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iPLA$_2$β and its Role in Male Fertility, Neurological Disorders, Metabolic Disorders, and Inflammation

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Abstract

The Ca^{2+}-independent phospholipases, designated as group VI iPLA$_2$s, also referred to as PNPLAs due to their shared homology with patatin, include the β, γ, δ, ε, ζ, and η forms of the enzyme. The iPLA$_2$s are ubiquitously expressed, share a consensus GXSXG catalytic motif, and exhibit organelle/cell-specific localization. Among the iPLA$_2$s, iPLA$_2$β has received wide attention as it is recognized to be involved in membrane remodeling, cell proliferation, cell death, and signal transduction. Ongoing studies implicate participation of iPLA$_2$β in a variety of disease processes including cancer, cardiovascular abnormalities, glaucoma, and peridonditis. This review will focus on iPLA$_2$β and its links to male fertility, neurological disorders, metabolic disorders, and inflammation.

Keywords

iPLA2β-derived lipids; signaling; membrane remodeling; immune responses; diabetes

I. iPLA$_2$β: The Beginning

Phospholipase enzymes catalyze hydrolysis of phospholipid substrates and Figure 1 illustrates the site of action of various classes of phospholipases. Phospholipases C cleave the diglyceride moiety from the phospho-headgroup. Phospholipases D cleave the polar head group from the phosphatidic acid moiety. Phospholipases A$_1$ hydrolyze the *sn*-1 fatty acid substituent from the glycerol backbone to yield a free fatty acid and a 1-lysophospholipid, and phospholipases A$_2$ (PLA$_2$) cleave the *sn*-2 substituent from the glycerol backbone to yield a free fatty acid and a 2-lysophospholipid. The PLA$_2$s include (s)ecretory, (c)ytosolic and Ca^{2+}-(i)ndependent PLA$_2$s and their products of action on the PC species are a fatty

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acid, such as arachidonic acid, and 2-lysophosphatidylcholine (LPC). Downstream effects of PLA_2 action may arise from intrinsic actions of the fatty acid product or of its metabolites, such as oxygenated eicosanoids, or from actions of the lysophospholipid products or its metabolites. In the case of LPC, such metabolites could include the bioactive product lysophosphatidic acid (LPA), and an alkyl ether LPC species could also be acetylated to yield the lipid mediator platelet activating factor (PAF), *e.g.*, 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. Other downstream effects of PLA_2 action can arise from remodeling or loss of the phospholipid substrate. With Group VI PLA_2, examples of downstream effects attributable to each of these possibilities are thought to occur.

Ca^{2+}-independent PLA_2 ($iPLA_2$) enzymes.

Although mammalian Groups I, II, III, V, X and XII $sPLA_2$ enzymes all require Ca^{2+} for catalysis, and the Group IV $cPLA_2$ requires Ca^{2+} for membrane-association, Ca^{2+}-independent PLA_2 activities had been described in some cells and tissues before the proteins responsible for these activities were identified[1,2] (Table 1). The Greek letter designations for the $iPLA_2$ isoforms were first suggested by Mancuso et al. in 2000[3] but have subsequently come into wide usage, including in recent reviews of the PLA_2 area[4–7]. All of the $iPLA_2$ isoforms are members of the Group VI PLA_2 family in the numbering system proposed by Dennis et al.[4,7], and distinct family members are designated by letters of the Roman alphabet in the order in which they were discovered in mammals. Group VIA PLA_2 was discovered first in 1997[8–10], and Group VIB was described next in 2002[3]. By that time, Greek letter designations for $cPLA_2$ isoforms of the Group IV PLA_2 family has come into widespread usage, and the first discovered was designated $cPLA_2\alpha$[11] and corresponded to Group IVA PLA_2[4,7]. The next two recognized $cPLA_2$ paralogs were designated $cPLA_2\beta$ and $cPLA_2\gamma$[11], respectively, and corresponded to Group IVB and Group IVC PLA_2[4,7]. Subsequently discovered Group IV paralogs were designated $cPLA_2$ δ, ε, and ζ[12] and corresponded to Group IVD, IVE, and IVF PLA_2. Mancuso et al.[3] proposed Greek letter designations of the Group VI PLA_2 family members to mimic the conventions that had been applied to the Group IV PLA_2 members[11]. The proposal of Mancuso et al. was that these designations be based on the first recognized phospholipase with both a GXSXG lipase consensus motif and a GXGXXG nucleotide-binding motif, which was the potato enzyme patatin[13]. The first recognized mammalian Group VI PLA_2, i.e. Group VIA PLA_2, was thus designated $iPLA_2\beta$, and Group VIB PLA_2 was designated $iPLA_2\gamma$[3]. Subsequently recognized Group VI PLA_2 family members[14,15] were designated $iPLA_2\delta$, $iPLA_2\varepsilon$, $iPLA_2\zeta$, and $iPLA_2\eta$[14], and corresponded to Group VI C, D, E, and F PLA_2[4,7], respectively. Because the plant enzyme patatin was considered the founding enzyme of the $iPLA_2$ family but is not assigned a letter designation in the Group VI PLA_2 naming conventions, there is a discrepancy in the ordinal positions of the letter designations of the mammalian $iPLA_2$/Group VI PLA_2 family members in the Greek and Roman alphabets, respectively. The relationships between these designations and the patatin-like phospholipase domain-containing protein numbering system[16,17] are specified in Table 1.

Ca^{2+}-independent Group VIA PLA_2 in rodents.

In myocardium, P388D1 macrophage-like cells, and pancreatic islet β-cells, such Ca^{2+}-independent PLA_2 activities shared the properties of activation by ATP and susceptibility to

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inhibition by a bromoenol lactone suicide substrate designated BEL[18–23]. This led Jones *et al.* at Genetics Institute to survey a variety of cells for Ca^{2+}-independent PLA_2 activity[10]. This activity was designated $iPLA_2$ to denote independence of Ca^{2+} for catalysis, and such $iPLA_2$ activity was found to be widely distributed[10].

The Genetics Institute group then focused on Chinese Hamster Ovary (CHO) cells[10], which expressed abundant $iPLA_2$ activity and could be readily grown in large quantities. The protein responsible for $iPLA_2$ activity was purified from the cytosolic fraction of 500 L of CHO cells by sequential chromatographic analyses involving ion exchange, hydrophobic interaction, heparin affinity, chromatofocusing, and gel filtration steps to yield an 85 kDa protein upon SDS-PAGE analyses, although catalytic activity migrated with an apparent molecular mass of 250–450 kDa on gel filtration chromatography. This was taken to suggest that the active form of $iPLA_2$ might be a multimer. The 85 kDa SDS-PAGE band was excised and digested with trypsin, and tryptic peptides isolated by reverse-phase HPLC, were sequenced by Edman degradation. Their sequences were used to design degenerate oligonucleotide probes with which to screen a CHO cell cDNA library to obtain full-length clones that were then sequenced. The cDNA encoded a protein with a calculated molecular mass of 85 kDa containing 752 amino acid residues that included a GXSXG serine lipase consensus motif ($GTS^{465}TG$) and eight strings of an ankyrin-like repetitive motif. The $iPLA_2$ sequence lacked homology with $cPLA_2$ or $sPLA_2$ enzymes. Northern blotting analyses revealed ubiquitous tissue expression of $iPLA_2$ mRNA, with the highest levels in testis and liver.

The $iPLA_2$ cDNA was subcloned into a mammalian expression plasmid and transiently expressed in COS (monkey kidney-derived fibroblast-like) cells, which resulted in over a 300-fold rise in Ca^{2+}-independent PLA_2 activity. A truncated form of $iPLA_2$ that lacked the N-terminal 150 amino acids and the ankyrin-repeat (AR) region lacked $iPLA_2$ activity upon expression as a FLAG epitope fusion protein, as did fusion proteins lacking C-terminal sequence from residue 416 to 752. An S465A mutant lacked catalytic activity when expressed as a FLAG fusion protein, but an S252A mutant was fully active, consistent with S^{465} of the $GTS^{465}TG$ sequence representing the active site nucleophile. Studies with model substrates indicated that $iPLA_2$ was selective for the *sn*-2 over *sn*-1 fatty acid substituent. Apparent selectivity for the identity of the *sn*-2 substituent depended on the mode of substrate presentation, but there was no significant preference for any particular fatty acid. Recombinant $iPLA_2$ was found to hydrolyze *sn*-2 acyl linkages in phospholipid substrates with *sn*-1 O-alkyl ether linkages readily, including platelet activating factor (PAF) with its short chain *sn*-2 acetyl substituent[10], including platelet activating factor (PAF) with its short chain *sn*-2 acetyl substituent. In contrast, $cPLA_2$ and Group IB, Group IIA, Group V $sPLA_2$ enzymes do not exhibit PAF acetylhydrolase activity, although PAF is a substrate for Group X $sPLA_2$[24]. Expressed $iPLA_2$ did not exhibit a preference for choline over ethanolamine substrate head-groups, but PI substrates were hydrolyzed 5-fold more slowly than the corresponding PC species, while PA substrates were hydrolyzed 20-fold more rapidly than the corresponding PC species.

Reports of cloning of $iPLA_2$ from mouse P388D1 cells[8] and from rat pancreatic islet β-cells[9] appeared virtually simultaneously with the report on cloning of the hamster $iPLA_2$ and

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exploited the CHO cell sequence[10]. An antiserum generated against the CHO cell $iPLA_2$ recognized a purified P388D1 cell protein of corresponding molecular mass on SDS-PAGE analysis, and the BEL concentration-dependences for inhibition of the CHO cell and P388D1 cell $iPLA_2$ activities were identical[8]. Both $iPLA_2$ proteins were also labeled by [^{3}H]BEL. Using PCR primers designed from the CHO cell $iPLA_2$ sequence and cDNA prepared from P388D1 cell mRNA as template, the nucleotide sequence of the P388D1 cell $iPLA_2$ mRNA was determined, and the mouse nucleotide sequence exhibited 92% homology with the hamster sequence. There was 95% sequence identity at the amino acid level between the CHO cell and P388D1 cell $iPLA_2$ proteins.

