Ether-lipids and cellular signaling: A differential role of alkyl- and alkenyl-ether-lipids?

Marion Papin [a], [*], Ana Maria Bouchet [b], Aurélie Chantôme [a], Christophe Vandier [a, b]

[a] Nutrition, Croissance, Cancer (N2C) UMR 1069, University of Tours, INSERM, 37000, Tours, France
[b] Lifesome Therapeutics, López de Hoyos 42, 28006, Madrid, Spain

ARTICLE INFO

ABSTRACT

Ether-lipids (EL) are specific lipids bearing a characteristic *sn*-1 ether bond. Depending on the ether or vinyl-ether nature of this bond, they are present as alkyl- or alkenyl-EL, respectively. Among EL, alkenyl-EL, also referred as plasmalogens in the literature, attract most of the scientific interest as they are the predominant EL species in eukaryotic cells, thus less is known about alkyl-EL. EL have been implicated in various signaling pathways and alterations in their quantity are frequently observed in pathologies such as neurodegenerative and cardiovascular diseases or cancer. However, it remains unknown whether both alkyl- and alkenyl-EL play the same roles in these processes. This review summarizes the roles and mechanisms of action of EL in cellular signaling and tries to discriminate between alkyl- and alkenyl-EL. We also focus on the involvement of EL-mediated alterations of cellular signaling in diseases and discuss the potential interest for EL in therapy.

© 2023 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.

Contents

* Corresponding author.
  E-mail address: marion.papin@etu.univ-tours.fr (M. Papin).

Abbreviations:

|			
AD	Alzheimer's disease	mTOR	Mammalian target of rapamycin
AGPS	Alkylglycerone phosphate synthase	MyD88	Myeloid differentiation primary response 88
Akt	Protein kinase B	NF-κB	Nuclear factor-kappa B
AMPK	AMP-activated protein kinase	PA	Phosphatidic acid
AP-1	Activator protein 1	PAF	Platelet activating factor
Bdnf	Brain-derived neurotrophic factor	PAF-R	Platelet activating factor receptor
cAMP	Cyclic adenosine monophosphate	PC	Phosphatidylcholine
CREB	Cyclic adenosine monophosphate response element-binding protein	PD	Parkinson's disease
		PE	Phosphatidylethanolamine
CD14	Cluster of differentiation 14	PEDS1	Plasmanylethanolamine desaturase 1
CD44	Cluster of differentiation 44	Pex	Peroxin
DAG	Diacylglycerol	PI3K	Phosphoinositide 3-kinase
DHAP	Dihydroxyacetone phosphate	PKA	Protein kinase A
EL	Ether-Lipid	PKC	Protein kinase C
EMT	Epithelial-mesenchymal transition	PLA2	Phospholipase A2
ERK	Extracellular signal-regulated kinase	PLC	Phospholipase C
FAR1	Fatty acid reductase 1	PPAR	Peroxisome proliferator-activated receptor
GNPAT	Glycerone phosphate O-acyltransferase	PRT	Plasmalogen replacement therapy
GPCR	G-protein coupled receptor	PUFA	Polyunsaturated fatty acid
GSK3β	Glycogen synthase kinase-3 β	RDCP	Rhizomelic chondrodysplasia punctate
IP3	Inositol triphosphate	ROS	Reactive oxygen species
KO	Knockout	STAT5	Signal transducer and activator of transcription 5
LPA	Lysophosphatidic acid	TLR4	Toll-like receptor 4
LPA-R	Lysophosphatidic acid receptor	TrkB	Tropomyosin receptor kinase B
LPS	Lipopolysaccharide	TRAAK	TWIK-related arachidonic acid-stimulated K+ channel
MAPK	Mitogen-activated protein kinase	TREK-1	Potassium channel subfamily K member 2
MD2	Myeloid Differentiation factor 2	TRPC4	Transient Receptor Potential Cation Channel Subfamily C Member 4
MMP2	Matrix metalloproteinase 2		
MMP9	Matrix metalloproteinase 9	ULK1	Unc-51-like kinase 1

1. Introduction

Compared to the usual endogenous glycerophospholipids composed of two fatty acids esterified to the *sn*-1 and *sn*-2 positions of a glycerol backbone, ether-lipids (EL) bear a fatty alcohol predominantly attached by an ether bond in their *sn*-1 position (Fig. 1A) [1]. This change in their structure gives them unique biophysical and biochemical properties, discussed in this review, which are hypothesized to be implicated in various cellular processes, although, to date, the extent of their role remains unclear [1—4]. Among EL, two subgroups exist depending on the nature of their *sn*-1 ether bond. Alkyl-EL (also called plasmanyl phospholipids) have an ether-bond while alkenyl-EL (also called plasmenyl phospholipids or plasmalogens) have a *cis* vinyl ether-bond. Their complex biosynthesis requires numerous enzymes, which have already been extensively reviewed [5]. Briefly, it starts in the peroxisome where glycerone phosphate O-acyltransferase (GNPAT) converts dihydroxyacetone phosphate (DHAP) to acyl-DHAP. The characteristic *sn*-1 ether-bond is then introduced by alkylglycerone phosphate synthase (AGPS) using acyl-DHAP and a fatty alcohol produced by fatty acid reductase 1 (FAR1) [1,2], thus forming the first ether-lipid precursor. After export to the endoplasmic reticulum, a series of enzymatic reactions takes place, generating alkyl-EL with various *sn*-2 and *sn*-3 groups such as alkyl-phosphatidylcholine (PC), -phosphatidylethanolamine (PE), -phosphatidic acid (PA) and their lyso-alkyl-EL counterparts with truncated *sn*-2: LPC, LPE, LPA. Plasmanylethanolamine desaturase 1 (PEDS1), encoded by TMEM189, then forms alkenyl-PE by desaturation of alkyl-PE, which can be remodelled into different alkenyl-EL species by further enzymatic reactions. To circumvent these crucial enzymes, EL can also be supplemented either as alkyl-EL, which are precursors for both alkyl- and alkenyl-EL, or directly as alkenyl-EL (Fig. 1B) [6—8].

Owing to their characteristic ether-bond, EL catabolism also requires specific enzymes. Alkylglycerol monooxygenase is responsible for the degradation of alkyl-EL as it cleaves the ether bond from alkyl-monoacylglycerol and alkyl-lysophospholipids [9,10]. Alkenyl-EL degradation is ensured by enzymes with either plasmalogenase or lysoplasmalogenase activity. Under specific conditions, oxidative stress can activate the plasmalogenase activity of cytochrome *c*, enabling the degradation of alkenyl-PE [11]. Alkyl-EL can also be degraded by lysoplasmalogenases after prior *sn*-2 deacylation, with TMEM86A and TMEM86B identified as lysoplasmalogenases [12,13].

In humans, early studies have shown that in total from all tissues, about 20% of phospholipids are alkenyl-EL [14]. EL distribution varies greatly across tissues with alkenyl-EL being the most abundant EL subgroup. In the brain, heart and adipose tissue, containing the most EL, they represent 20—27% of phospholipids while alkyl-EL remain under 5%. Other tissues such as lung, kidneys, muscle are described to contain mild amount of EL while the liver is particularly poor [14,15]. More recent studies using lipidomics analysis, although not as complete as the early ones, seem to confirm these results [16]. Alterations of EL quantities are frequently observed in pathologies, such as neurodegenerative and cardiovascular diseases or cancer [2,17], yet their role remains unclear and the possible differences between alkyl- and alkenyl-EL have been understudied. There are many studies involving alkenyl-EL as they are described to be important membrane constituents and show protective properties against
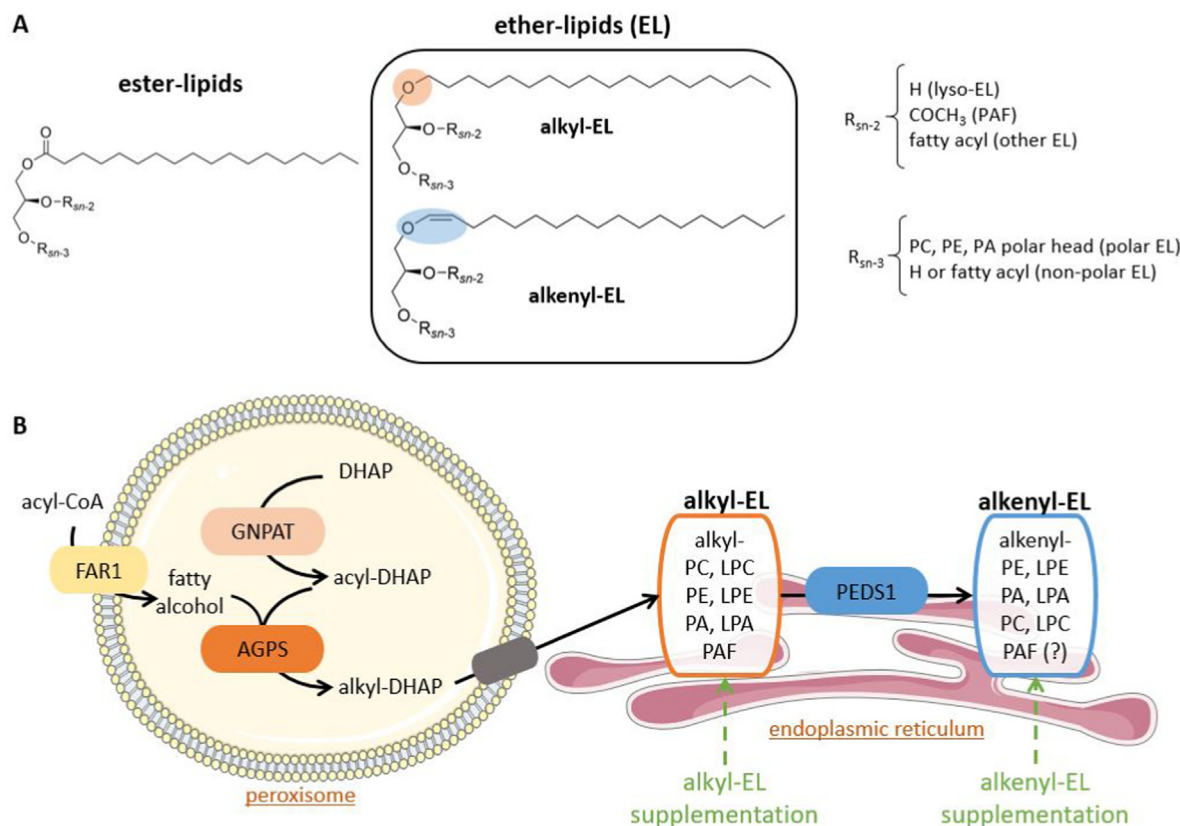
Fig. 1. (A) Example structures of endogenous ester- and ether-glycerolipids. Ester-lipids posess two fatty acids linked by an ester bond to the *sn*-1 and *sn*-2 positions of a glycerol backbone. Ether-lipids (EL) have at their *sn*-1 position a fatty alcohol attached by an ether bond. Depending on the nature of the ether bond, EL are either alkyl-EL (ether bond) or alkenyl-EL (vinyl-ether bond). EL have a fatty acid at their *sn*-2 position. Ester-lipids and EL can exist as glycerophospholipids bearing a polar head (mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE) or phosphatidic acid (PA)), as neutral lipids bearing another fatty acid (triglycerides) or a hydrogen (diglycerides). (B) Schematic overview of the EL biosynthesis. EL synthesis is initiated in the peroxisomes, dihydroxyacetone phosphate (DHAP) is acylated by glycerone phosphate O-acyltransferase (GNPAT) forming acyl-DHAP. The fatty acid of acyl-DHAP is next replaced by a fatty alcohol produced by fatty acid reductase 1 (FAR1) forming alkyl-DHAP in a reaction catalyzed by alkylglycerone phosphate synthase (AGPS). In the endoplasmic reticulum, alkyl-DHAP is catalyzed by several enzymes to form diverse alkyl-EL species. From alkyl-PE, plasmanylethanolamine desature 1 (PEDS1) forms alkenyl-PE and other enzymes enable the formation of other alkenyl-EL species. Alkyl- and alkenyl-EL can also be supplemented by treatment or diet (green dotted arrows).

