79–94.

[142] Hata, A. N., Breyer, R. M., Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol. Ther.* 2004, *103*, 147–166.

[143] Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y. et al., Cloning and expression of cDNA for a human thromboxane A_2 receptor. *Nature* 1991, *349*, 617–620.

[144] Capra, V., Habib, A., Accomazzo, M. R., Ravasi, S. et al., Thromboxane prostanoid receptor in human airway smooth muscle cells: a relevant role in proliferation. *Eur. J. Pharmacol.* 2003, *474*, 149–159.

[145] Hardy, C. C., Robinson, C., Tattersfield, A. E., Holgate, S. T., The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic men. *N. Engl. J. Med.* 1984, *311*, 209–213.

[146] Barr, R. M., Koro, O., Francis, D. M., Black, A. K. et al., The release of prostaglandin D_2 from human skin in vivo and in vitro during immediate allergic reactions. *Br. J. Pharmacol.* 1988, *94*, 773–780.

[147] Gardiner, P. J., Characterization of prostanoid relaxant/inhibitory receptors (ψ) using a highly selective agonist, TR4979. *Br. J. Pharmacol.* 1986, *87*, 45–56.

[148] Minami, T., Nakano, H., Kobayashi, T., Sugimoto, Y. et al., Characterization of EP receptor subtypes responsible for prostaglandin E_2-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br. J. Pharmacol.* 2001, *133*, 438–444.

[149] Martin, J. G., Suzuki, M., Maghni, K., Pantano, R. et al., The immunomodulatory actions of prostaglandin E_2 on allergic airway responses in the rat. *J. Immunol.* 2002, *169*, 3963–3969.

[150] Doi, Y. M. T., Nishizawa, M., Mabuchi, T., Mori, H. et al., Central nociceptive role of prostacyclin (IP) receptor induced by peripheral inflammation. *Neuroreport* 2002, *13*, 93–96.

[151] Murata, T., Ushikubi, F., Matsuoka, T., Hirata, M. et al., Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 1997, *388*, 678–682.

[152] Baba, H., Kohno, T., Moore, K. A., Woolf, C. J., Direct activation of rat spinal dorsal horn neurons by prostaglandin E_2. *J. Neurosci.* 2001, *21*, 1750–1756.

[153] Abrams, P. H., Feneley, R. C. L., The actions of prostaglandins on the smooth muscle of the human urinary tract in vitro. *Br. J. Urol.* 1975, *47*, 909–915.

[154] Kuehl, F. A., Egan, R. W., Prostaglandins, arachidonic acid, and inflammation. *Science* 1980, *210*, 978–984.

[155] Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M. et al., Prostaglandin E_2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* 2005, *310*, 1504–1510.

[156] MacKenzie, I. Z., Embrey, M. P., A comparison of PGE_2 and PGF_{2a} vaginal gel for ripening the cervix before induction of labour. *Br. J. Obstet. Gynaecol.* 1979, *86*, 167–170.

[157] Dong, M.-F., Ma, Z.-S., Ma, S.-J., Chai, S.-D. et al., Effect of prostaglandin E_1 on pulmonary arterial hypertension following corrective surgery for congenital heart disease. *J. Cardiovasc. Pharmacol. Ther.* 2012, *17*, 303–307.

[158] von Scheidt, W., Costard-Jaeckle, A., Stempfle, H. U., Deng, M. C. et al., Prostaglandin E_1 testing in heart failure-associated pulmonary hypertension enables transplantation: the PROPHET study. *J. Heart Lung Transplant.* 2006, *25*, 1070–1076.

[159] Nehra, A., Blute, M. L., Barrett, D. M., Moreland, R. B., Rationale for combination therapy of intraurethral prostaglandin E_1 and sildenafil in the salvage of erectile dysfunction patients desiring noninvasive therapy. *Int. J. Impot. Res.* 2002, *14*(Suppl 1), S38–S42.

[160] Grosser, T., Yu, Y., FitzGerald, G. A., Emotion recollected in tranquility: lessons learned from the COX-2 saga. *Annu. Rev. Med.* 2010, *61*, 17–33.

[161] Fries, S., Grosser, T., Price, T. S., Lawson, J. A. et al., Marked interindividual variability in the response to selective inhibitors of cyclooxygenase-2. *Gastroenterology* 2006, *130*, 55–64.

