SUPPLEMENTAL MATERIAL

Supplemental Table 1A. Free (non-esterified) fatty acid composition of human plasma. A total of 31 fatty acids were included in the GC/MS screen. All measurements were performed in triplicate.

Common Name	Chain Length[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)
Lauric acid	12:0	0.719	0.029	14.4	0.6
Myristic acid	14:0	6.06	0.069	138	2
Pentadecanoic acid	15:0	0.653	0.004	15.8	0.1
Palmitic acid	16:0	63.8	0.400	1634	10
cis-9-palmitoleic acid	16:1	14.7	0.169	372	4
Margaric acid	17:0	1.20	0.003	32.3	0.1
10Z-heptadecenoic acid	17:1	1.03	0.057	27.7	1.5
Stearic acid	18:0	22.1	0.035	628	1
Oleic acid	18:1	80.3	9.331	2267	263
Linoleic acid	18:2	15.2	0.437	427	12
α-linolenic acid	18:3(ω-3)	0.115	0.004	3.20	0.11
γ-Linolenic acid	18:3(ω-6)	1.03	0.005	28.7	0.1
Stearidonic acid	18:4	0.016	0.001	0.44	0.03
Arachidic acid	20:0	0.238	0.002	7.43	0.06
11,14-eicosadienoic acid	20:2	0.352	0.029	10.9	0.9
11,14,17-eicosatrienoic acid	20:3 (ω-3)	0.341	0.016	10.4	0.5
Bishomo-γ-linolenic acid	20:3 (ω-6)	0.542	0.005	16.6	0.2
5,8,11-eicosatrienoic acid	20:3 (ω-9)	0.095	0.012	2.91	0.37
Arachidonic acid	20:4	2.94	0.058	89.4	1.8
5,8,11,14,17-eicosapentaenoic acid	20:5	0.435	0.010	13.1	0.3
Behenic acid	22:0	0.160	0.007	5.45	0.24
cis-erucic acid	22:1	0.028	0.002	0.95	0.07
13,16-docosadienoic acid	22:2	0.011	0.001	0.37	0.03
13,16,19-docosatrienoic acid	22:3	0.004	0.001	0.13	0.03
7Z,10Z,13Z,16Z-docosatetraenoic acid	22:4	0.364	0.005	12.1	0.2
7,10,13,16,19-docosapentaenoic acid	22:5	0.400	0.005	13.2	0.2
4, 7,10,13,16,19-docosahexaenoic acid	22:6	0.990	0.009	32.5	0.3
Tricosanoic acid	23:0	0.033	0.004	1.17	0.14
Lignoceric acid	24:0	0.262	0.014	9.65	0.52
cis-selacholeic acid	24:1	0.070	0.006	2.56	0.22
Cerotic acid	26:0	0.110	0.006	4.36	0.24

[1] The chain length denotes the number of carbons followed by the number of double bonds

Supplemental Table 1B. Eicosanoids and related metabolites in human plasma. A total of 143 metabolites were included in the LC/MS screen. All measurements were performed in triplicate.

Metabolite[1]	Mean (pmol/ml)	SEM (pmol/ml)	Mean (ng/dl)	SEM (ng/dl)
COX Biosynthetic Pathway				
PGD1	0.010	0.001	0.354	0.035
15-keto-PGF1a	0.026	0.009	0.921	0.319
PGD2	0.200	0.065	7.04	2.29
PGE2	0.037	0.012	1.30	0.42
PGF2a	0.053	0.012	1.88	0.43
PGJ2	0.027	0.003	0.902	0.100
TXB2	0.037	0.007	1.37	0.26
15-keto-PGF2a	0.209	0.006	7.36	0.21
13,14-dihydro-15-keto-PGD2	0.053	0.003	1.87	0.11
13,14-dihydro-15-keto-PGE2	0.397	0.062	14.0	2.2
13,14-dihydro-15-keto-PGF2a	0.235	0.028	8.32	0.99
2,3-dinor-11ß-PGF2a	0.038	0.008	1.24	0.26
15d PGD2	1.94	0.645	64.8	21.6
15d PGJ2	0.083	0.003	2.62	0.10
11b-PGE2	0.130	0.006	4.58	0.21
11b-PGF2a	0.213	0.048	7.55	1.70
bicyclo-PGE2	0.039	0.002	1.30	0.07
11-HETE	0.727	0.060	23.3	1.9
12-HHTrE	2.03	0.278	56.9	7.8
PGD3	0.123	0.015	4.31	0.53
18-HEPE	0.138	0.024	4.39	0.76
TXB3	0.017	0.003	0.626	0.110
LOX Biosynthetic Pathway				
9-HODE	6.8	1.67	201	50
13-HODE	10.6	1.28	314	38
13-OxoODE	0.490	0.040	14.4	1.2
9-HOTrE	0.503	0.052	14.8	1.5
13-HOTrE	0.493	0.017	14.5	0.5
13-HOTrE(y)	0.503	0.044	14.8	1.3
5-HETrE	0.253	0.018	8.15	0.58
8-HETrE	0.123	0.003	3.96	0.10
15-HETrE	0.421	0.015	13.6	0.5
5-HETE	11.9	1.40	381	45
6-trans-LTB4	0.110	0.030	3.70	1.01
12-epi-LTB4	0.220	0.070	7.40	2.35
6-trans-12-epi-LTB4	0.097	0.037	3.26	1.24
LTB4	0.037	0.007	1.24	0.24
LTC4	0.047	0.023	2.94	1.44
11-trans-LTC4	0.009	0.002	0.56	0.13
LTE4	0.020	0.010	0.88	0.44
8-HETE	0.749	0.033	24.0	1.1
12-HETE	4.22	0.292	135	9
Tetranor 12-HETE	0.310	0.031	8.25	0.83
15-HETE	0.800	0.023	25.6	0.7
15-oxoETE	0.069	0.033	2.20	1.05
5,15-DiHETE	0.527	0.017	17.70	0.60

5S,6S-Lipoxin A4	0.053	0.013	1.87	0.46
5-HEPE	0.67	0.121	21.3	3.9
12-HEPE	0.517	0.084	16.5	2.7
15-HEPE	0.197	0.019	6.27	0.61
CYP Biosynthetic Pathway				
12,13-DiHOME	5.02	0.19	158	6
5,6-EpETrE	0.110	0.021	3.52	0.67
14,15-EpETrE	1.750	0.043	56.0	1.4
5,6-DiHETrE	0.610	0.057	20.6	1.9
8,9-DiHETrE	0.510	0.066	17.3	2.2
11,12-DiHETrE	0.513	0.094	17.4	3.2
14,15-DiHETrE	1.449	0.071	49.0	2.4
16-HETE	0.277	0.086	8.87	2.75
17-HETE	0.113	0.021	3.62	0.67
18-HETE	0.273	0.047	8.74	1.51
20-HETE	0.83	0.150	26.6	4.8
16(17)-EpDPE	0.239	0.048	8.23	1.65
19,20-DiHDPA	1.23	0.389	44.6	14.1
Non-Enzymatic Pathway				
5-iPF2a-VI	0.381	0.015	13.5	0.5
8-iso-PGF2a	0.151	0.018	5.35	0.64
9-HETE	1.704	0.081	54.6	2.6
9-HEPE	0.066	0.014	2.10	0.45
4-HDoHE	5.3	1.31	182	45
7-HDoHE	0.131	0.022	4.51	0.76
8-HDoHE	0.294	0.076	10.1	2.6
10-HDoHE	0.075	0.025	2.58	0.86
11-HDoHE	0.175	0.005	6.02	0.17
13-HDoHE	0.172	0.040	5.92	1.38
14-HDoHE	1.64	0.128	56.5	4.4
16-HDoHE	0.202	0.041	6.95	1.41
17-HDoHE	0.194	0.007	6.68	0.24
20-HDoHE	0.228	0.015	7.85	0.52

[1] For appreviations see http://www.lipidmaps.org

Supplemental Table 2A: Triacylglyceride isobaric molecular subsets in human plasma. All measurements were performed in triplicate.

