Electronic supplementary material

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Differences between serum polar lipid profile of male and female rheumatoid arthritis patients in response to glucocorticoid treatment

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Method—lipidomics profiling

For the detection of polar lipids, each 20 µL serum was spiked with internal standard (ISTD) mix and lipids were extracted by 440 µL methanol. After centrifugation, the supernatant was transferred to an Eppendorf tube and the solvent was evaporated using a Speedvac. The dried lipids were reconstituted in 145 µL isopropanol, 0.1% formic acid and transferred to autosampler vials. In total 8 µL was injected into ultra-performance liquid chromatography coupled to electrospray ionization-quadrupole time-of-flight (Agilent 6530 San Jose,A, USA) with an ACQUITY UPLC™ HSS T3 column (1.8 µm, 2.1×100mm). Lipids were detected in full scan in the negative mode.

Figures

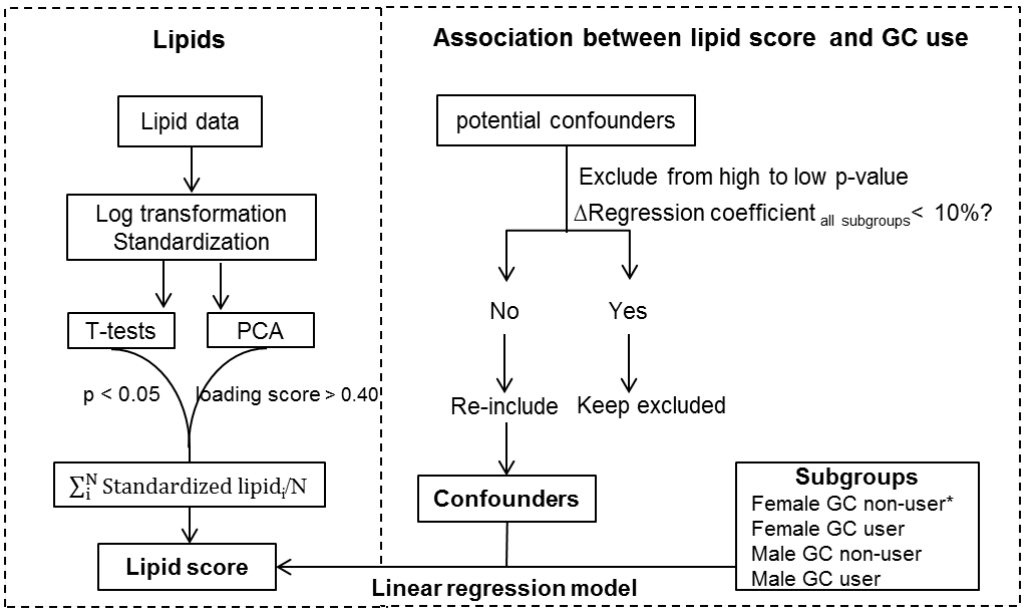

Fig S1. A schematic overview of the statistical analyses. * the subgroup “Female-GC non-user” is set as the reference group in the linear regression analysis.