To clone the $iPLA_2$ cDNA from a rat pancreatic islet cDNA library, an oligonucleotide probe was generated by PCR using HIT-T15 insulinoma cell RNA as template[9]. HIT-T15 cells are a proliferating β-cell line of hamster origin that also express an $iPLA_2$ similar to that in pancreatic islets[25]. A [^{32}P]-labeled form of the resultant 820 bp PCR product was generated by randomly primed labeling and used to screen an islet cDNA library that yielded five clones that were sequenced from both 5'- and 3'-ends and found to contain a single long open reading frame predicted to encode a protein with 751 amino acid residues and a calculated molecular mass of 83,591 daltons. The islet $iPLA_2$[9] exhibited 90% identity of nucleotide sequence and 95% identity of amino acid sequence with the CHO cell $iPLA_2$[10]. The deduced amino acid sequence included a region (residues 150–414) containing 8 strings of a repetitive sequence motif of about 33 residues in length similar to that in ankyrin-related proteins[9]. One absolutely conserved region of sequence is that between His^{421} and Glu^{551}, which is identical to the corresponding region in the CHO cell $iPLA_2$ and flanks and includes the GXSXG serine lipase consensus sequence of $GTS^{464}TG$. The islet $iPLA_2$ cDNA was subcloned into a mammalian expression vector and transiently expressed in COS-7 cells and CHO cells, which resulted in the appearance of Ca^{2+}-independent PLA_2 activity that was sensitive to inhibition by BEL with a concentration-dependence identical to that for the native $iPLA_2$ activity in HIT cell and islet cytosol. A similar pH dependence and stimulation by ATP were also observed for the $iPLA_2$ activity expressed from islet $iPLA_2$ cDNA and that in native HIT cell or islet cytosol. Studies with FACS-purified populations of islet β-cells and non-β- (predominantly α-) cells indicated that $iPLA_2$ mRNA resided specifically in β-cells[20].

These observations[8–10] established that the $iPLA_2$ enzymes cloned from CHO cells, P388D1 cells, and pancreatic islets represented species homologs of the same Ca^{2+}-independent PLA_2 enzyme from hamster, mouse, and rat, respectively, and these cytosolic enzymes were designated Group VI PLA_2 members[4,6,7]. They are now designated Group VIA PLA_2 or $iPLA_2\beta$ enzymes, distinguishing them from subsequently described membrane-associated Ca^{2+}-independent PLA_2 activity[3], designated Group VIB PLA_2 or $iPLA_2\gamma$ enzymes. Later observations indicated that the *R*-BEL enantiomer selectively inhibited Group VIB PLA_2 ($iPLA_2\gamma$) and the *S*-BEL enantiomer selectively inhibited Group VIA PLA_2 ($iPLA_2\beta$)[5], providing means to distinguish the role of each in biological processes.

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Human Group VIA PLA_2 enzymes, splice variants, and the Group VIA PLA_2 gene.

Two groups independently cloned the human Group VIA PLA_2 through different approaches[26,27]. In one, PCR experiments were performed using total RNA from two monoclonal B-lymphocyte cultured cell lies as template[26]. Sequences used for design of the RT-PCR primers were obtained from a TBLASTN database search using amino acid sequence derived from the CHO cell Group VIA PLA_2 sequence. Two human EST clones (46450 and 30643) thought to represent the human form of Group VIA PLA_2 exhibited 65–84% identify with the CHO cell sequence. The Group VIA PLA_2 primers amplified at least three distinct products representing splice variants. To obtain a full-length cDNA clone, 5'-RACE experiments were performed with cDNAs from several human tissues, including testes, which resulted in amplification of a 2.2 kb DNA fragment that was then subcloned. Five different clones were obtained a sequenced, and three of them had identical sequence and contained an open reading frame encoding the human Group VIA PLA_2 sequence. One clone had an insertion before the catalytic domain that resulted in a frame-shift that changed the reading frame and yielded a truncated sequence without the catalytic center designated ankyrin-iPLA_2-1 to denote that it coded only for the AR domain (Fig. 2). Another clone contained two inserts that again resulted in a truncated sequence without the catalytic domain and was designated ankyrin-iPLA_2-2. The full-length human Group VI PLA_2 cDNA sequence obtained by combining the 3'-end of EST 46450 with the appropriate 5'-RACE fragment exhibited 90% overall identity to the hamster, rat, and mouse sequences with the major difference that the human sequence had a 54 amino acid insert that interrupted the last AR in the rodent sequences. This insert was also present in the ankyrin-iPLA_2-1 and −2 sequences. An additional isoform contained the ARs and the catalytic domain but had a truncated C-terminus, and all four human isoforms appeared to represent variants produced by alternative splicing of the transcript. Expression of the full-length human Group VIA PLA_2 cDNA in COS cells resulted in a substantial increase in Ca^{2+}-independent PLA_2 activity, while expression of ankyrin-iPLA_2-1 cDNA alone did not. Co-expression of full-length human Group VIA PLA_2 cDNA with ankyrin-iPLA_2-1 cDNA resulted in less Ca^{2+}-independent PLA_2 activity than with the full-length cDNA alone, which was taken to suggest that hetero-oligomer formation might represent a means to regulate and diminish Group VIA PLA_2 activity. All of the observed human Group VIA PLA_2 cDNA sequences contained the 54 amino acid insert interrupting the last AR, and no short human isoform corresponding to the rodent Group VIA PLA_2 cDNA sequences was observed.

In the second approach to cloning the human Group VIA PLA_2 cDNA, a human insulinoma cell cDNA library was screened with a [^{32}P]-labeled rat Group VIA PLA_2 cDNA probe[27]. Two clones (NS0C1 and INS-C2) of 1.59 kb and 1.80 kb, respectively, hybridized to the probe and were sequenced. Both clones contained identical 3'-sequences that included a presumptive polyadenylation sequence and a poly(A) tail, and their sequences were identical except for additional 5'-sequence in the longer clone not contained in the shorter clone. Alignment of the rat Group VIA cDNA sequence indicated that both clones contained the 3'-end of the human Group VIA cDNA but that neither contained the full 5'-coding sequence. RNA from human islets was then used as template in PCR experiments with primers designed from the 5'-sequence of rat Group VIA cDNA and from sequence in the INS-C1 and INS-C2 clones. The primer sets were designed to amplify cDNA from the

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initiator methionine codon at the 5'-end through the region of sequence at the 3'-end recognized by primers designed from the INS-C1 and INS-C2 sequences. A nested primer approach was used with the 3'-end primers to verify the specificity of amplification products. PCR with a given set of primers using human pancreatic islet RNA as template yielded two products. With one primer set, a 1.65 kb PCR band was observed upon agarose gel electrophoresis that was expected based on the rat Group VIA cDNA sequence. In addition, a longer product of 1.85 kb was observed[27]. The 1.65 kb and 1.85 kb human islet PCR products were subcloned and sequenced, and each contained a 5'-coding sequence that specified an amino acid sequence highly homologous to the N-terminal portion of rat Group VIA PLA_2. The 1.65 kb and 1.85 kb sequences were identical except for a 162 bp insert in the longer product that was not contained in the shorter one. Similar PCR experiments using RNA from human U937 promonocytic cells as template indicated that U937 cells also express two distinct Group VIA PLA_2 mRNA species, and the lengths of the PCR products corresponded exactly to those from human islet RNA. Upon subcloning and sequencing, the U937 and islet products were found to be identical. Those findings indicate that human islets and U937 cells express mRNA species encoding two distinct isoforms of Group VIA PLA_2. No evidence for truncated Group VIA PLA_2 species such as those observed in human lymphocyte lines[26] was observed with human islet or U937 cell RNA.

The nucleotide and deduced amino acids of the long and short isoforms of human Group VI PLA_2 cloned from islets indicated that the shorter isoform was highly homologous to the hamster, mouse, and rat Group VIA PLA_2 sequences and that the longer isoform contained a 54 amino acid residue insert interrupting the eighth AR[27]. This insert is proline-rich, and a BLAST search revealed similarities to the proline-rich middle linker domain of the DAF-3 Smad protein from *Caenorhabditis elegans*, which is most closely related to mammalian Smad 4. The proline-rich middle linker domains of Smad4, DAF-3, and the human Group VIA insert share a $PX_5PX8HHPX_{12}Q$ motif, and this region mediates protein interactions of Smad4 with signaling partners.

The 5'-fragments of human islet Group VIA PLA_2 cDNA obtained from RT-PCR experiments with human islet RNA overlapped the 3'-fragments obtained from screening the insulinoma cell cDNA library, and within the region of overlap was an *Nco*I restriction endonuclease site, although there were no other *Nco*I sites in the sequences. To obtain cDNA species with the full coding sequences for the long and short human islet Group VIA PLA_2, appropriate 5'- and 3'- fragments were digested with *Nco*I, and ligation reactions were performed. The resultant plasmids were used to transform bacterial host cells and sequenced, and the cDNA species contained the full coding sequences of the human Group VIA PLA_2 isoforms and were inserted into vectors for expression in bacteria and in Sf9 insect cells and used to prepare a [^{32}P]-labeled Group VIA PLA_2 cDNA to generate a probe for genomic screening. Upon expression in bacteria the short and long isoform cDNA species yielded proteins of the expected 85 kDa and 88 kDa sizes, respectively. Uninfected Sf9 cells exhibited no measurable Ca^{2+}-independent PLA_2 activity, but expression of either short or long isoform human islet Group VIA PLA_2 resulted in appearance of such activities in cytosol and membranes that were sensitive to inhibition by the $iPLA_2$ suicide substrate BEL. PLA_2 activity of the long isoform in both cytosol and membranes was stimulated by ATP, but neither cytosolic nor membranous activity of the short isoform was affected by

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ATP. Both human Group VIA isoforms were thus catalytically active Ca^{2+}-independent PLA_2 enzymes inhibitable by BEL, but they exhibited differential sensitivity to ATP.

To determine the structure of the human Group VIA PLA_2 gene a [^{32}P]-labeled cDNA of the long isoform was prepared and used to screen a human genomic DNA library[27]. Eight genomic clones with overlapping regions of sequence were isolated and analyzed by Southern blotting and restriction endonuclease digestion. The cloned sequence spanned over 60 kb of DNA and included 16 exons representing 5'-untranslated region, the entire coding sequence, and 3'-untranslated region. Intron sizes were estimated from the length of PCR fragments produced by using genomic DNA as template and primers that hybridize with sequences in adjacent exons. Sequences of intron-exon boundaries were determined by comparing the sequences of genomic DNA and cDNA. In each case, the intron sequence at the 5'-boundary of the exon ended in the dinucleotide AG and that at the 3'-boundary of the exon began with the dinucleotide GT, conforming to recognized rules for sequences at such junctions. The 54 amino acid-insert interrupting the last AR in the long isoform of human Group VIA PLA_2 was found to correspond exactly to the amino acid sequence encoded by exon 8 of the human gene, indicating that the mRNA encoding the short isoform arises by an exon-skipping mechanism of alternative splicing. Different mechanisms of alternative splicing account for the Group VIA PLA_2 truncation variants observed in human B-lymphocyte lines, which contain additional sequence arising from introns that result in premature stop codons that yield proteins that contain the AR region but lack the catalytic domain[26]. To determine the chromosomal location of the human Group VIA PLA_2 gene, a clone identified in screening the human genomic DNA library with long isoform cDNA was biotinylated to generate a probe for Fluorescence *In Situ* Hybridization (FISH) experiments with human lymphocyte chromosomes[27]. Human chromosomes were identified from their DAPI (4′,6-diamidine-2′-phenylindole)-banding pattern, which were correlated with the sites of fluorescent signal from the biotinylated probe and indicated that the human Group VIA PLA_2 gene resides on chromosome 22. Detailed positional assignment from analyses of multiple photographs indicated that the gene resides in region q13.1 of chromosome 22.