neurodegeneration [3,18]. They have also been implicated in various signaling pathways while less data are available on the role of alkyl-EL. Oftentimes though, results on alkenyl-EL were obtained by knocking down GNPAT, meaning that one could not exclude a role of alkyl-EL. However, differentiating alkyl-from alkenyl-EL presents some difficulties as alkyl-EL supplementation can result in the production of alkenyl-EL [19]. In addition to that, since the structural difference between alkyl- and alkenyl-EL only resides in their ether/vinyl-ether bond nature, there has always been analytical difficulties to discriminate both EL subgroups [1]. Different analytical techniques have been developed, however none of them enable the quantification and complete identification of both alkyl- and alkenyl-EL. Indeed, with gas chromatography, the *sn*-1 group from alkyl-EL can be analysed as diacetates and the one from alkenyl-EL as dimethyl acetals in two parallel analyses, however in both cases only the *sn*-1 ether-linked chain can be identified while *sn*-2 and *sn*-3 information is lost [1,20,21]. We recently developed a high performance thin layer chromatography method to quantify alkyl- and alkenyl-EL from the same sample, however it only indicates the total quantity of each subgroup without any further identification information [22]. Recent advances in lipidomics by liquid chromatography-mass spectrometry benefited EL studies although some challenges still remain, the trickiest part being that mass spectrometry cannot identify the position of double bond and thus cannot clearly differentiate an alkyl-EL with an

unsaturation from an alkenyl-EL, unless developing very specific and thus rarely available methods [1,23,24]. All these issues to correctly differentiate alkyl-from alkenyl-EL have led to generalizations and possible misidentifications of EL subgroups.

In this review, we outline the known roles for EL in cellular signaling and try to discriminate between alkyl- and alkenyl-EL.

2. Biological relevance of ether-lipids (EL)

2.1. Effects of EL deficiency

The biological relevance of EL can clearly be seen with the EL-deficient rare peroxisomal disease Rhizomelic chondrodysplasia punctata (RCDP) which originates from mutations in genes directly coding for EL synthesis enzymes (GNPAT, AGPS, FAR1) or for peroxins 7 and 5L, needed for the peroxisomal import of AGPS [3,25]. Clinical features of severe RCDP include shortening of the proximal bones, growth retardation, myelination deficit, cardiac defects and cataracts as well as reduced life expectancy [3,25]. The severity of the disease was shown to be linked to the residual activity of the enzymes [26], displaying the important biological role of EL. To study it further, EL-deficient mouse models have been generated. It was observed that Gnpat knockout (KO), Pex7 KO and Agps KO mice all display growth retardation, brain, ocular and skeletal abnormalities, infertility and reduced survival [27—31], mimicking

human RCDP clinical features. In addition, Agps KO mice show important embryonic lethality, making this model difficult to use [31], although it appears to be the most appropriate to study EL deficiency since AGPS is responsible for the introduction of the ether bond and thus the formation of the first precursor to all EL. In comparison, Tmem189 KO mice, impairing specifically the alkenyl-EL synthesis also exhibit ocular and skeletal abnormalities and growth retardation. No infertility, brain abnormalities or reduced survival were described [32], suggesting an importance of both EL subgroups as well as a potential differential role for alkyl- and alkenyl-EL. It should however be noted that since PEDS1 has only very recently been attributed to its coding gene *TMEM189* [33–35], few studies has focused on Tmem189 KO mice.

2.2. Animal models showing an implication of EL on cellular signaling

Despite the apparent biological importance of EL, there has only been a few studies focusing on cellular signaling using EL-deficient animal models. In Gnpat KO mice, EL deficiency resulted in an impaired protein kinase B (Akt) activation and downstream signaling. The consequent lack of phosphorylation of glycogen synthase kinase-3 β (GSK3β) led to its constitutive activity, inhibiting Schwann cell differentiation and axon myelination [36]. In mice cortical tissues where sh-Gnpat were integrated, the reduced phosphorylation of Akt was also described, as well as a reduced phosphorylation of extracellular signal-regulated kinase (ERK) [37]. Similar results on Akt and ERK were observed in mice that had their hippocampus injected with sh-Gnpat, inhibiting the expression of memory-related genes such as the brain-derived neurotrophic factor (*Bdnf), synapsin-1,* and *SYT-1*. The reduced phosphorylation of cyclic AMP (cAMP) response element-binding protein (CREB) was also observed, leading to a downregulation of *Bdnf gene* [38].

Akt and ERK signaling pathways appear to be dysregulated in EL deficient mouse models and this was also observed in mice models treated or supplemented with EL. Alkenyl-EL diet resulted in an increase of pAkt, pERK and pCREB in mice hippocampal tissue [38]. However since these studies have only focused on the nervous system known to be rich in alkenyl-EL or used alkenyl-EL diet [36–39], it is hard to apprehend whether alkyl-EL are also involved. Moreover, without knocking down the enzyme PEDS1 responsible for the desaturation of the alkyl into an alkenyl group, forming alkenyl-EL, the supplementation with alkyl-EL ultimately results in a supplementation with alkenyl-EL as well [3]. In addition, it should be noted that even though the upregulation and downregulation of these signaling pathways was observed upon EL deficiency and EL enrichment, it is not a sufficient proof of a direct implication of EL in these signaling pathways, as their effect could be indirect. More mechanistic experiments are needed to determine precisely how EL exert their functions.

3. Molecular targets of EL

3.1. Toll-like receptor 4 (TLR4)

In addition to Akt, in the brain cortex of alkenyl-EL-fed mice, toll-like receptor 4 (TLR4) endocytosis was reduced [39], showing another signaling pathway that could be affected by alkenyl-EL. TLR4 endocytosis, involved in neuroinflammation, can trigger nuclear factor-kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) signaling, either through the canonical MyD88 (myeloid differentiation primary response 88)-dependent pathway or the independent one [18,40]. The effect of alkenyl-EL on these signaling pathways was confirmed by *in vitro* experiments in sh-GNPAT microglial cells that showed an increase of TLR4

endocytosis induced by bacterial toxin lipopolysaccharide (LPS) while alkenyl-EL treatment inhibited it, reducing the inflammation signals. Alkenyl-EL were able to inhibit both the LPS-induced MyD88-dependent and MyD88-independent pathways, resulting in an inhibition of NF-κB and p38MAPK activities as well as subsequent c-Fos/c-Jun transcriptional activity [39]. Interestingly, the fatty acid composition of alkenyl-EL could be of importance, since alkenyl-EL containing oleic acid did not inhibit inflammation [18]. It was proposed that alkenyl-EL could represent ligands of proteins CD14 or MD2, involved in the binding of LPS to TLR4, to block the induction of TLR4 by LPS. They might also be ligands for G-protein coupled receptor (GPCRs) or indirectly modulate GPCRs activation (see 3.2) so as to either inhibit the recruitment of CD14 and MD2 to TLR4 or inhibit endosomal recruitment of TLR4 [18,39]. Only alkenyl-EL were tested and reported to interfere with TLR4 endocytosis, probably by an indirect mechanism.

