

## SUPPORTING INFORMATION

### **Nontargeted quantitation of lipid classes using hydrophilic interaction liquid chromatography - electrospray ionization mass spectrometry with single internal standard and response factor approach**

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**Tables: 3, figures: 3**

**Table S-1.** Retention times of lipid species and their  $m/z$  values for SRM transitions using QqQ MS. The collision energy was 25 V for PE, and 30 V for PC and LPC. The cone voltage was 30 V for PE, and 40V for PC and LPC. All observed ions are protonated molecules  $[M+H]^+$ , unless indicated as sodium adducts  $[M+Na]^+$

<b>Phosphatidylethanolamines (PE)</b>		
Individual lipid species	SRM transition	Retention time [min]
(17:0/17:0) IS	721.1 - 580.1	26.3
(16:0/16:1)	691.8 - 550.8	26.3
(16:1/18:1); (16:0/18:2)	717.4 - 576.2	26.1
(16:0/18:1)	719.1 - 578.1	26.1
(16:0/20:4)	741.2 - 600.2	25.3
(18:1/18:2)	743.2 - 603.8	25.6
(18:1/18:1); (18:0/18:2)	745.2 - 604.2	25.6
(18:0/18:1)	747.2 - 606.1	25.7
(16:0/22:6)	765.0 - 624.1	25.0
(16:0/22:5)	767.2 - 626.1	24.8
(18:0/20:4)	769.0 - 628.1	24.8
(18:0/22:6)	793.1 - 652.2	24.3

**Phosphatidylcholines (PC)**

(17:0/17:0) IS	763.2 - 184.3	40.4
(16:0/16:1)	733.2 - 184.3	40.8
(16:0/16:0)	735.2 - 184.3	40.8
(16:0/16:1 + Na)	755.2 - 184.3	40.4
(16:0/16:0 + Na)	757.2 - 184.3	40.4
(16:1/18:1); (16:0/18:2)	759.2 - 184.3	40.3
(16:0/18:1)	761.2 - 184.3	40.3
(16:1/18:1 + Na); (16:0/18:2 + Na)	781.3 - 184.3	39.4
(16:0/20:4); (16:0/18:1 + Na)	783.3 - 184.3	39.4
(18:1/18:2)	785.2 - 184.3	39.8
(18:1/18:1); (18:0/18:2)	787.4 - 184.3	39.8
(18:0/18:1)	789.3 - 184.3	39.7
(16:0/20:4 + Na)	805.3 - 184.3	39.0
(16:0/22:6); (18:1/18:2 + Na)	807.3 - 184.3	38.9
(16:0/22:5); (18:1/18:1 + Na); (18:0/18:2 + Na)	809.3 - 184.3	38.8
(16:0/22:4); (18:0/18:1 + Na)	811.3 - 184.3	38.8
(16:0/22:6 + Na)	829.2 - 184.3	38.6

(16:0/22:5 + Na)	831.1 - 184.3	38.6
(16:0/22:4 + Na)	833.2 - 184.3	38.6
(18:0/22:5)	837.4 - 184.3	38.8
<b>Lysophosphatidylcholines (LPC)</b>		
(17:0/17:0) IS	510.9 - 184.3	51.2
(16:1)	495.2 - 184.3	51.6
(16:0)	497.2 - 184.3	51.8
(18:3); (16:0 + Na)	518.8 - 184.3	51.5
(18:2)	520.8 - 184.3	51.9
(18:1)	522.8 - 184.3	51.1
(18:0)	524.8 - 184.3	51.1
(18:2 + Na)	542.2 - 184.3	50.3
(20:4); (18:1 + Na)	544.2 - 184.3	50.9
(18:0) + Na	546.7 - 184.3	51.1

**Table S-2.** Comparison of concentrations ( $\mu\text{mol/g}$  egg yolk) of phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LPE), phosphatidylcholines (PC), sphingomyelins (SM) and lysophosphatidylcholines (LPC) in egg yolk using the nontargeted quantitation with single internal standard (IS) and response factor (RF) approach in HILIC-HPLC/ESI-MS, quantitation using  $^{31}\text{P}$  NMR spectrometry and the quantitation using SRM scans on QqQ MS

Lipid class	Nontargeted quantitation with single IS and RFs ( $\mu\text{mol/g}$ egg yolk)	Quantitation using $^{31}\text{P}$ NMR spectrometry ( $\mu\text{mol/g}$ egg yolk)	Quantitation with SRM scans ( $\mu\text{mol/g}$ egg yolk)
PE	$13.81 \pm 0.47$	12.77	$12.45 \pm 0.65$
LPE	$1.24 \pm 0.17$	1.13	not determined
PC	$50.64 \pm 2.55$	49.52	$47.78 \pm 2.30$
SM	$1.28 \pm 0.20$	1.44	not determined
LPC	$1.86 \pm 0.14$	2.00	$1.85 \pm 0.25$