Triacylglycerol[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (mg/dl)	SEM (mg/dl)
TG(48:1)	27.0	2.9	2.17	0.23
TG(48:2)	20.2	4.1	1.62	0.33
TG(50:0)	11.6	2.8	0.97	0.23
TG(50:1)	63.6	1.8	5.30	0.15
TG(50:2)	79.8	8.9	6.63	0.74
TG(50:3)	57.1	5.5	4.73	0.46
TG(50:4)	18.9	3.2	1.56	0.26
TG(52:1)	29.6	1.9	2.55	0.16
TG(52:2)	139.5	10.0	12.0	0.9
TG(52:3)	214.8	22.0	18.4	1.9
TG(52:4)	90.9	9.6	7.77	0.82
TG(52:5)	32.0	1.4	2.73	0.12
TG(54:2)	21.5	4.3	1.91	0.38
TG(54:3)	69.1	9.5	6.11	0.84
TG(54:4)	68.5	10.3	6.05	0.91
TG(54:5)	53.6	6.0	4.72	0.53
TG(54:6)	36.5	2.6	3.21	0.23
TG(56:6)	23.5	2.6	2.13	0.24

[1] TG(total acyl carbons:total double bonds).

Supplemental Table 2B: 1,2-Diacylglyceride isobaric molecular species in human plasma. All measurements were performed in triplicate.

1,2-Diacylglcerol[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)
1,2-DG(30:0)	0.58	0.21	31.3	11.3
1,2-DG(30:1)	0.231	0.054	12.4	2.9
1,2-DG(30:2)	0.032	0.009	1.72	0.48
1,2-DG(32:0)	1.42	0.42	80.7	23.9
1,2-DG(32:1)	1.07	0.14	60.6	7.9
1,2-DG(32:2)	0.415	0.069	23.4	3.9
1,2-DG(32:3)	0.060	0.024	3.37	1.35
1,2-DG(34:0)	1.37	0.53	81.7	31.6
1,2-DG(34:1)	3.87	0.62	230	37
1,2-DG(34:2)	2.82	0.36	167	21
1,2-DG(34:3)	1.65	0.17	97.4	10.0
1,2-DG(34:4)	0.173	0.081	10.2	4.8
1,2-DG(36:0)	0.69	0.19	43.1	11.9
1,2-DG(36:1)	1.24	0.33	77.2	20.5
1,2-DG(36:2)	4.06	0.54	252	34
1,2-DG(36:3)	8.1	1.0	501	62
1,2-DG(36:4)	5.28	0.65	326	40
1,2-DG(36:5)	0.961	0.023	59.1	1.4
1,2-DG(38:0)	0.003	0.000	0.20	0.00
1,2-DG(38:1)	0.013	0.005	0.85	0.33
1,2-DG(38:2)	0.022	0.010	1.43	0.65
1,2-DG(38:3)	0.28	0.02	18.1	1.3
1,2-DG(38:4)	0.94	0.11	60.6	7.1
1,2-DG(38:5)	1.61	0.20	103.0	13.0
1,2-DG(38:6)	1.137	0.073	72.8	4.7
1,2-DG(40:4)	0.036	0.004	2.42	0.27
1,2-DG(40:6)	0.254	0.032	17.0	2.1
1,2-DG(40:7)	0.310	0.068	20.7	4.5

[1] 1,2-DG(total acyl carbons:total double bonds).

Supplemental Table 2C: 1,3-Diacylglyceride isobaric molecular species in human plasma. All measurements were performed in triplicate.

1,3-Diacylglycerol[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)
1,3-DG(30:0)	0.243	0.012	13.1	0.6
1,3-DG(30:1)	0.105	0.018	5.65	0.97
1,3-DG(30:2)	0.013	0.003	0.70	0.16
1,3-DG(32:0)	0.677	0.027	38.5	1.5
1,3-DG(32:1)	0.411	0.037	23.3	2.1
1,3-DG(32:2)	0.161	0.021	9.09	1.19
1,3-DG(32:3)	0.017	0.004	0.96	0.23
1,3-DG(34:0)	1.04	0.11	62.2	6.5
1,3-DG(34:1)	1.48	0.10	88.0	5.8
1,3-DG(34:2)	1.16	0.11	69.0	6.5
1,3-DG(34:3)	0.571	0.045	33.7	2.7
1,3-DG(34:4)	0.046	0.010	2.71	0.59
1,3-DG(36:0)	0.769	0.064	48.0	4.0
1,3-DG(36:1)	0.361	0.056	22.5	3.5
1,3-DG(36:2)	1.36	0.15	84.6	9.6
1,3-DG(36:3)	1.84	0.18	114	11
1,3-DG(36:4)	1.42	0.14	87.5	8.4
1,3-DG(36:5)	0.312	0.031	19.2	1.9
1,3-DG(38:0)	0.040	0.021	2.61	1.37
1,3-DG(38:2)	0.002	0.000	0.13	0.00
1,3-DG(38:3)	0.007	0.000	0.45	0.00
1,3-DG(38:4)	0.036	0.016	2.32	1.03
1,3-DG(38:5)	0.253	0.025	16.3	1.6
1,3-DG(38:6)	0.399	0.061	25.6	3.9
1,3-DG(40:4)	0.360	0.033	24.2	2.2
1,3-DG(40:6)	0.044	0.017	2.94	1.14
1,3-DG(40:7)	0.118	0.017	7.86	1.13

[1] 1,3-DG(total acyl carbons:total double bonds).

Supplemental Table 3A: Glycerophospholipids in human plasma samples. The measurements for LPE, LPC, PS, PG, and PA species were performed in quadruplicate; the measurements for PE, PC, and PI species were performed in quintuplicate.

