thematic review series

Thematic Review Series: Phospholipases: Central Role in Lipid Signaling and Disease

Calcium-independent phospholipases A_2 and their roles in biological processes and diseases

Sasanka Ramanadham,[1,*,†] Tomader Ali,[§] Jason W. Ashley,[**] Robert N. Bone,[*,†] William D. Hancock,[*,†] and Xiaoyong Lei[*,†]

Cell, Developmental, and Integrative Biology,* Comprehensive Diabetes Center,† and Undergraduate Research Office,§ University of Alabama at Birmingham, Birmingham, AL 35294; and Department of Orthopaedic Surgery,** University of Pennsylvania, Philadelphia, PA 19104

Abstract Among the family of phospholipases A_2 (PLA_2s) are the Ca^{2+}-independent PLA_2s ($iPLA_2$s) and they are designated group VI $iPLA_2$s. In relation to secretory and cytosolic PLA_2s, the $iPLA_2$s are more recently described and details of their expression and roles in biological functions are rapidly emerging. The $iPLA_2$s or patatin-like phospholipases (PNPLAs) are intracellular enzymes that do not require Ca^{2+} for activity, and contain lipase (GXSXG) and nucleotide-binding (GXGXXG) consensus sequences. Though nine PNPLAs have been recognized, PNPLA8 (membrane-associated $iPLA_2\gamma$) and PNPLA9 (cytosol-associated $iPLA_2\beta$) are the most widely studied and understood. The $iPLA_2$s manifest a variety of activities in addition to phospholipase, are ubiquitously expressed, and participate in a multitude of biological processes, including fat catabolism, cell differentiation, maintenance of mitochondrial integrity, phospholipid remodeling, cell proliferation, signal transduction, and cell death. As might be expected, increased or decreased expression of $iPLA_2$s can have profound effects on the metabolic state, CNS function, cardiovascular performance, and cell survival; therefore, dysregulation of $iPLA_2$s can be a critical factor in the development of many diseases. This review is aimed at providing a general framework of the current understanding of the $iPLA_2$s and discussion of the potential mechanisms of action of the $iPLA_2$s and related involved lipid mediators.—Ramanadham, S., T. Ali, J. W. Ashley, R. N. Bone, W. D. Hancock, and X. Lei. **Calcium-independent phospholipases A_2 and their roles in biological processes and diseases. *J. Lipid Res*. 2015.** 56: **1643–1668.**

Supplementary key words signaling • membrane homeostasis • arachidonic acid • eicosanoids • docosahexaenoic acid • inflammation • immune responses • alternate splicing • β-cells • central nervous system disorders • cancers

OVERVIEW OF THE GROUP VIA Ca^{2+}-INDEPENDENT PHOSPHOLIPASES A_2

The Ca^{2+}-independent phospholipases A_2 ($iPLA_2$s) are part of a diverse family of PLA_2s that hydrolyze the *sn*-2 substituent from membrane phospholipids to release a free fatty acid and a lysolipid (1, 2). These enzymes are ubiquitously expressed, and in contrast to secretory PLA_2s ($sPLA_2$s) and cytosolic PLA_2s ($cPLA_2$s), do not require Ca^{2+} for either translocation or activity. Some of the first descriptions of $iPLA_2$ activity were in the mid- to late-1980s with the identification of a plasmalogen-selective PLA_2 in

Our group's data was supported by lipidomic analyses through the Mass Spectrometry Resource at Washington University School of Medicine and Virginia Commonwealth University; human islets from and Juvenile Diabetes Research Foundation and National Institutes of Health sponsored islet distribution centers; islet morphometry and histology cores at Washington University School of Medicine and University of Alabama at Birmingham; and funding from the National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK69455), American Diabetes Association, Iacocca Family Foundation, National Science Foundation, Seed/Pilot & Feasibility awards from the University of Alabama at Birmingham (Diabetes Research Center, Comprehensive Diabetes Center, Comprehensive Cancer Center, and Center for Metabolic Bone Diseases).

Manuscript received 18 February 2015 and in revised form 28 May 2015.

Published, JLR Papers in Press, May 28, 2015
DOI 10.1194/jlr.R058701

Abbreviations: AA, arachidonic acid; Ank, ankyrin; ATGL, adipose triglyceride lipase; BEL, bromoenol lactone; CGI-58, comparative gene identification-58; COX, cyclooxygenase; $cPLA_2$, cytosolic phospholipase A_2; CT, cytidyltransferase; ER, endoplasmic reticulum; FK, fluoroketone; FKGK18, fluoroketone-based inhibitor of $iPLA_2\beta$; GPx, glutathione peroxidase; INAD, infantile neuroaxonal dystrophy; $iPLA_2$, independent phospholipase A_2; $iPLA_2\beta$, cytosol-associated Ca^{2+}-independent phospholipase A_2; $iPLA_2\beta$-KO, global $iPLA_2\beta$-null mice; $iPLA_2\gamma$, membrane-associated Ca^{2+}-independent phospholipase A_2; LO, lipoxygenase; LPA, lysophosphatidic acid; LPC lysophosphatidylcholine; NBIA, neurodegeneration with brain iron accumulation; NOD, nonobese diabetic; NSMase-2, neutral SMase-2; NTE, neuropathy target esterase; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; PNPLA, patatin-like phospholipase; PSA, prostate-specific antigen; RIP-$iPLA_2\beta$-Tg, mice which overexpress $iPLA_2\beta$ only in β-cells; *R*-BEL, enantiomer of BEL selective for $iPLA_2\gamma$; ROS, reactive oxygen species; *S*-BEL, enantiomer of BEL selective for $iPLA_2\beta$; $sPLA_2$, secretory phospholipase A_2; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein; Tg, transgenic.

[1] To whom correspondence should be addressed.
e-mail: sramvem@uab.edu

the cytosol of canine myocardium (3) that migrated with a molecular mass of 40 kDa. Analogous activity was subsequently described in insulinoma cells (4) and renal proximal tubules (5), as well as in a macrophage cell line (6). The Ca^{2+}-independent PLA_2s are designated as group VI $iPLA_2$s (7, 8) and now include seven members, as described in **Table 1**: $iPLA_2\beta$ (VIA-1 and -2), $iPLA_2\gamma$ (VIB), $iPLA_2\delta$ (VIC), $iPLA_2\varepsilon$ (VID), $iPLA_2\zeta$ (VIE), and $iPLA_2\eta$ (VIF). Three others ($iPLA_2\varphi$, $iPLA_2\iota$, and $iPLA_2\kappa$) have been recognized, but very little is known about them and they are not yet assigned to group VI (9, 10). Due to their shared homology with patatin, the $iPLA_2$s are included in the patatin-like protein family and are also referred to as PNPLAs. The $iPLA_2$s also share a consensus GXSXG catalytic motif contained within a patatin-like lipase domain. This review discusses the current understanding of the various $iPLA_2$s, starting with the less-described $iPLA_2\delta$, $iPLA_2\varepsilon$, $iPLA_2\zeta$, and $iPLA_2\eta$, followed by emerging reports relating to $iPLA_2\gamma$, and ending with the most widely examined, $iPLA_2\beta$.

$iPLA_2\delta$

The group VIC $iPLA_2\delta$ (PNPLA6), also known as neuropathy target esterase (NTE), was recognized for manifesting $iPLA_2$ and lysophospholipase activities in 2002 (11). The gene for $iPLA_2\delta$ is located at chromosome 19p13.3-13.2, and encodes a protein containing 1,366 amino acids with a molecular mass of 146 kDa and an active site at S^{1005}. Expressed predominantly in neurons, $iPLA_2\delta$ localizes to the endoplasmic reticulum (ER) and Golgi apparatus (12), and its inhibition or deletion leads to axonal degeneration. It is in this context that NTE was discovered during studies for causes of organophosphorus ester-induced paralysis in the late 1960s (13, 14). Whereas conventional KO of NTE is embryonic lethal (15), conditional KO of NTE in the CNS leads to neurodegeneration (16, 17), suggesting loss of function as a causative factor in the development of neurological diseases. However, mutations in the catalytic site of NTE lead to hereditary spastic paraplegia (18, 19), a symptom of NTE-motor neuron disorder. Clinical manifestations of NTE-motor neuron disorder are only evident when mutations are carried by both alleles, suggesting that the neurodegeneration results from production of abnormal NTE, rather than due to reduction in NTE activity (20, 21). Among the syndromes associated with mutations in PNPLA6 are: Gordon Holmes (22, 23) and Boucher-Neuhauser (23), characterized by early-onset ataxia and hypogonadism; Oliver-McFarlane (24), characterized by trichomegaly, congenital hypopituitarism, retinal degeneration, and choroidal atrophy; Laurence-Moon (24), characterized by progressive spinocerebellar ataxia and spastic paraplegia; and photoreceptor degeneration and childhood blindness (25). An NTE-related $iPLA_2$ (PNPLA7) awaits further characterization (9, 10).

$iPLA_2\varepsilon$

The group VID $iPLA_2\varepsilon$ (PNPLA3), also referred to as adiponutrin, was described in 2001 (12). The gene for $iPLA_2\varepsilon$ is located at chromosome 22q13.31 and encodes a protein containing 481 amino acids with a molecular mass of 52 kDa and an active site at S^{47}. PNPLA3 is mainly expressed in intracellular membrane fractions in hepatocytes (26) and was originally described as a nutritionally-regulated adipose-specific transcript in 3T3-L1 adipocytes (12). In addition to phospholipase activity, $iPLA_2\varepsilon$ manifests TG lipase and acylglycerol transacylase activities (27), leading to the suggestion that it facilitates energy/lipid mobilization and storage in adipocytes. In this regard, $iPLA_2\varepsilon$ correlates highly with the development and progression of nonalcoholic fatty liver disease and has been identified as a genetic determinant of liver fibrosis (28, 29). Whereas the WT *PNPLA3* exhibits lipolytic activity toward TGs, the rs738409 variant *PNPLA3*, where isoleucine^{148} is replaced by methionine (L148M), reduces the access of substrates and activity of PNPLA3 toward glycerolipids. This leads to development of macrovesicular steatosis (26, 30), simple steatosis to steatohepatitis and progressive cirrhosis (31), and hepatic fibrinogenesis by a sterol regulatory element-binding protein (SREBP)-1c-PNPLA3 pathway (32). A greater impact of the L148M variant on hepatic lipid content is unmasked in the presence of other risk factors such as obesity (33), visceral adiposity (34), increased intake of sugars (35), omega-6 PUFAs (36), glucokinase regulatory protein gene variant (37), chronic hepatitis B (38), and hepatocellular carcinoma (39). In contrast to these reports, PNPLA3 has been reported to restore lipid homeostasis (40) by mediating acylation of lysophospholipids and hydrolyzing TGs in the liver in a direct manner or by regulation by cofactors (41, 42).

TABLE 1. Listing of the group VI family of $iPLA_2$s

Group VI PLA_2s	Described	Other Names	Chromosome	Amino Acids	kDa	Ank Repeats	Localization	Active Site
VIA-1, $iPLA_2\beta$	1994 (6)	PNPLA9	22q13.1	752	85	8	Cytosol	S^{465}
VIA-2, $iPLA_2\beta$	1999 (96)	PNPLA9	22q13.1	804	88	7	Cytosol	S^{519}
VIB, $iPLA_2\gamma$	2000 (61, 62)	PNPLA8	7q31	782	90	0	Membrane	S^{483}
VIC, $iPLA_2\delta$	2002 (11)	PNPLA6, NTE	19p13.3-13.2	1,366	146	0	Neurons (ER, Golgi)	S^{1005}
VID, $iPLA_2\varepsilon$	2001 (12)	PNPLA3, adiponutrin	22q13.31	481	52	0	Liver adipocytes	S^{47}
VIE, $iPLA_2\zeta$	2004 (27, 43, 44)	PNPLA2, desnutrin, ATGL	11p15.5	504	55	0	White and brown adipocytes (lipid droplets)	S^{47}
VIF, $iPLA_2\eta$	1994 (60)	PNPLA4, gene sequence-2 (GS2)	xp22.3	253	27	0	ubiquitous	S^{43}

iPLA$_2$ζ

The group VIE iPLA$_2$ζ (PNPLA2), also known as TST2.2, desnutrin, and adipose TG lipase (ATGL), was described in 2004 (27, 43, 44). The gene for iPLA$_2$ζ is located at chromosome 11p15.5 and encodes a protein containing 504 amino acids with a molecular mass of 55 kDa and an active site at S^{47}. Similar to iPLA$_2$ε, iPLA$_2$ζ also exhibits TG lipase and acylglycerol transacylase activities (27). For optimal activity, ATGL requires the cofactor comparative gene identification-58 (CGI-58), which amplifies the hydrolase activity 20-fold (45). Mutations in CGI-58, as in Chanarin-Dorfman syndrome (46), lead to TGs in various tissues and decreases in both CGI-58 and ATGL have been reported to exacerbate myocardial steatosis and oxidative stress to promote cardiac apoptosis in a rodent T2D model (47). Analogously, ATGL deficiency in mice promotes tissue accumulation of lipids and leads to premature death due to cardiomyopathy, as a consequence of reductions in fatty acid oxidative gene expression, mitochondrial fatty acid oxidation, and reduced oxygen consumption (48). Macrophages with ATGL deficiency are more susceptible to ceramide-mediated mitochondrial dysfunction and programmed cell death (49). β-Cell-specific ATGL-deficiency has been demonstrated to lead to hyperglycemia due to impaired insulin secretion, as a consequence of increased islet TG content with lower fatty acid levels. These mice also have decreased expression of PPARδ genes that encode enzymes required in mitochondrial oxidation, and this is reflected by impaired mitochondrial respiration and ATP production needed for glucose-stimulated insulin secretion (50). While polymorphisms in *PNPLA2* are reported to highly correlate with T2D (51), the contribution of ATGL to insulin secretion and signaling has been challenged (52, 53). In addition to its links to CGI-58 and PPARδ, ATGL has been reported to interact with TNFα in adipocytes (54), estrogen receptor α (ERα) in bone marrow (55), fat-specific protein 27 (FSP27) in human adipocytes (56), sirtuin 1 (SIRT1) during β-adrenergic signaling (57), hepatic PPARα (58), AMPK during thermogenesis (59), and to be a candidate for transcriptional control by PPARγ-mediated signals (54).

iPLA$_2$η

The group VIF iPLA$_2$η (PNPLA4), also known as gene sequence-2 (GS2), was described in 1994 (60). The gene for iPLA$_2$η is located at xp22.3 and encodes a protein containing 253 amino acids with a molecular mass of 27 kDa and an active site at S^{43}. Similar to iPLA$_2$ε and iPLA$_2$ζ, iPLA$_2$η exhibits TG lipase and acylglycerol transacylase activities (27). Though expression of iPLA$_2$η in a variety of tissues (liver, brain, skeletal muscle, lung, placenta, kidney, and pancreas) was identified in 1994, and more recently in adipose tissue (27), to date, very little is known about its biology or its role in metabolic diseases. Similar to iPLA$_2$ε and iPLA$_2$ζ, iPLA$_2$η activation is proposed to contribute to regulation of anabolic and catabolic fluxes of acyl equivalents in tissues. It has been suggested that the TG lipase activity of iPLA$_2$ε, iPLA$_2$ζ, and iPLA$_2$η play roles in serum fatty acid accumulations associated with metabolic syndrome and T2D. A related GS2-like iPLA$_2$ (PNPLA5) has yet to be characterized (9, 10).

iPLA$_2$γ

The group VIB iPLA$_2$γ (PNPLA8) genomic organization and mRNA sequence were first described in a variety of tissues (skeletal muscle, heart, placenta, brain, liver, and pancreas) in 2000 (61) and later in the same year in lymphocytes (62). The gene for iPLA$_2$γ is located at 7q31 and encodes a protein containing 782 amino acids with a molecular mass of 90 kDa and an active site at S^{483}. Recognition of the similarity in the catalytic domain between human iPLA$_2$γ, cPLA$_2$, and plant PLA$_2$ patatin and conservation of sequence surrounding Asp^{627}, and noting that substitution of alanine for either Ser^{483} of Asp^{627} caused loss of iPLA$_2$γ activity, led to the suggestion that the Ser-Asp dyad constitutes the active site in human iPLA$_2$γ (63). Initially recognized as membrane associated (61, 62), dual-competing subcellular localization signals have been identified in discrete isoforms of iPLA$_2$γ (64) that promote its accumulation and expression of activity in the peroxisomes and mitochondria (65), leading to the suggestion that iPLA$_2$γ plays a role in integration of lipid and energy metabolism. Further, iPLA$_2$ activity in the ER of rabbit and rat kidney (66) and ventricular myocyte membranes (67) has been demonstrated to be due to iPLA$_2$γ.

The iPLA$_2$γ protein contains four methionine residues that can act as potential translational initiation sites (60, 63) to generate the full-length (~88 kDa) and three truncated products (77, 74, and 63 kDa). Attempts at expression of the truncated products in HEK293 cells, however, led to the predominant expression of the 63 kDa product (68), the isoform reported earlier to be expressed in peroxisomes (64). Further examination of parental cells revealed that the 63 kDa isoform was much more abundant than the full-length iPLA$_2$γ in HEK293 and human colorectal cancer cell lines, HCA-7 and WiDr, while in human bronchial epithelial (BEAS-2B) and rat fibroblastic (3YI) cells, the full-length iPLA$_2$γ was the predominant isoform (68). These authors suggested that iPLA$_2$γ potentiates arachidonic acid (AA) release from various subclasses of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) to increase prostaglandin E$_2$ (PGE$_2$) production via cyclooxygenase (COX)-1 and -2, and this contributes to cell growth and tumorigenesis. In contrast, comparative substrate preference studies revealed that unlike cPLA$_2$, which generates predominantly 1-palmitoyl lysophosphatidylcholine (LPC) and AA from 1-palmitoyl-2-arachidonoyl-*sn*-phosphatidylcholine hydrolysis, and iPLA$_2$β, which exhibits mixed PLA$_1$/PLA$_2$ activities and generates 1-palmitoyl LPC at an initial 3-fold rate greater than 2-arachidonoyl LPC, iPLA$_2$γ overexpressed in and purified from Sf9 cells hydrolyzed saturated and monounsaturated fatty acids at equal rates from the *sn*-1 or *sn*-2 position in diacyl PC substrates. However, it was less effective in releasing

PUFAs from the *sn*-2 position, as reflected by generation of 2-arachidonoyl LPC at a 10-fold faster rate than 1-palmitoyl LPC (69).

Understanding the role of iPLA$_2$γ-derived lipid signals has substantially advanced following the generation of mice with tissue-specific overexpression or global KO of iPLA$_2$γ. Cardiac-specific overexpression of iPLA$_2$γ presented multiple phenotypes that included reductions in myocardial phospholipid mass in fasted and fed states, accumulation of TGs with caloric restriction, acute fasting-induced hemodynamic dysfunction (that was accompanied by loosely packed and disorganized mitochondrial cristae), and elevated levels of 2-arachidonoyl-*sn*-glycero-3-phosphocholine and 2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (65). These findings were associated with increased expression not of the full-length, but of the 70 and 63 kDa iPLA$_2$γ isoforms. Impairment in mitochondrial function is also evidenced in iPLA$_2$γ-null mice, which exhibit growth retardation, cold intolerance, and increased mortality due to aortic stress that were associated with decreased myocardial function and O_2 consumption (70). The iPLA$_2$γ-null mice also become resistant to Western diet-induced increases in body weight, adiposity, circulating levels of cholesterol, glucose, and insulin; and insulin resistance, and glucose intolerance (71). Ability to utilize fat and carbohydrates is also affected in these mice in association with severe impairment in skeletal muscle mitochondrial oxidation of fatty acids. Subsequent studies revealed that marked decreases in cardiolipin molecular species containing 22:6 were an underlying cause for the mitochondrial uncoupling evident in the iPLA$_2$γ-null mice (72). Similarly, hippocampal phospholipid metabolism was found to be severely compromised with iPLA$_2$γ-deficiency leading to a mitochondrial neurodegenerative disorder characterized by degenerating mitochondria, autophagy, and cognitive dysfunction, that was associated with alterations in the compositions and content of PCs, PEs, oxidized PEs, and ceramides and a shift in cardiolipins to shorter chain molecular species (73). Increased lipid peroxidation was also evident in the skeletal muscles of iPLA$_2$γ-null mice, which exhibited abnormal mitochondrial function, oxidative stress, growth retardation, and loss of skeletal muscle structure and function (74). These findings are consistent with a previous report of the protective effects of iPLA$_2$γ, deduced using a selective inhibitor of iPLA$_2$γ against oxidant-induced lipid peroxidation and necrosis of renal proximal tubular cells (75). In contrast to the earlier report in the heart (65), the full-length iPLA$_2$γ was the major isoform detected in the last two studies (74, 75).

The identification of iPLA$_2$γ in the mitochondria and ER paved the way for studies that demonstrated a protective role for iPLA$_2$γ against cell death. Utilizing knockdown protocols, global iPLA$_2$γ-null mouse model, or by selective inhibition with bromoenol lactone (BEL) (suicide substrate) of iPLA$_2$γ (*R*-BEL) versus iPLA$_2$β (*S*-BEL), several studies reported protective effects of iPLA$_2$γ against oxidant- and cytokine-induced cell death. Human astrocytes exposed to hydrogen peroxide or tert-butyl hydroperoxide exhibited cell death, and pretreatment with *S*-BEL, but not *R*-BEL, amplified loss of ATP levels and cell necrosis (76). Similarly, knockdown of iPLA$_2$γ in renal proximal tubular cells resulted in increased susceptibility to oxidant-induced cell death, elevations in lipid peroxidation, and uncoupled oxygen consumption (77, 78). Analogous findings were reported in INS-1 insulinoma cells, where knockdown of iPLA$_2$γ promoted increases in cytokine- and oxidant-induced membrane peroxidation and apoptosis (79). Cytoprotective effects of iPLA$_2$γ were also demonstrated in glomerular epithelial cells, and these were attributed to iPLA$_2$γ-mediated upregulation of ER chaperones (80). A general conclusion derived from these studies is that the lack of iPLA$_2$γ decreases substrate availability for reacylation, leading to increases in lipid peroxidation. In apparent contrast to these reports, genetic ablation of iPLA$_2$γ or its inhibition with *R*-BEL attenuated calcium-, reactive oxygen species (ROS)-, or oxidized lipid-mediated increases in liver mitochondrial swelling, mitochondrial permeability transition pore opening, and cytochrome *c* release from mitochondria, which trigger the intrinsic apoptotic pathway (81, 82).

The more recent description of iPLA$_2$γ, to date, has limited wide studies of its role in clinical diseases, but a few reports suggest a role for iPLA$_2$γ in certain clinical-related disorders. Chagas' disease is caused by protozoan parasite *Trypanosoma cruzi*, which infects cardiac myocytes promoting release of inflammatory mediators such as eicosanoids. Inhibition of iPLA$_2$γ attenuated AA and PGE_2 release and platelet-activating factor (PAF) production from HL-1 cardiac myocytes infected with *T. cruzi* (83), and these effects were alleviated by pretreatment with *R*-BEL. Consistent with a protective role of iPLA$_2$γ in this process, the survival rates were lowered and tissue parasitism amplified in *T. cruzi*-infected iPLA$_2$γ-null mice, suggesting that iPLA$_2$γ activity affords protection against acute state cardiomyopathy in Chagas' disease (83). In contrast, iPLA$_2$γ-deficiency has been shown to increase bleeding time and provide resistance to thromboembolism (84), raising the possibility of targeting iPLA$_2$γ for antithrombotic drug development. To date, the only reported clinical manifestation relating to iPLA$_2$γ is a report that its absence is associated with myocardial dysfunction, cognitive defects, and mitochondrial degeneration (85) in a case study that closely parallels the phenotype in iPLA$_2$γ-null mice (73).

iPLA$_2$β

The group VIA iPLA$_2$β (PNPLA9) is the most widely described of the iPLA$_2$s and expression of its activity was first described in P388D1 macrophage-like cells in 1994 (6) and later shown to be the same enzyme (86) as that cloned from Chinese hamster ovary cells in 1997 (86–88). Unlike cPLA$_2$, which exhibits preference for hydrolysis of AA from the *sn*-2 position (89), the iPLA$_2$s demonstrate no substrate specificity and manifest PLA$_2$/PLA$_1$, lysophospholipase (90, 91), transacylase (27, 91), and thioesterase (92, 93) activities. The extensively studied iPLA$_2$β was

cloned from hamster (87), mouse (86), and rat (94), and they represent species homologs that are 85 kDa proteins (752 amino acids) with a serine lipase consensus sequence (GTSGT), preceded by eight N-terminal ankyrin (Ank) repeats (87, 94).

A homologous 88 kDa iPLA$_2$β was cloned from human lymphocyte lines and testis (95) that contains a 54-amino acid insert interrupting the eighth Ank repeat. Subsequent analyses with human pancreatic islet mRNA identified cDNA species that encoded two distinct 85 kDa (VIA-1) and 88 kDa (VIA-2) human iPLA$_2$β isoforms (96). Analogous transcripts were also identified in human promonocytic U937 cells. The human iPLA$_2$β gene resides on chromosome 22 in region q13.1 and contains 16 exons in the VIA-2 transcript. Exon 8 is not present in the VIA-1 transcript, indicating that it arises by an exon-skipping mechanism of alternative splicing.

Additional alternate splicing events generate iPLA$_2$β variants that differ in their subcellular localization, catalytic activity, and likely cellular function (95). Splice variants (Ank-1 and Ank-2) encode premature stop codons due to alternatively spliced exon 10a. The proteins encoded by these splice variants, VIA Ank-1 (53 kDa, ~479 amino acids) and VIA Ank-2 (47 kDa, ~427 amino acids), terminate after the seventh Ank repeat domain and before the active site, whereas VIA-3 (~70 kDa, 640 amino acids) terminates just after the lipase active site. Two additional active iPLA$_2$β isoforms have been recognized to arise from proteolytic cleavage: a ~63 kDa isoform (VIA-4, 623 amino acids) arising from caspase-3-catalyzed cleavage at the N terminal (97, 98) and a ~70 kDa (VIA-5, ~640 amino acids) isoform arising from C-terminal cleavage (99). Proteomic analyses by mass spectrometry further reveal that the iPLA$_2$β is a candidate for numerous truncations at the N-terminal end (100, 101), but the activities and biological roles manifested by these products have not yet been discerned.

