

## Review

Phospholipase A<sub>2</sub> regulation of lipid droplet formationCarlos Guijas<sup>a,b</sup>, Juan P. Rodríguez<sup>a,c</sup>, Julio M. Rubio<sup>a,b</sup>, María A. Balboa<sup>a,b</sup>, Jesús Balsinde<sup>a,b,\*</sup><sup>a</sup> Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Valladolid, 47003 Valladolid, Spain<sup>b</sup> Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), 28029 Madrid, Spain<sup>c</sup> Laboratorio de Investigación en Proteínas, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, 3400 Corrientes, Argentina

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## ABSTRACT

The classical regard of lipid droplets as mere static energy-storage organelles has evolved dramatically. Nowadays these organelles are known to participate in key processes of cell homeostasis, and their abnormal regulation is linked to several disorders including metabolic diseases (diabetes, obesity, atherosclerosis or hepatic steatosis), inflammatory responses in leukocytes, cancer development and neurodegenerative diseases. Hence, the importance of unraveling the cell mechanisms controlling lipid droplet biosynthesis, homeostasis and degradation seems evident. Phospholipase A<sub>2</sub>s, a family of enzymes whose common feature is to hydrolyze the fatty acid present at the sn-2 position of phospholipids, play pivotal roles in cell signaling and inflammation. These enzymes have recently emerged as key regulators of lipid droplet homeostasis, regulating their formation at different levels. This review summarizes recent results on the roles that various phospholipase A<sub>2</sub> forms play in the regulation of lipid droplet biogenesis under different conditions. These roles expand the already wide range of functions that these enzymes play in cell physiology and pathophysiology.

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## 1. Introduction

Lipid droplets (LDs) are organelles present practically in all cell types. LDs are spherical particles with a surface composed of a phospholipid monolayer decorated by a variety of proteins that encases a neutral lipid core consisting primarily of triacylglycerol (TAG) and cholesteryl esters (CEs). Over a hundred distinct molecular species of phospholipids have been identified in the LD phospholipid monolayer [1–3]. In mammalian cells and yeast, phosphatidylcholine (PC) is the most abundant phospholipid, comprising up to 60% of total phospholipid content, followed by phosphatidylethanolamine (PE) (24%) and phosphatidylinositol (PI) (8%) [4].

Specific populations of proteins that are more or less tightly bound to the LD surface play different roles in LD biogenesis, trafficking and mobilization. Prominent proteins of mammalian LDs are the so-called PAT family, which includes perilipin, adipophilin and TIP47 (Tail-Interacting Protein of 47 kDa) [5]. Additionally, numerous enzymes

related to lipid metabolism have been described, including adipose triglyceride lipase, hormone-sensitive lipase, CGI-58 (comparative gene identification-58) [6,7], CTP:phosphocholine cytidylyltransferase [8], lipin-1 [9,10], and group IVA phospholipase A<sub>2</sub> (cytosolic phospholipase A<sub>2</sub>α, cPLA<sub>2</sub>α) [11–13].

LD biogenesis needs to be contemplated in the context of the synthesis and turnover of its major components: TAG and CEs. These neutral lipids can be formed using fatty acids arising from various origins, i.e. exogenous sources, other cellular compartments, or via stimulation of the de novo fatty acid biosynthetic pathway. Once synthesized, the neutral lipids are first clamped between the two layers of the endoplasmic reticulum membrane. Phospholipid bilayers can accommodate about 3% TAG or 5% CEs, on a molar basis, before the neutral lipid phase separates to form spherical domains of about 25–28 nm diameter that remain sandwiched between the bilayer leaflets [14]. Such TAG microdomains are probably the precursors of the LD that are released from the endoplasmic membrane to the cytosol; however there is much controversy about the origin of the LDs and various hypotheses have been put forward to explain the experimental evidence. These have recently been reviewed in detail [15].

LD number and size varies dynamically from one cell type to another, or among cells of the same population, and the activation state of the cells appears to be a key factor [16,17]. It has recently been shown in hepatocytes that the LDs within a given cell or among cells of the same tissue show a remarkable heterogeneity [18]. Thus it has been suggested that the existence of cells containing high lipid levels within a cell population could potentially reduce lipotoxicity to the average cell without impairing overall lipid homeostasis [18].

**Abbreviations:** LD, lipid droplet; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; cPLA<sub>2</sub>, cytosolic phospholipase A<sub>2</sub>; iPLA<sub>2</sub>, Ca<sup>2+</sup>-independent phospholipase A<sub>2</sub>; sPLA<sub>2</sub>, secreted phospholipase A<sub>2</sub>; Lp-PLA<sub>2</sub>, lipoprotein-associated PLA<sub>2</sub>; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; lysoPE, lysophosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; lysoPA, lysophosphatidic acid; TAG, triacylglycerol; CEs, cholesteryl esters; AA, arachidonic acid; HCV, hepatitis C virus; LDL, low-density lipoprotein; ox-LDL, oxidized low-density lipoprotein; PAF, platelet-activating factor

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The impressive number of recent studies and review articles dealing with LDs illustrates the dramatic change in the way these organelles are currently perceived. From being regarded as mere storage depots for metabolic fuel and membrane-building blocks, LDs are now recognized

as key players in the cellular regulation of lipid homeostasis, signaling, protein sorting, extra- and intracellular trafficking, and gene transcription regulation [15,19,20]. Furthermore, the formation of LDs may also constitute a sign of stress and of apoptotic cell death [21].

