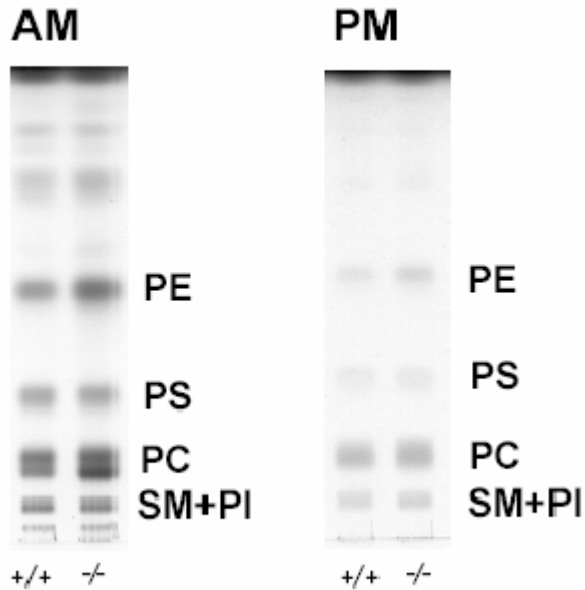


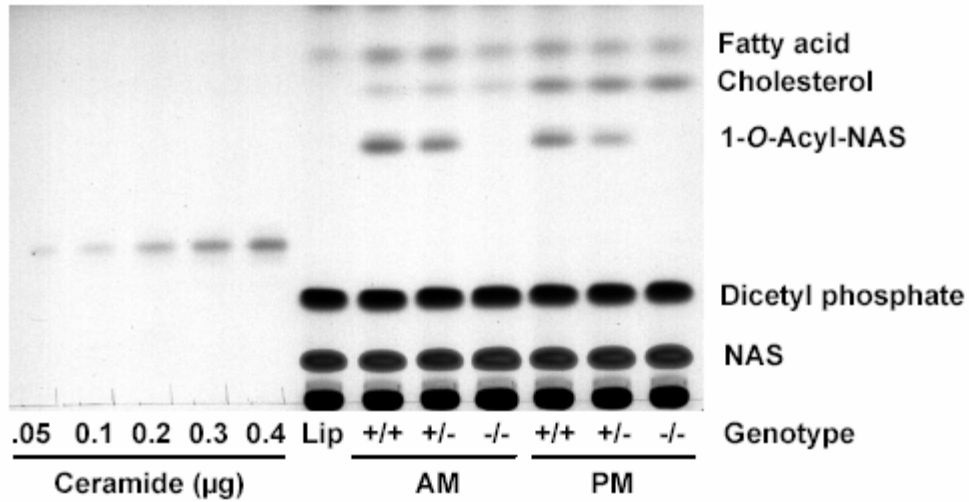
Lysosomal phospholipase A2 and phospholipidosis Supplemental data

Phospholipid profile in alveolar (AM) and peritoneal (PM) macrophages



Three month-old $Lpla2^{+/+}$ and $Lpla2^{-/-}$ mice were used. Each cell homogenate prepared from AM and PM was dispersed in chloroform/methanol mixture and lipid extraction was carried out as described in the Materials and Methods. In this study, lipid extracts obtained from 32 μg of protein of AM homogenate and 12 μg of protein of PM homogenate were developed by an HPTLC. PE, PS, PC, PI and SM denote phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and sphingomyelin, respectively.

