Supplementary data

Supplementary Figure 1. Representative mass spectra of OxPLs generated by oxidation of a single molecular species of PC. PAPC (*m/z* 782) and PLPC (*m/z* 758) were oxidized by exposure of dry lipids to air and analyzed by mass spectrometry in positive mode using flow injection. The data illustrate that non-enzymatic oxidation of a single PC produces dozens of oxidized species. Note that *m/z* values of all major oxidized products present in these spectra are monitored by the HPLC-MS/MS method.

Supplementary Figure 2. OxPCs elute from reversed-phase column significantly earlier than the bulk of unoxidized PCs. The total ion current chromatogram shows the sum of intensities of 99 *m/z* values monitored at every time point during elution from the column. Air-oxidized pure PCs were used to estimate the time window when oxidized species were eluted. In addition to fibroblast extract, lipid extracts of mouse lung and liver were taken as representative samples obtained in experiments in vivo (generous gift of Dr. Konstantin Birukov, Univ. of Chicago). Based on these data, OxPCs were quantified in further experiments within 8 minutes (dashed line).

Supplementary Figure 3. Variability of patterns of OxPLs in different biological samples. OxPCs were detected by HPLC-MS/MS in LLE-purified lipid extracts from sham-treated (control) fibroblasts (left column) or lungs of untreated mice (right column, generous gift of Dr. Konstantin Birukov, Univ. of Chicago). Selected *m/z* values are shown to illustrate significant differences between the samples in relative abundances of isobaric peaks.

Supplementary Figure 4. Identification of selected endogenous OxPCs by tandem mass spectrometry in negative mode. Commercial standards or lipid extracts from UVA-irradiated human dermal fibroblasts were analyzed in positive or negative mode using transitions characteristic of each OxPC.

Supplementary Table 1.

Extraction efficiency of the LLE procedure.

Samples of lipids extracted from fibroblasts by methanol/acetic acid (3%)/BHT (0.01%) (lipid amount equivalent of two wells in a 6-well dish) were spiked with POVPC, PGPC, PONPC and PAzPC (50 ng each) either before or after the LLE procedure. Lipid extract without added standards was used for determination of endogenous levels of these PCs; endogenous values were subtracted from the levels obtained for spiked samples. The yield was expressed as the ratio of analytes in samples spiked before the LLE to those spiked after. In half of the samples precipitated protein was removed from the methanolic extract by centrifugation (10' at 12000 g) prior to hexane/BHT (0.01%) extraction.

	Extraction yield, % ± SD	
Analyte	**In the presence of precipitated protein**	**Precipitated protein removed by centrifugation before LLE**
DNPC	89.0 ± 1.8	88.7 ± 3.7
POVPC	66.7 ± 9.5	70.2 ± 5.0
PGPC	88.1 ± 3.6	88.7 ± 2.9
PONPC	61.5 ± 11.4	65.9 ± 6.8
PAzPC	88.6 ± 2.1	87.1 ± 2.5

Supplementary Table 2. Analytical parameters for quantification of OxPLs. Internal standard (DNPC, 3.1 pmol) was applied on the column together with increasing amounts of calibrants. The lowest calibrant has a signal-to-noise ratio ≥ 6 for each analyte. Calibrants having signal heights > $3.5x10^6$ cps were not considered for calibration due to non-linear detector response. Acceptance range for back-calculated accuracy of calibrants was 80-120%. Equation for calculation was obtained using 1/x weighted linear regression.

Phospholipid	**Equation for calculation**	**Linear range, pmol on column (number of calibrants)**	**r value**
POVPC	y = 0.0575x + 0.00147	0.08-80 (*n* = 11)	0.9981
PGPC	y = 0.566x - 0.0000465	0.08-10 (*n* = 8)	0.9996
PONPC	y = 0.279x + 0.000801	0.08-80 (*n* = 11)	0.9991
PAzPC	y = 0.65x + 0.00249	0.08-10 (*n* = 8)	0.9997

Supplementary Table 3

A list of *m/z* values monitored by the procedure.

m/z values were calculated based on the structures of known and predicted oxidized molecular species generated from PAPC, PLPC, SAPC and SLPC. In addition, a few major fragmented species generated from PDHPC, as well as lysoPCs and internal standards are included. The right column contains selected references to publications describing the presence of corresponding OxPC species in cells and tissues. Four molecular species produced in vivo (POVPC, PGPC, PONPC and PAzPC) were unequivocally identified using commercial standards and tandem mass-spectrometry in negative ion mode. Note that identification of peaks for which no commercial standards were available was beyond the scope of this work.