Phospholipid	Mean (nmol/ml)	SEM (nmol/ml)	Mean (mg/dl)	SEM (mg/dl)
PE(32:1)	0.904	0.065	0.062	0.004
PE(34:0)	0.801	0.128	0.058	0.009
PE(34:1)	5.95	0.18	0.427	0.013
PE(34:1p)[1]	3.93	0.13	0.276	0.009
PE(34:2)	12.0	0.8	0.857	0.057
PE(34:2p)[1]	7.32	0.23	0.512	0.016
PE(36:0)	14.1	0.6	1.05	0.04
PE(36:1)	8.21	0.37	0.612	0.028
PE(36:2)	29.5	0.9	2.19	0.07
PE(36:2e)/PE(36:1p)[1]	5.46	0.18	0.398	0.013
PE(36:3)	16.0	0.5	1.18	0.04
PE(36:3e)/PE(36:2p)[1]	13.7	0.5	0.995	0.036
PE(36:4)	22.9	0.5	1.69	0.04
PE(36:4e)/PE(36:3p)[1]	11.7	0.3	0.851	0.022
PE(36:5)	4.10	0.20	0.302	0.015
PE(36:5e)/PE(36:4p)[1]	19.6	0.9	1.42	0.07
PE(38:1)	10.3	0.3	0.800	0.023
PE(38:2)	0.763	0.142	0.059	0.011
PE(38:3)	6.83	0.73	0.525	0.056
PE(38:4)	48.1	2.0	3.69	0.15
PE(38:5)	20.9	0.8	1.60	0.06
PE(38:5e)/PE(38:4p)[1]	54.6	0.3	4.10	0.02
PE(38:6)	19.5	0.7	1.49	0.05
PE(38:6e)/PE(38:5p)[1]	32.7	0.6	2.45	0.04
PE(40:1)	1.73	0.40	0.138	0.032
PE(40:4)	2.34	0.06	0.186	0.005
PE(40:5)	4.84	0.44	0.384	0.035
PE(40:5e)[1]	7.7	0.25	0.600	0.019
PE(40:6)	10.0	0.5	0.792	0.040
PE(40:6e)[1]	11.5	0.3	0.897	0.023
PE(40:7)	2.57	0.18	0.203	0.014
PE(40:7e)[1]	15.9	0.4	1.23	0.03
PE(42:1)	0.862	0.120	0.071	0.010
PE(42:5)	1.47	0.36	0.121	0.030
PE(42:5p)[1]	1.83	0.18	0.148	0.014
PE(42:6)	1.64	0.34	0.134	0.028
PE(42:6p)[1]	1.70	0.22	0.136	0.018
PE(42:7)	1.20	0.21	0.098	0.017
LPE(16:0)	3.45	0.42	0.156	0.019
LPE(18:0)	7.41	0.76	0.356	0.037
LPE(18:1)	6.44	0.71	0.308	0.034
LPE(18:2)	7.85	1.06	0.374	0.051
LPE(20:4)	7.32	0.75	0.367	0.038
LPE(22:1)	0.311	0.099	0.017	0.005
LPE(22:6)	3.86	0.44	0.203	0.023

PC(30:1)	1.12	0.11	0.078	0.008
PC(32:0)	11.4	0.6	0.839	0.043
PC(32:1)	28.6	1.8	2.09	0.13
PC(32:2)	9.80	0.45	0.714	0.032
PC(34:0)	7.66	2.05	0.583	0.153
PC(34:1)	89.3	8.1	6.78	0.60
PC(34:1e)[1]	2.80	0.13	0.209	0.01
PC(34:2)	188	14	14.2	1.0
PC(34:2e)[1]	3.99	0.21	0.296	0.015
PC(34:3)	13.8	1.0	1.04	0.07
PC(36:0)	7.95	1.51	0.627	0.117
PC(36:1)	99.8	13.0	7.85	1.00
PC(36:1e)/PC(36:0p)[1]	2.57	0.55	0.199	0.042
PC(36:2)	254	18	19.9	1.39
PC(36:2e)/PC(36:1p)[1]	6.25	0.55	0.482	0.042
PC(36:3)	165	13	12.9	1.0
PC(36:4)	172	11	13.4	0.8
PC(36:4e)/PC(36:3p)[1]	29.0	1.5	2.23	0.11
PC(36:5)	12.8	1.2	0.997	0.092
PC(38:2)	37.6	5.7	3.06	0.46
PC(38:3e)/PC(38:2p)[1]	11.6	0.9	0.921	0.07
PC(38:4)	254	21	20.6	1.67
PC(38:5)	86.3	9.0	6.97	0.71
PC(38:5e)/PC(38:4p)[1]	49.9	2.8	3.96	0.22
PC(38:6)	62.9	4.9	5.07	0.39
PC(40:2)	133	16	11.2	1.3
PC(40:4)	36.7	5.5	3.08	0.45
PC(40:5)	66.6	10.1	5.56	0.83
PC(40:6)	79.4	10.3	6.61	0.84
PC(40:7)	23.1	4.5	1.92	0.37
PC(40:8)	27.0	2.5	2.24	0.20
LPC(16:0)	29.8	4.9	1.48	0.23
LPC(16:0e)[1]	0.491	0.089	0.023	0.004
LPC(16:0p)[1]	1.73	0.26	0.080	0.012
LPC(16:1)	3.77	0.68	0.181	0.033
LPC(18:0)	23.3	3.5	1.22	0.18
LPC(18:0e)[1]	0.911	0.149	0.045	0.007
LPC(18:1)	14.8	2.3	0.751	0.114
LPC(18:2)	16.9	2.2	0.851	0.113
LPC(20:3)	3.26	0.43	0.173	0.023
LPC(20:4)	5.73	0.73	0.303	0.039
LPC(22:5)	1.00	0.11	0.056	0.006
LPC(22:6)	1.57	0.18	0.087	0.010
PS(32:1)	0.239	0.017	0.018	0.001
PS(34:0)	0.307	0.062	0.023	0.005
PS(34:1)	0.186	0.029	0.014	0.002
PS(34:2)	0.151	0.021	0.011	0.002
PS(36:0)	2.090	0.190	0.165	0.015
PS(36:1)	0.733	0.149	0.058	0.012
PS(36:2)	0.316	0.065	0.025	0.005
PS(36:3)	0.123	0.020	0.010	0.002

PS(36:4)	0.179	0.031	0.014	0.002
PS(38:1)	0.264	0.075	0.022	0.006
PS(38:2)	0.188	0.023	0.015	0.002
PS(38:3)	0.194	0.043	0.016	0.003
PS(38:4)	0.481	0.083	0.039	0.007
PS(38:5)	0.281	0.054	0.023	0.004
PS(38:6)	0.162	0.017	0.013	0.001
PS(40:3)	0.090	0.015	0.008	0.001
PS(40:4)	0.101	0.029	0.008	0.002
PS(40:5)	0.114	0.045	0.010	0.004
PS(40:6)	0.637	0.109	0.053	0.009
PS(40:7)	0.169	0.028	0.014	0.002
PG(34:1)	0.604	0.044	0.045	0.003
PG(34:2)	0.080	0.010	0.006	0.001
PG(36:1)	1.59	0.12	0.123	0.009
PG(36:2)	0.194	0.010	0.015	0.001
PG(36:3)	0.338	0.052	0.026	0.004
PG(36:4)	0.217	0.036	0.017	0.003
PG(36:5)	0.351	0.025	0.027	0.002
PG(38:4)	0.191	0.026	0.015	0.002
PG(38:5)	0.605	0.127	0.048	0.010
PG(38:6)	0.919	0.137	0.073	0.011
PG(40:4)	0.323	0.019	0.027	0.002
PG(40:5)	0.159	0.029	0.013	0.002
PG(40:6)	0.145	0.021	0.012	0.002
PG(40:7)	0.181	0.024	0.015	0.002
PG(40:8)	0.105	0.018	0.009	0.001
PG(40:9)	0.116	0.019	0.009	0.002
PA(32:0)	0.186	0.037	0.012	0.002
PA(32:1)	0.199	0.048	0.013	0.003
PA(34:0)	0.225	0.021	0.015	0.001
PA(34:1)	0.147	0.012	0.010	0.001
PA(34:2)	0.144	0.015	0.010	0.001
PA(36:0)	0.231	0.032	0.016	0.002
PA(36:1)	0.118	0.012	0.008	0.001
PA(36:2)	0.223	0.047	0.016	0.003
PA(36:3)	0.117	0.017	0.008	0.001
PA(36:4)	0.208	0.042	0.014	0.003
PA(38:2)	0.138	0.029	0.010	0.002
PA(38:3)	0.121	0.011	0.009	0.001
PA(38:4)	0.164	0.018	0.012	0.001
PA(38:5)	0.212	0.043	0.015	0.003
PA(38:6)	0.070	0.010	0.005	0.001
PI(32:1)	0.746	0.165	0.060	0.013
PI(34:0)	0.473	0.101	0.040	0.008
PI(34:1)	1.78	0.14	0.149	0.012
PI(34:2)	2.64	1.30	0.220	0.108
PI(36:0)	0.269	0.015	0.023	0.001
PI(36:1)	1.77	0.14	0.153	0.012
PI(36:2)	4.29	0.39	0.370	0.034