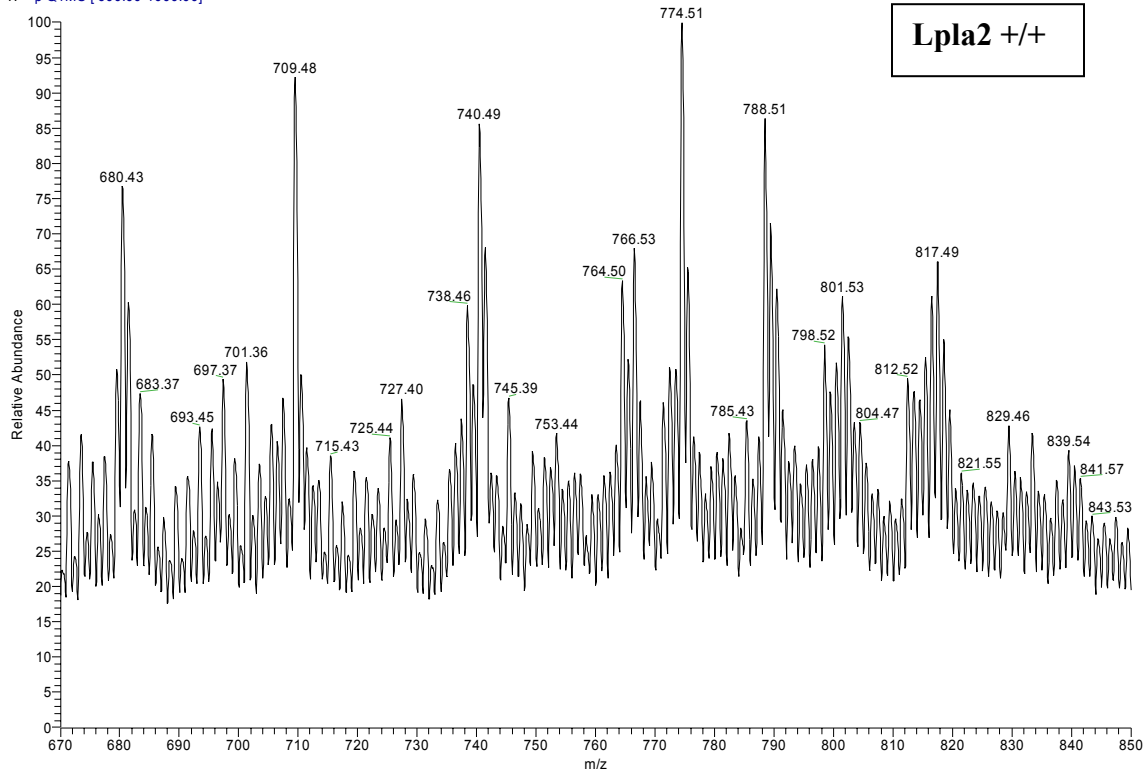
Cholesterol levels and *Lpla2* activities in alveolar (AM) and peritoneal (PM) macrophages



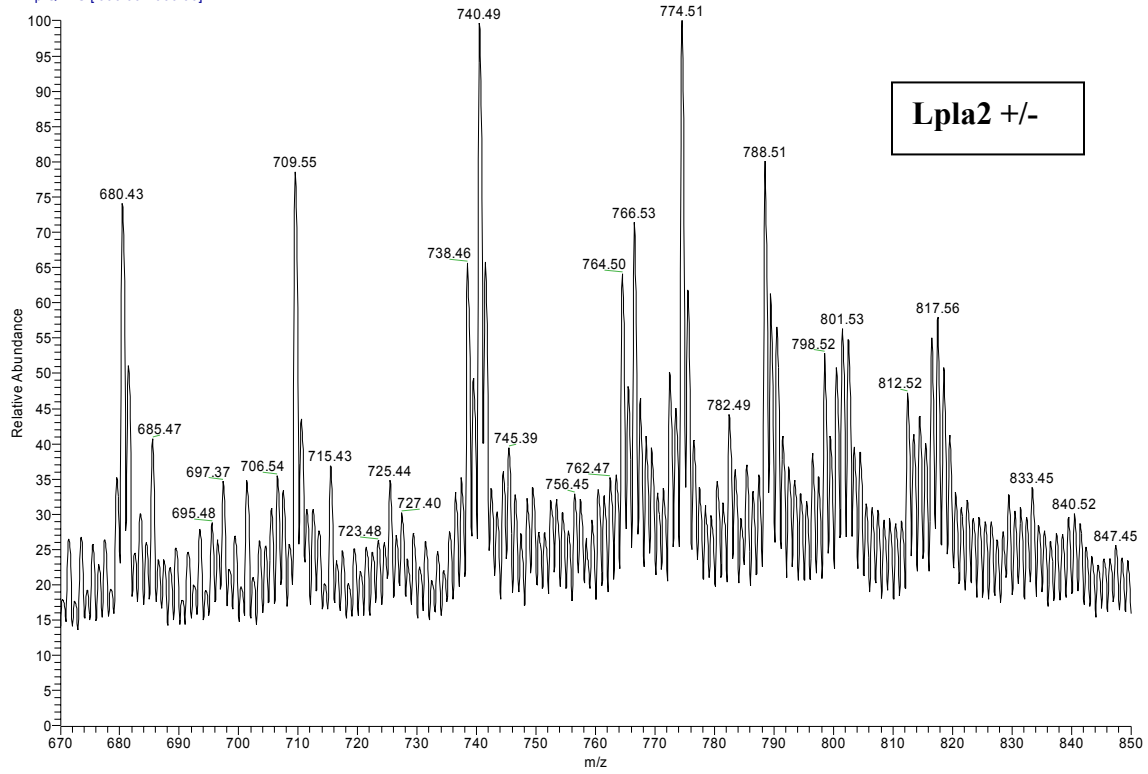
Enzyme activity measurements were carried out using cell extracts of alveolar and peritoneal macrophages from wild type, heterozygous, and null 3 month old mice as described in the Methods section. The reaction products were separated by HPTLC using a solvent system consisting of chloroform:acetic acid, 9:1.