[162] Duffield-Lillico, A. J., Boyle, J. O., Zhou, X. K., Ghosh, A. et al., Levels of prostaglandin E metabolite and leukotriene E_4 are increased in the urine of smokers: evidence that celecoxib shunts arachidonic acid into the 5-lipoxygenase pathway. *Cancer Prev. Res.* 2009, *2*, 322–329.

[163] Hamberg, M., Samuelss, B., On the metabolism of prostaglandins E_1 and E_2 in man. *J. Biol. Chem.* 1971, *246*, 6713–6721.

[164] Song, W. L., Lawson, J. A., Wang, M., Zou, H. et al., Noninvasive assessment of the role of cyclooxygenases in cardiovascular health: A detailed HPLC/MS/MS method. *Methods Enzymol.* 2007, *433*, 51–72.

[165] Samuelsson, B., The discovery of the leukotrienes. *Am. J. Respir. Crit. Care Med.* 2000, *161*, S2–S6.

[166] Briscoe, C. P., Tadayyon, M., Andrews, J. L., Benson, W. G. et al., The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* 2003, *278*, 11303–11311.

[167] Kliewer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J. et al., Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 4318–4323.

[168] Murphy, R. C., Gijón, M. A., Biosynthesis and metabolism of leukotrienes. *Biochem. J.* 2007, *405*, 379–395.

[169] Bisgaard, H., Pathophysiology of the cysteinyl leukotrienes and effects of leukotriene receptor antagonists in asthma. *Allergy* 2001, *56*, 7–11.

[170] Shastri, S., McNeill, J. R., Wilson, T. W., Poduri, R. et al., Cysteinyl leukotrienes mediate enhanced vasoconstriction to angiotensin II but not endothelin-1 in SHR. *Am. J. Physiol. Heart Circ. Physiol.* 2001, *281*, H342–H349.

[171] Parameswaran, K., Cox, G., Radford, K., Janssen, L. J. et al., Cysteinyl leukotrienes promote human airway smooth muscle migration. *Am. J. Respir. Crit. Care Med.* 2002, *166*, 738–742.

[172] Paruchuri, S., Tashimo, H., Feng, C., Maekawa, A. et al., Leukotriene E_4–induced pulmonary inflammation is mediated by the P2Y12 receptor. *J. Exp. Med.* 2009, *206*, 2543–2555.

[173] Kumlin, M., Dahlen, B., Bjorck, T., Zetterstrom, O. et al., Urinary excretion of leukotriene E_4 and 11-dehydrothromboxane B_2 in response to bronchial provocations with allergen, aspirin, leukotriene D_4, and histamine in asthmatics. *Am. Rev. Respir. Dis.* 1992, *146*, 96–103.

[174] Boyce, J. A., Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol. Rev.* 2007, *217*, 168–185.

[175] Turner, C. R., Breslow, R., Conklyn, M. J., Andresen, C. J. et al., In vitro and in vivo effects of leukotriene B4 antagonism in a primate model of asthma. *J. Clin. Invest.* 1996, *97*, 381–387.

[176] Sharon, P., Stenson, W. F., Enhanced synthesis of leukotriene B4 by colonic mucosa in inflammatory bowel disease. *Gastroenterology* 1984, *86*, 453–460.

[177] Sánchez-Galán, E., Gómez-Hernández, A., Vidal, C., Martín-Ventura, J. L. et al., Leukotriene B4 enhances the activity of nuclear factor-κB pathway through BLT1 and BLT2 receptors in atherosclerosis. *Cardiovasc. Res.* 2009, *81*, 216–225.

[178] Janakiram, N. B., Mohammed, A., Rao, C. V., Role of lipoxins, resolvins, and other bioactive lipids in colon and pancreatic cancer. *Cancer Metastasis Rev.* 2011, *30*, 507–523.

[179] Serhan, C. N., Hamberg, M., Samuelsson, B., Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc. Natl. Acad. Sci. USA* 1984, *81*, 5335–5339.

[180] Kohli, P., Levy, B. D., Resolvins and protectins: mediating solutions to inflammation. *Br. J. Pharmacol.* 2009, *158*, 960–971.

[181] Serhan, C. N., Petasis, N. A., Resolvins and protectins in inflammation resolution. *Chem. Rev.* 2011, *111*, 5922–5943.