**Table 1**  
PLA<sub>2</sub> classification and roles in LD formation.

Group	Subgroup	Trivial name	MW (kDa)	Type of enzyme	Other catalytic activities	Role in LD formation	Ref.
I	A	–	13–15	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	B	Pancreatic PLA <sub>2</sub>	13–15	Secreted, Ca <sup>2+</sup> dependent	–	–	–
II	A	–	13–15	Secreted, Ca <sup>2+</sup> dependent	–	EA: Promotes LD formation	[48]
	B	–	13–15	Secreted, Ca <sup>2+</sup> dependent	Inactive	OE: Induces foam cell formation	[125]
	C	–	15	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	D	–	14–15	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	E	–	14–15	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	F	–	16–17	Secreted, Ca <sup>2+</sup> dependent	–	–	–
III	–	–	15–18; 55	Secreted, Ca <sup>2+</sup> dependent	–	OE: Promotes foam cell formation	[117]
IV	A	cPLA <sub>2</sub> α	85	Cytosolic, Ca <sup>2+</sup> dependent	Lysophospholipase, transacylase	KD: decreases LD formation	[20,46,52,53]
	B	cPLA <sub>2</sub> β	114	Cytosolic, Ca <sup>2+</sup> dependent	–	–	–
	C	cPLA <sub>2</sub> γ	64	Cytosolic, Ca <sup>2+</sup> dependent	Lysophospholipase	–	–
	D	cPLA <sub>2</sub> δ	91	Cytosolic, Ca <sup>2+</sup> dependent	–	–	–
	E	cPLA <sub>2</sub> ε	95	Cytosolic, Ca <sup>2+</sup> dependent	–	–	–
	F	cPLA <sub>2</sub> ζ	95	Cytosolic, Ca <sup>2+</sup> dependent	–	–	–
V	–	–	14	Secreted, Ca <sup>2+</sup> dependent	–	EA: Promotes foam cell formation	[50,124]
VI	A	iPLA <sub>2</sub> , iPLA <sub>2</sub> β	88–90	Ca <sup>2+</sup> independent	PLA <sub>1</sub> , lysophospholipase, PAF acetylhydrolase, and transacylase	OE: Accumulation of TAG in myocardium.	[113]
	B	iPLA <sub>2</sub> γ	88	Ca <sup>2+</sup> independent	–	–	–
VII	A	PAF-AH (lipoprotein associated PLA <sub>2</sub> )	45	Secreted, Ca <sup>2+</sup> independent	Lipase, esterase and transacylase	OE: Attenuation of foam cell formation.	[144]
	B	PAF-AH (II)	40	Intracellular, Ca <sup>2+</sup> independent	–	–	–
VIII	A	PAF-AH Ib (α1)	26	Ca <sup>2+</sup> independent	–	–	–
	B	PAF-AH Ib (α2)	26	Ca <sup>2+</sup> independent	–	–	–
IX	–	Conodipine-M	14	Secreted, Ca <sup>2+</sup> dependent	–	–	–
X	–	–	14	Secreted, Ca <sup>2+</sup> dependent	–	EA: Promotes foam cell formation. EA/OE: Induces LD formation.	[49,118] [51,122]
XI	A	–	12.4	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	B	–	12.9	Secreted, Ca <sup>2+</sup> dependent	–	–	–
XII	A	–	19	Secreted, Ca <sup>2+</sup> dependent	–	–	–
	B	–	19.7	Secreted, Ca <sup>2+</sup> dependent	Inactive	KO: Increases hepatic TAG	[140]
XIII	–	–	<10	Secreted, Ca <sup>2+</sup> dependent	–	–	–
XIV	–	–	13–19	Secreted, Ca <sup>2+</sup> dependent	–	–	–
XV	–	Lysosomal PLA <sub>2</sub>	45	Lysosomal, Ca <sup>2+</sup> independent	Transacylase	KO: Foam cell formation	[148]
XVI	–	Adipose-specific PLA <sub>2</sub>	18	Ca <sup>2+</sup> dependent/independent	PLA <sub>1</sub>	–	–

KO: knock out; KD: knock down (siRNA); OE: overexpression; EA: external addition.

## 2. Phospholipase A<sub>2</sub> enzymes

### 2.1. Classification

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes constitute a heterogeneous group of proteins which have been systematically classified according to sequence homology criteria. The latest update to this classification, published in 2011 [22], included 16 groups, most of them with several subgroups, comprising more than 30 proteins (Table 1). Only PLA<sub>2</sub>s whose nucleotide sequence has been determined are included in the classification. Although the common feature of all these proteins is that they possess PLA<sub>2</sub> activity, that is, they hydrolyze the fatty acid at the sn-2 position of glycerophospholipids, recent additions to the classification display very low PLA<sub>2</sub> activity or no PLA<sub>2</sub> activity at all. Also some members of the PLA<sub>2</sub> superfamily frequently display other activities in addition to PLA<sub>2</sub>, such as phospholipase A<sub>1</sub>, lysophospholipase, transacylase or TAG lipase [22,23].

There is an alternative PLA<sub>2</sub> classification, sometimes more useful, which groups these enzymes into 6 major classes on the basis of biochemical commonalities and/or cell regulation properties. These are the Ca<sup>2+</sup>-dependent cytosolic PLA<sub>2</sub>s (cPLA<sub>2</sub>), the Ca<sup>2+</sup>-dependent secreted PLA<sub>2</sub>s (sPLA<sub>2</sub>), the Ca<sup>2+</sup>-independent cytosolic PLA<sub>2</sub>s (iPLA<sub>2</sub>), the platelet-activating factor acetyl hydrolases (PAF-AH), the lysosomal PLA<sub>2</sub> (LPLA<sub>2</sub>) and the adipose-specific PLA<sub>2</sub> (AdPLA) [22,23]. Note that despite its evident usefulness, this classification is not devoid of inconsistencies, e.g. despite being referred to as secreted enzymes, some sPLA<sub>2</sub>s may indeed act inside the cells and cPLA<sub>2</sub>γ is grouped among the Ca<sup>2+</sup>-dependent cytosolic enzymes despite it being completely Ca<sup>2+</sup>-independent.

Of these classes, the first two have been repeatedly implicated in AA mobilization leading to eicosanoid synthesis in response to a variety of immunoinflammatory stimuli [24–26]. The pivotal enzyme is the cPLA<sub>2</sub>α, an enzyme that exhibits marked preference towards AA-containing phospholipids [27]. Some sPLA<sub>2</sub> enzymes, i.e. those from groups IIA, V and X may amplify the cPLA<sub>2</sub>α-mediated AA release and eicosanoid production either directly by effecting the AA release themselves or by modulating the extent of cPLA<sub>2</sub>α activation [28–30]. cPLA<sub>2</sub>α-independent AA release and eicosanoid response by group V and group X sPLA<sub>2</sub>s acting directly on the extracellular surface are also known to occur in some instances [31]. There is no clear evidence that iPLA<sub>2</sub> enzymes play major effector roles in AA mobilization in response to most stimuli although in some circumstances and in response to specific agonists, these enzymes may serve such role [22,32]. iPLA<sub>2</sub> enzymes may also participate in the formation of cellular AA pools by providing lysophospholipid acceptors for AA reacylation reactions [33–35].

The presence of PLA<sub>2</sub> enzymes in practically all cells and tissues examined stresses the important roles they play in many physiological and pathophysiological processes. A number of comprehensive reviews have recently been published covering various aspects of PLA<sub>2</sub> biochemistry, including structure, catalysis, cellular regulation and physiological/pathophysiological functions [22,23,36–38], and the reader is kindly referred to them for further details.

### 2.2. PLA<sub>2</sub> and lipid droplets

Recent evidence suggests that LDs may function as intracellular sites for eicosanoid biosynthesis. LDs of different cell populations such as eosinophils, macrophages, neutrophils or mast cell avidly incorporate exogenous AA. Lipid analyses of LDs isolated from blood leukocytes have shown that AA is mainly esterified in phospholipids [39], whereas in mast cells and macrophages it is found primarily in neutral lipids [40]. Whether these differences in localization have a physiological consequence or are due to other factors, i.e. differences in the total content of AA present in the cells [41], is unknown.