3.2. G-protein-coupled receptors (GPCRs)

Alkenyl-EL have already been proposed to mediate the activation of GPCRs, which could be involved in numerous signaling pathways. Notably, knocking down orphan GPCRs GPR1, 19, 21, 27 or 61 prevented the alkenyl-EL-induced increase of pERK and pAkt [41]. In neuronal cells, this activation of ERK and Akt was shown to be important for survival. In addition, the alkenyl-EL-mediated activation of GPR21 was shown to induce the activation of Perforin-1 *via* STAT5, participating to the activation of NK cells. Interestingly, the conservation of the extracellular glycosylation site of GPR21 was necessary for alkenyl-EL-mediated NK cells activation [42]. Additional studies are however needed to determine whether alkenyl-EL act directly as GPCRs ligands or indirectly, for instance by modulating membrane dynamics (see 4.2). Note that again only alkenyl-EL were tested on these orphan GPCRs.

Some notorious GPCRs are however receptors for alkyl-EL. For instance, the alkyl-EL PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) has its own receptor, the PAF receptor (PAF-R) which belongs to the family of GPCRs and is involved in inflammation and numerous biological effects. PAF-R is coupled to Gαq and Gαi G-proteins. Gαq activates phospholipase C (PLC), converting phosphatidylinositol 4,5-bisphosphate to inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 transiently increases calcium release from the endoplasmic reticulum to the cytosol, which can trigger store-operated calcium entry while calcium favours protein kinase C (PKC) activation by DAG [43]. Of note, ether-linked diglycerides also bind PKC, however, in contrast to ester-linked DAG they reduce PKC activity and decrease PKC downstream signaling [3]. Gαi inhibits adenylate cyclase, reducing cAMP concentration and the activation of cAMP-dependent kinases such as PKA [4]. PAF-R activation was also reported to activate ERK and p38MAPK signaling pathways [44]. Alkyl-EL with similar structures such as 1-*O*-alkyl-2-acetyl-*sn*-glycerol or 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphate have also been reported to activate PAF-R, although it could be indirectly considering that these molecular species represent precursors for PAF [4]. Comparatively, the ester form of PAF was reported to be a weaker activator of PAF-R compared to PAF, although it was observed to exert opposite effects in inflammation [45]. An antagonist role was also proposed for the alkenyl form of PAF based on results obtained with synthetic compounds [46], however more studies need to be done to confirm these differential roles especially considering that there have not been any recent report of a presence of an alkenyl form of PAF endogenously. It seems however that even though PAF-R can be activated by other ligands than PAF, PAF is the most potent one, thus eliciting the strongest activation for the lowest concentration.

Another GPCR that can be the target of both alkyl- and alkenyl-

EL is the LPA receptor (LPA-R) [3]. Signaling through LPA-R can notably activate PI3K/Akt, ERK/MAPK, PLC/IP3/DAG signaling and was reported to be involved in cell migration, survival, proliferation and changes in the cytoskeleton [47]. Both alkyl- and alkenyl-LPA were shown to be ligands for LPA-R, although they represent weaker ligands than acyl-LPA [48]. In ovarian cancer cells, both alkyl- and alkenyl-LPA were independently able to increase the phosphorylation of ERK and PI3K/Akt, supposedly through LPA-R signaling [3], showing that they detain a biological effect despite they weaker affinity for LPA-R than acyl-LPA.

3.3. Peroxisome proliferator-activated receptor (PPAR)

EL are also known to be ligands for PPARs. Alkyl-EL with an oxidized fatty acid in their sn-2 position were shown to be high affinity ligands to PPARγ, interacting with its ligand-bind pocket [49]. In addition, the intact alkyl-EL PC(O-18:1/16:0) was found to bind to and activate PPARγ in adipocytes, although this activation was observed with a concentration of alkyl-EL about 20 times more important than the one reported for oxidized alkyl-EL [49,50]. Alkyl-LPA(O-18:1) was observed to be a partial agonist of PPARγ [51], however in this case as acyl-LPA were also shown to be PPARγ agonists [52], it seems arguable that alkyl-LPA have a specific role through PPARγ activation. Interestingly, alkyl-EL could not activate PPARα [50], in contrast to alkenyl-derived halogenated fatty aldehydes [53,54]. There are however no mention of intact alkenyl-EL activating any PPAR. PPARs are involved in many cellular processes, they notably regulate glucose and lipid metabolism and PPARγ activation was reported to reduce inflammation and to be protective against neurodegeneration [55]. Although direct activation of PPARs by some molecular species of EL was shown, more studies are needed to confirm whether EL-induced PPAR activation plays a significant biological role.

4. Indirect effect of EL

4.1. EL as reservoirs of polyunsaturated fatty acids (PUFA)

EL are particularly enriched in polyunsaturated fatty acids (PUFA), which can be liberated from the sn-2 position of EL, by the action of phospholipases A2 [3]. These PUFA can act as second messengers or form other signaling molecules, for instance arachidonic acid can activate PKC signaling or represent a ligand for PPARs like other PUFAs and it can also form eicosanoids [3,56,57]. The importance of sn-2 PUFA from EL in cellular signaling still needs to be properly assessed, as there is no proof that this PUFA pool is any different from the one originating from their ester counterpart. However, there could be a difference between alkyl- and alkenyl-EL PUFA, as an alkenyl-EL-selective PLA2 exists [3], suggesting that the PUFA pool from alkenyl-EL could be used specifically. To date, no PLA2 has been reported to be selective for alkyl-EL.

4.2. EL-mediated modulation of membrane dynamics

One strong hypothesis for the mechanism of action of EL is that they could also regulate signaling pathways through the modulation of membrane dynamics, alkenyl-EL in particular are structural components of cell membranes and EL variation among the tissues [25] leads to a different dipolar membrane potential as compared to membranes containing ester lipids [58]. The absence of the carbonyl from the sn-1 ester bond but also a reduction in hydrogen bonding between the phospholipids may be responsible of these dipolar potential changes [59]. Among other differences between an ester-lipid and the corresponding EL, it is worth mentioning the

difference in lipid packing. Experiments carried out on monolayers revealed the larger area per lipid occupied by ester-lipid with PC polar head compared with its alkyl or alkenyl analogues with identical ester-linked sn-2 chain, demonstrating that having an ether or a vinyl-ether bond can make the difference [60]. In addition, among EL, alkyl-EL were shown to occupy a larger area per lipid than alkenyl-EL. The larger area occupied by ester-lipids compared with EL, and by alkyl-EL compared with alkenyl-EL could be the consequence of a better hydration of the ester bond compared with the ether bond and/or a reorientation of the acyl chain. This increased packing leads to a network of more ordered acyl chains and consequently a more rigid bilayer [61]. Despite the water effect on carbonyl, it is known that the interaction of different ions at the level of the head group and up to the glycerol plays a particular role in modifying the hydration. One should also consider changes in area per molecule due to the interaction not only with water but also with ions such as Na^+ or K^+. Saunders et al. concluded that an ether-linked lipid bilayer has lower affinity for ions than the ester-linked analogue, with a distinct peak in solvent density suggesting that the waters in EL are more strongly coordinated to the lipid head groups [62].

The nanodomains, key in signalling processing and particularly enriched in alkenyl-EL [63], suggest a specific importance of alkenyl-EL homeostasis in membrane dynamics. The rigidity and increased lateral packing together with the findings that EL are located in membrane structures rich in sphingomyelin and cholesterol infer that alkenyl-PEs may exert their physiological role through changing the biophysical properties of lipid rafts. The fact that cholesterol is able to increase the ordering of membranes independently of the presence of ester-lipids or EL, demonstrates that the interaction of cholesterol with the carbonyl group is not essential for the condensing effect [60]. Alkenyl-EL, the predominant EL species, are indeed important membrane constituents, playing a role in membrane fluidity and curvature [3,64]. It is hypothesized that it could either impair or improve the recruitment of different membrane-associated proteins, with the examples of EL deficiency leading to impaired Akt recruitment and, supposedly, facilitated PKC recruitment [3,65]. Alkenyl-EL diet was also found to enhance the localisation of tropomyosin receptor kinase B (TrkB) in the lipid rafts of mice hippocampus tissues, likely facilitating TrkB signaling, while Gnpat knockdown reduced its localisation [38].

Among EL, alkenyl-EL were also showed to be particularly important for processes involving membrane fusion [3]. PE alkenyl-EL play a role in vesicle fission and fusion processes, as well as in the formation of other curved architectures that facilitate the fusion of membranes. This view is supported by experimental evidence of the increased tendency of alkenyl-PE to form inverted hexagonal phases at lower temperatures compared to their ester counterparts. For instance, PE alkenyl-EL stabilize hexagonal non-lamellar phases decreasing the phase transition temperatures around 38 °C (lamellar to hexagonal), while choline based phospholipids prefer the lamellar phase [66]. As a consequence, it can be speculated that PE alkenyl-EL could induce the formation of non-bilayer intermediates and thus could be important for membrane remodelling processes in cells.

While the biophysical properties of homogenous EL bilayers and simple mixtures compared to their ester-lipid counterparts are relatively well understood, how these properties are affecting different membrane cellular processes in combination with other lipids is far from understood. The changes in the proportions of EL in a biological membrane are inevitably accompanied by changes in the composition of the polar head groups and hydrophobic moieties of the plasma membranes. Even though all the changes attributed to the membrane physical properties due its altered EL content may be only speculative, they can still open a fruitful way

and contribute to the deciphering of the specific role of EL in physiological and biochemical mechanisms.

