Lysophospholipids: Potential Markers of Diseases and Infertility?

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Abstract: The *in vivo* concentration of lysophospholipids (LPL) such as lysophosphatidylcholine (LPC) increases under different pathological conditions and, thus, LPL attract nowadays considerable diagnostic and pharmacological interest. LPL are particularly interesting because they possess pro- and anti-inflammatory properties and can be generated by two completely different pathways: either by the influence of (a) phospholipases and (b) different reactive oxygen species (ROS) that are generated in significant amounts under inflammatory conditions. This review provides a summary of the mechanisms by which LPL can be generated under *in vitro* and *in vivo* conditions. The focus will be on lysophosphatidylcholine (LPC) because this LPL is most abundant among all LPL and was, thus, most intensively studied so far. Additionally, biochemical, chromatographic and spectroscopic methods of LPL and LPC determinations will be discussed. Finally, the effects of LPL as signaling molecules and their roles in different pathologies such as infertility, cancer, atherosclerosis or inflammatory diseases are discussed. Special emphasis will be on the role of LPL in reproduction failures related to poor semen quality and, in that context, the potential role of LPC as a disease-indicative molecule.

Keywords: Disease Markers, Inflammation, Lysophospholipids, Lysophosphatidylcholine, Phospholipids, Phospholipase, Reactive Oxygen Species, Spermatozoa.

1. INTRODUCTION

The aim of this review is the discussion of the generation of lysophospholipids (LPL) under *in vivo* conditions and their roles in certain pathologies such as atherosclerosis or infertility and - consequently - their diagnostic relevance as well as methods of LPL determination. Particular attention will be paid to lysophosphatidylcholine (LPC), although other LPL such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are also of high relevance but will not be discussed here due to the limited space. This review should be considered as an update of a previously published review dealing with the same subject [1].

LPL are basically derived from phospholipids (PLs) by the selective loss of one fatty acyl residue induced by enzymes and/or reactive oxygen species (ROS). Although details of LPL generation are still unknown, PLs are thus the unequivocal precursors. Therefore, this review starts with an overview of relevant PLs [2].

1.1. Important Phospholipids

PLs constitute a highly important class of biomolecules, of which glycerophospholipids (GPLs) are of particular relevance [2]. All GPL consist of a glycerol backbone, where two hydroxyl groups are esterified with two varying organic fatty acids (termed "R" in Fig. (**1**)). The third hydroxyl group is esterified with phosphoric acid. The resulting molecule is termed "phosphatidic acid" (PA). *Via* ester condensation with different alcohols such as choline or ethanolamine, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are generated. These compounds represent the most abundant zwitterionic GPLs of biological membranes (Fig. (**1**)).

Additionally, there are also acidic (negatively charged at pH 7.4) GPLs, such as phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI) and polyphosphoinositides (PPI) with several phosphate residues at the inositol ring. The most important PL without the glycerol backbone is sphingomyelin (SM) and the LPL derived thereof are sphingosine-1-phosphate (S1P) and ceramide (Cer). Although these compounds are also assumed to possess important cellular functions [3] both will be treated here only very loosely.

The majority of PLs occurring under *in vivo* conditions is characterized by a saturated fatty acyl residue in *sn*-1 position, while the second fatty acyl residue is often moderately (e.g. oleic acid) or even highly unsaturated [2]. The arachidonyl residue with four double bonds (20:4) is of particular relevance due to the molecules with regulatory function derived thereof (e.g. prostaglandins). It should be explicitly noted that in addition to diacyl compounds there are also alkyl-acyl and alkenyl-acyl compounds. Especially

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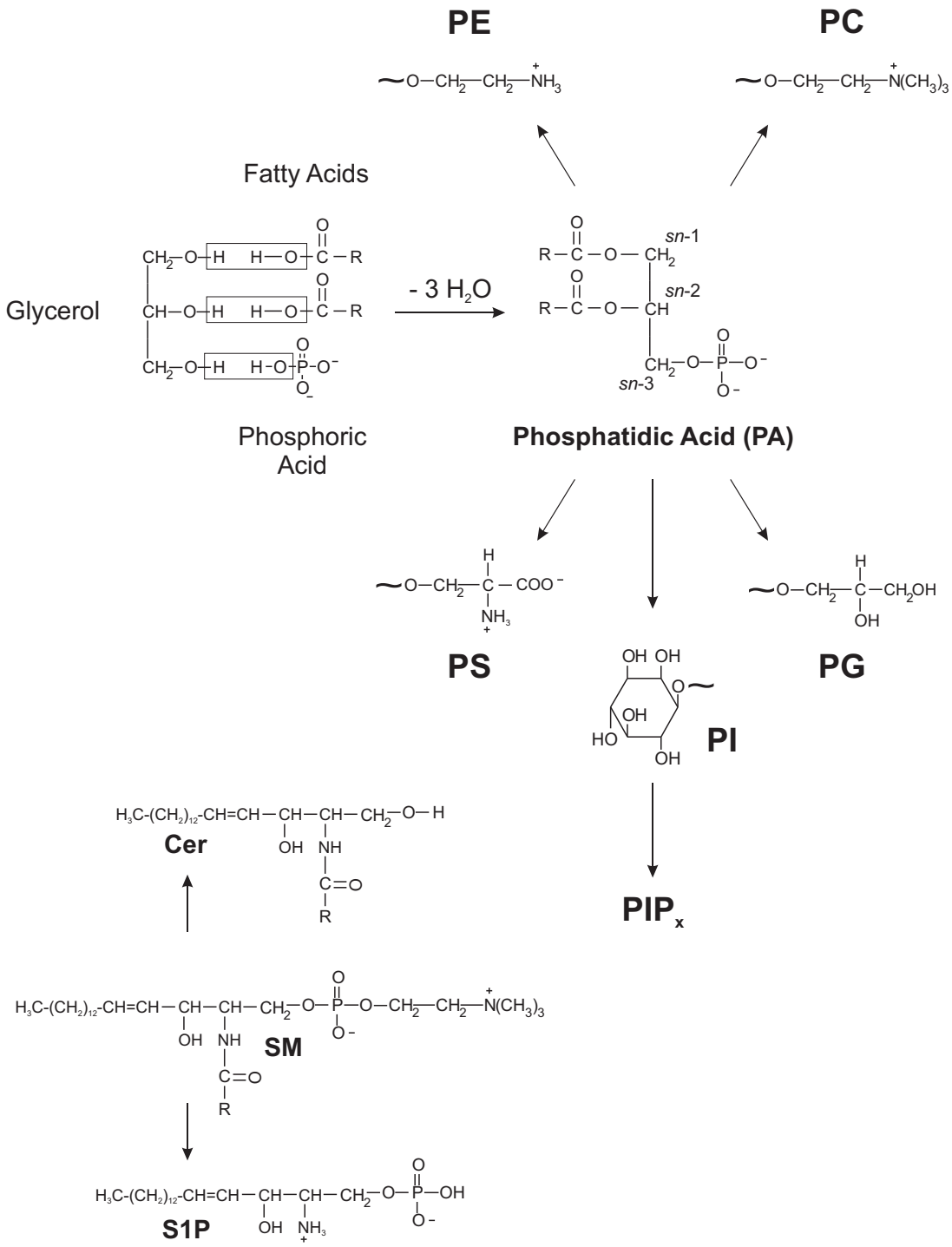