This assignment was confirmed by a different approach based on computational gene identification[28]. A PAC human genomic library was screened with human Group VIA PLA_2 cDNA, and a parallel BLASTN computational search of the GenBank database was performed using the Group VIA PLA_2 cDNA sequence. The comparison revealed segments of identical sequences in two genomic clones (HS228A9 and HS447A4). The total coding sequence and the 3'-UTR of the Group VIA PLA_2 mRNA was contained in clone HS228A9, and the 5'-UTR was contained in clone HS447C4, both of which had been localized to chromosome 22q13.1 between genetic markers DS426 and DS272. Ad additional non-coding exon was identified in the 5'-untranslated region that had not been appreciated in the earlier study[27], resulting in the assignment of 17 exons that included the 162 bp sequence encoding the 54 amino acid-insert in the long human isoform of Group VI PLA_2 in exon 9[28].

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II. iPLA$_2$β: Early Links to Male Infertility and Neurological Disorders

iPLA$_2$β and male fertility.

In early studies of iPLA$_2$β physiological functions, its gene was disrupted by homologous recombination to generate mice that do not express iPLA$_2$β[29]. Heterozygous iPLA$_2$β$^{+/-}$ breeding pairs yielded a Mendelian 1:2:1 ratio of *iPLA$_2$β*$^{+/+}$, *iPLA$_2$β*$^{+/-}$, and *iPLA$_2$β*$^{-/-}$ pups and a 1:1 male:female gender distribution of *iPLA$_2$β*$^{-/-}$ pups. Several tissues of wild-type mice were found to express iPLA$_2$β mRNA, immunoreactive protein, and activity, and testes expressed the highest levels. Testes or other tissues of *iPLA$_2$β*$^{-/-}$ mice expressed no iPLA$_2$β mRNA or protein. The most striking phenotype initially observed with *iPLA$_2$β*$^{-/-}$ mice was impaired male fertility. Spermatozoa from *iPLA$_2$β*$^{-/-}$ mice exhibited reduced motility and impaired ability to fertilize mouse oocytes *in vitro* and *in vivo*. Mating iPLA$_2$β$^{-/-}$ male mice with *iPLA$_2$β*$^{+/+}$, *iPLA$_2$β*$^{+/-}$, or *iPLA$_2$β*$^{-/-}$ female mice yielded only about 7% of the number of pups produced by mating pairs with an *iPLA$_2$β*$^{+/+}$ or *iPLA$_2$β*$^{+/-}$ male, but *iPLA$_2$β*$^{-/-}$ female mice had nearly normal fertility[29]. These findings indicate that iPLA$_2$β plays an important functional role in spermatozoa and stand in contrast to earlier reports that disruption of the Group IVA PLA$_2$ (cPLA$_2$α) gene impairs female but not male reproductive ability[30,31].

More recent observations indicate that iPLA$_2$β participates in the acrosome reaction of spermatozoa[32]. The roles of two intracellular (iPLA$_2$β and cytosolic PLA$_2$α) and one secreted (group X) PLA$_2$s were examined in spontaneous and progesterone-induced acrosome reactions by using knock-out mice and specific inhibitors[32]. These studies revealed that iPLA$_2$β is critical for the spontaneous acrosome reaction and that both iPLA$_2$β and Group X sPLA$_2$ are involved in progesterone-induced acrosomal exocytosis[32]. In contrast, cytosolic PLA$_2$α was not required for either type of acrosome reaction. Progesterone-induced acrosomal exocytosis was found to spread over 30 min in the mouse, and kinetic analyses suggested the presence of different subpopulations of spermatozoa that utilized, separate PLA$_2$ pathways to achieve acrosomal exocytosis[32]. At progesterone concentrations below 2 μM, spermatozoa undergoing early acrosome reactions (0–5 min) relied on iPLA$_2$β, while those undergoing late acrosome reactions (20–30 min) relied on Group X sPLA$_2$[32].

iPLA$_2$β and neurological disorders.

Despite readily apparent phenotypic abnormalities in male fertility[29,32] and glucose tolerance[33] in iPLA$_2$β-null mice, initial experience with these mice revealed no obvious neurologic impairment, but in 2006 mutations in the human iPLA$_2$β gene were reported in patients with childhood genetic disorders categorized as neuroaxonal dystrophies[34,35]. A locus for infantile neuroaxonal dystrophy (INAD), neurodegeneration with brain iron accumulation (NBIA), and Karak syndrome was mapped to human chromosome 22q12-q13 and associated with iPLA$_2$β mutations[34,35]. INAD and NBIA share the distinctive pathologic feature of axonal degeneration with distended axons (spheroid bodies) throughout the central nervous system (CNS)[36]. INAD is characterized by progressive motor and sensory impairment, and spheroids are also found in peripheral nerves[35]. NBIA comprises a clinically and genetically heterogeneous group of disorders with high basal

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ganglia iron. MRI imaging in INAD typically shows cerebellar atrophy with signal hyperintensity in the cerebellar cortex and hypointensity in the pallida and substantia nigra[34]. The designation *PLA2G6*-associated neurodegeneration (PLAN) was proposed for these disorders in 2008[37], and accumulated experience by that date indicated that 80% of INAD patients and 20% of NBIA patients had associated iPLA$_2$β mutations[38]. Mutations predicted to lead to absent protein were associated with the INAD profile of early onset and rapid progression, while compound heterozygous missense mutations correlated with the less severe phenotype of NBIA, consistent with residual function in the mutant protein[38]. iPLA$_2$β mutations were subsequently recognized to be associated with adult-onset dystonia-Parkinsonism[39], and PARK14-linked Parkinsonism associated with iPLA$_2$β mutations is clinically heterogeneous with respect to age of onset and association with fronto-temporal dementia and other features[40–43]. Hereditary spastic paraplegia is also associated with iPLA$_2$β mutations[44]. A current perspective is that the PLAN spectrum comprises a continuum of three predominant human phenotypes with overlapping clinical and radiologic features: 1.) INAD usually begins between ages 6 months and 3 years with psychomotor regression or delay, hypotonia, and progressive spastic tetraparesis. Disease progression is rapid, and survival past age 10 years infrequent[38]. 2.) Atypical NAD has more phenotypic variability and onset can be as late as the end of the second decade with presenting signs of gait instability, ataxia, speech delay, and autistic features. The course is a fairly stable static encephalopathy initially that after several years is followed by neurologic deterioration[38]. 3.) *PLA2G6*-related dystonia parkinsonism has a variable age of onset but most individuals present in early adulthood with gait disturbance or neuropsychiatric manifestations. Dystonia and parkinsonism typically develop in late teens to early twenties and are accompanied by rapid cognitive decline[38].

Recognition of the association of human neurologic disorders with iPLA$_2$β prompted reexamination of *iPLA$_2$β-null* mice for evidence of neurologic impairment, and this revealed that such mice did in fact exhibit an INAD-like syndrome[45]. *iPLA$_2$β-null* mice were found to develop age-dependent neurological impairment that was evident in rotarod, balance, and climbing tests by 13 months of age[45]. The primary abnormality underlying this neurological impairment was the formation of spheroids containing tubulovesicular membranes remarkably similar to human INAD[45]. Spheroids were strongly labeled with anti-ubiquitin antibodies. Accumulation of ubiquitinated protein in spheroids was evident in some brain regions as early as 4 months of age, and the onset of motor impairment correlated with a dramatic increase in ubiquitin-positive spheroids throughout the neuropil in nearly all brain regions. Furthermore accumulating ubiquitinated proteins were observed primarily in insoluble fractions of brain tissue, implicating protein aggregation in this pathogenic process[45]. These results indicate that loss of iPLA$_2$β causes age-dependent impairment of axonal membrane homeostasis and protein degradation pathways, leading to age-dependent neurological impairment[45]. This *iPLA$_2$β-null* mouse line was also studied by another group who demonstrated that these mice develop cerebellar atrophy by the age of 13 months[46]. Atrophied cerebella exhibited significant loss of Purkinje cells, as well as reactive astrogliosis, the activation of microglial cells, and pronounced upregulation of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Glial

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cell activation and the elevation in TNF-α and IL-1β expression occurred before apparent cerebellar atrophy[46]

Another group who independently generated a separate *iPLA$_2$β-null* mouse line using a different targeting construct to generate the iPLA$_2$β-null allele also reported that those mice developed similar neurologic abnormalities[47]. They developed normally and grew to maturity, but all showed evidence of severe motor dysfunction, including a hind limb clasping reflex during tail suspension, abnormal gait, and poor performance in the hanging wire grip test. Neuropathological examination of the nervous system revealed widespread degeneration of axons and/or synapses, accompanied by the presence of numerous spheroids (swollen axons) and vacuoles. Those findings provided additional, independent evidence that lack of iPLA$_2$β causes neuroaxonal degeneration is mice[47]. A third group reported development of a mouse line with a point mutation in the AR domain of iPLA$_2$β that had been generated by N-ethyl-N-nitrosourea mutagenesis[48]. These mutant mice developed severe motor dysfunction, including abnormal gait and poor performance in the hanging grip test, as early as 7 to 8 weeks of age. Neuropathological examination revealed widespread formation of spheroids containing tubulovesicular membranes similar to human INAD. Molecular and biochemical analysis revealed that the mutant mice expressed *iPLA$_2$β* mRNA and protein, but the mutated iPLA$_2$β protein had no glycerophospholipid-hydrolyzing enzyme activity[48]. The significantly earlier onset of disease in this model compared to those reported earlier[45,46] was suggested to be related to production of iPLA$_2$β protein, albeit catalytically inactive as a PLA$_2$[48].

A fourth group independently developed an additional iPLA$_2$β-deficient mouse strain with an INAD-like neurologic disorder[49]. This strain carried a hypomorphic allele of iPLA$_2$β that reduced transcript levels to 5–10% of that observed in wild-type mice. Homozygous mice from this strain developed pathology analogous to that observed in INAD patients[49]. In addition to its PLA$_2$ activity in phospholipid hydrolysis, iPLA$_2$β had been suggested to have non-canonical functions, including regulating store-operated Ca^{2+} entry into cells and modulation of mitochondrial function, and deficiency in this functions was postulated to contribute to INAD development[49]. This group studied changes in ATP-induced Ca^{2+} signaling in astrocytes derived from the mouse strain with the hypomorphic iPLA$_2$β allele and from the strain developed earlier with an inactivating point mutation[48] because ATP is an important transmitter inducing Ca^{2+} signals in astroglial networks[49]. Severe disturbances in Ca^{2+} responses to ATP were observed in astrocytes derived from both mutant mouse strains. The durations of the Ca^{2+} responses in mutant astrocytes were significantly reduced when compared with values observed in control cells and the reduced Ca^{2+} responses appeared to be attributable to a 2.3-fold reduction in capacitative Ca^{2+} entry. Others have reported that in a mouse model in which exon 2 of the iPLA$_2$β gene is deleted, there is defective store-operated Ca^{2+} entry that mimics the defects observed in cells from human subjects with idiopathic Parkinson's disease[50].