Mass Spectra of Choline Glycerophospholipids

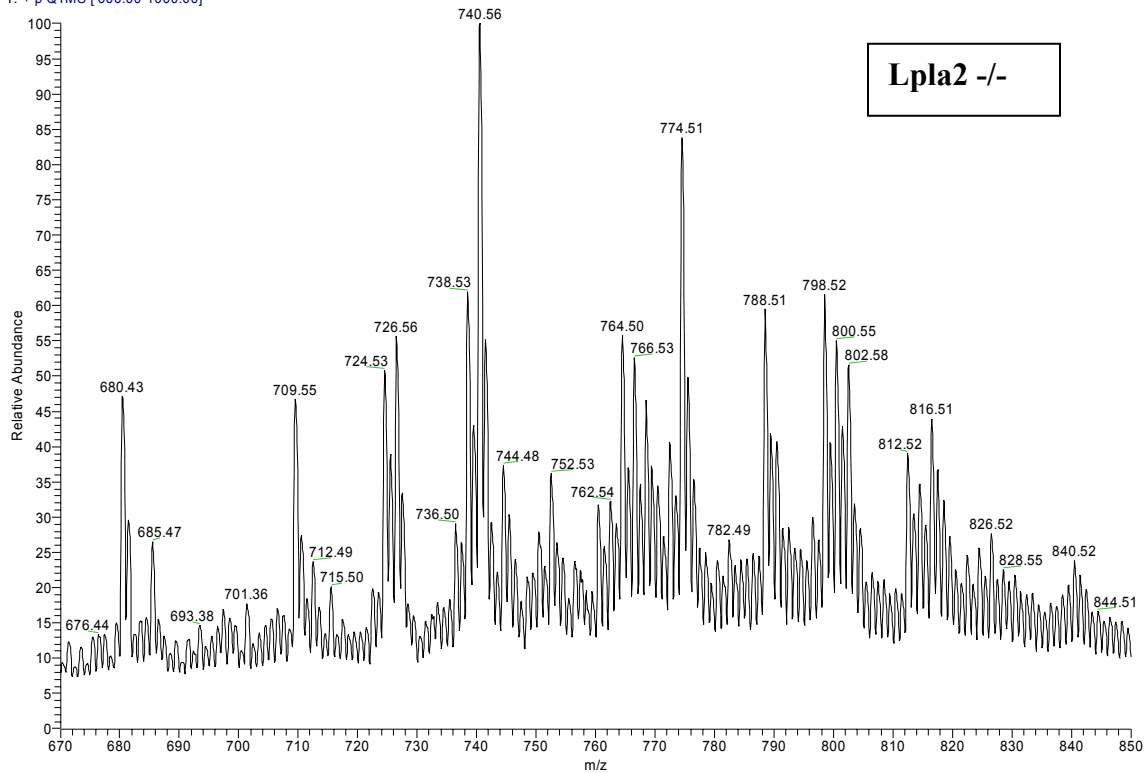
Shay1_pc#4-298 RT: 0.08-6.41 AV: 295 SM: 7G NL: 3.52E6
T: + p Q1MS [600.00-1000.00]



