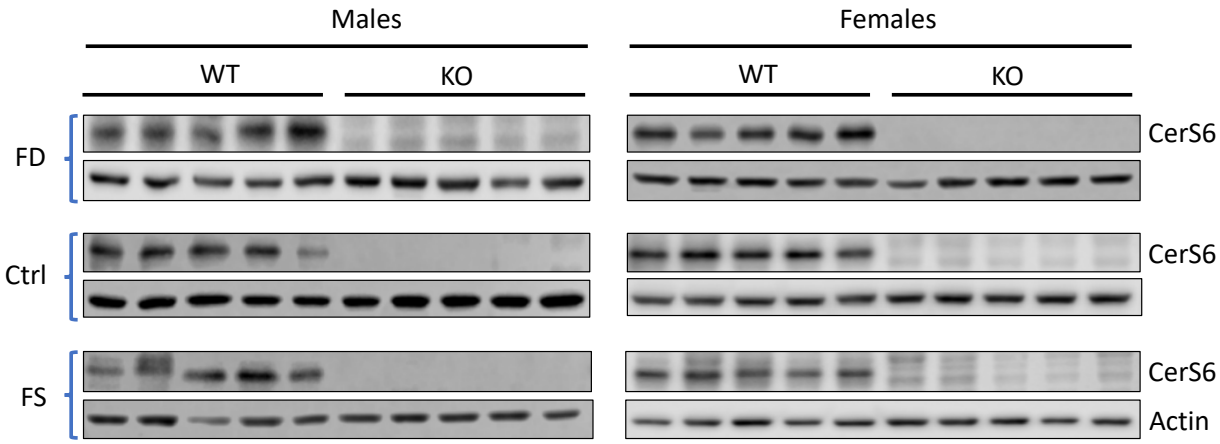
Supplementary Information

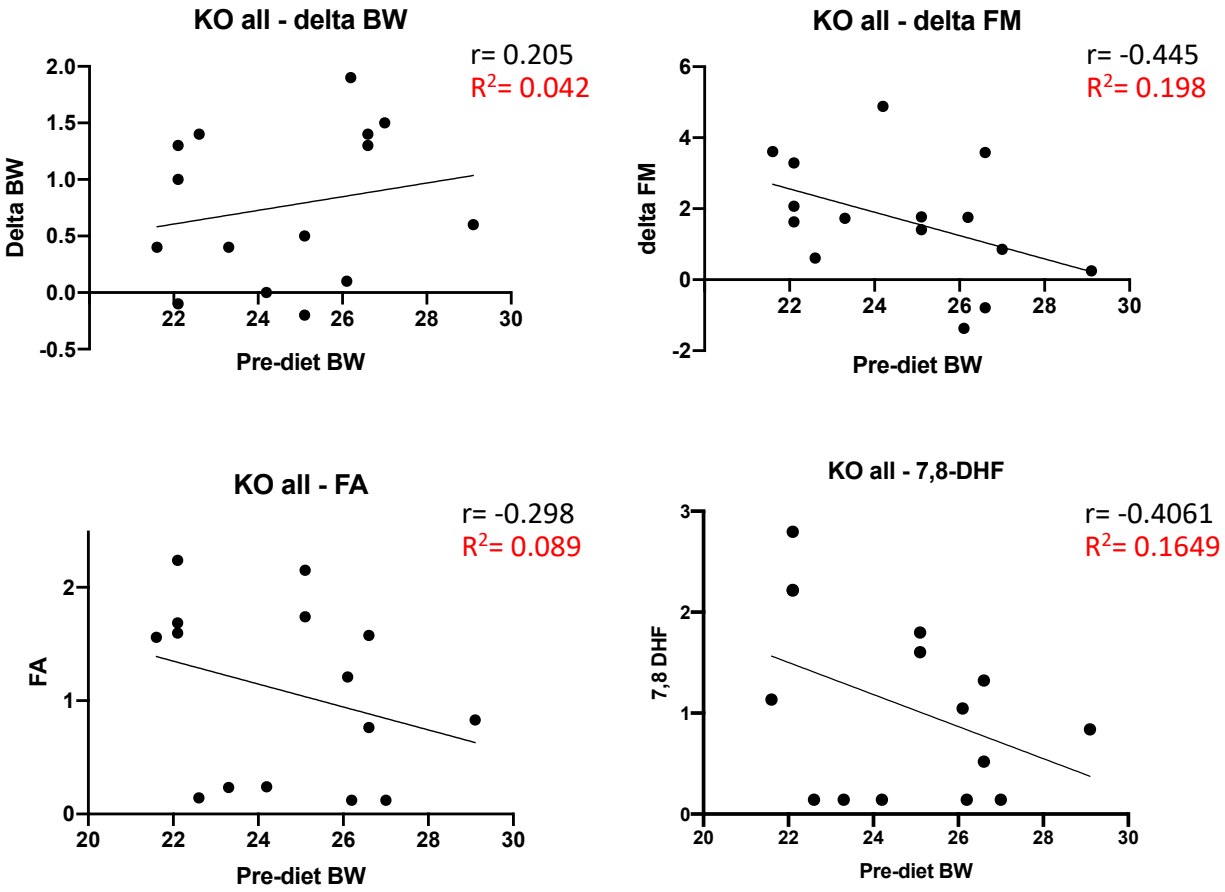
Supplementary Figures 1 – 12

Supplementary Tables 1, 3-14

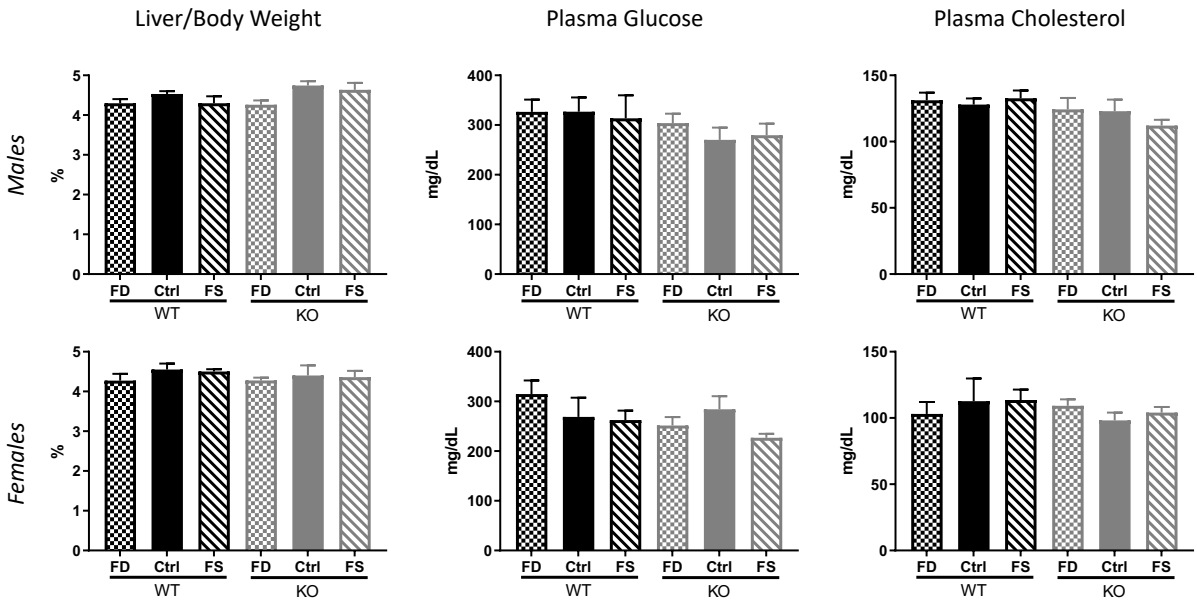
Supplementary File: Supplementary Table 2



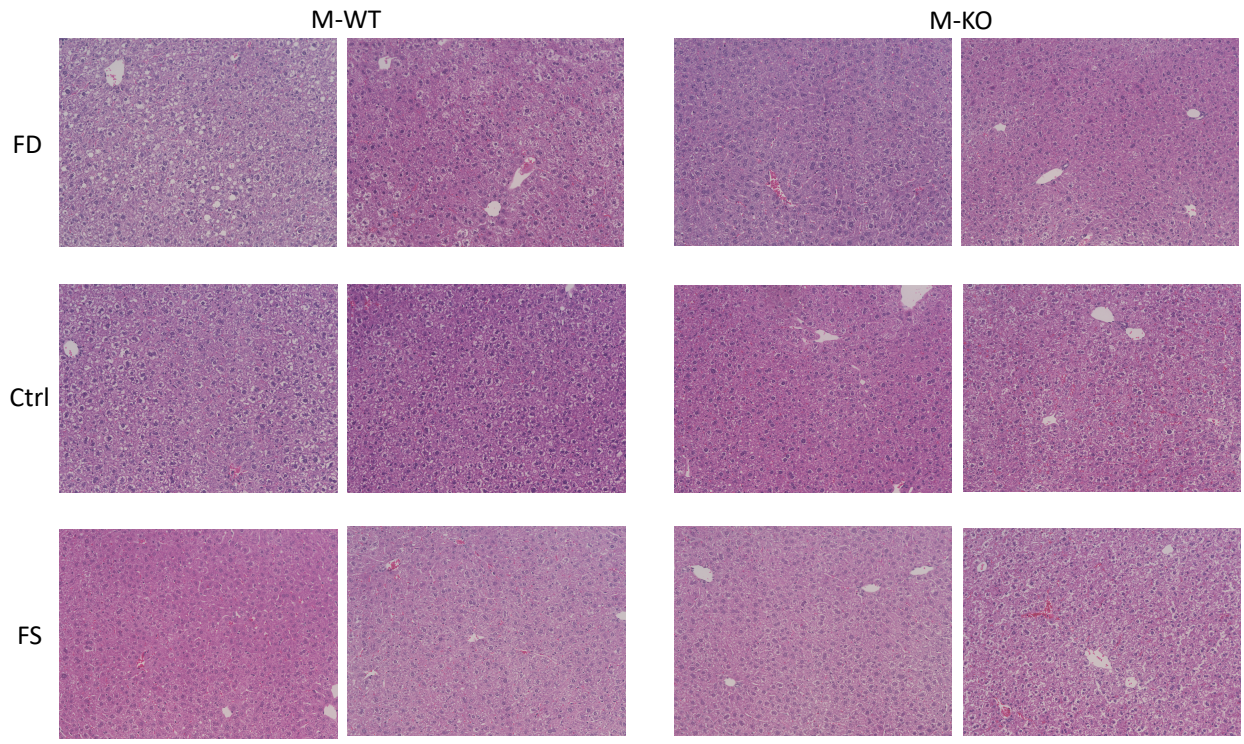
Supplementary Figure 1. Western blot analysis confirms lack of CerS6 expression in animals randomized to CerS6 KO groups based on -/- genotype determined by tail lysate PCR.



