

Individuals with Metabolic Syndrome show altered fecal lipidomic profiles with no signs of intestinal inflammation or increased intestinal permeability

Coleman *et al.*,

Contents: Figure legends S1-3, Figure S1-3, Tables S1-4

Figure S1. Body Composition and Insulin resistances calculation. Graph showing (A) weight (kg); (B) body mass index (BMI); (C) Waist-to-Hip Ratio; (D) Height (cm); (E) percent of body fat; (F) Fat mass (kg); (G) Lean mass (kg); (H) HOMA-IR; and (I) QUICKI. Graphs indicate median (\pm minimum and maximum). Graphs indicate median (\pm minimum and maximum). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns, not significant. Two-tailed unpaired Student's t tests (Figure S1 A,C,D,G,I) or two-tailed Mann–Whitney U (Figure S1 B,E,F,H).

Figure S2. Fecal lipidomics. (A) Plot showing the top 30 LMSD identified fecal lipids found to be the most significantly different (mean; p -value range: 0.008 – 0.003). SP, sphingolipids. (B) Three component Principal components analysis (PCA) model of hybrid metabolites. Color represents sample group, please see figure legend. (C) Heatmap showing unsupervised clustering analysis of samples (HC vs MetS) using the significant metabolites ($p < 0.05$) from the comparison, samples were clustered with the Complete method and Euclidian distance function.

Figure S3. Pearson's correlation coefficients between Metabolic Syndrome risk factors and fecal metabolites. Heatmap showing Pearson's r between triglyceride, HDL cholesterol, TNF α , fasting insulin, and fecal metabolites that included the

top 10 lipids identified in figure 3B, carnosine, and orotic acid. Pearson's r , 0.5 – 1 and (-0.5) – (-1) were found to be significant, $p < 0.05$ (Table S4).