Fig. (1). Chemical structures of relevant glycerophospholipids (**GPL**) and their (simplified) generation under *in vivo* conditions. Arrows from PA assign esterified GPL headgroups. Although SM does not represent a GPL, it is also an important membrane constituent and the compounds derived thereof (S1P and Cer) are important signaling molecules. Abbreviations: **Cer**, Ceramide; **PC**, Phosphatidylcholine; **PE**, Phosphatidylethanolamine; **PG**, Phosphatidylglycerol; **PI**, Phosphatidylinositol; **PPI**, Poly-phosphoinositides; **PS**, Phosphatidylserine; **S1P**, Sphingosine-1-phosphate; **SM**, Sphingomyelin.

the last lipid class will be discussed in the context of spermatozoa where they are particularly abundant.

GPLs are converted into LPLs by the action of phospholipases and the reactions catalyzed by these enzymes are illustrated in (Fig. (**2**)).

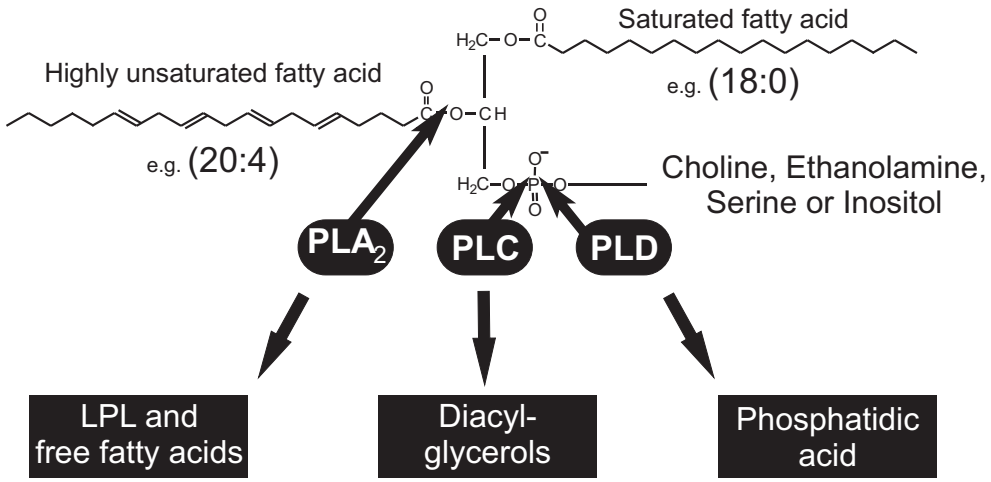

Fig. (2). Schema of the LPL generation under *in vivo* conditions from a selected PL molecule together with the involved enzymes. Abbreviations: **PLA_2**, phospholipase A_2; **PLC**, phospholipase C; **PLD**, phospholipase D.

The focus of this review is the action of phospholipase A_2 (PLA_2) as this is (to our best knowledge) the most important pathway of LPL formation under *in vivo* conditions. Therefore, PLC and PLD leading to the generation of diacylglycerols (DAGs) and PAs, respectively, will not be comprehensively discussed in this paper. The interested reader is referred to an excellent recently published review [4]. Finally, it should be noted that the free (often highly unsaturated) fatty acids that are released under the influence of PLA_2 have also considerable physiological significance: unsaturated fatty acids such as arachidonic acid are very sensitive to oxidation and the metabolic (oxidation) products such as thromboxanes or leukotrienes have a considerable biological impact [5].

1.2. Generation of LPL under the Influence of Phospholipases

Phospholipase "A" is represented by a group of enzymes that catalyze the hydrolysis of one fatty acyl residue from the glycerol backbone of a PL. The basic reaction is illustrated in (Fig. (**2**)). A free fatty acid is released by this reaction and the corresponding LPL left in the membrane [6]. Both, the LPL as well as the free fatty acid are considered as important molecules with potential messenger functions. Additionally, free fatty acids as well as LPL represent detergents and destabilize the cellular membrane [7].

Based on the stereospecificity of the reaction, PLA_1 (phospholipase A_1, E.C. 3.1.1.32) and PLA_2 (phosphatidyl-choline 2-acylhydrolase, E.C. 3.1.1.4) activities can be differentiated. Please note that PLA_1 enzymes play a much smaller physiological role than PLA_2 [6]. Accordingly, PLA_2 enzymes will be emphasized in this review. This group comprises 12 families some of which may also express PLA_1, or lysophospholipase A_1/A_2 activity [8]. Individual families of PLA_2 differ with respect to their molecular weights (MW). The MW may range between 13-18 kDa (the secretory enzyme ($sPLA_2$)) and about 85 kDa (the cytosolic enzyme ($cPLA_2$)) [8]. Additionally, their requirements for Ca^{2+} and/or phosphorylation to become active are different. For instance, $cPLA_2$ requires μM amounts of Ca^{2+}, whereas $sPLA_2$ is only active in the presence of mM amounts of Ca^{2+} [6].

1.3. Generation of LPL under the Influence of Reactive Oxygen Species (ROS)

It is commonly accepted that the concentration of LPL (in particular that of LPC) increases under inflammatory conditions. This has been shown, for instance, in the case of the joint fluids from patients suffering from rheumatoid arthritis [9] or atherosclerosis patients [10, 11]. Some other examples will be discussed below in more detail. The prevailing opinion is that LPL are generated under *in vivo* conditions by the release and/or activation of PLA_2 that is particularly present in neutrophilic granulocytes, important "cellular" mediators of inflammation. However, neutrophils do not only secrete PLA_2, but are also capable of generating ROS [12] (cf. Fig. (**3**)).