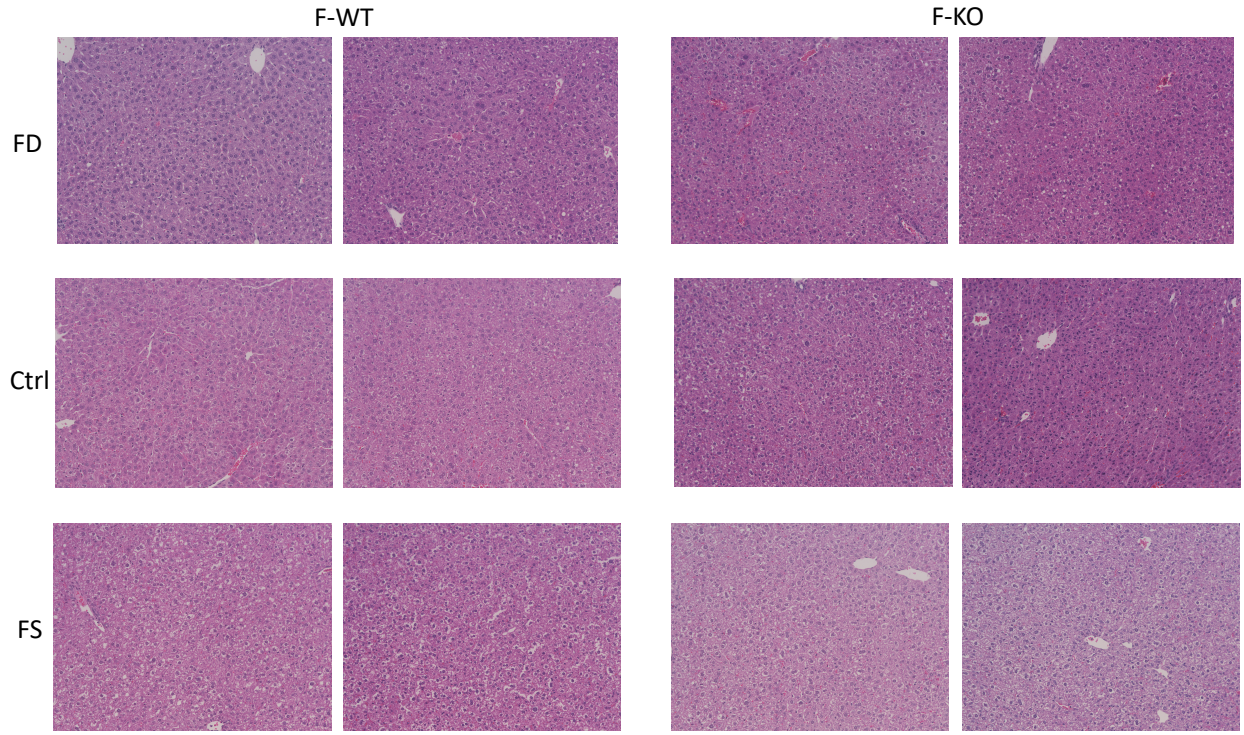
Supplementary Figure 2. Simple regression analysis revealed no covariation with pre-diet body weight for multiple measured parameters: body weight increase (delta BW), fat mass increase (delta FM), as well as for liver FA, DHF and 5-MTHFconcentrations. Data are shown for all dietary groups including CerS6 KO male mice only.



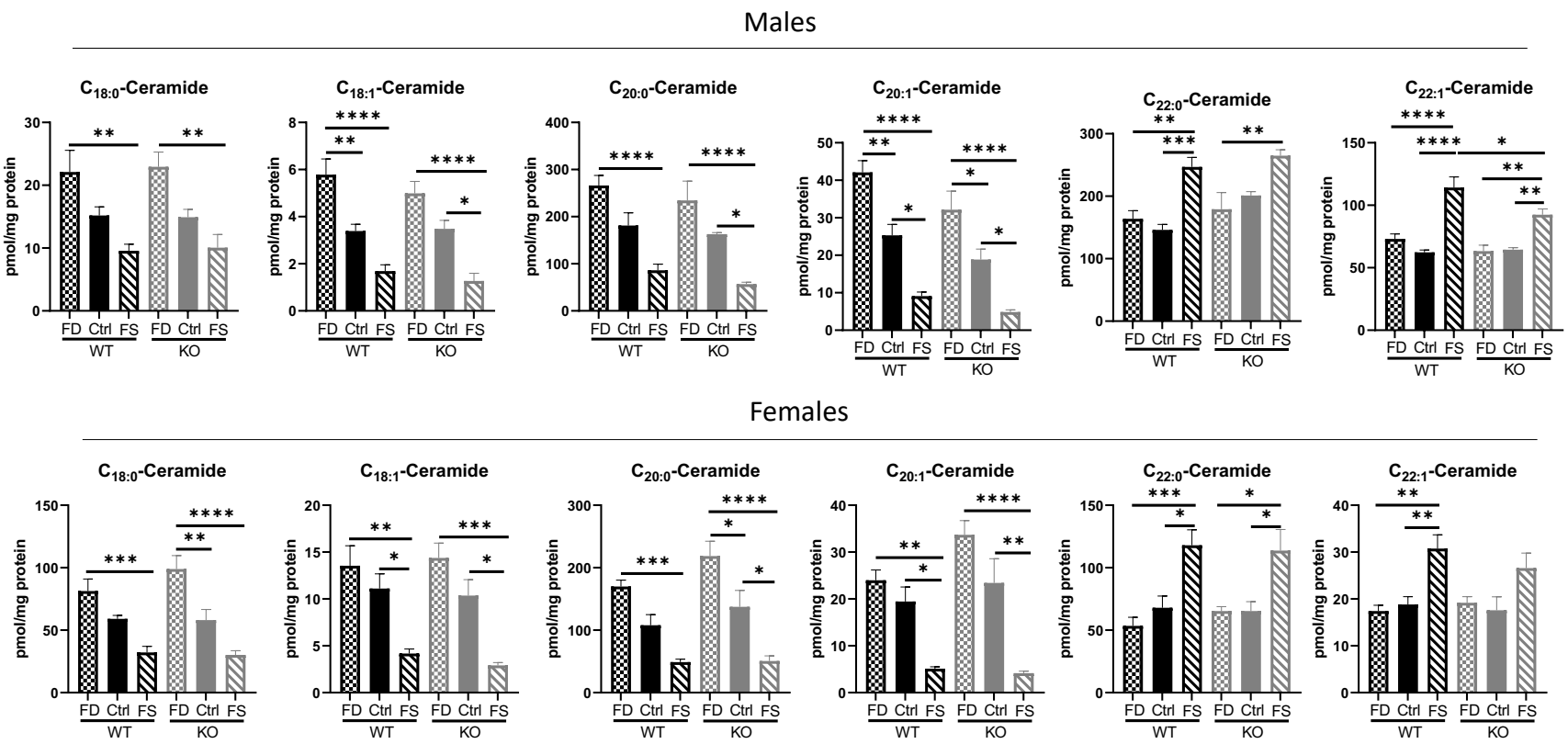
Supplementary Figure 3. Neither CerS6 knockout, nor dietary FA had significant effects on liver/body weight ratio, plasma glucose or plasma cholesterol. Data are shown as mean ± SEM, n=5. Checkered bars, FD diet; solid bars, Control diet; striped bars, FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ determined by One-way ANOVA with Sidak's multiple comparisons test.



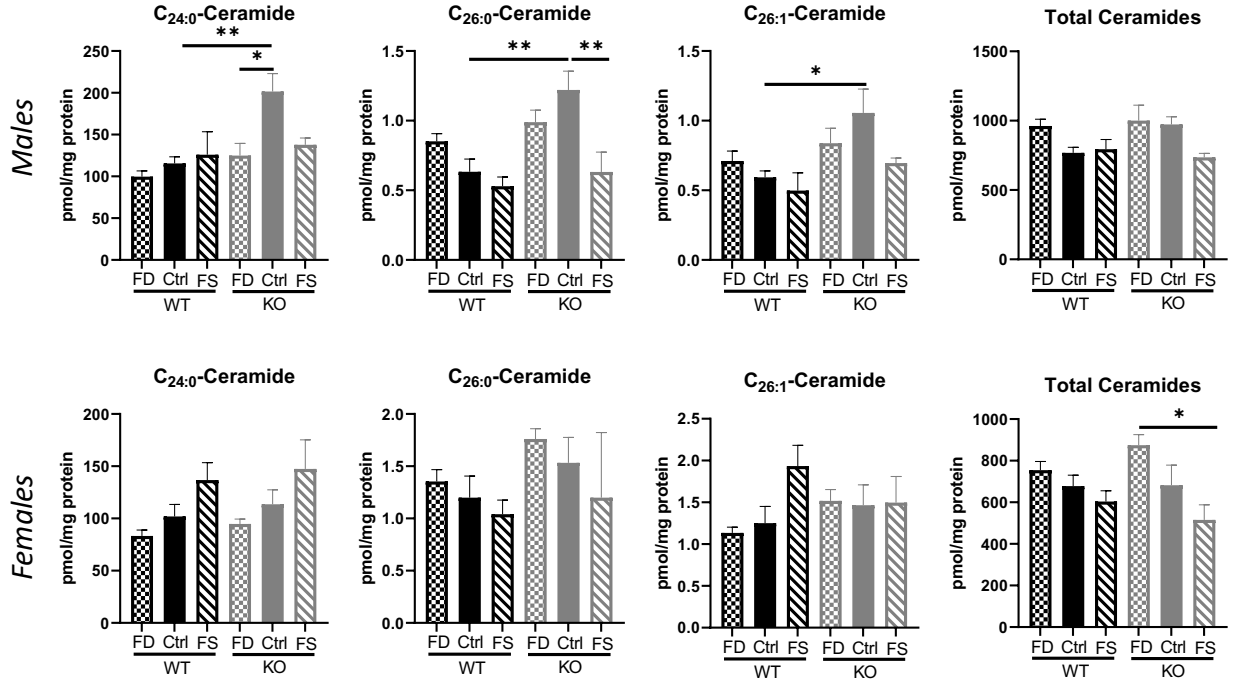
Supplementary Figure 4. Liver sections stained with H&E revealed no significant differences between male WT and KO mice for any diet.