That iPLA$_2$β plays an evolutionarily conserved role in neuronal function is also supported by the observations that: 1.) Mutations in the *Drosophila* homolog of human iPLA$_2$β results in age-dependent loss of psychomotor activity and neurodegeneration[51]; 2.) An iPLA$_2$β missense mutation near the active site that is associated with human INAD also results in

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neuroaxonal dystrophy in the Papillon dog[52]; and 3.) iPLA$_2$β-deficiency in Zebrafish leads to dopaminergic cell death, axonal degeneration, increased β-synuclein expression, and defects in brain functions[53]. Taken together with the human and mouse data, these observations point to important and highly conserved functions of iPLA$_2$β in the nervous system.

Comparison of the PLA$_2$ catalytic activity of wild type iPLA$_2$β and of various iPLA$_2$β mutants that had been expressed and assayed *in vitro* revealed that human wild type iPLA$_2$β enzyme hydrolyzes both phospholipids and lysophospholipids to release free fatty acids from radiolabeled phospholipid substrates, as expected[54]. Mutations associated with different disease phenotypes were found to have different effects on catalytic activity. Mutations associated with INAD/NBIA caused loss of enzyme activity, with mutant proteins exhibiting less than 20% of the specific activity of WT protein in both lysophospholipase and phospholipase assays. These findings were taken to suggest that INAD/NBIA is caused by loss of iPLA$_2$β-catalyzed fatty acid release from phospholipids that results in accumulation of phospholipid substrates and provides a mechanistic explanation for the accumulation of membranes in neuroaxonal spheroids[54]. In contrast, mutations associated with dystonia-parkinsonism did not impair catalytic activity, and two mutations produced a significant increase in specific activity for phospholipid but not lysophospholipid substrates[54]. Although dystonia-parkinsonism mutations did not appear to directly impair catalytic function, such mutations may modify substrate preferences or iPLA$_2$β regulatory mechanisms[54]. These findings[54] stand in contrast to another report[55] in which iPLA$_2$β sequence variants were screened in 250 patients with Parkinson's Disease and 550 controls in Chinese Han populations. Four iPLA$_2$β sequence mutants were observed that included a coding synonymous 1959T>A transition of exon 13 in one patient and two missense mutations c.1966C>G in exon 13 and c.2077C>G in exon 14 in two different patients, which resulted in the amino acid changes Leu656Val and Leu693Val respectively[55]. A frame-shift mutation P.His597fx69 in exon 12 was also observed in one patient. These four rare variants were not observed in 550 control individuals. Upon expression and assay *in vitro* wild type iPLA$_2$β hydrolyzed phospholipid substrates, but the frame-shift P.His597fx69 iPLA$_2$β mutant exhibited less than 6% of wild type phospholipase specific activity The Leu656Val and Leu693Val iPLA$_2$β mutants exhibited 45% and 35% reductions in phospholipase assay, respectively. Thus, in this Chinese population iPLA$_2$β mutations associated with Parkinson's Disease did result in diminished or absent PLA$_2$ activity[55].

How alterations in iPLA$_2$β might affect neuronal function is not clearly established. Loss of iPLA$_2$β activity has been reported to result in an increase in ceramide levels in *Drosophila* central nervous system with adverse consequences[56], although iPLA$_2$β activity has been found to promote ceramide accumulation in mammalian cells[57]. In iPLA$_2$β-null mice decreased brain docoshexaenoic acid metabolism and signaling have been observed that may play a role in neuronal dysfunction[58,59]. It has also been suggested that neuroaxonal dystrophy in iPLA$_2$β deficiency results from insufficient remodeling and degeneration of mitochondrial and presynaptic membranes[60], and there is evidence of mitochondrial injury and dysfunction in iPLA$_2$β-null mice[61,62] and of increased mitochondrial lipid peroxidation and dysfunction in brains of iPLA$_2$β-null *Drosophila* and in fibroblasts of human subjects with iPLA$_2$β gene mutations that cause INAD[63]. iPLA$_2$β PARK14 mutants are also

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defective in preventing rotenone-induced mitochondrial dysfunction, generation of reactive oxygen species, and activation of the mitochondrial apoptotic pathway under conditions where WT iPLA$_2$β does prevent these events[64]. There is also growing evidence that iPLA$_2$β participates in the repair of oxidatively damaged mitochondrial membranes[65–69].

III. iPLA$_2$β: Current Understanding of its Role in Metabolic Disorders and Inflammation

Metabolic Disorders.

Among the roles ascribed to iPLA$_2$β is its function in signal transduction [6]. Early studies revealed that iPLA$_2$β participates in insulin secretion, where glucose-stimulated insulin secretion (GSIS) is attenuated by iPLA$_2$β-deficiency and amplified by overexpression of iPLA$_2$β in β-cells[33]. The impact of iPLA$_2$β on GSIS has been linked to iPLA$_2$β-mediated accumulation of arachidonic acid, which negatively regulates the activity of the delayed rectifier potassium channel Kv2.1, thereby prolonging glucose-induced depolarization of the β-cell membrane and allowing for greater Ca^{2+} influx to support higher insulin secretion. Imposition of stress (i.e. diabetogenic agent streptozotocin or high fat diet) on iPLA$_2$β-knockout mice leads to greater impairment in glucose tolerance, relative to WT mice[33]. However, this was not found to be associated with reduced insulin sensitivity, suggesting that the glucose intolerance is a consequence of reduced insulin secretory capacity.

Another role proposed for iPLA$_2$β is its functioning as a housekeeping enzyme to regulate cellular levels of 2-lysophosphatidylcholine (LPC) and arachidonate incorporation into PC[70]. Type 2 diabetes (T2D) is characterized by peripheral insulin resistance and the ultimate failure of islet β-cells is typically associated with elevations in plasma free fatty acids[71]. Various lipid-related factors are reported to contribute to the development of insulin resistance, including triglycerides, ceramides, and diacylglycerols[72–75]. Fatty acid-induced insulin resistance is coupled to activation of C-Jun N-terminal kinase (JNK) and subsequent phosphorylation of the insulin receptor substrate 1 (IRS-1)[76]. Utilizing L6 myotubes and the *db/db* mouse, which develops obesity, hyperlipidemia, and diabetes due to deficiency in leptin receptor activity, Han et al[77] identified a role for LPC in the development of insulin resistance. They determined that palmitate-induced increase in intracellular LPC in the myotubes was inhibited by BEL, an iPLA$_2$-selective inhibitor, as were JNK activation and IRS-1 phosphorylation. Further, administration of BEL to the *db/db* mice reduced LPC abundance in both the liver and muscle along with JNK activation and IRS-1 phosphorylation. These changes were accompanied by a reduction in plasma insulin levels and an increase in β-cell volume, most likely due to BEL-mediated decrease in β-cell stress. While exogenous addition of LPC increased JNK activation and IRS-1 phosphorylation and rescued their decreases from siRNA-mediated reduction in iPLA$_2$β, inhibition of ceramide synthesis did not prevent palmitate-induced insulin resistance. These observations coupled with the finding that BEL increased diacyglycerides, while mitigating insulin resistance, highlighted a potential role of LPC in insulin resistance. A caveat in this study is that the BEL used was a racemic mixture of *S*- and *R*-enantiomers, which inhibit iPLA$_2$β and iPLA$_2$γ, respectively. In fact, siRNA knockdown of both iPLA$_2$β and iPLA$_2$γ reduced LPC and JNK activation. As other studies have reported that genetic ablation of iPLA$_2$γ counters

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development of obesity, insulin resistance, and metabolic abnormalities[78,79], it is likely that both iPLA$_2$β and iPLA$_2$γ generate lipid signals that contribute to the development of insulin resistance. Collectively, these studies reveal that iPLA$_2$ (β and γ)-derived lipids (iDLs) can impair the ability to preserve glucose tolerance in the presence of added stress.

Genome wide meta-analysis revealed a significant association between iPLA$_2$β and plasma lipids that are recognized to be important mediators of metabolic syndrome[80]. Surprisingly, neither arachidonate-containing PC species of islets, brain, and testis[81,82] nor the rate of incorporation of arachidonate into PC[29,83] in iPLA$_2$β-deficient mice maintained on a normal chow diet are altered. Further, the content of triacylglycerides and total cholesterol in plasma, liver, muscle, and adipose tissue was similar in iPLA$_2$β-deficient mice fed a normal chow diet or high fat diet[84]. In contrast, when iPLA$_2$β-deficiency was introduced into *ob/ob* mice, homozygous for leptin mutation, these mice were protected against becoming obese and developing hepatic steatosis[85]. This improved phenotype was associated with reductions in serum lipid, insulin resistance, and *de novo* lipogenesis. It was suggested that iPLA$_2$β and cPLA$_2$α, by providing mono- and poly-unsaturated fatty acid substrates that are directed towards incorporation into triacylglycerides participate in the development of hepatic steatosis in the *ob/ob*. With iPLA$_2$β-deficiency, however, recovery of arachidonate- and DHA-containing PC and PE species is observed[85].

A more recent genome wide meta-analysis[86] identified novel genetic loci that were linked with body mass index (BMI) and body fat percentage (BF%). Among these was a loci in or near *PLA2G6* that associated with BF%, rather than BMI, and this effect was more pronounced in men (2-fold) than in women. The BF% increasing allele was found to be linked with lower insulin and triglyceride levels and a reduced T2D risk. Further analyses of single nucleotide polymorphisms in *PLA2G6* loci identified a strong association between BF % and higher birth weight and greater pre-pubertal height. While this study identified novel linkage between *PLA2G6* and adiposity, the authors noted the potential relevance of other genes present in the *PLA2G6* locus. Candidate genes included *PICK1*, which encodes a membrane sculpting BAR domain protein that facilitates storage and secretion of hormones (i.e., insulin from beta-cell) and *SOX10*, which encodes SRY-related HMG-box family of transcription factors and is an important regulator of embryonic development. The similarity in phenotypes resulting from mutations in *PICK1* and *SOX10* suggested that they may be the driving force of the observed *PLA2G6*-associations.

Studies performed by the Gross group using transgenic mice, selectively overexpressing iPLA$_2$β in cardiac myocytes[87], reveal that myocardial ischemia promotes activation of iPLA$_2$β leading to accumulation of acylcarnitines in the ischemic heart. Similarly, diabetic hearts also accumulate long chain acylycarnitines (LCACs) due to higher expression and activity of iPLA$_2$β, which provides free fatty acids that can be used to generate LCACs. The increases in LCACs are amplified by overexpression of iPLA$_2$β in the heart and reduced by selective inhibition of iPLA$_2$β[88]. LCACs are generated via carnitine palmitoyl transferase I (CPT-I), which is regulated through cytosolic malonyl CoA, and the combination of increased iPLA$_2$β and CPT-1 and reduced malony CoA in the diabetic myocardium appears to be central to the LCAC accumulation. This may be expected to alter membrane properties and give rise to diabetes-induced arrhythmias.