Shortly, ROS are derived from "normal" (atmospheric) oxygen that is initially converted into superoxide anion radicals ($O_2^{\bullet-}$) that dismutate spontaneously (or much more readily in the presence of the enzyme superoxide dismutase (SOD) that is also present in neutrophils) into hydrogen peroxide. H_2O_2 is the starting material for the generation of further, much more reactive species, for instance, hydroxyl radicals ($HO^{\bullet}$). $HO^{\bullet}$ are extremely reactive and react diffusion-controlled with virtually all compounds containing C-H groups. $HO^{\bullet}$ are generated under *in vivo* conditions (and in the presence of low-valent transition metals such as Fe^{2+} or Cu^{+}) by the "famous" Fenton reaction that means the transition metal-catalyzed, homolytic scission of H_2O_2. However, even more than one hundred years after the first description of the Fenton reaction there is still no agreement about the actually generated species [13].

Despite the high reactivity of $HO^{\bullet}$, another ROS seems primarily responsible for the increased levels of LPC under pathological conditions [14]: Hypochlorous acid (HOCl) [11]. HOCl is generated under *in vivo* conditions from H_2O_2 and Cl^- ions under the catalytic influence of the enzyme myeloperoxidase (MPO) [14] that is nearly exclusively found in neutrophils, where it makes out about 5% of the total amount of proteins [12 and references cited therein]:

$$H_2O_2 + Cl^- \rightarrow HOCl + HO^-$$

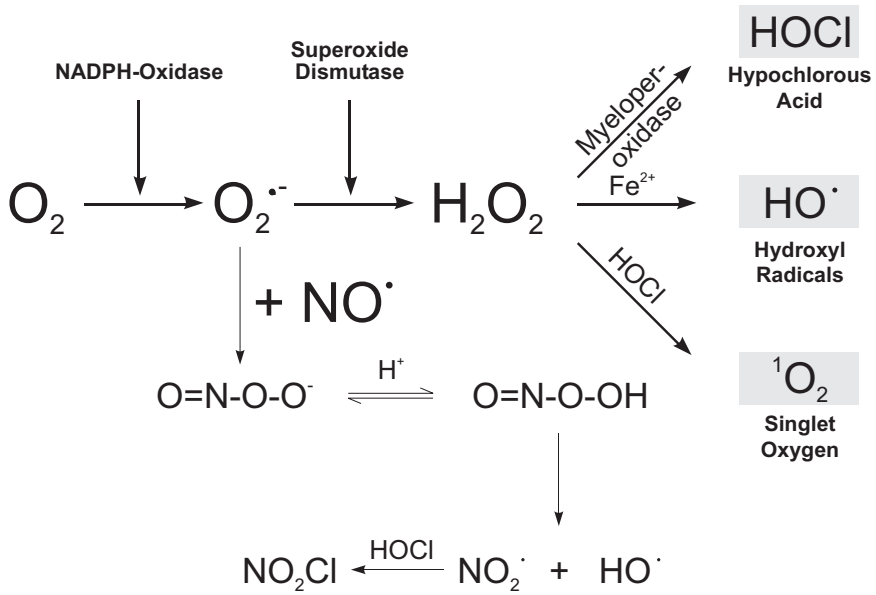

Fig. (3). Simplified schema of ROS and reactive nitrogen species (RNS) generation under *in vivo* conditions. Please note that this is a very simplified summary of potential chemical reactions that does not take the different locations of enzymes and their substrates into account. RNS will not be discussed here.

As the number of neutrophilic granulocytes increases massively under inflammatory conditions, the role of MPO and its products are obvious [15]. Further ROS such as singlet oxygen ($^1O_2$), nitric oxide ($^{\bullet}NO$) or peroxynitrite ($ONOO^-$) (cf. Fig. (**3**)) may be also involved in LPL generation but their contribution will not be discussed here because the so far available data related to these processes are rather limited.

The generation of LPC from PC under *in vivo* conditions is normally discussed by an increased activity of PLA_2 in the presence of HOCl [16]. However, it could be shown on a model level that LPC is also generated in the absence of PLA_2, i.e. under the exclusive influence of HOCl: Using PC vesicles with different acyl compositions it could be proven that LPC is an abundant reaction product if PC reacts with either HOCl alone or with the products of the complete $MPO/H_2O_2/Cl^-$ system [17].

Although chlorohydrins (addition products of HOCl to the double bonds of unsaturated fatty acyl residues, cf. Fig. (**4**)) are the most abundant products, LPC is also generated in a significant yield [17].

Please note that exclusively saturated LPC species are generated indicating that the unsaturated, but not the saturated fatty acyl residue is fragmented during the reaction: The yield of LPC increases if the saturation degree of the applied PC decreases. This makes this pathway of LPC generation particularly relevant for cells and tissues with high moieties of highly unsaturated fatty acids such as spermatozoa or brain, respectively, and suggest LPC as a marker of oxidative stress. A mechanism to explain this behavior has been recently proposed (Fig. (**4**)) [18]: Oxygen and chlorine are rather electronegative elements and weaken the ester bonds of lipids by the withdrawal of electrons. Therefore, the acyl residues in the chlorohydrins are more sensitive to hydrolysis than the unmodified acyl residues of the original lipids [18]. This has been also very recently confirmed by the investigation of oxidized lipoproteins [19]. The slow conversion of PC into LPC in the presence of atmospheric oxygen might also be the reason why PL solutions do normally contain small amounts of LPC [20].

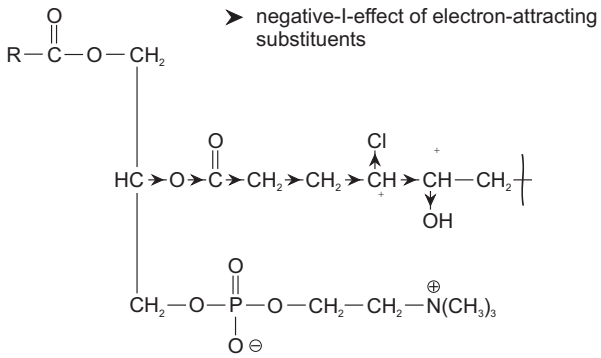

Fig. (4). Proposed weakening of the ester bond in unsaturated phosphatidylcholines by the introduction of electron-withdrawing substituents upon chlorohydrin formation. Reproduced with permission from Arnhold *et al*. [18]. Copyright (2002) by Elsevier.

Surprisingly, however, the expected LPL were not obtained if PE or PS vesicles were treated with HOCl, even if a significant excess of HOCl over the PL was used [21, 22]. This is a clear indication that the value of the above-mentioned mechanism (Fig. (**4**)) is limited and further parameters (for instance the structure of the PL headgroup) influence the LPL yield. Another important source of LPL in biological systems is the oxidative modification of plasmalogen species that possess (normally in *sn*-1 position) a vinyl ether linkage instead of the common ester linkage. This important topic has been recently reviewed [23] and it

seems very likely that plasmalogens represent highly important antioxidative compounds.