Basic characteristics of iPLA$_2$β

In addition to the Ank repeats, the iPLA$_2$β protein contains an ATP binding consensus motif (GGGVKG), an N-terminal caspase-3 cleavage site (DVTD), and a putative bipartite nuclear localization sequence (KREFGEHTKMTDVKKPK). Though under basal conditions iPLA$_2$β is predominantly localized in the cytosol (102), translocation of iPLA$_2$β to the Golgi, ER, mitochondria, and nucleus is evident under stimulatory conditions (98, 100, 103–110). The iPLA$_2$β and iPLA$_2$γ share signature ATP binding motif, serine lipase site, and a region of 9 amino acids (627-635 in iPLA$_2$γ), whose biological significance is not known, but otherwise lack any additional homology (61, 87).

Modulation of iPLA$_2$β

Oligomerization. A unique distinction between iPLA$_2$β and other PLA$_2$s is the presence of a variable number of Ank repeats in iPLA$_2$β, which are absent in other PLA$_2$s. Several lines of study suggest that the Ank regions confer iPLA$_2$β protein activity. The active form of iPLA$_2$β appears to be an oligomer of interacting protein subunits, as supported by radiation inactivation and gel filtration chromatography analyses that reveal association of the 85 kDa iPLA$_2$β activity with an apparent molecular mass of 250–350 kDa (6, 87, 111). This has led to the speculation that the active form of iPLA$_2$β is an oligomer of 85 kDa subunits and that the subunits associate with each other via their Ank repeat regions (87), similar to the involvement of Ank repeats in other protein-protein interactions (112). Consistent with this possibility are the observations that iPLA$_2$β deletion mutants lacking the Ank repeat domain, but retaining the catalytic domain, are catalytically inactive (87) and that activity of the full-length protein is reduced when it is coexpressed with truncated iPLA$_2$β-like proteins that retain the Ank repeat domain, but lack the catalytic domain (95). In the long isoform of human iPLA$_2$, a proline-rich insert interrupts the last iPLA$_2$β Ank repeat with some similarities to the Smad4 domain that mediates interactions with signaling partners (113). This raises the possibility that the proline-rich insert in human iPLA$_2$β allows it to interact with proteins not recognized by the short isoform of iPLA$_2$β.

Oxidation. It has been suggested that iPLA$_2$β inactivation can occur by a mechanism involving oxidation of sulfhydryl groups within the iPLA$_2$β (78). Subsequently, oligomerization of iPLA$_2$β in INS-1 cells in response to oxidative stress was demonstrated (114). Oxidant-induced oligomerization alters the subcellular localization of iPLA$_2$β and results in reduced release of AA, suggesting inhibition of iPLA$_2$β catalytic activity. These nonproductive oligomers are DTT-sensitive and therefore likely generated through intermolecular disulfide bonds. Like iPLA$_2$β, iPLA$_2$γ activity is suppressed by oxidants, but restored when oxidant-inhibited enzyme is treated with a reducing agent (78, 115).

Together, these studies indicate that iPLA$_2$β monomers are capable of assembling into both productive and nonproductive oligomers. The productive oligomerization is mediated through the N-terminal Ank repeat domain, while inactive oligomers are formed through intramolecular disulfide bonds.

Activation. The iPLA$_2$β protein contains a consensus nucleotide-binding motif (GGGVKG) that is homologous to those of protein kinases (61, 116). This feature mediates regulation and stabilization of iPLA$_2$β activity by ATP (6, 91, 117, 118), which is independent of enzyme phosphorylation (6, 117, 118). Also contained in iPLA$_2$β are a C-terminal 1-9-14 calmodulin-binding motif (IRKGQGNKVKK LSI) and a calmodulin-binding peptide (AWSEMVGIQYFR) (106, 116, 119). These facilitate formation of a signaling complex between iPLA$_2$β and CaMKIIβ and enhancement of both activities is evident upon their association (120). This has been offered as one explanation of why Ca^{2+} store depletion activates iPLA$_2$ (121) and may occur in vascular myocytes (90), pancreatic islet β-cells (122), and human granulocytes (123). Ca^{2+} store depletion also activates hydrolysis of arachidonate from phospholipids in differentiated human U937 promonocytic cells by a mechanism that

does not require a rise in cytosolic [Ca^{2+}] (124). The iPLA$_2$β protein also contains a consensus sequence site for caspase-3-mediated cleavage within the first Ank repeat (125). The truncated product manifests higher activity (125) and localizes to the nucleus under high glucose stimulation and prolonged stress (98, 107), suggesting that it may amplify hydrolysis of nuclear membrane phospholipids and lead to nuclear membrane lysis, or that the iPLA$_2$β and/or iPLA$_2$β-derived products accumulate in an environment that can potentially participate in transcriptional induction of favorable and nonfavorable genes.

Gene induction. The iPLA$_2$β gene contains a sterol regulatory element (SRE) (126). Under stressful conditions SREBPs are processed to mature forms of SREBPs (127–135), which translocate to the nucleus and bind to SRE. This leads to induction of iPLA$_2$β transcription and protein expression, which are suppressed in the presence of a dominant negative form of SREBP-1 (136-138). Intriguingly, the iPLA$_2$β gene exhibits remarkable cross-species homology in the promoter region, which contains putative consensus sequences for a number of stress-related transcriptional factors, suggesting that the iPLA$_2$β gene is a candidate for modulation during periods of stress. Confirmation of iPLA$_2$β induction by these stress-related transcriptional factors will lead to a better understanding of the role of iPLA$_2$β in stress responses and disease manifestation.

iPLA$_2$β inactivation. Inhibitors of iPLA$_2$β include arachidonyl trifluoromethyl ketone (AACOCF$_3$), methyl arachidonyl fluorophosphonate, and palmitoyl trifluoromethyl ketone (PACOCF$_3$), which are sometimes used for "selective" inhibition of cPLA$_2$ (139–141). While siRNAs directed at iPLA$_2$β and, now available, iPLA$_2$β-KO and iPLA$_2$β-Tg mice (142–144) have provided insight into biological processes impacted by iPLA$_2$β, the majority of studies to assess the role of the iPLA$_2$β isoform have utilized a selective inhibitor of iPLA$_2$ (145). This inhibitor, (E)-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one, was synthesized in 1991 and was originally designated as a haloenol lactone substrate (146), but is now referred to as BEL. The BEL compound is an irreversible inhibitor that selectively targets iPLA$_2$ enzymes and has little or no effect on cPLA$_2$ or sPLA$_2$ activity at concentrations that inhibit iPLA$_2$β or iPLA$_2$γ (27, 146, 147). Over the years, BEL has been used to discern the involvement of iPLA$_2$ in biological processes and, to date, is still considered the only available specific irreversible inhibitor of iPLA$_2$. Because the *S*- and *R*-enantiomers of BEL exhibit selective inhibition of iPLA$_2$β and iPLA$_2$γ, respectively (148), comparison of outcomes using racemic and enantiomers of BEL facilitates distinction of effects due to the β versus the γ isoform. Studies of the mechanism of inhibition reveal that the binding of BEL to iPLA$_2$β leads to generation of a diffusible bromoketomethyl acid, which promotes covalent modification of cysteine residues and not the active site serine of iPLA$_2$β (139, 146, 149, 150). In the presence of DTT, C651 was the only cysteine residue that was modified by BEL, leading to the suggestion that DTT protects iPLA$_2$β from inactivation by BEL. However, using different isolation protocols and higher iPLA$_2$β-specific activity, an additional interaction between C651 and active site serine, S465, was suggested to account for substantial BEL-mediated inhibition (151). While the use of BEL continues to enhance our knowledge of iPLA$_2$β-mediated effects, its irreversible inhibitory profile, potential cytotoxicity (152, 153), and several examples of inhibition of nonPLA$_2$ enzymes (11, 27, 152, 154, 155) render it unfeasible for in vivo iPLA$_2$ inhibition.

To improve selectivity and reduce toxicity, other compounds are being developed and fluoroketone (FK)-based inhibitors are proving to be as potent as BEL, while being more selective for iPLA$_2$β and also exhibiting reversible inhibitory kinetics (1, 156, 157). Because FK inhibitors target serine active sites, they could potentially also inhibit cPLA$_2$s. However, modification of the FK group along with addition of a hydrophobic terminus, connected by a medium-length carbon chain to mimic the fatty acid chain, conferred selectivity of the FK compounds for iPLA$_2$ versus sPLA$_2$ or cPLA$_2$ (157), and earlier generation FK compounds (FKGK11 and FKGK2) were found to be beneficial in an experimental autoimmune encephalomyelitis animal model of multiple sclerosis (158). Subsequently, the FK-based inhibitor of iPLA$_2$β (FKGK18) was found to be 7-fold more potent than FKGK11 toward iPLA$_2$β; 195 and >455 times more potent for iPLA$_2$β than for group IVA cPLA$_2$ and group V sPLA$_2$, respectively (159); and effective in cell-based studies (160) and in countering T1D (161). Recently developed and awaiting characterization is an even more selective inhibitor (GK187) of iPLA$_2$β (162). On-going deuterium exchange mass spectrometry and molecular dynamics analyses suggest that FKGK inhibitor binding to iPLA$_2$β causes changes in the loops surrounding the active site of iPLA$_2$β in the catalytic domain, blocking access to phospholipid substrates and reducing solvent accessibility (163). As the development of chemical inhibitors continues, newer structurally dissimilar and smaller compounds (164) with even greater selectivity for iPLA$_2$β are forthcoming, as described at the 6th International Conference on PLA$_2$s in 2015 (Kokotos et al., unpublished observations).

Proposed roles for iPLA$_2$β

Membrane remodeling. One of the earliest proposed functions for iPLA$_2$β was a "housekeeping" role that involves generation of lysophospholipid acceptors for incorporation of AA into phospholipids, based on experiments involving inhibition of iPLA$_2$β activity in P388D1 cells with BEL or with an antisense oligonucleotide (165, 166). Inhibition of iPLA$_2$β activity in P388D1 cells suppressed (∼60%) incorporation of [^{3}H]AA into phospholipids while reducing (∼60%) [^{3}H]LPC levels in [^{3}H]choline-labeled P388D1 cells. However, [^{3}H]palmitic acid incorporation was only slightly reduced. This is thought to represent the mechanism whereby iPLA$_2$β inhibition reduces incorporation of [^{3}H]AA into P388D1 cell phospholipids. Such incorporation reflects a deacylation/reacylation cycle (167) of phospholipid remodeling rather than de novo synthesis (168), and the level of LPC acceptors is thought to limit

the rate of [^{3}H]AA incorporation into P388D1 cell PC (165, 166).

A second housekeeping function for iPLA$_2$β is suggested from studies with CTP:PC cytidyltransferase (CT)-overexpressing Chinese hamster ovary cells (169). CT catalyzes the rate-limiting step in PC biosynthesis via the Kennedy pathway, and cells overexpressing CT exhibit increased rates of PC biosynthesis and degradation and little net change in PC accumulation (169). Immunoreactive iPLA$_2$β protein and activity increase in the CT overexpressors and the increased PC degradation is prevented by BEL, suggesting that iPLA$_2$β is upregulated in response to CT overexpression (169). In general, this could represent an important role for iPLA$_2$β in cell biology because PC biosynthesis is involved in regulation of cell cycle and apoptosis (170).

More recently, in a study examining the effects of lipotoxicity in β-cells, the monolysocardiolipin content was reported to correlate with iPLA$_2$β expression level (171). The authors suggested that iPLA$_2$β contributed to cardiolipin remodeling by excising oxidized PUFA residues from cardiolipin to yield monolysocardiolipin species for reacylation with unoxidized C18:2-CoA to regenerate the native cardiolipin structure and function. This facilitated stabilization of association of cytochrome *c* with mitochondrial membranes and decreasing its appearance in the cytosol, thereby reducing ROS-mediated apoptosis. The authors concluded that participation of iPLA$_2$β in such an excision-reacylation mechanism of repair of oxidized phospholipids represents a special case of the originally proposed function of the enzyme in phospholipid remodeling.

Cell proliferation. Studies utilizing chemical inhibition or genetic modification protocols reveal a positive correlation between maintenance of iPLA$_2$β activity and cell proliferation. In the presence of BEL, human promonocytic U937 (172) and ovarian carcinoma (173) cells exhibit a decreased rate of proliferation and this is rescued in Caco-2 (174) and endothelial (175) cells by addition of AA. Consistently, knockdown of iPLA$_2$β suppresses and overexpression of iPLA$_2$β accelerates proliferation of insulinoma cells (176, 177). Further, while proliferation of vascular smooth muscle cells from iPLA$_2$-null mice is severely reduced, it is reversed upon addition of AA or PGE$_2$ (178). Other studies suggest that iPLA$_2$β is required for cell cycle progression, through both p53-dependent and -independent mechanisms (173, 175, 179–181). The molecular mechanism whereby iPLA$_2$β promotes cell cycle progression and proliferation remains unclear, but is likely to be related to bioactive lipid mediators that are generated by the enzyme. For example, the products of iPLA$_2$β activity may activate genes involved in cell division (87, 173, 182, 183). Arachidonate and eicosanoids have been linked to iPLA$_2$β-dependent proliferation (174, 175). Ovarian cancer cells produce lysophosphatidic acid (LPA) in an iPLA$_2$β-dependent manner, and this potent mitogen acts in an autocrine fashion to induce proliferation and migration (173, 182). These observations imply that regulation of iPLA$_2$β activity may need to be considered in the context of countering tumorigenesis.

Bone formation. AA and its metabolites are important mediators of bone remodeling. The 5-lipoxygenase (LO) products, leukotrienes and 5-HETE, function as negative modulators of bone formation by inhibiting osteoblast differentiation and bone formation (184). In contrast, PGE$_2$ enhances bone formation and mass by increasing osteoblast replication and differentiation and/or by inhibiting osteoclastic resorption (185–188), although high concentrations of PGE$_2$ can stimulate bone resorption (189). Dietary supplementation with AA promotes increases in bone mass and volume (190, 191), reflecting a beneficial role of eicosanoids in bone formation. iPLA$_2$β-null female mice exhibit an age-related low bone/high bone adiposity phenotype that is independent of changes in estradiol levels (192). Osteoblasts and adipocytes share a common mesenchymal stem cell origin, and treatment of WT bone marrow stromal cells with BEL recapitulates the in vivo phenotype in promoting differentiation of the cells in favor of adipocytes away from osteoblasts (192). The higher adiposity in the bone marrow would be expected to compromise bone integrity, as in osteoporosis or diabetes, and this indeed is evidenced in the iPLA$_2$β-null mice, as reflected by increased fragility and decreased strength of their bones (192). These findings suggest that iPLA$_2$β-derived lipids play a critical role in deciding the fate of stem cells toward becoming osteoblasts or adipocytes, and raise the possibility that cell differentiation may be modulated by differential iPLA$_2$β activation.

Male fertility. Disruptions in PLA$_2$ are often associated with impairment in normal reproduction. For instance, deficiency in sPLA$_2$-III, which is expressed in proximal epididymal epithelium, causes defects in sperm maturation and impairment in the ability of the spermatozoa to fertilize intact eggs (193). These were associated with a compromised shift in acyl groups from oleic acid, linoleic acid, and AA to docosapentaenoic and docosahexaenoic acids during epididymal transit. Another sPLA$_2$ (group X) expressed in the acrosome of spermatozoa is released in an active form during capacitation through spontaneous acrosome reaction. Deficiency in sPLA$_2$-X promoted lower rates of spontaneous acrosome reaction and decreased in vitro fertilization efficiency (194). In contrast, only the females are affected by cPLA$_2$ deficiency and they exhibit problems with ovulation, oocyte transport, and oocyte implantation (195). Consistent with this, female patients experiencing decreased implantation rates in in vitro fertilization have reduced cPLA$_2$α expression and PGE$_2$ levels (196). Similar to absences in sPLA$_2$s, male mice with homozygous iPLA$_2$β gene disruption have impaired reproductive ability that is marked by reduced sperm motility and ability to fertilize mouse oocytes (143). Analogous phenotype is exhibited in WT spermatozoa treated with BEL. Based on earlier reports of involvement of LPC in acrosome reaction (197–199) and induction of capacitation (200), it was suggested that LPC levels are decreased

due to reduction in hydrolysis of spermatozoa membrane phospholipids in the absence of iPLA$_2$β. Females deficient in iPLA$_2$β, however, do not appear to suffer any adverse fertility consequences (143).

iPLA$_2$β and islet β-cells

The continued description of activity and related biochemistry of a Ca^{2+}-iPLA$_2$ in myocardium in the early 1990s coincided with observations that fuel secretagogue-stimulated accumulations in AA in islet β-cells occurred, in part, in the absence of Ca^{2+}. This led to the first description of a similar Ca^{2+}-iPLA$_2$ activity in pancreatic islet β-cells (102) that resided predominantly in islet β-cells, with very little such activity in islet nonβ-cells. This was followed by the first demonstration of a functional role for iPLA$_2$β activity in a biological process, where activation of iPLA$_2$β was a requisite for optimal glucose-stimulated insulin secretion from islet β-cells (201, 202), and implicated a signaling role for iPLA$_2$β. In contrast to reports using P338D1 cells, findings utilizing multiple approaches in a variety of β-cell models indicated that the iPLA$_2$β did not serve a membrane remodeling role, but rather, it had a signaling role in β-cells (105, 142, 144, 176, 177, 203–205). Further, the failure of iPLA$_2$β manipulations to modulate membrane remodeling in native macrophages (206) suggested that the housekeeping role of iPLA$_2$β may be cell-specific and is predominant in the macrophage-like cell line P338D1. However, the demonstration of iPLA$_2$β activity protecting β-cells against lipotoxicity by preserving cardiolipin content (171) raises the possibility that iPLA$_2$β may manifest an organelle- and stimuli-specific protective/remodeling role in β-cells.

Glucose-stimulated insulin secretion. The β-cell is the primary sensor of glucose and when circulating levels of glucose rise, it is transported into the β-cell where it undergoes glycolytic metabolism (207–210). During this process, ATP is generated and its binding to plasma membrane ATP-sensitive potassium (K_{ATP}) channels leads to inactivation of the channels and membrane depolarization (211–214). This activates voltage-operated Ca^{2+} channels promoting Ca^{2+} influx and a rise in cytosol [Ca^{2+}] (215–219), which is a critical signal of insulin release from secretory granules (215–217, 220). It has been recognized for a long time that fuel secretagogues, such as glucose, also induce hydrolysis of membrane phospholipids leading to accumulations in inositol 1,4,5-trisphosphate, free AA, and AA metabolites (221–225). AA, at concentrations that accumulate following stimulation with glucose, induces a rise in β-cell cytosolic Ca^{2+} concentrations, due in part to Ca^{2+} influx and Ca^{2+} release from intracellular (i.e., ER) stores (170, 202, 222, 226, 227). Whereas activation of phosphoinositide-phospholipase C requires Ca^{2+} (221), a component of the accumulation of AA and its metabolites do not (170). This led to the hypothesis that a Ca^{2+}-independent phospholipase activity may be manifested in β-cells that is activated upon glucose stimulation, and the resulting generation of AA serves to amplify the Ca^{2+} signal necessary for optimal insulin secretion. Utilization of various insulinoma cell lines, rodent and human islets, molecular biological protocols, and genetically-modified mice, collectively, provided a mechanism by which stimulation of β-cells increases hydrolysis of AA from β-cell membrane phospholipids, in parallel with insulin secretion, in an iPLA$_2$β-dependent manner (4, 105, 177, 201, 203, 204). Chemical inhibition, siRNA knockdown, or genetic ablation of iPLA$_2$β activity attenuated glucose-stimulated insulin secretion, while the opposite was evident with increased expression of β-cell iPLA$_2$β (99, 111, 142, 144, 176, 201, 203–205). As shown earlier in myocardial studies (87, 118), the predominant lipid pools that serve as substrates for iPLA$_2$β in β-cells are plasmalogens, and their abundance is decreased within minutes of glucose stimulation (4, 203, 204). Interestingly, islet β-cell membranes are enriched in AA, in particular, plasmenylethanolamine molecular species that contain AA (4, 204). The presence of such a membrane phospholipid composition lends itself to accumulations in AA in the β-cells upon activation of β-cell iPLA$_2$β. Modulation of the islet β-cell-delayed rectifier potassium channel, Kv2.1, by AA amplifies Ca^{2+} influx into the β-cells and enhances glucose-stimulated insulin secretion (142, 228). While AA itself manifests biological activity, the accompanying lysolipids and metabolites of AA, or eicosanoids, significantly impact various cellular processes to promote a multitude of effects (229, 230).

Pancreatic islets also express Ca^{2+}-dependent PLA$_2$s that are involved in the insulin secretory process. The cPLA$_2$ is much more abundant in human islets than rodent islets, but very little is expressed in insulinoma cells (147, 231–235). cPLA$_2$ is activated by influx of Ca^{2+} into the cell (232) and is subject to glucose-mediated phosphorylation (235). A variety of sPLA$_2$s (1B, IIC, IIF, XIIA/B) have also been described in whole pancreas (236), islets (233, 234, 237), and in β-cell secretory granules (234, 238, 239) from which sPLA$_2$-1B is co-released with insulin (236). When observations from these studies are taken together, a sequential participation of PLA$_2$s in the insulin secretory process is suggested. Activation of iPLA$_2$β following glucose stimulation amplifies the Ca^{2+} signal within the β-cell to promote insulin secretion, which can be returned to basal levels by inhibition of iPLA$_2$β (201, 204). Activation of cPLA$_2$ due to accumulations in intracellular Ca^{2+} may then serve to maintain insulin secretion and inhibition of cPLA$_2$ appears to reduce secretion only 50% (233). The role of sPLA$_2$ may reside in its ability to enhance the ability of plasma membranes to bind secretory granules (238, 240–242), though a role for sPLA$_2$ in inactivating the K_{ATP} channels has also been described (237). In view of expression of membrane receptors for sPLA$_2$ in β-cells (238, 243, 244), the role of sPLA$_2$s in β-cells may be to serve as feedback modulators to maintain/amplify insulin secretion.

iPLA$_2$β and β-cell death and T1D

Among the roles ascribed to iPLA$_2$β, its contribution to apoptosis was initially recognized in studies performed by Kudo's group (97, 125). Their work with human leukemic monocyte lymphoma U937 cells revealed that activation of iPLA$_2$β, as opposed to cPLA$_2$, enhanced cell death and

that iPLA$_2$β undergoes caspase-3 (apoptosis executioner)-catalyzed cleavage to generate a more active shorter isoform of iPLA$_2$β. During the same period, Polonsky's group reported that ER stress-induced death of insulinoma cells occurs independently of Ca^{2+} and is mediated by a metabolite of AA (245). Intriguingly, while short-term (minutes) stimulation of iPLA$_2$β in β-cells has a beneficial effect of enhancing glucose-stimulated insulin secretion (4, 105, 177, 201, 203, 204), long-term (hours) exposures to pro-inflammatory cytokines, hyperglycemia, and ER stress induce iPLA$_2$β at the message, protein, and activity levels in β-cells (98, 136–138, 246) and lead to deleterious consequences that ultimately cause β-cell death (98, 103, 104, 136–138, 246). β-Cell apoptosis is a critical contributor to the onset and progression of diabetes and it is in this context that our group has focused on iPLA$_2$β biology to understand the underlying molecular mechanisms by which iPLA$_2$β-derived lipids promote β-cell death during T1D development, and our current understanding is illustrated in **Fig. 1**.

iPLA$_2$β and sphingolipids. Stress stimuli induce the intrinsic (mitochondrial) apoptotic pathway, which is mitigated by suppressing iPLA$_2$β expression/activity (103, 104, 136, 138, 246). Intriguingly, the primary lipid signals promoting mitochondrial decompensation were ceramides, derived through hydrolysis of sphingomyelins by neutral sphingomyelinase-2 (NSMase-2) (103, 104). Ceramides are part of the sphingolipid family and act as lipid messengers that can suppress cell growth and induce apoptosis (247–249), and as expected, inhibition or knockdown of NSMase-2-mitigated ceramide accumulation (103, 104, 137). Unexpectedly, inhibition, knockdown, or iPLA$_2$β deficiency attenuated, and iPLA$_2$β overexpression exacerbated, NSMase-2 induction and ceramide accumulation (103, 104, 136, 138, 246). These findings indicated that NSMase-2-catalyzed ceramide accumulations during β-cell apoptosis occur via an iPLA$_2$β-dependent mechanism. A recent report suggests that iPLA$_2$β-derived AA stimulates p38 MAPK and that this may serve as an additional intervening modulator of iPLA$_2$β-mediated increase in ceramide accumulations in β-cells (250).

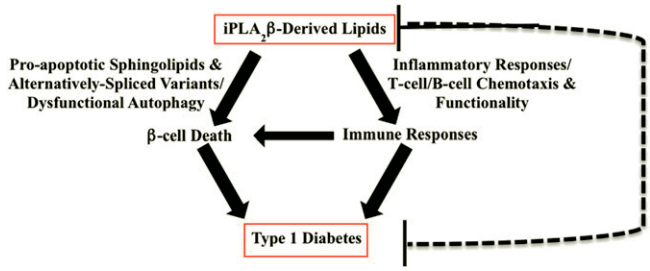

Fig. 1. Proposed roles for iPLA$_2$β-derived lipid signals in promoting β-cell death leading to T1D. Our collection of studies reveal that iPLA$_2$β activation is associated with processes that lead to β-cell apoptosis. The bioactive lipids (and their metabolites), derived from iPLA$_2$β-catalyzed hydrolysis of membrane phospholipids, are proposed to trigger: *a*) generation of pro-apoptotic sphingolipids, pro-apoptotic variants of apoptotic factors, and autophagy dysfunction to promote β-cell death; and *b*) inflammatory responses, immune cell functionality, and chemotaxis to promote immune responses, which serve to amplify the β-cell death process. We suggest that these effects of iPLA$_2$β-derived lipids, working in concert, contribute to the onset and progression of β-cell death, which eventually leads to the development of T1D.

iPLA$_2$β and alternate splicing. β-Cell apoptosis due to ER stress and pro-inflammatory cytokines is mediated through the intrinsic pathway, which is dependent on mitochondrial dysfunction and activation of caspase-9 (103, 104, 136, 138, 246, 251). The intrinsic apoptosis pathway is regulated by members of the Bcl-2 family of proteins that can be pro- or anti-apoptotic, depending on the spectrum of Bcl-2 homology domains that they contain. Among the anti-apoptotic Bcl-2 family members is Bcl-x(L), which associates with mitochondrial membranes and prevents their permeabilization, an early step in the intrinsic apoptosis pathway (252, 253). Overexpression of Bcl-x(L) has been correlated with increased survival of a variety of cells and tissues (254), including islet β-cells (255–257). Bcl-x(L)-null β-cells are hypersensitive to pro-apoptotic stimuli and reduced expression of Bcl-x(L) protein correlates with β-cell apoptosis in response to immunosuppressive drugs or high glucose (255–258). Conversely, overexpression of exogenous Bcl-x(L) protects β-cells from pro-inflammatory cytokine- and thapsigargin-induced apoptosis (258, 259).