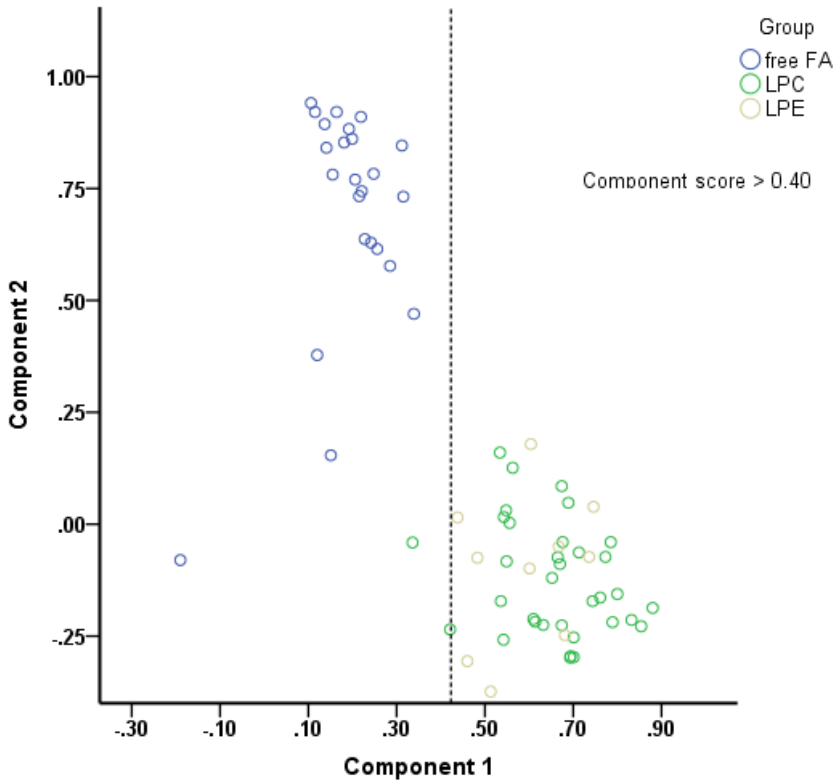

Fig. S2 Graphical representation of loading scores of 68 lipids in component 1 and 2 by PCA analysis. All the lysophospholipids (green and yellow circles) are with loading scores > 0.40 in component 1 (on the right side of the dash line). FA: fatty acid; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines .

Table S1. Baseline characteristics of patients in the cohort (n=281), split for GC non-users and users, females and males.

Item	Females		Males		p-value
	GC non-users (n = 136)	**GC users (n = 77)**	**GC non-users (n = 26)**	**GC users (n = 42)**	
Age, years, mean ± SD [a]	53.2 ± 12.8	53.8 ± 14.1	54.2 ± 12.2	57.0 ± 11.7	0.428
Body mass index, kg/m^2, mean ± SD [a]	27.2 ± 5.8	27.0 ± 5.2	26.7 ± 3.4	25.0 ± 3.2	0.115
Current smoker, n (%) [b]	28 (20.6)	11 (14.3)	12 (46.2)	19 (45.2)	**<0.001**
Positive RF, n (%) [b]	91 (66.9)	38 (49.4)	15 (57.7)	34 (81.0)	**0.004**
Positive ACPA, n (%) [b]	90 (66.2)	49 (63.6)	17 (65.4)	31 (73.8)	0.726
CRP, mg/dL, median (IQR) [c]	6.0 (2.7-15.0)	5.0 (3.0-16.5)	5.5 (2.3-12.0)	10 (2.3-35.3)	0.487
Use of other DMARDs, n (%) [b]					
Methotrexate, n (%) [b]	99 (72.8)	48 (62.3)	22 (84.6)	32 (76.2)	0.115
Leflunomide, n (%) [b]	17 (12.5)	9 (11.7)	2 (7.7)	7 (16.7)	0.737
Hydroxychloroquine, n (%) [b]	21 (15.4)	22 (28.6)	9 (34.6)	10 (23.8)	0.050
Use of non-DMARDs					
NSAIDs, n (%) [b]	94 (69.1)	39 (50.6)	20 (76.9)	22 (52.4)	**0.010**
Anticoncepcion, n (%) [b]	10 (7.4)	5 (6.5)	0 (0.0)	0 (0.0)	0.162
Antidiabetics, n (%) [b]	5 (3.7)	4 (5.2)	1 (3.8)	1 (2.4)	0.892
Antihyperlipedemia, n (%) [b]	18 (13.2)	13 (16.9)	6 (23.1)	1 (2.4)	0.065
Bisphosphonates, n (%) [b]	34 (25.0)	58 (75.3)	3 (11.5)	30 (71.4)	**<0.001**
DAS28, mean ± SD [a]	4.6 ± 1.2	4.6 ± 1.2	3.9 ± 1.1	4.4 ± 1.4	0.071
TJC, median (IQR) [c]	8.0 (2.3-12.0)	7.0 (2.0-15.0)	4.0 (1.0-6.3)	6.0 (0.0-14.0)	0.069
SJC, median (IQR) [c]	2.0 (0.0-4.0)	2.0 (0.0-4.0)	1.0 (0.0-3.0)	1.0 (0.0-4.3)	0.885
VAS-GH, mean ± SD [a]	58.6 ± 24.2	56.4 ± 22.7	49.2 ± 25.1	57.1 ± 23.3	0.324
ESR, mm/h, median (IQR) [c]	19.0 (11.0-37.0)	22.0 (6.5-39.0)	15.5 (6.0-34.3)	19.0 (9.5-47.5)	0.786

P-values for comparisons among four subgroups were calculated by one-way ANOVA [a], Chi-square tests [b], and Kruskal wallis H tests [c]) based on distribution of the clinical parameter. Bold values indicate significant p-values ($p < 0.05$).