Figure S1

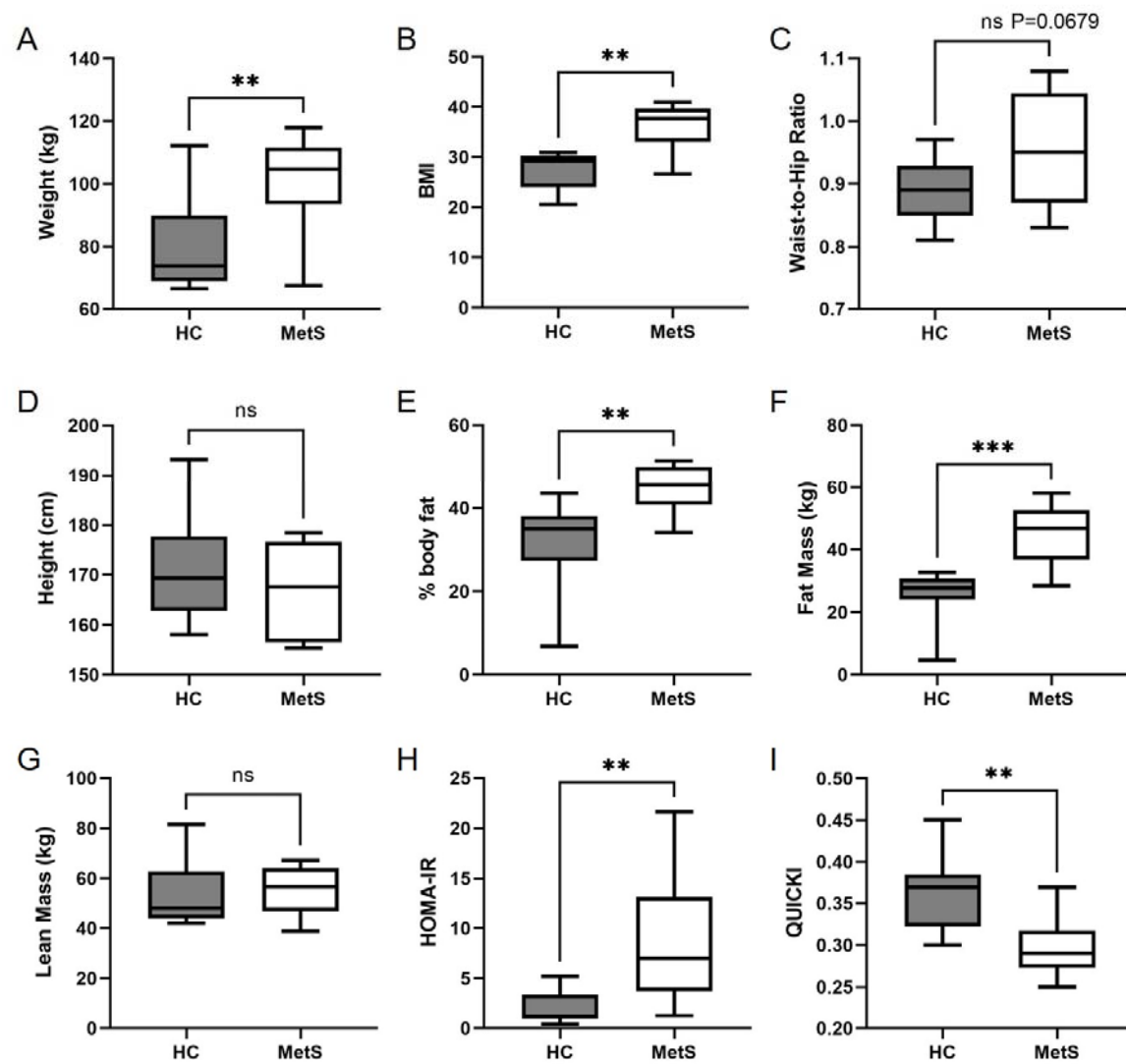
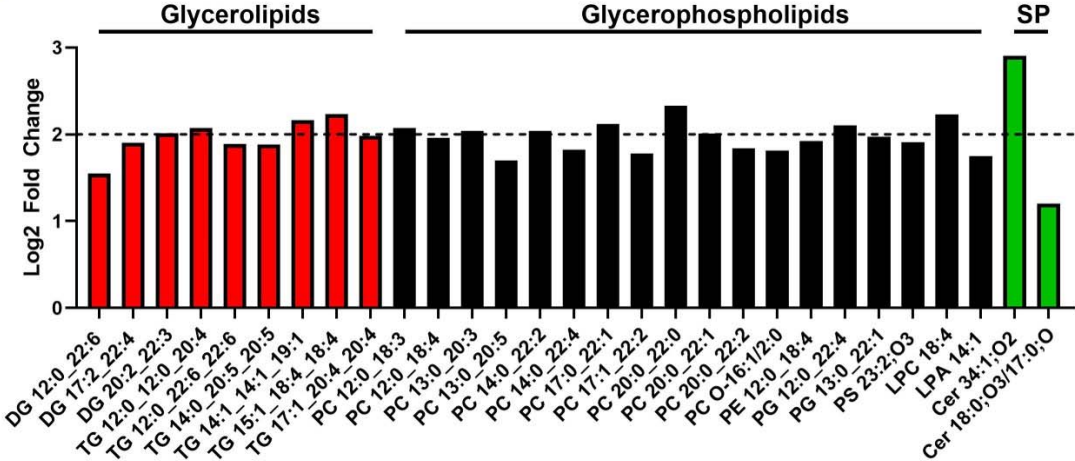
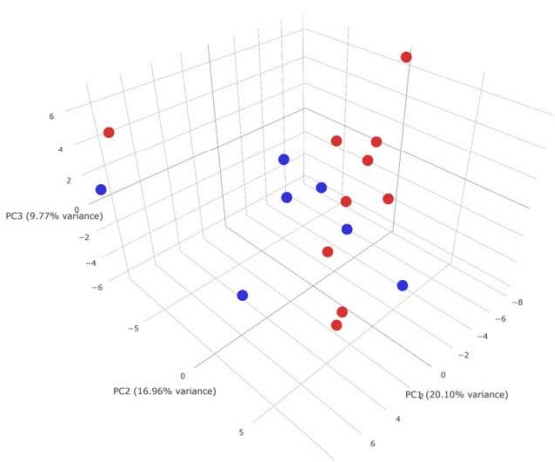


