Lysophospholipids: Their Generation, Physiological Role and Detection. Are They Important Disease Markers?

Beate Fuchs and Jürgen Schiller*

Institute of Medical Physics and Biophysics, Faculty of Medicine, University of Leipzig, Härtelstr. 16-18, D-04107 Leipzig, Germany

Abstract: The concentration of lysophospholipids (LPL) increases under pathological conditions and, thus, LPL attract diagnostic and pharmacological interest. LPL are particularly interesting because they possess pro- and anti-inflammatory properties and can be generated by phospholipases and reactive oxygen species (ROS).

This review provides an overview of the mechanisms by which LPL are generated and how they can be determined. The effects of LPL as signaling molecules and their roles in different pathologies are discussed. The focus will be on lysophosphatidylcholine (LPC).

Key Words: Lysophospholipids, Phospholipids, Lysophosphatidylcholine, Phospholipase, Reactive Oxygen Species, Inflammation, Disease Markers, Signaling Molecules.

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 as well as their diagnostic relevance. 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. LPL are basically derived from phospholipids (PL) 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, PL are the unequivocal starting materials. Therefore, this review starts with an overview of relevant PL [1].

1.1. Important Phospholipids

PL constitute a highly important class of biomolecules [2], of which glycerophospholipids (GPL) are of particular relevance [3]. All GPL consist of a glycerol backbone, esterified with two varying organic fatty acids (termed "R" in Fig. (**1**)) and one molecule of phosphoric acid. The resulting molecule is termed "phosphatidic acid" (PA). Via ester condensation with different alcohols such as, for instance, choline and ethanolamine, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are generated. These compounds represent the most important and most abundant zwitterionic GPL of biological membranes (Fig. (**1**)) [1].

Additionally, there are also acidic (negatively charged at pH 7.4) GPL, such as phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI) and polyphosphoinositides (PPI) with several phosphate residues at the inositol ring. The most important (non-GPL) 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 [4] both will be treated here only very loosely due to the restricted available space.

The majority of PL 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 or even highly unsaturated. The arachidonyl residue with four double bonds is of particular relevance due to the regulatory molecules derived thereof (e.g. prostaglandins).

GPL are converted into LPL by the action of phospholipases and the reactions that they are catalyzing are schematically shown in (Fig. (**2**)).

The focus of this review is the action of phospholipase A_2 (PLA_2) as this is (to the current state of knowledge) the most important pathway of LPL formation under *in vivo* conditions. Therefore, PLC and PLD leading to the generation of diacylglycerols and phosphatidic acids, respectively, will not be treated here. The interested reader is referred to an excellent recently published review [5].

1.2. Generation of LPL under the Influence of Phospholipases

Phospholipase "A" is represented by a group of enzymes that catalyze the cleavage of one fatty acyl residue from the glycerol backbone of a given PL. The basic reaction is schematically given in (Fig. (**2**)). Thereby, a free fatty acid is released and the corresponding LPL left in the membrane [6]. Both, the LPL as well as the fatty acid (that is often a highly unsaturated one that may easily undergo further oxidative modification) are considered as important molecules with messenger functions [7].

*Address correspondence to this author at the Universität Leipzig, Medizinische Fakultät, Institut für Medizinische Physik und Biophysik, Härtelstr. 16-18, D-04107 Leipzig, Germany; Tel: +49-341-97-15733; Fax: +49-341-97-15709; E-mail: juergen.schiller@medizin.uni-leipzig.de