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The development of diabetes is associated with β-cell death (type 1 diabetes, T1D) or β-cell dysfunction preceding their death (T2D). Participation in apoptotic processes is another role suggested for $iPLA_2β$[6] and we have reported that $iPLA_2β$ participates in β-cell death due to ER stress and pro-inflammatory cytokines, which are critical contributors to β-cell death leading to T1D. Our collection of work[57,89–91] reveals that $iPLA_2β$ participation in mediating β-cell death is through (a) induction of ceramides generation via neutral sphingomyelinase-catalyzed hydrolysis of sphingomyelins, which leads to disruption of mitochondrial integrity, caspse-3 activation and ultimately β-cell death and by (b) modulating alternate splicing of Bcl-x that promotes a decrease in the ratio of anti-apoptotic variant Bcl-x(L)/pro-apoptotic variant Bcl-x(s), that occurs via a nonceramide dependent pathway, possible involving arachidonic acid.

$iPLA_2β$ and inflammation.

As recognition of the roles for $iPLA_2β$ is expanding, there are now a number of studies that demonstrate a critical role for $iPLA_2β$-derived lipids (iDLs) in promoting inflammatory responses, which include chemokine-mediated recruitment of leukocytes to inflamed sites, and the production of cytokines and reactive oxygen species (ROS). An early-demonstrated signaling role for iDLs in this process is their impact on monocyte migration[92,93]. The chemokine, monocyte chemoattractant protein (MCP-1), plays a critical role in inflammatory disorders by stimulating chemotaxis to promote migration of monocytes towards inflamed sites[94]. The Cathcart group linked monocyte migration with PLA_2 activity and demonstrated that the chemotactic response to MCP-1 involved both $iPLA_2β$ and $cPLA_2α$. Utilizing chemical and siRNA approaches, they identified different roles for the two PLA_2s. With MCP-1 stimulation, $cPLA_2α$ was found to translocate to the endoplasmic reticulum where its activity leads to the generation of arachidonic acid, which functions to regulate the speed of monocyte migration. In contrast, $iPLA_2β$ mobilizes to the membrane to increase the generation of lysophosphatidic acid (LPA), whose function appears to be to provide migration directionality by targeting F-actin polymerization. These findings led to their suggestion that $iPLA_2β$ functions as a “cellular compass” to affect monocyte migration in response to MCP-1.

Production of ROS is a critical step in the propagation of the inflammatory response. The NADPH oxidases (NOX) catalyze ROS generation in macrophages[95], which express both NOX2 and NOX4[96]. The NOX is comprised of cytosolic components Rac2, and $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$, which are tightly associated, and membrane flavocytochrome b_{568}[97]. Activation of NOX requires translocation of the cytosolic components to the membrane, facilitating transfer of electrons from NOX to O_2 to generate superoxide anion (O_2^-)[98,99]. Moreover, the activation and superoxide generation steps appear to require the presence of arachidonic acid[98,100,101]. It has been reported that in neutrophils from diabetic subjects and when exposed to high glucose, there is a prominent translocation of the NADPH complex to the membrane resulting in increased superoxide generation[102]. It has seen been shown that neutrophils from diabetic subjects manifest greater expression and activity of $iPLA_2β$. Superoxide production from these neutrophils was found to require $iPLA_2β$-mediated generation of arachidonic acid. This was supported by inhibition of superoxide production with an inhibitor and siRNA directed against $iPLA_2β$ and subsequent rescue of production

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with addition of arachidonic acid[103]. While not directly assessed in this study, the role of arachidonic acid in this process has been proposed to include promoting NOX binding and translocation of cytosolic components to the membrane[104].

The activity of NOX2 is regulated by $[Ca^{2+}]i$[105] and S100 Ca^{2+}-binding proteins, S100A8 and S100A9, are thought to participate[106]. This process requires translocation of S100A8/9 complex to the membrane that is promoted following p38 MAPK-mediated phosphorylation of S100A9[107]. Previously, it was reported that p38 MAPK activity can be modulated by $iPLA_2\beta$[108], giving rise to studies demonstrating that $iPLA_2\beta$-mediated phosphorylation and translocation of S100A8/9 through activation of p38 MAPK[109]. This led to regulation of NOX2 activity and subsequent ROS production. These outcomes were prevented by chemical inhibition of $iPLA_2\beta$ or siRNA directed against $iPLA_2\beta$. While $iPLA_2\beta$ activity was implicated in this process, the study did not assess the relevant iDL required to cause affect.

While the major isoforms of NOX in macrophages are NOX2 and NOX4[96], the latter has been reported to promote myocytes chemotaxis and macrophage recruitment during diabetic metabolic stress[110]. Lipoprotein receptor-knockout mice ($LDLR^{-/-}$), a model of diet-induced atherosclerosis, exhibit temporal increases in $iPLA_2\beta$ and aortic atherosclerotic lesions[111]. In this model, MCP-1-induced macrophage migration in response to high glucose or low-density lipoprotein (LDL) was mitigated by an inhibitor or siRNA directed against $iPLA_2\beta$. Inhibiting $iPLA_2\beta$ prevented subsequent H_2O_2 production and this was associated with reduction in NOX4, but not NOX2, expression. These outcomes were rescued by LPA, suggesting that $iPLA_2\beta$-mediated generation of LPA was required for NOX4 activation it macrophages.

When the above observations are considered along with findings linking $cPLA_2\alpha$ with NOX activation[101,112], a scenario evolves where coordinated activities of $iPLA_2\beta$ and $cPLA_2\alpha$ in concert promote NOX activation. In those studies, the Levy group utilizing neutrophils and granulocyte-like PLB-385 cells and employing immunoprecipitation and microscopy approaches reported that upon stimulation, $cPLA_2\alpha$ is recruited to the plasma membrane after assembly of NOX. The association of $cPLA_2\alpha$ with NOX is thought to require the phosphorylated form of $cPLA_2\alpha$. Subsequent generation of $cPLA_2\alpha$ -mediated hydrolysis of arachidonic acid coincided with the production of superoxide. It was suggested that the arachidonic acid facilitates association of the NOX complex resulting in optimal oxidase activation. They went on further to determine using blot overlay and Forster resonance energy analyses that the binding between $cPLA_2\alpha$ and NOX at the membrane is mediated by $p47^{phox}$ and that the anchoring of $cPLA_2\alpha$ is facilitated by the $cPLA_2\alpha$ -C2 domain, which exhibits specificity for phosphatidylserine-rich plasma membranes [113]. Collectively, it might be surmised that $iPLA_2\beta$ activity induces translocation of the NOX components to the membrane and that $cPLA_2\alpha$ activity promotes optimal membrane-associated oxidase activity.

Other instances of interplay between $iPLA_2\beta$ and $cPLA_2\alpha$ have also been suggested. An earlier report had suggested that thapsigargin-triggered activation of store-operated channels and capacitative Ca^{2+} influx is mediated by lysophospholipids generated via $iPLA_2\beta$

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activation[114]. This study relied exclusively on chemical inhibition of iPLA$_2$β to discern its role. Subsequently, utilizing iPLA$_2$β-deficient mice to preclude non-specific effects of chemical inhibitors, and stimulants (thapsigargin or ionophore A231987), the Gross group described sequential roles of iPLA$_2$β and cPLA$_2$α in promoting arachidonic acid release and eicosanoid generation[115], and contributing to vascular tone, signaling, and in smooth muscle cell proliferation and migration. Based on the difference in their mode of action, where thapsigargin promotes Ca^{2+}-mediated depletion of internal stores and A23187-mediated transmembrane Ca^{2+} influx can lead to activation of cPLA$_2$α, they proposed that an initial iPLA$_2$β-mediated release of arachidonic acid is followed by a later induction of cPLA$_2$α via iPLA$_2$β-mediated activation of capacitative Ca^{2+} influx.

An underlying process in atherosclerosis is foam cell formation and ROS-mediated oxidation of low density lipoproteins are critical for the conversion of macrophages to foam cells[116]. The generation of ROS appears to be dependent on toll-like receptor (TLR), in particular TLR9 signaling[117,118] through induction of NOX. A study done by the Lee group[116] revealed that *S*-BEL (selective for iPLA$_2$β) and iPLA$_2$β siRNA, but not *R*-BEL (selective for iPLA$_2$γ) or iPLA$_2$γ siRNA, more strongly reduced lipopolysaccharide (LPS)-induced ROS production, NOX1 expression, and inhibited foam cell formation. They further observed that inhibition of iPLA$_2$β reduced LPS-stimulated Akt phosphorylation. These studies identified a role for iPLA$_2$β in mediating the atherosclerosis process via Akt signaling to induce NOX1 expression and generation of ROS by a TLR4-dependent pathway. However, this study did not extend to discerning the specific lipids generated by iPLA$_2$β activity and are critical in promoting foam cell formation.

Macrophage adhesion to modified extracellular matrix (ECM) components and their retention is another requisite for the inflammatory process. The Class A Scavenger Receptors (SR-A) present on macrophages are homotrimeric membrane glycoproteins and they function in Ca^{2+}-independent macrophage adhesion to modified ECM proteins[119]. This also involves intracellular signaling cascades and the assembly and organization of the actin cytoskeleton to allow for spreading and firm cell adhesion. These processes are regulated by Rac and Cdc42, members of the Rho-like GTPase family[120]. *In vitro* studies with peritoneal macrophages revealed that inhibition of iPLA$_2$β, but not cPLA$_2$α, decreased cell adhesion and spreading[121]. Such inhibition was recapitulated by inactivation of 12/15-lipoxygenase (LOX), but not of cyclooxygenase or CytP450 epoxygenase, and rescued by arachidonic acid addition. Further, Rac and Cdc42 activation were also mitigated by inhibitors of iPLA$_2$β and 12/15-LOX. These findings suggest that 12/15-LOX metabolites of arachidonic acid, derived from iPLA$_2$β activation, participate in macrophage adhesion and spreading by coupling SR-A to Rac and Cdc42 activation. A subsequent study demonstrated that *Trypanosoma cruzi* infection, which results in inflammation of myocardial tissue, increases polymorphonuclear (PMN) leukocyte adhesion that was associated with increased iPLA$_2$β and iPLA$_2$γ activity, arachidonic acid release, and PAF production[122]. While Inhibition of iPLA$_2$β or iPLA$_2$γ reduced arachidonic acid release, only inhibition of iPLA$_2$β decreased PAF production and PMN adhesion. These outcomes were recapitulated by the inability of RAW 264.7 murine macrophage/monocyte cell adherence to cardiac endothelia cells obtained from iPLA$_2$β-deficient mice. These findings suggest a role for PAF derived from iPLA$_2$β activity in promoting cell adhesion during tissue inflammation.