Several enzymes implicated in the biosynthesis of eicosanoids have been found to be associated with LDs under activation conditions,

including cPLA<sub>2</sub>α [11–13]. Extracellular signal-regulated kinases-1 and -2, which can phosphorylate and activate cPLA<sub>2</sub>α, have also been found in LDs [11], as well as the major AA-converting enzymes 5-lipoxygenase, 15-lipoxygenase, 5-lipoxygenase-activating protein, and cyclooxygenase-2 [16,42]. Lipin-1, a phosphatidic acid phosphatase that acts as an upstream regulator of cPLA<sub>2</sub>α and attendant AA release and eicosanoid production [43–45], has also been found in LDs [9].

Aside from the role of LDs as platforms for compartmentalized regulation of AA mobilization and eicosanoid synthesis, various PLA<sub>2</sub> forms have also emerged as critical regulators of the biogenesis of LDs. PLA<sub>2</sub>s may act at several levels, namely (i) as providers of free fatty acids from membrane phospholipids for the synthesis of neutral lipids [46–48], (ii) as modifiers of phospholipid-containing particles to facilitate their uptake and internalization by cells [49,50], (iii) as generators of metabolites that may control LD formation [51], and (iv) as direct regulators of the formation of the organelle [52–54].

### 3. cPLA<sub>2</sub>

To date, 6 cPLA<sub>2</sub>s have been identified in mammals and classified as group IV PLA<sub>2</sub>s. These enzymes possess a high molecular weight (61–114 kDa) and, with the exception of cPLA<sub>2</sub>γ, a C2 domain at their N-terminal region which binds Ca<sup>2+</sup>, allowing these enzymes to translocate to intracellular membranes. Contrary to sPLA<sub>2</sub>s, only micromolar concentrations of Ca<sup>2+</sup> are required for activity [22,23,27].

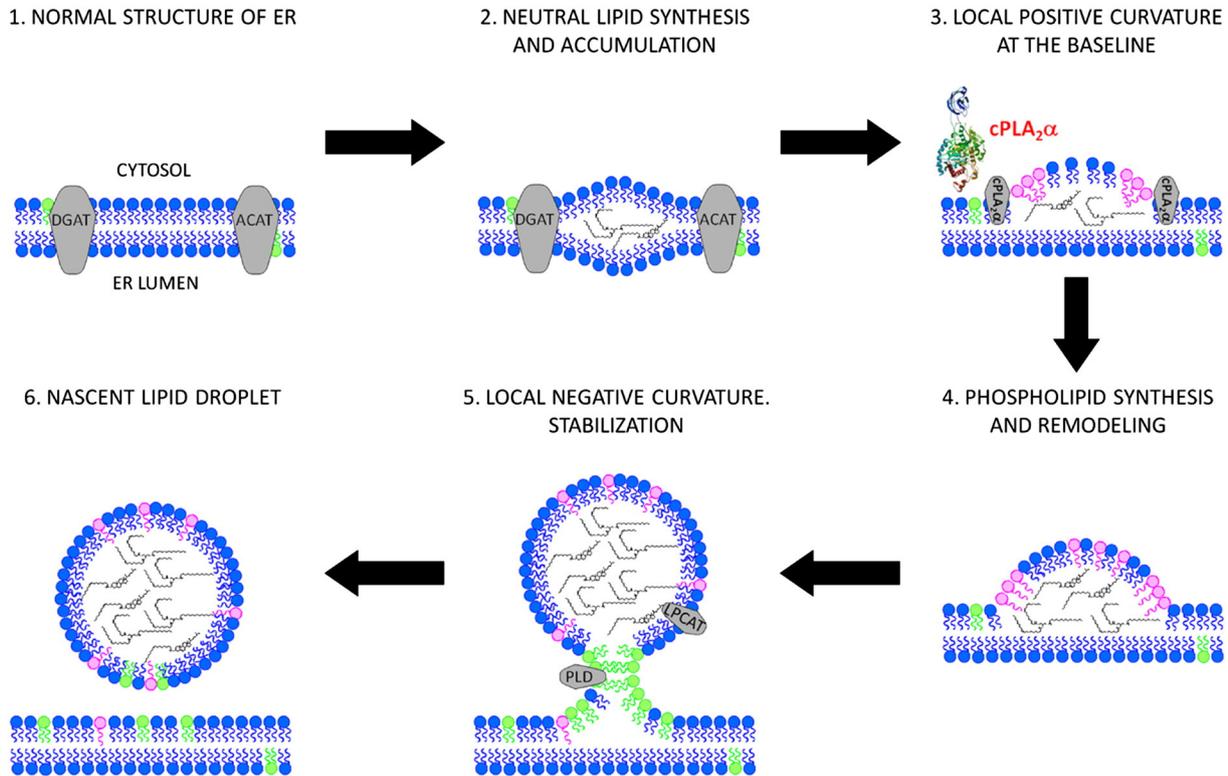
The best characterized of these enzymes is group IVA PLA<sub>2</sub> or cPLA<sub>2</sub>α, which may further require phosphorylation by several kinases and association with phosphoinositides or ceramide-1 phosphate for full activation [55]. After stimulation of the cells with a variety of agonists, the enzyme translocates to multiple intracellular compartments, including the nuclear envelope, Golgi, endoplasmic reticulum, phagosomes, plasma membrane and LDs [11–13,23,27,36,56].

#### 3.1. Mechanisms of LD biogenesis involving cPLA<sub>2</sub>α

The most accepted, albeit not the only, hypothesis for the biogenesis of LDs proposes that these organelles are generated in between the two leaflets of the smooth endoplasmic reticulum, and bud out to the cytoplasm after a very tightly regulated process which includes generation of free fatty acids, thioesterification of the latter with CoA, neutral lipid synthesis, remodeling of endoplasmic reticulum membrane phospholipids, and synthesis of new phospholipids and LD-associated proteins [15]. While various cellular PLA<sub>2</sub>s may contribute to generate the pool of free fatty acids initially needed for LD synthesis, strong evidence is emerging that the PLA<sub>2</sub> form that is centrally involved in the remodeling of endoplasmic reticulum phospholipids and LD expansion processes involving deacylation/reacylation reactions is the cPLA<sub>2</sub>α [15,57,58] (Fig. 1).

DGAT and ACAT, the terminal enzymes for TAG and CE biosynthesis, are homogeneously distributed along the endoplasmic reticulum and are able to synthesize a large amount of neutral lipid in response to different situations such as external lipid overload, endoplasmic reticulum stress or cellular activation [59]. Initially, the neutral lipids formed can be accommodated into the hydrophobic space between the two leaflets of the endoplasmic reticulum up to a point where the two leaflets of the endoplasmic reticulum separate, and TAG and CE become sequestered inside a phospholipid monolayer to form a cytoplasmic LD [14,15,60]. Small amounts of phospholipid are required for LD monolayer expansion, but a tight regulation of the ratio of phospholipid to neutral lipid in LDs is essential to maintain the round shape and biophysical properties of the nascent organelle [4].