1389-5575/09 $55.00+.00

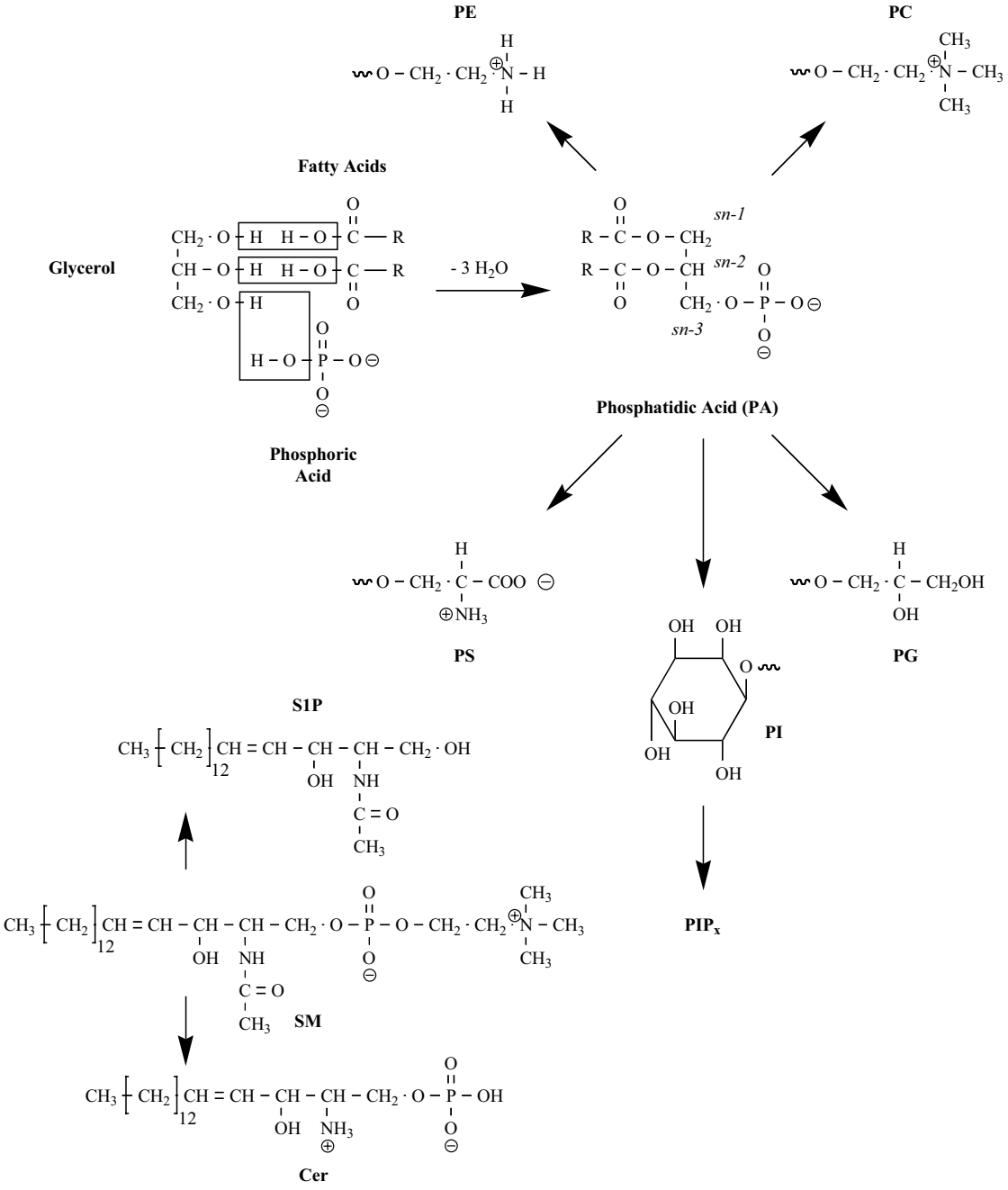

Fig. (1). Chemical structures of relevant glycerophospholipids and their (simplified) generation under *in vivo* conditions. For details see text. Although sphingomyelin does not represent a glycerophospholipid, it is also an important membrane constituent and the compounds derived thereof (S1P and Cer) 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.

Based on the stereospecificity of the reaction, one can distinguish between PLA_1 (phospholipase A_1, E.C. 3.1.1.32) and PLA_2 (phosphatidylcholine 2-acylhydrolase, E.C. 3.1.1.4) activity. Please note that PLA_1 enzymes play a much smaller physiological role than PLA_2 [6]. Accordingly, PLA_2 enzymes will be primarily discussed. This group comprises 12 families some of which may also express PLA_1, or lysophospholipase A_1/A_2 activity [7]. 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 ($cPLA_2$)) [7]. 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]. As $sPLA_2$ is massively involved in inflammatory processes (e.g. in rheumatoid arthritis) this enzyme (obtained from the synovial (joint) fluids of patients suffering from arthritic joint diseases) was primarily investigated [8].

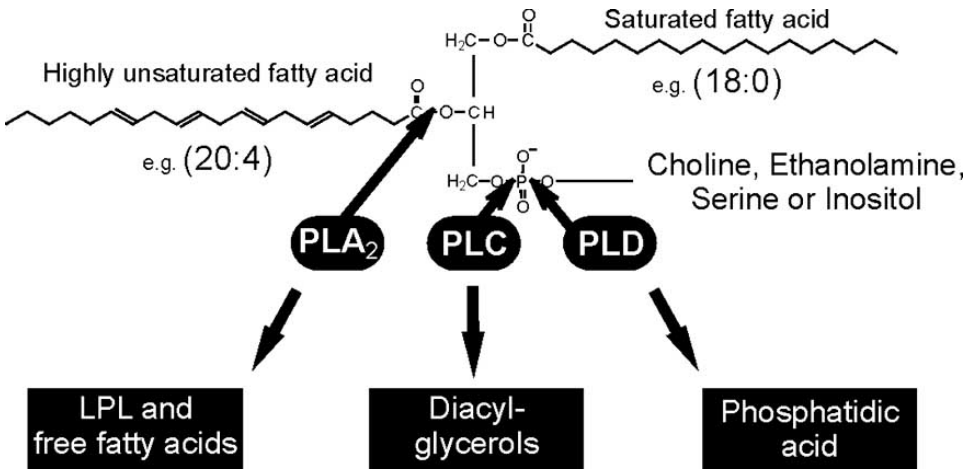

Fig. (2). Schema of the generation of lipid-derived signaling molecules under *in vivo* conditions from a selected PL molecule along with the enzymes involved. Abbreviations: PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D.

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 patients suffering from rheumatoid arthritis [9], atherosclerosis [10], lung infection [11] and many others. The prevailing opinion is that LPL are generated under *in vivo* conditions by the release and/or activation of PLA_2 that is present in e.g. 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**)).

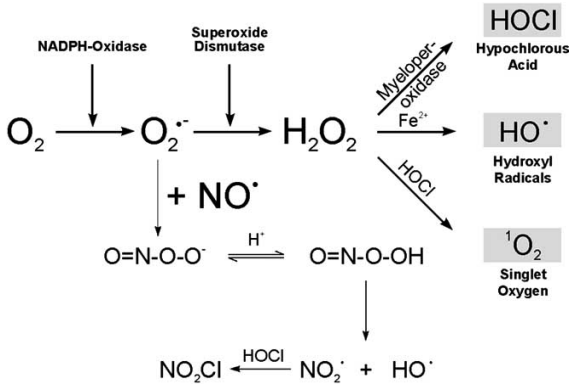

Fig. (3). Simplified Schema of ROS and RNS generation under *in vivo* conditions. Please note that this is a very simplified summary of potential chemical reactions that does not take the different locations of enzymes and their substrates into account.

Shortly, ROS are derived from "normal" (atmospheric) oxygen that is initially converted into superoxide anion radicals ($O_2^{\bullet-}$) that dismutate spontaneously (or much faster 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 among the most reactive species on earth and react diffusion-controlled with 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+}) by the "famous" Fenton reaction: Even more than one hundred years after its first description 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). HOCl is generated under *in vivo* conditions from H_2O_2 and Cl^- ions under the catalytic influence of the enzyme myeloperoxidase (MPO) [15] that is nearly exclusively found in neutrophils, where it makes out about 5% of the total amount of proteins [12 and references cited therein]:

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

As the number of neutrophilic granulocytes increases massively under inflammatory conditions, the role of MPO and its products are obvious [16]. 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 as 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 [17]. 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 [18].