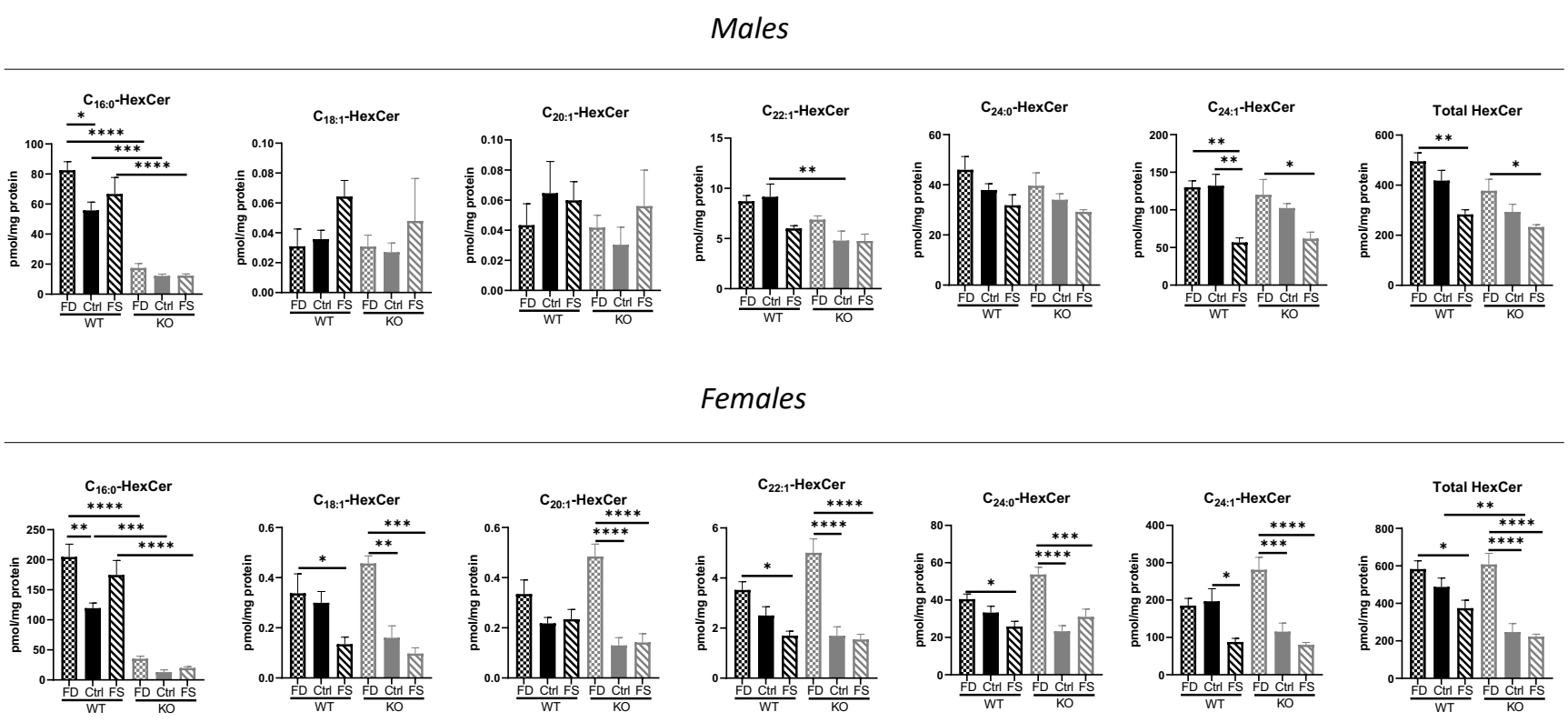
Supplementary Figure 5. Liver sections stained with H&E revealed no significant differences between female WT and KO mice for any diet.



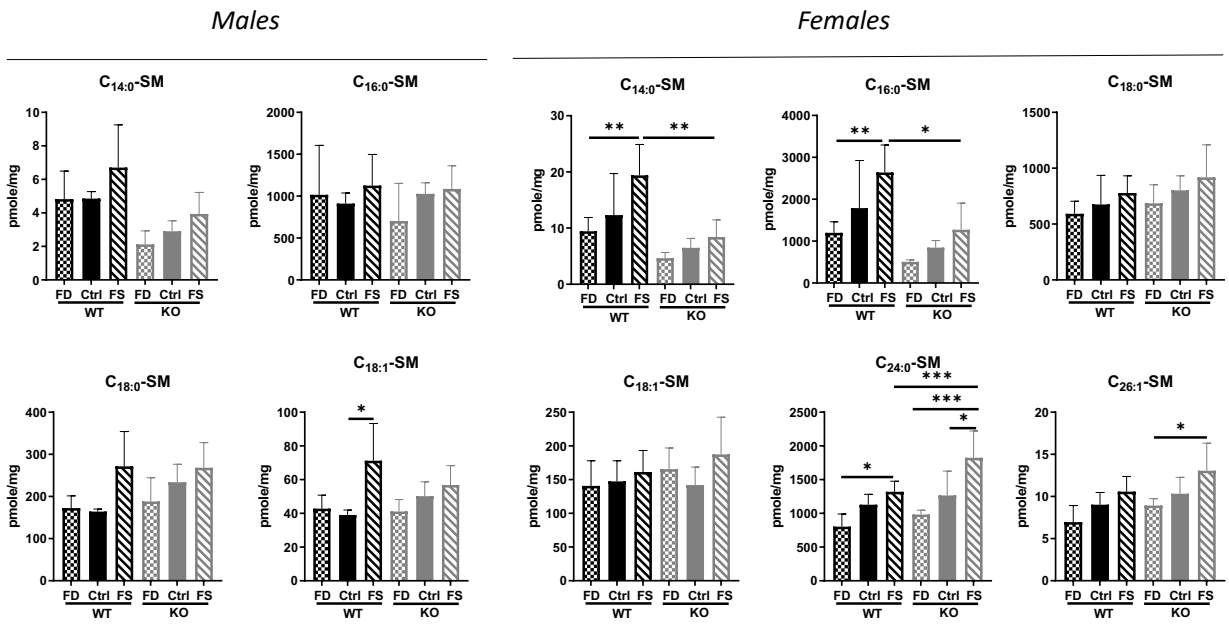
Supplementary Figure 6. Male and female mice demonstrated similar response to dietary FA, with long chain ceramides decreasing with increase of dietary FA while very long chain ceramide (C_{22}- and $C_{22:1}$-) increased on FS diet. Data are shown as mean ± SEM, n=5. Checkered bars, FD diet; solid bars, Control diet; striped bars, FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ determined by One-way ANOVA with Sidak's multiple comparisons test.



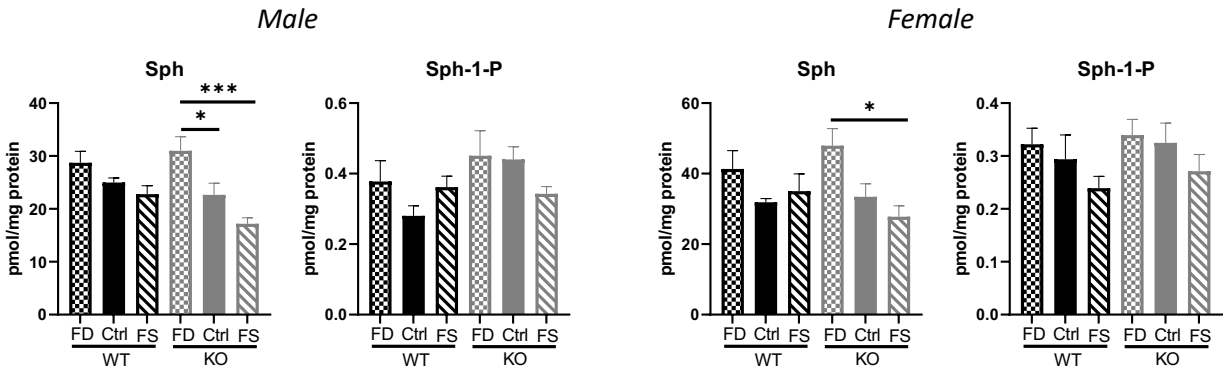
Supplementary Figure 7. Very long chain ceramides and the total ceramide concentrations in male and female WT and CerS6 KO mice. In male mice, an increase in very-long-chain ceramides was seen in CerS6 KO compared to WT mice on CTRL diet only, with no effect of FD diet. No significant differences in response to FA were seen in females. Data are shown as mean $\pm$ SEM, n=5. Checkered bars, FD diet; solid bars, Control diet; striped bars, FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ determined by One-way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure 8. Hexosyl ceramide species were less responsive to dietary FA in male mice, with the most pronounced change in $C_{16:0}$-HexCer as well as $C_{24:1}$-HexCer. In female mice, FD diet increased several HexCer species compared to CTRL and FS diets. Data are shown as mean $\pm$ SEM, n=5. Checkered bars, FD diet; solid bars, Control diet; striped bars, FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ determined by One-way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure 9. Sphingomyelins show tendency to increase on FA over-supplemented diet. No significant changes in in sphingomyelins on FS diet was seen for both WT and KO male mice but females showed significant increases of C_{14}-, C_{16}-, C_{24}- and $C_{24:1}$-ceramides. Data are shown as mean values $\pm$ SEM. Checkered bars: FD diet, Solid bars: Control diet, Striped bars: FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, ****, $p<0.0001$ according to One-way ANOVA with Sidak's multiple comparisons test.