PI(36:3)	1.17	0.06	0.101	0.005
PI(36:4)	1.27	0.08	0.109	0.007
PI(36:5)	0.570	0.065	0.049	0.006
PI(38:2)	0.189	0.059	0.017	0.005
PI(38:3)	1.830	0.140	0.163	0.012
PI(38:4)	11.0	0.7	0.975	0.061
PI(38:5)	1.28	0.14	0.113	0.012
PI(38:6)	0.498	0.130	0.044	0.011
PI(40:3)	0.217	0.019	0.020	0.002
PI(40:4)	0.451	0.030	0.041	0.003
PI(40:5)	0.475	0.046	0.043	0.004
PI(40:6)	0.540	0.053	0.049	0.005

[1] Ether-linked isobaric species of plasmanyl (e) and plasmenyl (p) analogues of glycerophospholipids. When chromatographic separation was not achieved, both analogues are listed.

Supplemental Table 3B: Concentration and composition of *N*-acyl-PS in human plasma after acidic Folch extraction. All measurements were performed in triplicate.

N-acyl-PS	Mean (pmol/ml)	SEM (pmol/ml)	Mean (µg/dl)	SEM (µg/dl)
52:1	9.60	1.20	0.987	0.123
54:2	2.54	0.41	0.268	0.043

Supplemental Table 4A: Sphingomyelins in human plasma samples. All measurements were performed in quadruplicate.

SM	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)	Sphingoid base[1]	Fatty acid[2]	%[2]
C31:0	0.303	0.004	20.0	0.3			
					d18:0	C13:0	100
C32:2	0.544	0.016	36.6	1.1			
					d18:2	C14:0	100
C32:1	9.17	0.18	620	12			
					d18:1	C14:0	66
					d16:1	C16:0	34
C32:0	1.00	0.01	68.1	0.7			
					d18:0	C14:0	100
C33:2	0.201	0.005	13.8	0.4			
					d18:2	C15:0	16
					d17:2	C16:0	84
C33:1	6.22	0.12	429	8			
					d18:1	C15:0	12
					d16:1	C17:0	88
C33:0	0.590	0.008	40.8	0.5			
					d18:0	C15:0	100
C34:2	0.702	0.021	49.3	1.5			
					d18:2	C16:0	85
					d18:1	C16:1	7
					d16:1	C18:1	7
C34:1	81.0	0.9	5700	66			
					d18:1	C16:0	96
					d16:1	C18:0	4
C34:0	16.7	0.2	1180	17			
					d18:0	C16:0	100
C35:1	3.75	0.36	269	26			
					d18:1	C17:0	36
					d17:1	C18:0	34
					d19:1	C16:0	30
C35:0	1.24	0.33	88.9	23.5			
					d18:0	C17:0	100
C36:3	1.05	0.07	76.5	4.9			
					d18:2	C18:1	100
C36:2	10.8	0.2	791	13			
					d18:2	C18:0	52
					d18:1	C18:1	31
					d18:0	C18:2	4
					d16:1	C20:1	4
					d19:1	C17:1	7
					d20:2	C16:0	2
C36:1	16.2	0.4	1180	26			
					d18:1	C18:0	81
					d16:1	C20:0	19
C36:0	4.11	0.08	301	6			
					d18:0	C18:0	63
					d16:0	C20:0	9
					d19:0	C17:0	29

C37:1	0.902	0.055	67.3	4.1			
					d18:1	C19:0	64
					d19:1	C18:0	36
C38:3	0.511	0.044	38.6	3.3			
					d18:2	C20:1	44
					d18:1	C20:2	28
					d16:1	C22:2	28
C38:2	6.60	0.24	500	18			
					d18:2	C20:0	75
					d18:1	C20:1	12
					d17:2	C21:0	6
					d16:1	C22:1	7
C38:1	9.98	0.24	758	18			
					d18:1	C20:0	49
					d16:1	C22:0	51
C38:0	2.78	0.03	212	2			
					d18:0	C20:0	60
					d16:0	C22:0	40
C39:2	0.894	0.044	69.0	3.4			
					d18:2	C21:0	50
					d16:2	C23:0	50
C39:1	4.34	0.13	336	10			
					d18:1	C21:0	18
					d17:1	C22:0	35
					d16:1	C23:0	28
					d19:1	C20:0	11
					d20:1	C19:0	9
C40:3	1.47	0.10	115	8			
					d18:2	C22:1	46
					d18:1	C22:2	39
					d16:1	C24:2	15
C40:2	15.7	0.6	1240	44			
					d18:2	C22:0	59
					d18:1	C22:1	16
					d16:1	C24:1	25
C40:1	15.0	0.4	1180	31			
					d18:1	C22:0	86
					d16:1	C24:0	14
C40:0	3.32	0.10	262	8			
					d18:0	C22:0	100
C41:3	0.672	0.011	53.6	0.9			
					d18:2	C23:1	100
C41:2	5.85	0.15	468	12			
					d18:2	C23:0	55
					d18:1	C23:1	11
					d17:1	C24:1	31
					d20:1	C21:1	2
C41:1	7.00	0.27	562	22			
					d18:1	C23:0	84
					d17:1	C24:0	11
					d16:1	C25:0	5
C42:4	3.67	0.17	298	14			
					d18:2	C24:2	84

					d18:1	C24:3	16
C42:3	21.2	0.6	1720	49			
					d18:2	C24:1	66
					d18:1	C24:2	32
					d20:1	C23:2	2
C42:2	33.0	0.8	2680	66			
					d18:2	C24:0	29
					d18:1	C24:1	71
C42:1	12.5	0.6	1020	53			
					d18:1	C24:0	100
C43:3	0.509	0.012	42.1	1.0			
					d18:2	C25:1	60
					d16:1	C27:2	40
C43:2	1.73	0.07	143	6			
					d18:2	C25:0	6
					d18:1	C25:1	28
					d17:1	C26:1	37
					d19:1	C24:1	17
					d20:1	C23:1	11
C43:1	1.30	0.05	108	4			
					d18:1	C25:0	25
					d19:1	C24:0	10
					d19:0	C24:1	50
					d20:1	C23:0	15
C44:3	0.468	0.007	39.3	0.6			
					d18:1	C26:2	20
					d18:0	C26:3	30
					d17:1	C27:2	30
					d20:1	C28:2	20
C44:2	0.276	0.011	23.2	0.9			
					d18:1	C26:1	36
					d18:0	C26:2	36
					d19:1	C25:1	28
C44:1	0.216	0.005	18.2	0.4			
					d18:0	C26:1	21
					d17:0	C27:1	19
					d16:0	C28:1	18
					d19:0	C25:1	22
					d20:0	C24:1	19

[1] The sphingoid base nomenclature (e.g., d18:1 for sphingosine) gives the number of hydroxyl groups (d for two), followed by the chain length (number of carbons) and number of double bonds. Quantitation of these backbones assumes similar ion yields as for the corresponding d18 species.

[2] Determined during SM analysis by ion trap as outlined under Experimental Procedures.

Supplemental Table 4B: Monohexosylceramides in human plasma samples. All measurements were performed in quadruplicate.