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 [18]. 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 LPCs increases if the saturation degree of the applied PC decreases and a mechanism to explain this behavior has been recently proposed (Fig. (**4**)) [19]: Oxygen and

chlorine are rather electronegative elements and weaken the ester bond 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 [19].

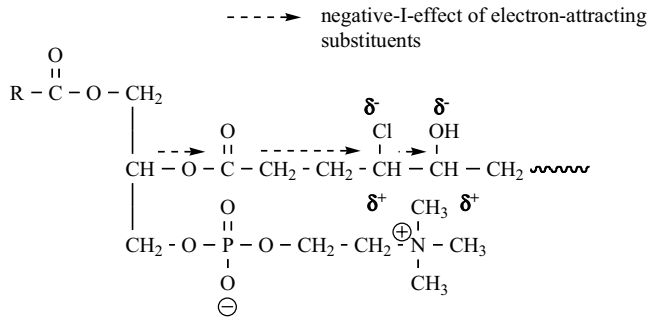

Fig. (4). Proposed weakening of the ester bond in unsaturated phosphatidylcholines caused by the introduction of electron-withdrawing substituents upon chlorohydrin formation. Reproduced with permission from Arnhold *et al.* [19]. Copyright (2002) by Elsevier.

Chlorohydrins and LPCs were also obtained in significant yields if e.g. lipoproteins from human blood were incubated with HOCl [20]. However, due to the high protein content of lipoproteins and the considerable reactivity of the thiol and amino groups of proteins with HOCl, a considerable excess of HOCl was necessary to induce alterations of the lipid constituents [21]. Surprisingly, 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 [22]. This is a clear indication that the value of the above-mentioned mechanism (Fig. (**4**)) is limited and further parameters (maybe also 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].

1.4. "Unwanted" Generation of LPL from PL

It must be emphasized that the ester linkage of lipids is relatively labile and can be easily hydrolyzed. Therefore, upon storage of PL solutions time-dependent hydrolysis occurs even at low temperatures [24] resulting (among other products) in the generation of LPL. This hydrolysis is surely dependent on the oxygen concentration [25] and can, thus, be most efficiently minimized if all solutions are handled under an atmosphere of inert gas.

The problem of unwanted LPL generation is particularly important if the effects of LPL on e.g. cellular functions are of interest and clearly emphasizes the need of reliable analytical methods allowing the sensitive determination of small amounts of LPL in order to exclude misleading effects of LPL present as impurity in the PL stock solution.

2. METHODS OF LPL DETERMINATIONS

Due to the importance of phospholipases, the determination of LPL concentrations [26] is normally connected with the determination of PLA_2 activities. There are many different methods available and a comprehensive review of this topic is available in [27].

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 normally used in order to avoid opaque "solutions". This approach must be critically regarded as (a) the bilayer structure of PL is neglected and (b) potential effects of the detergent moiety on the activity of the enzyme cannot be excluded.

2.1. Methods Based on UV- or Fluorescence Spectroscopy

The hydrolysis of PL catalyzed by PLA_2 can be monitored by an assay developed by Aarsman *et al.* [28]. This method is based on the use of a certain thiol ester as 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, simple to use and highly standardized.

An additional interesting method was developed in 1989 by Rawyler and Sigenthaler [29]. 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 (e.g. in a mixture) can be made as 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 and that these methods are not suitable for the determination of the LPL concentration in a given PL sample.

2.2. Methods Based on Chromatography

Chromatographic methods (particularly high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC)) are nowadays highly established in PL research [30] and also widely used for the determination of phospholipase activities [31]. TLC was so far primarily applied because it can be very easily implemented and is 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) followed by a separation according to differences in the fatty acyl composition in the second dimension. Although this is a powerful method it has the disadvantage that only a single sample can be analyzed simultaneously [30]. 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 [31].

Due to the limited space, only a few selected examples can be given here: The PLA_2 activity in stimulated macro-

phages 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 [32]. Unfortunately, the introduction of artificial labels may influence the membrane structure and, thus, the obtained results do not absolutely reflect the real biological conditions, i.e. the determined enzyme activities can be different from that determined with the native substrate. The same disadvantage holds if spin labels for the determination of PLA_2 activities by means of electron spin resonance are used [33]. 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 debate [27].

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 [34] 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 $sPLA_2$ [35]. Using this approach, peaks arising from residual substrate and from 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 because no specific label is required under these conditions. In this case the cells of interest are grown in the presence of a radioactively-labeled substrate. Separation of the lipid metabolites of interest can be accomplished, for instance, by HPLC and the radioactivity afterwards determined in the individual fractions [36].

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 [37]. Due to its comparably high sensitivity and the high natural abundance of the phosphorous nucleus, ^{31}P NMR is unequivocally the method of choice for the detection and determination of PL by NMR.

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, 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**)).

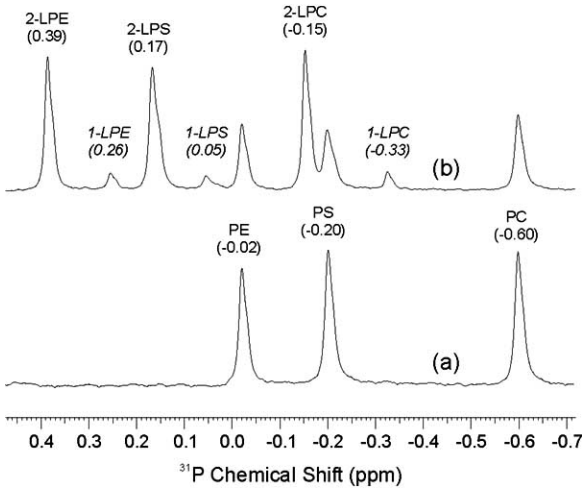

Fig. (5). 242.94 MHz ^{31}P NMR spectra of three selected PL (a) and their corresponding LPL generated by PLA_2-induced cleavage (b). Although exclusively the lyso compounds with the free hydroxyl group in *sn*-2 position are generated by the enzyme, there are also minor signals of the corresponding isomers, i.e. in the used detergent, migration of the acyl residue from the *sn*-1 to the *sn*-2 position occurs. Spectra are calibrated with respect to external 85% H_3PO_4. Reprinted with permission and with modification from [37].

