

Lipidomic Approaches to Study HDL Metabolism in Patients with Central Obesity Diagnosed with Metabolic Syndrome

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Supplementary materials

List of Supplementary Files:

1. Supplementary Figures 1-2

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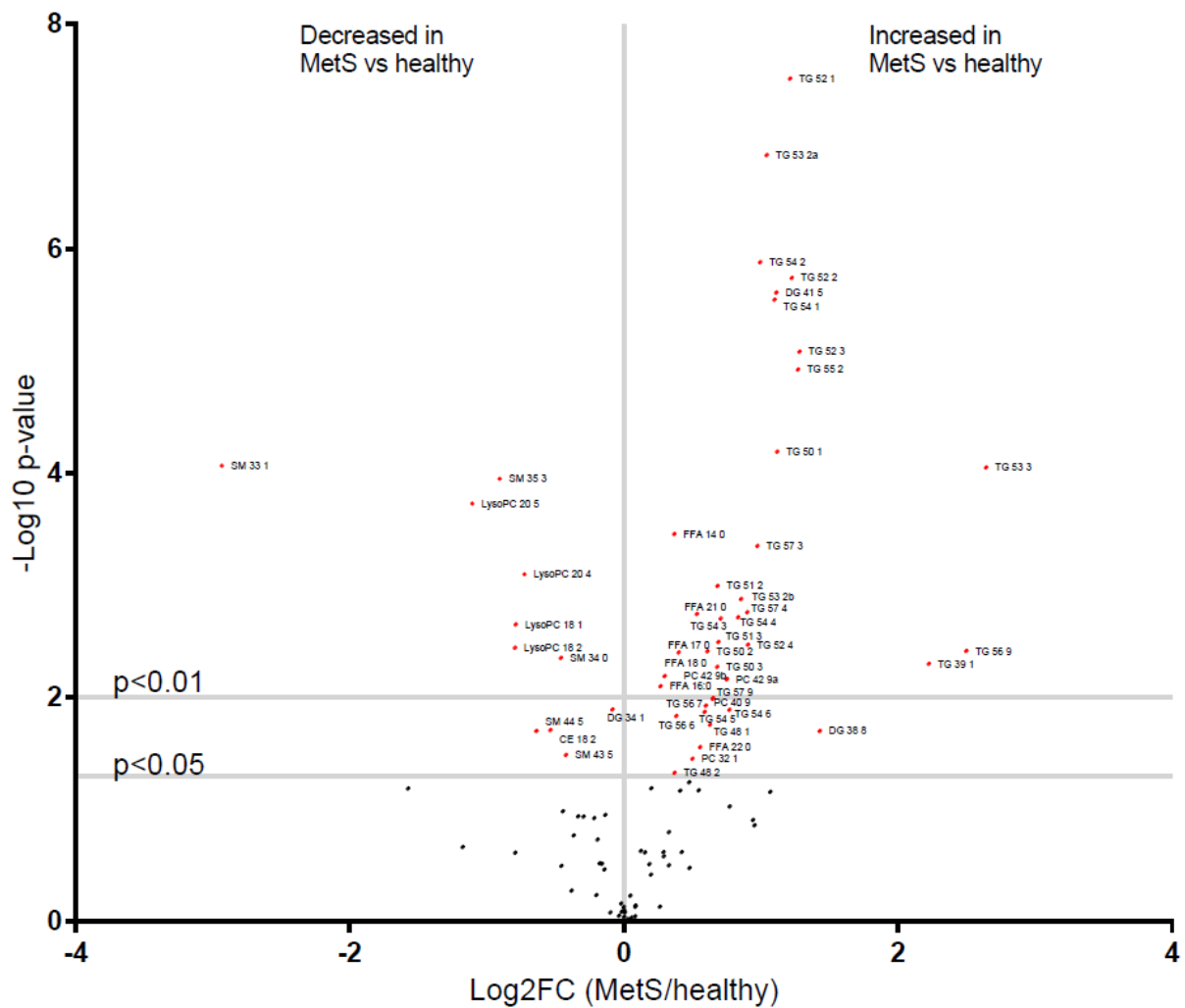


Figure S1. Volcano plot of whole serum lipidome. The whole serum lipidome of MetS participants ($n = 14$) showed lower levels of specific sphingomyelins (SM), lysophosphatidylcholines (LysoPC), cholesteryl esters (CE) and higher triglycerides (TG), diglycerides (DG) and some phosphatidylcholines (PC) when compared to healthy subjects ($n = 11$). Statistical significance was assessed by student two-sided T-Test. Lipid species were analysed by liquid chromatography-mass spectrometry as reported in the method section. Mean value of each lipid in healthy and MetS participants along with P-values and FDR are reported in Supplementary Table 4.