By controlling the ratio of phospholipid to lysophospholipid in the endoplasmic reticulum and Golgi complex, PLA<sub>2</sub> enzymes determine the shape and curvature of the membranes of these organelles, and allow the proper execution of a number of events such as membrane tubule formation in the Golgi, cargo delivery from the endoplasmic



**Fig. 1.** LD synthesis from the cytosolic face of the smooth endoplasmic reticulum membranes. The essential role of cPLA<sub>2</sub>α in inducing a positive membrane curvature is indicated in step 3. Lysophospholipids are highlighted in pink, and phosphatidic acid in green. All other phospholipids are shown in blue. For further details see text. DGAT, diacylglycerol:acyl-CoA acyl transferase; ACAT, acyl-CoA:cholesterol acyl transferase; ER, endoplasmic reticulum; PLD, phospholipase D; LPCAT, lysophosphatidylcholine:acyl-CoA acyl transferase.

reticulum to the Golgi, or membrane fusion processes [61–63]. Thus cPLA<sub>2</sub>α may exert similar regulatory effects on the membranes of the smooth endoplasmic reticulum at the time that neutral lipid concentration reaches its critical concentration in between the leaflets. As a matter of fact, inhibition of cPLA<sub>2</sub>α activity by various means does not change the cellular levels of neutral lipid but results in distorted endoplasmic reticulum membranes with tubulovesicular structure [52].

The nascent LD needs a local positive membrane curvature in the endoplasmic reticulum membrane baseline to grow up. The normal endoplasmic reticulum membrane is composed primarily of phosphatidylcholine (PC), which presents a cylindrical shape and generates a neutral curvature, and to a lesser extent, phosphatidylethanolamine (PE), which generates a slight negative curvature [4,19]. Biophysical determinations have unveiled that accumulation of lysophospholipids with a wedge shape conformation such as lysoPC, lysoPE and, especially, lysoPA, generates a local positive membrane curvature, beneficial in terms of nascent LD budding [4,15,19,58,64–66]. Since lysophospholipids are generated by PLA<sub>2</sub>s, the key role that cPLA<sub>2</sub>α appears to play in these stages of LD formation can be easily explained [21,46,52–54] (Fig. 1).

Once the required positive curvature at the LD baseline is generated, more neutral lipids can be accommodated inside the phospholipid monolayer, either synthesized in the endoplasmic reticulum or directly in the forming LD, where the enzymes necessary for TAG and CE biosynthesis are localized. This is accompanied by the synthesis of phospholipid to maintain the appropriate phospholipid to neutral lipid, in a process occurring via both the de novo phospholipid biosynthesis and fatty acid recycling by the Lands pathway [8,15], a route in which PLA<sub>2</sub> enzymes are essential [37,55]. In this regard, the PC molecules on the LD surface are particularly enriched in monounsaturated fatty acids, something that does not apply to the PC molecules at the endoplasmic reticulum [4], and highlights the important role of phospholipid fatty acid recycling to achieve the proper phospholipid composition of LDs at this stage.

Once the LD buds from the endoplasmic reticulum membrane, conversion of the conical lysophospholipids into cylindrical phospholipids

is necessary to establish a neutral curvature that confers stability and protects the hydrophobic core from lipolysis. At this stage, participating lysophospholipid acyltransferases using acyl-CoA as donor are the essential enzymes [15,58]. Some of these enzymes have also been found to be associated with LDs [4,67].

Finally, before LD is completely formed, a local negative curvature in the baseline of the endoplasmic reticulum membrane is required [15, 58]. This is promoted by the accumulation of PA, which induces a spontaneous strong negative curvature [19,68] (Fig. 1). PA is thought to be generated primarily by phospholipase D [69,70], but achieving the optimal levels of this metabolite may also involve the participation of the PA phosphatase lipin-1 [9,10]. Inhibition of lipin-1 results in LD fragmentation and reduced size and number of LDs without changes in total TAG content [9,10]. While some of these effects are likely due to modification of cellular PA levels, lipin-1 is also known to regulate cPLA<sub>2</sub>α activation [9], which raises the possibility that the latter enzyme may also be involved in the regulation of these events.

### 3.2. Pathways leading to cPLA<sub>2</sub>α activation that impact on LD formation

Recent studies have suggested that cPLA<sub>2</sub>α activation by phosphorylation is necessary for LD formation [21,46,52–54]. The relevant phosphorylation with regard to LD formation is the one that occurs at Ser<sup>505</sup>, catalyzed by different members of the mitogen-associated protein kinase (MAPK) family of enzymes [55]. Depending on cell type and stimulus, this phosphorylation is carried out by the extracellular-regulated kinases p42 and p48, p38, and/or SAPK/JNK [55]. This phosphorylation occurs much before the cells start making LDs in response to stimulation and its inhibition strongly blocks LD formation [21,46,52–54].

cPLA<sub>2</sub>α phosphorylation at Ser<sup>505</sup> in cells subjected to different stress conditions appears to be necessary and sufficient for LD formation even at Ca<sup>2+</sup> levels similar to those found in unstimulated cells [46]. This is consistent with results showing that pathogens such as bacteria,

fungi or virus, which are recognized by Toll-like receptors and activate MAPK signaling pathways and cPLA<sub>2</sub>α in inflammatory cells in the absence of sustained intracellular Ca<sup>2+</sup> elevations [30,71,72], are strong inducers of LD formation in these cells [16,64,73–75]. This LD induction is accompanied by the translocation of cPLA<sub>2</sub>α to the LD surface, an event that may serve to modulate and/or amplify the inflammatory response, as it allows the enzyme to reach the AA-containing phospholipids on the LD surface [13,16]. This translocation is likely necessary as well to regulate the biogenesis of the organelle itself, as discussed in the previous section. Coincidentally, phosphorylation of cPLA<sub>2</sub>α on Ser<sup>505</sup> is also required for translocation of the enzyme to phagosomes in macrophages responding to phagocytic stimuli [56]. Since a tight association between LDs and phagosomes appears to exist in immunoinflammatory cells [64], it is possible that cPLA<sub>2</sub>α plays similar regulatory roles for both LD formation and phagosome maturation in cells involved in innate immune and inflammatory reactions.

Although the addition of serum to serum-starved cells leads to the rapid activation of all three major MAPK family members, namely, p44/p42, p38, and JNK, only JNK activation is involved in cPLA<sub>2</sub>α phosphorylation at Ser<sup>505</sup> [52]. Moreover, serum-induced LD formation triggers the activation of ceramide kinase, leading to increased levels of ceramide 1-phosphate, which in turn regulates the phosphorylation activation of cPLA<sub>2</sub>α [53]. Interestingly, down-regulation of ceramide kinase blunts not only cPLA<sub>2</sub>α phosphorylation and LD formation but also JNK activation [21,53], suggesting that, with independence of any direct effect of ceramide 1-phosphate on cPLA<sub>2</sub>α [76], the lipid may also affect other events that lie well upstream of the phospholipase.