2. METHODS OF LPL DETERMINATIONS

Due to the importance of phospholipases, the determination of LPL concentrations is nearly a synonym of the determination of PLA_2 activities. There are many different methods available and a comprehensive review of this topic has been published some time ago [24].

However, please note that one important structural aspect of amphiphilic PL, the bilayer structure, is normally neglected in the majority of PLA_2 assays. Instead, simplified "mixed" micelles of PL and detergents are often used in order to avoid opaque "solutions". This approach must be critically regarded because (a) the bilayer structure of PL is completely neglected under these conditions and (b) potential effects of the detergent moiety on the activity of the enzyme cannot be excluded.

2.1. Methods Based on UV-, Fluorescence or ESR Spectroscopy

The hydrolysis of PL catalyzed by PLA_2 can be monitored by an assay developed by Aarsman *et al.* [25]. This method is based on the use of a certain thiol ester as the enzyme substrate. After the release of the thiol by enzymatic cleavage, the sulfhydryl (-SH) group is determined colorimetrically by *Ellman's* reagent (5,5'-dithiobis-(2-nitrobenzoic acid)) which forms a yellow colored product if it reacts with thiols. This assay is nowadays commercially available and, thus, simply to use and highly standardized.

An additional interesting method was developed in 1989 by Rawyler and Sigenthaler [26]. The method is based on the property of the cationic dye *Safranine* to change its spectroscopic properties in the presence of negatively charged molecules. Taking into account that fatty acids are negatively charged at physiological pH (7.4) this assay can be generally used to monitor the activities of lipases - not only phospholipases. It is a particular advantage that natural (non-labeled) lipids can be used. On the negative side, however, no differentiation between the individual lipases (particularly in mixtures) can be made because the actually measured quantity is the amount of released free fatty acids. Please also note that the lack of structural information is a serious drawback of spectrophotometric methods in general. Therefore, these methods are not the techniques of choice to determine the LPL concentration in a given PL sample.

Another method to follow the hydrolysis of membrane PL *in situ* uses spin-labeled PL with a NO-group on a short fatty acyl residue in *sn*-2 position. These analogues incorporate readily into the outer leaflet of cell membranes and are further physiologically re-distributed between the membrane layers. Their susceptibility to hydrolytic processes results in freely diffusible spin-labeled fatty acids [27]. These produce an isotropic (narrow) signal in the ESR spectra that is clearly distinguishable from the (broad) signal of the intact PL analogue and allows to calculate the relative amount of hydrolyzed labels as for instance in spermatozoa membranes [28]. It has been shown that spin-labeled PL analogues basically reflect the transmembrane re-distribution of intrinsic PL [29]. Therefore, PL analogues that are asymmetrically distributed between the inner and outer membrane leaflet might be applied to localize the site of hydrolytic activity.

To observe and quantify hydrolytic activities in intact membranes, several fluorescent lipid molecules are commercially available (cf. the offers by Invitrogen and Molecular Probes) although the incorporation of these PL into the membranes is often a laborious process. Therefore, their potential to reflect endogenous conditions has to be critically scrutinized in the respective case.

2.2. Methods Based on Chromatography

Chromatographic methods (particularly high-performance liquid chromatography (HPLC) [30] and thin-layer chromatography (TLC)) [31] are nowadays highly established in PL research and also widely used for the determination of phospholipase activities. TLC was so far primarily applied because it can be very easily implemented and is quite inexpensive. Moreover, it is possible by using two-dimensional TLC to separate a given mixture into the individual lipid classes (according to differences in the headgroups) in the first dimension followed by a separation according to differences in the fatty acyl composition in the second dimension (for instance, by means of silver ion chromatography) [31]. Although this is a powerful method it has the disadvantage that only a single sample can be analyzed at one time [31]. HPLC is used in a similar way as TLC. However, one major disadvantage of HPLC is its limited reproducibility because even very small impurities of water in the mobile phase influence the retention times significantly [32].

Due to the limited space, only a few selected examples can be given here: The PLA_2 activity in stimulated macrophages could be determined by HPLC using the synthetic NBD-labeled ether lipid 1-O-(12-NBD-aminododecyl)-2-acyl-*sn*-glycero-3-phosphocholine: The released LPC contains the fluorescence label that can be identified by monitoring the fluorescence at 450 nm [33]. Unfortunately, the introduction of artificial labels may influence the membrane structure and, thus, the obtained results do not necessarily reflect the real biological conditions, i.e. the determined enzyme activities can be different from that determined with the native substrate. It is also important to note that the use of substrates that contain one or more alkyl linkages (instead of acyl linkages in natural PL) is still a matter of discussion [34].

Labeling of cellular lipids is, however, not always an absolute prerequisite. One can also use natural substrates and study the activities of the cellular phospholipases by measuring the PL/LPL ratio subsequent to separation by TLC. An assay for the determinations of PA, PE and diacylglycerols (DAG) in cultured cells has been developed [35] and is based on the densitometric determination of charred spots obtained after two-dimensional separation of the related PL and LPL classes.

Finally, capillary electrophoresis (CE) can be also used for the continuous monitoring of the PC digestion by $sPLA_2$ [36]. Using this approach, peaks arising from residual

substrate and released arachidonic acid can be simultaneously detected and quantified.

2.3. Methods Based on Radioactivity

Due to the above-mentioned drawbacks, methods based on radioactivity are still highly popular and widely used. An additional advantage is the high sensitivity of this method. The assay is performed by growing the cells in the presence of a radioactively-labeled substrate such as ^{32}P phosphoinositide. Separation of the lipid metabolites of interest can be accomplished, for instance, by HPLC or TLC and the radioactivity afterwards determined in the individual fractions [37].

2.4. Methods Based on NMR Spectroscopy

Although nuclear magnetic resonance (NMR) spectroscopy is a relatively insensitive method, the determination of phospholipase activities (or generally the PL/LPL ratio in a mixture) was among the first applications of this method and has been comprehensively reviewed [38]. Due to its relatively high sensitivity and the high natural abundance of the phosphorous nucleus, ^{31}P NMR is unequivocally the NMR method of choice for the detection and determination of PLs and the corresponding LPLs.

Using ^{31}P NMR in combination with a suitable detergent that suppresses the broad resonances of PLs, LPC and PC can be simultaneously detected and from the ratio of the integral intensities of both resonances [38], the determination of the related phospholipase activities is possible. An additional advantage of this method is that all relevant PL classes as well as their LPL analogues (that can be additionally differentiated according to the position of the fatty acyl residue) can be simultaneously determined in mixtures [38]. An illustrative example is shown in (Fig. (**5**)).