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An additional link between iPLA$_2\beta$ and a GTP binding protein was identified in aortic and mesenteric arteries of type 1 and type 2 diabetic mouse models[123]. Diabetic vascular complications encompass increase in vasoconstrictive and decrease in vasodilatory responses and G-protein coupled receptor agonism can modulate vascular smooth muscle (VSM) contraction by eliciting Ca^{2+}-sensitization[124]. Dysregulation of this process can lead to severe cardiovascular complications. The GTP-binding protein RhoA, RHO kinase (ROCK), protein kinase C (PKC), and CPI-17 (protein kinase C-potentiated phosphatase inhibitor) function as Ca^{2+}-sensitizers. Elevations in glucose, as in T1D and T2D, were found to induce iPLA$_2\beta$ via PKC activation leading to activation of RhoA/ROCK/CPI-17 and diabetes-induced hypercontractility of VSM, which was ameliorated with inhibition or knockout of iPLA$_2\beta$, but not iPLA$_2\gamma$. Inhibition of 12/15-LOX was also effective in reducing VSM hypercontractility, suggesting that iPLA$_2\beta$, through generation of 12/15-LOX products, participates in the evolution of VSM abnormalities that are prevalent in diabetes.

Proinflammatory cytokines produced by immune cells are integral to the inflammatory process. We reported that a selective inhibitor of iPLA$_2\beta$ reduces T1D incidence in the non-obese diabetic (NOD) mouse model[125]. This was associated with reduced infiltration of leukocytes into the islets and decreased generation of TNFα from CD4$^+$ T-cells and antibodies from B-cells isolated from NOD. Further, iPLA$_2\beta$-deficiency favored macrophage polarization towards an M2 anti-inflammatory phenotype[126], concurrent with reduced macrophage IFNγ+LPS-activated production of TNFα and IL-1β. Decreased production of the pro-inflammatory cytokines in iPLA$_2\beta$-deficient macrophages was recapitulated in WT macrophages exposed to inhibitors of iPLA$_2\beta$, COX, or 12-LOX. These inhibitors also mitigated M1 polarization of WT macrophages with IFNγ+LPS activation. An impact of iPLA$_2\beta$ on cytokine production was also recognized in a neointima formation, which can lead to development of atherosclerosis[127]. That study revealed an increase in iPLA$_2\beta$ expression in arteries following coronary artery ligation that preceded neointima formation. Inhibition, knockdown, or genetic deletion of iPLA$_2\beta$ inhibited and overexpression of iPLA$_2\beta$ exacerbated macrophage vascular infiltration and neointima formation. Activation of iPLA$_2\beta$ was also linked to higher production of inflammatory markers TNFα, IL-6, IL-1β, and MCP-1. These outcomes were again reduced in iPLA$_2\beta$-deficient macrophages and by inhibition of 12/15-LOX, but not COX, 5-LOX, or cytochrome P450. Collectively, these findings suggest that COX and 12-LOX products generated downstream of iPLA$_2\beta$ activation affect immune cell function and responses under different disease settings.

Other studies suggest protective effects of iPLA$_2\beta$, as its deletion exacerbated experimental colitis[128], autoimmune hepatitis[129], and age-related liver and intestinal disorders[130]. These were noted to be related to defective membrane remodeling, and reductions in LPC, which provides the eat-me signal for recruitment of phagocytes to facilitate clearance of apoptotic cellular debris, and in LPA, which promotes macrophage migration. iPLA$_2\beta$-deficiency also has been reported to result in higher abundance of parasite pseudocysts in the hearts of mice infected with *Trypanosoma cruzi*[131]. In WT hearts, the infection induced NO production to facilitate parasite clearance by macrophages. With iPLA$_2\beta$ deficiency or inhibition of iPLA$_2\beta$, but not iPLA$_2\gamma$, NO production was reduced leading to accumulation of the pseudocysts.

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These reports suggest that iDLs are important contributors to inflammation and as such could be critical in the development of autoimmune disorders[125,129,132]. While the focus of this review is iPLA$_2$β, it must be recognized that other PLA$_2$s, working in concert with iPLA$_2$β, may also be required to produce the inflammatory sequela. In this regard, a study of pleurisy provides insight into involvement of multiple PLA$_2$s that may apply to the etiology of other inflammatory landscapes as well[133]. They report that the inflammatory process encompasses two stages of arachidonic acid release. The release at onset leads to generation of pro-inflammatory eicosanoids and release at the later stage to generation of resolving eicosanoids. At the onset of pleurisy, iPLA$_2$β activity is predominant and leads to increased production of PGE$_2$, LTB$_4$, PAF, and IL-1β to drive the inflammatory process. Subsequently, a resolution phase evolves during which sPLA$_2$s (Groups IIA and V) mediate generation of PAF and lipoxin A$_4$, which in turn induce cPLA$_2$α and the second stage of arachidonic acid release. This pool of arachidonic acid is metabolized to anti-inflammatory PGD$_2$ and its breakdown product 15deoxy delta 12–14 PGJ$_2$, as a means to continue the resolution phase. Interestingly, corticosterone is released at the beginning of inflammation to limit inflammatory responses. It inhibits cPLA$_2$α, but not iPLA$_2$β, and its levels decrease during resolution, which could lead to an increase in cPLA$_2$α and the emergence of a more dynamic resolution phase.

IV. iPLA$_2$β – Moving Forward

Ongoing studies are greatly increasing our understanding of the multitude of roles that iPLA$_2$β and iDLs have in a variety of biological processes and diseases. Continued studies are facilitated by the availability of genetically-modified mice with iPLA$_2$β-deficiency[29] and the significant advances recently reported in the following areas:

Crystal structure of iPLA$_2$β.

The iPLA$_2$β protein sequence consists an N-terminal domain, an AR domain, and a catalytic domain (CAT)[134]. To examine the structure of the short isoform iPLA$_2$β, its cDNA cloned from CHO cells was used to express the enzyme with a C-terminal 6XHisTag, and the recombinant enzyme was purified and crystalized for selenomethione single-wavelength anomalous diffraction (SAD) studies combined with molecular replacement (MR) studies using two different protein models. The model proteins were patatin (32% sequence identity to the CAT domain) and four ARs (20% sequence identity to the four C-terminal ARs of iPLA$_2$β. The core secondary structure elements of the CAT domain are similar to patatin and the fold structure resembles that of iPLA$_2$β. The active site is located within the globular domain as in patatin, but the catalytic residues are more solvent accessible. The active site cavity is wide open and can accommodate phospholipids with long polyunsaturated fatty acid chains. A long C-terminal α-helix is kinked and participates in dimer formation. The electron density map reveals nine ARs in the ANK domain, and its orientation surprisingly reflects attachment to the CAT domain on the side opposite to the membrane-binding surface. The outer helices of AR$_7$ and AR$_8$ form an extensive hydrophobic interface with the CAT domain. ATP was found to bind near Trp[293] rather than in the previously suspected glycine-rich domain. Trp[293] lies within AR$_6$ (residues 282–308), which contains a helix two amino acids shorter than a conventional AR helix that creates a kink of the ANK domain.

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Potential ATP binding at this location suggests that nucleotides can regulate ANK domain conformation. The crystalized iPLA$_2$β is a dimer formed through association of the CAT domains, and the ANK domains previously suspected to be involved in oligomerization are oriented outwards in opposite directions for form an elongated structure. The CAT domains interact through an extended hydrophobic dimerization interface formed by three α-helices, several loops (including residues 554–570), and part of the central β-sheet. Trp^{695} forms extensive hydrophobic interactions with the opposite monomer, including a stacking interaction with its counterpart, and substitution of Glu at this position (W695E) by mutagenesis causes the resultant protein to behave as a monomer in solution. In the dimer, two active centers and the predicted membrane binding loops are oriented in the same direction. The active sites are close to the dimerization interface and to each other, and the catalytic Asp^{598} is at the beginning of the π-helical loop (residues 599–603). Two leucines of this loop form contacts with the long α-helix (residues 604–624) of the opposite monomer[134]. These features suggest strong allosteric association of the two active sites and dependence of the catalytic activity on the dimer conformation. Binding studies indicate that calmodulin (CaM) [135–137] associates with the iPLA$_2$β dimer rather than a monomer, and, consistent with this, two 1–9-14 CaM binding motifs lie on the same side of the dimer in close proximity.

Surprising features of these findings include the open conformation of the active site in the absence of membrane interaction with both sites of the dimer providing sufficient space for phospholipids to access the catalytic center[134]. In addition, the dimer is formed by interaction of the CAT domains, while the ANK domains are oriented outwards and do not mediate interactions of the monomers. Disruption of the dimer in the W695E mutant yields an inactive enzyme. Intimate allosteric interaction of the active sites and the dimerization interface also provides a potential mechanism for inhibition by CaM, the binding of which stabilizes a closed conformation of the active sites. The outward-facing ANK domains of the model suggest potential interaction sites for cytosolic Ca^{2+}/calmodulin-dependent protein kinase IIβ (CaMKIIβ) and for the cytosolic C-terminus of transmembrane calnexin, both of which are known to interact with iPLA$_2$β[10,138]. The knowledge of the crystal structure for iPLA$_2$β will allow further studies on how iPLA$_2$β may be regulated in the absence and presence of potential binding partners.

Substrate specificity and molecular dynamics of iPLA$_2$β.

The Dennis group utilizing lipidomics-based LC/MS protocols assessed substrate preference of iPLA$_2$β versus cPLA$_2$α and Group V sPLA$_2$ and noted several features that were unique to iPLA$_2$β[139]. In the presence of equimolar mixture of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphate (PAPA), 1-palmitoyl-2-arachidonoyl-*sn*-phosphatidylcholine (PAPC), 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (PAPE), 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (PAPG), and 1-palmitoyl-2arachidonoyl-*sn*-glycero-3-phospho-L-serine (PAPS)**,** in contrast to cPLA$_2$α which showed no preference and Group V sPLA$_2$ was more active towards PAPG, iPLA$_2$β was found to have the greatest activity towards PAPE. They further demonstrated that with respect to the *sn*-2 substituent on membrane phospholipids, cPLA$_2$α preferred arachidonic acid, Group V sPLA$_2$ was active towards most fatty acids with linoleic and myristic acids

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slightly better preferred, but that $iPLA_2\beta$ was much more selective for linoleate and myristate. Also noted were differences in the preference for *sn*-1 fatty acid, with $cPLA_2\alpha$ and Group V $sPLA_2$ preferring steric acid, while $iPLA_2\beta$ was more active with phospholipids containing palmitic acid in the *sn*-1 position. The differences in substrate preference were very nicely rationalized with atomistic molecular dynamics analyses. Using simulations with each enzyme in the presence of optimal substrates, they were able to describe distinguishing features about the active site for each enzyme. The active site was described as containing two binding regions, a hydrophobic one where the headgroup binds and a hydrophilic one where the two acyl chains bind. Substrate specificity is conferred by the recruitment of the optimal substrate (phospholipid + *sn*-2 fatty acid) to its select hydrophobic binding subsite. Their simulations revealed that the active site of $cPLA_2\alpha$ is a channel that is deep and rigid and able to take up the whole phospholipid molecule, lending to its specificity for arachidonate-containing phospholipids. However, the active site for Group V $sPLA_2$ is a shallow cavity that can only take up the *sn*-2 fatty acid, reflected by its similar activity towards various *sn*-2 fatty acids. The active site of $iPLA_2\beta$ on the other hand is more flexible and is capable of changing volume, thus facilitating activity towards different phospholipids containing different *sn*-2 fatty acids. Analyses such as these can increase our understanding of how enzyme activity might be affected by changes in the enzyme molecular structure in a diseased state, relative to a healthy environment.