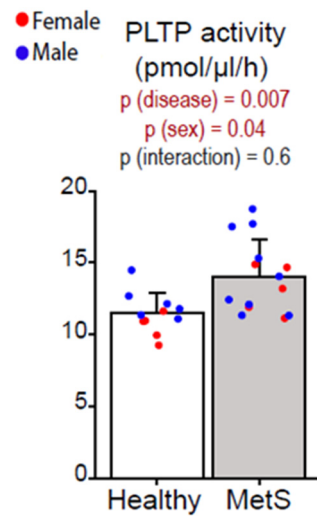


Figure S2. MetS is associated with higher PLTP activity. Phospholipid transfer protein (PLTP) activity was higher in the MetS group when compared with control. PLTP assays were performed on serum from healthy ($n = 11$) and MetS ($n = 14$) participants as detailed in the method section. Data are expressed as mean $\pm$ standard deviation. Statistical significance was assessed by two-way ANOVA using disease stage and sex as covariates; a p -value < 0.05 was considered significant.

Table S1. Clinical characteristics of the study cohort.

	Healthy	MetS	<i>p</i> -value	
			Sex	MetS
N (M/F)	6/5	9/5	-	0.6
Age (years)	29 ± 2	42 ± 10	0.4	0.0004
Body mass index (Kg/m ²)	22.8 ± 1.9	32.7 ± 3.4	0.1	0.00000003
Waist circumference (cm)	86 ± 8	111 ± 7	0.08	0.00000004
SBP (mm/Hg)	116 ± 8	122 ± 12	0.02	0.2
DBP (mmHg)	78 ± 6	80 ± 7	0.3	0.6
Total cholesterol (mmol/L)	5.5 ± 1	5 ± 1.1	0.2	0.4
HDL-C (mmol/L)	1.6 ± 0.4	0.9 ± 0.2	0.001	0.000002
LDL-C (mmol/L)	3.5 ± 0.9	3.4 ± 1.1	0.4	0.8
Triglycerides (mmol/L)	0.8 ± 0.3	1.5 ± 0.3	0.003	0.00006
Glucose (mmol/L)	5 ± 0.4	5.7 ± 0.7	0.7	0.006
Insulin (pmol/L)	53 ± 17	101 ± 61	0.4	0.002
HOMA2-IR	1 ± 0.3	1.9 ± 1.1	0.5	0.01
ApoB-100/ApoA-1 ratio	1.3 ± 0.3	2.1 ± 0.6	0.1	0.002

Data are expressed as mean ± standard deviation. Differences between the two groups were analysed with 2-way ANOVA using disease stage and sex as co-variables; a *p*-value < 0.05 was considered significant. Abbreviations: M = Male, F = Female, BMI = Body Mass Index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TG = triglycerides, HOMA2-IR = Homeostasis Model Assessment 2 of Insulin Resistance; ApoB-100, apolipoprotein B-100; ApoA-1 apolipoprotein A-1.

Table S2. Average value for lipid species in the whole serum of Healthy and MetS participants.