4.3. EL and ion channel activity

Another indirect effect of EL on cellular signaling consists in their regulation of ion channels activity, which could lead to alterations in cellular signaling. The effect of EL on ion channels and its implication in diseases has already been described extensively elsewhere [17]. To our knowledge, an effect on ion channel expression was not established. Different mechanisms of action could be involved, notably the modulation of membrane dynamics or the binding of EL to specific GPCRs. Recently, alkenyl-PE were proposed to increase TRPC4 activity leading to a transient increase of calcium influx and phosphorylation of AMPK [67]. Alkyl-PE and lyso-alkenyl-PE were tested and they did not elicit such effect, meaning that both the vinyl-ether bond and the *sn*-2 group are necessary for the regulation of TRPC4 activity. The absence of activation by alkyl-PE underlines the essential role of the vinyl-ether bond in such interaction but one cannot rule out the possibility that the channel activity is also modulated by the membrane microviscosity dictated by its lipid composition. In the same line but for alkyl-EL, they also have been involved in the modulation of ion channels activity, with the example of TREK-1 and TRAAK that opened after application of PAF and lyso-alkyl-PC [68]. Since they are stretch-sensitive channels, the effect of alkyl-EL was likely through the modulation of membrane dynamics. In addition to the modulation of their activity by EL, ion channels can be targets of cellular signaling, considering that they can be phosphorylated by kinases such as PKC and cAMP-dependent kinases, which affects their gating and/or conducting properties [69]. This means that an EL-mediated alteration of cellular signaling could affect some ion channels activities, leading to further dysregulations of excitation-responses coupling due to altered ion fluxes [17].

4.4. Antioxidant properties of alkenyl-EL and EL implication in ferroptosis

A specificity of alkenyl-EL is their antioxidant properties, owing to their chemical structure. Their *sn*-1 vinyl-ether bond is described to be preferentially oxidized notably by reactive oxygen species, thus protecting the other surrounding phospholipids from oxidation [2,46]. This might confer to alkenyl-EL a specific role compared to alkyl-EL. In addition, a role for EL in ferroptosis, an oxidative stress-induced form of regulated cell death has recently emerged [70,71]. It is triggered by an excess of lipid peroxidation which results in damages in the cell membrane [71]. With EL being rich in PUFA, they are both susceptible to peroxidation and thus could contribute to ferroptosis, but since alkenyl-EL are oxidative sinks, it is hypothesized that they could protect against ferroptosis. In accordance with this hypothesis, alkenyl-EL were indeed shown to protect cells against the induction of ferroptosis [72]. However, another study reported that both EL species promoted cell susceptibility to ferroptosis with no protective role for alkenyl-EL [73]. Further studies are thus needed, although the discordance in the results is thought to be due to the cellular models used and their inherent EL metabolism [70].

5. Role of EL-mediated signaling in disease ?

Interestingly, aside from RCDP, altered EL levels have been observed in several disorders, such as peroxisomal, neurological, cardiovascular and metabolic disorders or cancers [2,17]. It is unclear whether EL levels disruption is only a consequence of the diseases without further implication or if they play a role in them.

Considering that EL can mediate several signaling pathways, one can question whether the alterations in EL levels participate to the disease by affecting cell signaling.

5.1. EL and neurodegenerative diseases

Decreased levels of EL have been associated to Alzheimer's disease (AD) and other neurodegenerative diseases, like Parkinson's disease (PD) and Huntington's in many studies [3]. More specifically, oral supplementation with alkenyl-EL was shown to improve cognitive functions of AD patients [18]. Loss of alkenyl-EL is thought to aggravate neurodegenerative diseases through dysregulated alkenyl-EL-mediated signaling, notably *via* impaired Akt/GSK3β signaling [3,36]. There is almost no data concerning the concentration of alkyl-EL in these diseases and their involvement, although one study showed a reduction of alkyl-EL in AD [74]. Based on the known roles of alkyl-EL on signaling, it has been hypothesized that reduced levels of alkyl-EL could also contribute to aggravating neurodegenerative diseases, for instance through reduced PPARγ activation [3]. In addition, considering the antioxidative properties of plasmalogens, it has been proposed that their diminution could lead to increased oxidative stress and thus aggravate neurodegenerative diseases [75]. More studies are needed to confirm these suppositions.

5.2. EL and cardiovascular diseases

EL quantities are altered in cardiovascular diseases such as ischemia-reperfusion injury where there is a downregulation of alkenyl-EL. This decrease could notable be caused by an enhanced activity of the alkenyl-EL-specific PLA2, increasing the level of lyso-plasmalogens which were shown to increase PKA activity [17]. Similarly, to neurodegenerative diseases, this reduction of alkenyl-EL could moreover aggravate the disease due to increased oxidative stress. Interestingly, an increase of PAF was also observed in ischemia, which suggests that different classes of EL could have different roles in ischemia [17].

5.3. EL and cancers

Dysregulation of cell signaling is typically known to drive the progression of cancer, by promoting cell growth, proliferation and survival as well as cell migration and invasion [76]. We focus here on dysregulated pathways that are also reported to be EL-mediated. These include notably PI3K/Akt, ERK/MAPK and NF-κB pathways, which have been reported to be over-activated in various cancers. In cancer cells, PI3K/Akt is known to promote cell survival and proliferation, ERK/MAPK and NF-κB pathways are reported to promote several cancer features such as migration, invasion, adhesion, proliferation and survival [76]. EL quantities are also disturbed in cancer, it is in fact one of the very rare disease in which an increase in EL has been reported and associated with cancer aggressiveness [2]. It is thus legitimate to investigate if there is a contribution of the increased quantity of EL and the dysregulation of these signaling pathways. Interestingly, targeting AGPS to reduce EL was able to reduce pathogenicity in various cancer cell lines [57,77,78]. In glioma and hepatic carcinoma EL reduction induced by AGPS knockdown resulted in a reduction of cell adhesion and invasion, and stopped cell cycle [77]. They observed a reduced activity of the MAPK pathways, with a reduction of the epithelial-mesenchymal transition (EMT) transcription factors Twist, AP-1 and Snail, as well as a reduction of their target genes such as MMP2, MMP9, CD44 and E-cadherin. This effect is thought to occur through LPA-R signaling, although signaling through prostaglandin E2, reduced after AGPS knockdown, could also be involved.

Similarly, in another study, AGPS knockdown effectively reduced cell migration and invasion, and this effect was reversed by treatment with ether-LPA as well as with PGE2. Noteworthily, acyl-LPA, also lowered by AGPS knockdown, was able to rescue cell migration [57]. This similar effect of alkyl- and acyl-LPA was also observed in sh-AGPS glioma and hepatic carcinoma cell lines, where both lipid species activated PI3K/Akt pathways, supposedly through LPA-R signaling [79]. These results could suggest a non-specific role of ether-LPA-induced LPA-R signaling. However, it is also proposed that increasing either acyl- or alkyl-LPA would ultimately rescue LPA-R signaling considering that both lipid species are reduced upon AGPS knockdown [57], thus not excluding a specific role for alkyl-LPA in addition to the role of acyl-LPA. Using an inhibitor of AGPS in breast and prostate cancer cells reduced cell proliferation, migration and impaired EMT through the reduction of Snail transcription factor [78]. Both alkyl- and alkenyl-LPA treatments promoted proliferation and cell invasion through the increased activity of ERK/MAPK and Akt pathways in ovarian cancer cells [3], implying in this case a specific role for ether-LPA. Interestingly, EL that compose the membrane promote the production and release of exosomes in cancer cells [80]. The differential role of alkyl- and alkenyl-EL was not investigated. Exosome, a subset of extracellular vesicles, function as a node of intercellular communication and molecular transfer and cancer cells secrete more exosomes than healthy cells with more microRNAs than healthy cells. This suggests that one mode of action of EL may be the regulation of protein expression. In addition, the ROS scavenger properties of alkenyl-EL could protect cancer cells against oxidative stress [81].

From these results, it appears that the increased levels of EL in cancer cells could possibly contribute to the pathogenicity through increasing the activity of diverse cellular signaling pathways. Both alkyl- and alkenyl-EL could be at play, to date there are unfortunately not enough data to confirm their involvement and to differentiate their roles.

6. EL and therapeutic strategies

Because of their implication in cellular processes and diseases and as they are natural product found to enriched in marine and land animals' meat [82], EL represent interesting therapeutic agents. Indeed, EL have been implicated in promoting autophagy. Mixes of purified alkyl-PC and alkenyl-PE were both shown to enhance autophagy, through the concomitant activation of the AMPK-ULK1 pathway and inhibition of the mTOR-ULK1 pathway [83]. Interestingly, in this study, the mix of alkenyl-PE was more effective than the one of alkyl-PC, which can suggest that the vinyl-ether bond of alkenyl-EL is more efficient to enhance autophagy than the simple ether bond. However, since alkenyl-PE were compared to alkyl-PC and since the sn-2 chains were also different, the polar heads and sn-2 groups could also explain the difference of effect. Since a dysregulation of autophagy has been linked notably to neurodegenerative diseases, carcinogenesis, liver and heart diseases, EL appear to have a potential therapeutic interest as autophagy-enhancers.

EL could also represent promoters of ferroptosis susceptibility. Since ferroptosis is involved notably in neurodegenerative diseases, ischemia/reperfusion injury and cancer [71], it appears as a promising therapeutic target and modulating it with natural EL supplementation could be interesting. First results have suggested a beneficial effect of promoting ferroptosis in cancer therapy, either alone or cumulated with chemotherapy [71].

Finally, since a deficiency in EL is observed in several diseases and thought notably to alter cellular signaling, it is expected that restoring EL levels could reverse the deleterious effects observed. This is the goal of alkenyl-EL replacement therapy (called plasmalogen replacement therapy, PRT), currently under study. In a clinical trial, PRT was able to increase alkenyl-EL quantities and to improve cognitive functions of patients with AD and PD. These promising results obtained so far are well documented elsewhere [8].