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 separation techniques - is simultaneously possible.

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" [40] methods was a real breakthrough regarding lipid research 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 [40]. However, FAB is nowadays rarely used because considerable fragmentation of the analyte is observed anyway and only a single application of FAB MS for the determination of the activity of PLA_2 was described [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 (MW), head group, degree of saturation of the fatty acyl residues, the analyte concentration and the presence of inorganic salts and further impurities [42]. Therefore, internal standards (e.g. deuterated lipids) are normally required to make concentration measures reliable.

For instance, the LPC concentration in human plasma was determined by ESI-MS [43] demonstrating that the quantification of LPC can be accomplished in a two minute assay giving a detection limit of less than 1 μM LPC. A

similar study was also performed with the blood from patients suffering from diabetes [44]. Other quantitative applications of ESI-MS are described in the excellent review by Pulfer and Murphy [45].

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 [46] (cf. Fig. (**6**)) and the method also works with 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 concentration measure of LPL [47].

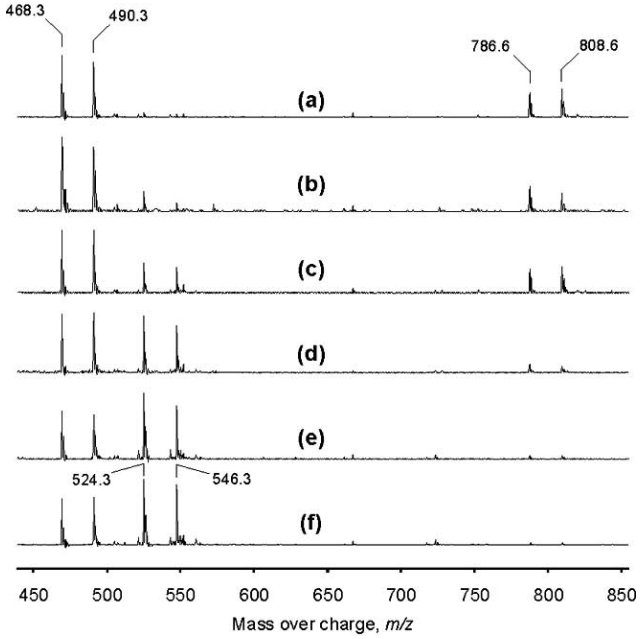

Fig. (6). Positive ion MALDI-TOF mass spectra of the digestion products of PC 18:0/18:2 with pancreatic PLA_2. In (a) the spectrum of the starting material is given; (b), (c), (d), (e) and (f) represent the digestion products after 30s and after 1, 10, 40 and 60 min of incubation with $pPLA_2$, respectively. Peaks are labeled according to their *m/z* ratio: 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 [39]. 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.* [46]. Copyright (2002) by Elsevier.

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 lines and the immunological effects induced by these compounds have been recently comprehensively reviewed [48]. It is particularly remarkable that - although many papers demonstrated the pro-inflammatory effects of LPC - there is also evidence of anti-inflammatory effects of these compounds [48], 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 arachidonic acid that is readily converted into compounds with strong immunomodulating effects [48].

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 as prostaglandins must be inhibited [49]. Another fact was the recently discovered occurrence of LPC-chlorohydrins in human atherosclerotic lesions [50], making the situation still more complex. Due to these problems, *in vitro* effects of LPL on selected cell lines will be initially discussed.

3.1. G-Protein Coupled Receptors

Over the last decade, LPL cell-cell signalling has been recognized to be mediated by membrane-bound receptors. Due the enormous physiological and medical relevance, this represents a rapidly growing area of research. 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) named LPA 1-4 and S1P 1-5 [51,52]. They use classic G protein signaling pathways including phospholipase C activation, Ca^{2+} mobilization, phosphoinositide-3-kinase (PI3K) and inhibition of adenylate cyclase (AC). These pathways have been already covered in several excellent reviews [53,54] and will not be discussed further here due to the limited space.

3.2. Effects of LPL on Selected Cell Lines

As polymorphonuclear leukocytes (PMNs) are highly abundant under inflammatory conditions, effects of LPC on these cells will be emphasized: For instance, it could be shown by means of chemiluminescence that LPC 16:0 is able to inhibit the ROS production in stimulated PMNs [55] by modulating the signaling pathways leading to ROS generation [56]. 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 [57]. It could also be shown that LPC enhances the generation of $O_2^{\bullet-}$ [58] as well as H_2O_2 [59]. Similar observations were also made for other lymphocytes, whereby also an increased number of apoptotic cells could be observed in the presence of LPL [60].

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 reason 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 [61]. In Fig. (**7**) an overview of the PC and LPC pathways in mammalian cells is schematically given [62,63].