ACPA: anti citrullinated protein antibody,RP: c-reactive protein, DAS28: disease activity score based on a 28-joint count, DMARDs: disease-modifying antirheumatic drugs, ESR: erythrocyte sedimentation rate, IQR: interquartile range, NASID: non-steroid anti-inflammatory drug, RF: rheumatoid factor, SD: standard deviation, SJC: swollen joint count, TJC: tender joint count, VAS-GH: visual analogue scale-general health.

Table S2. List of detected metabolites in lipidomics profiling

Lipid Class	Lipid Maps	Metabolite species	Amount (n)
Free fatty acids (FA)	FA01	C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C17:1,C18:0, C18:1, C18:2, C18:3-ω3/ω6, C20:0, C20:1, C20:2, C20:3-ω3/ω6, C20:3-ω9, C20:4-ω6, C20:5-ω3, C22:4, C22:5-ω3,C 22:5-ω6, C22:6, C24:0, C24:1	24
Lysophosphatidylcholine (LPC)	GP0105	*sn1:* C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3-ω3/ω6, C19:0, C20:1, C20:2, C20:3-ω3/ω6, C20:3-ω9, C20:4, C20:5, C22:4, C22:5-ω3, C22:5-ω6, C22:6 *sn2:* C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3-ω3/ω6, C20:3-ω3/ω6,C 20:4, C20:5, C22:6	30
Lysophosphatidylethanolamine (LPE)	GP0205	C16:0, C18:0, C18:1, C18:2, C20:3-ω3/ω6, C20:4, C20:5, C22:5-ω3, C22:5-ω6, C22:6	10
Plasmalogen Lysophosphatidylcholine (LPC-O) [a]	GP0106	C16:0; C18:0; C18:1; C18:2	4

The "*sn*1-" or "*sn*2-" prefix is used to indicate the position of the fatty acid chain esterified to the glycerol backbone of a lysophosphatidylcholine.

[a] The 'LPC-O' is used to identify a plasmalogen lysophosphatidylcholine, where the fatty acid chain is attached via a vinyl ether linkage to the glycerol backbone. The 'ω3', 'ω6', 'ω9' are used to indicate double bond (C=C) at the third, sixed or ninth carbon atom from the end of the fatty acid chain.

Table S3. Differences in 68 serum lipid levels between GC users and non-users in males and females, respectively. The mean differences between GC non-users and users are shown. *P*-values were calculated by means of an independent sample t-test.