Modulation of Bcl-x(L) expression is a complex mechanism consisting of both transcriptional and posttranscriptional processes and often leads to generation of both pro- and anti-apoptotic proteins from a single pre-mRNA (254, 260). Bcl-x(L) is the most abundant variant of the Bcl-x pre-mRNA, but other species can be generated at the expense of the mature mRNA encoding this anti-apoptotic protein (254). To date, Bcl-x RNA splicing has not been investigated in the β-cell, especially in the context of β-cell apoptosis and diabetes mellitus. We find that increased expression of iPLA$_2$β in β-cells is associated with a lower ratio of Bcl-x(L) to Bcl-x(s), while the opposite is true with iPLA$_2$β inhibition or deficiency (261). Lipidomic analyses by mass spectrometry revealed that the ratio of Bcl-x(L)/x(s) was directly proportional to the ratio of 5-HETE to EPA, suggesting that prolonged iPLA$_2$β activation in β-cells promotes generation of lipids, AA- and nonAA-derived, away from 5-HETE, thus disfavoring generation of the anti-apoptotic Bcl-x(L) variant. These findings raise the possibility that among the mechanisms by which iPLA$_2$β promotes β-cell apoptosis is one in which its activation triggers alternate splicing events favoring apoptotic processes. Of note, β-cell death due to direct activation of the mitochondrial apoptotic pathway by staurosporine is mitigated by iPLA$_2$β activity and this was related to preservation of repair mechanisms to sustain mitochondrial membrane components, such as cardiolipins (262, 263).

iPLA$_2$β and immune responses. β-Cell subcellular membranes are enriched in AA-containing phospholipids (144, 203, 204) and iPLA$_2$β activation leads to hydrolysis of AA, which can be metabolized by COX and LO enzymes to

generate eicosanoids (97, 125, 245, 264). These bioactive lipids act as paracrine and autocrine factors and greatly contribute to inflammation (264–266) and autoimmune diseases (267–274). PGE_2 has been reported to reduce debris clearance (275), and apoptotic clearance defects in non-obese diabetic (NOD) macrophages and dendritic cells are attributed to high PGE_2 levels (276, 277). It might be speculated that suppression of PGE_2 or other COX/LO-derived metabolite (278) generation can prevent the spread of β-cell apoptosis. Further, lysolipids and eicosanoids act as "chemoattractants" (230, 279–281), and increased generation of these could promote migration of immune cells toward the islet and subsequent infiltration, raising the possibility that in vivo inhibition of $iPLA_2\beta$ could mitigate immune responses leading to T1D. Autoimmune insulitis is marked by degradation of the islet basement membrane, which presents a physical barrier to infiltrating leukocytes (282). Remarkably, the abundance of infiltrating cells is significantly reduced in NOD mice treated with FKGK18 (161).

It is well-established that pancreatic islet β-cells are subject to pro-inflammatory cytokine-induced apoptosis during the pathogenesis of T1D (283). Our group has observed that in addition to inducing $iPLA_2\beta$, pro-inflammatory cytokines promote ER stress, LPC generation, caspase-3 activation, and β-cell apoptosis and these outcomes were prevented following inhibition of $iPLA_2\beta$ with *S*-BEL (136). Further, addition of the cytokines to islets overexpressing $iPLA_2\beta$ resulted in exacerbated ER stress and β-cell apoptosis, which were blunted in islets devoid of $iPLA_2\beta$ (136). Taken together, these findings provided evidence of $iPLA_2\beta$ participation in β-cell apoptosis due to pro-inflammatory cytokines. This prompted further investigation of the role of $iPLA_2\beta$ in the pathogenesis in the NOD mouse, a model of spontaneous autoimmune-mediated T1D (161). In this study, we found that following administration of $iPLA_2\beta$-selective inhibitor FKGK18, NOD mice had significantly reduced incidence of diabetes and reduced insulitis, higher circulating insulin, and preservation of β-cell mass (161). Additionally, FKGK18 reduced TNFα production from $CD4^+$ T-cells and antibodies from B-cells, independent of $iPLA_2\gamma$ activation, suggesting modulation of immune cell responses by $iPLA_2\beta$ or $iPLA_2\beta$-derived products (161). Furthermore, adoptive transfer of diabetes by $CD4^+$ T-cells to immunodeficient and nondiabetogenic NOD.*scid* mice was mitigated by FKGK18 pretreatment and TNFα production from $CD4^+$ T-cells was reduced by inhibitors of COX and 12-LO (161). These observations suggest that modulation of immune cell function may be a mechanism by which $iPLA_2\beta$ and $iPLA_2\beta$-derived lipid signals participate in autoimmune-mediated β-cell death. In support of involvement of $iPLA_2\beta$-derived lipid signals is the recent observation that 12-LO products contribute to pro-inflammatory cytokine-mediated β-cell dysfunction and apoptosis (284).

$iPLA_2\beta$ and autophagy. Whereas apoptosis is a well-studied process in β-cells, autophagy in β-cells has not received significant attention. Autophagy is a constitutively active process of cellular degradation in all cell types and is regarded as a generally protective process to prolong cell survival. However, under increased stress, dysregulation of autophagy can lead to cell death (285). In view of evidence linking ceramides with autophagy (286, 287) and our collective observations that ceramide generation can occur via an $iPLA_2\beta$-mediated mechanism, we addressed the possibility that $iPLA_2\beta$ expression modulates β-cell autophagy (246). We found that thapsigargin-induced ER stress promoted a greater conversion of LC3-I to LC3-II, reflecting activation of the autophagic response in islets from RIP-$iPLA_2\beta$-Tg mice relative to islets from age-matched WT mice. In contrast, LC3-II generation was decreased in islets deficient in $iPLA_2\beta$. Thapsigargin is thought to specifically block fusion of autophagosomes with lysosomes (288) and in the presence of inhibitors of autolysosomal activity, LC3-II accumulations in the RIP-$iPLA_2\beta$-Tg and $iPLA_2\beta$-KO mice are similar to their corresponding WT groups. Interestingly, inhibition of NSMase-2 in the presence of thapsigargin had minimal effect in WT islets, but modestly reduced LC3-II flux in the Tg. These findings suggest that differential $iPLA_2\beta$ activation can impact β-cell autophagy and that the most likely point of effect is beyond the induction step, in part, mediated by NSMase-2.

While this was the first demonstration of a link between $iPLA_2\beta$ and autophagy, it has been reported that mice deficient in $iPLA_2\gamma$ had enlarged hippocampal mitochondria, and that their degeneration led to an increase in autophagy (73). Those authors concluded that $iPLA_2\gamma$-deficiency decreased mitochondrial membrane remodeling, resulting in loss of membrane potential and subsequent mitochondrial dysfunction leading to cognitive dysfunction and increased autophagy in the hippocampus. However, more detailed studies are needed to identify the precise location of $iPLA_2\beta$ impact on the autophagic responses in β-cells.

$iPLA_2\beta$ and diseases

Neurodegenerative. PLA_2s, including $iPLA_2\beta$, are widely expressed in different regions of the brain (289–291), and $iPLA_2\beta$ immunoreactivity has been reported to be predominant in the cytosol and of less abundance in the nuclear fraction (292), suggesting a role for the enzyme and its products in nuclear signaling. $iPLA_2\beta$ has been purified from rat brain cytosol (293) and identified in dendrites and neurons, microglia, Purkinje cells, and astrocytes (290, 294–296), suggesting that it also participates in neuronal signaling. Advances in lipidomic analyses, coupled with kinetic studies in rodents and positron emission tomography protocols in humans, reveal that the turnover of AA in the brain is associated with $cPLA_2$ and COX-2 activities, whereas that of DHA (22:6n-3) was related to $iPLA_2\beta$ and COX-1 activities (297). This is consistent with the reports that in contrast to the islets, which are enriched in AA-containing glycerophospholipids, the most prominent *sn*-2 substituent in brain glycerophospholipids is DHA (297, 298), and $iPLA_2\beta$-deficiency results in reduced DHA metabolism and content in the brain (299). Considering

its ubiquitous expression in the brain, dysregulation of iPLA$_2$β, as opposed to or in association with other PLA$_2$s, has been recognized to play critical roles in a variety of neurological disorders.

Schizophrenia. Magnetic resonance spectroscopy studies reveal a higher than normal turnover of brain membrane phospholipids in patients with schizophrenia (300, 301). Whereas serum Ca^{2+}-dependent PLA$_2$ activity appears to be unchanged, iPLA$_2$β is significantly higher in patient sera relative to control subjects (300). Consistent with these findings, iPLA$_2$β activity was increased (nearly 45%) in the temporal cortex of patients and proposed to be a causative factor in abnormal fatty acid metabolism and oxidative stress in schizophrenia (302). The increased breakdown in membrane phospholipids due to higher increases in iPLA$_2$β activity, may be reflected by alterations in neuronal membrane properties leading to hypodopaminergy (303). In contrast, Ca^{2+}-dependent PLA$_2$ activity was decreased in the patients with schizophrenia (302). Another report suggests that increases in serum iPLA$_2$β activity are only evident in first-episode or acute early phase schizophrenia, and not apparent at chronic stages or in patients experiencing multi-episode schizophrenia (304). Brain samples from epilepsy patients with schizophreniform symptoms also express higher iPLA$_2$β activity (305). Genetic studies to identify PLA$_2$ polymorphisms associated with schizophrenia are, however, unclear. While allelic association between *PLA2G6* gene polymorphism has been reported, in the absence of similar links to PLA2G1A or PLA2GIIA-D (sPLA$_2$s), PLA2GIVA-C (cPLA$_2$), PNPLA3 (iPLA$_2$ε), or PNPLA8 (iPLA$_2$γ) genes (306, 307), others have reported potential association between schizophrenia and PLA2GIVA (303) and PLA2GIVC (308).

Alzheimer's disease. This disease is characterized by the polymerization of amyloid β and especially tau proteins, leading to the formation of senile plaques and neurofibrillary tangles, and such polymerization is stimulated by AA and other unsaturated fatty acids (309). All groups of PLA$_2$s have been proposed to play a role in the development of Alzheimer's disease, which is a leading cause of dementia in the elderly. For instance, sPLA$_2$s (IIA, V, IVA) are thought to be involved in neuronal death, sPLA$_2$s (III and X) with neurogenesis, and cPLA$_2$ and iPLA$_2$ in both neuronal death and neurogenesis (304). A frontal variant of Alzheimer's disease, in which there is a high occurrence of neurofibrillary tangles in the frontal cortex, is accompanied by a decrease in iPLA$_2$, but not cPLA$_2$, activity in the dorsolateral prefrontal cortex (310). This report suggests that this may be a compensatory response to accelerated phospholipid metabolism early in the disorder. Consistently, the predominant PLA$_2$ activity in rodent hippocampal slices was reported to be manifested by iPLA$_2$ and to be essential for synaptic plasticity, as reflected in iPLA$_2$ inhibition studies (311). This was later confirmed in whole animal studies (141), leading to the suggestion that reductions in cPLA$_2$ and iPLA$_2$ activities can have adverse effects on memory in patients with Alzheimer's (312). These studies, however, did not distinguish which iPLA$_2$ (β or γ) isoform activity was critical for the dementia associated with Alzheimer's. However, a more recent report suggests that the early-onset, but not the later-onset, fronto-temporal type of dementia may be linked to *PLA2G6* mutations (313). Further, sPLA$_2$, cPLA$_2$, and iPLA$_2$ activities were reported to be reduced in serum from patients with bipolar disorders, who have a 5-fold increased risk for developing Alzheimer's disease (314).

Parkinson's disease. Selective degeneration of dopaminergic neurons in the substantia nigra has been suggested to be related to low activity of phospholipid catabolic/anabolic enzymes, which may promote the oxidative membrane damage associated with Parkinson's disease that begins at 15–30 years of age (315). Many recessive loci have been linked to Parkinson's disease; however, they all do not associate with the disease. Homozygosity mapping and mutational analyses over the past 5 years reveals a complicated connection with mutations in the *PLA2G6* gene at the PARK14 locus. Initially recognized in adult-onset parkinsonism (316), *PLA2G6* gene mutations are now linked to levodopa-responsive parkinsonism with severe impairments in swallowing, dystonia, and pyramidal weakness (317, 318), and to autosomal early-onset parkinsonism (313, 318–322). However, studies among Asian populations are conflicting, where a link between *PLA2G6* mutations and Parkinson's disease was reported in Singapore (323), but not in China (324, 325) or Japan (326).

INAD and NBIA. Infantile neuroaxonal dystrophy (INAD) and neurodegeneration with brain iron accumulation (NBIA) are two additional autosomal recessive neurodegenerative diseases, which begin within 1–2 years of life and are characterized by widespread neurodegeneration that includes psychomotor regression, spasticity, and optic atrophy. INAD is characterized by the presence of axonal spheroids throughout the central and peripheral systems. NBIA patients are a subset of INAD patients with brain iron accumulation and they feature idiopathic neurodegeneration. A potential link between INAD and *PLA2G6* mutations was first reported in two unrelated Bedouin Israeli kindreds (327) and in a 2-year-old boy with psychomotor regression (328), and the pathogenesis is thought to begin prior to birth (329). The accompanying clinical INAD symptoms were found to recapitulate in subsequently generated global iPLA$_2$β-null mice (330, 331). Screening of DNA from human patients with INAD and NBIA identified 80% of INAD and 20% of NBIA patients with mutations in the *PLA2G6* gene, and neuropathologic changes that were analogous to those associated with Parkinson's and Alzheimer's diseases (332, 333). Studies using iPLA$_2$β-null mice suggest degeneration of mitochondrial inner membranes and presynaptic membranes (334), reductions in capacitative Ca^{2+} entry in astrocytes (335), point mutation in the Ank repeat producing an inactive iPLA$_2$β protein (336), and neuro-inflammation and Purkinje cell loss (337) as potential underlying mechanisms that lead to pathogenesis in neurodegenerative disorders associated with brain iron accumulations such as INAD, NBIA (338), Karak syndrome (339), and Parkinson's and Alzheimer's diseases accompanied by iron accumulations. In this regard, it should be noted that common

mutations and combination of mutations in *PLA2G6*-associated neurodegeneration may be associated with Parkinson's and Alzheimer's diseases in the absence or presence of iron accumulations (316, 339–347).

Other roles in the CNS. Additional evidence of iPLA$_2$β involvement in neurodegenerative disorder include: association of a subset of Shindler's disease in infants with *PLA2G6* mutations (348); elevations in iPLA$_2$β in patients with bipolar 1 disorder and a history of psychosis (349); neuro-inflammation and associated neuropathology with motor dysfunction in later life due to iPLA$_2$β-deficiency (350); and roles for iPLA$_2$β during early beneficial stages of myelin breakdown following peripheral nerve injury (351) and detrimental demyelination due to spinal cord injury (352), brain endothelial cell migration and proliferation (353), antidepressant-like effects of maprotiline (354), and pro-oxidative signaling related to ethanol-induced neurotoxicity (355).

Cancers. The ability of iPLA$_2$β to promote cell proliferation becomes prominent in the context of tumorigenesis. Several in vitro studies reveal higher expression of iPLA$_2$β in stimulated immortal cell lines and that chemical inhibition or siRNAs targeted against iPLA$_2$β reduces proliferation and promotes apoptosis of the cells (97, 108, 125, 356–364). Subsequent studies targeting specific cancers suggest that iPLA$_2$β promotes cancer cell growth via signal transduction pathways involving epidermal growth factor receptors, MAPKs, E3 ubiquitin-protein ligase mdm2, tumor suppressor protein p53, and cell cycle regulator p21 (365–367). In addition, there is increasing support for a role of iPLA$_2$β and iPLA$_2$β-derived LPA in promoting cancer cell proliferation and metastasis.

Ovarian. A significant number of studies have explored the impact of PLA$_2$ activation in the context of ovarian cancer development. A potential involvement of cPLA$_2$ and iPLA$_2$ activities was recognized in women with endometrial dysfunction, who had a 4-fold increase in PLA$_2$ activity (368). Studies with ovarian carcinoma cells demonstrated a role for iPLA$_2$β, but not for cPLA$_2$, in promoting S and G2/M cell cycle phases, that was independent of p53 (173). BEL prevented these effects, but they were rescued with addition of LPA, and knockdown of iPLA$_2$β inhibited cell proliferation in culture and tumorigenicity of cancer cell lines in nude mice. Consistent with these findings, tumorigenesis and ascites formation associated with the epithelial ovarian cancer cell line ID8 administration were reduced by nearly 50% in iPLA$_2$β-null mice, as were the levels of LPA and LPC (370). Such inhibition was elevated to 95% when ID8 cells in which iPLA$_2$β was knocked down were used, suggesting the importance of iPLA$_2$β activity in host and tumor cells for cancer progression. Further, LPA, but not LPC, enhanced in vivo ascites formation and tumorigenesis in the iPLA$_2$β-null mice. Similar inhibition of cell adhesion, migration, and invasion of epithelial ovarian cancer cells was demonstrated with a structurally dissimilar iPLA$_2$β inhibitor, FKGK11 (371), confirming a role for iPLA$_2$β in these processes. In contrast, reports using nonselective (372) and selective PLA$_2$ inhibitors combined with targeted genetic knockdowns (373) suggest that both cPLA$_2$ and iPLA$_2$β activities contribute to elevations in LPA and to ovarian cancer development.

Other cancers. Presently, there are limited numbers of studies linking iPLA$_2$β activity to development of other cancers; however, this area has experienced growth over the past 5 years. Comparison of LNCaP prostate cancer cells with normal prostate epithelial RWPE-1 cells revealed decreased expression of inhibitory Ank-iPLA$_2$β, but increases in expression (VIA-1 and -2) and activity of iPLA$_2$β in the cancer cells. Both inhibition of iPLA$_2$β with BEL and iPLA$_2$β knockdown reduced prostate-specific antigen (PSA) secretion from and apoptosis of the cancer cells (374). Exogenous PSA rescued inhibition of apoptosis by BEL, suggesting that iPLA$_2$β modulates PSA secretion, which in turn provides an autocrine survival function. Inhibition of iPLA$_2$β, but not iPLA$_2$γ, in p53-positive LNCaP cells was also found to be associated with activation of p38 and induction of reactive species, which leads to cell cycle arrest and cytostasis (375). Lung tumor growth in an in vivo allograft model is promoted by PRDX6, which is a bifunctional protein with glutathione peroxidase (GPx) and iPLA$_2$β activities (376). Nude mice bearing PRDX6-overexpressing lung cancer cells exhibited increases in tumor size and weight and increased expression of both GPx and iPLA$_2$β, and these outcomes were inhibited when a mutant PRDX6 was used, suggesting that PRDX6 promotes lung tumor growth via GPx and iPLA$_2$β. Occurrence of breast cancer is often accompanied by metastasis to the lung and injection of E0771 breast cancer cells in the mouse mammary pads resulted in an 11-fold higher number of breast cancer cells in WT lungs, relative to lungs from iPLA$_2$β-null mice (377). Further, production of thrombin-stimulated platelet-activating factor in lung endothelial cells was increased in the WT lungs and absent in the iPLA$_2$β-null mice. Taken together with the finding that inhibition of iPLA$_2$β, but not iPLA$_2$γ, prevented increases in PAF in WT cells suggests that iPLA$_2$β modulation of PAF production is a factor in cancer cell metastasis. Consistent with these findings, cigarette smoke extract increased PAF accumulations and increased cell motility in MDA-MB-231 breast tumor cells, and these effects were mitigated by iPLA$_2$β inhibition (378). Recently, unicortin, a member of the corticotrophin-releasing factor family, was shown to promote hepatic cancer cell line migration by upregulating cPLA$_2$ and suppress migration by downregulating iPLA$_2$β at the transcriptional level (379). Polymorphisms in *PLA2G6* have also been linked to increased risks for colorectal (380) and invasive cutaneous melanoma (381, 382).

Cardiovascular. The myocardial 40 kDa iPLA$_2$, described as a plasmalogen-selective PLA$_2$ (3, 118), was inhibited by BEL (146), stabilized by ATP (117), and formed an oligomeric regulatory complex with phosphofructokinase (384). From the collection of related studies, it can be ascertained that iPLA$_2$β activation in the heart may be beneficial and also detrimental (265, 385, 386). For instance, increases in membrane-iPLA$_2$ (387) and mitochondrial-iPLA$_2$ (388) activities were associated with irreversible cell damage

during myocardial ischemia and reperfusion; cardiomyopathy associated with HIV infection may involve an $iPLA_2$ signaling pathway (389); and $iPLA_2\beta$ activation contributing to arrhythmogenic conduction slowing due to ischemia in diabetic hearts (390) have been reported. In contrast, $iPLA_2$ activation or its preference for plasmalogens was evident during global ischemia (391). However, $iPLA_2$ activation facilitated incorporation of 18:2 into $(18:2)_4$-cardiolipin to maintain mitochondrial bioenergetics during heart failure (392), protected against oxidant-induced cardiotoxicity (393), and contributed to cardiac endothelial cell production of PGI2, which manifests protective effects in the heart (394). In Barth syndrome, a metabolic disorder caused by mutations in the mitochondrial transacylase tafazzin that is characterized with disturbances in cardiolipin abundance and molecular species, cardiolipin deacylation by $iPLA_2\beta$, but not $iPLA_2\gamma$, is thought to play a role (395, 396). There remains some uncertainty about which $iPLA_2$ isoform is expressed in the myocardium, with both $iPLA_2\gamma$ (385) and $iPLA_2\beta$ (265) reported to be the predominant isoform, and their relative contribution to myocardial PLA_2 activity.

$iPLA_2\beta$ has also been implicated in activation of store-operated Ca^{2+}-channels and Ca^{2+} release-activated Ca^{2+}-channels in a variety of cells, and it has been suggested that an endogenous calcium influx factor activates $iPLA_2\beta$ when applied to cell homogenates (397–399). In this context, inhibition of $iPLA_2\beta$ reduces endothelial agonist-induced intracellular Ca^{2+} release and extracellular Ca^{2+} influx, suggesting that $iPLA_2\beta$ is an important mediator of vascular relaxation (400).

Inflammatory and autoimmune. $iPLA_2\beta$ activity has been reported in many components of the immune system, including macrophages (6, 401–404), monocytes (405), neutrophils (406, 407), mast cells (408), and T-cells and B-cells (409). Much like different immune cells can have differing roles within the immune system; $iPLA_2\beta$ can have cell-specific roles. Among its proposed roles in macrophages, $iPLA_2\beta$ has been implicated in playing a major role in free fatty acid accumulation in macrophages (165, 410–412) leading to apoptosis. $iPLA_2\beta$, but not $cPLA_2$, has also been reported to promote macrophage proliferation (413). Interestingly, this phenomenon may be cell-specific, as transfection of a kidney cell line with $iPLA_2\beta$ reduced cell proliferation (125), whereas, mature T- and B-lymphocytes do not express $iPLA_2\beta$ and inhibition of $iPLA_2\beta$ in immature T- and B-cells reduces cell viability and proliferation (409). Altered proliferation may work in tandem with increased apoptosis due to $iPLA_2\beta$-derived free fatty acids. Further, $iPLA_2\beta$-derived LPC during apoptosis has been reported to promote binding of IgM antibodies to dying cells and promote their clearance (414). Thus, $iPLA_2\beta$-derived free fatty acids may have both proliferative and apoptotic effects dependent on the specific cell system and/or stimuli.

More clearly evidenced, however, is the contribution of $iPLA_2\beta$ in macrophage adhesion during inflammation. The $iPLA_2\beta$ is required for maintenance of macrophage spreading and adhesion (415). Further, adhesion of macrophages is coupled by $iPLA_2\beta$-mediated AA release and 12/15-LO activity on receptors for macrophages within the extracellular matrix, contributing to inflammation by increasing macrophage retention at inflammation sites (416). Interestingly, the lack of $iPLA_2\beta$ resulted in macrophage buildup late after nerve injury, suggesting $iPLA_2\beta$ is involved in late state macrophage clearance (351). In support of this phenomenon, macrophage phagocytosis was blunted in $iPLA_2\beta$-depleted cells and increased by overexpression of $iPLA_2\beta$ (417). Furthermore, chemical inhibition of $iPLA_2\beta$ resulted in decreased macrophage IgG-mediated phagocytosis, which was associated

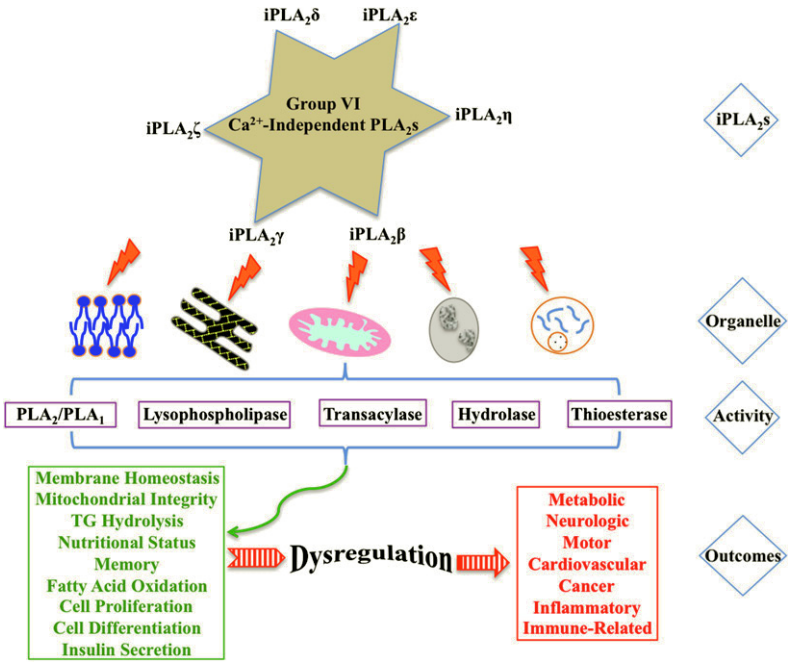

Fig. 2. Biological roles and consequences of $iPLA_2$ activation. The different isoforms of $iPLA_2$ (δ, ε, ζ, η, γ, and β) manifest cell/organelle-specific roles by expressing a variety of activities at the plasma membrane, ER, mitochondria, peroxisomes, and nucleus. The outcomes can be homeostatic and beneficial under normal conditions, but when the expression and/or activity are dysregulated (increased or decreased), they can be detrimental and lead to a variety of disorders. While the illustration describes the involvement of $iPLA_2$s, it might be expected that $cPLA_2$s and $sPLA_2$s, in a cell/organelle-specific manner, participate in a sequential manner or in concert with the $iPLA_2$s to produce the various outcomes.

with decreased AA release from the macrophages (418, 419). Thus, iPLA$_2$β likely influences macrophage activity, translocation, and attachment to the site of inflammation, and clearance of debris and apoptotic cells at the end of inflammation.