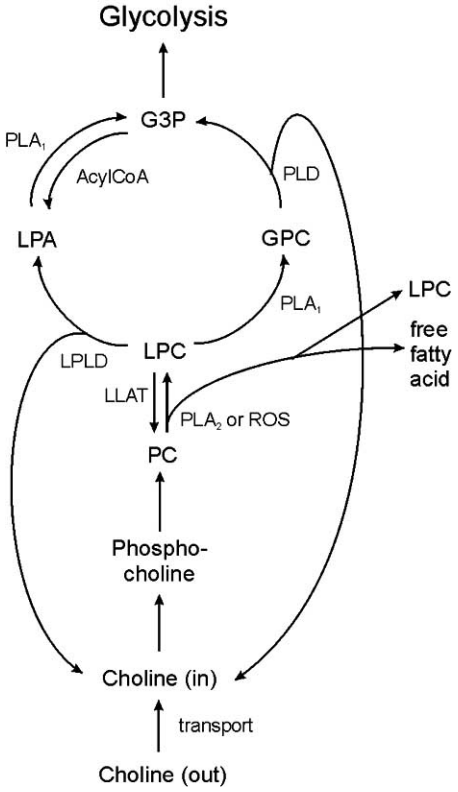

Fig. (7). Phosphatidylcholine metabolism in mammalian cells [62,63]. Abbreviations: Acyl CoA, acyl-coenzym A; G3P, glycerol-3-phosphate; GPC, glycerophosphocholine; LLAT, lyso-lecithin-acyltransferase; LPA, lyso-phosphatidic acid; LPC, lyso-phosphatidylcholine; LPLD, lyso-phospholipase D; PC, phosphatidylcholine; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; PLD, phospholipase D; ROS, reactive oxygen species.

3.3. 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, for instance, induces enhanced phagocytic activity of macrophages [64]. Intracutaneous injection of LPC in healthy volunteers similarly elicited acute inflammation with the accumulation of T lymphocytes, monocytes and neutrophils [65].

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 to LPC in oxidized low-density lipoprotein (oxLDL) [66]. The concentration of LPC in plasma is also very high (about 200-300 μM) with most LPC bound to albumin and lipoproteins [67].

Over the past 20 years abundant evidence has accumulated of direct pro-inflammatory and atherogenic effects of LPC and an excellent review of this important topic is available in [10]. However, there is increasing evidence that LPC has also anti-inflammatory actions, 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 [68].

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

3.4. 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 [49] and ROS. Therefore, a single drug molecule with both - antioxidant 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 as quercetin (a flavonoid) might be useful as potent anti-inflammatory drugs [80]. 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 detailed review see [49]).

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.

CONCLUSIONS AND OUTLOOK

Of course, this review could provide only a very small insight into this complex topic and many questions remain to be answered. However, 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 been 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

Tab. 1. Selected Diseases Characterized by Alterations of the LPC Concentration. Of Course this Table must not be Regarded to Give a Complete Survey but Only some Selected Examples

Disease	Remarks	References
Renal Failure on Hemo-dialysis	It could be shown that LPC has a prominent effect on the function of the kidneys. Additionally, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) have also prominent effects.	[69]
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.	[70]
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 lysolipid 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.	[71]
Asthma	LPC is most probably an important trigger of asthma.	[72]
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	[59] [73]
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 significant effect on LPC concentration.	[74] [75]
Asthma and Rhinitis	Leukocyte PLA_2 activity and plasma LPC levels are highly correlated and were found significantly raised in both, patients suffering from asthma and rhinitis.	[72]
Ischemia	The appearance of LPC in blood and lymph in animals and in venous effluents in man in response to ischemia could be proven. This suggests a vascular site for the production of LPC.	[76]
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.	[77]
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.	[78]
Reproduction Failure	It could be shown that low-quality or apoptotic spermatozoa are characterized by an enhanced content of LPC. Due to the extremely high contents of highly unsaturated fatty acyl residues of the PL of spermatozoa, it is very likely that ROS are involved in LPC generation.	[79]

with variations in the headgroup, fatty acyl lengths and degrees of unsaturation. Therefore, efforts may not only be focused on medical or cell biological issues, but must also comprise chemical as well as analytical aspects.

ACKNOWLEDGEMENTS

This work was supported by the German Research Council (DFG Schi 476/5-1 and FU 771/1-1) and the Federal Ministry of Education and Research (Grant BMBF 0313836).

ABBREVIATIONS

AC = Adenylate Cyclase

AcylCoA = Acyl-Coenzym A

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

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

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

REFERENCES

[1] Larsson, K.; Sato, K.; Tiberg, F. *Lipids: Structure, Physical Properties and Functionality*, The Oily Press Lipid Library: Bridgwater, **2006**.

[2] Stryer, L. *Biochemie*, Vieweg: Braunschweig, **1988**.

[3] Gunstone, F.D.; *Phospholipid Technology and Applications*, The Oily Press Lipid Library: Bridgwater, **2008**.

[4] Billich, A.; Baumruker, T. Sphingolipid metabolizing enzymes as novel therapeutic targets. *Subcell. Biochem.*, **2008**, *49*, 487-522.

[5] Wymann, M.P.; Schneiter, R. Lipid signalling in disease. *Nat. Rev. Mol. Cell. Biol.*, **2008**, *9*, 162-76.

[6] Kaiser, E. Phospholipase A_2: its usefulness in laboratory diagnostics. *Clin. Lab.*, **1999**, *36*, 65-163.

[7] Six, D.A.; Dennis, E.A. The expanding superfamily of phospholipase A_2 enzymes: Classification and characterization. *Biochim. Biophys. Acta*, **2000**, *1488*, 1-19.

[8] Seilhamer, J.J.; Pruzanski, W.; Vadas, P.; Plant, S.; Miller, J.A.; Kloss, J.; Johnson, L.K. Cloning and recombinant expression of phospholipase A_2 present in rheumatoid arthritic synovial fluid. *J. Biol. Chem.*, **1989**, *264*, 5335-8.

[9] Fuchs, B.; Schiller, J.; Wagner, U.; Häntzschel, H.; Arnold, K. The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis: investigations by ^{31}P NMR and MALDI-TOF MS. *Clin. Biochem.*, **2005**, *38*, 925-33.

[10] Matsumoto, T.; Kobayashi, T.; Kamata, K. Role of lysophosphatidylcholine (LPC) in atherosclerosis. *Curr. Med. Chem.*, **2007**, *14*, 3209-20.

[11] Hammerschmidt, S.; Büchler, N.; Wahn, H. Tissue lipid peroxidation and reduced glutathione depletion in hypochlorite-induced lung injury. *Chest*, **2002**, *121*, 573-81.