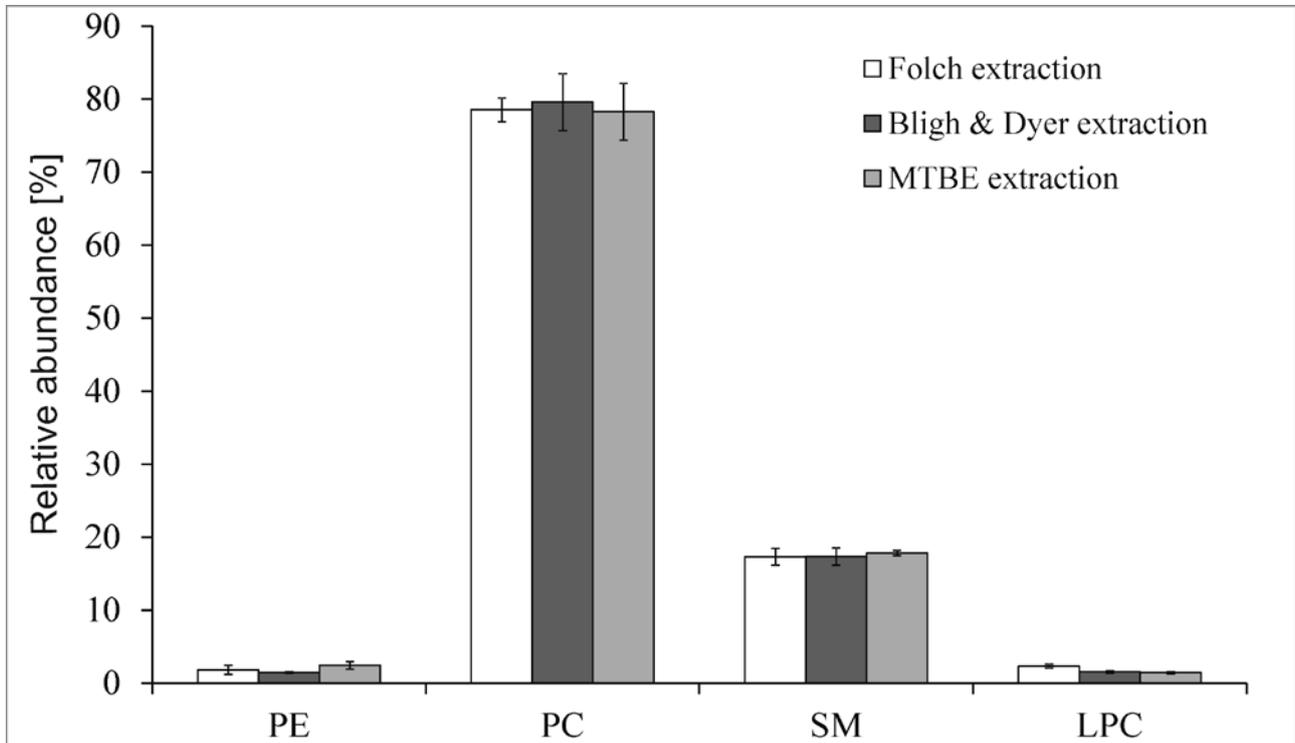
**Table S-3.** Comparison of concentrations ( $\mu\text{mol/mL}$ ) of phosphatidylethanolamines (PE), phosphatidylcholines (PC), sphingomyelins (SM) and lysophosphatidylcholines (LPC) in the NIST human plasma using the nontargeted quantitation with single internal standard (IS) and response factor (RF) approach in HILIC-HPLC/ESI-MS, quantitation using  $^{31}\text{P}$  NMR spectrometry and published data<sup>1</sup>

Lipid class	Nontargeted quantitation with single IS and RFs ( $\mu\text{mol/mL}$ )	Quantitation using $^{31}\text{P}$ NMR spectrometry ( $\mu\text{mol/mL}$ )	Published data <sup>1</sup> ( $\mu\text{mol/mL}$ )
PE	$0.491 \pm 0.033$	0.458	0.435
PC	$2.009 \pm 0.106$	1.961	1.974
SM	$0.326 \pm 0.005$	0.307	0.303
LPC	$0.109 \pm 0.021$	0.111	0.103