As might be expected, products derived from iPLA$_2$β activation can participate in inflammatory responses, which are also triggered during development of autoimmune diseases. Several factors, including ER stress (420, 421), ROS (422, 423), and NF-κB (424, 425), contribute to inflammatory diseases (i.e., autoimmune, metabolic, cancers, rheumatoid arthritis). NADPH oxidases generate ROS and cytokines induce it in a 12-LO product-dependent manner (230). ROS induce iPLA$_2$β (82, 426–432) and ROS generation is increased in neutrophils by iPLA$_2$β activation (433) and reduced in macrophages with iPLA$_2$β deficiency (434). Various stresses activate NF-κB (435–438), and iPLA$_2$β likely modulates NF-κB via ROS generation (439). ROS subsequently induce the chemoattractant, MCP-1 (434), raising the possibility that there is crosstalk between NF-κB and iPLA$_2$β. Further, chemotaxis in response to MCP-1 requires iPLA$_2$β activation (440). It has been suggested that iPLA$_2$β activity drives the initial inflammatory response through synthesis of PGE$_2$, LTB4, and IL-1β, and that sPLA$_2$s and cPLA$_2$ are activated during the resolution phase (441).

The development of FK reversible inhibitors is facilitating testing the impact of PLA$_2$s in autoimmune diseases. Comparisons of earlier generation FKGK11 (iPLA$_2$) and FKGK2 (strong pan) with cPLA$_2$ inhibition in an experimental autoimmune encephalomyelitis animal model of multiple sclerosis revealed that cPLA$_2$ was involved in early onset, iPLA$_2$β-deficiency in the onset and progression, and sPLA$_2$ in later remission stages of experimental autoimmune encephalomyelitis (158).

Metabolic. Studies in iPLA$_2$β-null mice revealed that while fasting and fed blood glucose are unchanged from WT mice, iPLA$_2$β-null mice develop more severe hyperglycemia than WT mice after administration of multiple low doses of streptozotocin (144). This led to the suggestion that iPLA$_2$β results in an impaired ability to compensate for metabolic stresses. Consistent with this, high-fat diet exacerbates glucose tolerance in the iPLA$_2$β-null mice, relative to WT mice. However, in spite of a global iPLA$_2$β-deficiency, these mice do not develop dyslipidemia in response to high-fat diet (442).

CONCLUDING COMMENTS

In addition to the above-discussed iPLA$_2$s, other smaller mass isoforms with different substrate preferences and susceptibility to BEL have been reported in the kidney (443, 444) and rat parotid gland (445). It is likely that continued studies will reveal additional novel iPLA$_2$ activities that are products of different genes or are generated via alternate splicing or proteolytic cleavage of the currently identified isoforms. Regardless, it is clear that the iPLA$_2$s participate in numerous biological processes and that modulation of their cell and/or organelle-specific expression or activity can have profound beneficial or detrimental consequences on membrane integrity and signal transduction. As global and tissue-specific iPLA$_2$-KO or Tg models continue to be developed, so will our understanding of the specific roles of iPLA$_2$s in vivo. Studies incorporating genetic analyses will no doubt aid in identifying further associations between diseases and polymorphisms in the iPLA$_2$ genes. While the focus of this review is on iPLA$_2$s, it is readily apparent that cPLA$_2$s and sPLA$_2$s may act sequentially or in concert with iPLA$_2$s in a cell/organelle-specific manner to produce relevant effects, and this should be a consideration in further exploration of the roles of PLA$_2$ in biological processes. Over the past decade, important links between iPLA$_2$ dysregulation and various diseases have come to the forefront, as illustrated in **Fig. 2**, and with continued studies of this family of PLA$_2$s, a greater understanding of the importance of iPLA$_2$-derived lipid signaling in disease development can be attained and this will facilitate identification of novel pathways that can potentially be targeted for drug therapy. Herein, every attempt was made to provide a comprehensive current understanding of iPLA$_2$s and omission of any relevant citations was unintentional. Readers interested in further elaboration of the areas addressed here are directed to other reviews (1, 153, 289, 291, 365–367, 447–451).

REFERENCES

1. Dennis, E. A., J. Cao, Y. H. Hsu, V. Magrioti, and G. Kokotos. 2011. Phospholipase A$_2$ enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev.* **111:** 6130–6185.
2. Gijón, M. A., and C. C. Leslie. 1997. Phospholipases A$_2$. *Semin. Cell Dev. Biol.* **8:** 297–303.
3. Wolf, R. A., and R. W. Gross. 1985. Identification of neutral active phospholipase C which hydrolyzes choline glycerophospholipids and plasmalogen selective phospholipase A$_2$ in canine myocardium. *J. Biol. Chem.* **260:** 7295–7303.
4. Ramanadham, S., M. J. Wolf, P. A. Jett, R. W. Gross, and J. Turk. 1994. Characterization of an ATP-stimulatable Ca^{2+}-independent phospholipase A$_2$ from clonal insulin-secreting HIT cells and rat pancreatic islets: a possible molecular component of the beta-cell fuel sensor. *Biochemistry.* **33:** 7442–7452.
5. Portilla, D., S. V. Shah, P. A. Lehman, and M. H. Creer. 1994. Role of cytosolic calcium-independent plasmalogen-selective phospholipase A$_2$ in hypoxic injury to rabbit proximal tubules. *J. Clin. Invest.* **93:** 1609–1615.
6. Ackermann, E. J., E. S. Kempner, and E. A. Dennis. 1994. Ca^{2+}-independent cytosolic phospholipase A$_2$ from macrophage-like P388D1 cells. Isolation and characterization. *J. Biol. Chem.* **269:** 9227–9233.
7. Dennis, E. A. 1997. The growing phospholipase A$_2$ superfamily of signal transduction enzymes. *Trends Biochem. Sci.* **22:** 1–2.
8. Balsinde, J., and E. A. Dennis. 1997. Function and inhibition of intracellular calcium-independent phospholipase A$_2$. *J. Biol. Chem.* **272:** 16069–16072.
9. Kienesberger, P. C., M. Oberer, A. Lass, and R. Zechner. 2009. Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. *J. Lipid Res.* **50(Suppl):** S63–S68.
10. Wilson, P. A., S. D. Gardner, N. M. Lambie, S. A. Commans, and D. J. Crowther. 2006. Characterization of the human patatin-like phospholipase family. *J. Lipid Res.* **47:** 1940–1949.

11. van Tienhoven, M., J. Atkins, Y. Li, and P. Glynn. 2002. Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. *J. Biol. Chem.* **277:** 20942–20948.
12. Baulande, S., F. Lasnier, M. Lucas, and J. Pairault. 2001. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. *J. Biol. Chem.* **276:** 33336–33344.
13. Johnson, M. K. 1969. A phosphorylation site in brain and the delayed neurotoxic effect of some organophosphorus compounds. *Biochem. J.* **111:** 487–495.
14. Johnson, M. K. 1969. The delayed neurotoxic effect of some organophosphorus compounds. Identification of the phosphorylation site as an esterase. *Biochem. J.* **114:** 711–717.
15. Winrow, C. J., M. L. Hemming, D. M. Allen, G. B. Quistad, J. E. Casida, and C. Barlow. 2003. Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. *Nat. Genet.* **33:** 477–485.
16. Akassoglou, K., B. Malester, J. Xu, L. Tessarollo, J. Rosenbluth, and M. V. Chao. 2004. Brain-specific deletion of neuropathy target esterase/swisscheese results in neurodegeneration. *Proc. Natl. Acad. Sci. USA.* **101:** 5075–5080.
17. Read, D. J., Y. Li, M. V. Chao, J. B. Cavanagh, and P. Glynn. 2009. Neuropathy target esterase is required for adult vertebrate axon maintenance. *J. Neurosci.* **29:** 11594–11600.
18. Rainier, S., M. Bui, E. Mark, D. Thomas, D. Tokarz, L. Ming, C. Delaney, R. J. Richardson, J. W. Albers, N. Matsunami, et al. 2008. Neuropathy target esterase gene mutations cause motor neuron disease. *Am. J. Hum. Genet.* **82:** 780–785.
19. Rainier, S., J. W. Albers, P. J. Dyck, O. P. Eldevik, S. Wilcock, R. J. Richardson, and J. K. Fink. 2011. Motor neuron disease due to neuropathy target esterase gene mutation: clinical features of the index families. *Muscle Nerve.* **43:** 19–25.
20. Hein, N. D., J. A. Stuckey, S. R. Rainier, J. K. Fink, and R. J. Richardson. 2010. Constructs of human neuropathy target esterase catalytic domain containing mutations related to motor neuron disease have altered enzymatic properties. *Toxicol. Lett.* **196:** 67–73.
21. Hein, N. D., S. R. Rainier, R. J. Richardson, and J. K. Fink. 2010. Motor neuron disease due to neuropathy target esterase mutation: enzyme analysis of fibroblasts from human subjects yields insights into pathogenesis. *Toxicol. Lett.* **199:** 1–5.
22. Topaloglu, A. K., A. Lomniczi, D. Kretzschmar, G. A. Dissen, L. D. Kotan, C. A. McArdle, A. F. Koc, B. C. Hamel, M. Guclu, E. D. Papatya, et al. 2014. Loss-of-function mutations in PNPLA6 encoding neuropathy target esterase underlie pubertal failure and neurological deficits in Gordon Holmes syndrome. *J. Clin. Endocrinol. Metab.* **99:** E2067–E2075.
23. Synofzik, M., M. A. Gonzalez, C. M. Lourenco, M. Coutelier, T. B. Haack, A. Rebelo, D. Hannequin, T. M. Strom, H. Prokisch, C. Kernstock, et al. 2014. PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. *Brain.* **137:** 69–77.
24. Hufnagel, R. B., G. Arno, N. D. Hein, J. Hersheson, M. Prasad, Y. Anderson, L. A. Krueger, L. C. Gregory, C. Stoetzel, T. J. Jaworek, et al. 2015. Neuropathy target esterase impairments cause Oliver-McFarlane and Laurence-Moon syndromes. *J. Med. Genet.* **52:** 85–94.
25. Kmoch, S., J. Majewski, V. Ramamurthy, S. Cao, S. Fahiminiya, H. Ren, I. M. MacDonald, I. Lopez, V. Sun, V. Keser, et al. 2015. Mutations in PNPLA6 are linked to photoreceptor degeneration and various forms of childhood blindness. *Nat. Commun.* **6:** 5614.
26. He, S., C. McPhaul, J. Z. Li, R. Garuti, L. Kinch, N. V. Grishin, J. C. Cohen, and H. H. Hobbs. 2010. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J. Biol. Chem.* **285:** 6706–6715.
27. Jenkins, C. M., D. J. Mancuso, W. Yan, H. F. Sims, B. Gibson, and R. W. Gross. 2004. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A_2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J. Biol. Chem.* **279:** 48968–48975.
28. Romeo, S., J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L. A. Pennacchio, E. Boerwinkle, J. C. Cohen, and H. H. Hobbs. 2008. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **40:** 1461–1465.
29. Yuan, X., D. Waterworth, J. R. Perry, N. Lim, K. Song, J. C. Chambers, W. Zhang, P. Vollenweider, H. Stirnadel, T. Johnson, et al. 2008. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am. J. Hum. Genet.* **83:** 520–528.
30. Pirazzi, C., M. Adiels, M. A. Burza, R. M. Mancina, M. Levin, M. Stahlman, M. R. Taskinen, M. Orho-Melander, J. Perman, A. Pujia, et al. 2012. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. *J. Hepatol.* **57:** 1276–1282.
31. Valenti, L., A. Alisi, and V. Nobili. 2012. I148M PNPLA3 variant and progressive liver disease: a new paradigm in hepatology. *Hepatology.* **56:** 1883–1889.
32. Krawczyk, M., F. Grunhage, and F. Lammert. 2013. Identification of combined genetic determinants of liver stiffness within the SREBP1c-PNPLA3 pathway. *Int. J. Mol. Sci.* **14:** 21153–21166.
33. Romeo, S., F. Sentinelli, S. Dash, G. S. Yeo, D. B. Savage, F. Leonetti, D. Capoccia, M. Incani, C. Maglio, M. Iacovino, et al. 2010. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of European descent. *Int. J. Obes. (Lond).* **34:** 190–194.
34. Giudice, E. M., A. Grandone, G. Cirillo, N. Santoro, A. Amato, C. Brienza, P. Savarese, P. Marzuillo, and L. Perrone. 2011. The association of PNPLA3 variants with liver enzymes in childhood obesity is driven by the interaction with abdominal fat. *PLoS ONE.* **6:** e27933.
35. Davis, J. N., K. A. Le, R. W. Walker, S. Vikman, D. Spruijt-Metz, M. J. Weigensberg, H. Allayee, and M. I. Goran. 2010. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am. J. Clin. Nutr.* **92:** 1522–1527.
36. Santoro, N., M. Savoye, G. Kim, K. Marotto, M. M. Shaw, B. Pierpont, and S. Caprio. 2012. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS ONE.* **7:** e37827.
37. Santoro, N., C. K. Zhang, H. Zhao, A. J. Pakstis, G. Kim, R. Kursawe, D. J. Dykas, A. E. Bale, C. Giannini, B. Pierpont, et al. 2012. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. *Hepatology.* **55:** 781–789.
38. Viganò, M., L. Valenti, P. Lampertico, F. Facchetti, B. M. Motta, R. D'Ambrosio, S. Romagnoli, P. Dongiovanni, B. Donati, S. Fargion, et al. 2013. Patatin-like phospholipase domain-containing 3 I148M affects liver steatosis in patients with chronic hepatitis B. *Hepatology.* **58:** 1245–1252.
39. Friedrich, K., A. Wannhoff, S. Kattner, M. Brune, J. R. Hov, K. H. Weiss, C. Antoni, M. Dollinger, C. Neumann-Haefelin, T. Seufferlein, et al. 2014. PNPLA3 in end-stage liver disease: alcohol consumption, hepatocellular carcinoma development, and transplantation-free survival. *J. Gastroenterol. Hepatol.* **29:** 1477–1484.
40. Chamoun, Z., F. Vacca, R. G. Parton, and J. Gruenberg. 2013. PNPLA3/adiponutrin functions in lipid droplet formation. *Biol. Cell.* **105:** 219–233.
41. Huang, Y., J. C. Cohen, and H. H. Hobbs. 2011. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J. Biol. Chem.* **286:** 37085–37093.
42. Kumari, M., G. Schoiswohl, C. Chitraju, M. Paar, I. Cornaciu, A. Y. Rangrez, N. Wongsiriroj, H. M. Nagy, P. T. Ivanova, S. A. Scott, et al. 2012. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab.* **15:** 691–702.
43. Villena, J. A., S. Roy, E. Sarkadi-Nagy, K. H. Kim, and H. S. Sul. 2004. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J. Biol. Chem.* **279:** 47066–47075.
44. Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, et al. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science.* **306:** 1383–1386.
45. Lass, A., R. Zimmermann, G. Haemmerle, M. Riederer, G. Schoiswohl, M. Schweiger, P. Kienesberger, J. G. Strauss, G. Gorkiewicz, and R. Zechner. 2006. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell Metab.* **3:** 309–319.
46. Yamaguchi, T., and T. Osumi. 2009. Chanarin-Dorfman syndrome: deficiency in CGI-58, a lipid droplet-bound coactivator of lipase. *Biochim. Biophys. Acta.* **1791:** 519–523.

47. Inoue, T., K. Kobayashi, T. Inoguchi, N. Sonoda, Y. Maeda, E. Hirata, Y. Fujimura, D. Miura, K. Hirano, and R. Takayanagi. 2013. Downregulation of adipose triglyceride lipase in the heart aggravates diabetic cardiomyopathy in db/db mice. *Biochem. Biophys. Res. Commun.* **438:** 224–229.
48. Haemmerle, G., A. Lass, R. Zimmermann, G. Gorkiewicz, C. Meyer, J. Rozman, G. Heldmaier, R. Maier, C. Theussl, S. Eder, et al. 2006. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science.* **312:** 734–737.
49. Aflaki, E., P. Doddapattar, B. Radovic, S. Povoden, D. Kolb, N. Vujic, M. Wegscheider, H. Koefeler, T. Hornemann, W. F. Graier, et al. 2012. C16 ceramide is crucial for triacylglycerol-induced apoptosis in macrophages. *Cell Death Dis.* **3:** e280.
50. Tang, T., M. J. Abbott, M. Ahmadian, A. B. Lopes, Y. Wang, and H. S. Sul. 2013. Desnutrin/ATGL activates PPARdelta to promote mitochondrial function for insulin secretion in islet beta cells. *Cell Metab.* **18:** 883–895.
51. Schoenborn, V., I. M. Heid, C. Vollmert, A. Lingenhel, T. D. Adams, P. N. Hopkins, T. Illig, R. Zimmermann, R. Zechner, S. C. Hunt, et al. 2006. The ATGL gene is associated with free fatty acids, triglycerides, and type 2 diabetes. *Diabetes.* **55:** 1270–1275.
52. Kienesberger, P. C., D. Lee, T. Pulinilkunnil, D. S. Brenner, L. Cai, C. Magnes, H. C. Koefeler, I. E. Streith, G. N. Rechberger, G. Haemmerle, et al. 2009. Adipose triglyceride lipase deficiency causes tissue-specific changes in insulin signaling. *J. Biol. Chem.* **284:** 30218–30229.
53. Peyot, M. L., C. Guay, M. G. Latour, J. Lamontagne, R. Lussier, M. Pineda, N. B. Ruderman, G. Haemmerle, R. Zechner, E. Joly, et al. 2009. Adipose triglyceride lipase is implicated in fuel- and non-fuel-stimulated insulin secretion. *J. Biol. Chem.* **284:** 16848–16859.
54. Kim, J. Y., K. Tillison, J. H. Lee, D. A. Rearick, and C. M. Smas. 2006. The adipose tissue triglyceride lipase ATGL/PNPLA2 is downregulated by insulin and TNF-alpha in 3T3–L1 adipocytes and is a target for transactivation by PPARgamma. *Am. J. Physiol. Endocrinol. Metab.* **291:** E115–E127.
55. Wend, K., P. Wend, B. G. Drew, A. L. Hevener, G. A. Miranda-Carboni, and S. A. Krum. 2013. ERα regulates lipid metabolism in bone through ATGL and perilipin. *J. Cell. Biochem.* **114:** 1306–1314.
56. Grahn, T. H., R. Kaur, J. Yin, M. Schweiger, V. M. Sharma, M. J. Lee, Y. Ido, C. M. Smas, R. Zechner, A. Lass, et al. 2014. Fat-specific protein 27 (FSP27) interacts with adipose triglyceride lipase (ATGL) to regulate lipolysis and insulin sensitivity in human adipocytes. *J. Biol. Chem.* **289:** 12029–12039.
57. Khan, S. A., A. Sathyanarayan, M. T. Mashek, K. T. Ong, E. E. Wollaston-Hayden, and D. G. Mashek. 2015. ATGL-catalyzed lipolysis regulates SIRT1 to control PGC-1α/PPAR-α signaling. *Diabetes.* **64:** 418–426.
58. Ong, K. T., M. T. Mashek, N. O. Davidson, and D. G. Mashek. 2014. Hepatic ATGL mediates PPAR-alpha signaling and fatty acid channeling through an L-FABP independent mechanism. *J. Lipid Res.* **55:** 808–815.
59. Ahmadian, M., M. J. Abbott, T. Tang, C. S. Hudak, Y. Kim, M. Bruss, M. K. Hellerstein, H. Y. Lee, V. T. Samuel, G. I. Shulman, et al. 2011. Desnutrin/ATGL is regulated by AMPK and is required for a brown adipose phenotype. *Cell Metab.* **13:** 739–748.
60. Lee, W. C., E. Salido, and P. H. Yen. 1994. Isolation of a new gene GS2 (DXS1283E) from a CpG island between STS and KAL1 on Xp22.3. *Genomics.* **22:** 372–376.
61. Mancuso, D. J., C. M. Jenkins, and R. W. Gross. 2000. The genomic organization, complete mRNA sequence, cloning, and expression of a novel human intracellular membrane-associated calcium-independent phospholipase A_2. *J. Biol. Chem.* **275:** 9937–9945.
62. Tanaka, H., R. Takeya, and H. Sumimoto. 2000. A novel intracellular membrane-bound calcium-independent phospholipase A_2. *Biochem. Biophys. Res. Commun.* **272:** 320–326.
63. Tanaka, H., R. Minakami, H. Kanaya, and H. Sumimoto. 2004. Catalytic residues of group VIB calcium-independent phospholipase A_2 (iPLA$_2$gamma). *Biochem. Biophys. Res. Commun.* **320:** 1284–1290.
64. Mancuso, D. J., C. M. Jenkins, H. F. Sims, J. M. Cohen, J. Yang, and R. W. Gross. 2004. Complex transcriptional and translational regulation of iPLA$_2$gamma resulting in multiple gene products containing dual competing sites for mitochondrial or peroxisomal localization. *Eur. J. Biochem.* **271:** 4709–4724.
65. Mancuso, D. J., X. Han, C. M. Jenkins, J. J. Lehman, N. Sambandam, H. F. Sims, J. Yang, W. Yan, K. Yang, K. Green, et al. 2007. Dramatic accumulation of triglycerides and precipitation of cardiac hemodynamic dysfunction during brief caloric restriction in transgenic myocardium expressing human calcium-independent phospholipase A_2gamma. *J. Biol. Chem.* **282:** 9216–9227.
66. Kinsey, G. R., B. S. Cummings, C. S. Beckett, G. Saavedra, W. Zhang, J. McHowat, and R. G. Schnellmann. 2005. Identification and distribution of endoplasmic reticulum iPLA$_2$. *Biochem. Biophys. Res. Commun.* **327:** 287–293.
67. Beckett, C. S., and J. McHowat. 2008. Calcium-independent phospholipase A_2 in rabbit ventricular myocytes. *Lipids.* **43:** 775–782.
68. Murakami, M., S. Masuda, K. Ueda-Semmyo, E. Yoda, H. Kuwata, Y. Takanezawa, J. Aoki, H. Arai, H. Sumimoto, Y. Ishikawa, et al. 2005. Group VIB Ca^{2+}-independent phospholipase A_2gamma promotes cellular membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A_2. *J. Biol. Chem.* **280:** 14028–14041.
69. Yan, W., C. M. Jenkins, X. Han, D. J. Mancuso, H. F. Sims, K. Yang, and R. W. Gross. 2005. The highly selective production of 2-arachidonoyl lysophosphatidylcholine catalyzed by purified calcium-independent phospholipase A_2gamma: identification of a novel enzymatic mediator for the generation of a key branch point intermediate in eicosanoid signaling. *J. Biol. Chem.* **280:** 26669–26679.
70. Mancuso, D. J., H. F. Sims, X. Han, C. M. Jenkins, S. P. Guan, K. Yang, S. H. Moon, T. Pietka, N. A. Abumrad, P. H. Schlesinger, et al. 2007. Genetic ablation of calcium-independent phospholipase A_2gamma leads to alterations in mitochondrial lipid metabolism and function resulting in a deficient mitochondrial bioenergetic phenotype. *J. Biol. Chem.* **282:** 34611–34622.
71. Song, H., M. Wohltmann, S. Bao, J. H. Ladenson, C. F. Semenkovich, and J. Turk. 2010. Mice deficient in group VIB phospholipase A_2 (iPLA$_2$gamma) exhibit relative resistance to obesity and metabolic abnormalities induced by a Western diet. *Am. J. Physiol. Endocrinol. Metab.* **298:** E1097–E1114.
72. Mancuso, D. J., H. F. Sims, K. Yang, M. A. Kiebish, X. Su, C. M. Jenkins, S. Guan, S. H. Moon, T. Pietka, F. Nassir, et al. 2010. Genetic ablation of calcium-independent phospholipase A_2gamma prevents obesity and insulin resistance during high fat feeding by mitochondrial uncoupling and increased adipocyte fatty acid oxidation. *J. Biol. Chem.* **285:** 36495–36510.
73. Mancuso, D. J., P. Kotzbauer, D. F. Wozniak, H. F. Sims, C. M. Jenkins, S. Guan, X. Han, K. Yang, G. Sun, I. Malik, et al. 2009. Genetic ablation of calcium-independent phospholipase A_2{gamma} leads to alterations in hippocampal cardiolipin content and molecular species distribution, mitochondrial degeneration, autophagy, and cognitive dysfunction. *J. Biol. Chem.* **284:** 35632–35644.
74. Yoda, E., K. Hachisu, Y. Taketomi, K. Yoshida, M. Nakamura, K. Ikeda, R. Taguchi, Y. Nakatani, H. Kuwata, M. Murakami, et al. 2010. Mitochondrial dysfunction and reduced prostaglandin synthesis in skeletal muscle of Group VIB Ca^{2+}-independent phospholipase A_2gamma-deficient mice. *J. Lipid Res.* **51:** 3003–3015.
75. Kinsey, G. R., J. McHowat, C. S. Beckett, and R. G. Schnellmann. 2007. Identification of calcium-independent phospholipase A_2gamma in mitochondria and its role in mitochondrial oxidative stress. *Am. J. Physiol. Renal Physiol.* **292:** F853–F860.
76. Peterson, B., T. Knotts, and B. S. Cummings. 2007. Involvement of Ca^{2+}-independent phospholipase A_2 isoforms in oxidant-induced neural cell death. *Neurotoxicology.* **28:** 150–160.
77. Kinsey, G. R., J. L. Blum, M. D. Covington, B. S. Cummings, J. McHowat, and R. G. Schnellmann. 2008. Decreased iPLA$_2$gamma expression induces lipid peroxidation and cell death and sensitizes cells to oxidant-induced apoptosis. *J. Lipid Res.* **49:** 1477–1487.
78. Cummings, B. S., A. K. Gelasco, G. R. Kinsey, J. McHowat, and R. G. Schnellmann. 2004. Inactivation of endoplasmic reticulum bound Ca^{2+}-independent phospholipase A_2 in renal cells during oxidative stress. *J. Am. Soc. Nephrol.* **15:** 1441–1451.
79. Bao, S., H. Song, M. Tan, M. Wohltmann, J. H. Ladenson, and J. Turk. 2012. Group VIB phospholipase A_2 promotes proliferation of INS-1 insulinoma cells and attenuates lipid peroxidation and apoptosis induced by inflammatory cytokines and oxidant agents. *Oxid. Med. Cell. Longev.* **2012:** 989372.
80. Elimam, H., J. Papillon, T. Takano, and A. V. Cybulsky. 2015. Calcium-independent phospholipase A_2gamma enhances activation of the ATF6 transcription factor during endoplasmic reticulum stress. *J. Biol. Chem.* **290:** 3009–3020.