[182] Serhan, C. N., Yang, R., Martinod, K., Kasuga, K. et al., Maresins: novel macrophage mediators with potent anti-inflammatory and proresolving actions. *J. Exp. Med.* 2009, *206*, 15–23.

[183] Serhan, C. N., Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? *Am. J. Pathol.* 2010, *177*, 1576–1591.

[184] Samuelsson, B., Dahlén, S. E., Lindgren, J. A., Rouzer, C. A. et al., Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987, *237*, 1171–1176.

[185] Chiang, N., Arita, M., Serhan, C. N., Anti-inflammatory circuitry: lipoxin, aspirin-triggered lipoxins and their receptor ALX. *Prostagl. Leukot. Essent. Fatty Acids* 2005, *73*, 163–177.

[186] Zhou, M., Chen, B., Sun, H., Deng, Z. et al., The protective effects of lipoxin A_4 during the early phase of severe acute pancreatitis in rats. *Scand. J. Gastroenterol.* 2011, *46*, 211–219.

[187] Arita, M., Ohira, T., Sun, Y.-P., Elangovan, S. et al., Resolvin E_1 selectively interacts with leukotriene B_4 receptor BLT_1 and ChemR23 to regulate Inflammation. *J. Immunol.* 2007, *178*, 3912–3917.

[188] Zhang, M. J., Spite, M., Resolvins: anti-inflammatory and proresolving mediators derived from omega-3 polyunsaturated fatty acids. *Annu. Rev. Nutr.* 2012, *32*, 203–227.

[189] Levy, B. D., De Sanctis, G. T., Devchand, P. R., Kim, E. et al., Multi-pronged inhibition of airway hyper-responsiveness and inflammation by lipoxin A_4. *Nat. Med.* 2002, *8*, 1018–1023.

[190] Levy, B. D., Kohli, P., Gotlinger, K., Haworth, O. et al., Protectin D_1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J. Immunol.* 2007, *178*, 496–502.

[191] Levy, B. D., Lukacs, N. W., Berlin, A. A., Schmidt, B. et al., Lipoxin A_4 stable analogs reduce allergic airway responses via mechanisms distinct from $CysLT_1$ receptor antagonism. *FASEB J.* 2007, *21*, 3877–3884.

[192] Neilson, A. P., Ren, J., Hong, Y. H., Sen, A. et al., Effect of fish oil on levels of R- and S-enantiomers of 5-, 12-, and 15-hydroxyeicosatetraenoic acids in mouse colonic mucosa. *Nutr. Cancer* 2011, *64*, 163–172.

[193] Liu, C., Xu, D., Liu, L., Schain, F. et al., 15-lipoxygenase-1 induces expression and release of chemokines in cultured human lung epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2009, *297*, L196–L203.

[194] Chanez, P., Bonnans, C., Chavis, C., Vachier, I., 15-lipoxygenase: a Janus enzyme? *Am. J. Respir. Cell Mol. Biol.* 2002, *27*, 655–658.

[195] Morin, C., Sirois, M., Echave, V., Gomes, M. M. et al., Functional effects of 20-HETE on human bronchi: hyperpolarization and relaxation due to BKCa channel activation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2007, *293*, L1037–L1044.

[196] Katoh, T., Takahashi, K., Capdevila, J., Karara, A. et al., Glomerular stereospecific synthesis and hemodynamic actions of 8,9-epoxyeicosatrienoic acid in rat kidney. *Am. J. Physiol.* 1991, *261*, F578–F586.

[197] Harder, D. R., Campbell, W. B., Roman, R. J., Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *J. Vasc. Res.* 1995, *32*, 79–92.

[198] Roman, R. J., P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.* 2002, *82*, 131–185.

[199] Pokreisz, P., Fleming, I., Kiss, L., Barbosa-Sicard, E. et al., Cytochrome P450 epoxygenase gene function in hypoxic pulmonary vasoconstriction and pulmonary vascular remodeling. *Hypertension* 2006, *47*, 762–770.

[200] Pascual, J. M. S., McKenzie, A., Yankaskas, J. R., Falck, J. R. et al., Epoxygenase metabolites of arachidonic acid affect electrophysiologic properties of rat tracheal epithelial cells1. *J. Pharmacol. Exp. Ther.* 1998, *286*, 772–779.

[201] Jacobs, E. R., Zeldin, D. C., The lung HETEs (and EETs) up. *Am. J. Physiol. Heart Circ. Physiol.* 2001, *280*, H1–H10.