Supplementary Figure 10. No significant differences between diets were observed in Sphingosine-1-phosphate concentrations, but sphingosine was elevated in KO livers of both male and female mice on the FD diet with no changes in WT mice. Data are shown as mean values ± SEM. Checkered bars: FD diet, Solid bars: Control diet, Striped bars: FS diet. WT shown in black and KO shown in grey. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, ****, $p<0.0001$ according to One-way ANOVA with Sidak's multiple comparisons test.

a

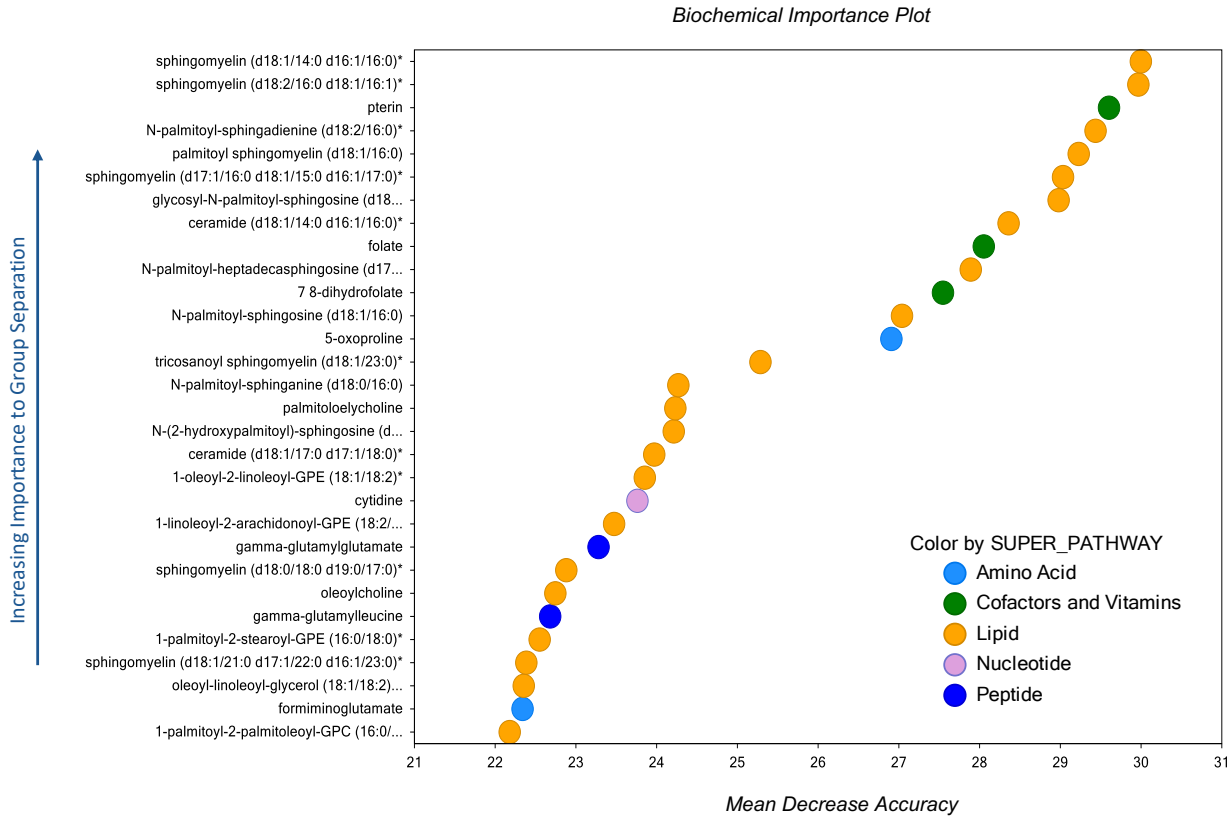
Statistically Significant Biochemicals	ANOVA Main Effects						
	Genotype Main Effect	Sex Main Effect	Diet Main Effect	Genotype: Sex Interaction	Genotype: Diet Interaction	Sex: Diet Interaction	Genotype: Sex: Diet Interaction
Total biochemicals p≤0.05	273	550	298	143	86	112	44
Total biochemicals 0.05<p<0.10	47	35	68	57	44	70	49

b

Predicted group

Actual group	CerS6 KO Ctrl F	CersS6 KO FD F	CerS6Ko FS F	WT Ctrl F	WT FD F	WT FS F	CerS6 KO Ctrl M	CersS6 KO FD M	CerS6Ko FS M	WT Ctrl M	WT FD M	WT FS M	Class Error
CerS6 KO Ctrl F	1	1	2	0	0	1	0	0	0	0	0	0	80%
CersS6 KO FD F	0	5	0	0	0	0	0	0	0	0	0	0	0%
CerS6Ko FS F	0	0	5	0	0	0	0	0	0	0	0	0	0%
WT Ctrl F	0	0	0	1	2	2	0	0	0	0	0	0	80%
WT FD F	0	2	0	1	2	0	0	0	0	0	0	0	60%
WT FS F	0	0	0	0	0	5	0	0	0	0	0	0	0%
CerS6 KO Ctrl M	0	0	0	0	0	0	3	1	1	0	0	0	40%
CersS6 KO FD M	0	0	0	0	0	0	2	2	0	1	0	0	60%
CerS6Ko FS M	0	0	0	0	0	0	0	1	4	0	0	0	20%
WT Ctrl M	0	0	0	0	0	0	0	0	0	4	1	0	20%
WT FD M	0	0	0	0	0	0	0	1	0	1	2	1	60%
WT FS M	0	0	0	0	0	0	0	0	1	0	0	4	20%
Predictive accuracy = 64%													

Supplementary Figure 11. Metabolomics data summary. (a) Numbers of biochemicals out of total 736 measured metabolites that show differences between genotypes, gender and diets, as well as interactions between genotype and gender, genotype and diet, gender and diet. (b) Random forest analysis of the metabolomic data based on the biochemicals that provide best separation between the groups gives predicted accuracy of 64% when 12 experimental groups were compared. Predictive accuracy when separation into groups is done by random chance alone is 8.3%.



Supplementary Figure 12. Top 30 biochemicals ranked by their importance in separating metabolic profiles of 12 groups.

Supplementary Table 1. Primers used for genotyping offspring from CerS6$^{+/-}$ crosses.

Primer name	Sequence
CerS6 forward	5’-TTCGGTTAAGAATGGCCTTG-3’
CerS6 KO reverse	5’-CCAATAAACCCTCTTGCAGTTGC-3’
Wild type reverse	5’-CACACCCATATGGAACTCTTACA-3’

Supplementary Table 2. Metabolomics data file: Pathways Heatmap.

Supplementary Table 3. CerS6 KO livers have significantly lower levels of C_{16}-acyl chain-based ceramide (a) and sphingomyelin (b) species and significantly higher levels of very-long-chain sphingomyelins in both male and female mice.

a

		Fold Change											
		Female			Male			Female / Male					
		KO / WT			KO / WT			WT			KO		
Sub Pathway	Biochemical Name	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	0.75	0.63	0.62	1.05	1.00	0.75	1.07	1.28	1.25	0.76	0.81	1.03
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.37	0.35	0.39	0.74	0.74	0.48	1.41	1.58	1.43	0.70	0.74	1.16
	N-stearoyl-sphingosine (d18:1/18:0)*	1.08	0.90	1.00	1.20	1.31	0.89	2.70	3.99	2.57	2.43	2.74	2.89
	ceramide (d18:1/14:0, d16:1/16:0)*	0.47	0.47	0.40	0.29	0.39	0.27	1.51	1.94	1.44	2.46	2.37	2.11
	ceramide (d18:1/17:0, d17:1/18:0)*	0.77	0.60	0.77	1.00	0.94	0.70	1.91	2.66	1.96	1.48	1.70	2.17
	ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*	1.10	1.10	1.20	1.08	1.17	0.96	0.82	0.84	0.71	0.83	0.79	0.90
	ceramide (d18:2/24:1, d18:1/24:2)*	1.08	1.03	1.17	1.06	1.24	0.97	0.83	1.02	0.93	0.84	0.85	1.12
	N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.53	0.49	0.71	0.69	0.85	0.49	0.88	1.05	0.94	0.68	0.61	1.37
	N-erucoyl-sphingosine (d18:1/22:1)*	1.06	1.03	1.09	0.86	1.15	0.78	0.18	0.27	0.19	0.22	0.24	0.27
	N-palmitoyl-sphingadienine (d18:2/16:0)*	0.43	0.36	0.44	0.59	0.61	0.50	0.65	0.79	0.68	0.47	0.47	0.59
	N-behenoyl-sphingadienine (d18:2/22:0)*	0.92	0.94	1.22	0.96	0.92	1.03	0.29	0.25	0.26	0.27	0.25	0.31
	N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	0.07	0.07	0.07	0.09	0.12	0.08	1.30	1.68	1.51	1.03	0.95	1.28
Hexosylceramides (HCER)	glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.15	0.13	0.15	0.27	0.23	0.22	2.24	2.73	2.90	1.30	1.52	1.94
	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	0.90	0.75	0.69	1.02	1.09	0.74	12.57	20.19	11.77	11.08	13.95	10.95
	glycosyl-N-arachidoyl-sphingosine (d18:1/20:0)*	1.02	1.03	0.97	0.81	0.93	0.86	1.45	1.69	1.55	1.81	1.87	1.76
	glycosyl-N-erucoyl-sphingosine (d18:1/22:1)*	0.88	0.91	0.85	0.50	0.81	0.65	0.27	0.40	0.36	0.48	0.44	0.47
	glycosyl ceramide (d18:1/23:1, d17:1/24:1)*	0.86	0.82	0.89	0.65	0.82	0.82	0.98	1.26	1.28	1.30	1.26	1.40
	glycosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	0.93	1.00	0.94	0.92	1.14	0.94	1.55	1.87	1.85	1.57	1.64	1.86
	glycosyl ceramide (d18:2/24:1, d18:1/24:2)*	1.12	0.99	1.11	1.03	1.28	1.07	1.52	2.17	2.11	1.64	1.69	2.19
Lactosylceramides (LCER)	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.52	0.35	0.43	0.36	0.45	0.52	1.06	1.21	1.17	1.54	0.93	0.96
	lactosyl-N-nervonoyl-sphingosine (d18:1/24:1)*	1.06	1.06	0.98	1.14	1.27	1.10	1.05	1.25	1.34	0.97	1.05	1.20