81. Moon, S. H., C. M. Jenkins, M. A. Kiebish, H. F. Sims, D. J. Mancuso, and R. W. Gross. 2012. Genetic ablation of calcium-independent phospholipase A_2gamma (iPLA$_2$gamma) attenuates calcium-induced opening of the mitochondrial permeability transition pore and resultant cytochrome c release. *J. Biol. Chem.* **287:** 29837–29850.
82. Brustovetsky, T., B. Antonsson, R. Jemmerson, J. M. Dubinsky, and N. Brustovetsky. 2005. Activation of calcium-independent phospholipase A_2 (iPLA$_2$) in brain mitochondria and release of apoptogenic factors by BAX and truncated BID. *J. Neurochem.* **94:** 980–994.
83. Sharma, J., C. S. Eickhoff, D. F. Hoft, D. A. Ford, R. W. Gross, and J. McHowat. 2013. The absence of myocardial calcium-independent phospholipase A_2gamma results in impaired prostaglandin E_2 production and decreased survival in mice with acute Trypanosoma cruzi infection. *Infect. Immun.* **81:** 2278–2287.
84. Yoda, E., K. Rai, M. Ogawa, Y. Takakura, H. Kuwata, H. Suzuki, Y. Nakatani, M. Murakami, and S. Hara. 2014. Group VIB calcium-independent phospholipase A_2 (iPLA$_2$gamma) regulates platelet activation, hemostasis and thrombosis in mice. *PLoS ONE.* **9:** e109409.
85. Saunders, C. J., S. H. Moon, X. Liu, I. Thiffault, K. Coffman, J. B. LePichon, E. Taboada, L. D. Smith, E. G. Farrow, N. Miller, et al. 2015. Loss of function variants in human PNPLA8 encoding calcium-independent phospholipase A_2 gamma recapitulate the mitochondriopathy of the homologous null mouse. *Hum. Mutat.* **36:** 301–306.
86. Balboa, M. A., J. Balsinde, S. S. Jones, and E. A. Dennis. 1997. Identity between the Ca^{2+}-independent phospholipase A_2 enzymes from P388D1 macrophages and Chinese hamster ovary cells. *J. Biol. Chem.* **272:** 8576–8580.
87. Tang, J., R. W. Kriz, N. Wolfman, M. Shaffer, J. Seehra, and S. S. Jones. 1997. A novel cytosolic calcium-independent phospholipase A_2 contains eight ankyrin motifs. *J. Biol. Chem.* **272:** 8567–8575.
88. Jones, S. S., J. Tang, R. Kriz, M. Shaffer, J. Knopf, and J. Seehra. 1996. Isolation, molecular cloning and expression of a novel calcium-independent phospholipase A_2. *FASEB J.* **10:** A977.
89. Ghosh, M., D. E. Tucker, S. A. Burchett, and C. C. Leslie. 2006. Properties of the group IV phospholipase A_2 family. *Prog. Lipid Res.* **45:** 487–510.
90. Wolf, M. J., and R. W. Gross. 1996. Expression, purification, and kinetic characterization of a recombinant 80-kDa intracellular calcium-independent phospholipase A_2. *J. Biol. Chem.* **271:** 30879–30885.
91. Lio, Y. C., and E. A. Dennis. 1998. Interfacial activation, lysophospholipase and transacylase activity of group VI Ca^{2+}-independent phospholipase A_2. *Biochim. Biophys. Acta.* **1392:** 320–332.
92. Jenkins, C. M., W. Yan, D. J. Mancuso, and R. W. Gross. 2006. Highly selective hydrolysis of fatty acyl-CoAs by calcium-independent phospholipase A_2beta. Enzyme autoacylation and acyl-CoA-mediated reversal of calmodulin inhibition of phospholipase A_2 activity. *J. Biol. Chem.* **281:** 15615–15624.
93. Carper, M. J., S. Zhang, J. Turk, and S. Ramanadham. 2008. Skeletal muscle group VIA phospholipase A_2 (iPLA$_2\beta$): expression and role in fatty acid oxidation. *Biochemistry.* **47:** 12241–12249.
94. Ma, Z., S. Ramanadham, K. Kempe, X. S. Chi, J. Ladenson, and J. Turk. 1997. Pancreatic islets express a Ca^{2+}-independent phospholipase A_2 enzyme that contains a repeated structural motif homologous to the integral membrane protein binding domain of ankyrin. *J. Biol. Chem.* **272:** 11118–11127.
95. Larsson, P. K. A., H-E. Claesson, and B. P. Kennedy. 1998. Multiple splice variants of the human calcium-independent phospholipase A_2 and their effect on enzyme activity. *J. Biol. Chem.* **273:** 207–214.
96. Ma, Z., X. Wang, W. Nowatzke, S. Ramanadham, and J. Turk. 1999. Human pancreatic islets express mRNA species encoding two distinct catalytically active isoforms of group VI phospholipase A_2 (iPLA$_2$) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA$_2$ gene on chromosome 22q13.1. *J. Biol. Chem.* **274:** 9607–9616.
97. Atsumi, G., M. Tajima, A. Hadano, Y. Nakatani, M. Murakami, and I. Kudo. 1998. Fas-induced arachidonic acid release is mediated by Ca^{2+}-independent phospholipase A_2 but not cytosolic phospholipase A_2, which undergoes proteolytic inactivation. *J. Biol. Chem.* **273:** 13870–13877.
98. Ramanadham, S., F. F. Hsu, S. Zhang, C. Jin, A. Bohrer, H. Song, S. Bao, Z. Ma, and J. Turk. 2004. Apoptosis of insulin-secreting cells induced by endoplasmic reticulum stress is amplified by overexpression of group VIA calcium-independent phospholipase A_2 (iPLA$_2\beta$) and suppressed by inhibition of iPLA$_2\beta$. *Biochemistry.* **43:** 918–930.
99. Ramanadham, S., H. Song, F. F. Hsu, S. Zhang, M. Crankshaw, G. A. Grant, C. B. Newgard, S. Bao, Z. Ma, and J. Turk. 2003. Pancreatic islets and insulinoma cells express a novel isoform of group VIA phospholipase A_2 (iPLA$_2\beta$) that participates in glucose-stimulated insulin secretion and is not produced by alternate splicing of the iPLA$_2\beta$ transcript. *Biochemistry.* **42:** 13929–13940.
100. Song, H., S. Bao, X. Lei, C. Jin, S. Zhang, J. Turk, and S. Ramanadham. 2010. Evidence for proteolytic processing and stimulated organelle redistribution of iPLA$_2\beta$. *Biochim. Biophys. Acta.* **1801:** 547–558.
101. Song, H., S. Hecimovic, A. Goate, F. F. Hsu, S. Bao, I. Vidavsky, S. Ramanadham, and J. Turk. 2004. Characterization of N-terminal processing of group VIA phospholipase A_2 and of potential cleavage sites of amyloid precursor protein constructs by automated identification of signature peptides in LC/MS/MS analyses of proteolytic digests. *J. Am. Soc. Mass Spectrom.* **15:** 1780–1793.
102. Gross, R. W., S. Ramanadham, K. K. Kruszka, X. Han, and J. Turk. 1993. Rat and human pancreatic islet cells contain a calcium ion independent phospholipase A_2 activity selective for hydrolysis of arachidonate which is stimulated by adenosine triphosphate and is specifically localized to islet beta-cells. *Biochemistry.* **32:** 327–336.
103. Lei, X., S. Zhang, A. Bohrer, S. Bao, H. Song, and S. Ramanadham. 2007. The group VIA calcium-independent phospholipase A_2 participates in ER stress-induced INS-1 insulinoma cell apoptosis by promoting ceramide generation via hydrolysis of sphingomyelins by neutral sphingomyelinase. *Biochemistry.* **46:** 10170–10185.
104. Lei, X., S. Zhang, A. Bohrer, and S. Ramanadham. 2008. Calcium-independent phospholipase A_2 (iPLA$_2\beta$)-mediated ceramide generation plays a key role in the cross-talk between the endoplasmic reticulum (ER) and mitochondria during ER stress-induced insulin-secreting cell apoptosis. *J. Biol. Chem.* **283:** 34819–34832.
105. Ma, Z., S. Ramanadham, M. Wohltmann, A. Bohrer, F. F. Hsu, and J. Turk. 2001. Studies of insulin secretory responses and of arachidonic acid incorporation into phospholipids of stably transfected insulinoma cells that overexpress group VIA phospholipase A_2 (iPLA$_2\beta$) indicate a signaling rather than a housekeeping role for iPLA$_2\beta$. *J. Biol. Chem.* **276:** 13198–13208.
106. Turk, J., and S. Ramanadham. 2004. The expression and function of a group VIA calcium-independent phospholipase A_2 (iPLA$_2\beta$) in beta-cells. *Can. J. Physiol. Pharmacol.* **82:** 824–832.
107. Ma, Z., S. Zhang, J. Turk, and S. Ramanadham. 2002. Stimulation of insulin secretion and associated nuclear accumulation of iPLA$_2\beta$ in INS-1 insulinoma cells. *Am. J. Physiol. Endocrinol. Metab.* **282:** E820–E833.
108. Shinzawa, K., and Y. Tsujimoto. 2003. PLA2 activity is required for nuclear shrinkage in caspase-independent cell death. *J. Cell Biol.* **163:** 1219–1230.
109. Bao, S., C. Jin, S. Zhang, J. Turk, Z. Ma, and S. Ramanadham. 2004. Beta-cell calcium-independent group VIA phospholipase A_2 (iPLA$_2\beta$): tracking iPLA$_2\beta$ movements in response to stimulation with insulin secretagogues in INS-1 cells. *Diabetes.* **53(Suppl 1):** S186–S189.
110. Ramanadham, S., H. Song, S. Bao, F-F. Hsu, S. Zhang, Z. Ma, C. Jin, and J. Turk. 2004. Islet complex lipids: Involvement in the actions of group VIA calcium-independent phospholipase A_2 in β-cells. *Diabetes.* **53:** S179–S185.
111. Ramanadham, S., M. J. Wolf, Z. Ma, B. Li, J. Wang, R. W. Gross, and J. Turk. 1996. Evidence for association of an ATP-stimulatable Ca^{2+}-independent phospholipase A_2 from pancreatic islets and HIT insulinoma cells with a phosphofructokinase-like protein. *Biochemistry.* **35:** 5464–5471.
112. Bennett, V. 1992. Ankyrins. Adaptors between diverse plasma membrane proteins and the cytoplasm. *J. Biol. Chem.* **267:** 8703–8706.
113. de Caestecker, M. P., P. Hemmati, S. Larisch-Bloch, R. Ajmera, A. B. Roberts, and R. J. Lechleider. 1997. Characterization of functional domains within Smad4/DPC4. *J. Biol. Chem.* **272:** 13690–13696.
114. Song, H., S. Bao, S. Ramanadham, and J. Turk. 2006. Effects of biological oxidants on the catalytic activity and structure of group VIA phospholipase A_2. *Biochemistry.* **45:** 6392–6406.
115. McHowat, J., L. M. Swift, A. Arutunyan, and N. Sarvazyan. 2001. Clinical concentrations of doxorubicin inhibit activity of myocardial membrane-associated, calcium-independent phospholipase A_2. *Cancer Res.* **61:** 4024–4029.

116. Ma, Z., and J. Turk. 2001. The molecular biology of the group VIA Ca^{2+}-independent phospholipase A_2. *Prog. Nucleic Acid Res. Mol. Biol.* **67:** 1–33.
117. Hazen, S. L., and R. W. Gross. 1991. ATP-dependent regulation of rabbit myocardial cytosolic calcium- independent phospholipase A_2. *J. Biol. Chem.* **266:** 14526–14534.
118. Hazen, S. L., D. A. Ford, and R. W. Gross. 1991. Activation of a membrane-associated phospholipase A_2 during rabbit myocardial ischemia which is highly selective for plasmalogen substrate. *J. Biol. Chem.* **266:** 5629–5633.
119. Jenkins, C. M., M. J. Wolf, D. J. Mancuso, and R. W. Gross. 2001. Identification of the calmodulin-binding domain of recombinant calcium-independent phospholipase A_2beta. Implications for structure and function. *J. Biol. Chem.* **276:** 7129–7135.
120. Wang, Z., S. Ramanadham, Z. A. Ma, S. Bao, D. J. Mancuso, R. W. Gross, and J. Turk. 2005. Group VIA phospholipase A_2 forms a signaling complex with the calcium/calmodulin-dependent protein kinase IIbeta expressed in pancreatic islet beta-cells. *J. Biol. Chem.* **280:** 6840–6849.
121. Wolf, M. J., J. Wang, J. Turk, and R. W. Gross. 1997. Depletion of intracellular calcium stores activates smooth muscle cell calcium-independent phospholipase A_2. A novel mechanism underlying arachidonic acid mobilization. *J. Biol. Chem.* **272:** 1522–1526.
122. Nowatzke, W., S. Ramanadham, Z. Ma, F. F. Hsu, A. Bohrer, and J. Turk. 1998. Mass spectrometric evidence that agents that cause loss of Ca^{2+} from intracellular compartments induce hydrolysis of arachidonic acid from pancreatic islet membrane phospholipids by a mechanism that does not require a rise in cytosolic Ca^{2+} concentration. *Endocrinology.* **139:** 4073–4085.
123. Larsson Forsell, P. K., G. Runarsson, M. Ibrahim, M. Bjorkholm, and H. E. Claesson. 1998. On the expression of cytosolic calcium-independent phospholipase A_2 (88 kDa) in immature and mature myeloid cells and its role in leukotriene synthesis in human granulocytes. *FEBS Lett.* **434:** 295–299.
124. Rzigalinski, B. A., P. F. Blackmore, and M. D. Rosenthal. 1996. Arachidonate mobilization is coupled to depletion of intracellular calcium stores and influx of extracellular calcium in differentiated U937 cells. *Biochim. Biophys. Acta.* **1299:** 342–352.
125. Atsumi, G., M. Murakami, K. Kojima, A. Hadano, M. Tajima, and I. Kudo. 2000. Distinct roles of two intracellular phospholipase A_2s in fatty acid release in the cell death pathway. Proteolytic fragment of type IVA cytosolic phospholipase A_2alpha inhibits stimulus-induced arachidonate release, whereas that of type VI Ca^{2+}-independent phospholipase A_2 augments spontaneous fatty acid release. *J. Biol. Chem.* **275:** 18248–18258.
126. Seashols, S. J., A. del Castillo Olivares, G. Gil, and S. E. Barbour. 2004. Regulation of group VIA phospholipase A_2 expression by sterol availability. *Biochim. Biophys. Acta.* **1684:** 29–37.
127. Akarte, A. S., B. P. Srinivasan, and S. Gandhi. 2012. Vildagliptin selectively ameliorates GLP-1, GLUT4, SREBP-1c mRNA levels and stimulates β-cell proliferation resulting in improved glucose homeostasis in rats with streptozotocin-induced diabetes. *J. Diabetes Complications.* **26:** 266–274.
128. Boslem, E., G. MacIntosh, A. M. Preston, C. Bartley, A. K. Busch, M. Fuller, D. R. Laybutt, P. J. Meikle, and T. J. Biden. 2011. A lipidomic screen of palmitate-treated MIN6 beta-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. *Biochem. J.* **435:** 267–276.
129. Chan, J. Y., G. J. Cooney, T. J. Biden, and D. R. Laybutt. 2011. Differential regulation of adaptive and apoptotic unfolded protein response signalling by cytokine-induced nitric oxide production in mouse pancreatic beta cells. *Diabetologia.* **54:** 1766–1776.
130. Chin, H. J., Y. Y. Fu, J. M. Ahn, K. Y. Na, Y. S. Kim, S. Kim, and D. W. Chae. 2010. Omacor, n-3 polyunsaturated fatty acid, attenuated albuminuria and renal dysfunction with decrease of SREBP-1 expression and triglyceride amount in the kidney of type II diabetic animals. *Nephrol. Dial. Transplant.* **25:** 1450–1457.
131. Heller, J. J., J. Qiu, and L. Zhou. 2011. Nuclear receptors take center stage in Th17 cell-mediated autoimmunity. *J. Clin. Invest.* **121:** 519–521.
132. Kaplan, M., M. Aviram, and T. Hayek. 2012. Oxidative stress and macrophage foam cell formation during diabetes mellitus-induced atherogenesis: Role of insulin therapy. *Pharmacol. Ther.* **136:** 175–185.
133. Véret, J., N. Coant, E. V. Berdyshev, A. Skobeleva, N. Therville, D. Bailbe, I. Gorshkova, V. Natarajan, B. Portha, and H. Le Stunff. 2011. Ceramide synthase 4 and de novo production of ceramides with specific N-acyl chain lengths are involved in glucolipotoxicity-induced apoptosis of INS-1 β-cells. *Biochem. J.* **438:** 177–189.
134. Wang, H., G. Kouri, and C. B. Wollheim. 2005. ER stress and SREBP-1 activation are implicated in beta-cell glucolipotoxicity. *J. Cell Sci.* **118:** 3905–3915.
135. Yano, M., K. Watanabe, T. Yamamoto, K. Ikeda, T. Senokuchi, M. Lu, T. Kadomatsu, H. Tsukano, M. Ikawa, M. Okabe, et al. 2011. Mitochondrial dysfunction and increased reactive oxygen species impair insulin secretion in sphingomyelin synthase 1-null mice. *J. Biol. Chem.* **286:** 3992–4002.
136. Lei, X., R. N. Bone, T. Ali, S. Zhang, A. Bohrer, H. M. Tse, K. R. Bidasee, and S. Ramanadham. 2014. Evidence of contribution of $iPLA_2\beta$-mediated events during islet beta-cell apoptosis due to proinflammatory cytokines suggests a role for $iPLA_2\beta$ in T1D development. *Endocrinology.* **155:** 3352–3364.
137. Lei, X., S. Zhang, S. E. Barbour, A. Bohrer, E. L. Ford, A. Koizumi, F. R. Papa, and S. Ramanadham. 2010. Spontaneous development of endoplasmic reticulum stress that can lead to diabetes mellitus is associated with higher calcium-independent phospholipase A_2 expression: a role for regulation by SREBP-1. *J. Biol. Chem.* **285:** 6693–6705.
138. Lei, X., S. Zhang, A. Bohrer, S. E. Barbour, and S. Ramanadham. 2012. Role of calcium-independent phospholipase A_2beta in human pancreatic islet beta-cell apoptosis. *Am. J. Physiol. Endocrinol. Metab.* **303:** E1386–E1395.
139. Ackermann, E. J., K. Conde-Frieboes, and E. A. Dennis. 1995. Inhibition of macrophage Ca^{2+}-independent phospholipase A_2 by bromoenol lactone and trifluoromethyl ketones. *J. Biol. Chem.* **270:** 445–450.
140. Magrioti, V., and G. Kokotos. 2010. Phospholipase A2 inhibitors as potential therapeutic agents for the treatment of inflammatory diseases. *Expert Opin. Ther. Pat.* **20:** 1–18.
141. Schaeffer, E. L., and W. F. Gattaz. 2005. Inhibition of calcium-independent phospholipase A_2 activity in rat hippocampus impairs acquisition of short- and long-term memory. *Psychopharmacology (Berl.).* **181:** 392–400.
142. Bao, S., D. A. Jacobson, M. Wohltmann, A. Bohrer, W. Jin, L. H. Philipson, and J. Turk. 2008. Glucose homeostasis, insulin secretion, and islet phospholipids in mice that overexpress $iPLA_2\beta$ in pancreatic β-cells and in $iPLA_2\beta$-null mice. *Am. J. Physiol. Endocrinol. Metab.* **294:** E217–E229.
143. Bao, S., D. J. Miller, Z. Ma, M. Wohltmann, G. Eng, S. Ramanadham, K. Moley, and J. Turk. 2004. Male mice that do not express group VIA phospholipase A_2 produce spermatozoa with impaired motility and have greatly reduced fertility. *J. Biol. Chem.* **279:** 38194–38200.
144. Bao, S., H. Song, M. Wohltmann, S. Ramanadham, W. Jin, A. Bohrer, and J. Turk. 2006. Insulin secretory responses and phospholipid composition of pancreatic islets from mice that do not express group VIA phospholipase A_2 and effects of metabolic stress on glucose homeostasis. *J. Biol. Chem.* **281:** 20958–20973.
145. Lei, X., S. E. Barbour, and S. Ramanadham. 2010. Group VIA Ca^{2+}-independent phospholipase A_2 ($iPLA_2\beta$) and its role in beta-cell programmed cell death. *Biochimie.* **92:** 627–637.
146. Hazen, S. L., L. A. Zupan, R. H. Weiss, D. P. Getman, and R. W. Gross. 1991. Suicide inhibition of canine myocardial cytosolic calcium-independent phospholipase A_2. Mechanism-based discrimination between calcium- dependent and -independent phospholipases A_2. *J. Biol. Chem.* **266:** 7227–7232.
147. Ma, Z., S. Ramanadham, Z. Hu, and J. Turk. 1998. Cloning and expression of a group IV cytosolic Ca^{2+}-dependent phospholipase A_2 from rat pancreatic islets. Comparison of the expressed activity with that of an islet group VI cytosolic Ca^{2+}-independent phospholipase A_2. *Biochim. Biophys. Acta.* **1391:** 384–400.
148. Jenkins, C. M., X. Han, D. J. Mancuso, and R. W. Gross. 2002. Identification of calcium-independent phospholipase A_2 ($iPLA_2$) beta, and not $iPLA_2$gamma, as the mediator of arginine vasopressin-induced arachidonic acid release in A-10 smooth muscle cells. Enantioselective mechanism-based discrimination of mammalian $iPLA_2$s. *J. Biol. Chem.* **277:** 32807–32814.
149. Song, H., S. Ramanadham, S. Bao, F. F. Hsu, and J. Turk. 2006. A bromoenol lactone suicide substrate inactivates group VIA phospholipase A_2 by generating a diffusible bromomethyl keto acid that alkylates cysteine thiols. *Biochemistry.* **45:** 1061–1073.
150. Song, H., H. Rohrs, M. Tan, M. Wohltmann, J. H. Ladenson, and J. Turk. 2010. Effects of endoplasmic reticulum stress on group VIA phospholipase A_2 in beta cells include tyrosine phosphorylation