[202] Hsi, L. C., Wilson, L., Nixon, J., Eling, T. E., 15-lipoxygenase-1 metabolites down-regulate peroxisome proliferator-activated receptor gamma via the MAPK signaling pathway. *J. Biol. Chem.* 2001, *276*, 34545–34552.

[203] Gardner, H. W., Nelson, E. C., Tjarks, L. W., England, R. E., Acid-catalyzed transformation of 13(S)-hydroperoxylinoleic acid into epoxyhydroxyoctadecenoic and trihydroxyoctadecenoic acids. *Chem. Phys. Lipids* 1984, *35*, 87–101.

[204] Koeners, M. P., Wesseling, S., Ulu, A., Sepúlveda, R. L. et al., Soluble epoxide hydrolase in the generation and maintenance of high blood pressure in spontaneously hypertensive rats. *Am. J. Physiol. Endocrinol. Metab.* 2011, *300*, E691–E698.

[205] Deems, R., Buczynski, M. W., Bowers Gentry, R., Harkewicz, R. et al., in: Brown, H. A. (Ed.), *Methods in Enzymology*, Academic Press, San Diego, CA 2007, pp. 59–82.

[206] Shearer, G. C., Harris, W. S., Pedersen, T. L., Newman, J. W., Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters. *J. Lipid Res.* 2010, *51*, 2074–2081.

[207] Jahn, U., Galano, J. M., Durand, T., Beyond prostaglandins – Chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty acids. *Angew. Chem. Int. Ed.* 2008, *47*, 5894–5955.

[208] Lee, S. H., Blair, I. A., Targeted chiral lipidomics analysis of bioactive eicosanoid lipids in cellular systems. *BMB Rep.* 2009, *42*, 401–410.

[209] Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G. et al., Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992, *258*, 1946–1949.

[210] Hanus, L., Gopher, A., Almog, S., Mechoulam, R., Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J. Med. Chem.* 1993, *36*, 3032–3034.

[211] Zoerner, A. A., Gutzki, F. M., Batkai, S., May, M. et al., Quantification of endocannabinoids in biological systems by chromatography and mass spectrometry: a comprehensive review from an analytical and biological perspective. *Biochim. Biophys. Acta* 2011, *1811*, 706–723.

[212] De Petrocellis, L., Cascio, M. G., Di Marzo, V., The endocannabinoid system: a general view and latest additions. *Br. J. Pharmacol.* 2004, *141*, 765–774.

[213] Hansen, H. S., Diep, T. A., *N*-acylethanolamines, anandamide and food intake. *Biochem. Pharmacol.* 2009, *78*, 553–560.

[214] Hansen, H. S., Moesgaard, B., Hansen, H. H., Petersen, G., *N*-acylethanolamines and precursor phospholipids – relation to cell injury. *Chem. Phys. Lipids* 2000, *108*, 135–150.

[215] McKinney, M. K., Cravatt, B. F., *Annual Review of Biochemistry*, Annual Reviews, Palo Alto 2005, pp. 411–432.

[216] Ueda, N., Tsuboi, K., Uyama, T., *N*-acylethanolamine metabolism with special reference to *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). *Prog. Lipid Res.* 2010, *49*, 299–315.

[217] Ueda, N., Tsuboi, K., Uyama, T., Enzymological studies on the biosynthesis of *N*-acylethanolamines. *Biochim. Biophys. Acta* 2010, *1801*, 1274–1285.

[218] Mechoulam, R., Benshabat, S., Hanus, L., Ligumsky, M. et al., Identification of an endogenous 2-monoglyceride, present in the canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 1995, *50*, 83–90.

[219] Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S. et al., 2-Arachidonoylglycerol – A possible endogenous cannabinoid receptor-ligand in brain. *Biochem. Biophys. Res. Commun.* 1995, *215*, 89–97.

[220] Bisogno, T., Ligresti, A., Di Marzo, V., The endocannabinoid signalling system: biochemical aspects. *Pharmacol. Biochem. Behav.* 2005, *81*, 224–238.

[221] Blankman, J. L., Simon, G. M., Cravatt, B. F., A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* 2007, *14*, 1347–1356.

[222] Hanus, L., Abu-Lafi, S., Fride, E., Breuer, A. et al., 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 3662–3665.