7. Conclusion and perspectives

EL have been reported to be involved in the regulation of various signaling pathways, summarised in Fig. 2. This regulation could occur notably through different receptors such as TLR4, GPCRs, PPARs (Fig. 2A, B and G) and ion channels (Fig. 2C), either directly as their ligands or indirectly through the regulation of other targets, the formation of signaling molecules or the modulation of membrane dynamics and anti-oxidative potential (Fig. 2D, E and F). To date however, no study has really focused on determining whether alkyl-EL and alkenyl-EL play the same or different role. Studies on central nervous system tissues target mainly alkenyl-EL as they are the predominant EL species in these tissues, so alkyl-EL are only seen as precursors for alkenyl-EL. Generally, studies using alkyl-EL involve almost exclusively alkyl-glycerols, the non-polar form of EL, used as precursors for PAF or alkenyl-EL. From these studies, it can look like alkyl-EL are only a means to an end and not an interesting bioactive lipid class. Considering that alkyl- and alkenyl-EL differ structurally only by their ether bond type and that they share similar properties and targets, on can wonder why studying alkyl-EL (as a final EL species and not a precursor for alkenyl-EL) and their difference from alkenyl-EL does not gather more scientific interest. They are assuredly less abundant in tissues than alkenyl-EL, yet metabolically speaking their ether bond is more stable than an ester one and chemically speaking it is more stable than the vinyl-ether one. As the antioxidant role of alkenyl-EL alone cannot possibly explain all the effects of alkenyl-EL, it seems curious that this only difference in their properties would justify the lack of interest in alkyl-EL compared to alkenyl-EL.

One can then wonder whether it is actually technically possible to test out the differences between alkyl- and alkenyl-EL. When performing supplementation in vivo or in vitro, alkyl-EL are described to be ultimately metabolized into alkenyl-EL. The only way to avoid this transformation would be to knock down the enzyme PEDS, responsible for the formation of the characteristic vinyl-ether bond of alkenyl-EL. PEDS1, encoded by TMEM189 has only been assigned recently so hopefully more studies using this genetic tool will emerge in the next years. However, independently from its role in alkenyl-EL synthesis, PEDS1 has been shown to regulate negatively autophagy by interacting with ULK1 [84]. PEDS1 knockdown results in an increased cell autophagy, resulting in a possible bias when implementing it to reduce alkenyl-EL levels. Moreover, as there are some interconnections between EL metabolism and other lipids, as well as possible compensations, knocking down PEDS also results in alterations among other lipid classes. One notable example is the fact that cholesterol synthesis was shown to be impacted by alkenyl-EL homeostasis [85]. Finally, as alkenyl-EL are supposed to be structurally important due to their non-negligible quantity in cell membranes, it seems likely that reducing their quantity would affect cell membrane dynamics and cell signaling indirectly.

One interesting in vitro model is the RAW.12 mutant strain, derived from the RAW264.7 murine macrophage-like cell line. This strain, deficient in both DHAPAT and PEDS1, was reported to show a 90%-decrease in alkenyl-EL and, presumably, also in alkyl-EL. Alkyl-EL supplementation of this cell line bypasses GNPAT and restores alkyl-EL without increasing alkenyl-EL, while supplementation with alkenyl-EL directly bypasses PEDS1 and restores only alkenyl-EL, which should enable the study of their differential effects
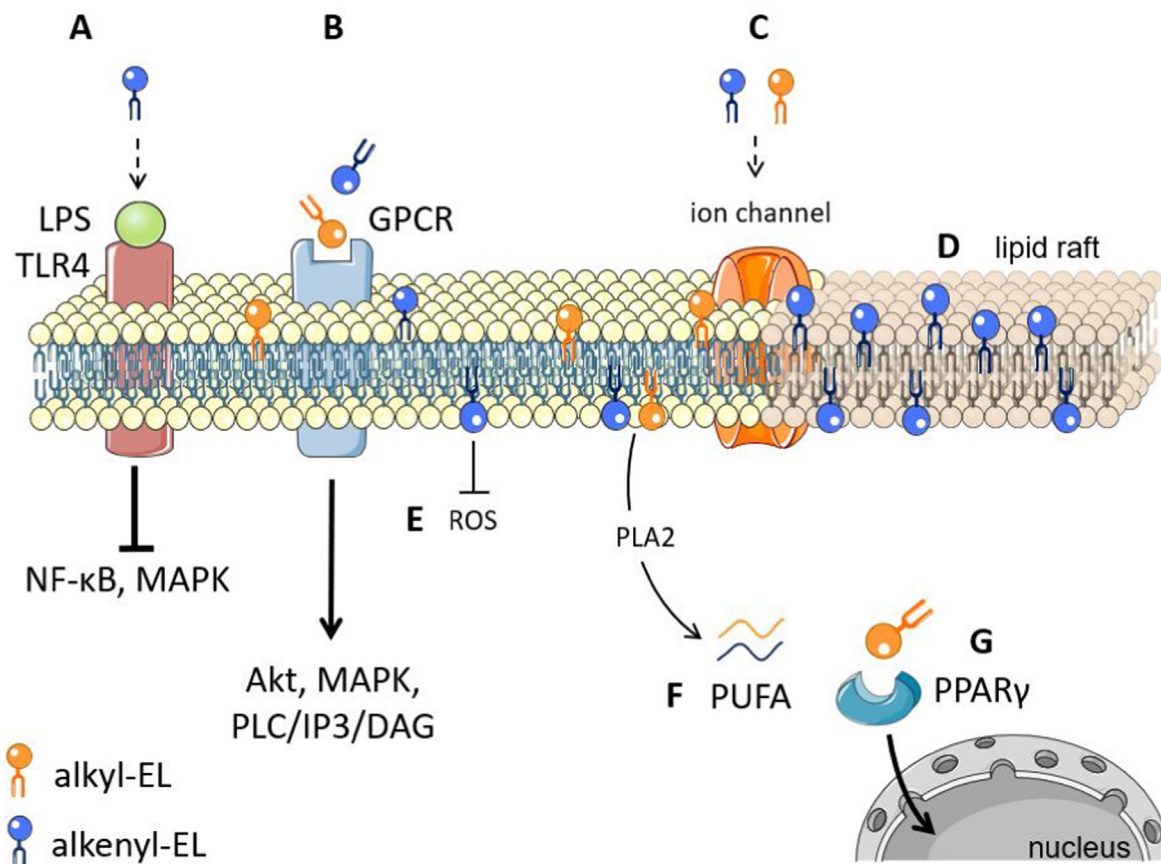
Fig. 2. Summary of alkyl- and alkenyl-EL roles in cellular signaling pathways. Alkyl-EL are displayed in orange and alkenyl-EL in blue. Dotted arrows represent indirect mechanisms. (A) Alkenyl-EL promote LPS-mediated TLR4 endocytosis, downregulating NF-κB and MAPK signaling pathways. (B) Alkyl- and alkenyl-EL are ligands of GPCRs, which can activate various signaling pathways (Akt, MAPK, PLC/IP3/DAG). (C) EL can indirectly regulate ion channels' activities. (D) Alkenyl-EL can modulate the biophysical properties of membrane, they are notably important constituents of lipid rafts, nanodomains representing platforms for cellular signaling. (E) Alkenyl-EL have antioxidant properties. (F) EL can serve as reservoirs of second messengers, notably PLA2 can liberate their *sn-2* PUFA, which for example acts as ligands for PPARs. (G) Some alkyl-EL are ligands of PPARγ.

[86,87]. To date, no such study design has been implemented to study the differential role of alkyl- and alkenyl-EL on cell signaling.

In short, EL and their role on cellular signaling still remains for now mysterious as there are still many questions to be asked, with one of them being: do alkyl- and alkenyl-EL play differential role? We believe that in order to clearly identify the biological role of alkyl- and alkenyl-EL, suppression of the expression of key alkyl/alkenyl-EL synthesis enzymes or alkyl/alkenyl-EL supplementations are not sufficient and a combination of both approaches is required.

If a differential role of alkyl and alkenyl lipids in various pathologies is demonstrated, it could be proposed that some of these lipids become biomarkers of these pathologies and modifiable through nutritional approaches (having properly quantified the quantity of alkyl- and alkenyl-EL).

Acknowledgments

References

[1] J. Koch, K. Watschinger, E.R. Werner, M.A. Keller, Tricky isomers—the evolution of analytical strategies to characterize plasmalogens and plasmanyl ether lipids, Front. Cell Dev. Biol. 10 (2022) 864716, https://doi.org/10.3389/fcell.2022.864716.

[2] J.M. Dean, I.J. Lodhi, Structural and functional roles of ether lipids, Protein Cell 9 (2018) 196–206, https://doi.org/10.1007/s13238-017-0423-5.

[3] F. Dorninger, S. Forss-Petter, I. Wimmer, J. Berger, Plasmalogens, platelet-activating factor and beyond — ether lipids in signaling and neuro-degeneration, Neurobiol. Dis. 145 (2020) 105061, https://doi.org/10.1016/j.nbd.2020.105061.

[4] N. Rangholia, T.M. Leisner, S.P. Holly, Bioactive ether lipids: primordial Modulators of cellular signaling, Metabolites 11 (2021) 41, https://doi.org/10.3390/metabo11010041.

[5] K. Watschinger, E.R. Werner, Orphan enzymes in ether lipid metabolism, Biochimie 95 (2013) 59–65, https://doi.org/10.1016/j.biochi.2012.06.027.

[6] A.K. Das, A.K. Hajra, High incorporation of dietary 1-O-heptadecyl glycerol into tissue plasmalogens of young rats, FEBS (Fed. Eur. Biochem. Soc.) Lett. 227 (1988) 187–190, https://doi.org/10.1016/0014-5793(88)80895-0.

[7] S. Paul, A.A. Rasmiena, K. Huynh, A.A.T. Smith, N.A. Mellett, K. Jandeleit-Dahm, G.I. Lancaster, P.J. Meikle, Oral supplementation of an alkylglycerol mix comprising different alkyl chains effectively modulates multiple endogenous plasmalogen species in mice, Metabolites 11 (2021) 299, https://doi.org/10.3390/metabo11050299.