and increased association with calnexin. *J. Biol. Chem.* **285:** 33843–33857.
151. Jenkins, C. M., J. Yang, and R. W. Gross. 2013. Mechanism-based inhibition of iPLA$_2$β demonstrates a highly reactive cysteine residue (C651) that interacts with the active site: mass spectrometric elucidation of the mechanisms underlying inhibition. *Biochemistry.* **52:** 4250–4263.
152. Fuentes, L., R. Perez, M. L. Nieto, J. Balsinde, and M. A. Balboa. 2003. Bromoenol lactone promotes cell death by a mechanism involving phosphatidate phosphohydrolase-1 rather than calcium-independent phospholipase A$_2$. *J. Biol. Chem.* **278:** 44683–44690.
153. Wilkins 3rd, W. P., and S. E. Barbour. 2008. Group VI phospholipases A$_2$: homeostatic phospholipases with significant potential as targets for novel therapeutics. *Curr. Drug Targets.* **9:** 683–697.
154. Balsinde, J., and E. A. Dennis. 1996. Bromoenol lactone inhibits magnesium-dependent phosphatidate phosphohydrolase and blocks triacylglycerol biosynthesis in mouse P388D1 macrophages. *J. Biol. Chem.* **271:** 31937–31941.
155. Daniels, S. B., E. Cooney, M. J. Sofia, P. K. Chakravarty, and J. A. Katzenellenbogen. 1983. Haloenol lactones. Potent enzyme-activated irreversible inhibitors for alpha-chymotrypsin. *J. Biol. Chem.* **258:** 15046–15053.
156. Ali, T., G. Kokotos, V. Magrioti, R. N. Bone, J. A. Mobley, W. Hancock, and S. Ramanadham. 2013. Characterization of FKGK18 as inhibitor of group VIA Ca^{2+}-independent phospholipase A$_2$ (iPLA$_2$β): candidate drug for preventing beta-cell apoptosis and diabetes. *PLoS ONE.* **8:** e71748.
157. Kokotos, G., Y. H. Hsu, J. E. Burke, C. Baskakis, C. G. Kokotos, V. Magrioti, and E. A. Dennis. 2010. Potent and selective fluoroketone inhibitors of group VIA calcium-independent phospholipase A$_2$. *J. Med. Chem.* **53:** 3602–3610.
158. Kalyvas, A., C. Baskakis, V. Magrioti, V. Constantinou-Kokotou, D. Stephens, R. Lopez-Vales, J. Q. Lu, V. W. Yong, E. A. Dennis, G. Kokotos, et al. 2009. Differing roles for members of the phospholipase A$_2$ superfamily in experimental autoimmune encephalomyelitis. *Brain.* **132:** 1221–1235.
159. Stephens, D., E. Barbayianni, V. Constantinou-Kokotou, A. Peristeraki, D. A. Six, J. Cooper, R. Harkewicz, R. A. Deems, E. A. Dennis, and G. Kokotos. 2006. Differential inhibition of group IVA and group VIA phospholipases A$_2$ by 2-oxoamides. *J. Med. Chem.* **49:** 2821–2828.
160. Gil-de-Gómez, L., A. M. Astudillo, C. Guijas, V. Magrioti, G. Kokotos, M. A. Balboa, and J. Balsinde. 2014. Cytosolic group IVA and calcium-independent group VIA phospholipase A$_2$s act on distinct phospholipid pools in zymosan-stimulated mouse peritoneal macrophages. *J. Immunol.* **192:** 752–762.
161. Bone, R. N., Y. Gai, V. Magrioti, M. G. Kokotou, T. Ali, X. Lei, H. M. Tse, G. Kokotos, and S. Ramanadham. 2015. Inhibition of Ca^{2+}-independent phospholipase A$_2$beta (iPLA$_2$β) ameliorates islet infiltration and incidence of diabetes in NOD mice. *Diabetes.* **64:** 541–554.
162. Magrioti, V., A. Nikolaou, A. Smyrniotou, I. Shah, V. Constantinou-Kokotou, E. A. Dennis, and G. Kokotos. 2013. New potent and selective polyfluoroalkyl ketone inhibitors of GVIA calcium-independent phospholipase A$_2$. *Bioorg. Med. Chem.* **21:** 5823–5829.
163. Hsu, Y. H., D. Bucher, J. Cao, S. Li, S. W. Yang, G. Kokotos, V. L. Woods, Jr., J. A. McCammon, and E. A. Dennis. 2013. Fluoroketone inhibition of Ca^{2+}-independent phospholipase A$_2$ through binding pocket association defined by hydrogen/deuterium exchange and molecular dynamics. *J. Am. Chem. Soc.* **135:** 1330–1337.
164. Barbayianni, E., D. Stephens, A. Grkovich, V. Magrioti, Y. H. Hsu, P. Dolatzas, D. Kalogiannidis, E. A. Dennis, and G. Kokotos. 2009. 2-Oxoamide inhibitors of phospholipase A$_2$ activity and cellular arachidonate release based on dipeptides and pseudodipeptides. *Bioorg. Med. Chem.* **17:** 4833–4843.
165. Balsinde, J., M. A. Balboa, and E. A. Dennis. 1997. Antisense inhibition of group VI Ca^{2+}-independent phospholipase A$_2$ blocks phospholipid fatty acid remodeling in murine P388D1 macrophages. *J. Biol. Chem.* **272:** 29317–29321.
166. Balsinde, J., I. D. Bianco, E. J. Ackermann, K. Conde-Frieboes, and E. A. Dennis. 1995. Inhibition of calcium-independent phospholipase A$_2$ prevents arachidonic acid incorporation and phospholipid remodeling in P388D1 macrophages. *Proc. Natl. Acad. Sci. USA.* **92:** 8527–8531.
167. Chilton, F. H., A. N. Fonteh, M. E. Surette, M. Triggiani, and J. D. Winkler. 1996. Control of arachidonate levels within inflammatory cells. *Biochim. Biophys. Acta.* **1299:** 1–15.
168. Dennis, E. A. 1992. The biosynthesis of phospholipids. *Methods Enzymol.* **209:** 1–4.
169. Barbour, S. E., A. Kapur, and C. L. Deal. 1999. Regulation of phosphatidylcholine homeostasis by calcium-independent phospholipase A2. *Biochim. Biophys. Acta.* **1439:** 77–88.
170. Wolf, B. A., S. M. Pasquale, and J. Turk. 1991. Free fatty acid accumulation in secretagogue-stimulated pancreatic islets and effects of arachidonate on depolarization-induced insulin secretion. *Biochemistry.* **30:** 6372–6379.
171. Song, H., M. Wohltmann, M. Tan, J. H. Ladenson, and J. Turk. 2014. Group VIA phospholipase A$_2$ mitigates palmitate-induced beta-cell mitochondrial injury and apoptosis. *J. Biol. Chem.* **289:** 14194–14210.
172. Balboa, M. A., Y. Sáez, and J. Balsinde. 2003. Calcium-independent phospholipase A$_2$ is required for lysozyme secretion in U937 promonocytes. *J. Immunol.* **170:** 5276–5280.
173. Song, Y., P. Wilkins, W. Hu, K. S. Murthy, J. Chen, Z. Lee, R. Oyesanya, J. Wu, S. E. Barbour, and X. Fang. 2007. Inhibition of calcium-independent phospholipase A$_2$ suppresses proliferation and tumorigenicity of ovarian carcinoma cells. *Biochem. J.* **406:** 427–436.
174. Sanchez, T., and J. J. Moreno. 2002. Calcium-independent phospholipase A$_2$ through arachidonic acid mobilization is involved in Caco-2 cell growth. *J. Cell. Physiol.* **193:** 293–298.
175. Herbert, S. P., and J. H. Walker. 2006. Group VIA calcium-independent phospholipase A$_2$ mediates endothelial cell S phase progression. *J. Biol. Chem.* **281:** 35709–35716.
176. Bao, S., A. Bohrer, S. Ramanadham, W. Jin, S. Zhang, and J. Turk. 2006. Effects of stable suppression of group VIA phospholipase A$_2$ expression on phospholipid content and composition, insulin secretion, and proliferation of INS-1 insulinoma cells. *J. Biol. Chem.* **281:** 187–198.
177. Ma, Z., A. Bohrer, M. Wohltmann, S. Ramanadham, F-F. Hsu, and J. Turk. 2001. Studies of phospholipid metabolism, proliferation, and secretion of stably transfected insulinoma cells that overexpress group VIA phospholipase A2. *Lipids.* **36:** 689–700.
178. Moon, S. H., C. M. Jenkins, D. J. Mancuso, J. Turk, and R. W. Gross. 2008. Smooth muscle cell arachidonic acid release, migration, and proliferation are markedly attenuated in mice null for calcium-independent phospholipase A$_2$beta. *J. Biol. Chem.* **283:** 33975–33987.
179. Berti-Mattera, L. N., S. Harwalkar, B. Hughes, P. L. Wilkins, and K. Almhanna. 2001. Proliferative and morphological effects of endothelins in Schwann cells: roles of p38 mitogen-activated protein kinase and Ca^{2+}-independent phospholipase A$_2$. *J. Neurochem.* **79:** 1136–1148.
180. Zhang, X. H., C. Zhao, K. Seleznev, K. Song, J. J. Manfredi, and Z. A. Ma. 2006. Disruption of G1-phase phospholipid turnover by inhibition of Ca^{2+}-independent phospholipase A$_2$ induces a p53-dependent cell-cycle arrest in G1 phase. *J. Cell Sci.* **119:** 1005–1015.
181. Sanchez, T., and J. J. Moreno. 2001. The effect of high molecular phospholipase A$_2$ inhibitors on 3T6 fibroblast proliferation. *Biochem. Pharmacol.* **61:** 811–816.
182. Sengupta, S., Y. J. Xiao, and Y. Xu. 2003. A novel laminin-induced LPA autocrine loop in the migration of ovarian cancer cells. *FASEB J.* **17:** 1570–1572.
183. Turk, J., R. W. Gross, and S. Ramanadham. 1993. Amplification of insulin secretion by lipid messengers. *Diabetes.* **42:** 367–374.
184. Traianedes, K., M. R. Dallas, I. R. Garrett, G. R. Mundy, and L. F. Bonewald. 1998. 5-Lipoxygenase metabolites inhibit bone formation in vitro. *Endocrinology.* **139:** 3178–3184.
185. Akamine, T., W. S. Jee, H. Z. Ke, X. J. Li, and B. Y. Lin. 1992. Prostaglandin E$_2$ prevents bone loss and adds extra bone to immobilized distal femoral metaphysis in female rats. *Bone.* **13:** 11–22.
186. Weinreb, M., D. Shamir, M. Machwate, G. A. Rodan, S. Harada, and S. Keila. 2006. Prostaglandin E$_2$ (PGE$_2$) increases the number of rat bone marrow osteogenic stromal cells (BMSC) via binding the EP4 receptor, activating sphingosine kinase and inhibiting caspase activity. *Prostaglandins Leukot. Essent. Fatty Acids.* **75:** 81–90.
187. Weinreb, M., I. Suponitzky, and S. Keila. 1997. Systemic administration of an anabolic dose of PGE$_2$ in young rats increases the osteogenic capacity of bone marrow. *Bone.* **20:** 521–526.

188. Yang, R. S., T. K. Liu, and S. Y. Lin-Shiau. 1993. Increased bone growth by local prostaglandin E_2 in rats. *Calcif. Tissue Int.* **52:** 57–61.
189. Miyaura, C., M. Inada, C. Matsumoto, T. Ohshiba, N. Uozumi, T. Shimizu, and A. Ito. 2003. An essential role of cytosolic phospholipase A_2alpha in prostaglandin E_2-mediated bone resorption associated with inflammation. *J. Exp. Med.* **197:** 1303–1310.
190. Weiler, H. A. 2000. Dietary supplementation of arachidonic acid is associated with higher whole body weight and bone mineral density in growing pigs. *Pediatr. Res.* **47:** 692–697.
191. Sato, Y., N. Arai, A. Negishi, and K. Ohya. 1997. Expression of cyclooxygenase genes and involvement of endogenous prostaglandin during osteogenesis in the rat tibial bone marrow cavity. *J. Med. Dent. Sci.* **44:** 81–92.
192. Ramanadham, S., K. E. Yarasheski, M. J. Silva, M. Wohltmann, D. V. Novack, B. Christiansen, X. Tu, S. Zhang, X. Lei, and J. Turk. 2008. Age-related changes in bone morphology are accelerated in group VIA phospholipase A_2 (iPLA$_2\beta$)-null mice. *Am. J. Pathol.* **172:** 868–881.
193. Sato, H., Y. Taketomi, Y. Isogai, Y. Miki, K. Yamamoto, S. Masuda, T. Hosono, S. Arata, Y. Ishikawa, T. Ishii, et al. 2010. Group III secreted phospholipase A_2 regulates epididymal sperm maturation and fertility in mice. *J. Clin. Invest.* **120:** 1400–1414.
194. Escoffier, J., I. Jemel, A. Tanemoto, Y. Taketomi, C. Payre, C. Coatrieux, H. Sato, K. Yamamoto, S. Masuda, K. Pernet-Gallay, et al. 2010. Group X phospholipase A_2 is released during sperm acrosome reaction and controls fertility outcome in mice. *J. Clin. Invest.* **120:** 1415–1428.
195. Bonventre, J. V., Z. Huang, M. R. Taheri, E. O'Leary, E. Li, M. A. Moskowitz, and A. Sapirstein. 1997. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A_2. *Nature.* **390:** 622–625.
196. Achache, H., A. Tsafrir, D. Prus, R. Reich, and A. Revel. 2010. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. *Fertil. Steril.* **94:** 1271–1278.
197. Ho, H. C., and S. S. Suarez. 2001. An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca^{2+} store is involved in regulating sperm hyperactivated motility. *Biol. Reprod.* **65:** 1606–1615.
198. Marquez, B., and S. S. Suarez. 2004. Different signaling pathways in bovine sperm regulate capacitation and hyperactivation. *Biol. Reprod.* **70:** 1626–1633.
199. Yuan, Y. Y., W. Y. Chen, Q. X. Shi, L. Z. Mao, S. Q. Yu, X. Fang, and E. R. Roldan. 2003. Zona pellucida induces activation of phospholipase A_2 during acrosomal exocytosis in guinea pig spermatozoa. *Biol. Reprod.* **68:** 904–913.
200. Wu, C., T. Stojanov, O. Chami, S. Ishii, T. Shimizu, A. Li, and C. O'Neill. 2001. Evidence for the autocrine induction of capacitation of mammalian spermatozoa. *J. Biol. Chem.* **276:** 26962–26968. [Erratum. 2001. *J. Biol. Chem.* **276:** 47746.]
201. Ramanadham, S., R. W. Gross, X. Han, and J. Turk. 1993. Inhibition of arachidonate release by secretagogue-stimulated pancreatic islets suppresses both insulin secretion and the rise in beta-cell cytosolic calcium ion concentration. *Biochemistry.* **32:** 337–346.
202. Ramanadham, S., R. Gross, and J. Turk. 1992. Arachidonic acid induces an increase in the cytosolic calcium concentration in single pancreatic islet beta cells. *Biochem. Biophys. Res. Commun.* **184:** 647–653.
203. Ramanadham, S., A. Bohrer, R. W. Gross, and J. Turk. 1993. Mass spectrometric characterization of arachidonate-containing plasmalogens in human pancreatic islets and in rat islet beta-cells and subcellular membranes. *Biochemistry.* **32:** 13499–13509.
204. Ramanadham, S., A. Bohrer, M. Mueller, P. Jett, R. W. Gross, and J. Turk. 1993. Mass spectrometric identification and quantitation of arachidonate-containing phospholipids in pancreatic islets: prominence of plasmenylethanolamine molecular species. *Biochemistry.* **32:** 5339–5351.
205. Ramanadham, S., F. F. Hsu, A. Bohrer, Z. Ma, and J. Turk. 1999. Studies of the role of group VI phospholipase A_2 in fatty acid incorporation, phospholipid remodeling, lysophosphatidylcholine generation, and secretagogue-induced arachidonic acid release in pancreatic islets and insulinoma cells. *J. Biol. Chem.* **274:** 13915–13927.
206. Bao, S., Y. Li, X. Lei, M. Wohltmann, W. Jin, A. Bohrer, C. F. Semenkovich, S. Ramanadham, I. Tabas, and J. Turk. 2007. Attenuated free cholesterol loading-induced apoptosis but preserved phospholipid composition of peritoneal macrophages from mice that do not express group VIA phospholipase A_2. *J. Biol. Chem.* **282:** 27100–27114.
207. Ashcroft, S. J. 1980. Glucoreceptor mechanisms and the control of insulin release and biosynthesis. *Diabetologia.* **18:** 5–15.
208. Hedeskov, C. J. 1980. Mechanism of glucose-induced insulin secretion. *Physiol. Rev.* **60:** 442–509.
209. Malaisse, W. J., A. Sener, A. Herchuelz, and J. C. Hutton. 1979. Insulin release: the fuel hypothesis. *Metabolism.* **28:** 373–386.
210. Meglasson, M. D., and F. M. Matschinsky. 1986. Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab. Rev.* **2:** 163–214.
211. Ashcroft, F. M., D. E. Harrison, and S. J. Ashcroft. 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature.* **312:** 446–448.
212. Cook, D. L., and C. N. Hales. 1984. Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature.* **311:** 271–273.
213. Rorsman, P., and G. Trube. 1985. Glucose dependent K+-channels in pancreatic beta-cells are regulated by intracellular ATP. *Pflugers Arch.* **405:** 305–309.
214. Sturgess, N. C., M. L. Ashford, D. L. Cook, and C. N. Hales. 1985. The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet.* **2:** 474–475.
215. Arkhammar, P., T. Nilsson, P. Rorsman, and P. O. Berggren. 1987. Inhibition of ATP-regulated K^+ channels precedes depolarization-induced increase in cytoplasmic free Ca^{2+} concentration in pancreatic beta-cells. *J. Biol. Chem.* **262:** 5448–5454.
216. Gylfe, E. 1988. Nutrient secretagogues induce bimodal early changes in cytoplasmic calcium of insulin-releasing ob/ob mouse beta-cells. *J. Biol. Chem.* **263:** 13750–13754.
217. Gylfe, E. 1988. Glucose-induced early changes in cytoplasmic calcium of pancreatic beta-cells studied with time-sharing dual-wavelength fluorometry. *J. Biol. Chem.* **263:** 5044–5048.
218. Keahey, H. H., A. S. Rajan, A. E. Boyd 3rd, and D. L. Kunze. 1989. Characterization of voltage-dependent Ca^{2+} channels in beta-cell line. *Diabetes.* **38:** 188–193.
219. Vacher, P., J. McKenzie, and B. Dufy. 1989. Arachidonic acid affects membrane ionic conductances of GH3 pituitary cells. *Am. J. Physiol.* **257:** E203–E211.
220. Wollheim, C. B., and G. W. Sharp. 1981. Regulation of insulin release by calcium. *Physiol. Rev.* **61:** 914–973.
221. Biden, T. J., B. Peter-Riesch, W. Schlegel, and C. B. Wollheim. 1987. Ca2+-mediated generation of inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetrakisphosphate in pancreatic islets. Studies with K+, glucose, and carbamylcholine. *J. Biol. Chem.* **262:** 3567–3571.
222. Metz, S. A. 1988. Membrane phospholipid turnover as an intermediary step in insulin secretion. Putative roles of phospholipases in cell signaling. *Am. J. Med.* **85:** 9–21.
223. Prentki, M., and F. M. Matschinsky. 1987. Ca2+, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol. Rev.* **67:** 1185–1248.
224. Robertson, R. P. 1988. Eicosanoids as pluripotential modulators of pancreatic islet function. *Diabetes.* **37:** 367–370.
225. Turk, J., B. A. Wolf, and M. L. McDaniel. 1987. The role of phospholipid-derived mediators including arachidonic acid, its metabolites, and inositoltrisphosphate and of intracellular Ca^{2+} in glucose-induced insulin secretion by pancreatic islets. *Prog. Lipid Res.* **26:** 125–181.
226. Turk, J., M. Mueller, A. Bohrer, and S. Ramanadham. 1992. Arachidonic acid metabolism in isolated pancreatic islets. VI. Carbohydrate insulin secretagogues must be metabolized to induce eicosanoid release. *Biochim. Biophys. Acta.* **1125:** 280–291.
227. Metz, S. A., B. Draznin, K. E. Sussman, and J. W. Leitner. 1987. Unmasking of arachidonate-induced insulin release by removal of extracellular calcium. Arachidonic acid mobilizes cellular calcium in rat islets of Langerhans. *Biochem. Biophys. Res. Commun.* **142:** 251–258.
228. Jacobson, D. A., C. R. Weber, S. Bao, J. Turk, and L. H. Philipson. 2007. Modulation of the pancreatic islet beta-cell-delayed rectifier potassium channel Kv2.1 by the polyunsaturated fatty acid arachidonate. *J. Biol. Chem.* **282:** 7442–7449.
229. Nanda, B. L., A. Nataraju, R. Rajesh, K. S. Rangappa, M. A. Shekar, and B. S. Vishwanath. 2007. PLA_2 mediated arachidonate free radicals: PLA_2 inhibition and neutralization of free radicals by anti-oxidants–a new role as anti-inflammatory molecule. *Curr. Top. Med. Chem.* **7:** 765–777.

230. Weaver, J. R., T. R. Holman, Y. Imai, A. Jadhav, V. Kenyon, D. J. Maloney, J. L. Nadler, G. Rai, A. Simeonov, and D. A. Taylor-Fishwick. 2012. Integration of pro-inflammatory cytokines, 12-lipoxygenase and NOX-1 in pancreatic islet beta cell dysfunction. *Mol. Cell. Endocrinol.* **358:** 88–95.
231. Loweth, A. C., J. H. Scarpello, and N. G. Morgan. 1994. A comparison of cytosolic phospholipase A_2 expression in human islets of Langerhans and rodent insulin-secreting cells. *Biochem. Soc. Trans.* **22:** 430S.
232. Jolly, Y. C., C. Major, and B. A. Wolf. 1993. Transient activation of calcium-dependent phospholipase A_2 by insulin secretagogues in isolated pancreatic islets. *Biochemistry.* **32:** 12209–12217.
233. Loweth, A. C., J. H. Scarpello, and N. G. Morgan. 1995. Phospholipase A_2 expression in human and rodent insulin-secreting cells. *Mol. Cell. Endocrinol.* **112:** 177–183.
234. Chen, M., Z. Yang, A. Naji, and B. A. Wolf. 1996. Identification of calcium-dependent phospholipase A_2 isoforms in human and rat pancreatic islets and insulin secreting beta-cell lines. *Endocrinology.* **137:** 2901–2909.
235. Dunlop, M., and S. Clark. 1995. Glucose-induced phosphorylation and activation of a high molecular weight cytosolic phospholipase A_2 in neonatal rat pancreatic islets. *Int. J. Biochem. Cell Biol.* **27:** 1191–1199.
236. Eerola, L. I., F. Surrel, T. J. Nevalainen, M. H. Gelb, G. Lambeau, and V. J. Laine. 2006. Analysis of expression of secreted phospholipases A_2 in mouse tissues at protein and mRNA levels. *Biochim. Biophys. Acta.* **1761:** 745–756.
237. Juhl, K., A. M. Efanov, H. L. Olsen, and J. Gromada. 2003. Secretory phospholipase A_2 is released from pancreatic beta-cells and stimulates insulin secretion via inhibition of ATP-dependent K^+ channels. *Biochem. Biophys. Res. Commun.* **310:** 274–279.
238. Ramanadham, S., Z. Ma, H. Arita, S. Zhang, and J. Turk. 1998. Type IB secretory phospholipase A_2 is contained in insulin secretory granules of pancreatic islet beta-cells and is co-secreted with insulin from glucose-stimulated islets. *Biochim. Biophys. Acta.* **1390:** 301–312.
239. Metz, S., D. Holmes, R. P. Robertson, W. Leitner, and B. Draznin. 1991. Gene expression of type I phospholipase A_2 in pancreatic beta cells. Regulation of mRNA levels by starvation or glucose excess. *FEBS Lett.* **295:** 110–112.
240. Karli, U. O., T. Schafer, and M. M. Burger. 1990. Fusion of neurotransmitter vesicles with target membrane is calcium independent in a cell-free system. *Proc. Natl. Acad. Sci. USA.* **87:** 5912–5915.
241. Nagao, T., T. Kubo, R. Fujimoto, H. Nishio, T. Takeuchi, and F. Hata. 1995. Ca^{2+}-independent fusion of secretory granules with phospholipase A_2-treated plasma membranes in vitro. *Biochem. J.* **307:** 563–569.
242. Nishio, H., T. Takeuchi, F. Hata, and O. Yagasaki. 1996. Ca(2+)-independent fusion of synaptic vesicles with phospholipase A2-treated presynaptic membranes in vitro. *Biochem. J.* **318:** 981–987.
243. Ishizaki, J., K. Hanasaki, K. Higashino, J. Kishino, N. Kikuchi, O. Ohara, and H. Arita. 1994. Molecular cloning of pancreatic group I phospholipase A_2 receptor. *J. Biol. Chem.* **269:** 5897–5904.
244. Lambeau, G., P. Ancian, J. Barhanin, and M. Lazdunski. 1994. Cloning and expression of a membrane receptor for secretory phospholipases A_2. *J. Biol. Chem.* **269:** 1575–1578.
245. Zhou, Y. P., D. Teng, F. Dralyuk, D. Ostrega, M. W. Roe, L. Philipson, and K. S. Polonsky. 1998. Apoptosis in insulin-secreting cells. Evidence for the role of intracellular Ca2+ stores and arachidonic acid metabolism. *J. Clin. Invest.* **101:** 1623–1632.
246. Lei, X., R. N. Bone, T. Ali, M. Wohltmann, Y. Gai, K. J. Goodwin, A. E. Bohrer, J. Turk, and S. Ramanadham. 2013. Genetic modulation of islet beta-cell $iPLA_2\beta$ expression provides evidence for its impact on beta-cell apoptosis and autophagy. *Islets.* **5:** 29–44.
247. Jayadev, S., B. Liu, A. E. Bielawska, J. Y. Lee, F. Nazaire, M. Y. Pushkareva, L. M. Obeid, and Y. A. Hannun. 1995. Role for ceramide in cell cycle arrest. *J. Biol. Chem.* **270:** 2047–2052.
248. Obeid, L. M., and Y. A. Hannun. 1995. Ceramide: a stress signal and mediator of growth suppression and apoptosis. *J. Cell. Biochem.* **58:** 191–198.
249. Venable, M. E., J. Y. Lee, M. J. Smyth, A. Bielawska, and L. M. Obeid. 1995. Role of ceramide in cellular senescence. *J. Biol. Chem.* **270:** 30701–30708.
250. Song, H., M. Wohltmann, M. Tan, S. Bao, J. H. Ladenson, and J. Turk. 2012. Group VIA PLA_2 ($iPLA_2$beta) is activated upstream of p38 mitogen-activated protein kinase (MAPK) in pancreatic islet beta-cell signaling. *J. Biol. Chem.* **287:** 5528–5541.
251. Vannuvel, K., P. Renard, M. Raes, and T. Arnould. 2013. Functional and morphological impact of ER stress on mitochondria. *J. Cell. Physiol.* **228:** 1802–1818.
252. Adams, J. M., and S. Cory. 2007. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene.* **26:** 1324–1337.
253. Michels, J., O. Kepp, L. Senovilla, D. Lissa, M. Castedo, G. Kroemer, and L. Galluzzi. 2013. Functions of Bcl-xL at the interface between cell death and metabolism. *Int. J. Cell Biol.* **2013:** 705294.
254. Yip, K. W., and J. C. Reed. 2008. Bcl-2 family proteins and cancer. *Oncogene.* **27:** 6398–6406.
255. Carrington, E. M., M. D. McKenzie, E. Jansen, M. Myers, S. Fynch, C. Kos, A. Strasser, T. W. Kay, C. L. Scott, and J. Allison. 2009. Islet beta-cells deficient in Bcl-xL develop but are abnormally sensitive to apoptotic stimuli. *Diabetes.* **58:** 2316–2323.
256. Federici, M., M. Hribal, L. Perego, M. Ranalli, Z. Caradonna, C. Perego, L. Usellini, R. Nano, P. Bonini, F. Bertuzzi, et al. 2001. High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes.* **50:** 1290–1301.
257. Hui, H., N. Khoury, X. Zhao, L. Balkir, E. D'Amico, A. Bullotta, E. D. Nguyen, A. Gambotto, and R. Perfetti. 2005. Adenovirus-mediated XIAP gene transfer reverses the negative effects of immunosuppressive drugs on insulin secretion and cell viability of isolated human islets. *Diabetes.* **54:** 424–433.
258. Klein, D., M. M. Ribeiro, V. Mendoza, S. Jayaraman, N. S. Kenyon, A. Pileggi, R. D. Molano, L. Inverardi, C. Ricordi, and R. L. Pastori. 2004. Delivery of Bcl-XL or its BH4 domain by protein transduction inhibits apoptosis in human islets. *Biochem. Biophys. Res. Commun.* **323:** 473–478.
259. Zhou, Y-P., J. C. Pena, M. W. Roe, A. Mittal, M. Levisetti, A. C. Baldwin, W. Pugh, D. Ostrega, N. Ahmed, V. P. Bindokas, et al. 2000. Overexpression of Bcl-xL in beta -cells prevents cell death but impairs mitochondrial signal for insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **278:** E340–E351.
260. Schwerk, C., and K. Schulze-Osthoff. 2005. Regulation of apoptosis by alternative pre-mRNA splicing. *Mol. Cell.* **19:** 1–13.
261. Barbour, S. E., P. T. Nguyen, M. Park, B. Emani, X. Lei, M. Kambalapalli, J. C. Shultz, D. Wijesinghe, C. E. Chalfant, and S. Ramanadham. 2015. Group VIA phospholipase A_2 ($iPLA_2\beta$) modulates Bcl-x 5′-splice site selection and suppresses anti-apoptotic Bcl-x(L) in β-cells. *J. Biol. Chem.* **290:** 11021–11031.
262. Seleznev, K., C. Zhao, X. H. Zhang, K. Song, and Z. A. Ma. 2006. Calcium-independent phospholipase A_2 localizes in and protects mitochondria during apoptotic induction by staurosporine. *J. Biol. Chem.* **281:** 22275–22288.
263. Zhao, Z., X. Zhang, C. Zhao, J. Choi, J. Shi, K. Song, J. Turk, and Z. A. Ma. 2010. Protection of pancreatic beta-cells by group VIA phospholipase A_2-mediated repair of mitochondrial membrane peroxidation. *Endocrinology.* **151:** 3038–3048.
264. Luo, P., and M. H. Wang. 2011. Eicosanoids, beta-cell function, and diabetes. *Prostaglandins Other Lipid Mediat.* **95:** 1–10.
265. Jenkins, C. M., A. Cedars, and R. W. Gross. 2009. Eicosanoid signalling pathways in the heart. *Cardiovasc. Res.* **82:** 240–249.
266. Khanapure, S. P., D. S. Garvey, D. R. Janero, and L. G. Letts. 2007. Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers. *Curr. Top. Med. Chem.* **7:** 311–340.
267. Adibhatla, R. M., and J. F. Hatcher. 2008. Phospholipase A(2), reactive oxygen species, and lipid peroxidation in CNS pathologies. *BMB Rep.* **41:** 560–567.
268. Bending, D., P. Zaccone, and A. Cooke. 2012. Inflammation and type one diabetes. *Int. Immunol.* **24:** 339–346.
269. Kim, N., and A. D. Luster. 2007. Regulation of immune cells by eicosanoid receptors. *ScientificWorldJournal.* **7:** 1307–1328.
270. Korotkova, M., and P. J. Jakobsson. 2014. Persisting eicosanoid pathways in rheumatic diseases. *Nat. Rev. Rheumatol.* **10:** 229–241.
271. Li, H., M. L. Edin, A. Gruzdev, J. Cheng, J. A. Bradbury, J. P. Graves, L. M. DeGraff, and D. C. Zeldin. 2013. Regulation of T helper cell subsets by cyclooxygenases and their metabolites. *Prostaglandins Other Lipid Mediat.* **104–105:** 74–83.
272. Mirshafiey, A., and F. Jadidi-Niaragh. 2010. Prostaglandins in pathogenesis and treatment of multiple sclerosis. *Immunopharmacol. Immunotoxicol.* **32:** 543–554.
273. Willcox, A., S. J. Richardson, A. J. Bone, A. K. Foulis, and N. G. Morgan. 2009. Analysis of islet inflammation in human type 1 diabetes. *Clin. Exp. Immunol.* **155:** 173–181.