Development of novel and more selective inhibitors of $iPLA_2\beta$.

In view of the similar functions of the PLA_2s, selective inhibitors of the enzymes would significantly enhance our understanding of the specific role of each PLA_2 in a biological event. In this regard, the work done by the Kokotos group has made major strides in generating more potent and selective inhibitors of the PLA_2s. Utilizing computational chemistry and organic synthesis in conjunction with SAR and *in vitro* activity assays, they identified fluoroketone compounds with high potency and selectivity towards $iPLA_2\beta$, as compared against $cPLA_2\alpha$ or Group V $sPLA_2$[140]. Additionally, one such compound, designated FKGK18, was demonstrated to be more selective for $iPLA_2\beta$ than $iPLA_2\gamma$[141]. Unlike BEL, FKGK18 is a reversible inhibitor without significant cytotoxic effects and has proven to be effective in reducing T1D in a spontaneous diabetes rodent model[125]. Analogously, the earlier generation FKGK11 was able to ameliorate clinical scores associated with experimental autoimmune encephalomyelitis [132]. Studies such as these highlight the potential of utilizing these inhibitors to mitigate complications associated with autoimmune- and inflammation-based disorders. More recently, modification of the fluoroketone backbone has led to identification of even more potent and selective $iPLA_2$ inhibitors (*in revision*). Moreover, integration of molecular dynamics stimulations, SAR assays, and hydrogen/deuterium exchange mass spectrometry has led to identification of new fluoroketone compounds and one based on a keto-1,2,4 cadaizole functionality with a thioether that manifested selective potency against $iPLA_2\beta$, relative to $cPLA_2\alpha$ or Group V $sPLA_2$[142,143]. Those studies were able to further establish that the sulfur atom in the beta-thioether analogues is vital for the potency and that the hydrophobic chain for the selectivity. In the future, coupling these approaches with the now available crystal structure of $iPLA_2\beta$ would most definitely lead to the generation of very selective and potent inhibitors of $iPLA_2\beta$ that will be amenable for therapeutics.

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Supplementary Material

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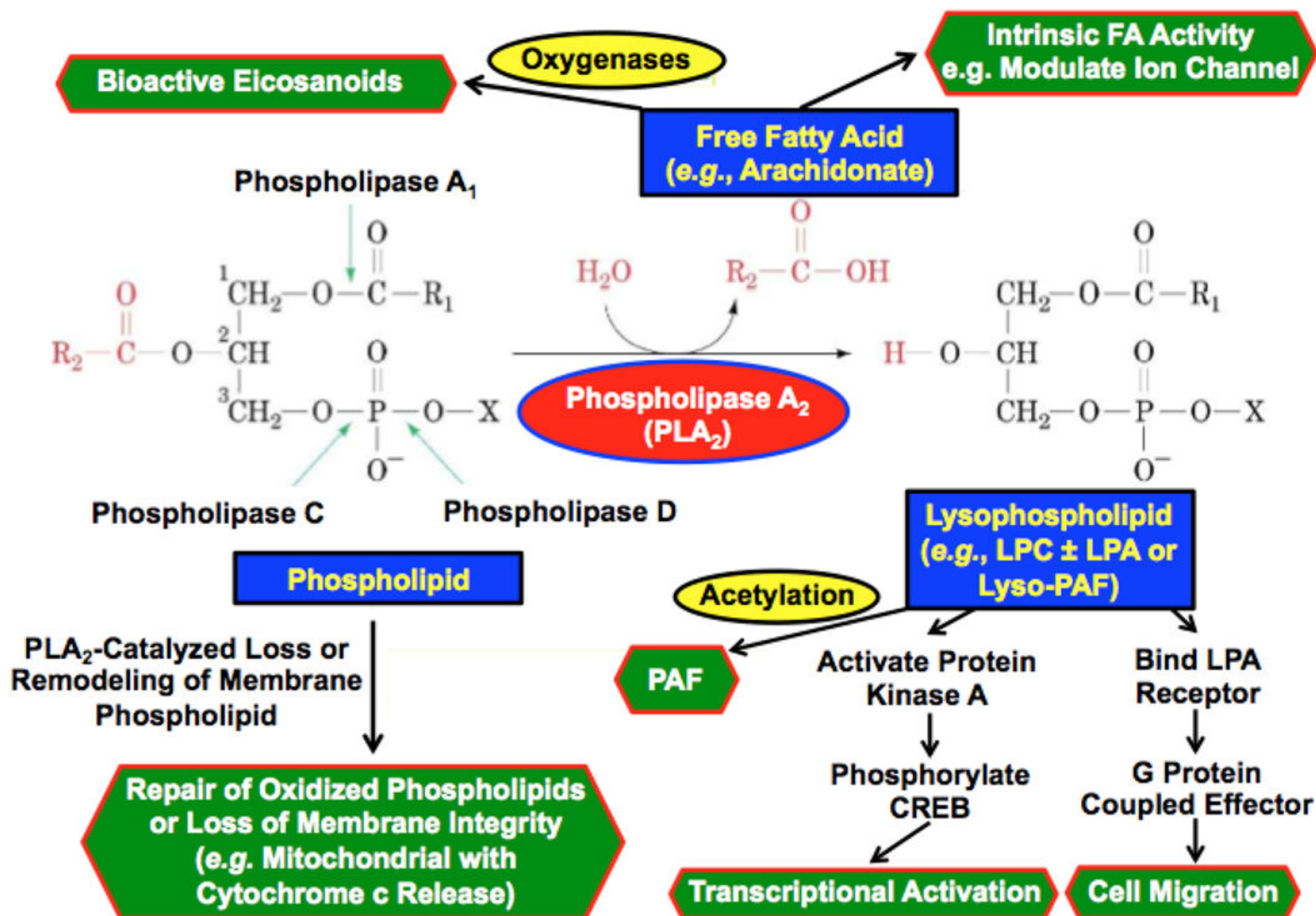

Figure 1. The phospholipase A_2 reaction and actions of its products.

Phospholipids have a glycerol backbone and an esterified phosphate moiety at the *sn*-3 position that may also be esterified to a polar head group, such as choline, ethanolamine, glycerol, serine, inositol or phosphatidylglycerol. In the *sn*-2 position, a fatty acid moiety is esterified to the glycerol backbone. In the *sn*-1 position, there may be a second esterified fatty acid residue or there may be a saturated ether linkage to a fatty alcohol residue or a vinyl ether linkage to a fatty aldehdye residue. Phospholipases C cleave the diglyceride moiety from the phospho-headgroup. Phospholipases D cleave the polar head group from the phosphatidic acid moiety. Phospholipases A_1 hydrolyze the *sn*-1 fatty acid substituent from the glycerol backbone to yield a free fatty acid and a 1-lysophospholipid, and Phospholipases A_2 (PLA_2) cleave the *sn*-2 substituent from the glycerol backbone to yield a free fatty acid and a 2-lysophospholipid. The products of PLA_2 action on the phosphatidylcholine (PC) species illustrated in the figure are arachidonic acid and 2-lysophosphatidylcholine (LPC). Downstream effects of PLA_2 action may arise from intrinsic actions of the fatty acid product or of its metabolites, such as oxygenated eicosanoids, or from actions of the lysophospholipid products or its metabolites. In the case of LPC, such metabolites could include the bioactive product lysophosphatidic acid (LPA), and an alkyl ether LPC species could also be acetylated to yield the lipid mediator Platelet Activating Factor (PAF), *e.g.*, 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. Other downstream

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effects of PLA_2 action can arise from remodeling or loss of the phospholipid substrate. With Group VIA PLA_2 ($iPLA_2\beta$) examples of downstream effects attributable to each of these possibilities are thought to occur. The free fatty acid arachidonic acid derived from $iPLA_2\beta$ action in pancreatic islet β-cells is thought to activate voltage-operated K^+ channels[83,153]. A 12/15lipoxygenase product from $iPLA_2\beta$-derived free arachidonic acid is thought to activate a RhoA kinase pathway to vascular smooth muscle cell activation in diabetic vasculopathy[123]. LPC derived from $iPLA_2\beta$ action is thought to participate in CREB-mediated activation of transcriptional responses to viral infection in macrophages[154], and LPA derived from $iPLA_2\beta$ action is thought to stimulate macrophage migration into sites of vascular inflammation in diabetes[111]. Ether linked LPC derived from $iPLA_2\beta$ action appears to provide substrate for PAF biosynthesis in inflamed endothelial cells[122,131,155]. $iPLA_2\beta$ also appears to participate in the repair of oxidized cardiolipin species in mitochondria by excising oxygenated fatty acid residues, and with overwhelming mitochondrial injury this may lead to cytochrome c release and initiation of apoptosis[67–69].