[223] Porter, A. C., Sauer, J. M., Knierman, M. D., Becker, G. W. et al., Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.* 2002, *301*, 1020–1024.

[224] Bisogno, T., Melck, D., Bobrov, M. Y., Gretskaya, N. M. et al., *N*-acyl-dopamines: novel synthetic CB1 cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. *Biochem. J.* 2000, *351*, 817–824.

[225] Oka, S., Tsuchie, A., Tokumura, A., Muramatsu, M. et al., Ether-linked analogue of 2-arachidonoylglycerol (noladin ether) was not detected in the brains of various mammalian species. *J. Neurochem.* 2003, *85*, 1374–1381.

[226] Buczynski, M. W., Parsons, L. H., Quantification of brain endocannabinoid levels: methods, interpretations and pitfalls. *Br. J. Pharmacol.* 2010, *160*, 423–442.

[227] Pacher, P., Batkai, S., Kunos, G., The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* 2006, *58*, 389–462.

[228] Sugiura, T., Kondo, S., Kishimoto, S., Miyashita, T. et al., Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor – Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *J. Biol. Chem.* 2000, *275*, 605–612.

[229] Starowicz, K., Nigam, S., Di Marzo, V., Biochemistry and pharmacology of endovanilloids. *Pharmacol. Ther.* 2007, *114*, 13–33.

[230] Balvers, M. G. J., Verhoeckx, K. C. M., Meijerink, J., Bijlsma, S. et al., Time-dependent effect of in vivo inflammation on eicosanoid and endocannabinoid levels in plasma, liver, ileum and adipose tissue in C57BL/6 mice fed a fish-oil diet. *Int. Immunopharmacol.* 2012, *13*, 204–214.

[231] Schmid, H. H. O., Schmid, P. C., Natarajan, V., *N*-acylated glycerophopholipids and their derivatives. *Prog. Lipid. Res.* 1990, *29*, 1–43.

[232] Darmani, N. A., Izzo, A. A., Degenhardt, B., Valenti, M. et al., Involvement of the cannabimimetic compound, *N*-palmitoyl-ethanolamine, in inflammatory and neuropathic conditions: review of the available pre-clinical data, and first human studies. *Neuropharmacology* 2005, *48*, 1154–1163.

[233] Matias, I., Gonthier, M. P., Petrosino, S., Docimo, L. et al., Role and regulation of acylethanolamides in energy balance: focus on adipocytes and beta-cells. *Br. J. Pharmacol.* 2007, *152*, 676–690.

[234] Jean-Gilles, L., Feng, S., Tench, C. R., Chapman, V. et al., Plasma endocannabinoid levels in multiple sclerosis. *J. Neurol. Sci.* 2009, *287*, 212–215.

[235] Zoerner, A. A., Stichtenoth, D. O., Engeli, S., Batkai, S. et al., Allergen challenge increases anandamide in bronchoalveolar fluid of patients with allergic asthma. *Clin. Pharmacol. Ther.* 2011, *90*, 388–391.

[236] Hermanson, D. J., Marnett, L. J., Cannabinoids, endocannabinoids, and cancer. *Cancer Metastasis Rev.* 2011, *30*, 599–612.

[237] Kondo, S., Kondo, H., Nakane, S., Kodaka, T. et al., 2-arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca2+-dependent and -independent mechanisms. *FEBS Lett.* 1998, *429*, 152–156.

[238] Balvers, M. G. J., Verhoeckx, K. C. M., Witkamp, R. F., Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* 2009, *877*, 1583–1590.

[239] Skonberg, C., Artmann, A., Cornett, C., Hansen, S. H. et al., Pitfalls in the sample preparation and analysis of *N*-acylethanolamines. *J. Lipid Res.* 2010, *51*, 3062–3073.

[240] Zoerner, A. A., Batkai, S., Suchy, M. T., Gutzki, F. M. et al., Simultaneous UPLC-MS/MS quantification of the endocannabinoids 2-arachidonoyl glycerol (2AG), 1-arachidonoyl glycerol (1AG), and anandamide in human plasma: minimization of matrix-effects, 2AG/1AG isomerization and degradation by toluene solvent extraction. *J. Chromatogr. B* 2012, *883*, 161–171.

[241] Estruch, R., Ros, E., Salas-Salvado, J., Covas, M. I. et al., Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Eng. J. Med.* 2013, *368*, 1279–1290.