274. Yousefi, B., F. Jadidi-Niaragh, G. Azizi, F. Hajighasemi, and A. Mirshafiey. 2014. The role of leukotrienes in immunopathogenesis of rheumatoid arthritis. *Mod. Rheumatol.* **24:** 225–235.
275. Kim, R., M. Emi, and K. Tanabe. 2005. Cancer cell immune escape and tumor progression by exploitation of anti-inflammatory and pro-inflammatory responses. *Cancer Biol. Ther.* **4:** 924–933.
276. Atkinson, M. A. 1997. Mechanisms underlying the loss of self tolerance in NOD mice. *Res. Immunol.* **148:** 301–306.
277. Trudeau, J. D., J. P. Dutz, E. Arany, D. J. Hill, W. E. Fieldus, and D. T. Finegood. 2000. Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? *Diabetes.* **49:** 1–7.
278. Basu, S., A. Larsson, J. Vessby, B. Vessby, and C. Berne. 2005. Type 1 diabetes is associated with increased cyclooxygenase- and cytokine-mediated inflammation. *Diabetes Care.* **28:** 1371–1375.
279. Lauber, K., E. Bohn, S. M. Krober, Y. J. Xiao, S. G. Blumenthal, R. K. Lindemann, P. Marini, C. Wiedig, A. Zobywalski, S. Baksh, et al. 2003. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell.* **113:** 717–730.
280. Peter, C., M. Waibel, H. Keppeler, R. Lehmann, G. Xu, A. Halama, J. Adamski, K. Schulze-Osthoff, S. Wesselborg, and K. Lauber. 2012. Release of lysophospholipid 'find-me' signals during apoptosis requires the ATP-binding cassette transporter A1. *Autoimmunity.* **45:** 568–573.
281. Peter, C., M. Waibel, C. G. Radu, L. V. Yang, O. N. Witte, K. Schulze-Osthoff, S. Wesselborg, and K. Lauber. 2008. Migration to apoptotic "find-me" signals is mediated via the phagocyte receptor G2A. *J. Biol. Chem.* **283:** 5296–5305.
282. Irving-Rodgers, H. F., A. F. Ziolkowski, C. R. Parish, Y. Sado, Y. Ninomiya, C. J. Simeonovic, and R. J. Rodgers. 2008. Molecular composition of the peri-islet basement membrane in NOD mice: a barrier against destructive insulitis. *Diabetologia.* **51:** 1680–1688.
283. Eizirik, D. L., and T. Mandrup-Poulsen. 2001. A choice of death-the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia.* **44:** 2115–2133.
284. Taylor-Fishwick, D. A., J. Weaver, L. Glenn, N. Kuhn, G. Rai, A. Jadhav, A. Simeonov, A. Dudda, D. Schmoll, T. R. Holman, et al. 2015. Selective inhibition of 12-lipoxygenase protects islets and beta cells from inflammatory cytokine-mediated beta cell dysfunction. *Diabetologia.* **58:** 549–557.
285. Choi, K. S. 2012. Autophagy and cancer. *Exp. Mol. Med.* **44:** 109–120.
286. Scarlatti, F., C. Bauvy, A. Ventruti, G. Sala, F. Cluzeaud, A. Vandewalle, R. Ghidoni, and P. Codogno. 2004. Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. *J. Biol. Chem.* **279:** 18384–18391.
287. Tanida, I. 2011. Autophagosome formation and molecular mechanism of autophagy. *Antioxid. Redox Signal.* **14:** 2201–2214.
288. Ganley, I. G., P. M. Wong, and X. Jiang. 2011. Thapsigargin distinguishes membrane fusion in the late stages of endocytosis and autophagy. *Autophagy.* **7:** 1397–1399.
289. Farooqui, A. A., W. Y. Ong, and L. A. Horrocks. 2006. Inhibitors of brain phospholipase A_2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. *Pharmacol. Rev.* **58:** 591–620.
290. Molloy, G. Y., M. Rattray, and R. J. Williams. 1998. Genes encoding multiple forms of phospholipase A_2 are expressed in rat brain. *Neurosci. Lett.* **258:** 139–142.
291. Farooqui, A. A., and L. A. Horrocks. 2004. Brain phospholipases A_2: a perspective on the history. *Prostaglandins Leukot. Essent. Fatty Acids.* **71:** 161–169.
292. Ong, W. Y., J. F. Yeo, S. F. Ling, and A. A. Farooqui. 2005. Distribution of calcium-independent phospholipase A_2 ($iPLA_2$) in monkey brain. *J. Neurocytol.* **34:** 447–458.
293. Yang, H. C., M. Mosior, B. Ni, and E. A. Dennis. 1999. Regional distribution, ontogeny, purification, and characterization of the Ca^{2+}-independent phospholipase A_2 from rat brain. *J. Neurochem.* **73:** 1278–1287.
294. Zanassi, P., M. Paolillo, and S. Schinelli. 1998. Coexpression of phospholipase A_2 isoforms in rat striatal astrocytes. *Neurosci. Lett.* **247:** 83–86.
295. Balboa, M. A., I. Varela-Nieto, K. Killermann Lucas, and E. A. Dennis. 2002. Expression and function of phospholipase A_2 in brain. *FEBS Lett.* **531:** 12–17.
296. Shirai, Y., and M. Ito. 2004. Specific differential expression of phospholipase A_2 subtypes in rat cerebellum. *J. Neurocytol.* **33:** 297–307.
297. Rapoport, S. I. 2008. Arachidonic acid and the brain. *J. Nutr.* **138:** 2515–2520.
298. Ramanadham, S., F. F. Hsu, A. Bohrer, W. Nowatzke, Z. Ma, and J. Turk. 1998. Electrospray ionization mass spectrometric analyses of phospholipids from rat and human pancreatic islets and subcellular membranes: comparison to other tissues and implications for membrane fusion in insulin exocytosis. *Biochemistry.* **37:** 4553–4567.
299. Cheon, Y., H. W. Kim, M. Igarashi, H. R. Modi, L. Chang, K. Ma, D. Greenstein, M. Wohltmann, J. Turk, S. I. Rapoport, et al. 2012. Disturbed brain phospholipid and docosahexaenoic acid metabolism in calcium-independent phospholipase A_2-VIA ($iPLA_2\beta$)-knockout mice. *Biochim. Biophys. Acta.* **1821:** 1278–1286.
300. Ross, B. M., C. Hudson, J. Erlich, J. J. Warsh, and S. J. Kish. 1997. Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium-independent phospholipase A_2. *Arch. Gen. Psychiatry.* **54:** 487–494.
301. Smalheiser, N. R., and D. R. Swanson. 1998. Calcium-independent phospholipase A_2 and schizophrenia. *Arch. Gen. Psychiatry.* **55:** 752–753.
302. Ross, B. M., S. Turenne, A. Moszczynska, J. J. Warsh, and S. J. Kish. 1999. Differential alteration of phospholipase A_2 activities in brain of patients with schizophrenia. *Brain Res.* **821:** 407–413.
303. Schaeffer, E. L., W. F. Gattaz, and G. P. Eckert. 2012. Alterations of brain membranes in schizophrenia: impact of phospholipase A_2. *Curr. Top. Med. Chem.* **12:** 2314–2323.
304. Smesny, S., D. Kinder, I. Willhardt, T. Rosburg, J. Lasch, G. Berger, and H. Sauer. 2005. Increased calcium-independent phospholipase A_2 activity in first but not in multiepisode chronic schizophrenia. *Biol. Psychiatry.* **57:** 399–405.
305. Gattaz, W. F., K. D. Valente, N. R. Raposo, S. Vincentiis, and L. L. Talib. 2011. Increased PLA_2 activity in the hippocampus of patients with temporal lobe epilepsy and psychosis. *J. Psychiatr. Res.* **45:** 1617–1620.
306. Junqueira, R., Q. Cordeiro, I. Meira-Lima, W. F. Gattaz, and H. Vallada. 2004. Allelic association analysis of phospholipase A_2 genes with schizophrenia. *Psychiatr. Genet.* **14:** 157–160.
307. Kerr, D. S., L. L. Talib, V. J. Yamamoto, A. S. Ferreira, M. V. Zanetti, M. H. Serpa, G. F. Busatto, M. T. Van de Bilt, and W. F. Gattaz. 2013. Antipsychotic drugs decrease $iPLA_2$ gene expression in schizophrenia. *Schizophr. Res.* **147:** 203–204.
308. Yu, Y., R. Tao, J. Shi, X. Zhang, C. Kou, Y. Guo, X. Zhang, X. Lin, S. Liu, G. Ju, et al. 2005. A genetic study of two calcium-independent cytosolic PLA_2 genes in schizophrenia. *Prostaglandins Leukot. Essent. Fatty Acids.* **73:** 351–354.
309. Wilson, D. M., and L. I. Binder. 1997. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am. J. Pathol.* **150:** 2181–2195.
310. Talbot, K., R. A. Young, C. Jolly-Tornetta, V. M. Lee, J. Q. Trojanowski, and B. A. Wolf. 2000. A frontal variant of Alzheimer's disease exhibits decreased calcium-independent phospholipase A_2 activity in the prefrontal cortex. *Neurochem. Int.* **37:** 17–31.
311. Wolf, M. J., Y. Izumi, C. F. Zorumski, and R. W. Gross. 1995. Long-term potentiation requires activation of calcium-independent phospholipase A_2. *FEBS Lett.* **377:** 358–362.
312. Schaeffer, E. L., O. V. Forlenza, and W. F. Gattaz. 2009. Phospholipase A_2 activation as a therapeutic approach for cognitive enhancement in early-stage Alzheimer disease. *Psychopharmacology (Berl.).* **202:** 37–51.
313. Tomiyama, H., H. Yoshino, and N. Hattori. 2011. Analysis of PLA2G6 in patients with frontotemporal type of dementia. *Parkinsonism Relat. Disord.* **17:** 493–494.
314. Ikenaga, E. H., L. L. Talib, A. S. Ferreira, R. Machado-Vieira, O. V. Forlenza, and W. F. Gattaz. 2015. Reduced activities of phospholipases A_2 in platelets of drug-naive bipolar disorder patients. *Bipolar Disord.* **17:** 97–101.
315. Ross, B. M., A. Moszczynska, J. Erlich, and S. J. Kish. 1998. Low activity of key phospholipid catabolic and anabolic enzymes in human substantia nigra: possible implications for Parkinson's disease. *Neuroscience.* **83:** 791–798.
316. Paisan-Ruiz, C., K. P. Bhatia, A. Li, D. Hernandez, M. Davis, N. W. Wood, J. Hardy, H. Houlden, A. Singleton, and S. A. Schneider. 2009. Characterization of PLA2G6 as a locus for dystonia-parkinsonism. *Ann. Neurol.* **65:** 19–23.
317. Paisán-Ruiz, C., R. Guevara, M. Federoff, H. Hanagasi, F. Sina, E. Elahi, S. A. Schneider, P. Schwingenschuh, N. Bajaj, M. Emre,

et al. 2010. Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatacsin mutations. *Mov. Disord.* **25:** 1791–1800.
318. Yoshino, H., H. Tomiyama, N. Tachibana, K. Ogaki, Y. Li, M. Funayama, T. Hashimoto, S. Takashima, and N. Hattori. 2010. Phenotypic spectrum of patients with PLA2G6 mutation and PARK14-linked parkinsonism. *Neurology.* **75:** 1356–1361.
319. Shi, C. H., B. S. Tang, L. Wang, Z. Y. Lv, J. Wang, L. Z. Luo, L. Shen, H. Jiang, X. X. Yan, Q. Pan, et al. 2011. PLA2G6 gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. *Neurology.* **77:** 75–81.
320. Lu, C. S., S. C. Lai, R. M. Wu, Y. H. Weng, C. L. Huang, R. S. Chen, H. C. Chang, Y. H. Wu-Chou, and T. H. Yeh. 2012. PLA2G6 mutations in PARK14-linked young-onset parkinsonism and sporadic Parkinson's disease. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **159B:** 183–191.
321. Paisán-Ruiz, C., A. Li, S. A. Schneider, J. L. Holton, R. Johnson, D. Kidd, J. Chataway, K. P. Bhatia, A. J. Lees, J. Hardy, et al. 2012. Widespread Lewy body and tau accumulation in childhood and adult onset dystonia-parkinsonism cases with PLA2G6 mutations. *Neurobiol. Aging.* **33:** 814–823.
322. Gui, Y. X., Z. P. Xu, L. Wen, H. M. Liu, J. J. Zhao, and X. Y. Hu. 2013. Four novel rare mutations of PLA2G6 in Chinese population with Parkinson's disease. *Parkinsonism Relat. Disord.* **19:** 21–26.
323. Tan, E. K., P. Ho, L. Tan, K. M. Prakash, and Y. Zhao. 2010. PLA2G6 mutations and Parkinson's disease. *Ann. Neurol.* **67:** 148.
324. Tian, J. Y., B. S. Tang, C. H. Shi, Z. Y. Lv, K. Li, R. L. Yu, L. Shen, X. X. Yan, and J. F. Guo. 2012. Analysis of PLA2G6 gene mutation in sporadic early-onset parkinsonism patients from Chinese population. *Neurosci. Lett.* **514:** 156–158.
325. Lv, Z., J. Guo, Q. Sun, K. Li, R. Yu, J. Tian, X. Yan, and B. Tang. 2012. Association between PLA2G6 gene polymorphisms and Parkinson's disease in the Chinese Han population. *Parkinsonism Relat. Disord.* **18:** 641–644.
326. Tomiyama, H., H. Yoshino, K. Ogaki, L. Li, C. Yamashita, Y. Li, M. Funayama, R. Sasaki, Y. Kokubo, S. Kuzuhara, et al. 2011. PLA2G6 variant in Parkinson's disease. *J. Hum. Genet.* **56:** 401–403.
327. Khateeb, S., H. Flusser, R. Ofir, I. Shelef, G. Narkis, G. Vardi, Z. Shorer, R. Levy, A. Galil, K. Elbedour, et al. 2006. PLA2G6 mutation underlies infantile neuroaxonal dystrophy. *Am. J. Hum. Genet.* **79:** 942–948.
328. Biancheri, R., A. Rossi, G. Alpigiani, M. Filocamo, C. Gandolfo, R. Lorini, and C. Minetti. 2007. Cerebellar atrophy without cerebellar cortex hyperintensity in infantile neuroaxonal dystrophy (INAD) due to PLA2G6 mutation. *Eur. J. Paediatr. Neurol.* **11:** 175–177.
329. Polster, B., M. Crosier, S. Lindsay, and S. Hayflick. 2010. Expression of PLA2G6 in human fetal development: implications for infantile neuroaxonal dystrophy. *Brain Res. Bull.* **83:** 374–379.
330. Malik, I., J. Turk, D. J. Mancuso, L. Montier, M. Wohltmann, D. F. Wozniak, R. E. Schmidt, R. W. Gross, and P. T. Kotzbauer. 2008. Disrupted membrane homeostasis and accumulation of ubiquitinated proteins in a mouse model of infantile neuroaxonal dystrophy caused by PLA2G6 mutations. *Am. J. Pathol.* **172:** 406–416.
331. Wada, H., T. Yasuda, I. Miura, K. Watabe, C. Sawa, H. Kamijuku, S. Kojo, M. Taniguchi, I. Nishino, S. Wakana, et al. 2009. Establishment of an improved mouse model for infantile neuroaxonal dystrophy that shows early disease onset and bears a point mutation in PLA2G6. *Am. J. Pathol.* **175:** 2257–2263.
332. Gregory, A., S. K. Westaway, I. E. Holm, P. T. Kotzbauer, P. Hogarth, S. Sonek, J. C. Coryell, T. M. Nguyen, N. Nardocci, G. Zorzi, et al. 2008. Neurodegeneration associated with genetic defects in phospholipase A_2. *Neurology.* **71:** 1402–1409.
333. Carrilho, I., M. Santos, A. Guimaraes, J. Teixeira, R. Chorao, M. Martins, C. Dias, A. Gregory, S. Westaway, T. Nguyen, et al. 2008. Infantile neuroaxonal dystrophy: what's most important for the diagnosis? *Eur. J. Paediatr. Neurol.* **12:** 491–500.
334. Beck, G., Y. Sugiura, K. Shinzawa, S. Kato, M. Setou, Y. Tsujimoto, S. Sakoda, and H. Sumi-Akamaru. 2011. Neuroaxonal dystrophy in calcium-independent phospholipase A_2beta deficiency results from insufficient remodeling and degeneration of mitochondrial and presynaptic membranes. *J. Neurosci.* **31:** 11411–11420.
335. Strokin, M., K. L. Seburn, G. A. Cox, K. A. Martens, and G. Reiser. 2012. Severe disturbance in the Ca^{2+} signaling in astrocytes from mouse models of human infantile neuroaxonal dystrophy with mutated PLA2G6. *Hum. Mol. Genet.* **21:** 2807–2814.
336. Wada, H., S. Kojo, and K. Seino. 2013. Mouse models of human INAD by PLA2G6 deficiency. *Histol. Histopathol.* **28:** 965–969.
337. Zhao, Z., J. Wang, C. Zhao, W. Bi, Z. Yue, and Z. A. Ma. 2011. Genetic ablation of PLA2G6 in mice leads to cerebellar atrophy characterized by Purkinje cell loss and glial cell activation. *PLoS ONE.* **6:** e26991.
338. Kruer, M. C., R. Paudel, W. Wagoner, L. Sanford, E. Kara, A. Gregory, T. Foltynie, A. Lees, K. Bhatia, J. Hardy, et al. 2012. Analysis of ATP13A2 in large neurodegeneration with brain iron accumulation (NBIA) and dystonia-parkinsonism cohorts. *Neurosci. Lett.* **523:** 35–38.
339. Morgan, N. V., S. K. Westaway, J. E. Morton, A. Gregory, P. Gissen, S. Sonek, H. Cangul, J. Coryell, N. Canham, N. Nardocci, et al. 2006. PLA2G6, encoding a phospholipase A_2, is mutated in neurodegenerative disorders with high brain iron. *Nat. Genet.* **38:** 752–754.
340. Gregory, A., and S. J. Hayflick. 2011. Genetics of neurodegeneration with brain iron accumulation. *Curr. Neurol. Neurosci. Rep.* **11:** 254–261.
341. Hayflick, S. J. 2006. Neurodegeneration with brain iron accumulation: from genes to pathogenesis. *Semin. Pediatr. Neurol.* **13:** 182–185.
342. Engel, L. A., Z. Jing, D. E. O'Brien, M. Sun, and P. T. Kotzbauer. 2010. Catalytic function of PLA2G6 is impaired by mutations associated with infantile neuroaxonal dystrophy but not dystonia-parkinsonism. *PLoS ONE.* **5:** e12897.
343. Sina, F., S. Shojaee, E. Elahi, and C. Paisan-Ruiz. 2009. R632W mutation in PLA2G6 segregates with dystonia-parkinsonism in a consanguineous Iranian family. *Eur. J. Neurol.* **16:** 101–104.
344. Kurian, M. A., N. V. Morgan, L. MacPherson, K. Foster, D. Peake, R. Gupta, S. G. Philip, C. Hendriksz, J. E. Morton, H. M. Kingston, et al. 2008. Phenotypic spectrum of neurodegeneration associated with mutations in the PLA2G6 gene (PLAN). *Neurology.* **70:** 1623–1629.
345. Bower, M. A., K. Bushara, M. A. Dempsey, S. Das, and P. J. Tuite. 2011. Novel mutations in siblings with later-onset PLA2G6-associated neurodegeneration (PLAN). *Mov. Disord.* **26:** 1768–1769.
346. Salih, M. A., E. Mundwiller, A. O. Khan, A. AlDrees, S. A. Elmalik, H. H. Hassan, M. Al-Owain, H. M. Alkhalidi, I. Katona, M. M. Kabiraj, et al. 2013. New findings in a global approach to dissect the whole phenotype of PLA2G6 gene mutations. *PLoS ONE.* **8:** e76831.
347. Crompton, D., P. K. Rehal, L. MacPherson, K. Foster, P. Lunt, I. Hughes, A. F. Brady, M. G. Pike, S. De Gressi, N. V. Morgan, et al. 2010. Multiplex ligation-dependent probe amplification (MLPA) analysis is an effective tool for the detection of novel intragenic PLA2G6 mutations: implications for molecular diagnosis. *Mol. Genet. Metab.* **100:** 207–212.
348. Westaway, S. K., A. Gregory, and S. J. Hayflick. 2007. Mutations in PLA2G6 and the riddle of Schindler disease. *J. Med. Genet.* **44:** e64.
349. Ross, B. M., B. Hughes, S. J. Kish, and J. J. Warsh. 2006. Serum calcium-independent phospholipase A_2 activity in bipolar affective disorder. *Bipolar Disord.* **8:** 265–270.
350. Blanchard, H., A. Y. Taha, Y. Cheon, H. W. Kim, J. Turk, and S. I. Rapoport. 2014. iPLA$_2$beta knockout mouse, a genetic model for progressive human motor disorders, develops age-related neuropathology. *Neurochem. Res.* **39:** 1522–1532.
351. Lopez-Vales, R., X. Navarro, T. Shimizu, C. Baskakis, G. Kokotos, V. Constantinou-Kokotou, D. Stephens, E. A. Dennis, and S. David. 2008. Intracellular phospholipase A_2 group IVA and group VIA play important roles in Wallerian degeneration and axon regeneration after peripheral nerve injury. *Brain.* **131:** 2620–2631.
352. Lopez-Vales, R., N. Ghasemlou, A. Redensek, B. J. Kerr, E. Barbayianni, G. Antonopoulou, C. Baskakis, K. I. Rathore, V. Constantinou-Kokotou, D. Stephens, et al. 2011. Phospholipase A_2 superfamily members play divergent roles after spinal cord injury. *FASEB J.* **25:** 4240–4252.
353. Giurdanella, G., C. Motta, S. Muriana, V. Arena, C. D. Anfuso, G. Lupo, and M. Alberghina. 2011. Cytosolic and calcium-independent phospholipase A_2 mediate glioma-enhanced proangiogenic activity of brain endothelial cells. *Microvasc. Res.* **81:** 1–17.
354. Lee, L. H., C. H. Tan, G. Shui, M. R. Wenk, and W. Y. Ong. 2012. Role of prefrontal cortical calcium independent phospholipase A_2 in antidepressant-like effect of maprotiline. *Int. J. Neuropsychopharmacol.* **15:** 1087–1098.
355. Moon, K. H., N. Tajuddin, J. Brown 3rd, E. J. Neafsey, H. Y. Kim, and M. A. Collins. 2014. Phospholipase A_2, oxidative stress, and neurodegeneration in binge ethanol-treated organotypic