Sphingoid base[1]	Fatty acid	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)	%Glc[2]	%Gal[2]
d16:1[3]	C22:0	0.036	0.010	2.79	0.76	96	4
d18:0	C13:0	0.0025	0.0016	0.16	0.11		
d18:0	C14:0	0.0010	0.0007	0.07	0.05		
d18:0	C16:0	0.0026	0.0009	0.18	0.06	100	0
d18:0	C17:0	0.0042	0.0023	0.30	0.16		
d18:0	C18:1	0.0003	0.0002	0.02	0.02		
d18:0	C18:0	0.0006	0.0003	0.04	0.02		
d18:0	C20:0	0.0134	0.0051	1.05	0.38	100	0
d18:0	C22:0	0.0072	0.0018	0.57	0.14	80	20
d18:0	C24:1	0.0038	0.0012	0.31	0.10		
d18:0	C24:0	0.0078	0.0012	0.63	0.10	88	12
d18:0	C26:1	0.0071	0.0019	0.60	0.16	34	66
d18:0	C26:0	0.0090	0.0019	0.76	0.16	50	50
d18:1	C14:0	0.0011	0.0009	0.07	0.06		
d18:1	C16:0	0.336	0.029	23.5	2.0	95	5
d18:1	C17:0	0.0081	0.0014	0.58	0.10		
d18:1	C18:1	0.0127	0.0027	0.92	0.19		
d18:1	C18:0	0.0275	0.0037	2.00	0.27	97	3
d18:1	C19:0	0.0016	0.0008	0.12	0.06		
d18:1	C20:0	0.079	0.008	5.98	0.61	96	4
d18:1	C22:0	0.522	0.033	41.0	2.6	98	2
d18:1	C23:0	0.187	0.017	15.0	1.3	92	8
d18:1	C24:1	0.332	0.009	27.0	0.7	96	4
d18:1	C24:0	0.409	0.028	33.2	2.3	99	1
d18:1	C25:0	0.0093	0.0027	0.77	0.22		
d18:1	C26:1	0.0178	0.0018	1.50	0.15	73	27
d18:1	C26:0	0.0073	0.0016	0.62	0.13	100	0
d18:2[3]	C14:0	0.0024	0.0008	0.16	0.06		
d18:2	C15:0	0.0013	0.0008	0.09	0.05		
d18:2	C16:0	0.013	0.003	1.1	0.2	87	13
d18:2	C18:1	0.0022	0.0007	0.16	0.05		
d18:2	C20:1	0.0085	0.0022	0.64	0.17	100	0
d18:2	C20:0	0.170	0.082	12.8	6.2	82	18
d18:2	C21:0	0.0125	0.004	0.96	0.31	100	0
d18:2	C22:0	0.0577	0.0050	4.5	0.4	96	4

[1] The sphingoid base nomenclature (e.g., d18:1 for sphingosine) gives the number of hydroxyl groups (d for two), followed by the chain length (number of carbons) and number of double bonds.

[2] GlcCer and GalCer were determined by an additional LC MS/MS analysis under the LC conditions that resolve these monohexosylceramides.

[3] Quantitation of these backbones assumes similar ion yields as for the corresponding d18 species.

Supplemental Table 4C: Ceramides in human plasma samples. All measurements were performed in quadruplicate.

Sphingoid base[1]	Fatty acid	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)
d16:1[2]					
	C22:0	0.094	0.003	5.59	0.20
d18:0					
	C13:0	0.009	0.0003	0.44	0.02
	C14:0	0.088	0.0004	4.49	0.02
	C15:0	0.005	0.0004	0.28	0.02
	C16:0	0.261	0.0004	14.1	0.0
	C17:0	0.009	0.0004	0.52	0.02
	C18:1	0.038	0.001	2.13	0.05
	C18:0	0.146	0.002	8.31	0.10
	C20:0	0.089	0.001	5.29	0.03
	C22:0	0.685	0.003	42.8	0.2
	C24:1	0.552	0.003	35.9	0.2
	C24:0	1.22	0.004	79.6	0.3
	C26:2	0.196	0.002	13.2	0.1
	C26:1	0.031	0.001	2.08	0.05
	C26:0	0.041	0.002	2.78	0.11
d18:1					
	C14:0	0.012	0.001	0.60	0.06
	C16:0	0.331	0.029	17.8	1.6
	C17:0	0.008	0.001	0.45	0.05
	C18:1	0.022	0.001	1.22	0.06
	C18:0	0.128	0.012	7.23	0.70
	C19:0	0.040	0.002	2.34	0.11
	C20:0	0.145	0.007	8.62	0.44
	C22:0	1.22	0.046	76.2	2.9
	C23:0	0.281	0.033	17.9	2.1
	C24:1	1.00	0.029	65.0	1.9
	C24:0	3.00	0.107	195	7
	C25:0	0.271	0.013	18.0	0.9
	C26:1	0.036	0.002	2.41	0.12
	C26:0	0.061	0.0007	4.15	0.05
d18:2[2]					
	C14:0	0.005	0.001	0.23	0.03
	C15:0	0.001	0.001	0.07	0.03
	C16:0	0.048	0.004	2.60	0.20
	C18:1	0.006	0.001	0.31	0.06
	C20:1	0.006	0.003	0.35	0.17
	C20:0	0.034	0.005	1.99	0.32
	C21:0	0.004	0.001	0.27	0.07
	C22:0	0.200	0.009	12.4	0.5
	C23:1	1.08	0.109	67.8	6.8
	C23:0	0.086	0.013	5.41	0.82
	C24:2	0.096	0.007	6.20	0.47
	C24:1	0.001	0.0001	0.01	0.01

[1] The sphingoid base nomenclature (e.g., d18:1 for sphingosine) gives the number of hydroxyl groups (d for two), followed by the chain length (number of carbons) and number of double bonds.

[2] Quantitation of these backbones assumes similar ion yields as for the corresponding d18 species.

Supplemental Table 4D: Sphingoid bases in human plasma samples. All measurements were performed in quadruplicate.

Sphingoid base[1]	Mean (pmol/ml)	SEM (pmol/ml)	Mean (µg/dl)	SEM (µg/dl)
Sphinganine (d18:0)	64	8	1.9	0.2
Sphingosine (d18:1)	91	10	2.7	0.3
Sphinganine 1-phosphate (d18:0-P)	97	15	3.7	0.6
Sphingosine 1-phosphate (d18:1-P)	308	10	11.7	0.4
Sphingosine 1-phosphate (d16:1-P)[2]	3.3	1.2	0.12	0.04
Sphingosine 1-phosphate (d19:1-P)[2]	4.5	2.0	0.17	0.08

[1] The sphingoid base nomenclature (e.g., d18:1 for sphingosine) gives the number of hydroxyl groups (d for two), followed by the chain length (number of carbons) and number of double bonds.

[2] Quantitation of these backbones assumes similar ion yields as for the corresponding d18 species (d17 subspecies were not quantified because this chain length was used for the spiked internal standard).

Supplemental Table 5A. Total (free and esterified) sterol levels in human plasma. All measurements were performed in triplicate.

Sterol[1]	**Mean (nmol/ml)**	**SEM (nmol/ml)**	**Mean (µg/dl)**	**SEM (µg/dl)**
24S-hydroxycholesterol	0.049	0.002	1.97	0.08
25-hydroxycholesterol	0.020	0.003	0.81	0.12
27-hydroxycholesterol	0.538	0.040	21.6	1.6
4β-hydroxycholesterol	0.055	0.002	2.21	0.08
7-dehydrocholesterol	3.01	0.254	116	10
7-oxocholesterol	0.122	0.083	4.91	3.34
7α-hydroxycholesterol	0.335	0.068	13.5	2.7
Campesterol[2]	6.46	0.051	259	2.0
Cholestenone	0.079	0.022	3.04	0.85
Desmosterol[2]	1.81	0.067	69.6	2.6
Lanosterol[2]	0.480	0.114	20.5	4.9
Lathosterol[2]	5.38	0.038	208	1.0
Sitosterol[2]	5.53	0.021	229	1.0
	µmol/ml	**µmol/ml**	**mg/dl**	**mg/dl**
Cholesterol[2]	3.76	0.098	145	4.0

[1] In addition to the sterols shown, two known oxidation products of cholesterol, 5/6α- and 5/6β-epoxycholesterol were detected. These sterols form spontaneously in variable amounts during sample work-up and are thus not reported here.