b

		Fold Change											
		Female			Male			Female / Male					
		KO / WT			KO / WT			WT			KO		
Sub Pathway	Biochemical Name	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS
Sphingolipid Metabolism	sphinganine	1.22	1.09	0.94	1.30	1.28	0.97	1.54	1.82	1.85	1.44	1.54	1.79
	palmitoyl dihydrosphingomyelin (d18:0/16:0)*	0.92	0.79	0.91	0.96	1.04	0.93	1.44	1.73	1.53	1.38	1.32	1.50
	behenoyl dihydrosphingomyelin (d18:0/22:0)*	1.12	1.48	1.58	1.41	1.43	1.19	1.53	1.27	1.34	1.22	1.32	1.78
	palmitoyl sphingomyelin (d18:1/16:0)	0.56	0.46	0.53	0.70	0.71	0.64	2.09	2.30	1.98	1.67	1.47	1.62
	stearoyl sphingomyelin (d18:1/18:0)	1.17	1.04	1.28	1.01	1.14	1.05	3.55	4.60	2.84	4.12	4.18	3.47
	behenoyl sphingomyelin (d18:1/22:0)*	1.16	1.20	1.59	1.15	1.32	1.33	0.63	0.70	0.58	0.63	0.64	0.69
	tricosanoyl sphingomyelin (d18:1/23:0)*	1.25	1.46	1.65	1.09	1.34	1.33	1.97	2.33	2.13	2.26	2.54	2.64
	lignoceroyl sphingomyelin (d18:1/24:0)	1.01	1.33	1.49	1.10	1.46	1.56	1.21	1.32	1.36	1.12	1.20	1.30
	sphingomyelin (d18:1/14:0, d16:1/16:0)*	0.63	0.55	0.48	0.30	0.38	0.35	3.06	3.62	2.64	6.38	5.31	3.66
	sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*	0.08	0.07	0.08	0.10	0.10	0.11	2.26	2.21	2.31	1.95	1.65	1.73
	sphingomyelin (d18:2/16:0, d18:1/16:1)*	0.50	0.43	0.47	0.51	0.53	0.55	1.52	1.63	1.41	1.51	1.32	1.19
	sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	0.86	0.77	0.87	0.93	0.85	0.85	2.74	2.93	2.50	2.54	2.64	2.56
	sphingomyelin (d18:1/18:1, d18:2/18:0)	1.13	1.01	1.17	0.83	1.01	0.95	3.71	4.30	2.64	5.01	4.31	3.24
	sphingomyelin (d18:1/20:0, d16:1/22:0)*	1.28	1.14	1.52	1.09	1.21	1.05	1.27	1.36	0.95	1.49	1.28	1.37
	sphingomyelin (d18:1/20:1, d18:2/20:0)*	1.21	1.19	1.48	0.67	0.96	0.83	1.12	1.32	0.83	2.01	1.66	1.48
	sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	1.10	1.11	1.64	0.88	0.89	0.96	0.49	0.49	0.50	0.61	0.61	0.85
	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	1.16	1.05	1.33	0.86	1.13	0.90	0.37	0.46	0.30	0.49	0.43	0.45
	sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	1.08	1.05	1.11	0.86	1.04	0.92	0.97	1.16	0.99	1.21	1.17	1.20
	sphingomyelin (d18:1/24:1, d18:2/24:0)*	1.19	1.23	1.44	1.22	1.53	1.16	1.58	1.98	1.47	1.54	1.59	1.81
	sphingomyelin (d18:2/24:1, d18:1/24:2)*	1.34	1.14	1.42	1.04	1.28	1.10	1.55	1.92	1.53	1.99	1.70	1.97
	sphingosine	1.07	1.02	0.95	1.07	1.14	0.86	1.23	1.41	1.30	1.23	1.26	1.43
	phytosphingosine	1.04	1.04	1.00	0.94	1.02	1.00	0.90	0.93	1.13	1.00	0.95	1.13
	sphingomyelin (d18:2/23:1)*	1.08	0.95	1.27	0.68	0.89	0.86	1.43	1.79	1.40	2.28	1.91	2.06
	sphingomyelin (d18:2/24:2)*	0.96	1.43	0.84	1.09	1.03	1.48	2.39	1.73	2.48	2.11	2.40	1.41
	sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*	1.28	1.06	1.39	1.27	1.49	1.63	1.01	1.61	1.40	1.02	1.15	1.20
	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)*	1.25	1.13	1.35	0.59	0.95	0.87	0.44	0.62	0.43	0.93	0.75	0.67
	sphingomyelin (d18:0/20:0, d16:0/22:0)*	1.69	1.44	2.31	1.25	1.73	1.24	1.10	2.06	1.18	1.49	1.71	2.20
	sphingomyelin (d18:0/18:0, d19:0/17:0)*	1.46	1.38	1.62	1.15	1.40	1.17	2.70	4.45	2.80	3.42	4.39	3.87
	sphingomyelin (d18:1/19:0, d19:1/18:0)*	1.29	1.02	1.39	0.91	0.89	0.88	1.30	1.47	1.12	1.84	1.68	1.78
	heptadecasphingosine (d17:1)	0.70	0.67	0.61	0.57	0.61	0.58	0.91	0.91	1.00	1.10	1.00	1.05
	hexadecasphingosine (d16:1)*	0.77	0.82	0.83	0.73	0.83	0.79	0.89	0.91	0.99	0.93	0.90	1.03
	sphingadienine	1.02	0.95	0.89	0.77	0.88	0.79	0.72	0.77	0.81	0.95	0.84	0.92

Supplementary Table 4. CerS6 KO significant decreased diacylglycerol species in male mice and caused significantly fewer changes in females. Additionally, multiple diacylglycerols are found at significantly higher concentrations in the KO female vs male livers (irrespective of diet), while in WT animals significantly lower number of diacylglycerol species followed the same trend.

		Fold Change											
		Female			Male			Female / Male					
		KO / WT			KO / WT			WT			KO		
Sub Pathway	Biochemical Name	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [1]*	0.73	2.50	0.85	0.26	0.55	0.23	1.20	0.90	0.96	3.34	4.11	3.56
	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	0.76	1.23	0.71	0.39	0.61	0.39	0.96	0.84	0.86	1.86	1.71	1.56
	diacylglycerol (14:0/18:1, 16:0/16:1) [1]*	0.87	1.28	0.78	0.37	0.62	0.45	0.90	0.87	0.88	2.10	1.81	1.52
	diacylglycerol (14:0/18:1, 16:0/16:1) [2]*	0.86	1.19	0.86	0.47	0.69	0.51	0.87	0.85	0.81	1.61	1.47	1.37
	diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	0.91	1.13	0.69	0.48	0.80	0.62	0.89	0.96	1.01	1.69	1.35	1.13
	palmitoyl-myristoyl-glycerol (16:0/14:0) [1]*	0.98	1.47	0.83	0.35	0.58	0.45	0.94	1.06	1.19	2.66	2.70	2.19
	palmitoyl-myristoyl-glycerol (16:0/14:0) [2]	1.01	1.16	0.92	0.64	0.77	0.53	1.29	1.40	1.11	2.03	2.10	1.94
	palmitoyl-palmitoyl-glycerol (16:0/16:0) [2]*	1.02	1.03	0.91	0.77	0.82	0.78	1.13	1.11	1.18	1.51	1.39	1.39
	palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	0.92	1.18	0.78	0.45	0.74	0.60	1.27	1.38	1.31	2.60	2.19	1.73
	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	0.92	1.12	0.83	0.56	0.78	0.67	1.08	1.08	1.08	1.76	1.54	1.34
	palmitoyl-linoleoyl-glycerol (16:0/18:2) [1]*	1.00	1.08	0.89	0.78	0.97	0.77	1.24	1.26	1.07	1.59	1.41	1.23
	palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]*	1.01	0.99	0.89	0.86	0.97	0.89	1.15	1.15	1.07	1.35	1.17	1.07
	palmitoyl-linolenoyl-glycerol (16:0/18:3) [2]*	0.96	1.36	0.96	0.55	0.86	0.77	1.35	0.81	1.18	2.32	1.28	1.47
	palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]*	0.71	1.33	0.70	0.32	0.58	0.44	0.83	0.67	0.84	1.82	1.53	1.35
	palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]*	0.88	1.03	0.63	0.59	0.84	0.64	1.04	1.08	1.09	1.56	1.33	1.07
	palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]*	0.84	1.13	0.90	1.08	1.08	0.95	1.13	0.82	0.90	0.88	0.86	0.85
	palmitoleoyl-arachidonoyl-glycerol (16:1/20:4) [2]*	0.83	1.23	0.78	1.51	1.29	0.98	1.84	1.14	1.24	1.01	1.08	0.98
	palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [1]*	0.84	0.97	0.87	0.95	1.01	0.86	1.22	1.48	1.15	1.09	1.42	1.17
	palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [2]*	0.63	1.41	0.88	0.77	1.18	0.78	1.74	1.09	1.32	1.42	1.30	1.48
	stearoyl-linoleoyl-glycerol (18:0/18:2) [2]*	0.88	1.13	0.75	0.83	0.88	0.92	1.48	1.31	1.59	1.57	1.68	1.29
	oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	0.85	1.17	0.63	0.40	0.76	0.62	1.38	1.70	1.84	2.95	2.62	1.88
	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	0.85	1.13	0.74	0.46	0.76	0.64	1.16	1.24	1.37	2.16	1.86	1.59
	oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	1.00	1.05	0.71	0.74	0.99	0.91	1.35	1.48	1.69	1.83	1.58	1.31
	oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	0.95	1.05	0.78	0.64	0.96	0.93	1.24	1.37	1.50	1.84	1.50	1.26
	linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]*	1.24	0.90	0.69	1.24	1.17	1.23	1.35	1.37	1.64	1.35	1.05	0.92
	linoleoyl-linoleoyl-glycerol (18:2/18:2) [2]*	0.96	1.20	0.77	0.96	1.48	1.34	1.71	1.66	2.29	1.72	1.35	1.31
	linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]*	1.17	1.13	0.85	0.93	0.98	0.98	1.27	0.96	1.20	1.60	1.11	1.04
	stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]*	0.78	1.02	0.75	0.90	1.18	0.79	1.28	1.32	1.21	1.12	1.14	1.15
	stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]*	0.81	1.03	0.96	0.98	1.21	1.06	1.31	1.20	1.15	1.09	1.03	1.04
	oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]*	0.87	0.91	0.44	0.54	0.64	0.73	0.48	0.94	0.72	0.78	1.32	0.43
	oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	0.79	0.94	0.62	0.72	0.97	0.81	1.01	0.90	1.09	1.10	0.87	0.84
	linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]*	0.99	0.85	0.68	1.15	0.65	0.94	0.89	0.64	1.10	0.76	0.83	0.80
	linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]*	0.82	0.91	0.71	1.11	1.39	1.14	1.49	1.37	1.46	1.10	0.90	0.92
	stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [2]*	0.71	1.06	0.94	1.20	1.15	0.89	2.23	1.51	1.41	1.31	1.40	1.49
	linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]*	0.84	0.76	0.78	1.43	1.29	1.12	1.47	1.20	1.12	0.86	0.70	0.78