Whole serum lipidome	Average normalised peak intensity - Healthy	Average normalised peak intensity - MetS	<i>p</i> -value	FDR
CE 16 0	0.411	0.402	0.884534653	0.930604166
CE 18 0	0.325	0.373	0.380575892	0.463110422
CE 18 1	39.352	39.596	0.832339241	0.884908035
CE 18 2	51.843	35.758	0.0196652	0.043010482
CE 20 4	24.528	19.485	0.115112903	0.189336767
DG 32 0	0.159321086	0.092046459	0.243699636	0.33261707
DG 34 1	0.108778231	0.102718975	0.012856743	0.031837802
DG 37 0	0.084824089	0.069177777	0.11622653	0.189336767
DG 38 8	0.349389706	0.940988321	0.020014779	0.043010482
DG 41 5	0.082156665	0.177741432	2.46519E-06	4.81E-05
LysoPC 15 0	0.010360738	0.007563991	0.319470554	0.403331574
LysoPC 16 0	0.692126414	0.693633619	0.909608664	0.937363362
LysoPC 16 1	0.014687905	0.015281642	0.918801711	0.937363362
LysoPC 17 0	0.012790905	0.011126363	0.580806068	0.690134268
LysoPC 18 0	0.325172269	0.252231062	0.170994451	0.257767755
LysoPC 18 1	0.269313457	0.156068212	0.002245602	0.00986112
LysoPC 18 2	0.268527176	0.154704396	0.003621153	0.013592192
LysoPC 18 3	0.140838234	0.149435702	0.720441127	0.823681686
LysoPC 20 3	0.063191507	0.046477716	0.104020014	0.178068159
LysoPC 20 4	0.043007377	0.026013381	0.000801808	0.005061411
LysoPC 20 5	0.052824818	0.024530703	0.000186948	0.001452442
PC 30 0	0.080767222	0.085586673	0.898560647	0.935614694
PC 32 0	0.281899667	0.313913895	0.242038273	0.33261707
PC 32 1	0.228358318	0.323204525	0.035343376	0.071393619
PC 32 2	0.062821669	0.083520648	0.068165627	0.122941577
PC 34 1	3.535585472	4.929156768	0.331956372	0.413920908
PC 34 2	6.568506053	9.593782425	0.067592892	0.122941577
PC 34 4	0.025174981	0.026685511	0.737229718	0.823681686
PC 35 2	0.138002281	0.142590461	0.590082186	0.693003497
PC 36 4	3.98809162	4.884301395	0.261416843	0.352041349
PC 36 5	0.308982022	0.388173434	0.314094329	0.401563635
PC 36 6	0.039237277	0.082340383	0.06977506	0.12363651
PC 38 7	0.57729321	0.774130529	0.241262138	0.33261707
PC 38 8	0.059159191	0.07102255	0.740415753	0.823681686
PC 40 0	0.08085624	0.155468724	0.124382921	0.196291797
PC 40 5	0.093837589	0.160207878	0.094168001	0.163982209
PC 40 9	0.271965242	0.409207094	0.013470016	0.032392181
PC 42 8	0.026458309	0.032343396	0.239925001	0.33261707
PC 42 9a	0.064336461	0.108317521	0.006892067	0.019888537
PC 42 9b	0.064336461	0.108317521	0.006892067	0.019888537
SM 26 1	0.038403762	0.07439313	0.138833026	0.215725163
SM 32 1	0.093972905	0.092958474	0.816508278	0.884908035
SM 33 1	0.31081373	0.040715321	8.61905E-05	0.000818946
SM 34 0	0.044982104	0.03273963	0.00446445	0.015030316
SM 34 2	0.128144362	0.114845383	0.304990085	0.397366428
SM 34 4	0.060446233	0.026742823	0.217432725	0.318271091
SM 35 3	0.02886031	0.015415948	0.000113006	0.000951138
SM 35 4	0.054145167	0.018189352	0.065268148	0.122075611
SM 36 2	0.080053791	0.080049914	0.742129044	0.823681686
SM 36 4	0.359171212	0.36641058	0.947735205	0.957212557
SM 39 1	0.046499889	0.042180325	0.343569225	0.423176728
SM 40 1	0.45293963	0.630525155	0.057244268	0.111185981
SM 41 1	0.162961689	0.144313469	0.302269104	0.397366428
SM 41 2	0.076223142	0.080969042	0.976977997	0.976977997
SM 42 2	0.702713625	0.615523888	0.185853935	0.276047757