[2] Measured by gas chromatography-mass spectrometry; all other sterols measured by liquid chromatography-mass spectrometry.

Supplemental Table 5B. Free sterol levels in human plasma. All measurements were performed in triplicate.

Sterol[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)	% Free
24S-hydroxycholesterol	0.006	0.0003	0.255	0.012	12.9
25-hydroxycholesterol	0.001	0.0001	0.036	0.002	4.4
27-hydroxycholesterol	0.013	0.001	0.514	0.029	2.4
4β-hydroxycholesterol	0.014	0.0005	0.546	0.019	24.7
7-dehydrocholesterol	n.d.	n.d.	n.d.	n.d.	n.d.
7-oxocholesterol	0.014	0.008	0.551	0.316	11.2
7α-hydroxycholesterol	0.012	0.002	0.468	0.063	3.5
Campesterol[2]	1.35	0.014	54.0	0.577	20.8
Cholestenone	n.d.	n.d.	n.d.	n.d.	n.d.
Desmosterol[2]	0.790	0.017	30.3	0.667	43.6
Lanosterol[2]	n.d.	n.d.	n.d.	n.d.	n.d.
Lathosterol[2]	2.43	0.048	93.7	1.86	45.0
Sitosterol[2]	0.902	0.089	37.3	3.67	16.3
	µmol/ml	**µmol/ml**	**mg/dl**	**mg/dl**	
Cholesterol[2]	0.820	0.009	31.6	0.300	21.8

[1] In addition to the sterols shown, two known oxidation products of cholesterol, 5/6α- and 5/6β-epoxycholesterol were detected. These sterols form spontaneously during sample work-up and are thus not reported here.

[2] Measured by gas chromatography-mass spectrometry; all other sterols measured by liquid chromatography-mass spectrometry.

n.d., Not detected.

Supplemental Table 5C: Cholesteryl ester molecular species in human plasma. All measurements were performed in triplicate.

Cholesteryl ester[1]	Mean (nmol/ml)	SEM (nmol/ml)	Mean (mg/dl)	SEM (mg/dl)
CE(14:0)	80	2	4.77	0.12
CE(14:1)	30	1	1.78	0.06
CE(15:0)	30	1	1.83	0.06
CE(15:1)	30	1	1.83	0.06
CE(16:0)	190	6	11.9	0.4
CE(16:1)	111	4	6.91	0.25
CE(16:2)	31	1	1.92	0.06
CE(17:0)	32	1	2.04	0.06
CE(17:1)	31	1	1.97	0.06
CE(18:0)	59	3	3.85	0.20
CE(18:1)	533	13	34.7	0.8
CE(18:2)	1820	85	118	6
CE(18:3)	147	8	9.50	0.52
CE(20:0)	32	1	2.18	0.07
CE(20:1)	30	1	2.04	0.07
CE(20:2)	34	2	2.30	0.14
CE(20:3)	32	1	2.16	0.07
CE(20:4)	237	13	15.9	0.9
CE(22:0)	23	2	1.63	0.14
CE(22:1)	10	1	0.71	0.07
CE(22:2)	10	1	0.71	0.07
CE(22:6)	32	2	2.23	0.14

[1] CE(total acyl carbons:total double bonds).

Supplemental Table 6A: Concentration and composition of free dolichol in human plasma[1]. All measurements were performed in triplicate.

Dolichol	Mean (pmol/ml)	SEM (pmol/ml)	Mean (ng/dl)	SEM (ng/dl)
16	0.13	0.05	14.4	5.5
17	1.30	0.12	153	14
18	8.79	0.54	1094	67
19	13.2	1.3	1733	171
20	1.7	0.58	235	80
21	n.d.	n.d.	n.d.	n.d.

[1] Free alcohol forms of dolichols 16-20 were analyzed as outlined under Experimental Procedures. Nor-dolichols 16-21 were used as internal standards for quantitation.

n.d., Not detected.

Supplemental Table 6B: Concentration and composition of CoQ in human plasma[1]. All measurements were performed in triplicate.

Coenzyme-Q	Mean (nmol/ml)	SEM (nmol/ml)	Mean (µg/dl)	SEM (µg/dl)
9	0.29	0.04	23.1	3.2
10	4.31	0.42	371	35

[1] CoQ_9 and CoQ_{10} were detected as outlined under Experimental Procedures. Yeast CoQ_6 was used as an internal standard for quantitation.

EXPERIMENTAL PROCEDURES

Production of the standard plasma reference material

The plasma standard reference material was produced by NIH in collaboration with NIST. A total of 22 liter of plasma was obtained from 100 individuals between 40 and 50 years of age who have undergone overnight fasting prior to the blood draw. The blood was collected into cold lithium heparin used as an anticoagulant and immediately centrifuged at 4°C at 8000 x g. To minimize losses of metabolites, the samples were processed to -80°C within 30 min of collection and stored under nitrogen. A rapid glucose test was administered to exclude individuals who did not adhere to the fasting requirement. Plasma was derived from an equal number of men and woman in an age range between 40 to 50 years with a racial distribution that reflects the distribution in the US population. Individuals with overt conditions, diseases and disorders, with body-mass indices outside the 95th percentile and anyone adhering to extreme dietary or exercise regimen were disqualified. The plasma was thawed once, pooled and thoroughly blended at 4°C, aliquoted into 1 ml vials, processed to -80°C under nitrogen and distributed for analysis. Red blood cell contamination was minimized and heme-contaminated individual samples were not used to pool. All plasma was demonstrated to be non-reactive when tested by US Food and Drug Administration licensed procedures for hepatitis B surface antigen, hepatitis C virus and human immunodeficiency virus.

Analytical procedures

Fatty acyls – Free fatty acids were extracted and analyzed by gas chromatography mass spectrometry (GC/MS) essentially as described previously (1). Briefly, 200 µl of plasma was supplemented with deuterated internal standards (Cayman Chemical, Ann Arbor, MI) and extracted twice with 0.05 N methanolic HCl/isooctane (1:3, v/v) and the combined isooctane layers were evaporated to dryness. The extracted free fatty acids were dissolved in 1% diisopropylehylamine in acetonitril and derivatized with 1% pentafluorobenzyl bromide. The fatty acid esters were analyzed by GC/MS on an Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent, Santa Clara, CA). Fatty acid quantitation was achieved by the stable isotope dilution method (2).

Eicosanoids were isolated from 0.9 ml of plasma supplemented with deuterated internal standards (Cayman Chemical) by solid phase extraction and analyzed by liquid chromatography mass spectrometry (LC/MS) as described (3). The extracted samples were evaporated, reconstituted in a small volume and the eicosanoids were separated by reverse phase liquid chromatography (LC) on a Synergy C18 column (2.1 x 250 mm, 4 microns; Phenomenex, Torrance, CA). Eicosanoids were analyzed using a tandem quadrupole mass spectrometer (MDS SCIEX 4000 Q Trap; Applied Biosystems, Foster City, CA) via multiple-reaction monitoring (MRM) in negative-ionization mode. Quantitative eicosanoid determination was performed by the stable isotope dilution method (2). Data analysis was performed using MultiQuant 1.2 software (Applied Biosystems).