Supplementary Table 5. CerS6 KO resulted in significantly reduced levels of long chain fatty acids in male mice with only few changes in female mice. Both WT and KO females have significantly higher levels of multiple long-chain and polyunsaturated fatty acids on FD and CTRL diets.

		Fold Change											
		Female			Male			Female / Male					
		KO / WT			KO / WT			WT			KO		
Sub Pathway	Biochemical Name	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS
Long Chain Fatty Acid	myristate (14:0)	0.88	1.04	0.93	0.65	0.71	0.61	1.46	1.39	0.94	1.98	2.05	1.42
	myristoleate (14:1n5)	0.95	0.98	0.68	0.89	0.94	0.63	1.14	1.26	0.95	1.22	1.31	1.02
	palmitate (16:0)	0.93	0.99	0.94	0.77	0.75	0.78	1.36	1.28	0.99	1.65	1.68	1.20
	palmitoleate (16:1n7)	0.76	1.07	0.78	0.44	0.56	0.47	1.19	1.07	0.74	2.08	2.02	1.24
	margarate (17:0)	0.97	1.09	0.97	0.99	0.82	0.81	1.59	1.45	1.10	1.56	1.94	1.32
	10-heptadecenoate (17:1n7)	0.78	1.13	0.93	0.64	0.65	0.68	1.49	1.27	1.00	1.81	2.22	1.36
	stearate (18:0)	0.95	0.97	1.05	1.00	0.86	0.91	1.40	1.27	1.01	1.33	1.42	1.16
	oleate/vaccenate (18:1)	0.91	1.01	0.87	0.57	0.66	0.68	1.41	1.43	1.09	2.27	2.18	1.38
	10-nonadecenoate (19:1n9)	0.90	1.19	0.88	0.63	0.66	0.79	1.15	1.06	1.04	1.65	1.90	1.16
	arachidate (20:0)	0.98	1.05	1.13	0.78	0.69	0.98	0.40	0.36	0.37	0.50	0.54	0.43
	eicosenoate (20:1)	0.82	1.14	0.72	0.45	0.55	0.73	0.66	0.58	0.67	1.19	1.22	0.66
	erucate (22:1n9)	0.92	1.11	1.04	0.57	0.69	1.05	0.17	0.20	0.19	0.27	0.32	0.18
	nervonate (24:1n9)*	0.92	1.05	1.19	0.96	0.86	1.10	0.97	0.97	0.76	0.94	1.18	0.82
Polyunsaturated Fatty Acid (n3 and n6)	heneicosapentaenoate (21:5n3)	1.27	1.04	1.30	0.91	0.58	0.75	1.54	1.23	0.54	2.16	2.20	0.94
	hexadecadienoate (16:2n6)	0.94	0.83	0.65	0.97	0.90	0.87	1.62	1.49	1.10	1.57	1.38	0.83
	stearidonate (18:4n3)	0.99	0.90	0.78	1.32	1.08	0.90	2.32	2.16	1.15	1.75	1.80	1.00
	eicosapentaenoate (EPA; 20:5n3)	0.75	0.83	0.99	1.12	0.84	0.77	1.61	1.20	0.65	1.08	1.18	0.85
	docosapentaenoate (n3 DPA; 22:5n3)	0.80	0.81	0.99	1.25	0.88	0.92	1.51	1.13	0.60	0.97	1.04	0.65
	docosahexaenoate (DHA; 22:6n3)	0.77	0.88	1.09	1.02	0.86	0.87	1.69	1.44	0.79	1.27	1.47	0.98
	nisinate (24:6n3)	0.80	0.66	0.99	0.78	0.81	1.15	0.87	0.87	0.64	0.90	0.71	0.55
	linoleate (18:2n6)	0.96	0.93	0.98	0.89	0.83	0.93	1.72	1.61	1.09	1.86	1.80	1.14
	linolenate [alpha or gamma; (18:3n3 or 6)]	0.92	0.96	0.90	0.80	0.74	0.80	2.20	1.76	1.06	2.54	2.27	1.20
	dihomo-linolenate (20:3n3 or n6)	0.81	0.79	0.95	0.82	0.75	0.96	1.13	1.04	0.81	1.11	1.10	0.79
	arachidonate (20:4n6)	0.78	0.88	1.19	0.93	0.85	0.93	1.20	1.04	0.65	1.00	1.07	0.84
	adrenate (22:4n6)	0.78	0.86	0.83	0.99	0.88	1.21	1.65	1.42	1.06	1.30	1.38	0.73
	docosapentaenoate (n6 DPA; 22:5n6)	0.55	0.69	1.09	0.90	0.75	1.08	1.29	0.92	0.77	0.79	0.84	0.78
	docosadienoate (22:2n6)	0.84	0.90	0.97	0.74	0.66	1.31	0.45	0.39	0.43	0.51	0.53	0.32
	dihomo-linoleate (20:2n6)	0.87	0.89	0.81	0.63	0.67	0.87	1.17	1.07	1.03	1.63	1.41	0.96

Supplementary Table 6. Phosphatidylcholines show few differences between KO and WT livers of both sexes independent of diet. Several phosphatidylcholine species are significantly higher in females than males.

		Fold Change											
		Female			Male			Female / Male					
		KO / WT			KO / WT			WT			KO		
Sub Pathway	Biochemical Name	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS	CTRL	FD	FS
Phosphatidylcholine (PC)	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	1.18	1.12	1.18	0.95	1.12	0.97	1.11	1.25	1.23	1.38	1.25	1.51
	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)*	0.84	0.88	1.05	0.79	0.93	0.81	0.95	1.01	0.95	1.01	0.95	1.24
	1,2-dipalmitoyl-GPC (16:0/16:0)	1.00	0.95	1.05	0.94	1.05	0.97	1.34	1.38	1.28	1.41	1.25	1.39
	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	1.06	1.14	1.15	0.91	1.07	0.94	0.53	0.61	0.61	0.61	0.65	0.75
	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.85	0.90	0.95	0.94	1.07	0.94	1.71	1.61	1.58	1.56	1.36	1.59
	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	1.03	1.08	1.07	0.95	1.06	0.96	0.80	0.90	0.88	0.87	0.91	0.98
	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	1.12	0.99	1.10	1.15	1.10	1.10	0.84	0.90	0.88	0.82	0.81	0.87
	1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)*	0.95	0.87	0.97	1.67	1.27	1.26	0.88	0.92	0.85	0.50	0.64	0.66
	1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)*	1.10	1.04	1.13	0.91	0.91	0.94	0.68	0.70	0.77	0.82	0.81	0.93
	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	0.96	0.91	0.98	0.98	0.98	0.90	1.07	1.10	0.97	1.04	1.03	1.06
	1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	1.00	0.97	1.01	0.96	0.99	0.92	1.15	1.18	1.08	1.20	1.16	1.18
	1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	1.03	1.06	1.05	1.00	1.10	1.01	0.71	0.76	0.77	0.73	0.73	0.80
	1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	0.92	0.94	1.10	1.19	1.24	1.06	0.62	0.70	0.68	0.48	0.53	0.71
	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	0.88	0.98	0.92	0.92	0.97	0.83	1.11	1.22	1.24	1.05	1.24	1.37
	1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	0.98	0.89	0.98	1.03	0.98	1.05	1.11	1.18	1.21	1.06	1.08	1.13
	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.84	0.89	0.91	0.93	0.92	0.80	1.43	1.45	1.21	1.30	1.41	1.38
	1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	0.92	0.94	0.93	1.03	0.94	0.84	1.79	1.71	1.52	1.60	1.72	1.69
	1,2-dioleoyl-GPC (18:1/18:1)	0.81	1.08	0.94	0.64	0.88	0.79	0.74	0.80	0.93	0.93	0.97	1.10
	1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	0.94	1.02	0.95	0.66	0.96	0.96	0.72	0.90	1.03	1.02	0.95	1.02
	1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*	0.89	1.06	1.01	0.76	1.02	0.81	1.09	1.20	1.13	1.26	1.25	1.39
	1,2-dilinoleoyl-GPC (18:2/18:2)	1.03	0.97	1.10	1.07	0.98	1.21	0.75	0.70	0.90	0.73	0.69	0.82
	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	1.17	1.00	1.27	1.72	1.28	1.50	0.87	0.68	0.83	0.59	0.53	0.70
	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	0.84	0.91	0.98	0.90	0.95	0.97	0.94	0.86	0.97	0.88	0.82	0.98

Supplementary Table 7. The top metabolites (46) that were significantly different between male and female mice in untargeted metabolomics analysis belong to the amino acid, sugar and lipid metabolism. Metabolites are listed with sub-pathway to which they belong.