SM 42 4	0.157507504	0.143491559	0.111666428	0.18797182
SM 43 5	0.034128588	0.025486603	0.032657117	0.06731365
SM 44 4	0.114987985	0.099041189	0.119949413	0.192299852
SM 44 5	0.367182916	0.235831394	0.019932648	0.043010482
TG 39 1	0.278069549	1.300649016	0.005025656	0.016373912
TG 46 1	0.119341106	0.13000335	0.234485553	0.33261707
TG 48 1	0.394532932	0.610449585	0.017553444	0.040293133
TG 48 2	0.286131151	0.369823345	0.047246376	0.093566353
TG 50 1	0.836468903	1.815968634	6.45962E-05	0.000724912
TG 50 2	1.248247296	1.905620078	0.003902709	0.013592192
TG 50 3	0.495514687	0.794676077	0.005346223	0.016874017
TG 51 2	0.129254553	0.207446742	0.001014893	0.006029658
TG 51 3	0.076587605	0.123505963	0.003224022	0.01356776
TG 52 1	0.61358433	1.421168547	3.04712E-08	3.08E-06
TG 52 2	2.600571787	6.08536918	1.82628E-06	4.61E-05
TG 52 3	2.075300893	5.046089209	8.26253E-06	0.000119217
TG 52 4	0.950946332	1.782204882	0.003397666	0.013592192
TG 53 2 a	0.075540129	0.155602421	1.46336E-07	7.39E-06
TG 53 2 b	0.075760345	0.137174887	0.001333268	0.007481114
TG 53 3	0.081434785	0.508221769	8.91922E-05	0.000818946
TG 54 1	0.114106124	0.244500483	2.85585E-06	4.81E-05
TG 54 2	0.346611382	0.69036496	1.31754E-06	4.44E-05
TG 54 3	1.105323274	1.805550841	0.001989238	0.009132412
TG 54 4	0.898778359	1.602380526	0.00193844	0.009132412
TG 54 5	0.398020176	0.678646158	0.012924256	0.031837802
TG 54 6	0.143875205	0.217944487	0.011898269	0.030813465
TG 55 2	0.17100972	0.412848174	1.19937E-05	0.000151421
TG 56 6	0.219177678	0.286022492	0.014695336	0.034516952
TG 56 7	0.135282204	0.212061272	0.010284861	0.027336077
TG 56 9	0.261915077	1.481717308	0.00385904	0.013592192
TG 57 3	0.054153722	0.106533008	0.000447025	0.003009969
TG 57 4	0.048980741	0.091410204	0.001743356	0.009110976
TG 57 9	0.034622606	0.054390902	0.010198682	0.027336077
FFA 14 0	0.302	0.389	0.000351032	0.002532448
FFA 15 0	0.035	0.040	0.064928655	0.122075611
FFA 16 0	7.142106257	8.600472092	0.00797862	0.022384463
FFA 17 0	0.074683697	0.095778543	0.003683136	0.013592192
FFA 17 1	0.035861929	0.035933378	0.795237938	0.873032954
FFA 18 0	6.853645383	8.433201254	0.006469691	0.019801175
FFA 18 1	1.06005879	1.331500654	0.159480253	0.244053115
FFA 18 2	0.270751664	0.30841671	0.306877044	0.397366428
FFA 18 3	0.07722938	0.059335179	0.529180317	0.636276333
FFA 20 4	0.092097111	0.086052748	0.827656182	0.884908035
FFA 21 0	0.012456402	0.018018219	0.001804154	0.009110976
FFA 22 0	0.012527612	0.018430634	0.027877688	0.058659302
FFA 22 6	0.026136606	0.025782511	0.691882355	0.803219745

Statistical significance was calculated using a two-tailed Student-T test on log2-transformed lipids; the adjusted *p*-value was calculated using the false discovery rate (FDR) as described in the methods.

Table S3. Targeted (LC MS/MS) parameters for the measurements of circulating cholesteryl esters (CE).

Metabolite	HMDB ID	Column	Retention Time (RT)	Mode	Precursor	Product	Collision Energy (CE)	Lens
			(min)		<i>m/z</i>	<i>m/z</i>	(V)	(V)
CE15:0 d7		18 CSH	7.6	Positive	640.58	376.387	24.713	155
CE16:0	HMDB0000885	18 CSH	7.7	Positive	647.815	369.333	24.511	140
CE18:1	HMDB0005189	18 CSH	7.6	Positive	673.537	369.333	27.242	152
CE18:2	HMDB0000610	18 CSH	7.5	Positive	671.53	369.333	28.86	151
CE18:3	HMDB0010369	18 CSH	7.2	Positive	669.56	369.333	21	151
CE 20:4	HMDB0006726	18 CSH	7.4	Positive	695.567	369.333	30.124	148

Table S4. Targeted (LC MS/MS) parameters for the measurements of circulating apolipoproteins.