Figure S2

A



B



C

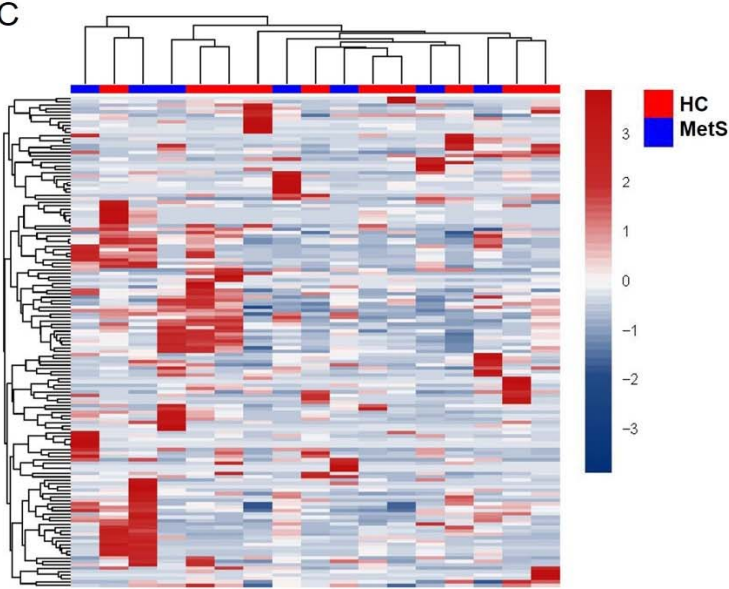


Figure S3

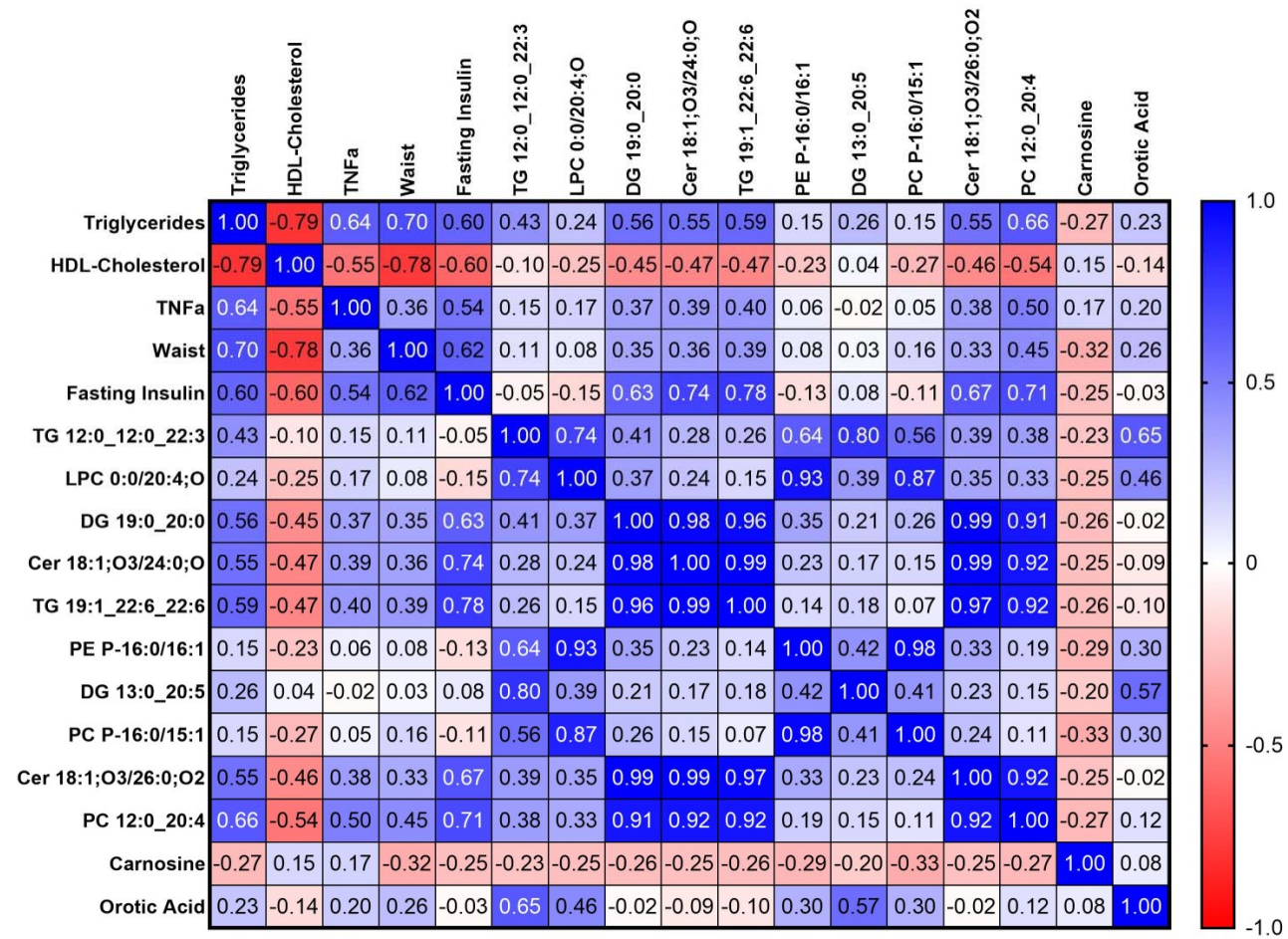


Table S1. Lipid Panel

<i>Lipid Panel</i>	Controls	Metabolic Syndrome	<i>p-value</i>
<i>Total Cholesterol (mg/dL)</i>	188.50 (51.20)	161.50 (70.70)	0.259
<i>HDL Cholesterol (mg/dL)</i>	60.50 (14.0)	42.50 (15.25)	**0.008
<i>LDL Cholesterol (mg/dL)</i>	111.00 (50.25)	78.50 (68.50)	0.217
<i>Triglycerides (mg/dL)</i>	100.50 (73.25)	183.50 (66.5)	**0.001