Glycerolipid and cholesteryl esters – Neutral lipids were extracted from plasma (1 µl diluted to 5ml with saline containing internal standards for each lipid class) using the immiscible solvent system isooctane/ethyl acetate as previously described (4). The triglycerides and cholesteryl esters were separated by a single normal phase HPLC column on-line to a tandem quadrupole mass spectrometer (MDS Sciex 4000 Q TRAP) and analyzed in a combined multiple reaction monitoring (cholesteryl esters) and mass spectral ($[M+NH_4]^+$ detection mode, triglyceride and diglycerides) as described (4). The 24 cholesteryl esters were quantified using a single internal standard and the calibration curve generated using 14 separate reference standard cholesteryl esters. The elution of the triglycerides was monitored after programmed change in instrument operation to the MS-only

mode occurred and the abundance of each triglyceride isobaric molecular species as $[M+NH_4]^+$ was used to calculate concentration by forming ratios between each abundance and the abundance of a single internal standard and applying a linear standard curve equation generated as the average from 4 separate reference triglycerides. A separate aliquot of the plasma (5 µl diluted with 75 µl of saline and containing the 1,3-diglyceride internal standard) was derivatized using difluorophenylisocyanate (DFPI) to form the difluorophenylurethane of each diglyceride lipid species. The isomeric 1,2-and 1,3-diglyceride derivatives could be separated on the normal phase HPLC column and the abundance of each isobaric molecular species detected as the $[M+NH_4]^+$ molecular adduct ion. Concentration of each diglyceride molecular species was calculated from a standard curve generated from a 1,3-diglyceride reference standard and a separate curve for the 1,2-diglyceride reference standard.

Glycerophospholipids – Samples were extracted and analyzed essentially as described with a slight modifications (5). Briefly, equal volumes of ice cold 0.1 N methanolic HCl and ice cold $CHCl_3$ were added to the NIST plasma aliquots. Following 1 min vortex at 4°C layers were separated by centrifugation (18,000g for 5 min, 4°C). After the extraction and standards addition solvent was evaporated. The resulting lipid film was dissolved in 100 µl of isopropanol (IPA):hexane:100 mM NH_4COOH(aq)=58:40:2 (mobile phase A). NIST human plasma glycerophospholipids were separated by normal phase LC on a Luna Silica column (Phenomenex) using a binary gradient program consisting of mobile phase A and IPA:hexane:100mM NH_4COOH(aq)=50:40:10 (mobile phase B), identified by MS/MS analysis (6) (MDS SCIEX 4000 Q Trap), and quantified by subsequent LC/MS analysis as described (5). Quantitative analysis of glycerophospholipid composition was performed in two stages, as many of the abundant lipids exceeded the maxima of the established standard curves. In the first stage of analysis, PA, PG, PS, LPC, and LPE lipids were quantified from four technical replicates. In the second stage of analysis, samples were diluted by 10 fold from the above protocol, and quantified for PC, PE, and PI lipids from five technical replicates. Each replicate above was prepared using 0.1 ml of human plasma SRM.

For N-acyl-PS analysis, an acidic Folch two-phase solution (7) of 8:4:3 chloroform:methanol:aqueous (v/v) was prepared with the aqueous portion composed of 0.33 ml human plasma SRM sample and 1.17 ml of 1.2 N HCl. Synthetic 1,2-dioleoyl-sn-glycero-3-phospho-L-serine-N-nonadecanoyl (Avanti Polar Lipids) was added as internal standard. The extracted N-acyl-PS was analyzed by normal-phase LC coupled with MRM, as described (8). Data acquisition and analysis were performed using Analyst 1.4 software (Applied Biosystems).

Sphingolipids – The sphingolipids were extracted from 10 µl of the NIST plasma and prepared for LC ESI-MS/MS as described previously (9), except a larger volume (0.6 ml) of LC mobile phase solvent was needed to redissolve the final dried residue. For this purpose, 0.3 ml of the LC mobile phase solvent was added to the residue in the extraction test tube, which was sonicated for approximately 15 s, then the suspension was transferred to a microcentrifuge tube. Next, the test tube was rinsed with an additional 0.3 ml of the mobile phase solvent, sonicated, and this was also added to the microcentrifuge tube; and finally, the pooled sample was centrifuged at 14,000 to 16,000 x g for several min and the clear supernatant was transferred to the autoinjector vial for analysis.

The LC conditions were the same as described previously (9), with the following minor modifications for the normal phase chromatography for complex sphingolipids (Cer, SM and monohexosylCer). These lipids were separated using a Supelco 2.1 (i.d.) x 50 mm LC-NH2 column at a flow rate of 0.75 ml/min and a binary solvent system. Prior to injection, the column was equilibrated for 1.0 min with 98% Mobile phase A2 ($CH_3CN/CH_3OH/CH_3COOH$, 97/2/1, v/v/v, with 5 mM ammonium acetate) and 2% Mobile phase B2 ($CH_3OH/H_2O/CH_3(CH_2)_3OH/CH_3COOH$, 64/15/20/1 v/v/v/v with 5 mM ammonium acetate) and, after sample injection, this mixture was continued for 3.2

min, followed by a 0.4-min linear gradient to 82% Mobile phase A2, which was held for 0.8 min. This was followed by a 1.6-min linear gradient to 100% B2 followed by a 1.0-min linear gradient to starting conditions. The temperature of the ion source was set to 350ºC for optimal ionization at this flow rate.

The sphingolipids were analyzed by LC ESI-MS/MS in positive ionization mode using an ABI 3000 triple quadrupole (QQQ) tandem mass spectrometer for complex sphingolipids (Cer, SM and monohexosylCer), and the sphingoid bases and sphingoid base 1-phosphates were analyzed using an MDS SCIEX 4000 Q Trap. Quantitation was performed by MRM and comparison with the spiked internal standard (9) as well as correction for differences in ion yield due to chain length, i.e., standard curves were prepared using Cer, SM and GlcCer with N-acyl-chain lengths from C12 to C24, and the other chain lengths were estimated by interpolation.

Since the monohexosylCer are comprised of both GlcCer and GalCer, the proportions of these were determined by analysis of the samples using a silica column (Supelco 2.1 (i.d.) x 250 mm LC-Si) eluted isocratically with Mobile phase A3 ($CH_3CN/CH_3OH/CH_3COOH$, 97/2/1, v/v/v, with 5 mM ammonium acetate) at 0.75 ml per min (run length, 10.5 min), as described previously (9), except that the temperature of the ion source was set to 350ºC. For the data shown in the tables, each chain length of monohexosylCer was quantified by LC ESI-MS/MS using the LC-NH2 column and the % that is comprised of GlcCer versus GalCer was determined using chromatography on this silica column.

Likewise, rigorous identification of the lipid backbones of the SM required additional analysis because in positive ionization mode, the product ion from SM arises from loss of the phosphocholine head group, which does not provide information about the specific sphingoid base(s) and fatty acid(s). To determine this information, the SM fraction was collected under the LC conditions described above, then the backbone subspecies were analyzed using the MDS SCIEX 4000 Q Trap in negative ionization mode, which produces cleavage products with backbone information (9). This is done by selecting the same precursor ion m/z for the first and second quadrupoles with Q2 offset by only 5 to 10 eV so fragmentation does not occur in the collision cell, then each precursor is induced to fragment in the ion trap by application of an amplitude frequency. This trap-induced fragmentation protocol enhances backbone structure specific fragments for the sphingoid base and fatty acids, allowing identification of the isomers that comprise each precursor m/z (for example, d16:1;C18:0 versus d18:1;C16:0). In our experience, the ions from the different subspecies are proportional to their amounts within a related series. Thus, the amounts of each SM subspecies were determined by first quantifying each isobaric mixture by LC ESI-MS/MS in positive ionization mode, then the ratios of the product ions from the trap-induced fragmentation in negative ionization mode were used to estimate their relative proportions. A signal to background ratio of 3 was used as a cut-off for quantitation, and any ions that were not positively identified by both methods were not included in the data set.