[8] J.C. Bozelli, R.M. Epand, Plasmalogen replacement therapy, Membranes 11 (2021) 838, https://doi.org/10.3390/membranes11110838.

[9] K. Watschinger, E.R. Werner, Alkylglycerol monooxygenase, IUBMB Life 65 (2013) 366–372, https://doi.org/10.1002/iub.1143.

[10] S. Sailer, M.A. Keller, E.R. Werner, K. Watschinger, The emerging physiological role of AGMO 10 Years after its gene identification, Life 11 (2021) 88, https://doi.org/10.3390/life11020088.

[11] C.M. Jenkins, K. Yang, G. Liu, S.H. Moon, B.G. Dilthey, R.W. Gross, Cytochrome c is an oxidative stress-activated plasmalogenase that cleaves plasmenylcholine

and plasmenylethanolamine at the sn-1 vinyl ether linkage, J. Biol. Chem. 293 (2018) 8693—8709, https://doi.org/10.1074/jbc.RA117.001629.

[12] L.-C. Wu, D.R. Pfeiffer, E.A. Calhoon, F. Madiai, G. Marcucci, S. Liu, M.S. Jurkowitz, Purification, identification, and cloning of lysoplasmalogenase, the enzyme that catalyzes hydrolysis of the vinyl ether bond of lysoplasmalogen, J. Biol. Chem. 286 (2011) 24916—24930, https://doi.org/10.1074/jbc.M111.247163.

[13] Y.K. Cho, Y.C. Yoon, H. Im, Y. Son, M. Kim, A. Saha, C. Choi, J. Lee, S. Lee, J.H. Kim, Y.P. Kang, Y.-S. Jung, H.K. Ha, J.K. Seong, J.G. Granneman, S.W. Kwon, Y.-H. Lee, Adipocyte lysoplasmalogenase TMEM86A regulates plasmalogen homeostasis and protein kinase A-dependent energy metabolism, Nat. Commun. 13 (2022) 4084, https://doi.org/10.1038/s41467-022-31805-3.

[14] L.A. Horrocks, M. Sharma, Plasmalogens and O-alkyl glycerophospholipids, in: J.N. Hawthorne, G.B. Ansell (Eds.), New Comprehensive Biochemistry, Elsevier, 1982, pp. 51—93, https://doi.org/10.1016/S0167-7306(08)60006-X.

[15] A. Diagne, J. Fauvel, M. Record, H. Chap, L. Douste-Blazy, Studies on ether phospholipids. II. Comparative composition of various tissues from human, rat and Guinea pig, Biochim. Biophys. Acta 793 (1984) 221—231, https://doi.org/10.1016/0005-2760(84)90324-2.

[16] I. Pradas, K. Huynh, R. Cabré, V. Ayala, P.J. Meikle, M. Jové, R. Pamplona, Lipidomics reveals a tissue-specific fingerprint, Front. Physiol. 9 (2018) 1165, https://doi.org/10.3389/fphys.2018.01165.

[17] D. Fontaine, S. Figiel, R. Félix, S. Kouba, G. Fromont, K. Mahéo, M. Potier-Cartereau, A. Chantôme, C. Vandier, Roles of endogenous ether lipids and associated PUFAs in the regulation of ion channels and their relevance for disease, JLR (J. Lipid Res.) 61 (2020) 840—858, https://doi.org/10.1194/jlr.RA120000634.

[18] M.S. Hossain, S. Mawatari, T. Fujino, Biological functions of plasmalogens, in: G. Lizard (Ed.), Peroxisome Biology: Experimental Models, Peroxisomal Disorders and Neurological Diseases, Springer International Publishing, Cham, 2020, pp. 171—193, https://doi.org/10.1007/978-3-030-60204-8_13.

[19] F. Dorninger, A. Brodde, N.E. Braverman, A.B. Moser, W.W. Just, S. Forss-Petter, B. Brügger, J. Berger, Homeostasis of phospholipids — the level of phosphatidylethanolamine tightly adapts to changes in ethanolamine plasmalogens, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1851 (2015) 117—128, https://doi.org/10.1016/j.bbalip.2014.11.005.

[20] M. Pinault, C. Guimaraes, H. Couthon, J. Thibonnet, D. Fontaine, A. Chantôme, S. Chevalier, P. Besson, P.-A. Jaffrès, C. Vandier, Synthesis of alkyl-glycerolipids standards for gas chromatography analysis: application for chimera and shark liver oils, Mar. Drugs 16 (2018) 101, https://doi.org/10.3390/md16040101.

[21] T.S. Oberg, R.E. Ward, J.L. Steele, J.R. Broadbent, Identification of plasmalogens in the cytoplasmic membrane of bifidobacterium animalis subsp. lactis, Appl. Environ. Microbiol. 78 (2012) 880—884, https://doi.org/10.1128/AEM.06968-11.

[22] M. Papin, C. Guimaraes, B. Pierre-Aue, D. Fontaine, J. Pardessus, H. Couthon, G. Fromont, K. Mahéo, A. Chantôme, C. Vandier, M. Pinault, Development of a high-performance thin-layer chromatography method for the quantification of alkyl glycerolipids and alkenyl glycerolipids from shark and chimera oils and tissues, Mar. Drugs 20 (2022) 270, https://doi.org/10.3390/md20040270.

[23] M.S. Blevins, S.W.J. Shields, W. Cui, W. Fallatah, A.B. Moser, N.E. Braverman, J.S. Brodbelt, Structural characterization and quantitation of ether-linked glycerophospholipids in peroxisome biogenesis disorder tissue by ultraviolet photodissociation mass spectrometry, Anal. Chem. 94 (2022) 12621—12629, https://doi.org/10.1021/acs.analchem.2c01274.

[24] J.M. Deeley, M.C. Thomas, R.J.W. Truscott, T.W. Mitchell, S.J. Blanksby, Identification of abundant alkyl ether glycerophospholipids in the human lens by tandem mass spectrometry techniques, Anal. Chem. 81 (2009) 1920—1930, https://doi.org/10.1021/ac802395d.

[25] N.E. Braverman, A.B. Moser, Functions of plasmalogen lipids in health and disease, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1822 (2012) 1442—1452, https://doi.org/10.1016/j.bbadis.2012.05.008.

[26] F. Dorninger, S. Forss-Petter, J. Berger, From peroxisomal disorders to common neurodegenerative diseases — the role of ether phospholipids in the nervous system, FEBS Lett. 591 (2017) 2761—2788, https://doi.org/10.1002/1873-3468.12788.

[27] C. Rodemer, T.-P. Thai, B. Brugger, T. Kaercher, H. Werner, K.-A. Nave, F. Wieland, K. Gorgas, W.W. Just, Inactivation of ether lipid biosynthesis causes male infertility, defects in eye development and optic nerve hypoplasia in mice, Hum. Mol. Genet. 12 (2003) 1881—1895, https://doi.org/10.1093/hmg/ddg191.

[28] F. Dorninger, R. Herbst, B. Kravic, B.Z. Camurdanoglu, I. Macinkovic, G. Zeitler, S. Forss-Petter, S. Strack, M.M. Khan, H.R. Waterham, R. Rudolf, S. Hashemolhosseini, J. Berger, Reduced muscle strength in ether lipid-deficient mice is accompanied by altered development and function of the neuromuscular junction, J. Neurochem. 143 (2017) 569—583, https://doi.org/10.1111/jnc.14082.

[29] P. Brites, A.M. Motley, P. Gressens, P.A.W. Mooyer, I. Ploegaert, V. Everts, P. Evrard, P. Carmeliet, M. Dewerchin, L. Schoonjans, M. Duran, H.R. Waterham, R.J.A. Wanders, M. Baes, Impaired neuronal migration and endochondral ossification in Pex7 knockout mice: a model for rhizomelic chondrodysplasia punctata, Hum. Mol. Genet. 12 (2003) 2255—2267, https://doi.org/10.1093/hmg/ddg236.

[30] W. Fallatah, W. Cui, E. Di Pietro, G.T. Carter, B. Pounder, F. Dorninger, C. Pifl, A.B. Moser, J. Berger, N.E. Braverman, A Pex7 deficient mouse series correlates biochemical and neurobehavioral Markers to genotype severity—implications

for the disease spectrum of rhizomelic chondrodysplasia punctata type 1, Front. Cell Dev. Biol. 10 (2022) 886316, https://doi.org/10.3389/fcell.2022.886316.

[31] R.P. Liegel, A. Ronchetti, D.J. Sidjanin, Alkylglycerone phosphate synthase (AGPS) deficient mice: models for rhizomelic chondrodysplasia punctata type 3 (RCDP3) malformation syndrome, Molecular Genetics and Metabolism Reports 1 (2014) 299—311, https://doi.org/10.1016/j.ymgmr.2014.06.003.

[32] N.J. Ingham, S.A. Pearson, V.E. Vancollie, V. Rook, M.A. Lewis, J. Chen, A. Buniello, E. Martelletti, L. Preite, C.C. Lam, F.D. Weiss, Z. Powis, P. Suwannarat, C.J. Lelliott, S.J. Dawson, J.K. White, K.P. Steel, Mouse screen reveals multiple new genes underlying mouse and human hearing loss, PLoS Biol. 17 (2019) e3000194, https://doi.org/10.1371/journal.pbio.3000194.

[33] A. Gallego-García, A. Monera-Girona, E. Pajares Martínez, E. Bastida-Martínez, R. Pérez-Castaño, A. Iniesta, M. Fontes, S. Padmanabhan, M. Elías-Arnanz, A bacterial light response reveals an orphan desaturase for human plasmalogen synthesis, Science 366 (2019) 128—132, https://doi.org/10.1126/science.aay1436.