Table shows median and interquartile range. Student's t test

Table S2. Comprehensive Metabolic Panel

Comprehensive Metabolic Panel	Controls	Metabolic Syndrome	<i>p</i>-value
<i>Sodium</i> (mmol/L)	139.50 (3.70)	140.50 (2.50)	0.795
<i>Potassium</i> (mmol/L)	4.20 (0.18)	4.35 (0.48)	0.592
<i>Chloride</i> (mmol/L)	108.50 (5.00)	109.00 (6.50)	0.961
<i>CO₂</i> (mmol/L)	23.00 (3.50)	24.50 (5.75)	0.455
<i>Anion Gap</i>	8.00 (3.00)	6.00 (2.75)	0.865
<i>Calcium</i> (mg/dL)	9.30 (0.30)	9.65 (0.83)	0.493
<i>BUN</i> (mg/dL)	13.50 (3.50)	12.00 (8.75)	0.683
<i>Creatinine</i> (mg/dL)	0.785 (0.30)	0.870 (0.32)	0.243
<i>Total Protein</i> (gm/dL)	7.10 (0.78)	7.55 (0.58)	0.441
<i>Albumin</i> (gm/dL)	3.95 (0.33)	3.90 (0.78)	0.915
<i>Globulin</i> (gm/dL)	3.20 (0.75)	3.50 (0.60)	0.380
<i>Bilirubin</i> (mg/dL)	0.65 (0.75)	0.60 (0.475)	0.306
<i>Alk Phos</i> (U/L)	67.00 (30.00)	92.50 (37.75)	0.079
<i>AST</i> (U/L)	19.50 (14.00)	21.50 (27.75)	0.770
<i>ALT</i> (U/L)	34.00 (22.25)	33.50 (58.00)	0.288

Table shows median and interquartile range. Student's t test

Table S3. Putative LMSD ID of the top 30 lipids with the lowest *p*-value.

Feature ID	Observed <i>m/z</i>	Log2 Fold Change	<i>p</i> -value	Putative ID* (Category)	Main Class
5728	785.4944	2.166	0.003	Glycerolipids	Triacylglycerols
5013	698.4442	2.075	0.003	Glycerophospholipids	Diacylglycerophosphocholines
2341	379.249	1.746	0.003	Glycerophospholipids	Monoacylglycerophosphates
7313	1370.813	2.903	0.003	Sphingolipids	Gangliosides
6227	870.5284	2.009	0.004	Glycerophospholipids	Diacylglycerophosphocholines
6242	872.5436	2.329	0.004	Glycerophospholipids	Diacylglycerophosphocholines
5992	828.5175	2.121	0.004	Glycerophospholipids	Diacylglycerophosphocholines
3763	583.4164	1.548	0.004	Glycerolipids	Diacylglycerols
5406	741.4679	2.077	0.004	Glycerolipids	Triacylglycerols
5393	740.4648	2.040	0.004	Glycerophospholipids	Diacylglycerophosphocholines
3496	543.3401	1.759	0.005	Fatty Acyls	N-acyl amines
5998	829.5205	2.336	0.005	Glycerolipids	Triacylglycerols
5717	784.4908	2.042	0.005	Glycerophospholipids	Diacylglycerophosphocholines
6217	869.516	1.884	0.005	Glycerolipids	Triacylglycerols
4984	696.439	1.957	0.005	Glycerophospholipids	Diacylglycerophosphocholines
5363	736.4338	1.696	0.006	Glycerophospholipids	Diacylglycerophosphocholines
3319	514.4841	2.231	0.006	Glycerophospholipids	Monoacylglycerophosphocholines
5965	824.4862	1.776	0.006	Glycerophospholipids	Diacylglycerophosphocholines
5676	780.4599	1.824	0.006	Glycerophospholipids	Diacylglycerophosphocholines
3773	584.4232	1.202	0.006	Sphingolipid	N-acyl-4-hydroxysphinganes
4482	654.4183	1.921	0.006	Glycerophospholipids	Diacylglycerophosphoethanolamines
4454	652.4126	1.910	0.006	Glycerophospholipids	Oxidized glycerophosphoserines