Since all PL and LPL classes can be easily differentiated, the estimation of potential selectivities of a given phospholipase against different substrates - in a single experiment and without the need to apply any labels or separation techniques - is simultaneously possible. This is a considerable advantage of ^{31}P NMR.

2.5. Methods Based on Mass Spectrometry (MS)

Although MS is a quite old analytical technique, it (with the exception of GC/MS) did not play a major role in lipid research over decades due to limitations of the ionization process, preventing the detection of intact lipids [39]. The invention of "soft ionization" methods was a real breakthrough and MS techniques may be nowadays considered as the most powerful tools of lipid analysis.

Fast atom bombardment (FAB), electrospray ionization (ESI) and matrix-assisted laser desorption and ionization (MALDI) are considered as the most important soft-ionization methods and a survey of their characteristics is available in [40]. FAB is nowadays only rarely used because considerable fragmentation of the analyte is observed and only a single application of FAB MS for the determination of the activity of PLA_2 was described to our best knowledge [41].

Nowadays, ESI is much more common in the field of lipid analysis although the achievable signal intensities depend on many different parameters, such as chain length,

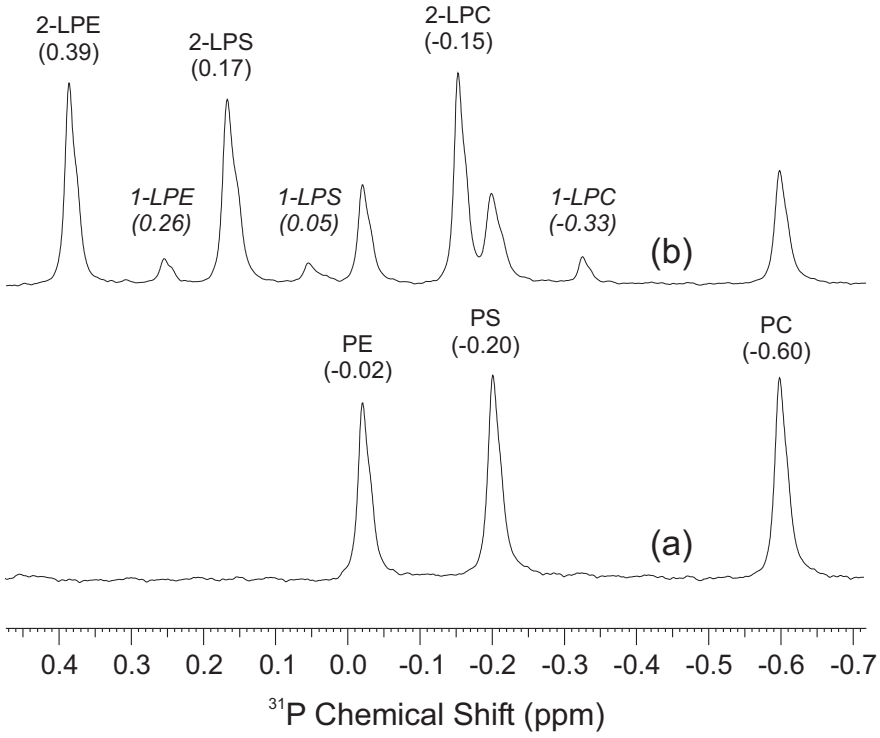

Fig. (5). 242.94 MHz ^{31}P NMR spectra of three selected PL (**a**) and their corresponding LPL generated by PLA_2-induced cleavage (**b**). Although exclusively the lyso compounds with the free hydroxyl group in *sn*-2 position are generated by the enzyme, there are also minor signals of the corresponding isomer, i.e. migration of the acyl residue from the *sn*-1 to the *sn*-2 position occurs in the used detergent.

head group, degree of saturation of the fatty acyl residues, the analyte concentration and the presence of inorganic salts and further impurities. Therefore, internal standards (e.g. deuterated lipids) are mandatory to make concentration measures reliable.

For instance, the LPC concentration in human plasma could be determined by ESI-MS [42] demonstrating that the quantification of LPC can be accomplished in a two minute assay giving a detection limit of less than 1 μM LPC. Other quantitative applications of ESI MS are described in the excellent reviews by Pulfer and Murphy [43] and Liebisch and Schmitz [44].

Another important soft-ionization MS technique is MALDI MS that is often - but not exclusively - combined with time-of-flight (TOF) mass analyzers [40]. Using MALDI-TOF MS it could be shown that the PC/LPC ratio can be easily determined in PC/PLC mixtures or PC in the presence of PLA_2 [45] (cf. Fig. (**6**)) and the method also works well with the more complex matrix of body fluids [9]. Please note that the addition of an internal standard is not absolutely necessary in MALDI-TOF MS but the signal-to-noise (*S/N*) ratio may be used instead as reliable measure of the LPL concentration [46].

Of course, MS approaches do not only allow the determination of enzyme activities but are also suitable for the determination of trace amounts of LPL in a given lipid solution. Previous separation is not absolutely necessary because the head group structure, that primarily determines the ion yield, is identical in the PL and the LPL [39].

3. ARE LPL SUITABLE INFLAMMATION AND/OR DISEASE MARKERS?

LPL and particularly LPC have significant effects on different cell lines and the immunological effects induced by these compounds have been recently comprehensively reviewed [47]. It is particularly remarkable that - although many papers demonstrated the pro-inflammatory effects of LPL such as LPC - there is also evidence of anti-inflammatory effects of these compounds [47], making their assessment as "bad" or "good" molecules difficult.

This complex behavior might be - at least partially - explained by the *in vivo* generation of two different compounds: LPL and free fatty acids. Under *in vivo* conditions the released free fatty acid is often highly unsaturated arachidonic acid that is readily converted into compounds with strong immuno-modulating effects.