[34] E.R. Werner, M.A. Keller, S. Sailer, K. Lackner, J. Koch, M. Hermann, S. Coassin, G. Golderer, G. Werner-Felmayer, R.A. Zoeller, N. Hulo, J. Berger, K. Watschinger, The TMEM189 gene encodes plasmanylethanolamine desaturase which introduces the characteristic vinyl ether double bond into plasmalogens, Proc. Natl. Acad. Sci. USA 117 (2020) 7792—7798, https://doi.org/10.1073/pnas.1917461117.

[35] M. Wainberg, R.A. Kamber, A. Balsubramani, R.M. Meyers, N. Sinnott-Armstrong, D. Hornburg, L. Jiang, J. Chan, R. Jian, M. Gu, A. Shcherbina, M.M. Dubreuil, K. Spees, W. Meuleman, M.P. Snyder, M.C. Bassik, A. Kundaje, A genome-wide atlas of co-essential modules assigns function to uncharacterized genes, Nat. Genet. 53 (2021) 638—649, https://doi.org/10.1038/s41588-021-00840-z.

[36] T.F. da Silva, J. Eira, A.T. Lopes, A.R. Malheiro, V. Sousa, A. Luoma, R.L. Avila, R.J.A. Wanders, W.W. Just, D.A. Kirschner, M.M. Sousa, P. Brites, Peripheral nervous system plasmalogens regulate Schwann cell differentiation and myelination, J. Clin. Invest. 124 (2014) 2560—2570, https://doi.org/10.1172/JCI72063.

[37] MdS. Hossain, Y. Abe, F. Ali, M. Youssef, M. Honsho, Y. Fujiki, T. Katafuchi, Reduction of ether-type glycerophospholipids, plasmalogens, by NF-κB signal leading to microglial activation, J. Neurosci. 37 (2017) 4074—4092, https://doi.org/10.1523/JNEUROSCI.3941-15.2017.

[38] MdS. Hossain, S. Mawatari, T. Fujino, Plasmalogens, the vinyl ether-linked glycerophospholipids, enhance learning and memory by regulating brain-derived neurotrophic factor, Front. Cell Dev. Biol. 10 (2022) 828282, https://doi.org/10.3389/fcell.2022.828282.

[39] F. Ali, MdS. Hossain, S. Sejimo, K. Akashi, Plasmalogens inhibit endocytosis of toll-like receptor 4 to attenuate the inflammatory signal in microglial cells, Mol. Neurobiol. 56 (2019) 3404—3419, https://doi.org/10.1007/s12035-018-1307-2.

[40] M. Youssef, A. Ibrahim, K. Akashi, M.S. Hossain, PUFA-plasmalogens attenuate the LPS-induced nitric oxide production by inhibiting the NF-kB, p38 MAPK and JNK pathways in microglial cells, Neuroscience 397 (2019) 18—30, https://doi.org/10.1016/j.neuroscience.2018.11.030.

[41] MdS. Hossain, K. Mineno, T. Katafuchi, Neuronal orphan G-protein coupled receptor proteins mediate plasmalogens-induced activation of ERK and Akt signaling, PLoS One 11 (2016) e0150846, https://doi.org/10.1371/journal.pone.0150846.

[42] M.S. Hossain, S. Mawatari, T. Fujino, Plasmalogen-mediated activation of GPCR21 regulates cytolytic activity of NK cells against the target cells, J. Immunol. 209 (2022) 310—325, https://doi.org/10.4049/jimmunol.2200183.

[43] X. Wang, J.H. Bae, S.U. Kim, J.G. McLarnon, Platelet-activating factor induced Ca2+ signaling in human microglia, Brain Res. 842 (1999) 159—165, https://doi.org/10.1016/S0006-8993(99)01849-1.

[44] S. Ishii, Platelet-activating factor receptor, in: XPharm: the Comprehensive Pharmacology Reference, Elsevier, 2007, pp. 1—13, https://doi.org/10.1016/B978-008055232-3.60082-0.

[45] V.H. Chaithra, S.P. Jacob, C.L. Lakshmikanth, M.S. Sumanth, K.V. Abhilasha, C.-H. Chen, A. Thyagarajan, R.P. Sahu, J.B. Travers, T.M. McIntyre, K. Kemparaju, G.K. Marathe, Modulation of inflammatory platelet-activating factor (PAF) receptor by the acyl analogue of PAF, JLR (J. Lipid Res.) 59 (2018) 2063—2074, https://doi.org/10.1194/jlr.M085704.

[46] P. Rong, J.-L. Wang, A. Angelova, Z.A. Almsherqi, Y. Deng, Plasmalogenic lipid analogs as platelet-activating factor antagonists: a potential novel class of anti-inflammatory compounds, Front. Cell Dev. Biol. 10 (2022) 859421, https://doi.org/10.3389/fcell.2022.859421.

[47] L.H.M. Geraldo, T.C.L. de, S. Spohr, R.F. do Amaral, A.C.C. da Fonseca, C. Garcia, F. de A. Mendes, C. Freitas, M.F. dosSantos, F.R.S. Lima, Role of lysophosphatidic acid and its receptors in health and disease: novel therapeutic strategies, Signal Transduct. Targeted Ther. 6 (2021) 45, https://doi.org/10.1038/s41392-020-00367-5.

[48] K. Bandoh, J. Aoki, A. Taira, M. Tsujimoto, H. Arai, K. Inoue, Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species: structure-activity relationship of cloned LPA receptors, FEBS (Fed. Eur. Biochem. Soc.) Lett. 478 (2000) 159—165, https://doi.org/10.1016/S0014-5793(00)01827-5.

[49] S.S. Davies, A.V. Pontsler, G.K. Marathe, K.A. Harrison, R.C. Murphy, J.C. Hinshaw, G.D. Prestwich, A.St Hilaire, S.M. Prescott, G.A. Zimmerman, T.M. McIntyre, Oxidized alkyl phospholipids are specific, high affinity

peroxisome proliferator-activated receptor γ ligands and agonists, J. Biol. Chem. 276 (2001) 16015—16023, https://doi.org/10.1074/jbc.M100878200.

[50] I.J. Lodhi, L. Yin, A.P.L. Jensen-Urstad, K. Funai, T. Coleman, J.H. Baird, M.K. El Ramahi, B. Razani, H. Song, F. Fu-Hsu, J. Turk, C.F. Semenkovich, Inhibiting adipose tissue lipogenesis reprograms thermogenesis and PPARγ activation to decrease diet-induced obesity, Cell Metabol. 16 (2012) 189—201, https://doi.org/10.1016/j.cmet.2012.06.013.

[51] T. Tsukahara, R. Tsukahara, S. Yasuda, N. Makarova, W.J. Valentine, P. Allison, H. Yuan, D.L. Baker, Z. Li, R. Bittman, A. Parrill, G. Tigyi, Different residues mediate recognition of 1-O-oleyllysophosphatidic acid and rosiglitazone in the ligand binding domain of peroxisome proliferator-activated receptor γ, J. Biol. Chem. 281 (2006) 3398—3407, https://doi.org/10.1074/jbc.M510843200.

[52] T.M. McIntyre, A.V. Pontsler, A.R. Silva, A. St Hilaire, Y. Xu, J.C. Hinshaw, G.A. Zimmerman, K. Hama, J. Aoki, H. Arai, G.D. Prestwich, Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARγ agonist, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 131—136, https://doi.org/10.1073/pnas.0135855100.

[53] E.N.D. Palladino, C.L. Hartman, C.J. Albert, D.A. Ford, The chlorinated lipidome originating from myeloperoxidase-derived HOCl targeting plasmalogens: metabolism, clearance, and biological properties, Arch. Biochem. Biophys. 641 (2018) 31—38, https://doi.org/10.1016/j.abb.2018.01.010.

[54] D.L. Ebenezer, P. Fu, R. Ramchandran, A.W. Ha, V. Putherickal, T. Sudhadevi, A. Harijith, F. Schumacher, B. Kleuser, V. Natarajan, S1P and plasmalogen derived fatty aldehydes in cellular signaling and functions, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1865 (2020) 158681, https://doi.org/10.1016/j.bbalip.2020.158681.

[55] B. Zhao, Z. Xin, P. Ren, H. Wu, The role of PPARs in breast cancer, Cells 12 (2022) 130, https://doi.org/10.3390/cells12010130.

[56] B. Grygiel-Górniak, Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications - a review, Nutr. J. 13 (2014) 17, https://doi.org/10.1186/1475-2891-13-17.

[57] D.I. Benjamin, A. Cozzo, X. Ji, L.S. Roberts, S.M. Louie, M.M. Mulvihill, K. Luo, D.K. Nomura, Ether lipid generating enzyme AGPS alters the balance of structural and signaling lipids to fuel cancer pathogenicity, Proc. Natl. Acad. Sci. USA 110 (2013) 14912—14917, https://doi.org/10.1073/pnas.1310894110.

[58] L. Wang, P.S. Bose, F.J. Sigworth, Using cryo-EM to measure the dipole potential of a lipid membrane, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 18528—18533, https://doi.org/10.1073/pnas.0608714103.

[59] F. Paltauf, Ether lipids in biomembranes, Chem. Phys. Lipids 74 (1994) 101—139, https://doi.org/10.1016/0009-3084(94)90054-X.

[60] J.M. Smaby, A. Hermetter, P.C. Schmid, F. Paltauf, H.L. Brockman, Packing of ether and ester phospholipids in monolayers. Evidence for hydrogen-bonded water at the sn-1 acyl group of phosphatidylcholines, Biochemistry 22 (1983) 5808—5813, https://doi.org/10.1021/bi00294a019.