Shay2_pc#3-324 RT: 0.05-5.89 AV: 322 SM: 7G NL: 3.96E6
T: + p Q1MS [600.00-1000.00]



Shay3_pc#3-311 RT: 0.04-5.64 AV: 309 SM: 7G NL: 4.64E6
T: + p Q1MS [600.00-1000.00]



[M+Li] ⁺	Assignment	Alveolar macrophage phosphatidylcholine (nmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
712.5	D14:0-16:0	4.38	3.41	11.01
722.5	P16:0-16:1	2.43	1.87	6.21
724.5	P16:0-16:0	4.59	1.79	32.76
726.5	A16:0-16:0	3.54	1.30	30.03
736.5	D16:1-16:1	5.86	3.75	9.35
738.5	D16:1-16:0	19.27	15.94	46.28
740.5	D16:0-16:0	32.15	26.91	78.78
748.5	P16:0-18:2	1.87	1.14	3.44
750.5	P16:0-18:1	2.66	1.59	9.06
752.5	A16:0-18:1	5.95	4.14	17.00
762.5	D16:1-18:2	4.92	4.31	9.76
764.5	D16:0-18:2	17.00	14.04	26.46
766.5	D16:0-18:1	16.79	14.07	23.29
772.5	P16:0-20:4	10.82	8.70	10.91
774.6	A16:0-20:4	28.69	21.84	41.87
776.5	P18:1-18:1	0.90	0.57	3.01
778.6	P18:0-18:1	2.66	2.17	4.80
780.5	A18:0-18:1	5.71	4.74	7.07
786.5	D16:1-20:4	3.79	3.07	5.45
788.6	D16:0-20:4	23.87	20.02	23.66
790.6	D18:1-18:2	8.94	7.49	11.44
792.6	D18:0-18:2/D18:1-18:1	4.50	3.50	6.15
794.6	D18:0-18:1	3.51	2.79	4.31
798.5	P18:1-20:4	12.14	9.58	29.73
800.6	P18:0-20:4	9.72	7.87	19.64
802.5	A18:0-20:4	8.83	6.76	16.86
810.5	A18:0-20:0	2.59	2.19	2.50
812.6	D16:0-22:6	9.84	9.65	13.10
814.6	D18:1-20:4	7.17	6.26	10.46
816.6	D18:0-20:4	10.50	9.19	13.87
836.6	D18:2-22:6	2.00	1.70	1.87
838.6	D18:1-22:6	2.46	2.26	2.40
840.6	D18:0-22:6	2.97	2.89	4.56
	Total PC	283.02	227.50	537.09
	PC-16:0	183.85	146.41	373.86
	PC-16:0/Total PC (%)	64.96	64.36	69.61