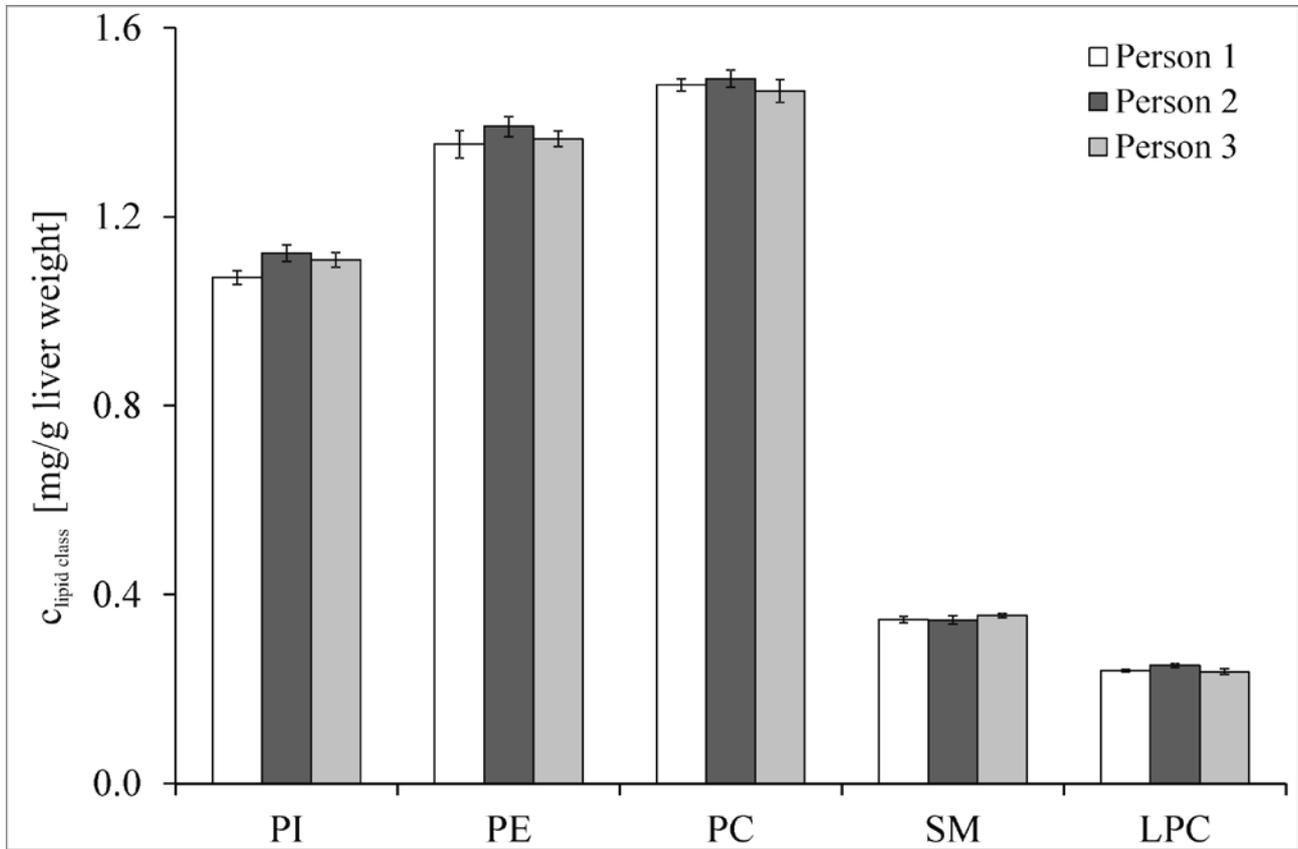
## Reference

- (1) Quehenberger, O.; Armando, A. M.; Brown, A. H.; Milne, S. B.; Myers, D. S.; Merrill, A. H.; Bandyopadhyay, S.; Jones, K. N.; Kelly, S.; Shaner, R. L.; Sullards, C. M.; Wang, E.; Murphy, R. C.; Barkley, R. M.; Leiker, T. J.; Raetz, C. R. H.; Guan, Z. Q.; Laird, G. M.; Six, D. A.; Russell, D. W.; McDonald, J. G.; Subramaniam, S.; Fahy, E.; Dennis, E. A. *J. Lipid Res.* **2010**, *51*, 3299.