Metabolite name	**Males (GC user vs. GC non-user)**		**Females (GC user vs. GC non-user)**	
	$Mean_{non-user}$-$Mean_{user}$	**p value**	**$Mean_{non-user}$-$Mean_{user}$**	**p value**
sn1-LPC (14:0)	-0.106	0.602	-0.444	0.001
sn1-LPC (15:0)	-0.116	0.633	-0.346	0.010
sn1-LPC (16:0)	0.027	0.796	-0.301	<0.001
sn1-LPC (16:1)	-0.181	0.422	-0.507	<0.001
sn1-LPC (18:0)	0.289	0.122	-0.148	0.304
sn1-LPC (18:1)	-0.091	0.512	-0.368	<0.001
sn1-LPC (18:2)	-0.283	0.027	-0.332	<0.001
sn1-LPC (18:3-ω3/ω6)	-0.343	0.077	-0.393	0.004
sn1-LPC (19:0)	0.252	0.153	0.091	0.494
sn1-LPC (20:1)	0.120	0.568	-0.207	0.099
sn1-LPC (20:2)	-0.153	0.408	-0.395	0.004
sn1-LPC (20:3-ω3/ω6)	0.152	0.342	-0.226	0.054
sn1-LPC (20:3-ω9)	0.284	0.276	-0.198	0.144
sn1-LPC (20:4)	0.183	0.244	-0.2	0.032
sn1-LPC (20:5)	-0.417	0.062	-0.237	0.074
sn1-LPC (22:4)	0.228	0.359	-0.283	0.047
sn1-LPC (22:5-ω3)	-0.161	0.369	-0.373	0.007
sn1-LPC (22:5-ω6)	0.357	0.128	-0.099	0.455
sn1-LPC (22:6)	0.107	0.610	-0.021	0.882
sn2-LPC (14:0)	-0.157	0.405	-0.427	0.001
sn2-LPC (16:0)	0.178	0.341	-0.501	<0.001
sn2-LPC (16:1)	-0.237	0.308	-0.487	<0.001
sn2-LPC (18:0)	0.211	0.098	-0.115	0.231
sn2-LPC (18:1)	-0.114	0.559	-0.443	<0.001
sn2-LPC (18:2)	-0.395	0.052	-0.533	<0.001
sn2-LPC (18:3-ω3/ω6)	-0.362	0.161	-0.411	0.001
sn2-LPC (20:3-ω3/ω6)	0.226	0.281	-0.263	0.062
sn2-LPC (20:4)	0.215	0.384	-0.331	0.021
sn2-LPC (20:5)	-0.421	0.075	-0.278	0.046
sn2-LPC (22:6)	0.092	0.666	-0.07	0.616
LPC (O-16:0)	0.157	0.466	-0.343	0.020
LPC (O-18:0)	-0.019	0.928	-0.419	0.003
LPC (O-18:1)	0.190	0.410	-0.152	0.299
LPC (O-18:2)	0.080	0.707	-0.398	0.004
LPE (16:0)	-0.279	0.143	-0.505	0.001
LPE (18:0)	-0.102	0.588	-0.587	<0.001
LPE (18:1)	0.031	0.883	-0.449	0.001
LPE (18:2)	-0.349	0.093	-0.497	<0.001
LPE (20:3-ω3/ω6)	-0.051	0.789	-0.469	0.001
LPE (20:4)	-0.062	0.785	-0.685	<0.001
LPE (20:5)	-0.463	0.029	-0.352	0.012
LPE (22:5-ω3)	-0.328	0.089	-0.694	<0.001

LPE (22:5-ω6)	0.294	0.215	-0.356	0.009
LPE (22:6)	-0.242	0.218	-0.512	0.001
FA (14:0)	0.089	0.654	-0.014	0.924
FA (14:1)	0.038	0.865	0.159	0.275
FA (15:0)	0.129	0.517	-0.027	0.855
FA (16:0)	-0.083	0.680	-0.006	0.966
FA (16:1)	0.113	0.600	0.042	0.770
FA (17:0)	-0.097	0.652	-0.057	0.692
FA (17:1)	0.041	0.852	0.023	0.872
FA (18:0)	-0.072	0.725	0.109	0.456
FA (18:1)	0.134	0.551	0.075	0.609
FA (18:2)	0.089	0.689	0.035	0.805
FA (18:3-ω3/ω6)	0.190	0.364	0.009	0.950
FA (20:0)	0.039	0.878	-0.071	0.620
FA (20:1)	0.054	0.803	-0.013	0.925
FA (20:2)	0.011	0.969	-0.042	0.761
FA (20:3-ω3/ω6)	0.377	0.109	-0.107	0.445
FA (20:3-ω9)	0.500	0.021	-0.074	0.595
FA (20:4-ω6)	0.250	0.212	-0.013	0.905
FA (20:5-ω3)	-0.105	0.628	-0.098	0.463
FA (22:4)	0.237	0.234	-0.077	0.543
FA (22:5-ω3)	-0.032	0.866	-0.143	0.271
FA (22:5-ω6)	0.247	0.261	0.017	0.901
FA (22:6)	0.371	0.107	0.072	0.585
FA (24:0)	0.071	0.783	0.028	0.838
FA (24:1)	-0.013	0.951	0.016	0.901

Table S4. Loading scores of 68 lipids in principle components analysis. Component loading scores of all lipids were presented for the first five components (with eigenvalue >1). Since the LPCs and LPEs showed high component loadings in one component (the first) and only with positive values, they all highly correlate in the same direction. Considering all LPCs and LPEs significantly different between GC users and non-users, all loading scores were ≥0.438.