In studies with human monocytes, it was found that not only JNK, but also p38 is required for the full phosphorylation activation of cPLA<sub>2</sub>α and subsequent LD formation [54]. Inhibition of one of these kinases but not the other reduces but does not abolish cPLA<sub>2</sub>α phosphorylation and LD formation, and simultaneous inhibition of both kinases is required for complete blockade of the responses [54]. Since both JNK and p38 regulate the phosphorylation of cPLA<sub>2</sub>α at the same residue, these data suggest the participation of an intermediate kinase, activated by both JNK and p38, which would be the one that directly phosphorylates cPLA<sub>2</sub>α at Ser<sup>505</sup> and facilitates LD synthesis. An analogous mechanism for the phosphorylation activation of cPLA<sub>2</sub>α by two distinct MAPK pathways has been reported in cardiomyocytes stimulated with ATP, where it was found that blockade of mitogen and stress-activated kinase-1, a kinase that is phosphorylated/activated by both p38 and p42/p44, results in inhibition of cPLA<sub>2</sub>α phosphorylation activation and of attendant cellular responses [77].

In some cellular systems, it has been reported that the regulatory effect of cPLA<sub>2</sub>α on LD formation is not exerted directly, but rather occurs after formation of downstream metabolites, i.e. eicosanoids generated by oxygenation of the cPLA<sub>2</sub>α product free AA [78]. However, when added exogenously, the free fatty acid is able to induce significant LD formation in cells in the presence of cyclooxygenase and lipoxygenase inhibitors [39,40,52,54]. Importantly, this process occurs in a cPLA<sub>2</sub>α-dependent manner because blockade of the enzyme strongly inhibits LD formation [52,54]. Moreover, free AA itself induces the phosphorylation activation of cPLA<sub>2</sub>α [12,54], thereby promoting more AA mobilization. A remarkable positive loop of activation would therefore be established that results in optimal LD formation.

### 3.3. cPLA<sub>2</sub>α role in LD formation in macrophages exposed to modified LDL

Circulating modified LDL, a major risk for the development of atherosclerosis, is taken up by macrophages and delivered to lysosomes, where part of its CE content is degraded by an acid lipase, and the resulting free cholesterol is utilized for membrane synthesis. When the amount of free cholesterol is excessive, it is re-acylated by ACAT and stored in cytoplasmic LDs, which would ultimately lead to foam cell formation. Cells use this energy-expensive mechanism to avoid

lipotoxicity, which is a major cause of macrophage apoptosis and atherosclerosis progression [79,80].

It is noteworthy that the fatty acid composition of foam cells, containing primarily oleic acid, differs from that of LDLs, in which linoleic acid predominates [47]. This finding constitutes a strong indication that a significant part of the acyl-CoA pool that is utilized to reacylate excess free cholesterol for LD storage must come from endogenous sources [47]. Studies aimed at delineating the intracellular sources of this fatty acyl-CoA in ox-LDL-treated macrophages came up with the finding that methyl arachidonoyl fluorophosphonate, a dual inhibitor of both cPLA<sub>2</sub> and iPLA<sub>2</sub> enzymes, but not bromoenol lactone, an iPLA<sub>2</sub> inhibitor [81–83], blocked CE synthesis, thus pointing to the involvement of a cPLA<sub>2</sub> form, most likely cPLA<sub>2</sub>α [47]. Independent studies had shown that macrophage treatment with oxLDL does induce the activation of cPLA<sub>2</sub>α and subsequent release of eicosanoids [84].

Two mechanisms have been proposed for the regulation of cPLA<sub>2</sub>-mediated CE formation and subsequent LD formation in ox-LDL-treated macrophages. The first one is the activation of the de novo pathway for ceramide synthesis. Elevated intracellular levels of ceramide and its phosphorylated product ceramide 1-phosphate are known to induce cPLA<sub>2</sub>α activation [76,85,86]. Murine macrophages increase their ceramide levels after exposure to ox-LDL without changes in sphingomyelin levels, suggesting activation of ceramide synthesis de novo, and inhibition of this pathway with fumonisin B1 significantly reduces CE synthesis [87, 88].

The second route that may regulate the cPLA<sub>2</sub>α-mediated CE formation in ox-LDL-treated macrophages is the availability of the oxidized fatty acid 13-hydroxyoctadecadienoic acid (13-HODE), formed by oxidation of linoleic acid, the major fatty acid in LDL. 13-HODE can be released from ox-LDL in atherosclerotic lesions via lipoprotein-associated PLA<sub>2</sub> (group VIIA PLA<sub>2</sub>) [89]. 13-HODE has been shown to activate cPLA<sub>2</sub>α by favoring translocation of the enzyme to the membrane, where its substrate resides [47,90]. This effect may be mediated by the anionic character of 13-HODE, which facilitates cPLA<sub>2</sub>α interaction with membranes, as has been reported with other anionic lipids [55,91,92] or perhaps also because 13-HODE incorporation into membranes might affect their physical properties, increasing the susceptibility of membrane phospholipids to cPLA<sub>2</sub>α [90]. Other major components of ox-LDL such as 7-ketocholesterol or 25-hydroxycholesterol do not mimic the effects of 13-HODE [90].

### 3.4. cPLA<sub>2</sub>α role in neutral lipid accumulation in liver

Studies on the regulation of LD synthesis and accumulation in the liver are of great pathophysiological interest, owing to the essential role of this organ in neutral lipid synthesis and accumulation, lipoprotein assembly and secretion and cholesterol biosynthesis. Abnormal LD formation in liver appears to be intimately linked with a number of pathologies, including steatosis, steatohepatitis, cirrhosis, and hepatitis C virus infection [93–95].

Studies using cPLA<sub>2</sub>α<sup>-/-</sup> mice have shown that this enzyme is required for TAG deposition in liver in animals fed a high-fat diet, thus suggesting that blockade of cPLA<sub>2</sub>α protects from hepatic steatosis under these conditions [96]. TAG blood levels were found to be similar in both cPLA<sub>2</sub>α<sup>+/+</sup> and cPLA<sub>2</sub>α<sup>-/-</sup> mice; however, PGE<sub>2</sub> levels were strongly reduced in the cPLA<sub>2</sub>α<sup>-/-</sup> animals, both under normal and high-fat diet [96]. PGE<sub>2</sub> is known to induce TAG accumulation in liver, acting via hepatocyte surface receptors [78], and to suppress TAG-rich VLDL secretion, resulting in its accumulation inside the hepatocyte [97]. Thus cPLA<sub>2</sub>α-mediated release of AA and its conversion into PGE<sub>2</sub> via COX-2 may constitute a straightforward pathway through which the phospholipase regulates in vivo TAG accumulation in liver cells [96].

