



Title	Determination of plasma lysophosphatidylethanolamines (lyso-PE) by LC-MS/MS revealed a possible relation between obesity and lyso-PE in Japanese preadolescent children : The Hokkaido study
Author(s)	Inoue, Nao; Gowda, Siddabasave Gowda B.; Gowda, Divyavani et al.
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Supplementary information

Determination of plasma lysophosphatidylethanolamines (lyso-PE) by LC-MS/MS revealed a possible relation between obesity and lyso-PE in Japanese preadolescent children: the Hokkaido study

Nao Inoue, Siddabasave Gowda B. Gowda, Divyavani Gowda, Toshihiro Sakurai, Atsuko Ikeda-Araki, Yu Ait Bamai, Rahel Mesfin Ketema, Reiko Kishi, Hitoshi Chiba, and Shu-Ping Hui*

* Correspondence: Prof. Shu-Ping Hui Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan E-mail: keino@hs.hokudai.ac.jp, Tel. & Fax: +81-11-706-3693.

Supplemental Table S1. Optimized ^aSRM parameters for ^blyso-PE species.

Species	Parent ion [M-H] ⁺ (m/z)	Product ion (m/z)	Collision energy (V)	Tube lens (V)
Lyso-PE 18:1(d7)	485.3	288.2	35	117
Lyso-PE 16:0	452.3	255.3	35	106
Lyso-PE 18:0	480.3	283.3	31	118
Lyso-PE 18:1	478.3	281.3	31	124
Lyso-PE 18:2	476.3	279.3	30	92
Lyso-PE 20:4	500.3	303.5	27	108
Lyso-PE 20:5	498.3	301.2	25	110
Lyso-PE 22:6	524.3	327.2	23	111

^aSRM, single reaction monitoring; ^bLyso-PE, Lysophosphatidylethanolamine.

Supplemental Table S2. Determination of linearity, ^aLOD, and ^bLOQ of ^clyso-PE species.

Species	Linearity	R ²	Range (pmol/μL)	LOD (pmol/μL)	LOQ (pmol/μL)
Lyso-PE 16:0	y = 0.6107x - 0.0022	0.9984	0.004-2.0	0.002	0.004
Lyso-PE 18:0	y = 1.0733x + 0.0093	0.9993	0.002-2.0	0.001	0.002
Lyso-PE 18:1	y = 0.9946x + 0.0133	0.9993	0.002-2.0	0.001	0.002
Lyso-PE 18:2	y = 0.6203x + 0.0083	0.9986	0.002-2.0	0.001	0.002
Lyso-PE 20:4	y = 0.1417x - 0.0004	0.9994	0.008-2.0	0.004	0.008
Lyso-PE 20:5	y = 0.0357x - 0.0074	0.9936	0.031-2.0	0.015	0.031
Lyso-PE 22:6	y = 0.1469x - 0.0029	0.9969	0.008-2.0	0.004	0.008

^aLOD, Limits of detection; ^bLOQ, Limits of quantification; ^cLyso-PE,

Lysophosphatidylethanolamine.

Supplemental Table S3. Results of recovery, matrix effect, and reproducibility.

		Recovery (n = 6)		Matrix effect (n = 6)		Intraday (n = 5)	Interday (n = 5)
		Mean $\pm$ ^a SD	^b CV	Mean $\pm$ SD	CV	CV (%)	CV (%)
		(%)	(%)	(%)	(%)		
^c Lyso-PE 16:0	High	92.4 $\pm$ 3.1	3.4	62.0 $\pm$ 2.5	4.1	6.3	4.6
	Medium	96.8 $\pm$ 4.0	4.2	69.3 $\pm$ 2.4	3.4	4.9	4.6
	Low	110.3 $\pm$ 8.4	7.6	76.2 $\pm$ 4.2	5.5	6.4	6.4
Lyso-PE 18:0	High	95.4 $\pm$ 1.5	1.6	64.5 $\pm$ 2.4	3.7	6.1	9.3
	Medium	102.8 $\pm$ 2.8	2.8	71.1 $\pm$ 1.6	2.2	4.5	7.5
	Low	104.4 $\pm$ 3.6	3.4	82.8 $\pm$ 2.5	3.0	6.6	12.0
Lyso-PE 18:1	High	95.3 $\pm$ 1.2	1.3	62.6 $\pm$ 2.4	3.8	3.6	5.9
	Medium	99.6 $\pm$ 2.5	2.5	64.6 $\pm$ 2.3	3.6	4.9	6.1
	Low	104.4 $\pm$ 7.6	7.3	68.7 $\pm$ 1.6	2.3	4.4	3.3
Lyso-PE 18:2	High	92.4 $\pm$ 1.9	2.1	61.6 $\pm$ 1.6	2.6	6.6	6.1
	Medium	101.0 $\pm$ 5.8	5.7	72.5 $\pm$ 2.2	3.1	4.1	4.8
	Low	110.5 $\pm$ 9.9	9	85.7 $\pm$ 3.2	3.7	5.7	7.9
Lyso-PE 20:4	High	98.1 $\pm$ 3.4	3.5	66.9 $\pm$ 1.3	2.0	4.9	7.6
	Medium	98.4 $\pm$ 7.7	7.8	75.7 $\pm$ 6.2	8.2	6.3	2.9
	Low	108.8 $\pm$ 10.4	9.5	92.2 $\pm$ 7.9	8.6	14.0	6.5
Lyso-PE 20:5	High	91.2 $\pm$ 12.7	13.9	63.7 $\pm$ 7.5	11.8	7.6	7.5
	Medium	95.1 $\pm$ 15.2	16	81.6 $\pm$ 12.6	15.4	9.8	11.5
	Low	120.3 $\pm$ 25.0	20.8	85.1 $\pm$ 12.2	14.4	16.3	17.8
Lyso-PE 22:6	High	93.6 $\pm$ 4.4	4.7	66.8 $\pm$ 2.7	4.1	7.6	5.4
	Medium	98.5 $\pm$ 8.2	8.3	78.0 $\pm$ 4.3	5.5	8.4	11.4
	Low	116.0 $\pm$ 5.3	4.5	96.5 $\pm$ 9.1	9.5	9.0	16.8