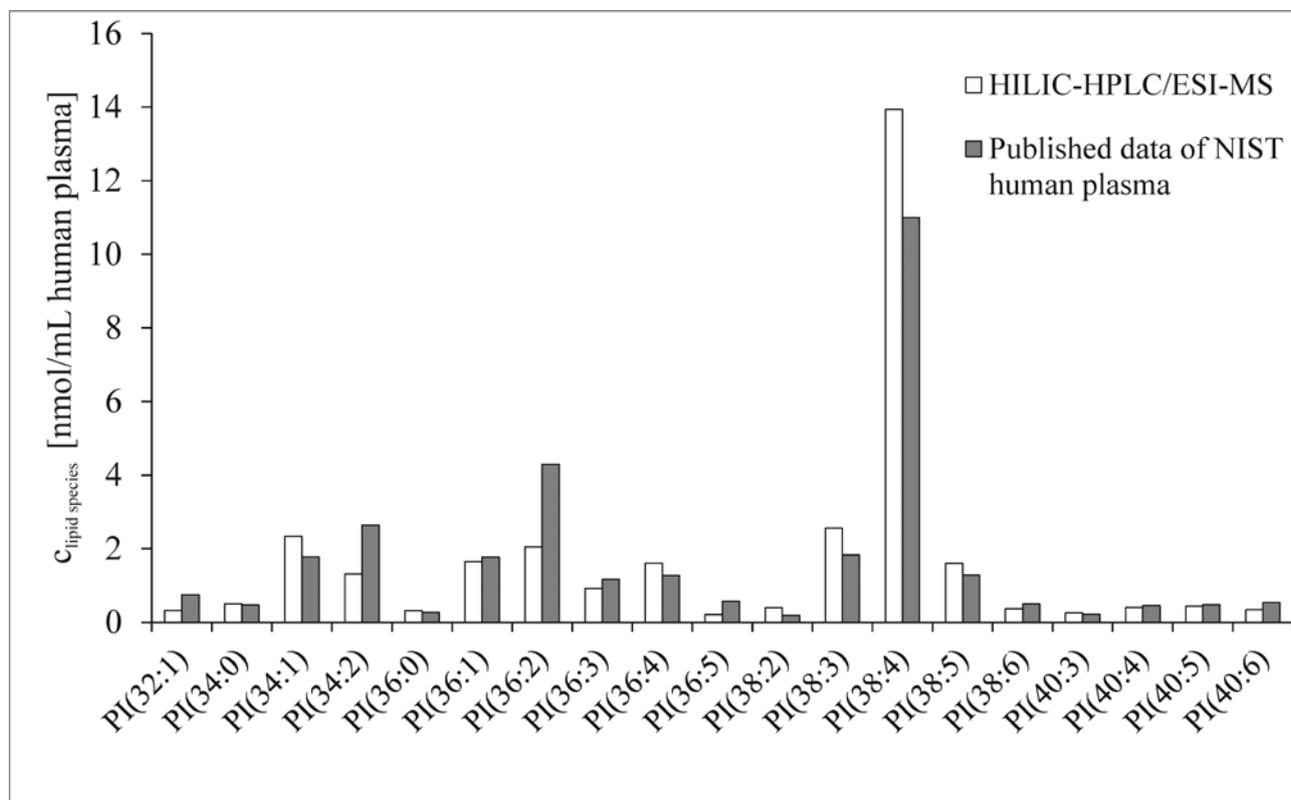
**Figure S-1.** Comparison of Folch (white columns), Bligh & Dyer (dark columns) and methyl-*tert*-butyl ether (MTBE) extraction (grey columns) for PE, PC, SM and LPC in the human plasma. Reported values are the standard mean of six measurements (three times extraction, each extract measured two times) together with standard deviations.



**Figure S-2.** Robustness test of extraction recoveries shown on the comparison of concentrations (mg/g) of PI, PE, PC, SM and LPC in the porcine liver extract performed by three persons. Reported values are the standard mean of three different extractions together with standard deviations.



**Figure S-3.** Comparison of concentrations (nmol/mL) of individual phosphatidylinositols (PI) in the NIST human plasma between our data obtained by HILIC-HPLC/ESI-MS in the negative ion-mode and published data<sup>1</sup>.



## Reference

- (1) Quehenberger, O.; Armando, A. M.; Brown, A. H.; Milne, S. B.; Myers, D. S.; Merrill, A. H.; Bandyopadhyay, S.; Jones, K. N.; Kelly, S.; Shaner, R. L.; Sullards, C. M.; Wang, E.; Murphy, R. C.; Barkley, R. M.; Leiker, T. J.; Raetz, C. R. H.; Guan, Z. Q.; Laird, G. M.; Six, D. A.; Russell, D. W.; McDonald, J. G.; Subramaniam, S.; Fahy, E.; Dennis, E. A. *J. Lipid Res.* **2010**, *51*, 3299.