Sex differences

p<0.01 487 metabolites

0.01<p<0.05 63 metabolites

Sub Pathway	Biochemical Name	p-value
Glutamate Metabolism	N-acetylglutamine	0.0000000
Histidine Metabolism	1-methylhistidine	0.0000000
Histidine Metabolism	N-acetyl-1-methylhistidine*	0.0000000
Histidine Metabolism	imidazole lactate	0.0000000
Histidine Metabolism	anserine	0.0000000
Histidine Metabolism	1-ribosyl-imidazoleacetate*	0.0000000
Histidine Metabolism	4-imidazoleacetate	0.0000000
Lysine Metabolism	N2-acetyllysine	0.0000000
Leucine, Isoleucine and Valine Metabolism	N-acetylleucine	0.0000000
Leucine, Isoleucine and Valine Metabolism	alpha-hydroxyisovalerate	0.0000000
Methionine, Cysteine, SAM and Taurine Metabolism	N-formylmethionine	0.0000000
Methionine, Cysteine, SAM and Taurine Metabolism	hypotaurine	0.0000000
Urea cycle; Arginine and Proline Metabolism	homoarginine	0.0000000
Urea cycle; Arginine and Proline Metabolism	N-acetylarginine	0.0000000
Urea cycle; Arginine and Proline Metabolism	trans-4-hydroxyproline	0.0000000
Gamma-glutamyl Amino Acid	gamma-glutamylglycine	0.0000000
Gamma-glutamyl Amino Acid	gamma-glutamylisoleucine*	0.0000000
Gamma-glutamyl Amino Acid	gamma-glutamylleucine	0.0000000
Fructose, Mannose and Galactose Metabolism	mannose	0.0000000
Aminosugar Metabolism	N-acetylglucosaminylasparagine	0.0000000
Long Chain Fatty Acid	arachidate (20:0)	0.0000000
Long Chain Fatty Acid	erucate (22:1n9)	0.0000000
Polyunsaturated Fatty Acid (n3 and n6)	docosadienoate (22:2n6)	0.0000000
Fatty Acid, Amino	2-aminooctanoate	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	oleoylcarnitine (C18:1)	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	arachidonoylcarnitine (C20:4)	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	behenoylcarnitine (C22)*	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	lignoceroylcarnitine (C24)*	0.0000000
Fatty Acid Metabolism(Acyl Carnitine)	nervonoylcarnitine (C24:1)*	0.0000000
Phospholipid Metabolism	trimethylamine N-oxide	0.0000000
Phosphatidylcholine (PC)	1,2-dipalmitoyl-GPC (16:0/16:0)	0.0000000
Phosphatidylcholine (PC)	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	0.0000000
Phosphatidylcholine (PC)	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.0000000
Phosphatidylcholine (PC)	1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	0.0000000
Phosphatidylcholine (PC)	1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	0.0000000
Phosphatidylcholine (PC)	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.0000000
Phosphatidylcholine (PC)	1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	0.0000000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*	0.0000000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.0000000
Phosphatidylethanolamine (PE)	1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.0000000
Phosphatidylethanolamine (PE)	1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)*	0.0000000
Phosphatidylserine (PS)	1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	0.0000000
Phosphatidylinositol (PI)	1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	0.0000000
Lysophospholipid	1-palmitoleoyl-GPC (16:1)*	0.0000000
Lysoplasmalogen	1-(1-enyl-stearoyl)-GPE (P-18:0)*	0.0000000

Supplementary Table 8. Fold-changes of the top 46 metabolites (from Supplementary Table 7) that were significantly different between male and female mice in untargeted metabolomics analysis.

Biochemical Name	Female/Male					
	WT			KO		
	CTRL/WT	FD	FS	CTRL/KO	FD	FS
N-acetylglutamine	0.56	0.52	0.45	0.58	0.50	0.56
1-methylhistidine	0.63	0.51	0.55	0.61	0.49	0.55
N-acetyl-1-methylhistidine*	0.36	0.36	0.27	0.52	0.31	0.36
imidazole lactate	0.16	0.13	0.18	0.17	0.12	0.15
anserine	0.40	0.41	0.42	0.46	0.35	0.42
1-ribosyl-imidazoleacetate*	0.15	0.21	0.32	0.23	0.38	0.24
4-imidazoleacetate	0.28	0.47	0.42	0.54	0.59	0.38
N2-acetyllysine	0.27	0.54	0.21	0.29	0.25	0.28
N-acetylleucine	0.61	0.53	0.35	0.29	0.46	0.37
alpha-hydroxyisovalerate	0.29	0.19	0.26	0.35	0.19	0.27
N-formylmethionine	0.28	0.35	0.47	0.19	0.24	0.41
hypotaurine	0.44	0.40	0.51	0.39	0.25	0.24
homoarginine	2.59	2.76	2.28	2.05	2.18	1.59
N-acetylarginine	0.28	0.23	0.28	0.24	0.18	0.24
trans-4-hydroxyproline	0.41	0.39	0.39	0.30	0.41	0.47
gamma-glutamylglycine	0.58	0.32	0.30	0.34	0.22	0.30
gamma-glutamylisoleucine*	0.71	0.57	0.68	0.59	0.48	0.56
gamma-glutamylleucine	0.57	0.50	0.66	0.50	0.40	0.52
mannose	0.66	0.54	0.62	0.65	0.56	0.69
N-acetylglucosaminylasparagine	2.56	2.12	2.51	2.42	2.19	2.27
arachidate (20:0)	0.40	0.36	0.37	0.50	0.54	0.43
erucate (22:1n9)	0.17	0.20	0.19	0.27	0.32	0.18
docosadienoate (22:2n6)	0.45	0.39	0.43	0.51	0.53	0.32
2-aminooctanoate	0.48	0.38	0.37	0.36	0.36	0.39
oleoylcarnitine (C18:1)	2.01	2.80	2.26	1.74	2.36	2.04
pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	0.45	0.36	0.40	0.22	0.16	0.21
arachidonoylcarnitine (C20:4)	2.43	2.81	1.67	3.21	2.86	2.30
behenoylcarnitine (C22)*	0.08	0.11	0.12	0.12	0.13	0.10
lignoceroylcarnitine (C24)*	0.46	0.66	0.55	0.34	0.46	0.44
nervonoylcarnitine (C24:1)*	0.17	0.22	0.21	0.21	0.22	0.14
trimethylamine N-oxide	20.24	21.64	13.01	18.10	18.58	9.35
1,2-dipalmitoyl-GPC (16:0/16:0)	1.34	1.38	1.28	1.41	1.25	1.39
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	0.53	0.61	0.61	0.61	0.65	0.75
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	1.71	1.61	1.58	1.56	1.36	1.59
1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	0.71	0.76	0.77	0.73	0.73	0.80
1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	0.62	0.70	0.68	0.48	0.53	0.71
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	1.43	1.45	1.21	1.30	1.41	1.38
1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	1.79	1.71	1.52	1.60	1.72	1.69
1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*	3.32	2.86	2.04	3.33	2.27	4.44
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.77	0.83	0.81	0.75	0.76	0.85
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.52	0.65	0.68	0.49	0.57	0.69
1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)*	0.74	0.74	0.83	0.53	0.60	0.77
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	1.36	1.54	1.41	1.26	1.32	1.41
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	0.51	0.51	0.44	0.48	0.47	0.45
1-palmitoleoyl-GPC (16:1)*	0.46	0.52	0.57	0.60	0.56	0.70
1-(1-enyl-stearoyl)-GPE (P-18:0)*	1.75	2.21	1.85	1.66	1.64	1.87

Supplementary Table 9. The top 46 metabolites exhibiting significant differences between genotypes belonged to the lipid super family, including sphingolipids, phosphatidylethanolamines, and diacylglycerol species. Metabolites are listed with sub-pathway to which they belong.

Genotype differences

p<0.01 173 metabolites

0.01<p<0.05 100 metabolites

Sub Pathway	Biochemical Name	p-value
Sphingolipid Metabolism	palmitoyl sphingomyelin (d18:1/16:0)	0.0000000
Sphingolipid Metabolism	sphingomyelin (d18:1/14:0, d16:1/16:0)*	0.0000000
Sphingolipid Metabolism	sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*	0.0000000
Sphingolipid Metabolism	sphingomyelin (d18:2/16:0, d18:1/16:1)*	0.0000000
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.0000000
Ceramides	ceramide (d18:1/14:0, d16:1/16:0)*	0.0000000
Ceramides	N-palmitoyl-sphingadienine (d18:2/16:0)*	0.0000000
Ceramides	N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	0.0000000
Hexosylceramides (HCER)	glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.0000000
Sphingolipid Metabolism	heptadecasphingosine (d17:1)	0.0000000
Ceramides	N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.0000000
Phosphatidylethanolamine (PE)	1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.0000000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.0000000
Sphingolipid Metabolism	tricosanoyl sphingomyelin (d18:1/23:0)*	0.0000000
Phosphatidylethanolamine (PE)	1,2-dilinoleoyl-GPE (18:2/18:2)*	0.0000000
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	0.0000000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.0000000
Phosphatidylethanolamine (PE)	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	0.0000000
Lactosylceramides (LCER)	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.0000000
Sphingolipid Metabolism	hexadecasphingosine (d16:1)*	0.0000000
Sphingolipid Metabolism	sphingomyelin (d18:0/20:0, d16:0/22:0)*	0.0000000
Phosphatidylcholine (PC)	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.0000000
Phosphatidylethanolamine (PE)	1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)*	0.0000000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.0000000
Fatty Acid Metabolism (Acyl Choline)	palmitoloelycholine	0.0000001
Sphingolipid Metabolism	behenoyl sphingomyelin (d18:1/22:0)*	0.0000001
Phosphatidylethanolamine (PE)	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.0000001
Sphingolipid Metabolism	lignoceroyl sphingomyelin (d18:1/24:0)	0.0000001
Fatty Acid Metabolism (Acyl Choline)	linoleoylcholine*	0.0000001
Sphingolipid Metabolism	sphingomyelin (d18:1/24:1, d18:2/24:0)*	0.0000001
Phosphatidylethanolamine (PE)	1,2-dipalmitoyl-GPE (16:0/16:0)*	0.0000002
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	0.0000004
Phosphatidylinositol (PI)	1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)*	0.0000007
Diacylglycerol	oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	0.0000007
Sphingolipid Metabolism	behenoyl dihydrosphingomyelin (d18:0/22:0)*	0.0000011
Diacylglycerol	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	0.0000014
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	0.0000016
Fatty Acid Metabolism (Acyl Choline)	oleoylcholine	0.0000017
Phosphatidylcholine (PC)	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	0.0000035
Ceramides	ceramide (d18:1/17:0, d17:1/18:0)*	0.0000035
Chemical	S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.0000036
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	0.0000038
Sphingolipid Metabolism	sphingomyelin (d18:0/18:0, d19:0/17:0)*	0.0000038
Sphingolipid Metabolism	sphingomyelin (d18:2/24:1, d18:1/24:2)*	0.0000046
Plasmalogen	1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	0.0000057
Hexosylceramides (HCER)	glycosyl-N-erucoyl-sphingosine (d18:1/22:1)*	0.0000065