Protein	Peptide sequence	Q1 <i>m/z</i>	Q3 <i>m/z</i>	Collision Energy (CE)
APOA-I	DYVSQFEGSALGK	700.8	808.4	20
APOA-IV	LGEVNTYAGDLQK	675.7	540.2	20
		675.7	575.6	20
APOB-100	TEVIPPLIENR	640.8	838.4	20
APOC-I	TPDVSSALDK	516.8	620.3	20
		516.8	719.4	20
APOC-II	TYLPAVDEK	518.2	658.2	15
		518.2	771.2	15
APOC-III	GWVTDGFSSLK	598.7	854.2	20
		598.7	953.3	20
APOD	NILTSNNIDVK	615.8	890.4	20
		615.8	1003.5	20
APOE	LGPLVEQGR	484.7	399.7	15
		484.7	588.2	20
APOM	EFPEVHLGQWYFIAGAAPT TK	754.4	615.8	30
BSA 1	LVNELTEFAK	582.3	951.29	20
BSA 2	LGEYGFQNALIVR	740.5	1017.4	25

Table S5. Average value for lipid species in the HDL of Healthy and MetS participants.

HDL lipidome	Average normalised peak intensity Healthy	Average normalised peak intensity MetS	<i>p</i> -value	FDR
CE 16 0	0.338540925	0.378028828	0.623829276	0.836281708
CE 18 1	16.21745037	11.25866694	0.052599514	0.173383585
CE 18 2	204.7446748	124.1649671	0.011707837	0.148856784
CE 20 4	80.78008219	61.23188783	0.231031364	0.489566461
LysoPC 16 0	0.820194003	0.794781016	0.855615207	0.961997015
LysoPC 16 1	0.004558758	0.003564052	0.35580107	0.620907749
LysoPC 17 0	0.021753407	0.021389974	0.967406142	0.983175306
LysoPC 18 0	0.570777088	0.580768708	0.775485956	0.932679055
LysoPC 18 1	0.234170864	0.214919709	0.649309269	0.837514854
LysoPC 18 2	0.129858771	0.083476155	0.031566421	0.158588981
LysoPC 18 3	0.200119696	0.189048574	0.720528086	0.890149461
LysoPC 20 3	0.144288832	0.139153262	0.981574291	0.983175306
LysoPC 20 4	0.081315063	0.071639247	0.50132205	0.769270042
LysoPC 20 5	0.0445405	0.029561901	0.050927484	0.173383585
PC 30 0	0.007740762	0.005115792	0.022315515	0.158588981
PC 30 1	0.001444872	0.001196408	0.606519372	0.836281708
PC 31 0	0.004614647	0.00178596	0.000152811	0.013600221
PC 32 0	0.092930064	0.063645056	0.004252601	0.064803468
PC 32 1	0.061851449	0.060558	0.983175306	0.983175306
PC 32 2	0.016996051	0.015416545	0.634268055	0.836281708
PC 33 0	0.00242063	0.001368813	0.022615386	0.158588981
PC 33 1	0.016056487	0.01289907	0.204304112	0.476465335
PC 33 2	0.031933493	0.023471885	0.042550242	0.158588981
PC 34 0	0.010579054	0.007266578	0.003270763	0.064803468
PC 34 1	2.034745873	1.73082767	0.164358477	0.39534877
PC 34 2	3.476053076	2.747097559	0.043013657	0.158588981
PC 34 3	0.082776371	0.076997158	0.665160513	0.838107651
PC 34 4	0.00462267	0.00455171	0.833586244	0.961997015
PC 35 1	0.024583278	0.019087934	0.100082534	0.276368772
PC 35 2	0.056418545	0.042990834	0.065223441	0.200168493
PC 35 3	0.005983456	0.005150018	0.496168175	0.769270042
PC 35 4	0.010493651	0.00865644	0.214856862	0.476465335
PC 36 1	0.187948922	0.145242462	0.089912285	0.266739778
PC 36 2	1.277281463	1.050659014	0.042284199	0.158588981
PC 36 3	0.812382053	0.721558791	0.292291274	0.553487732
PC 36 4	1.049740008	0.983048569	0.575740707	0.813347983
PC 37 3	0.004972606	0.004514469	0.386492592	0.658878302
PC 37 4	0.013861205	0.011413757	0.320410169	0.584942402
PC 37 5	0.003166099	0.003569474	0.828546422	0.961997015
PC 37 6	0.002658056	0.002709181	0.979677137	0.983175306
PC 38 2	0.024695643	0.02185884	0.430808477	0.710036194
PC 38 3	0.169732144	0.201896659	0.211208686	0.476465335
PC 38 4	0.74189748	0.695025313	0.552595888	0.806246459
PC 38 5	0.287121725	0.258353072	0.471846351	0.749898665
PC 38 6	0.434175289	0.454369	0.824929228	0.961997015
PC 39 6	0.001636547	0.002197552	0.63895681	0.836281708
PC 40 4	0.008522435	0.008554048	0.930665677	0.974461709
PC 40 5	0.027763257	0.027303223	0.922826934	0.974461709
PC 40 6	0.116564651	0.13085137	0.538332908	0.80490238
PC 40 7	0.015644849	0.015560681	0.883331027	0.961997015
PC 28 0	0.00109182	0.0006912	0.039453749	0.158588981
SM 32 0	0.000302537	0.000232601	0.219495267	0.476465335
SM 32 1	0.031888249	0.02608093	0.032467514	0.158588981
SM 33 0	0.002429473	0.00262276	0.61401929	0.836281708