m/z	**Compatible structures (precursor)**	**Number of isobaric peaks detected in this study**	**Selected publications showing the presence of these compounds in vitro or in vivo**
482	15:0-Lyso-PC, di-7:0-PC	2	
496	16:0-Lyso-PC	2	
518	18:3-Lyso-PC	1	
520	18:2-Lyso-PC	2	
522	18:1-Lyso-PC	2	
524	18:0-Lyso-PC	2	
538	di-9:0-PC (external standard)	2	
580	4-oxo-butyryl-PPC (PDHPC)	3	(1, 2)
594	POVPC (PAPC)	6	(2-10)
596	Succinoyl-PPC (PDHPC)	4	(1, 2)
610	PGPC (PAPC)	6	(4, 5, 7, 10, 11)
622	SOVPC (SAPC) 7-oxo-heptanoyl-PPC (PLPC) 4-hexenedioyl-PPC (PAPC)	8	(3, 4, 7, 12)
632	Furylbutanoyl-PPC (PAPC)	5	(13)
634	KOHA-PC (PDHPC)	5	(13)
636	8-oxo-octanoyl-PPC (PLPC) 4-OH-7-oxo-5-heptenoyl-PPC (PAPC) 5-heptenedioyl-PPC (PAPC) HOHA-PC (PDHPC)	2	(3, 10, 12, 13)
638	SGPC (SAPC)	4	(4, 7)
640	Acetal-POVPC	1	
648	KOOA-PPC (PAPC) 4-OOH-5-oxo-pentanoyl-PPC (PAPC)	5	(3, 6, 12, 13)

650	6-octenedioyl-PPC (PAPC) PONPC (PLPC) HOOA-PPC (PAPC) KHdiA-PC (PDHPC) 7-OH-5-heptaenoyl-SPC (SAPC)	6	(1-6, 10, 12, 13)
660	10-oxo-6,8-decedienoyl-PPC (PAPC) Furylbutanoyl-SPC (SAPC)	3	(10, 12)
664	KOdiA-PPC (PAPC)	5	(3, 5, 6, 10)
666	HOdiA-PPC (PAPC) PAzPC (PLPC)	7	(3-6, 10-12)
676	KOOA-SPC (SAPC) 11-oxo-9-undecenoyl-PPC (PLPC)	3	(12)
678	HOOA-SPC (SAPC) SONPC (SLPC)	6	(4)
682	Unknown	6	
688	Furyloctanoyl-PPC (PLPC)	7	(13)
692	KOdiA-SPC (SAPC) 8-OH-11-oxo-9- undecenoyl-PPC (PLPC)	10	(12)
694	HOdiA-SPC (SAPC) SAzPC (SLPC)	10	(4)
696	Acetal-PONPC (PLPC)	11	
704	KODA-PPC (PLPC) 8-OOH-9-oxo-nonanoyl-PPC (PLPC)	8	(5, 6, 10, 12, 13)
706	HODA-PPC (PLPC) 9-OH-12-oxo-10-dodecenoyl-PPC (PLPC)	9	(5, 6, 12, 13)
710	12-oxo-8,10-dodecendienoyl-PPC (PLPC)	4	(12)
716	Furyloctanoyl-SPC (SLPC)	4	
720	KDdiA-PPC (PLPC)	5	(5, 6, 10, 12)
722	5,10-diOH-6,8-undecedienedioic-PPC (PAPC) HDdiA-PPC (PLPC)	3	(5, 6, 10, 12)
724	Acetal-SONPC	4	
732	10-OH-5,8,11-tridecatrienoyl-PPC (PAPC) 8-oxo-9,11-tridecedienedioyl-PPC (PLPC)	3	(10, 12)
734	HODA-SPC (SLPC)	5	