Assignment	m/z	Alveolar macrophage Phosphatidylethanolamine (nmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
P14:0-18:1	672.50	1.31	0.94	6.43
P16:0-18:2	698.50	3.85	3.56	9.33
P16:0-18:1	700.50	7.90	4.25	13.34
D16:0-18:2/D16:1-18:1	714.50	1.20	1.15	2.24
D16:0-18:1/D16:1-18:0	716.50	2.49	2.03	2.62
P16:1-20:4	720.50	4.81	5.41	12.64
P16:0-20:4	722.50	57.16	43.26	100.61
P18:1-18:2/P16:0-20:3	724.50	0.00	0.00	0.00
P18:1-18:1/P18:0-18:2	726.50	0.80	0.51	3.55
P18:0-18:1	728.50	1.34	0.94	4.51
D16:0-20:4/D16:1-20:3	738.50	5.44	4.42	4.70
D18:1-18:2/D16:0-20:3	740.50	2.28	1.58	1.14
D18:1-18:1/D18:0-18:2	742.50	1.53	1.46	2.35
D18:0-18:1	744.50	2.77	2.64	3.08
P16:0-22:6	746.50	33.20	26.21	109.88
P18:1-20:4/P16:0-22:5	748.50	15.42	12.65	40.29
P18:0-20:4/P16:0-22:4	750.50	23.83	17.39	49.89
D16:1-22:6	760.50	2.16	1.87	3.96
D16:0-22:6	762.50	4.89	4.61	9.24
D16:0-22:5/D18:1-20:4	764.50	3.85	3.31	5.54
D18:0-20:4/D18:1-20:3	766.50	12.39	10.90	14.52
P18:2-22:6	770.50	1.16	1.24	2.03
P18:1-22:6	772.50	4.18	3.50	12.31
P18:0-22:6/P18:1-22:5	774.50	8.78	7.78	23.14
P18:0-22:5/P18:1-22:4	776.50	4.13	3.12	12.81
P20:0-20:4/P18:0-22:4	778.50	5.34	4.22	12.74
A20:0-20:4	780.50	9.50	10.87	12.28
D18:2-22:6	786.50	1.55	1.49	2.02
D18:1-22:6	788.50	2.98	2.68	4.29
D18:0-22:6/D18:1-22:5	790.50	8.75	8.97	19.74
D20:0-20:4/D18:0-22:4	794.50	10.14	6.58	14.29
D18:0-22:5/D18:1-22:4	792.50	5.11	4.83	8.72
Total PE		250.24	204.37	524.23
Total Plasmalogen		173.21	134.98	413.50
Plasmalogen (%)		69.22	66.05	78.88

[M+Li] ⁺	Assignment	Alveolar macrophage sphingomyelin (nmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
691.5	N15:2	0.22	0.08	0.01
695.5	N15:0	1.80	1.13	1.03
707.5	N16:1	3.55	2.58	2.37
709.5	N16:0	71.64	43.38	57.05
721.5	N17:1	0.00	0.07	0.02
723.5	N17:0	0.43	0.44	0.89
735.5	N18:1	0.17	0.18	0.00
737.5	N18:0	1.91	1.77	5.00
817.5	N24:2	0.00	0.76	1.05
819.5	N24:1	6.00	4.92	7.26
821.5	N24:0	1.65	0.90	1.30
Total SM		87.37	56.21	75.98

Assignment	m/z	Alveolar macrophage phospholipids (nmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
645.5	16:0-16:1PA	0.1	0.06	0.04
647.5	16:0-16:0PA	0.12	0.07	0.05
671.5	16:0-18:2PA	0.15	0.08	0.06
673.5	16:0-18:1PA	0.11	0.07	0.04
699.5	18:1-18:1PA	0.13	0.08	0.04
701.5	18:0-18:1PA	0.13	0.08	0.03
Total phosphatidic acid		0.74	0.44	0.26
719.5	16:0-16:1PG	1.5	1.14	3.02
721.5	16:0-16:0PG	2.49	1.87	4.84
745.5	16:0-18:2PG	3.88	2.74	7.14
747.6	16:0-18:1PG	5.43	3.86	9.05
767.5	18:2-18:3/16:1-20:4PG	1.27	0.86	2.04
769.5	18:2-18:2/16:0-20:4PG	4.06	2.73	5.98
771.5	18:1-18:2PG	4.06	2.61	6.33
773.5	18:1-18:1/16:0-20:2PG	3.7	2.45	5.53
775.6	18:0-18:1PG	0.3	0.22	0.33
791.5	16:1-22:6PG	2.01	1.47	3.37
793.5	18:2-20:4/16:0-22:6PG	8.53	5.98	13.37
795.5	18:1-20:4PG	5.53	3.54	9.96
817.5	20:4-20:4/18:2-22:6PG	7.41	5.55	12.89
819.5	18:1-22:6PG	10.67	7.96	16.25
841.5	20:4-22:6PG	6.48	5.09	11.52
843.5	20:3-22:6/20:4-22:5PG	3.08	1.94	3.63
865.5	22:6-22:6PG	4.57	4.14	6.54
867.5	22:5-22:6PG	3.27	2.35	5.09
869.5	22:4-22:6PG	1.57	1	2.11
Total phosphatidylglycerol		79.81	57.5	128.99
833.6	16:0-18:2PI	0.42	0.31	0.32
835.6	16:0-18:1PI	0.61	0.42	0.42