SM 33 1	0.014296825	0.010314505	0.004368773	0.064803468
SM 34 0	0.013058918	0.008003651	0.002458824	0.064803468
SM 34 1	0.626756539	0.425283243	0.001295217	0.057637155
SM 34 2	0.045148687	0.038144289	0.105579082	0.276368772
SM 35 1	0.010497086	0.007179481	0.015528282	0.153557454
SM 35 2	0.001136128	0.000937003	0.249748889	0.51692212
SM 36 1	0.082790498	0.065728927	0.099909629	0.276368772
SM 36 2	0.030235324	0.02608769	0.392365731	0.658878302
SM 36 3	0.001721493	0.00152746	0.565597702	0.811906379
SM 38 1	0.03565122	0.026333007	0.019850458	0.158588981
SM 38 2	0.030428857	0.02204447	0.028838667	0.158588981
SM 39 1	0.010290486	0.007721538	0.023973192	0.158588981
SM 40 0	0.000608204	0.000910179	0.34793977	0.619332791
SM 40 1	0.068845588	0.053772464	0.03351592	0.158588981
SM 40 2	0.064655861	0.048295186	0.013966115	0.153557454
SM 41 1	0.02287977	0.018455367	0.117246209	0.29814036
SM 42 1	0.049868766	0.03715174	0.040277282	0.158588981
SM 42 2	0.123749041	0.089865611	0.029189626	0.158588981
SM 46 0	0.001022669	0.00123924	0.103099405	0.276368772
SM 48 0	0.013629228	0.016802189	0.044547466	0.158588981
SM 30 1	0.002553294	0.002131579	0.32204694	0.584942402
TG 12 0	0.017840687	0.021320926	0.292154587	0.553487732
TG 18 0	0.002882784	0.003778043	0.886334328	0.961997015
TG 52 3	0.131747512	0.172092386	0.042095639	0.158588981
TG 52 4	0.050505198	0.063372905	0.12098313	0.299097182
TG 56 6	0.005339102	0.005903429	0.454233978	0.735033164
TG 56 7	0.00542369	0.007335577	0.266326986	0.526735595
TG 58 10	0.00158219	0.001589258	0.867994033	0.961997015
TG 58 12	0.000571951	0.000619179	0.730122592	0.890149461
TG 58 7	0.004268805	0.00472811	0.542630818	0.80490238
TG 59 8	0.001742913	0.002032943	0.261536373	0.526735595
TG 59 9	0.001496814	0.002174335	0.913253861	0.974461709

Statistical significance was calculated using a two-tailed Student-T test on log2-transformed lipids; the adjusted *p*-value was calculated using the false discovery rate (FDR) as described in the methods.