748	KDiA-SPC (SLPC)	2	
750	5,10-diOH-6,8-undecedienedioic-PPC (SAPC) HDiA-SPC (SLPC)	1	(10)
758	PLPC (only isobaric peaks eluting within 8 minutes; PLPC elutes later)	5	
760	10-OH-5,8,11-tridecatrienoyl-PPC (SAPC)	6	
772	PLPC-keto (PLPC)	2	(3, 10, 14, 15)
774	PLPC-OH (PLPC) PLPC-epoxy (PLPC)	2	(3, 4, 10, 14-16)
782	PAPC (only isobaric peaks eluting within 8 minutes; PAPC elutes later)	4	
786	SLPC (only isobaric peaks eluting within 8 minutes; SLPC elutes later)	3	
788	PLPC-epoxy,keto PLPC-OH,keto	3	(3, 15)
790	PLPC-OOH PLPC-diOH PLPC-OH,epoxy	4	(3, 4, 10, 15-17)
794	15-deoxy-$\Delta^{12,14}$-isoPGJ2-PPC (PAPC)	6	(18)
796	PAPC-keto	4	
798	PAPC-OH PAPC-epoxy	4	(4, 10, 14, 19)
800	SLPC-keto	2	(3, 14)
802	SLPC-OH SLPC-epoxy	1	(3, 4, 14)
804	PLPC-OOH,keto PLPC-diOH,keto	4	(3, 15)
806	PLPC-OOH,OH	5	(10)
808	PLPC-diOH,epoxy PLPC-triOH	8	(10, 14, 15)
810	PECPC (PAPC) SAPC (only isobaric peaks eluting within 8 minutes; SAPC elutes later)	4	(8)
812	isoPG(A2,J2)-PPC	6	(20, 21)
814	PAPC-OOH	4	(3, 4, 10)

	PAPC-diOH PAPC-OH,epoxy		
816	SLPC-epoxy,keto	6	(3)
818	SLPC-OOH SLPC-diOH SLPC-OH,epoxy	5	(3, 4)
820	2,3-dinor-isoTxB2-PPC (PAPC) PLPC-OOH,OH,keto PLPC-OOH,epoxy	5	(15)
822	15-deoxy-$\Delta^{12,14}$-isoPGJ2-PPC (SAPC) PLPC-diOOH PLPC-OOH,diOH PLPC-triOH,keto PLPC-triOH,epoxy	4	(15, 18)
824	SAPC-keto PLPC-tetraOH	2	(10, 15, 19)
826	SAPC-OH SAPC-epoxy	4	(4, 22)
828	PEIPC (PAPC) PAPC-OOH,keto	7	(3, 7-9, 23)
830	isoPG(E2,I2,D2)-PPC (PAPC) isoLG(E2,D2)-PPC (PAPC) PAPC-OOH,OH	6	(3, 20, 21, 24, 25)
832	isoPGF2α-PPC SLPC-OOH,keto	7	(26)
834	SLPC-OOH,OH	8	
836	SLPC-triOH	8	(14, 15)
838	SECPC	6	(7)
840	isoPG(A2,J2)-SPC (SAPC) SAPC-epoxy,keto SAPC-OH,keto	5	(20, 21)
842	SAPC-OOH SAPC-diOH SAPC-OH,epoxy	5	(3, 4)
844	PAPC-OOH,OH,keto PAPC-OOH,epoxy,keto	8	(3, 10)
846	PAPC-diOOH	9	(3, 10)

	PAPC-OOH,diOH		
848	Isofuran-PPC (PAPC) iso-TxB2-PPC (PAPC)	7	(3, 27-29)
850	SLPC-diOOH,epoxy SLPC-OOH,diOH SLPC-triOH,keto SLPC-triOH,epoxy	8	
852	SLPC-OOH,OH,keto SLPC-tetraOH	7	
856	SEIPC (SAPC) SAPC-OOH,keto	7	(7)
858	isoPG(E2,I2,D2)-SPC (SAPC) isoLG(E2,D2)-SPC (SAPC) SAPC-OOH,OH (SAPC)	7	(3, 20, 21, 24, 25)
860	isoPGF2α-SPC PAPC-OOH,OH,epoxy	7	(3)
862	PAPC-diOOH,OH	9	(10)
864	SLPC-diOOH,keto,epoxy	8	
866	SLPC-diOOH,OH,epoxy	8	
870	SAPC-OOH,diketo	10	
872	SAPC-OOH,OH,keto SAPC-OOH,keto,epoxy	8	
874	SAPC-diOOH	8	
876	Isofuran-PPC (SAPC) Iso-TxB2-SPC (SAPC)	5	(27-29)
878	PAPC-triOOH	6	(10)
882	SLPC-triOOH	7	
888	SAPC-OOH,OH,epoxy	3	
890	SAPC-diOOH,OH	6	
894	PAPC-triOOH,OH	3	(10)
906	SAPC-triOOH	5	
922	SAPC-triOOH,OH	4	

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