Metabolite name	Component				
	1	2	3	4	5
sn1*-LPC (14:0)	0.701	-0.253	0.057	0.147	-0.357
sn1*-LPC (15:0)	0.610	-0.212	-0.159	0.053	-0.066
sn1*-LPC (16:0)	0.880	-0.187	-0.077	-0.080	-0.009
sn1*-LPC (16:1)	0.773	-0.073	0.152	0.044	-0.319
sn1*-LPC (18:0)	0.744	-0.172	-0.249	-0.082	0.295
sn1*-LPC (18:1)	0.854	-0.228	0.023	0.131	0.135
sn1*-LPC (18:2)	0.701	-0.297	-0.036	0.181	0.429
sn1*-LPC (18:3-ω3/ω6)	0.694	-0.295	0.047	0.344	-0.100
***sn1*-LPC (19:0)**	0.421	-0.235	-0.315	-0.029	0.291
***sn1*-LPC (20:1)**	0.336	-0.041	-0.252	-0.132	0.203
sn1*-LPC (20:2)	0.800	-0.156	0.126	-0.108	0.108
***sn1*-LPC (20:3-ω3/ω6)**	0.665	-0.074	0.404	-0.312	-0.133
***sn1*-LPC (20:3-ω9)**	0.652	-0.120	0.345	-0.165	-0.213
sn1*-LPC (20:4)	0.674	0.085	0.154	-0.492	0.032
***sn1*-LPC (20:5)**	0.543	0.016	-0.668	0.069	-0.296
sn1*-LPC (22:4)	0.676	-0.040	0.539	-0.326	0.026
sn1*-LPC (22:5-ω3)	0.785	-0.040	-0.219	-0.022	0.014
***sn1*-LPC (22:5-ω6)**	0.556	0.003	0.569	-0.405	-0.068
***sn1*-LPC (22:6)**	0.534	0.160	-0.574	-0.254	-0.084
sn2*-LPC (14:0)	0.674	-0.226	0.057	0.173	-0.343
sn2*-LPC (16:0)	0.832	-0.214	-0.050	-0.057	-0.051
sn2*-LPC (16:1)	0.713	-0.063	0.183	0.026	-0.314
***sn2*-LPC (18:0)**	0.761	-0.164	-0.235	-0.079	0.283
sn2*-LPC (18:1)	0.789	-0.219	0.037	0.112	0.121
sn2*-LPC (18:2)	0.693	-0.298	-0.018	0.160	0.439
***sn2*-LPC (18:3-ω3/ω6) ***	0.614	-0.218	0.049	0.322	-0.104
***sn2*-LPC (20:3-ω3/ω6)**	0.670	-0.089	0.385	-0.302	-0.079
sn2*-LPC (20:4)	0.689	0.048	0.175	-0.463	0.024
sn2*-LPC (20:5)	0.548	0.031	-0.643	0.089	-0.315
***sn2*-LPC (22:6)**	0.563	0.126	-0.551	-0.251	-0.070
LPC (O-16:0)*	0.632	-0.225	-0.219	-0.301	0.408
LPC (O-18:0)*	0.542	-0.258	-0.252	-0.242	0.411
LPC (O-18:1)*	0.536	-0.172	-0.241	-0.290	0.544
LPC (O-18:2)*	0.549	-0.083	-0.283	-0.209	0.406
LPE (16:0)*	0.681	-0.248	0.066	0.176	-0.278
LPE (18:0)*	0.746	0.039	0.082	0.184	-0.072
LPE (18:1)*	0.460	-0.306	0.210	0.558	0.173
LPE (18:2)*	0.513	-0.374	0.219	0.588	0.217
LPE (20:3-ω3/ω6)*	0.601	-0.099	0.512	0.237	-0.167