The life cycle of hepatitis C virus (HCV) appears to be tightly related to hepatocyte LDs and VLDL secretion [98], hence, the PLA<sub>2</sub>-regulated LD formation could be exploited as a possible therapeutic strategy to

manage HCV infection [99,100]. HCV takes advantage of the host cell machinery leading to LD formation, including cPLA<sub>2</sub>α [74]. In accord with this, inhibition of cPLA<sub>2</sub>α with the selective inhibitor pyrrophenone results in dramatic decreases in both LD formation and HCV infectivity [74]. Interestingly a second cPLA<sub>2</sub> form, cPLA<sub>2</sub>γ, also appears to be involved in HCV replication and assembly, and its down-regulation also results in decreased HCV infectivity [101]. This effect of cPLA<sub>2</sub>γ is thought to occur via regulation of membranous web formation, an endoplasmic reticulum-derived structure with unique shape and curvature that is essential in HCV replication and assembly [102]. The finding that cPLA<sub>2</sub>γ is an endoplasmic reticulum-associated, inducible enzyme in hepatocytes [103] could help to explain its up-regulation following HCV infection and its role in endoplasmic reticulum-derived membranous web formation.

#### 4. Calcium-independent phospholipase A<sub>2</sub>

Human calcium-independent phospholipase A<sub>2</sub>s (iPLA<sub>2</sub>) constitute a diverse group of enzymes, referred to as the group VI in the classification of the PLA<sub>2</sub> superfamily of enzymes (Table 1). As all of the group VI iPLA<sub>2</sub> enzymes share a patatin domain, they are also referred to as patatin-like phospholipase domain-containing lipases (PNPLA), which is perhaps a more suitable denomination, inasmuch as more than a half of the enzymes of this group actually exhibit little PLA<sub>2</sub> activity [23].

PNPLA-1 to -5 are enzymes actively involved in the metabolism of LD and the development of adipose tissue. These include PNPLA-2 or adipose TAG lipase, also called desnutrin, and PNPLA-3 or adiponutrin. However, as these enzymes exhibit very low or no PLA<sub>2</sub> activity at all, they will not be further considered in this review. There are many recent excellent articles addressing different aspects of these enzymes and the interested reader is referred to them for details [104–108].

Group VIA PLA<sub>2</sub>, or iPLA<sub>2</sub>β, is the prototypic iPLA<sub>2</sub> enzyme. iPLA<sub>2</sub>β does not show any clear substrate preference in *in vitro* assays, being able to hydrolyze at comparable rates all kinds of phospholipids regardless of their fatty acid composition [23,109]. This lack of substrate specificity fits well with the originally proposed function of the enzyme as a major regulator of phospholipid fatty acyl chain remodeling under resting conditions [110–112]. Interestingly, recent results have suggested that under activation conditions, iPLA<sub>2</sub>β may show some selectivity for hydrolyzing PC molecules carrying palmitic acid at the sn-1 position [35]. The molecular determinants and physiological implications of this finding remain to be determined.

##### 4.1. iPLA<sub>2</sub>β role in LD formation

iPLA<sub>2</sub>β has been proposed as a key enzyme for TAG synthesis prior to stress-induced LD formation in serum deprived CHO-K1 cells [46]. The enzyme provides phospholipid-derived free fatty acids for esterification into neutral lipids [46]. The presence of lipid-rich serum in the cell culture medium also induces LDs in these cells, but this time in an iPLA<sub>2</sub>β-independent manner because there is an exogenous source free fatty acid [46,52]. This has led to the suggestion that LD formation from endogenous lipid sources upon starvation conditions is a cell

mechanism to convert structural components into energy-rich molecules and store them into LD to boost the survival of the cells [21,46,51].

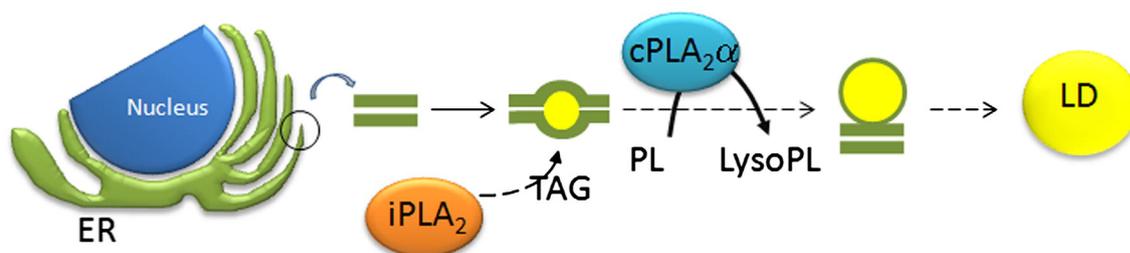
LD formation under stress conditions also involves participation of cPLA<sub>2</sub>α (see preceding section), and this enzyme appears to act after iPLA<sub>2</sub>β because both cPLA<sub>2</sub>α activity and AA release depend on an active iPLA<sub>2</sub>β [46] (Fig. 2). These data would be in accord with findings that TAG synthesis, mediated by iPLA<sub>2</sub>β, occurs prior to proper LD formation and, in turn, may suggest a regulatory role to iPLA<sub>2</sub>β beyond that of merely providing free fatty acids for TAG synthesis [46]. It is intriguing to speculate with possible mechanisms mediating cross-talk between iPLA<sub>2</sub>β and cPLA<sub>2</sub>α. A straightforward mechanism for this interplay could be one where products of iPLA<sub>2</sub>β action on phospholipids regulate, directly or indirectly, cPLA<sub>2</sub>α activation. For example, free fatty acids could directly activate cPLA<sub>2</sub>α to induce LD formation in monocytes [54], and the activation of MAPK-derived signaling, including cPLA<sub>2</sub>α phosphorylation, has been described to lie downstream of iPLA<sub>2</sub>β activation under certain conditions [113]. Coordinate actions of both iPLA<sub>2</sub>β and cPLA<sub>2</sub>α in regulating cellular signaling leading to LD formation have also recently been suggested in macrophages treated with a snake venom PLA<sub>2</sub> [48].

##### 4.2. iPLA<sub>2</sub>γ role in LD formation

The other widely-studied member of the iPLA<sub>2</sub> family is the group VIB enzyme, iPLA<sub>2</sub>γ. Its localization in peroxisomal and mitochondrial membranes suggests that this enzyme may be involved in the integration of lipid and energy metabolism [23]. A brief caloric restriction has been reported to induce phospholipid fatty acid turnover and TAG accumulation in murine myocardium as a mechanism to supply energy to cells [114]. iPLA<sub>2</sub>γ overexpression in these cells increases both phospholipid fatty acid turnover and TAG accumulation after a caloric restriction [115]. Under glucose deprivation, lipids are incorporated by cells more efficiently as a mechanism to obtain energy. Because iPLA<sub>2</sub>γ overexpression results in increased mitochondrial phospholipid turnover, these organelles appear morphologically disrupted, resulting in functional abnormalities. As a consequence of this, fatty acids are not efficiently β-oxidized, which leads to their storage in cytoplasmic LDs to avoid lipotoxicity [115].