Sterols – Sterols were analyzed as described previously with minor modifications (10-12). Total sterols (free sterols plus steryl esters) were extracted using two procedures dependent on the type of chromatography and mass spectrometry used for subsequent separation and detection. Sterols to be analyzed by HPLC-MS were extracted from the NIST plasma sample using a modified Bligh/Dyer extraction after addition of deuterated surrogate standards (Avanti Polar Lipids, Alabaster, AL). Extracts were dried under nitrogen and subjected to hydrolysis at high temperature (70ºC) for 1 hr with methanolic KOH. Samples were again subjected to modified Bligh/Dyer extraction, dried under nitrogen, and sterols isolated by solid-phase extraction on 100mg Isolute silica cartridges (Biotage, Charlotte, NC). Eluted samples were dried under nitrogen and reconstituted in 90% methanol. For sterols analyzed by GC-MS, aliquots of NIST plasma and surrogate standards were placed directly in methanolic KOH and hydrolyzed at 80-90ºC for 2 hrs. Samples were extracted with petroleum ether and dried under nitrogen. Alcoholic groups on sterols were derivatized with trimethylsilyl ethers by

treatment with Tri-Sil reagent (Pierce, Rockford, IL). Free sterols were extracted as above, but without base hydrolysis.

Oxysterols and several less abundant sterols were resolved by HPLC on a Luna C18 column (250x2mm, 3µm particle size; Phenomenex) using a binary gradient of 85% MeOH (A) and 100% MeOH (B), both supplemented with 5 mM ammonium acetate. Sterols were measured by tandem mass spectrometry using a triple quadrupole mass spectrometer (MDS Sciex 4000 Q TRAP) equipped with an electrospray interface. The source was operated in the positive mode, which predominantly formed ammonium adducts with sterols. The MS was operated in the multiple reaction monitoring mode (MRM) for maximum sensitivity. Abundant sterols were separated with an Agilent 6890 gas chromatograph equipped with a DB-5MS column (30m, 0.25mm i.d., 250µm film thickness). Sterols were measured with a 5973 MSD with electron ionization (Agilent, Santa Clara, CA).

Cardiolipin, Dolichol and ubiquinone analysis – For the analysis, neutral Folch extracts (7) were prepared from human plasma SRM samples to give a Folch two-phase solution of 8:4:3 chloroform:methanol:aqueous (v/v), with the aqueous portion composed of 0.33 ml of human plasma SRM sample and 1.17 ml phosphate-buffered saline. An internal standard mix composed of cardiolipin internal standard mixture 1 (Avanti Polar Lipids), nor-dolichol-[13-22] (Avanti Polar Lipids), and 0.05 mg/ml yeast coenzyme Q6 (Sigma) in chloroform was added for quantitation and the lipid extract contained in the organic phase was further processed for analysis.

Cardiolipin analysis was performed with normal-phase liquid chromatography coupled with tandem mass-spectrometry as described previously (13). Data acquisition and analysis were performed using Analyst QS software (Applied Biosystems). Dolichol and ubiquinone analyses were performed with reverse-phase chromatography coupled with MRM, as described (8). For dolichol analysis, samples were run in the negative ion mode and were monitored for singly-charged dolichol-acetate adduct ions $[M+CH_3CO_2]^-$ paired with the loss of the acetate ion (59.000 amu) for each analyte and nor-dolichol standard. For coenzyme Q analysis, samples were run in the positive ion mode and were monitored for singly-charged coenzyme Q-ammonium adduct ions $[M+NH_4]^+$ paired with the major fragment ion corresponding to a proton adduct of the quinone ring of coenzyme Q (197.000 amu) for each analyte and the internal standard. Data acquisition and analysis were performed using Analyst 1.4 software (Applied Biosystems).

Statistical Analyses – All experimental data are representative of at least three individual measurements. Data are expressed as mean ± standard error of the mean (SEM).

REFERENCES

1. Quehenberger, O., A. Armando, D. Dumlao, D. L. Stephens, and E. A. Dennis. 2008. Lipidomics analysis of essential fatty acids in macrophages. *Prostaglandins Leukot. Essent. Fatty Acids.* **79:** 123-129. Epub 2008 Nov 2008.
2. Hall, L. M., and R. C. Murphy. 1998. Electrospray mass spectrometric analysis of 5-hydroperoxy and 5-hydroxyeicosatetraenoic acids generated by lipid peroxidation of red blood cell ghost phospholipids. *J. Am. Soc. Mass. Spectrom.* **9:** 527-532.
3. Deems, R., M. W. Buczynski, R. Bowers-Gentry, R. Harkewicz, and E. A. Dennis. 2007. Detection and quantitation of eicosanoids via high performance liquid chromatography-electrospray ionization-mass spectrometry. *Methods Enzymol.* **432:** 59-82.
4. Hutchins, P. M., R. M. Barkley, and R. C. Murphy. 2008. Separation of cellular nonpolar neutral lipids by normal-phase chromatography and analysis by electrospray ionization mass spectrometry. *J. Lipid Res.* **49:** 804-813.
5. Ivanova, P. T., S. B. Milne, M. O. Byrne, Y. Xiang, and H. A. Brown. 2007. Glycerophospholipid identification and quantitation by electrospray ionization mass spectrometry. *Methods Enzymol.* **432:** 21-57.
6. Milne, S., P. Ivanova, J. Forrester, and H. Alex Brown. 2006. Lipidomics: an analysis of cellular lipids by ESI-MS. *Methods.* **39:** 92-103.
7. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497-509.
8. Guan, Z., S. Li, D. C. Smith, W. A. Shaw, and C. R. Raetz. 2007. Identification of N-acylphosphatidylserine molecules in eukaryotic cells. *Biochemistry.* **46:** 14500-14513.
9. Shaner, R. L., J. C. Allegood, H. Park, E. Wang, S. Kelly, C. A. Haynes, M. C. Sullards, and A. H. Merrill, Jr. 2009. Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers. *J. Lipid Res.* **50:** 1692-1707.
10. McDonald, J. G., B. M. Thompson, E. C. McCrum, and D. W. Russell. 2007. Extraction and analysis of sterols in biological matrices by high performance liquid chromatography electrospray ionization mass spectrometry. *Methods Enzymol.* **432:** 145-170.
11. Dzeletovic, S., O. Breuer, E. Lund, and U. Diczfalusy. 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal. Biochem.* **225:** 73-80.
12. Xu, F., S. D. Rychnovsky, J. D. Belani, H. H. Hobbs, J. C. Cohen, and R. B. Rawson. 2005. Dual roles for cholesterol in mammalian cells. *Proc. Natl. Acad. Sci. USA.* **102:** 14551-14556.
13. Garrett, T. A., C. R. Raetz, T. Richardson, R. Kordestani, J. D. Son, and R. L. Rose. 2009. Identification of phosphatidylserylglutamate: a novel minor lipid in Escherichia coli. *J. Lipid Res.* **50:** 1589-1599.