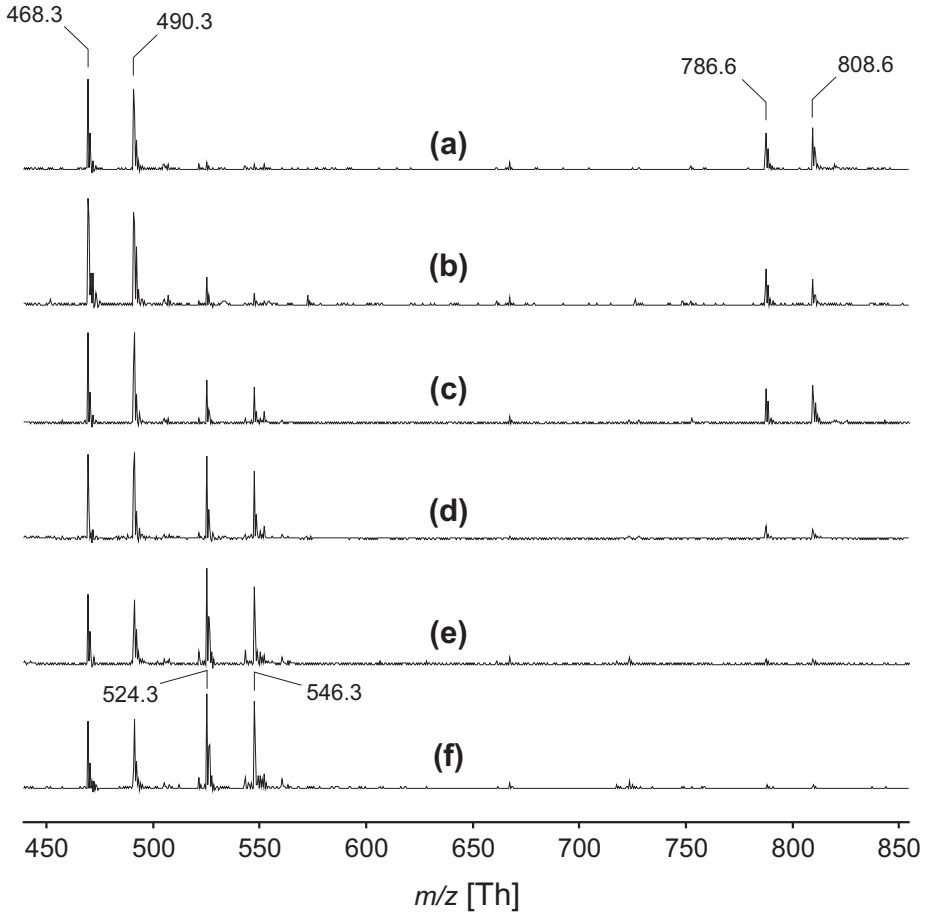

Fig. (6). Positive ion MALDI-TOF mass spectra of the digestion products of PC 18:0/18:2 with pancreatic PLA_2. In (**a**) the spectrum of the starting material is given; (**b**), (**c**), (**d**), (**e**) and (**f**) represent the digestion products after 30s and after 1, 10, 40 and 60 min of incubation with $pPLA_2$, respectively. Peaks are labeled according to their *m/z* ratios: Peaks at *m/z* = 468.3 and 490.3 arise from the H^+ and the Na^+ adduct of LPC14:0 that was added as internal standard [45]. Peaks at higher masses correspond to the PC, while those at lower masses represent the LPC generated upon the enzymatic digestion. Reproduced with permission from Petković *et al.* [45]. Copyright (2002) by Elsevier.

Therefore, it is quite difficult to judge which compound is primarily responsible for the *in vivo* effects but it seems clear from the pharmacological viewpoint that both, the generation of LPL and the formation of arachidonate-derived compounds such as prostaglandins must be inhibited [48]. Another fact was the recently discovered occurrence of LPC-chlorohydrins in human atherosclerotic lesions [49], making the situation even more complex. Due to these problems, *in vitro* effects of LPL on selected cell lines will be initially discussed prior to more complex systems.

3.1. G-Protein Coupled Receptors

Over the last decade, LPL cell-cell signaling has been recognized to be mediated by membrane-bound receptors. Due to the enormous physiological and medical relevance, this represents a rapidly growing area of research that has been already covered in several excellent reviews [50, 51] and, thus, will not be discussed in a very detailed fashion due to the limited space. Several LPLs have been analyzed for their intercellular signaling properties, but the so far best characterized are lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). These two lipids act through G-protein-coupled receptors (GPCRs) [52] named LPA 1-5, GPR87 [53] and P2Y5 [54], on the one hand, and S1P 1-5 [55, 56], on the other hand. They use classic G protein signaling pathways including phospholipase C activation, Ca^{2+} mobilization, phosphoinositide-3-kinase (PI3K) and inhibition of adenylate cyclase (AC) [57].

3.2. Effects of LPL on Selected Cell Lines

As polymorphonuclear leukocytes (PMNs) are one of the most important cell types under inflammatory conditions, effects of LPC on these cells will be emphasized: For instance, it could be shown by means of chemiluminescence (a quite simple technique that estimates the amount of generated ROS by measuring the emitted light) that LPC 16:0 is able to inhibit the ROS production in stimulated PMNs [58] by modulating the signaling pathways leading to ROS generation [59]. As the signaling cascade of PMNs is closely correlated with the Ca^{2+} ion concentration, many studies were dedicated to investigate by which pathway LPL influences the Ca^{2+} ion concentration and a clear increase could be monitored in the presence of LPC as well as other LPL derived from PE and PS [60]. It could also be shown that LPC enhances the generation of $O_2^{\bullet-}$ [61] as well as H_2O_2 [62], i.e. the educts of the generation of much more reactive ROS. Similar observations were made for other lymphocytes, whereby an increased number of apoptotic cells could also be observed in the presence of LPL [63].

Although LPC is, thus, clearly a chemotactic agent for PMNs, it is rather difficult to judge if these results are also relevant under *in vivo* conditions: The most serious problem is the presence of high concentrations of (often unknown) proteins under *in vivo* conditions: As LPC strongly binds to albumin and lipoproteins, the concentrations of available LPC may vary significantly. Therefore, data from different papers can be hardly compared. The second important problem is that LPC normally does not accumulate in the human body, but its concentration is carefully controlled by different mechanisms. The first pathway is the re-acylation of LPC to PC, whereas the second pathway comprises the degradation of LPC to glycerophosphorylcholine (GPC), i.e. the cleavage of the residual fatty acyl residue under the influence of lysophospholipases [64]. In Fig. (**7**), an overview of the PC and LPC pathways in mammalian cells is schematically given [65].