#### 5. Secreted phospholipase A<sub>2</sub>s

To date, 11 sPLA<sub>2</sub>s have been identified in mammals, classified into groups I, II, III, V, X and XII (Table 1). These enzymes are characterized by a low molecular weight (13–18 kDa), the requirement of millimolar concentrations of Ca<sup>2+</sup> to carry out their catalytic activity and the presence of multiple disulfide bonds in their structure [22,23]. Subtle differences exist regarding the substrate preference of these enzymes. Thus, some enzymes show preference for anionic phospholipids over PC (the group IIA enzyme), and vice versa (groups V and X). Regarding fatty acid specificity, the group V enzyme seems to prefer oleic acid and linoleic acid chains, group X shows some specificity for AA, and group III shows no specificity at all [22,23].



**Fig. 2.** Sequential role of PLA<sub>2</sub> forms in LD formation. iPLA<sub>2</sub> regulates neutral lipid synthesis first; then, cPLA<sub>2</sub>α regulates formation of the organelle itself. Whether there is cross-talk between the two PLA<sub>2</sub>s is not known. For details see text. ER, endoplasmic reticulum; PL, phospholipid; lysoPL, lysophospholipid.

### 5.1. sPLA<sub>2</sub> role in atherosclerosis

Atherosclerosis is a multifactorial pathology that constitutes the main risk factor for cardiovascular disease. Atherosclerosis is characterized by the retention of macrophages in the artery wall, and their transformation into foam cells due to the intracellular accumulation of lipoprotein-derived neutral lipids, primarily CEs, in the form of LDs [116].

sPLA<sub>2</sub>s have been found in atherosclerotic lesions, probably originating from the monocytes/macrophages, and a role for these enzymes in the emergence and progression of the disease has been repeatedly proposed [117,118].

From a general point of view, sPLA<sub>2</sub> forms may modify native circulating lipoproteins through the hydrolysis of their phospholipids, thereby generating free fatty acids and lysophospholipids that change the size, electric charge, oxidation susceptibility and/or aggregation state of the native lipoprotein particles. These changes facilitate their internalization by macrophages, resulting in LD accumulation and foam cell formation by different mechanisms depending on the affinity of sPLA<sub>2</sub> enzymes to the different substrates. In vitro studies have shown that among all sPLA<sub>2</sub>s putatively involved in atherosclerosis progression, the group X enzyme is the most potent hydrolytic enzyme, followed by groups V and III. Groups IIA, IIE and IIF exhibit low rates of hydrolysis of LDL particles [117]. Since PC species are the major phospholipid constituents of native LDL particles and the group X, V and III sPLA<sub>2</sub>s rapidly and efficiently hydrolyze this phospholipid, native LDL particles are converted into more aggregated small-dense particles because of the reduction in their phospholipid content [50,119,120]. These small dense LDL particles present a potent pro-atherogenic potential due, in part, to a higher oxidation susceptibility and also to their exposure to proteoglycan-binding regions [121], increasing their subendothelial accumulation, uptake by macrophages, LD formation and foam cell accumulation.

On the other hand, the accumulation of large amounts of sPLA<sub>2</sub>-derived fatty acids and lysophospholipids increases the negative electric charge of native LDL particles in a manner similar to when they become oxidized, and thus the particles acquire an ox-LDL-like phenotype that could constitute another potential mechanism for sPLA<sub>2</sub>-mediated foam cell formation. However, this is a subject of much debate. While ox-LDL particles have been found to be internalized via scavenger receptors (CD36, SR-A) by macrophages [122,123], the involvement of such receptors in sPLA<sub>2</sub>-modified LDL particle internalization is unclear [50,124]. These results, along with the different time-courses and localizations of internalized sPLA<sub>2</sub>-modified versus oxidized LDLs, suggest that the change in electric charge of LDL particles following sPLA<sub>2</sub> hydrolysis could not be of pathophysiological consequence for the accumulation of LDs in macrophages. Studies have been carried out in the presence of albumin to remove fatty acids and lysophospholipids from the sPLA<sub>2</sub>-modified LDL [50,125,126]. This strategy reduced the negative charge of the particles but did not affect LD accumulation [125,126]. Since albumin is abundant in atherosclerotic foci, the electric charge of the LDL particles may not change much after sPLA<sub>2</sub>-catalyzed phospholipid hydrolysis under pathophysiologically-relevant settings, which could explain the differences in compartmentalization and time-dependent accumulation of neutral lipids by macrophages compared with cells exposed to the strongly negatively charged ox-LDL [122].

Group IIA sPLA<sub>2</sub> constitutes a special case among sPLA<sub>2</sub>s because it only hydrolyzes a small amount of phospholipids within LDLs due to its preference for phospholipids containing anionic head groups [22, 23]. Group IIA sPLA<sub>2</sub>-overexpressing mice show minimal changes in the phospholipid composition of circulating LDLs compared with their wild-type littermates after 8 weeks on a high-fat diet [127]. Despite this reduced potential to hydrolyze LDLs compared to other sPLA<sub>2</sub>s [125], group IIA sPLA<sub>2</sub> has also been strongly related with atherosclerosis emergence and progression [128] due to the involvement of this enzyme in facilitating the migration of LDL particles to the subendothelium [119,

125]. Group IIA sPLA<sub>2</sub> is bound to intima proteoglycans [129], and promotes the interaction of ApoB-containing LDL with proteoglycans [130], thereby increasing the probability of the particle to undergo oxidation and to accumulate in the form of intima-associated 'extracellular LD' [131].

sPLA<sub>2</sub>-induced hydrolysis of lipoprotein phospholipids may lead to the release of significant amounts of AA [119,120,125]. Among the many biological activities of AA, there is its ability to potently induce LD formation in human monocytes [54], suggesting a proatherogenic role for AA, which may manifest even before monocyte extravasation and differentiation into macrophages has occurred [54,132].

It is well known that, contrary to LDL, HDL plays a protective role in atherosclerosis, contributing to the reverse efflux of CEs from foam cells to their degradation in the liver [133]. Group V and X sPLA<sub>2</sub>s can hydrolyze phospholipids in either HDL or LDL particles at similar rates [119]. Thus the pro-atherogenic role of some sPLA<sub>2</sub>s with regard to LD formation in macrophages could also be explained by the decreased reduction of LD levels that macrophages experience when exposed to sPLA<sub>2</sub>-modified HDL instead of native HDL [134]. The cholesterol efflux from macrophages to HDL is mediated by the expression of scavenger receptor B type I (SR-BI) on the surface of macrophages, and its up-regulation induces an increase in the rate of CE efflux, resulting in the reduction of LD formation [135]. The presence of phospholipids in HDL acceptor particles plays a key role in their CE efflux properties, because the more enriched in phospholipid the HDL particle, the better the interaction with the SR-BI receptor and hence, the higher the CE efflux [134,135]. Thus, a reduction in HDL phospholipid content due to sPLA<sub>2</sub> hydrolysis may explain the decrease in CE efflux from macrophages.