[61] T. Rog, A. Koivuniemi, The biophysical properties of ethanolamine plasmalogens revealed by atomistic molecular dynamics simulations, Biochim. Biophys. Acta Biomembr. 1858 (2016) 97—103, https://doi.org/10.1016/j.bbamem.2015.10.023.

[62] M. Saunders, M. Steele, W. Lavigne, S. Varma, S.A. Pandit, Interaction of salt with ether- and ester-linked phospholipid bilayers, Biochim. Biophys. Acta Biomembr. 1861 (2019) 907—915, https://doi.org/10.1016/j.bbamem.2019.01.016.

[63] L.J. Pike, X. Han, K.-N. Chung, R.W. Gross, Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis, Biochemistry 41 (2002) 2075—2088, https://doi.org/10.1021/bi0156557.

[64] A. Koivuniemi, The biophysical properties of plasmalogens originating from their unique molecular architecture, FEBS Lett. 591 (2017) 2700—2713, https://doi.org/10.1002/1873-3468.12754.

[65] R. Karadayi, J. Mazzocco, L. Leclere, B. Buteau, S. Gregoire, C. Belloir, M. Koudsi, P. Bessard, J.-B. Bizeau, E. Dubus, C. Fenech, L. Briand, L. Bretillon, A.M. Bron, X. Fioramonti, N. Acar, Plasmalogens regulate retinal connexin 43 expression and Müller glial cells gap junction intercellular communication and migration, Front. Cell Dev. Biol. 10 (2022) 864599, https://doi.org/10.3389/fcell.2022.864599.

[66] K. Lohner, P. Balgavy, A. Hermetter, F. Paltauf, P. Laggner, Stabilization of non-bilayer structures by the etherlipid ethanolamine plasmalogen, Biochim. Biophys. Acta Biomembr. 1061 (1991) 132—140, https://doi.org/10.1016/0005-2736(91)90277-F.

[67] M. Honsho, S. Mawatari, T. Fujino, Transient Ca2+ entry by plasmalogen-mediated activation of receptor potential cation channel promotes AMPK activity, Front. Mol. Biosci. 9 (2022) 1008626, https://doi.org/10.3389/fmolb.2022.1008626.

[68] F. Maingret, A.J. Patel, F. Lesage, M. Lazdunski, E. Honoré, Lysophospholipids open the two-pore domain Mechano-gated K+ channels TREK-1 and TRAAK, J. Biol. Chem. 275 (2000) 10128—10133, https://doi.org/10.1074/jbc.275.14.10128.

[69] I.B. Levitan, Phosphorylation of ion channels, J. Membr. Biol. 87 (1985)

[70] D. Balgoma, M. Hedeland, Etherglycerophospholipids and ferroptosis: structure, regulation, and location, Trends Endocrinol. Metabol. 32 (2021) 960—962, https://doi.org/10.1016/j.tem.2021.08.005.

[71] B.R. Stockwell, J.P.F. Angeli, H. Bayir, A.I. Bush, M. Conrad, S.J. Dixon, S. Fulda, S. Gascón, S.K. Hatzios, V.E. Kagan, K. Noel, X. Jiang, A. Linkermann, M.E. Murphy, M. Overholtzer, A. Oyagi, G.C. Pagnussat, J. Park, Q. Ran, C.S. Rosenfeld, K. Salnikow, D. Tang, F.M. Torti, S.V. Torti, S. Toyokuni, K.A. Woerpel, D.D. Zhang, Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease, Cell 171 (2017) 273—285, https://doi.org/10.1016/j.cell.2017.09.021.

[72] M.A. Perez, L. Magtanong, S.J. Dixon, J.L. Watts, Dietary lipids induce ferroptosis in caenorhabditiselegans and human cancer cells, Dev. Cell 54 (2020) 447—454, https://doi.org/10.1016/j.devcel.2020.06.019, e4.

[73] Y. Zou, W.S. Henry, E.L. Ricq, E.T. Graham, V.V. Phadnis, P. Maretich, S. Paradkar, N. Boehnke, A.A. Deik, F. Reinhardt, J.K. Eaton, B. Ferguson, W. Wang, J. Fairman, H.R. Keys, V. Dančík, C.B. Clish, P.A. Clemons, P.T. Hammond, L.A. Boyer, R.A. Weinberg, S.L. Schreiber, Plasticity of ether lipids promotes ferroptosis susceptibility and evasion, Nature 585 (2020) 603—608, https://doi.org/10.1038/s41586-020-2732-8.

[74] K. Huynh, W.L.F. Lim, C. Giles, K.S. Jayawardana, A. Salim, N.A. Mellett, A.A.T. Smith, G. Olshansky, B.G. Drew, P. Chatterjee, I. Martins, S.M. Laws, A.I. Bush, C.C. Rowe, V.L. Villemagne, D. Ames, C.L. Masters, M. Arnold, K. Nho, A.J. Saykin, R. Baillie, X. Han, R. Kaddurah-Daouk, R.N. Martins, P.J. Meikle, Concordant peripheral lipidome signatures in two large clinical studies of Alzheimer's disease, Nat. Commun. 11 (2020) 5698, https://doi.org/10.1038/s41467-020-19473-7.

[75] M. Jové, N. Mota-Martorell, È. Obis, J. Sol, M. Martín-Garí, I. Ferrer, M. Portero-Otin, R. Pamplona, Ether lipid-mediated antioxidant defense in Alzheimer's disease, Antioxidants 12 (2023) 293, https://doi.org/10.3390/antiox12020293.

[76] R. Sever, J.S. Brugge, Signal transduction in cancer, Cold Spring Harbor Perspectives in Medicine 5 (2015), https://doi.org/10.1101/cshperspect.a006098 a006098—a006098.

[77] Y. Zhu, L. Zhu, L. Lu, L. Zhang, G. Zhang, Q. Wang, P. Yang, Role and mechanism of the alkylglycerone phosphate synthase in suppressing the invasion potential of human glioma and hepatic carcinoma cells in vitro, Oncol. Rep. 32 (2014) 431—436, https://doi.org/10.3892/or.2014.3189.

[78] G. Stazi, C. Battistelli, V. Piano, R. Mazzone, B. Marrocco, S. Marchese, S.M. Louie, C. Zwergel, L. Antonini, A. Patsilinakos, R. Ragno, M. Viviano, G. Sbardella, A. Ciogli, G. Fabrizi, R. Cirilli, R. Strippoli, A. Marchetti, M. Tripodi, D.K. Nomura, A. Mattevi, A. Mai, S. Valente, Development of alkyl glycerone phosphate synthase inhibitors: structure-activity relationship and effects on ether lipids and epithelial-mesenchymal transition in cancer cells, Eur. J. Med. Chem. 163 (2019) 722—735, https://doi.org/10.1016/j.ejmech.2018.11.050.

[79] Y. Zhu, X.-J. Liu, P. Yang, M. Zhao, L.-X. Lv, G.-D. Zhang, Q. Wang, L. Zhang, Alkylglyceronephosphate synthase (AGPS) alters lipid signaling pathways and supports chemotherapy resistance of glioma and hepatic carcinoma cell lines, Asian Pac. J. Cancer Prev. APJCP 15 (2014) 3219—3226, https://doi.org/10.7314/APJCP.2014.15.7.3219.

[80] S. Phuyal, T. Skotland, N.P. Hessvik, H. Simolin, A. Øverbye, A. Brech, R.G. Parton, K. Ekroos, K. Sandvig, A. Llorente, The ether lipid precursor hexadecylglycerol stimulates the release and changes the composition of exosomes derived from PC-3 cells, J. Biol. Chem. 290 (2015) 4225—4237, https://doi.org/10.1074/jbc.M114.593962.

[81] M.S. Dahabieh, E. Di Pietro, M. Jangal, C. Goncalves, M. Witcher, N.E. Braverman, S.V. del Rincón, Peroxisomes and cancer: the role of a metabolic specialist in a disease of aberrant metabolism, Biochim. Biophys. Acta Rev. Canc 1870 (2018) 103—121, https://doi.org/10.1016/j.bbcan.2018.07.004.

[82] Y. Wu, Z. Chen, J. Jia, H. Chiba, S.-P. Hui, Quantitative and comparative investigation of plasmalogen species in daily foodstuffs, Foods 10 (2021) 124, https://doi.org/10.3390/foods10010124.

[83] X. Wang, X. Wang, P. Cong, L. Wu, Y. Ma, Z. Wang, T. Jiang, J. Xu, Sea cucumber ether-phospholipids improve hepatic steatosis and enhance hypothalamic autophagy in high-fat diet-fed mice, J. Nutr. Biochem. 106 (2022) 109032, https://doi.org/10.1016/j.jnutbio.2022.109032.

[84] J. Yu, L. Qu, Y. Xia, X. Zhang, J. Feng, M. Duan, P. guo, Y. Lou, P. Lv, W. Lu, Y. Chen, TMEM189 negatively regulates the stability of ULK1 protein and cell autophagy, Cell Death Dis. 13 (2022) 316, https://doi.org/10.1038/s41419-022-04722-y.

[85] M. Honsho, Y. Abe, Y. Fujiki, Dysregulation of plasmalogen homeostasis impairs cholesterol biosynthesis, J. Biol. Chem. 290 (2015) 28822—28833, https://doi.org/10.1074/jbc.M115.656983.

[86] N. Nagan, R.A. Zoeller, Plasmalogens: biosynthesis and functions, Prog. Lipid Res. 40 (2001) 199—229, https://doi.org/10.1016/S0163-7827(01)00003-0.

[87] S. Rangaswamy, R.A. Zoeller, Fatty acid desaturation in an animal cell mutant defective in plasmanylethanolamine desaturase, Biochim. Biophys. Acta Lipids Lipid. Metabol. 1211 (1994) 79—84, https://doi.org/10.1016/0005-2760(94)90141-4.