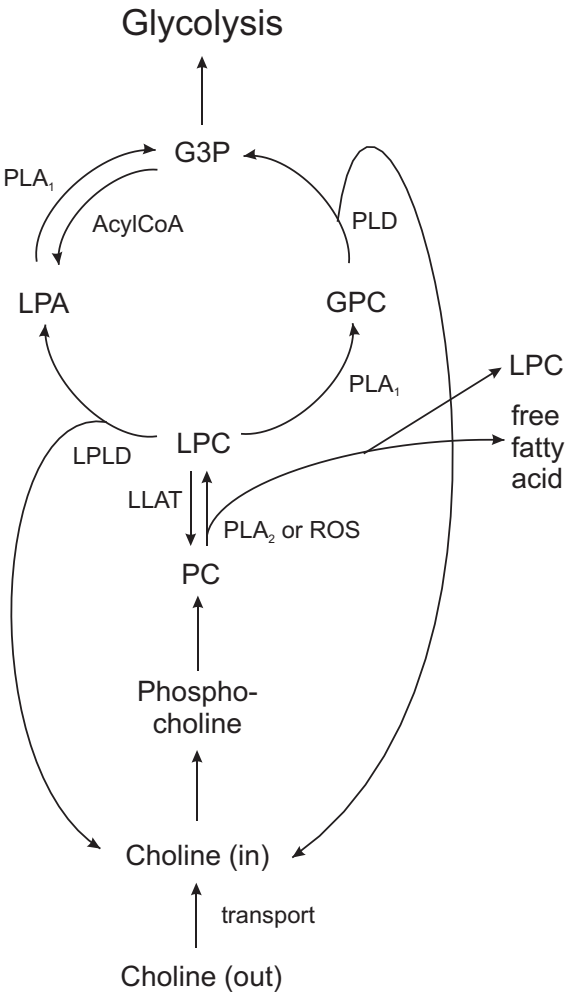

Fig. (7). Phosphatidylcholine metabolism in mammalian cells [65]. Abbreviations: **Acyl CoA**, acyl-coenzym A; **G3P**, glycerol-3-phosphate; **GPC**, glycerophosphocholine; **LLAT**, lyso-lecithinacyl-transferase; **LPA**, lyso-phosphatidic acid; **LPC**, lyso-phosphatidylcholine; **LPLD**, lyso-phospholipase D; **PC**, phosphatidylcholine; **PLA_1**, phospholipase A_1; **PLA_2**, phospholipase A_2; **PLD**, phospholipase D; **ROS**, reactive oxygen species.

3.3. Spermatozoa and Relations of LPL to Fertility

One of the important cell types where LPL are assumed to play a crucial role are spermatozoa. Mammalian spermatozoa have to undergo a complex series of cellular processes, termed capacitation, which prepares the male gametes in the female genital tract for the final fertilization events: During receptor mediated acrosome reaction (AR), acrosomal enzymes are released *via* exocytosis and allow the penetration through the protein coat of oocytes, the zona

pellucida (ZP). The latter actually triggers the acrosome reaction in spermatozoa *in vivo*. This again is the prerequisite for the subsequent sperm oocyte fusion.

As comprehensively reviewed by Ye [66] with the focus on LPA and S1P, numerous studies demonstrated the physiological functions of LPL in reproduction, from gametogenesis to parturition, as well as their pathological roles. LPC may cause erectile dysfunction [67] and it has been suggested that the pro-atherogenic effect of LPC signalling might have negative impact on penile function. The fertilizing ability of human spermatozoa strongly depends on the donor´s nutritional habits and obese man as well as diabetic subjects normally have reduced fertilizing potential [68].

It has been shown already in the 1980's that LPC is able to induce sperm acrosomal exocytosis *in vitro* [69]. The activation of PLA_2 prior to AR was suggested even before by Meizel [70] and has been comprehensively reviewed by Roldan [71, 72]. Moreover, a secretory PLA_2 is released during AR of mouse spermatozoa as an active enzyme involved in the subsequent fertilization [73]. PLA_2 metabolites may provide either second messengers for cellular signaling pathways and/or directly destabilize the membranes by changing their biophysical properties. As assessed by scanning electron microscopy, LPC also produced dramatic changes in the structure of the ZP surface which lost its typical rough aspect and became smooth [74].

Despite this physiological role of PLA_2 activation, spermatozoa avoid a premature destabilization, and LPL generation and reacylation are normally in a tightly-regulated equilibrium. Deviations from normal conditions may occur in pathological situations and an enhanced sperm LPL may be indicative for a reduced fertilizing potential.

The situation is similar for LPL generation after PL exposure to ROS. ROS do also play an important role in normal physiological processes [75] such as for instance the acrosome reaction [76]. However, the above mentioned results of the generation of LPC from PC under the influence of ROS, make LPC also most important as "bad" molecule in pathological situations or after *in vitro* challenges like gamete preservation where oxidative stress overrides the balancing cellular repair mechanisms.

There is significant evidence [77] that the LPC content reciprocally correlates with the sperm quality of human sperm. Using magnetic assisted cell sorting (MACS) to separate intact and apoptotic (annexin V positive) spermatozoa, it could be shown that the LPC content is much higher in the annexin V positive sperm and those sperm do more readily undergo capacitation and the acrosome reaction [78]. This, however, disturbs the physiological timing of these processes. Although it is not yet clear whether this is caused by an enhanced generation of ROS or enhanced PLA_2 activity, there are a lot of indications that oxidative stress has a pronounced influence on sperm behaviour [79]. Finally, it seems clear that the LPC content is a useful marker of sperm quality. In comparison to proteins, this confers the significant advantage that LPC (in contrast to specific proteins) may be regarded as a universal, non-specific marker that may be used to judge the quality of human [79] as well as animal spermatozoa [80].

3.4. Effects of LPL under *in vivo* Conditions

Compared to *in vitro* data, reliable *in vivo* data of the immuno-modulating actions of LPC are barely available. LPC-treatment of mice, however, induces enhanced phagocytic activity of macrophages [81]. Intracutaneous injection of LPC in healthy volunteers similarly elicited acute inflammation with the accumulation of T lymphocytes, monocytes and neutrophils [82].

Due to its socioeconomic significance, atherosclerosis is the *"par excellence"* disease to investigate LPC-induced effects - in particular as during LDL oxidation as much as 40% of PC present in the LDL is converted into LPC in oxidized low-density lipoprotein (oxLDL) [83]. The concentration of LPC in plasma is also very high (about 200-300 μM) with most LPC bound to albumin and lipoproteins [84].

Over the past 20 years abundant evidence of direct pro-inflammatory and atherogenic effects of LPC has accumulated and an excellent review of this important topic is available in [10]. However, there is increasing evidence that LPC has also anti-inflammatory properties, making its profile more complex than initially thought. It was beyond the focus of this paper to discuss these data in more detail because this is a focus of current research [85].

However, atherosclerosis is not the only disease where LPC plays a major role and additional further important diseases are summarized in Table **1**.