[12] Schiller, J.; Fuchs, B.; Arnhold, J.; Arnold, K. Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr. Med. Chem.*, **2003**, *10*, 2123-45.

[13] Koppenol, W.H. The centennial of the Fenton reaction. *Free Radic. Biol. Med.*, **1993**, *15*, 645-51.

[14] Fuchs, B.; Schober, C.; Richter, G.; Nimptsch, A.; Süß, R.; Schiller, J. The Reactions between HOCl and differently saturated phospholipids: Physiological relevance, products and methods of evaluation. *Mini-Rev. Org. Chem.*, **2008**, *5*, 254-61.

[15] Malle, E.; Furtmüller, P.G.; Sattler, W.; Obinger, C. Myeloperoxidase: a target for new drug development? *Br. J. Pharmacol.*, **2007**, *152*, 838-54.

[16] Halliwell, B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am. J. Med.*, **1991**, *91*, 14S-22S.

[17] Kougias, P.; Chai, H.; Lin, P.H.; Lumsden, A.B.; Yao, Q.; Chen, C. Lysophosphatidylcholine and secretory phospholipase A_2 in vascular disease: mediators of endothelial dysfunction and atherosclerosis. *Med. Sci. Monit.*, **2006**, *12*, RA5-16.

[18] Arnhold, J.; Osipov, A.N.; Spalteholz, H.; Panasenko, O.M.; Schiller, J. Effects of hypochlorous acid on unsaturated phosphatidylcholines. *Free Radic. Biol. Med.*, **2001**, *31*, 1111-9.

[19] Arnhold, J.; Osipov, A.N.; Spalteholz, H.; Panasenko, O.M.; Schiller, J. Formation of lysophospholipids from unsaturated phosphatidylcholines under the influence of hypochlorous acid. *Biochim. Biophys. Acta*, **2002**, *1572*, 91-100.

[20] Schiller, J.; Zschörnig, O.; Petković, M.; Müller, M.; Arnhold, J.; Arnold, K. Lipid analysis of human HDL and LDL by MALDI-TOF mass spectrometry and ^{31}P NMR. *J. Lipid Res.*, **2001**, *42*, 1501-8.

[21] Zschörnig, O.; Bergmeier, C.; Süß, R.; Arnold, K.; Schiller, J. Human low-density lipoprotein oxidation: Hypochlorous acid leads to the

generation of lysophospholipids under acidic conditions. *Lett. Org. Chem.*, **2004**, *1*, 381-90.

[22] Richter, G.; Schober, C.; Süß, R.; Fuchs, B.; Birkemeyer, C.; Schiller J. Comparison of the positive and negative ion electrospray ionization and matrix-assisted laser desorption ionization-time-of-flight mass spectra of the reaction products of phosphatidylethanolamines and hypochlorous acid. *Anal. Biochem.*, **2008**, *376*, 157-9.

[23] Leßig, J., Fuchs, B. Plasmalogens in biological systems: their role in oxidative processes in biological membranes and their contribution to pathological processes and aging. *Curr. Med. Chem.*, submited.

[24] Zuidam, N.J.; Gouw, H.K.; Barenholz, Y.; Crommelin, D.J. Physical (in) stability of liposomes upon chemical hydrolysis: the role of lysophospholipids and fatty acids. *Biochim. Biophys. Acta*, **1995**, *1240*, 101-10.

[25] Hernández-Caselles, T.; Villalaín, J.; Gómez-Fernández, J.C. Stability of liposomes on long term storage. *J. Pharm. Pharmacol.*, **1990**, *42*, 397-400.

[26] Christie, W.W. *Lipid Analysis* 3rd ed, The Oily Press Lipid Library: Bridgwater, **2003**.

[27] van den Bosch, H.; Aarsman, A.J. A review on methods of phospholipase A determination. *Agents Actions*, **1979**, *9*, 382-9.

[28] Aarsman, A.J.; van Deenen, L.L.M.; van den Bosch, H. Studies on lysophospholipases: VII. Synthesis of acylthioester analogs of lysolecithin and their use in a continuous spectrophotometric assay for lysophospholipases, a method with potential applicability to other lipolytic enzymes. *Bioorg. Chem.*, **1976**, *5*, 241-53.

[29] Rawyler, A.; Siegenthaler, P.A. A single and continuous spectrophotometric assay for various lipolytic enzymes, using natural, non-labelled lipid substrates. *Biochim. Biophys. Acta*, **1989**, *1004*, 337-44.

[30] Peterson, B.L.; Cummings, B.S. A review of chromatographic methods for the assessment of phospholipids in biological samples. *Biomed. Chromatogr.*, **2006**, *20*, 227-43.

[31] Hostetler, K,Y.; Gardner, M.F.; Aldern, K.A. Assay of phospholipases C and D in presence of other lipid hydrolases. *Methods Enzymol.*, **1991**, *197*, 125-34.

[32] Kleuser, B.; Meister, A.; Sternfeld, L.; Gercken, G. Measurement of phospholipase A_2 and 1-alkylglycerophosphocholine acetyltransferase activities in stimulated alveolar macrophages by HPLC analysis of NBD-labeled ether lipids. *Chem. Phys. Lipids*, **1996**, *79*, 29-37.

[33] Lai, C.S.; Zhang, J.Z.; Joseph, J. Spin-label assay for phospholipase A_2. *Anal. Biochem.*, **1988**, *172*, 397-402.

[34] Welsh, C.J.; Schmeichel, K. Assays for investigations of signal transduction mechanisms involving phospholipase D: mass measurements of phosphatidate, phosphatidylethanol, and diacylglycerol in cultured cells. *Anal. Biochem.*, **1991**, *192*, 281-92.

[35] Choi, S.; Lee, Y.S.; Na, D.S.; Yoo, Y.S. Determination of enzymatic activity and properties of secretory phospholipase A_2 by capillary electrophoresis. *J. Chromatogr. A*, **1999**, *853*, 285-93.