LPE (20:4)*	0.667	-0.050	0.375	0.126	0.044
LPE (20:5)*	0.483	-0.075	-0.496	0.458	-0.328
LPE (22:5-ω3)*	0.736	-0.073	0.161	0.340	-0.156
LPE (22:5-ω6)*	0.438	0.015	0.672	-0.080	-0.177
LPE (22:6)*	0.604	0.179	-0.323	0.128	-0.235
FA (14:0)	0.192	0.883	0.066	0.221	-0.006
FA (14:1)	0.141	0.841	0.023	0.089	-0.061
FA (15:0)	0.215	0.733	0.102	0.210	0.065
FA (16:0)	0.206	0.770	0.190	0.294	0.183
FA (16:1)	0.164	0.921	0.067	0.020	0.000
FA (17:0)	0.221	0.744	0.069	0.239	0.151
FA (17:1)	0.219	0.910	0.031	0.068	0.043
FA (18:0)	0.120	0.378	0.218	0.392	0.259
FA (18:1)	0.106	0.941	0.057	0.073	0.130
FA (18:2)	0.137	0.894	0.012	0.087	0.206
FA (18:3-ω3/ω6)	0.181	0.853	-0.044	0.183	0.119
FA (20:0)	0.151	0.154	0.105	0.336	0.209
FA (20:1)	0.115	0.921	-0.029	0.071	0.160
FA (20:2)	0.155	0.781	0.008	-0.129	0.112
FA (20:3-ω3/ω6)	0.315	0.732	0.216	-0.184	-0.111
FA (20:3-ω9)*	0.285	0.577	0.152	-0.208	-0.124
FA (20:4-ω6)	0.256	0.615	-0.019	-0.349	0.074
FA (20:5-ω3)	0.339	0.470	-0.686	0.085	-0.244
FA (22:4)	0.199	0.861	0.266	-0.164	-0.002
FA (22:5-ω3)	0.312	0.846	-0.275	0.076	-0.065
FA (22:5-ω6)	0.248	0.783	0.271	-0.237	-0.142
FA (22:6)	0.242	0.629	-0.494	-0.076	-0.163
FA (24:0)	-0.190	-0.080	0.319	0.392	0.553
FA (24:1)	0.228	0.637	-0.163	0.125	0.118

* Metabolites were also significantly different in independent t-tests between GC users and non-users, in either females or males (Figure 1, Table S3).

Table S5. The final regression model investigating the association between gender and GC use on the lysophospholipid score.

Variables	Coefficients [a]	95%-confidence interval	p value
Male patients no GC use (reference group)	0.149	(-0.491 to 0.789)	0.649
Male patients GC use	0.122	(-0.160 to 0.405)	0.397
Female patients no GC use	-0.310	(-0.604 to -0.015)	0.041
Female patients GC use	0.089	(-0.212 to 0.390)	0.565
Age	0.001	(-0.008 to 0.007)	0.937
Menopausal status	0.286	(0.053 to 0.519)	0.017
BMI (kg/m^2)	0.006	(-0.009 to 0.020)	0.447
RF positivity	-0.247	(-0.432 to -0.063)	0.009
ACPA positivity	0.182	(-0.008 to 0.372)	0.061
CRP (log transformed, mg/dL)	-0.122	(-0.192 to -0.052)	0.001
ESR (log transformed, mm/h)	0.058	(-0.031 to 0.147)	0.202
DAS28	-0.014	(-0.078 to 0.050)	0.663
Concomitant methotrexate	-0.023	(-0.181 to 0.134)	0.773
Concomitant hydroxychloroquin	-0.180	(-0.347 to -0.013)	0.035
Antidiabetics	-0.132	(-0.493 to 0.229)	0.474
NSAIDs	-0.068	(-0.215 to 0.078)	0.362

[a] Coefficients indicate the changes in mean lysophospholipid score after correcting for confounders—BMI, menopausal status, RF positive, log-transformed CRP, log transformed ESR, DAS28, and concomitant drugs (methotrexate, hydroxychloroquine, anti-diabetic drug and NSAIDs). BMI: body mass index; CRP: C-reactive protein; GC: glucocorticoid; RF: rheumatoid factor; ACPA: anti citrullinated protein antibody; ESR: erythrocyte sedimentation rate; DAS28: disease activity score based on a 28-joint count; NSAIDs: non-steroid anti-inflammatory drugs