3.5. Pharmacological Aspects

As LPC concentration is elevated in many pathologies (see above), different attempts were undertaken to decrease LPC concentration. Because of the obvious contribution of PLA_2, this has raised interest for pharmacologically-active substances capable of inhibiting PLA_2 activity. However, PLA_2 activation does not only result in LPC generation but also in arachidonate-derived free radical intermediates [48] and further ROS. Therefore, a single drug molecule with both - anti-oxidant and PLA_2 inhibition activity - would be useful since it could inhibit PLA_2 activity and simultaneously scavenge free radicals and lipid peroxides which are released during arachidonic acid metabolism. Molecules such as quercetin (a natural flavonoid) might be useful as potent anti-inflammatory drugs [93]. Quercetin obviously represents a "natural" remedy as it is also taken up by common nutrition. Flavonoids exhibit different mechanisms acting on multiple sites of cellular machinery depending on their chemical structure (for a detailed review see [48]).

In addition to the inhibition of PLA_2 and eicosanoid-generating enzymes, scavenging of free radicals and the reduction of pro-inflammatory molecules is also within the effect pattern of flavonoids. It seems that some of the structural requirements that are important for PLA_2 inhibition are also important for efficient antioxidative effects.

Table 1. Selected Diseases Characterized by Alterations of the LPC Concentration. This Table Must Not be Regarded to Give a Complete Survey but Summarizes Only Some Selected Examples

Disease	Remarks	References
Diabetes	Strongly increased concentrations of LPC could be monitored in the serum from patients suffering from diabetes. It could also be shown that the related PLA_2 activity contributes massively to the enhancement of the LPC concentration in circulating LDL.	[86]
Rheumatoid Arthritis	Synovial fluids as well as sera from patients with inflammatory joint diseases are characterized by reduced PC/LPC ratios. Using anti-inflammatory drugs, the PC/LPC ratio increases either by a reduced LPC generation or an enhanced re-acylation of LPC.	[9]
Cancer	Although still under intense research, ovarian cancer seems the *"par excellence"* type of cancer related to LPL. Elevated LPL levels were detected in plasma and ascites samples from patients with ovarian cancer. Other important LPL with diagnostic relevance are lysophosphatidylinositol (LPI) and sphingosylphosphorylcholine (SPC) as well as LPC and S1P.	[87]
Asthma and Rhinitis	LPC is most probably an important trigger of asthma. Leukocyte PLA_2 activity and plasma LPC levels are highly correlated and were found significantly raised in both, patients suffering from asthma and rhinitis.	[88]
Sepsis	In the context of sepsis, a potential pharmacological aspect of LPC was discovered: LPC can effectively attenuate sepsis effects (induced e.g. by *E. Coli* infiltration) by boosting the immune system. It was also found that plasma ceramide and LPC concentrations inversely correlate with mortality of sepsis patients	[89]
Hyperlipidemia	It was shown that LPC in oxidatively modified LDL from hyperlipidemic patients contains a higher proportion of long-chain acyl groups. It is suggested that particularly these LPC species promote the development of atherosclerosis in hyperlipidemic patients. It was also shown that the cholesterol intake has a considerable effect on LPC concentration.	[90]
Endometriosis	The concentration of LPC was found to be elevated in endometriosis. It is hypothesized hat LPC is responsible for the recruitment of leukocytes and the increase in macrophage activation.	[91]
Psoriatic Skin	It was demonstrated that PLA_2 activity as well as LPC concentration is increased in psoriatic skin. Surprisingly, however, the increased LPC levels were not accompanied by a corresponding increase in the activity of enzymes catabolizing LPC.	[92]

4. CONCLUSIONS AND OUTLOOK

Of course, this "minireview" could provide only a very small insight into this complex topic and many questions remain to be answered. Nevertheless, recent work indicates many important biological functions of LPL (as well as oxidatively modified fatty acids) in cellular signaling and the development of different important pathologies. However, even if there are many intriguing *in vitro* results, further efforts must be made to clarify the fate and details of the contribution of LPL under conditions of diseases. This particularly concerns the identification of the single central event in the LPC activity that likely triggers all further events on the cellular level. LPL receptors also need to be further characterized concerning response and ligand specificity because their selective pharmacological inhibition might represent a potential cure of many important diseases. Finally, little is so far known about the potentially different effects of LPL species with variations in the headgroup, fatty acyl lengths and degrees of unsaturation. Therefore, experimental efforts may not only be focused on medical or cell biological issues, but must also comprise chemical as well as analytical aspects.

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ABBREVIATIONS

AC = Adenylate Cyclase

AcylCoA = Acyl-Coenzym A

AR = Acrosome Reaction

CE = Capillary Electrophoresis

Cer = Ceramide

DAG = Diacylglycerol

EI = Electron Impact

ESI = Electrospray Ionization

ESR = Electron Spin Resonance

FAB = Fast Atom Bombardment

G3P = Glycerol-3-Phosphate

GC/MS = Gas Chromatography/Mass Spectrometry

GPC = Glycero-Phosphorylcholine

GPCR = G-Protein-Coupled Receptor

GPL = Glycerophospholipid

HDL = High-Density Lipoprotein

HPLC = High Performance Liquid Chromatography

LC = Liquid Chromatography

LDL = Low-Density Lipoprotein

LLAT = Lyso-Lecithinacyltransferase

LPA = Lysophosphatidic Acid

LPC = Lysophosphatidylcholine

LPE = Lysophosphatidylethanolamine

LPI = Lysophosphatidylinositol

LPL = Lysophospholipid

LPLD = Lyso-Phospholipase D

LPS = Lysophosphatidylserine

MACS = Magnetic-assisted Cell Sorting

MALDI = Matrix-assisted Laser Desorption and Ionization

MPO = Myeloperoxidase

MS = Mass Spectrometry

MW = Molecular Weight

m/z = Mass over Charge

NBD = 7-Nitrobenzo-2,1,3-oxadiazol

NMR = Nuclear Magnetic Resonance

PA = Phosphatidic Acid

PC = Phosphatidylcholine

PE = Phosphatidylethanolamine

PG = Phosphatidylglycerol

PI = Phosphatidylinositol

PI3K = Phosphoinositide-3-Kinase

PL = Phospholipid

PLA_1 = Phospholipase A_1

PLA_2 = Phospholipase A_2

PLC = Phospholipase C

PLD = Phospholipase D

PMNs = Polymorphonuclear Leukocytes

PPI = Poly-Phosphoinositides

PS = Phosphatidylserine

RNS = Reactive Nitrogen Species

ROS = Reactive Oxygen Species

S1P = Sphingosine-1-Phosphate

SM = Sphingomyelin

S/N = Signal-to-Noise (Ratio)

sn = Stereospecific Numbering

SPC = Sphingosylphosphorylcholine

SOD = Superoxide Dismutase

TLC = Thin-Layer Chromatography

TOF = Time-of-Flight

ZP = Zona Pelucida

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