[36] Catz, S.D.; Sterin-Speziale, N.B. Bradykinin stimulates phosphoinositide turnover and phospholipase C but not phospholipase D and NADPH oxidase in human neutrophils. *J. Leukoc. Biol.*, **1996**, *59*, 591-7.

[37] Schiller, J.; Arnold, K. Application of high resolution ^{31}P NMR spectroscopy to the characterization of the phospholipid composition of tissues and body fluids - a methodological review. *Med. Sci. Monit.*, **2002**, *8*, MT205-22.

[38] Schiller, J.; Müller, M.; Fuchs, B.; Arnold, K.; Huster, D. ^{31}P NMR spectroscopy of phospholipids: From micelles to membranes. *Curr. Anal. Chem.*, **2007**, *3*, 283-301.

[39] Schiller, J.; Süß, R.; Arnhold, J.; Fuchs, B.; Leßig, J.; Müller, M.; Petković, M.; Spalteholz, H.; Zschörnig, O.; Arnold, K. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. *Prog. Lipid Res.*, **2004**, *43*, 449-88.

[40] Fuchs, B.; Arnold, K.; Schiller, J. In *Encyclopedia of Analytical Chemistry*; Meyers, Ed.; John Wiley & Sons Ltd.: Chichester, **2008**; *Vol 1*, pp. 1-39.

[41] Isomura, S.; Ito, K.; Haruna, M. Quantitative analysis of the kinetics of phospholipase A_2 using fast atom bombardment mass spectrometry. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 337-40.

[42] Koivusalo, M.; Haimi, P.; Heikinheimo, L.; Kostiainen, R.; Somerharju, P. Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response. *J. Lipid Res.*, **2001**, *42*, 663-72.

[43] Liebisch, G.; Drobnik, W.; Lieser, B.; Schmitz, G. High-throughput quantification of lysophosphatidylcholine by electrospray ionization tandem mass spectrometry. *Clin. Chem.*, **2002**, *48*, 2217-24.

[44] Pang, L.Q.; Liang, Q.L.; Wang, Y.M.; Ping, L.; Luo, G.A. Simultaneous determination and quantification of seven major phospholipid classes in human blood using normal-phase liquid chromatography coupled with electrospray mass spectrometry and the application in diabetes nephropathy. *J. Chromatogr. B*, **2008**, *869*, 118-25.

[45] Pulfer, M.; Murphy, R.C. Electrospray mass spectrometry of phospholipids. *Mass Spectrom. Rev.*, **2003**, *22*, 332-64.

[46] Petković, M.; Müller, J.; Müller, M.; Schiller, J.; Arnold, K.; Arnhold, J. Application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for monitoring the digestion of phosphatidylcholine by pancreatic phospholipase A_2. *Anal. Biochem.*, **2002**, *308*, 61-70.

[47] Petković, M.; Schiller, J:; Müller, J.; Müller, M.; Arnold, K.; Arnhold, J. The signal-to-noise ratio as the measure for the quantification of lysophospholipids by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Analyst*, **2001**, *126*, 1042-50.

[48] Hong, C.-W.; Song, D.-K. Immunomodulatory actions of lysophosphatidylcholine. *Biomol. Ther.*, **2008**, *16*, 69-76.

[49] Nanda, B.L.; Nataraju, A.; Rajesh, R.; Rangappa, K.S.; Shekar, M.A.; Vishwanath, B.S. PLA_2 mediated arachidonate free radicals: PLA_2 inhibition and neutralization of free radicals by antioxidants - A new role as anti-inflammatory molecule. *Curr. Top. Med. Chem.*, **2007**, *7*, 765-77.

[50] Messner, M.C.; Albert, C.J.; McHowat, J.; Ford, D.A. Identification of lysophosphatidylcholine-chlorohydrin in human atherosclerotic lesions. *Lipids*, **2008**, *43*, 243-9.

[51] Chun, J.; Goetzl, E.J.; Hla, T.; Igarashi, Y.; Lynch, K.R.; Moolenaar, W.; Pyne, S.; Tigyi, G. International Union of Pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol. Rev.*, **2002**, *54*, 265-9.

[52] Noguchi, K.; Ishii, S.; Shimizu, T. Identification of p2y9/GPR23 as a novel G proteincoupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J. Biol. Chem.*, **2003**, *278*, 25600-6.

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

[54] Anliker, B.; Chun, J. Cell surface receptors in lysophospholipid signaling. *Semin. Cell Dev. Biol.*, **2004**, *15*, 457-65.

[55] Müller, J.; Petković, M.; Schiller, J.; Arnold, K.; Reichl, S.; Arnhold, J. Effects of lysophospholipids on the generation of reactive oxygen species by fMLP- and PMA-stimulated human neutrophils. *Luminescence*, **2002**, *17*, 141-9.

[56] Lin, P.; Welch, E.J.; Gao, X.P.; Malik, A.B.; Ye, R.D. Lysophosphatidylcholine modulates neutrophil oxidant production through elevation of cyclic AMP. *J. Immunol.*, **2005**, *174*, 2981-9.

[57] Frasch, S.C.; Zemski-Berry, K.; Murphy, R.C.; Borregaard, N.; Henson, P.M.; Bratton, D.L. Lysophospholipids of different classes mobilize neutrophil secretory vesicles and induce redundant signaling through G2A. *J. Immunol.*, **2007**, *178*, 6540-8.

[58] Englberger, W.; Bitter-Suermann, D.; Hadding, U. Influence of lysophospholipids and PAF on the oxidative burst of PMNL. *Int. J. Immunopharmacol.*, **1987**, *9*, 275-82.

[59] Yan, J.J.; Jung, J.S.; Lee, J.E.; Lee, J.; Huh, S.O.; Kim, H.S.; Jung, K.C.; Cho, J.Y.; Nam, J.S.; Suh, H.W.; Kim, Y.H.; Song, D.K. Therapeutic effects of lysophosphatidylcholine in experimental sepsis. *Nat Med.*, **2004**, *10*, 161-7.