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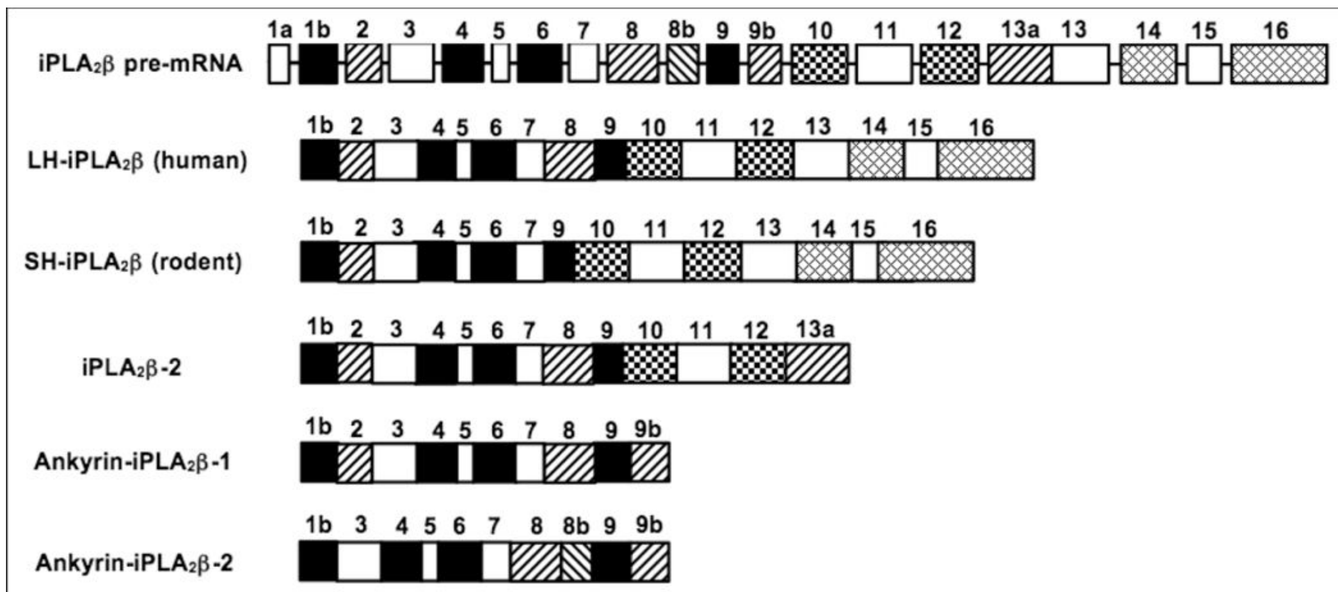

Figure 2. Schematic diagram of iPLA$_2$β pre-mRNA and transcripts derived from it by alterative splicing.

Ma *et al.* cloned the human iPLA$_2$β gene by screening a human Lambda FIX II genomic library and determined the gene structure by combining sequencing and PCR approaches. Larsson-Forsell *et al.*[28] analyzed the human iPLA$_2$β gene with two genomic clones (HS228A9 and HS447C4). Both groups found that the human iPLA$_2$β gene spans about 70 kb and consists of at least 17 exons, ranging from 74 to 811 bp in size, and 16 introns, ranging from 0.2 kb to 23 kb. Ma *et al.* designated the 5′-untranslated region (UTR) as exon 1a and considered the coding region to begin with exon 1b[27]. Larsson-Forsell designated the 5'-UTR as exon 1 and considered the coding region to begin with exon 2[28]. Exons 1b-16 in the report by Ma et al.[27] thus correspond to exons 2–17 in that by Larson-Forsell et al.[28]. The convention of Ma *et al.* [27] is used here. The human genomic clone HS447C4 contains exon 1a, and clone HS228A9 contains exon lb through exon 16. Exon 16 contains the iPLA$_2$β translational stop codon, the 3'-UTR, and the polyadenylation signal. At exon/intron boundaries, the 5'-donor and 3'-acceptor sequences conform to splicing sites consensus sequences[27,28]. Human islets and human U937 promonocytic cells express mRNA species encoding two iPLA$_2$β isoforms, designated LH-iPLA$_2$β and SH-iPLA$_2$β, respectively[27]. The latter corresponds to iPLA$_2$β isoforms cloned from rodent species[8–10]. A 162-bp in-frame insertion in the eighth AR of SHiPLA$_2$β corresponds to exon 8 of the human iPLA$_2$β gene, indicating that mRNA encoding the SHiPLA$_2$β isoform arises from an exon-skipping mechanism of alternative splicing. Splicing variants of human iPLA$_2$β have been identified from EST clones that reflect insertions of 52, 53, and 168 bp, respectively, that do not occur in the transcripts encoding LH- and SH-iPLA$_2$β isoforms[26]. These insertions arise from introns designated E8b, E9b, and E13a. The exon/intron boundary sequences of these alternative-splice sites demonstrate consensus splicing site sequences in the corresponding introns[27,28]. These alternative splicing events may yield three putative transcripts designated iPLA$_2$β2, Ankyrin-iPLA$_2$β−1, and Ankyrin-iPLA$_2$β−2 in the figure. These putative transcripts contain a polyadenylation signal encoded within exon 16 and therefore acquire a poly(A) tail required for translational competence. The iPLA$_2$β−2 transcript includes E13a

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that introduces a premature translational stop codon that results in a C-terminally truncated protein that retains the GXSXG lipase consensus sequence. The Ankyrin-iPLA$_2$β−1 transcript includes E9a, which introduces a premature translational stop codon that results in a C-terminally truncated protein that lacks the GXSXG lipase consensus sequence. The Ankyrin-iPLA$_2$β−2 transcript results from skipping exon 2 and inclusion of E8a and E9a. This transcript encodes a truncated protein that has a deletion near the N terminus and a C-terminal truncation from a premature stop codon within E9a and also lacks the GXSXG lipase consensus sequence[28].

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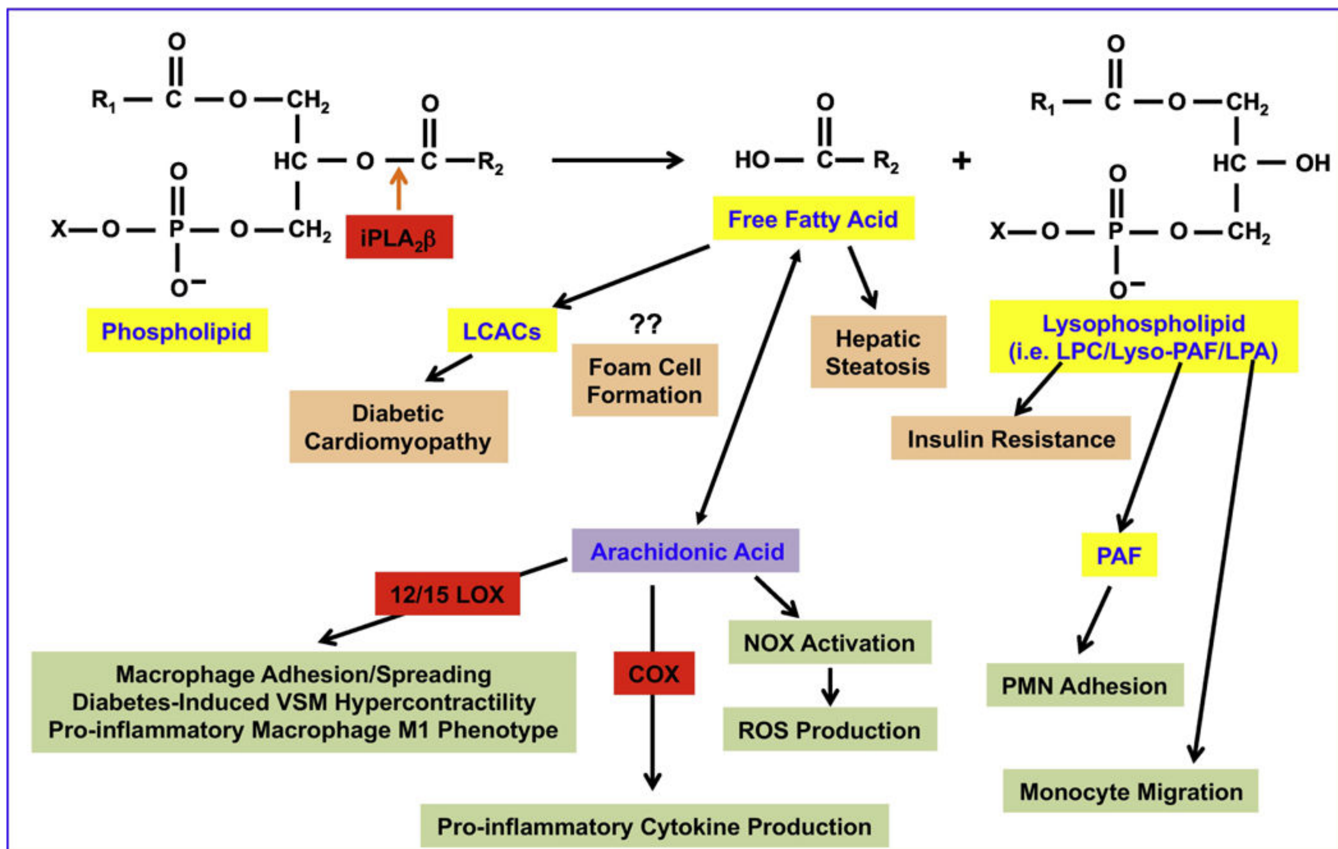

Figure 3. Participation of iPLA$_2$β-derived lipids in metabolic and inflammatory processes.
Activation of iPLA$_2$β leads to the release of the *sn*-2 fatty acid and and production of a lysophospholipid. The free fatty acids and LPC have been linked to metabolic disorders including cardiac abnormalities associated with diabetes, hepatic steatosis, foam cell formation, and insulin resistance. Further, arachidonic acid and 12/15-LOX- and COX-derived metabolites of arachidonic acid, along with PAF and LPA, have been implicated in several inflammatory processes.

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Table 1.

Patatin-Like Phospholipase (PNPLA) Protein Family Members

PNPLA Member	*PLA_2 Group*	*$iPLA_2$ Greek Letter*	*Other Designations*	*Tissues*	*Possible Functions*	*References*
PNPLA1	Not Applicable	Not Applicable	Not Applicable	Epidermal Keratinocytes	Acylceramide Synthase	144,145
PNPLA2	Group VIE PLA_2	$iPLA_{2\zeta}$	TTS-2.2, Adipose Triglyceride Lipase (ATGL), Desnutrin	Adipose, Heart	First step of TAG hydrolysis	6
PNPLA3	Group VID PLA_2	$iPLA_2\epsilon$	Adiponutrin	Adipose, Liver	May Regulate Hepatic Fat Content	6,146
PNPLA4	Group VIF PLA_2	$iPLA_{2\eta}$	GS2	Keratinocytes, Adipose, Liver, Muscle, Heart	May Regulate Cell all-*trans* retinoic acid levels	6
PNPLA5	NA	NA	GS2-like	Adipose, Lung, Brain, Pituitary	Unknown	6
PNPLA6	Group VIC PLA_2	$iPLA_{2\delta}$	Neuropathy Target Esterase (NTE)	Nervous System	Lysophospholipase Activity Maintains Axonal Integrity	6
PNPLA7	NA	NA	NTE-Related Esterase (NRE)	Adipose, pancreas, muscle, prostate	Lysophospholipase Activity May Modulate Energy Metabolism	6
PNPLA8	Group VIB PLA_2	$iPLA_2\gamma$	NA	Heart Predominant, Nervous System, Adipose, Other Tissues	Mitochondrial Bioenergetics Modulation	6,147–152
PNPLA9	Group VIA PLA_2	$iPLA_2\beta$	NA	Ubiquitous, Especially Testes, Nervous System, Pancreatic Islets	Spermatozoa Motility, Acrosome Exocytosis, Insulin Secretion, Apoptosis, Autophagy, Modulating Ion Channels, Axon Maintenance, Mitochondrial Repair	6