slice cultures of developing rat brain. *Alcohol. Clin. Exp. Res.* **38:** 161–169.
356. Liou, J. Y., N. Aleksic, S. F. Chen, T. J. Han, S. K. Shyue, and K. K. Wu. 2005. Mitochondrial localization of cyclooxygenase-2 and calcium-independent phospholipase A_2 in human cancer cells: implication in apoptosis resistance. *Exp. Cell Res.* **306:** 75–84.
357. Pérez, R., X. Matabosch, A. Llebaria, M. A. Balboa, and J. Balsinde. 2006. Blockade of arachidonic acid incorporation into phospholipids induces apoptosis in U937 promonocytic cells. *J. Lipid Res.* **47:** 484–491.
358. Pérez, R., R. Melero, M. A. Balboa, and J. Balsinde. 2004. Role of group VIA calcium-independent phospholipase A_2 in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide. *J. Biol. Chem.* **279:** 40385–40391.
359. Saavedra, G., W. Zhang, B. Peterson, and B. S. Cummings. 2006. Differential roles for cytosolic and microsomal Ca^{2+}-independent phospholipase A_2 in cell growth and maintenance of phospholipids. *J. Pharmacol. Exp. Ther.* **318:** 1211–1219.
360. Shin, K. J., C. Chung, Y. A. Hwang, S. H. Kim, M. S. Han, S. H. Ryu, and P. G. Suh. 2002. Phospholipase A_2-mediated Ca^{2+} influx by 2,2',4,6-tetrachlorobiphenyl in PC12 cells. *Toxicol. Appl. Pharmacol.* **178:** 37–43.
361. Wilson, H. A., D. V. Allred, K. O'Neill, and J. D. Bell. 2000. Activities and interactions among phospholipases A_2 during thapsigargin-induced S49 cell death. *Apoptosis.* **5:** 389–396.
362. Kim, S. Y., H. Y. Jo, M. H. Kim, Y. Y. Cha, S. W. Choi, J. H. Shim, T. J. Kim, and K. Y. Lee. 2008. H2O2-dependent hyperoxidation of peroxiredoxin 6 (Prdx6) plays a role in cellular toxicity via up-regulation of $iPLA_2$ activity. *J. Biol. Chem.* **283:** 33563–33568.
363. Lallemand, C., B. Blanchard, M. Palmieri, P. Lebon, E. May, and M. G. Tovey. 2007. Single-stranded RNA viruses inactivate the transcriptional activity of p53 but induce NOXA-dependent apoptosis via post-translational modifications of IRF-1, IRF-3 and CREB. *Oncogene.* **26:** 328–338.
364. Zhang, X. H., C. Zhao, and Z. A. Ma. 2007. The increase of cell-membranous phosphatidylcholines containing polyunsaturated fatty acid residues induces phosphorylation of p53 through activation of ATR. *J. Cell Sci.* **120:** 4134–4143.
365. Hooks, S. B., and B. S. Cummings. 2008. Role of Ca^{2+}-independent phospholipase A_2 in cell growth and signaling. *Biochem. Pharmacol.* **76:** 1059–1067.
366. Scott, K. F., M. Sajinovic, J. Hein, S. Nixdorf, P. Galettis, W. Liauw, P. de Souza, Q. Dong, G. G. Graham, and P. J. Russell. 2010. Emerging roles for phospholipase A_2 enzymes in cancer. *Biochimie.* **92:** 601–610.
367. Laye, J. P., and J. H. Gill. 2003. Phospholipase A_2 expression in tumours: a target for therapeutic intervention? *Drug Discov. Today.* **8:** 710–716.
368. Bonney, R. C., and S. Franks. 1988. The activity of calcium dependent and calcium independent phospholipase A2 in normal endometrium and in endometrium from women suffering from menorrhagia and polycystic ovary syndrome. *Gynecol. Endocrinol.* **2:** 131–138.
369. Deleted in proof.
370. Li, H., Z. Zhao, G. Wei, L. Yan, D. Wang, H. Zhang, G. E. Sandusky, J. Turk, and Y. Xu. 2010. Group VIA phospholipase A2 in both host and tumor cells is involved in ovarian cancer development. *FASEB J.* **24:** 4103–4116.
371. Li, H., Z. Zhao, C. Antalis, Z. Zhao, R. Emerson, G. Wei, S. Zhang, Z. Y. Zhang, and Y. Xu. 2011. Combination therapy of an inhibitor of group VIA phospholipase A_2 with paclitaxel is highly effective in blocking ovarian cancer development. *Am. J. Pathol.* **179:** 452–461.
372. Shen, Z., J. Belinson, R. E. Morton, Y. Xu, and Y. Xu. 1998. Phorbol 12-myristate 13-acetate stimulates lysophosphatidic acid secretion from ovarian and cervical cancer cells but not from breast or leukemia cells. *Gynecol. Oncol.* **71:** 364–368.
373. Cai, Q., Z. Zhao, C. Antalis, L. Yan, G. Del Priore, A. H. Hamed, F. B. Stehman, J. M. Schilder, and Y. Xu. 2012. Elevated and secreted phospholipase A2 activities as new potential therapeutic targets in human epithelial ovarian cancer. *FASEB J.* **26:** 3306–3320.
374. Nicotera, T. M., D. P. Schuster, M. Bourhim, K. Chadha, G. Klaich, and D. A. Corral. 2009. Regulation of PSA secretion and survival signaling by calcium-independent phopholipase A_2beta in prostate cancer cells. *Prostate.* **69:** 1270–1280.
375. Sun, B., X. Zhang, C. Yonz, and B. S. Cummings. 2010. Inhibition of calcium-independent phospholipase A_2 activates p38 MAPK signaling pathways during cytostasis in prostate cancer cells. *Biochem. Pharmacol.* **79:** 1727–1735.
376. Yun, H. M., K. R. Park, H. P. Lee, D. H. Lee, M. Jo, D. H. Shin, D. Y. Yoon, S. B. Han, and J. T. Hong. 2014. PRDX6 promotes lung tumor progression via its GPx and $iPLA_2$ activities. *Free Radic. Biol. Med.* **69:** 367–376.
377. McHowat, J., G. Gullickson, R. G. Hoover, J. Sharma, J. Turk, and J. Kornbluth. 2011. Platelet-activating factor and metastasis: calcium-independent phospholipase A_2beta deficiency protects against breast cancer metastasis to the lung. *Am. J. Physiol. Cell Physiol.* **300:** C825–C832.
378. Kispert, S., J. Marentette, and J. McHowat. 2015. Cigarette smoke induces cell motility via platelet-activating factor accumulation in breast cancer cells: a potential mechanism for metastatic disease. *Physiol. Rep.* **3:** e12318.
379. Zhu, C., Z. Sun, C. Li, R. Guo, L. Li, L. Jin, R. Wan, and S. Li. 2014. Urocortin affects migration of hepatic cancer cell lines via differential regulation of $cPLA_2$ and $iPLA_2$. *Cell. Signal.* **26:** 1125–1134.
380. Hoeft, B., J. Linseisen, L. Beckmann, K. Muller-Decker, F. Canzian, A. Husing, R. Kaaks, U. Vogel, M. U. Jakobsen, K. Overvad, et al. 2010. Polymorphisms in fatty-acid-metabolism-related genes are associated with colorectal cancer risk. *Carcinogenesis.* **31:** 466–472.
381. Kvaskoff, M., D. C. Whiteman, Z. Z. Zhao, G. W. Montgomery, N. G. Martin, N. K. Hayward, and D. L. Duffy. 2011. Polymorphisms in nevus-associated genes MTAP, PLA2G6, and IRF4 and the risk of invasive cutaneous melanoma. *Twin Res. Hum. Genet.* **14:** 422–432.
382. Scuderi, M. R., C. D. Anfuso, G. Lupo, C. Motta, L. Romeo, L. Guerra, A. Cappellani, N. Ragusa, G. Cantarella, and M. Alberghina. 2008. Expression of Ca^{2+}-independent and Ca^{2+}-dependent phospholipases A_2 and cyclooxygenases in human melanocytes and malignant melanoma cell lines. *Biochim. Biophys. Acta.* **1781:** 635–642.
383. Deleted in proof.
384. Hazen, S. L., and R. W. Gross. 1993. The specific association of a phosphofructokinase isoform with myocardial calcium-independent phospholipase A_2. Implications for the coordinated regulation of phospholipolysis and glycolysis. *J. Biol. Chem.* **268:** 9892–9900.
385. McHowat, J., and M. H. Creer. 2004. Catalytic features, regulation and function of myocardial phospholipase A_2. *Curr. Med. Chem. Cardiovasc. Hematol. Agents.* **2:** 209–218.
386. White, M. C., and J. McHowat. 2007. The therapeutic potential of phospholipase A_2 inhibitors in cardiovascular disease. *Cardiovasc. Hematol. Agents Med. Chem.* **5:** 91–95.
387. Ford, D. A., S. L. Hazen, J. E. Saffitz, and R. W. Gross. 1991. The rapid and reversible activation of a calcium-independent plasmalogen-selective phospholipase A_2 during myocardial ischemia. *J. Clin. Invest.* **88:** 331–335.
388. Williams, S. D., and R. A. Gottlieb. 2002. Inhibition of mitochondrial calcium-independent phospholipase A_2 ($iPLA_2$) attenuates mitochondrial phospholipid loss and is cardioprotective. *Biochem. J.* **362:** 23–32.
389. Kan, H., Z. Xie, and M. S. Finkel. 2006. $iPLA_2$ inhibitor blocks negative inotropic effect of HIV gp120 on cardiac myocytes. *J. Mol. Cell. Cardiol.* **40:** 131–137.
390. Rahnema, P., Y. Shimoni, and A. Nygren. 2011. Reduced conduction reserve in the diabetic rat heart: role of $iPLA_2$ activation in the response to ischemia. *Am. J. Physiol. Heart Circ. Physiol.* **300:** H326–H334.
391. Vesterqvist, O., C. A. Sargent, G. J. Grover, and M. L. Ogletree. 1996. Myocardial calcium-independent phospholipase A_2 activity during global ischemia in isolated rabbit hearts. *Cardiovasc. Res.* **31:** 932–940.
392. Zachman, D. K., A. J. Chicco, S. A. McCune, R. C. Murphy, R. L. Moore, and G. C. Sparagna. 2010. The role of calcium-independent phospholipase A_2 in cardiolipin remodeling in the spontaneously hypertensive heart failure rat heart. *J. Lipid Res.* **51:** 525–534.
393. McHowat, J., L. M. Swift, and N. Sarvazyan. 2001. Oxidant-induced inhibition of myocardial calcium-independent phospholipase A_2. *Cardiovasc. Toxicol.* **1:** 309–316.
394. Sharma, J., J. Turk, D. J. Mancuso, H. F. Sims, R. W. Gross, and J. McHowat. 2011. Activation of group VI phospholipase A_2 isoforms in cardiac endothelial cells. *Am. J. Physiol. Cell Physiol.* **300:** C872–C879.

395. Malhotra, A., I. Edelman-Novemsky, Y. Xu, H. Plesken, J. Ma, M. Schlame, and M. Ren. 2009. Role of calcium-independent phospholipase A_2 in the pathogenesis of Barth syndrome. *Proc. Natl. Acad. Sci. USA.* **106:** 2337–2341.
396. Kiebish, M. A., K. Yang, X. Liu, D. J. Mancuso, S. Guan, Z. Zhao, H. F. Sims, R. Cerqua, W. T. Cade, X. Han, et al. 2013. Dysfunctional cardiac mitochondrial bioenergetic, lipidomic, and signaling in a murine model of Barth syndrome. *J. Lipid Res.* **54:** 1312–1325.
397. Smani, T., S. I. Zakharov, E. Leno, P. Csutora, E. S. Trepakova, and V. M. Bolotina. 2003. Ca^{2+}-independent phospholipase A_2 is a novel determinant of store-operated Ca^{2+} entry. *J. Biol. Chem.* **278:** 11909–11915.
398. Csutora, P., V. Zarayskiy, K. Peter, F. Monje, T. Smani, S. I. Zakharov, D. Litvinov, and V. M. Bolotina. 2006. Activation mechanism for CRAC current and store-operated Ca^{2+} entry: calcium influx factor and Ca^{2+}-independent phospholipase A_2beta-mediated pathway. *J. Biol. Chem.* **281:** 34926–34935.
399. Bolotina, V. M. 2004. Store-operated channels: diversity and activation mechanisms. *Sci. STKE.* **2004:** pe34.
400. Dietrich, H. H., D. R. Abendschein, S. H. Moon, N. Nayeb-Hashemi, D. J. Mancuso, C. M. Jenkins, K. M. Kaltenbronn, K. J. Blumer, J. Turk, and R. W. Gross. 2010. Genetic ablation of calcium-independent phospholipase A_2beta causes hypercontractility and markedly attenuates endothelium-dependent relaxation to acetylcholine. *Am. J. Physiol. Heart Circ. Physiol.* **298:** H2208–H2220.
401. Balsinde, J. 2002. Roles of various phospholipases A_2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. *Biochem. J.* **364:** 695–702.
402. Balsinde, J., M. A. Balboa, and E. A. Dennis. 2000. Identification of a third pathway for arachidonic acid mobilization and prostaglandin production in activated P388D1 macrophage-like cells. *J. Biol. Chem.* **275:** 22544–22549.
403. Hsu, F. F., Z. Ma, M. Wohltmann, A. Bohrer, W. Nowatzke, S. Ramanadham, and J. Turk. 2000. Electrospray ionization/mass spectrometric analyses of human promonocytic U937 cell glycerolipids and evidence that differentiation is associated with membrane lipid composition changes that facilitate phospholipase A_2 activation. *J. Biol. Chem.* **275:** 16579–16589.
404. Pindado, J., J. Balsinde, and M. A. Balboa. 2007. TLR3-dependent induction of nitric oxide synthase in RAW 264.7 macrophage-like cells via a cytosolic phospholipase A_2/cyclooxygenase-2 pathway. *J. Immunol.* **179:** 4821–4828.
405. Pérez-Chacón, G., A. M. Astudillo, V. Ruiperez, M. A. Balboa, and J. Balsinde. 2010. Signaling role for lysophosphatidylcholine acyltransferase 3 in receptor-regulated arachidonic acid reacylation reactions in human monocytes. *J. Immunol.* **184:** 1071–1078.
406. Degousee, N., F. Ghomashchi, E. Stefanski, A. Singer, B. P. Smart, N. Borregaard, R. Reithmeier, T. F. Lindsay, C. Lichtenberger, W. Reinisch, et al. 2002. Groups IV, V, and X phospholipases A_2s in human neutrophils: role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis. *J. Biol. Chem.* **277:** 5061–5073.
407. Marshall, J., E. Krump, T. Lindsay, G. Downey, D. A. Ford, P. Zhu, P. Walker, and B. Rubin. 2000. Involvement of cytosolic phospholipase A_2 and secretory phospholipase A2 in arachidonic acid release from human neutrophils. *J. Immunol.* **164:** 2084–2091.
408. Ueno, N., Y. Taketomi, K. Yamamoto, T. Hirabayashi, D. Kamei, Y. Kita, T. Shimizu, K. Shinzawa, Y. Tsujimoto, K. Ikeda, et al. 2011. Analysis of two major intracellular phospholipases A_2 (PLA_2) in mast cells reveals crucial contribution of cytosolic PLA_2alpha, not Ca^{2+}-independent PLA_2beta, to lipid mobilization in proximal mast cells and distal fibroblasts. *J. Biol. Chem.* **286:** 37249–37263.
409. Roshak, A. K., E. A. Capper, C. Stevenson, C. Eichman, and L. A. Marshall. 2000. Human calcium-independent phospholipase A_2 mediates lymphocyte proliferation. *J. Biol. Chem.* **275:** 35692–35698.
410. Balboa, M. A., and J. Balsinde. 2002. Involvement of calcium-independent phospholipase A_2 in hydrogen peroxide-induced accumulation of free fatty acids in human U937 cells. *J. Biol. Chem.* **277:** 40384–40389.
411. Deleted in proof.
412. Martínez, J., and J. J. Moreno. 2001. Role of Ca^{2+}-independent phospholipase A_2 on arachidonic acid release induced by reactive oxygen species. *Arch. Biochem. Biophys.* **392:** 257–262.
413. Balboa, M. A., R. Perez, and J. Balsinde. 2008. Calcium-independent phospholipase A_2 mediates proliferation of human promonocytic U937 cells. *FEBS J.* **275:** 1915–1924.
414. Kim, S. J., D. Gershov, X. Ma, N. Brot, and K. B. Elkon. 2002. I-PLA_2 activation during apoptosis promotes the exposure of membrane lysophosphatidylcholine leading to binding by natural immunoglobulin M antibodies and complement activation. *J. Exp. Med.* **196:** 655–665.
415. Teslenko, V., M. Rogers, and J. B. Lefkowith. 1997. Macrophage arachidonate release via both the cytosolic Ca^{2+}-dependent and -independent phospholipases is necessary for cell spreading. *Biochim. Biophys. Acta.* **1344:** 189–199.
416. Nikolic, D. M., M. C. Gong, J. Turk, and S. R. Post. 2007. Class A scavenger receptor-mediated macrophage adhesion requires coupling of calcium-independent phospholipase A_2 and 12/15-lipoxygenase to Rac and Cdc42 activation. *J. Biol. Chem.* **282:** 33405–33411.
417. Pérez, R., M. A. Balboa, and J. Balsinde. 2006. Involvement of group VIA calcium-independent phospholipase A2 in macrophage engulfment of hydrogen peroxide-treated U937 cells. *J. Immunol.* **176:** 2555–2561.
418. Karimi, K., T. R. Gemmill, and M. R. Lennartz. 1999. Protein kinase C and a calcium-independent phospholipase are required for IgG-mediated phagocytosis by Mono-Mac-6 cells. *J. Leukoc. Biol.* **65:** 854–862.
419. Lennartz, M. R., J. B. Lefkowith, F. A. Bromley, and E. J. Brown. 1993. Immunoglobulin G-mediated phagocytosis activates a calcium-independent, phosphatidylethanolamine-specific phospholipase. *J. Leukoc. Biol.* **54:** 389–398.
420. Atkinson, M. A., J. A. Bluestone, G. S. Eisenbarth, M. Hebrok, K. C. Herold, D. Accili, M. Pietropaolo, P. R. Arvan, M. Von Herrath, D. S. Markel, et al. 2011. How does type 1 diabetes develop?: the notion of homicide or beta-cell suicide revisited. *Diabetes.* **60:** 1370–1379.
421. Satoh, T., N. Abiru, M. Kobayashi, H. Zhou, K. Nakamura, G. Kuriya, H. Nakamura, Y. Nagayama, E. Kawasaki, H. Yamasaki, et al. 2011. CHOP deletion does not impact the development of diabetes but suppresses the early production of insulin autoantibody in the NOD mouse. *Apoptosis.* **16:** 438–448.
422. Padgett, L. E., K. A. Broniowska, P. A. Hansen, J. A. Corbett, and H. M. Tse. 2013. The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. *Ann. N. Y. Acad. Sci.* **1281:** 16–35.
423. Thayer, T. C., M. Delano, C. Liu, J. Chen, L. E. Padgett, H. M. Tse, M. Annamali, J. D. Piganelli, L. L. Moldawer, and C. E. Mathews. 2011. Superoxide production by macrophages and T cells is critical for the induction of autoreactivity and type 1 diabetes. *Diabetes.* **60:** 2144–2151.
424. Baumann, B., H. H. Salem, and B. O. Boehm. 2012. Anti-inflammatory therapy in type 1 diabetes. *Curr. Diab. Rep.* **12:** 499–509.
425. Chan, J. Y., T. J. Biden, and D. R. Laybutt. 2012. Cross-talk between the unfolded protein response and nuclear factor-κB signalling pathways regulates cytokine-mediated beta cell death in MIN6 cells and isolated mouse islets. *Diabetologia.* **55:** 2999–3009.
426. Blanc, L., C. Barres, P. Bette-Bobillo, and M. Vidal. 2007. Reticulocyte-secreted exosomes bind natural IgM antibodies: involvement of a ROS-activatable endosomal phospholipase iPLA_2. *Blood.* **110:** 3407–3416.
427. Deleted in proof.
428. Deleted in proof.
429. Muralikrishna Adibhatla, R., and J. F. Hatcher. 2006. Phospholipase A_2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic. Biol. Med.* **40:** 376–387.
430. Osada-Oka, M., M. Takahashi, S. Akiba, and T. Sato. 2006. Involvement of Ca^{2+}-independent phospholipase A_2 in the translocation of hypoxia-inducible factor-1α to the nucleus under hypoxic conditions. *Eur. J. Pharmacol.* **549:** 58–62.
431. Deleted in proof.
432. Sun, G. Y., J. Xu, M. D. Jensen, S. Yu, W. G. Wood, F. A. Gonzalez, A. Simonyi, A. Y. Sun, and G. A. Weisman. 2005. Phospholipase A_2 in astrocytes: responses to oxidative stress, inflammation, and G protein-coupled receptor agonists. *Mol. Neurobiol.* **31:** 27–41.
433. Tithof, P. K., M. Peters-Golden, and P. E. Ganey. 1998. Distinct phospholipases A2 regulate the release of arachidonic acid for eicosanoid production and superoxide anion generation in neutrophils. *J. Immunol.* **160:** 953–960.
434. Tan, C., R. Day, S. Bao, J. Turk, and Q. D. Zhao. 2014. Group VIA phospholipase A_2 mediates enhanced macrophage migration

in diabetes mellitus by increasing expression of nicotinamide adenine dinucleotide phosphate oxidase 4. *Arterioscler. Thromb. Vasc. Biol.* **34:** 768–778.
435. Mollah, Z. U., S. Pai, C. Moore, B. J. O'Sullivan, M. J. Harrison, J. Peng, K. Phillips, J. B. Prins, J. Cardinal, and R. Thomas. 2008. Abnormal NF-kappa B function characterizes human type 1 diabetes dendritic cells and monocytes. *J. Immunol.* **180:** 3166–3175.
436. Devaraj, S., M. R. Dasu, J. Rockwood, W. Winter, S. C. Griffen, and I. Jialal. 2008. Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. *J. Clin. Endocrinol. Metab.* **93:** 578–583.
437. Deng, J., P. D. Lu, Y. Zhang, D. Scheuner, R. J. Kaufman, N. Sonenberg, H. P. Harding, and D. Ron. 2004. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol. Cell. Biol.* **24:** 10161–10168.
438. Jiang, H. Y., S. A. Wek, B. C. McGrath, D. Scheuner, R. J. Kaufman, D. R. Cavener, and R. C. Wek. 2003. Phosphorylation of the alpha subunit of eukaryotic initiation factor 2 is required for activation of NF-kappaB in response to diverse cellular stresses. *Mol. Cell. Biol.* **23:** 5651–5663.
439. Morgan, M. J., and Z. G. Liu. 2011. Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res.* **21:** 103–115.
440. Carnevale, K. A., and M. K. Cathcart. 2001. Calcium-independent phospholipase A_2 is required for human monocyte chemotaxis to monocyte chemoattractant protein 1. *J. Immunol.* **167:** 3414–3421.
441. Gilroy, D. W., J. Newson, P. Sawmynaden, D. A. Willoughby, and J. D. Croxtall. 2004. A novel role for phospholipase A_2 isoforms in the checkpoint control of acute inflammation. *FASEB J.* **18:** 489–498.
442. Zhang, L., S. Zhong, Y. Li, G. Ji, M. Sundaram, and Z. Yao. 2013. Global inactivation of the Pla2g6 gene in mice does not cause dyslipidemia under chow or high-fat diet conditions. *J. Cancer Prev.* **18:** 235–248.
443. Portilla, D., M. D. Crew, D. Grant, G. Serrero, L. M. Bates, G. Dai, M. Sasner, J. Cheng, and A. Buonanno. 1998. cDNA cloning and expression of a novel family of enzymes with calcium-independent phospholipase A_2 and lysophospholipase activities. *J. Am. Soc. Nephrol.* **9:** 1178–1186.
444. Portilla, D., and G. Dai. 1996. Purification of a novel calcium-independent phospholipase A_2 from rabbit kidney. *J. Biol. Chem.* **271:** 15451–15457.
445. Mizuno-Kamiya, M., H. Inokuchi, Y. Kameyama, K. Yashiro, and A. Fujita. 2001. Ca^{2+}-independent phospholipase A_2 activity in apical plasma membranes from the rat parotid gland. *Arch. Oral Biol.* **46:** 789–799.
446. Deleted in proof.
447. Akiba, S., and T. Sato. 2004. Cellular function of calcium-independent phospholipase A_2. *Biol. Pharm. Bull.* **27:** 1174–1178.
448. Hui, D. Y. 2012. Phospholipase A_2 enzymes in metabolic and cardiovascular diseases. *Curr. Opin. Lipidol.* **23:** 235–240.
449. Richardson, R. J., N. D. Hein, S. J. Wijeyesakere, J. K. Fink, and G. F. Makhaeva. 2013. Neuropathy target esterase (NTE): overview and future. *Chem. Biol. Interact.* **203:** 238–244.
450. Dongiovanni, P., B. Donati, R. Fares, R. Lombardi, R. M. Mancina, S. Romeo, and L. Valenti. 2013. PNPLA3 I148M polymorphism and progressive liver disease. *World J. Gastroenterol.* **19:** 6969–6978.
451. Dubuquoy, C., A. F. Burnol, and M. Moldes. 2013. PNPLA3, a genetic marker of progressive liver disease, still hiding its metabolic function? *Clin. Res. Hepatol. Gastroenterol.* **37:** 30–35.