[60] Zurgil, N.; Afrimzon, E.; Shafran, Y.; Shovman, O.; Gilburd, B.; Brikman, H.; Shoenfeld, Y.; Deutsch, M. Lymphocyte resistance to lysophosphatidylcholine mediated apoptosis in atherosclerosis. *Atherosclerosis*, **2007**, *190*, 73-83.

[61] Birgbauer, E.; Chun, J. New developments in the biological functions of lysophospholipids. *Cell. Mol. Life Sci.*, **2006**, *63*, 2695-701.

[62] Tome, M.E.; Lutz, N.W.; Briehl, M.M. Overexpression of catalase or Bcl-2 delays or prevents alterations in phospholipid metabolism

during glucocorticoid-induced apoptosis in WEHI7.2 cells. *Biochim. Biophys. Acta,* **2003**, *1642*, 149-62.

[63] Podo, F. Tumour phospholipid metabolism. *NMR Biomed.,* **1999**, *12*, 413-39.

[64] Ngwenya, B.Z.; Yamamoto, N. Activation of peritoneal macrophages by lysophosphatidylcholine. *Biochim Biophys Acta,* **1985**, *839*, 9-15.

[65] Ryborg, A.K.; Deleuran, B.; Sogaard, H.; Kragballe, K. Intracutaneous injection of lysophosphatidylcholine induces skin inflammation and accumulation of leukocytes. *Acta Derm. Venereol.*, **2000**, *80*, 242-6.

[66] Stiko, A.; Regnström, J.; Shah, P.K.; Cercek, B.; Nilsson, J. Active oxygen species and lysophosphatidylcholine are involved in oxidized low density lipoprotein activation of smooth muscle cell DNA synthesis. *Arterioscler. Thromb. Vasc. Biol.*, **1996**, *16*, 194-200.

[67] Kishimoto, T.; Soda, Y.; Matsuyama, Y.; Mizuno, K. An enzymatic assay for lysophosphatidylcholine concentration in human serum and plasma. *Clin. Biochem.*, **2002**, *35*, 411-6.

[68] Lavi, S.; McConnell, J.P.; Rihal, C.S.; Prasad, A.; Mathew, V.; Lerman, L.O.; Lerman, A. Local production of lipoprotein-associated phospholipase A_2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation*, **2007**, *115*, 2715-21.

[69] Sasagawa, T.; Suzuki, K.; Shiota, T.; Kondo, T.; Okita, M. The significance of plasma lysophospholipids in patients with renal failure on hemodialysis. *J. Nutr. Sci. Vitaminol. (Tokyo)*, **1998**, *44*, 809-18.

[70] Iwase, M.; Sonoki, K.; Sasaki, N.; Ohdo, S.; Higuchi, S.; Hattori, H.; Iida, M. Lysophosphatidylcholine contents in plasma LDL in patients with type 2 diabetes mellitus: relation with lipoprotein-associated phospholipase A_2 and effects of simvastatin treatment. *Atherosclerosis*, **2008**, *196*, 931-6.

[71] Okita, M.; Gaudette, D.C.; Mills, G.B.; Holub, B.J. Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine (lysoPC) in ovarian cancer patients. *Int. J. Cancer*, **1997**, *71*, 31-4.

[72] Mehta, D.; Gupta, S.; Gaur, S.N.; Gangal, S.V.; Agrawal, K.P. Increased leukocyte phospholipase A2 activity and plasma lysophosphatidylcholine levels in asthma and rhinitis and their relationship to airway sensitivity to histamine. *Am. Rev. Respir. Dis.*, **1990**, *142*, 157-61.

[73] Drobnik, W.; Liebisch, G.; Audebert, F.X.; Fröhlich, D.; Glück, T.; Vogel, P.; Rothe, G.; Schmitz, G. Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients. *J. Lipid Res.*, **2003**, *44*, 754-61.

[74] Chen, L.; Liang, B.; Froese, D.E.; Liu, S.; Wong, J.T.; Tran, K.; Hatch, G.M.; Mymin, D.; Kroeger, E.A.; Man, R.Y.; Choy, P.C. Oxidative modification of low density lipoprotein in normal and hyperlipidemic patients: effect of lysophosphatidylcholine composition on vascular relaxation. *J. Lipid Res.*, **1997**, *38*, 546-53.

[75] Zhang, B.; Fan, P.; Shimoji, E.; Itabe, H.; Miura, S.; Uehara, Y.; Matsunaga, A.; Saku, K. Modulating effects of cholesterol feeding and simvastatin treatment on plateletactivating factor acetylhydrolase activity and lysophosphatidylcholine concentration. *Atherosclerosis*, **2006**, *186*, 291-301.

[76] McHowat, J.; Corr, P.B. Thrombin-induced release of lysophosphatidylcholine from endothelial cells. *J. Biol. Chem.*, **1993**, *268*, 15605-10.

[77] Murphy, A.A.; Santanam, N.; Morales, A.J.; Parthasarathy, S. Lysophosphatidyl choline, a chemotactic factor for monocytes/T-lymphocytes is elevated in endometriosis. *J. Clin. Endocrinol. Metab.*, **1998**, *83*, 2110-3.

[78] Ryborg, A.K.; Grøn, B.; Kragballe, K. Increased lysophosphatidylcholine content in lesional psoriatic skin. *Br. J. Dermatol.*, **1995**, *133*, 398-402.

[79] Glander, H.-J.; Schiller, J.; Süß, R.; Paasch, U.; Grunewald, S.; Arnhold, J. Deterioration of spermatozoal plasma membrane is associated with an increase of sperm lyso-phosphatidylcholines. *Andrologia*, **2002**, *34*, 360-6.

[80] Lindahl, M.; Tagesson, C. Selective inhibition of group II phospholipase A_2 by quercetin. *Inflammation*, **1993**, *17*, 573-82.

Received: 23 September, 2008 Revised: 22 December, 2008 Accepted: 24 December, 2008