Results are expressed as mean $\pm$ SD. The recovery and matrix effect of the method were confirmed by spiking the known concentration of lyso-PE standards (Low: 0.5 pmol/ μ L; Medium: 1.0 pmol/ μ L; High: 2.0 pmol/ μ L) to 10 μ L human plasma (n = 6 replicates). ^aSD, Standard deviation; ^bCV, Coefficient of variation; ^cLyso-PE, Lysophosphatidylethanolamine.

Supplemental Table S4. Difference in lyso-PE concentrations according to age among the same BMI category.

Underweight (pmol/ μ L)					
(years old)	9	10	11	12	P value
Lyso-PE 16:0	1.26 (1.03–1.41))	1.24 (1.01–1.51)	1.32 (1.16–1.61)	1.18 (0.99–1.51)	0.701
Lyso-PE 18:0	1.22 (1.00–1.41)	1.22 (0.99–1.39)	1.25 (1.14–1.42)	1.16 (1.02–1.36)	0.782
Lyso-PE 18:1	1.42 (0.91–1.97)	1.58 (1.21–1.89)	1.34 (0.98–2.28)	1.32 (0.87–2.12)	0.671
Lyso-PE 18:2	3.50 (2.44–4.56)	3.64 (2.85–4.51)	3.39 (2.51–4.98)	3.16 (2.46–4.66)	0.811
Lyso-PE 20:4	1.55 (1.24–2.00)	1.61 (1.32–1.93)	1.51 (1.30–2.01)	1.61 (1.36–2.13)	0.943
Lyso-PE 20:5	0.79 (0.00–0.98)	0.91 (0.00–1.28)	0.88 (0.70–1.16)	0.84 (0.72–1.05)	0.267
Lyso-PE 22:6	1.40 (1.10–1.68)	1.42 (1.06–1.65)	1.60 (1.03–1.89)	1.58 (1.33–1.87)	0.390
Total lyso-PE	11.24 (8.55–13.59)	11.93 (9.41–13.49)	12.27 (9.57–14.60)	11.40 (8.65–13.75)	0.674

Normal range (pmol/ μ L)					
(years old)	9	10	11	12	P value
Lyso-PE 16:0	1.15 (1.00–1.53)	1.23 (1.05–1.39)	1.19 (1.15–1.40)	1.09 (0.86–1.45)	0.747
Lyso-PE 18:0	1.19 (0.93–1.40)	1.24 (0.99–1.35)	1.09 (1.00–1.49)	1.08 (0.98–1.39)	0.973
Lyso-PE 18:1	1.24 (0.91–1.62)	1.33 (1.03–1.90)	1.29 (1.12–1.51)	1.20 (0.89–1.48)	0.456
Lyso-PE 18:2	3.12 (2.12–3.95)	3.43 (2.63–4.12)	3.16 (2.97–3.48)	2.93 (2.53–3.31)	0.499
Lyso-PE 20:4	1.49 (1.09–1.98)	1.52 (1.28–1.88)	1.40 (1.20–1.82)	1.50 (1.39–1.65)	0.960
Lyso-PE 20:5	0.93 (0.70–1.23)	1.09 (0.72–1.24)	0.89 (0.76–1.06)	0.87 (0.68–1.07)	0.599
Lyso-PE 22:6	1.57 (1.25–1.88)	1.52 (1.25–1.85)	1.56 (1.27–1.87)	1.59 (1.23–1.91)	0.970
Total lyso-PE	10.31 (8.74–12.91)	10.97 (9.81–13.40)	10.80 (10.09–11.88)	10.14 (8.60–11.94)	0.490

Results are expressed as median (interquartile range). Nonparametric Kruskal-Wallis test was used for the data comparison.