Because of the wealth of data suggesting a prominent role for sPLA<sub>2</sub>s in the development and progression of atherosclerosis, pharmacological inhibition of these enzymes has been contemplated as a possible therapeutic approach to treating cardiovascular diseases [118]. Unfortunately, recent trials testing the effect of varespladib, an inhibitor of group IIA sPLA<sub>2</sub> which most likely also inhibits groups V and X, have provided discouraging results. sPLA<sub>2</sub> inhibition with this drug did not reduce the risk of recurrent cardiovascular events in patients with acute coronary syndrome and actually increased the risk of myocardial infarction [136,137]. Clearly, further studies are needed to establish whether targeting the production of bioactive lipids via sPLA<sub>2</sub> inhibition will have a clinical benefit.

### 5.2. Other sPLA<sub>2</sub> roles

A striking feature of tumor proliferating cells is their lipogenic phenotype, to provide building blocks for making new membrane or to supply energy for growth [138,139]. These cells also synthesize significant amounts of LDs as a survival mechanism under starvation conditions [51]. Some sPLA<sub>2</sub> enzymes have been related to cancer development by different mechanisms, including the release of AA and the synthesis of eicosanoids, and the stimulation of LD synthesis [140].

Incubation of MDA-MB-231 human breast cancer cells with group X sPLA<sub>2</sub> results in increased LD formation by a mechanism that fully depends on the activity of the enzyme [51]. These cells also show up-regulation of their  $\beta$ -oxidation related genes and repression of the de novo lipogenesis genes [51]. This is in accordance with other studies showing that the sole overexpression of group X sPLA<sub>2</sub> in cultured pre-adipocyte cells results in decreased transcription of genes encoding for lipogenic proteins such as SREBP-1c, fatty acid synthase or PPAR $\gamma$  [141]. Inhibition of fatty acid  $\beta$ -oxidation with etomoxir also reduces LD formation in response to group X sPLA<sub>2</sub>, highlighting the seemingly odd circumstance that anabolic –neutral lipid formation and storage in LD– and catabolic – $\beta$ -oxidation of fatty acids– processes take place simultaneously. Thus, fatty acids released by the action of group X sPLA<sub>2</sub> on membranes can follow two opposite routes, and the proposal has been made that one route may serve to support the other, e.g. fatty

acid  $\beta$ -oxidation products such as ATP or NADPH could be needed to support LD biogenesis [21,51].

The group XII of the PLA<sub>2</sub> family consists of two members, XIIA and XIIB. Both PLA<sub>2</sub>s have the same sequence except for the fact that the active site His in group XIIA is replaced with Leu in Group XIIB, which makes the latter catalytically inactive [22,23]. In spite of this, group XIIB sPLA<sub>2</sub>, which is quite abundant in hepatocytes, appears to play a prominent role in regulating LD size and number in these cells [142,143]. By acting downstream of the transcription factor hepatocyte nuclear factor-4 $\alpha$  (HNF-4 $\alpha$ ), group XIIB sPLA<sub>2</sub> regulates VLDL secretion from the liver to the bloodstream. Down-regulation of the enzyme results in reduced VLDL secretion and the accumulation of TAG in the form of LDs into hepatocytes, leading to a fatty liver phenotype as well as the concomitant decrease in circulating HDL, LDL and TAG levels. In the group XIIB sPLA<sub>2</sub><sup>-/-</sup> mice, the genes encoding for fatty acid synthase and stearyl-CoA desaturase-1 appear down-regulated, perhaps as a response to the excessive accumulation of LDs in the hepatocytes of these mice [143].

## 6. Roles of other phospholipases A<sub>2</sub>

PAF acetyl hydrolases are a group of PLA<sub>2</sub>s which hydrolyze acetate from the sn-2 position of PAF, releasing acetic acid and 1-O-alkyl-PC (i.e. lyso-PAF). To date, four of these enzymes have been described, and one of them, group VIIA PLA<sub>2</sub>, also known as Ca<sup>2+</sup>-independent secreted PAF acetyl hydrolase, or lipoprotein-associated PLA<sub>2</sub> (Lp-PLA<sub>2</sub>), has been related with foam cell formation [144]. In addition to PAF, this enzyme also hydrolyzes phospholipids containing a short-chain oxidized fatty acid at the sn-2 position [22,23].

In some studies, an anti-atherogenic function for Lp-PLA<sub>2</sub> has been assigned through the reduction of early foam cell formation in blood vessels. Lp-PLA<sub>2</sub> overexpression protects HDL and LDL from oxidation, on one hand converting LDL to a less proatherogenic particle and, on the other hand, preventing the reduction of the HDL-mediated CE reverse efflux transport from macrophages [134]. This results in the reduction of foam cell formation and atherosclerosis injury in in vivo models [145]. Moreover, foam cell formation in RAW264.7 macrophages is also decreased when the cells are incubated with ox-LDL particles that contain Lp-PLA<sub>2</sub> activity. Inhibition of the Lp-PLA<sub>2</sub> activity associated with the particles leads to increased foam cell formation [146].

On a different view, the products of Lp-PLA<sub>2</sub> action on lipoprotein phospholipids, lysoPC and oxidized fatty acids, are both well established proinflammatory mediators. Accordingly, and despite the protective role that this enzyme appears to exert on foam cell formation, a predominant pro-atherogenic role has been assigned to this enzyme owing to its ability to generate pro-inflammatory substances which may contribute to atherosclerosis progression by several mechanisms [145,147]. The presence of this enzyme in plasma has been deemed as a marker of coronary risk [148]. The pharmaceutical industry has developed a number of reversible inhibitors for this enzyme and one of them, darapladib, has been tested in clinical trials in humans with discouraging results [149].

Group XV PLA<sub>2</sub> or lysosomal PLA<sub>2</sub> hydrolyzes PC and PE substrates under acidic conditions in a Ca<sup>2+</sup>-independent manner [22,23,150]. This enzyme is highly expressed in alveolar macrophages and contributes to the metabolism of the pulmonary surfactant [150]. Macrophages from mice lacking group XV PLA<sub>2</sub> present a massive accumulation of phospholipids in the form of lamellar body inclusions, indicative of cellular phospholipidosis [150]. Phospholipidosis is related with a foam cell phenotype similar to that of macrophages challenged with ox-LDL. According with the protective role of group XV PLA<sub>2</sub> in foam cell formation, mice lacking this enzyme develop more severe lesions than their wild type littermates in a model of atherosclerosis [150].

## 7. Concluding remarks

The interest for LDs is rapidly increasing but still, little is known about the mechanisms governing their formation. The studies discussed

in this article regarding the involvement of PLA<sub>2</sub>s constitute a starting point for a detailed understanding of molecular mechanisms and participating pathways. Clearly, this is a very complex issue involving multiple enzymes, and there is still much to be learned about the interplay between some of the participant PLA<sub>2</sub> enzymes and the regulatory processes involved.

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