857.6	16:0-20:4PI	1.7	1.35	0.92
859.6	18:1-18:2PI	0.27	0.15	0.24
861.6	18:1-18:1PI	0.48	0.34	0.28
863.6	18:0-18:1PI	0.22	0.16	0.14
881.5	18:2-20:4/16:0-22:6PI	0.85	0.6	0.74
883.6	18:1-20:4PI	1	0.73	0.67
885.6	18:0-20:4PI	5.37	4.18	3.01
887.6	18:0-20:3PI	0	0	0
889.6	18:0-20:2PI	0.02	0	0
909.6	18:0-22:6PI	0.72	0.56	0.54
911.6	18:0-22:5PI	0.22	0.15	0.12
	Total phosphatidylinositol	11.24	8.49	6.96
732.5	16:0-16:1PS	0.32	0.32	0.31
734.5	16:0-16:0PS	0.48	0.38	0.63
758.5	16:0-18:2PS	0.42	0.43	0.28
760.5	16:0-18:1PS	6.84	5.26	6.11
762.5	16:0-18:0PS	0.65	0.9	1.28
782.5	18:2-18:2PS	1.13	0.9	0.6
784.5	18:1-18:2PS	0.94	0.38	0.5
786.5	18:1-18:1PS	6.89	4.59	4.51
788.5	18:0-18:1PS	9.32	7.16	8.54
806.5	18:2-20:4PS	0.83	0.71	1.1
808.5	18:1-20:4PS	0.97	0.84	0.85
810.5	18:0-20:4PS	7.04	3.98	6.26
812.5	18:0-20:3PS	2.02	1.45	1.73
814.5	18:0-20:2PS	0	0.02	0.06
834.5	18:0-22:6PS	14.4	11.3	25.02
836.5	18:0-22:5PS	8.2	5.77	9.39
838.5	20:0-20:4PS	6.92	4.76	8.68
878.5	22:6-22:6PS	0.01	0.02	0.07
	Total phosphatidylserine	67.38	49.17	75.92

[M+Na] ⁺	Assignment	Alveolar macrophage lysophosphatidylcholine (nmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
488.6	14:1 LPC	0.04	0.05	0.11
490.6	14:0 LPC	0.02	0.04	0.03
516.2	16:1 LPC	0.15	0.18	0.22
518.2	16:0 LPC	0.57	0.40	1.14
542.2	18:2 LPC	0.05	0.04	0.11
544.2	18:1 LPC	0.09	0.07	0.22
546.2	18:0 LPC	0.21	0.16	0.27
566.2	20:4 LPC	0.04	0.04	0.09
590.2	22:6 LPC	0.03	0.04	0.11
	Total LPC	1.20	1.02	2.30

m/z	Assignment	Alveolar macrophage ceramide (pmol/mg protein)		
		Lpla2 +/+	Lpla2 +/-	Lpla2 -/-
522.5	N15:0	2	1	1
536.5	N16:0	936	1225	856
564.5	hydro_N17:1 / N18:0	27	28	21
592.5	hydro_N19:1 / N20:0	7	14	10
616.5	N22:2	2	3	2
618.5	N22:1	2	5	5
620.5	N22:0	86	145	93
632.5	N23:1	3	3	3
634.5	N23:0	34	58	57
644.5	N24:2	44	93	78
646.5	N24:1/hydro-N23:2	339	664	535
648.5	N24:0/hydro-N23:1	375	633	532
674.7	N26:1	13	7	35
676.5	N26:0	3	7	10
	Total	1873	2886	2238