4998	697.4416	2.015	0.006	Glycerolipids	Diacylglycerols
5403	741.3887	2.106	0.006	Glycerophospholipids	Diacylglycerophosphoglycerols
6509	913.5423	1.983	0.006	Glycerolipids	Triacylglycerols
4468	653.4156	1.901	0.006	Glycerolipids	Diacylglycerols
6368	893.5571	1.889	0.007	Glycerolipids	Triacylglycerols
6211	868.5124	1.835	0.007	Glycerophospholipids	Diacylglycerophosphocholines
5549	761.4656	1.974	0.007	Glycerophospholipids	Diacylglycerophosphoglycerols
3344	520.4426	1.813	0.007	Glycerophospholipids	1-alkyl,2-acylglycerophosphocholines

* Putative ID derived from LIPID MAPS® Structure Database (LMSD) utilizing observed *m/z*.

Table S4. Pearson's coefficient correlation p -value corresponding to figure S3.

	TG	HDL	TNFa	Waist	Fasting Insulin	TG 12:0_12:0 22:3	LPC 0:0/20:4;O	DG 19:0_20:0	Cer 18:1;O3/24:0;O	TG 19:1_22:6 22:6	PE P-16:0/16:1	DG 13:0_20:5	PC P-16:0/15:1	Cer 18:1;O3/26:0;O2	PC 12:0_20:4	Carnosine	Orotic Acid
TG		0.001	0.005	0.001	0.011	0.083	0.350	0.020	0.020	0.012	0.560	0.304	0.577	0.021	0.004	0.293	0.383
HDL	0.001		0.022	0.001	0.010	0.702	0.335	0.071	0.054	0.054	0.367	0.892	0.285	0.064	0.026	0.567	0.598
TNFa	0.005	0.022		0.151	0.024	0.574	0.504	0.146	0.125	0.110	0.806	0.935	0.849	0.135	0.042	0.513	0.437
Waist	0.001	0.001	0.151		0.007	0.663	0.757	0.170	0.154	0.116	0.757	0.907	0.531	0.192	0.072	0.206	0.318
Fasting Insulin	0.011	0.010	0.024	0.007		0.855	0.552	0.006	0.001	0.001	0.615	0.754	0.670	0.003	0.001	0.324	0.924
TG 12:0_12:0 22:3	0.083	0.702	0.574	0.663	0.855		0.001	0.106	0.272	0.314	0.005	0.001	0.019	0.126	0.132	0.372	0.004
LPC 0:0/20:4;O	0.350	0.335	0.504	0.757	0.552	0.001		0.141	0.357	0.557	0.001	0.123	0.001	0.165	0.195	0.327	0.065
DG 19:0_20:0	0.020	0.071	0.146	0.170	0.006	0.106	0.141		0.001	0.001	0.164	0.428	0.307	0.001	0.001	0.323	0.927
Cer 18:1;O3/24:0;O	0.020	0.054	0.125	0.154	0.001	0.272	0.357	0.001		0.001	0.374	0.506	0.559	0.001	0.001	0.334	0.740
TG 19:1_22:6 22:6	0.012	0.054	0.110	0.116	0.001	0.314	0.557	0.001	0.001		0.583	0.497	0.780	0.001	0.001	0.316	0.699
PE P-16:0/16:1	0.560	0.367	0.806	0.757	0.615	0.001	0.001	0.164	0.374	0.583		0.091	0.001	0.190	0.455	0.264	0.236
DG 13:0_20:5	0.304	0.892	0.935	0.907	0.754	0.001	0.123	0.428	0.506	0.497	0.091		0.103	0.375	0.564	0.437	0.016
PC P-16:0/15:1	0.577	0.285	0.849	0.531	0.670	0.019	0.001	0.307	0.559	0.780	0.001	0.103		0.344	0.677	0.201	0.234
TG 18:3_18:3 20:0	0.021	0.064	0.135	0.192	0.001	0.126	0.165	0.001	0.001	0.001	0.190	0.375	0.344		0.001	0.341	0.930
DG 21:0_22:6	0.004	0.026	0.042	0.072	0.001	0.132	0.195	0.001	0.001	0.001	0.455	0.564	0.677	0.001		0.285	0.632
Carnosine	0.293	0.567	0.513	0.206	0.324	0.372	0.327	0.323	0.334	0.316	0.264	0.437	0.201	0.341	0.285		0.774
Orotic Acid	0.383	0.598	0.437	0.318	0.924	0.004	0.065	0.927	0.740	0.699	0.236	0.016	0.234	0.930	0.632	0.774	