Supplementary Table 10. The top 46 metabolites exhibiting the most significant difference between dietary groups belonged to several metabolic pathways including amino acid metabolism and TCA cycle intermediates. Metabolites are listed with sub-pathway to which they belong.

Diet differences

p<0.01 167 metabolites

0.01<p<0.05 131 metabolites

Sub Pathway	Biochemical Name	p-value
Folate Metabolism	folate	0.0000000
Folate Metabolism	7,8-dihydrofolate	0.0000000
Pterin Metabolism	pterin	0.0000000
Phenylalanine Metabolism	N-acetylphenylalanine	0.0000000
Gamma-glutamyl Amino Acid	gamma-glutamylglutamate	0.0000000
Glutathione Metabolism	5-oxoproline	0.0000000
Methionine, Cysteine, SAM and Taurine Metabolism	N-acetyltaurine	0.0000000
Histidine Metabolism	formiminoglutamate	0.0000001
Partially Characterized Molecules	glycine conjugate of C10H14O2 (1)*	0.0000008
Urea cycle; Arginine and Proline Metabolism	trans-4-hydroxyproline	0.0000017
Folate Metabolism	5-methyltetrahydrofolate (5MeTHF)	0.0000025
Riboflavin Metabolism	flavin adenine dinucleotide (FAD)	0.0000063
Glutamate Metabolism	N-acetylglutamate	0.0000103
Aminosugar Metabolism	glucuronate	0.0000121
Riboflavin Metabolism	riboflavin (Vitamin B2)	0.0000150
Lysophospholipid	1-stearoyl-GPE (18:0)	0.0000152
Fructose, Mannose and Galactose Metabolism	galactose 1-phosphate	0.0000157
Glutamate Metabolism	4-hydroxyglutamate	0.0000218
Eicosanoid	12-HHTrE	0.0000236
Hexosylceramides (HCER)	glycosyl-N-arachidoyl-sphingosine (d18:1/20:0)*	0.0000275
Pyrimidine Metabolism, Uracil containing	uridine	0.0000278
Methionine, Cysteine, SAM and Taurine Metabolism	N-acetylmethionine	0.0000398
Lysophospholipid	1-palmitoyl-GPE (16:0)	0.0000484
Phospholipid Metabolism	glycerophosphoserine*	0.0000547
Polyamine Metabolism	N-acetylputrescine	0.0000693
Fatty Acid Metabolism (Acyl Choline)	palmitoloelycholine	0.0000695
Urea cycle; Arginine and Proline Metabolism	homoarginine	0.0000790
Gamma-glutamyl Amino Acid	gamma-glutamylglutamine	0.0000793
Fatty Acid Metabolism(Acyl Carnitine)	3-hydroxybutyrylcarnitine (1)	0.0000855
Glutamate Metabolism	glutamate	0.0001000
Lysine Metabolism	N6,N6,N6-trimethyllysine	0.0001000
Glutathione Metabolism	glutathione, oxidized (GSSG)	0.0001000
Purine Metabolism, Guanine containing	7-methylguanine	0.0001000
Lysine Metabolism	lysine	0.0002000
TCA Cycle	isocitrate	0.0002000
Fatty Acid, Dihydroxy	14,15-DiHETrE	0.0002000
Sphingolipid Metabolism	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)*	0.0002000
Secondary Bile Acid Metabolism	taurolithocholate	0.0002000
Secondary Bile Acid Metabolism	taurohyodeoxycholic acid	0.0002000
Riboflavin Metabolism	flavin mononucleotide (FMN)	0.0002000
Chemical	S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.0002000
Glycine, Serine and Threonine Metabolism	betaine	0.0003000
Glycogen Metabolism	maltopentaose	0.0003000
Sphingolipid Metabolism	sphingomyelin (d18:1/14:0, d16:1/16:0)*	0.0003000
Vitamin A Metabolism	retinol (Vitamin A)	0.0003000
Vitamin B6 Metabolism	pyridoxal phosphate	0.0003000

Supplementary Table 11. Top 46 metabolites exhibiting the most significant genotype:sex interactions. Metabolites are listed with sub-pathway to which they belong.

Genotype:Sex interaction

p<0.01 73 metabolites

0.01<p<0.05 70 metabolites

Sub Pathway	Biochemical Name	p-value
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.0000000
Sphingolipid Metabolism	palmitoyl sphingomyelin (d18:1/16:0)	0.0000001
Chemical	S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.0000036
Sphingolipid Metabolism	sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)*	0.0000072
Phosphatidylcholine (PC)	1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)*	0.0000106
Hexosylceramides (HCER)	glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.0000136
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [1]*	0.0000142
Ceramides	N-palmitoyl-sphingadienine (d18:2/16:0)*	0.0000143
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	0.0000180
Diacylglycerol	diacylglycerol (14:0/18:1, 16:0/16:1) [2]*	0.0000608
Sphingolipid Metabolism	sphingomyelin (d18:1/14:0, d16:1/16:0)*	0.0000614
Diacylglycerol	diacylglycerol (14:0/18:1, 16:0/16:1) [1]*	0.0000652
Sphingolipid Metabolism	sphingomyelin (d18:1/20:1, d18:2/20:0)*	0.0000768
TCA Cycle	succinylcarnitine (C4-DC)	0.0001000
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [2]*	0.0001000
Diacylglycerol	palmitoyl-myristoyl-glycerol (16:0/14:0) [2]	0.0001000
Diacylglycerol	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	0.0001000
Sphingolipid Metabolism	sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	0.0001000
Diacylglycerol	palmitoyl-myristoyl-glycerol (16:0/14:0) [1]*	0.0002000
Diacylglycerol	palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	0.0002000
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	0.0003000
Phosphatidylethanolamine (PE)	1,2-dilinoleoyl-GPE (18:2/18:2)*	0.0004000
Diacylglycerol	palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]*	0.0004000
Long Chain Fatty Acid	oleate/vaccenate (18:1)	0.0005000
Purine Metabolism, Guanine containing	guanosine	0.0005000
Pentose Metabolism	ribulose/xylulose	0.0006000
Phosphatidylethanolamine (PE)	1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)*	0.0006000
Sphingolipid Metabolism	sphingomyelin (d18:1/19:0, d19:1/18:0)*	0.0007000
Pyrimidine Metabolism, Thymine containing	3-aminoisobutyrate	0.0010000
Glutathione Metabolism	ophthalmate	0.0012000
Long Chain Fatty Acid	palmitoleate (16:1n7)	0.0013000
Guanidino and Acetamido Metabolism	guanidinosuccinate	0.0014000
Phosphatidylcholine (PC)	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	0.0015000
Chemical	O-sulfo-L-tyrosine	0.0016000
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	pyruvate	0.0017000
Fructose, Mannose and Galactose Metabolism	galactonate	0.0018000
Fatty Acid, Monohydroxy	16-hydroxypalmitate	0.0018000
Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	0.0019000
Sterol	4-cholesten-3-one	0.0023000
Diacylglycerol	diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	0.0024000
Phosphatidylcholine (PC)	1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)*	0.0026000
Long Chain Fatty Acid	myristate (14:0)	0.0033000
Diacylglycerol	linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]*	0.0034000
Ceramides	ceramide (d18:1/14:0, d16:1/16:0)*	0.0034000
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	0.0035000
Long Chain Fatty Acid	eicosenoate (20:1)	0.0038000

Supplementary Table 12. Top 46 metabolites exhibiting the most significant sex: diet interactions.

Metabolites are listed with sub-pathway to which they belong.

Sex:diet interaction

p<0.01 45 metabolites

0.01<p<0.05 67 metabolites

Sub-pathway	Metabolite	p-value
Fatty Acid, Monohydroxy	3-hydroxylaurate	0.0001
Chemical	S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.0002
Polyunsaturated Fatty Acid (n3 and n6)	docosahexaenoate (DHA; 22:6n3)	0.0003
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	dihydroxyacetone phosphate (DHAP)	0.0005
Phosphatidylethanolamine (PE)	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.0006
Methionine, Cysteine, SAM and Taurine Metabolism	N-acetyltaurine	0.0008
Polyunsaturated Fatty Acid (n3 and n6)	linoleate (18:2n6)	0.0008
Polyunsaturated Fatty Acid (n3 and n6)	linolenate [alpha or gamma; (18:3n3 or 6)]	0.0009
Fatty Acid, Monohydroxy	16-hydroxypalmitate	0.0009
Sphingolipid Metabolism	sphingomyelin (d18:1/18:1, d18:2/18:0)	0.0012
Polyunsaturated Fatty Acid (n3 and n6)	docosapentaenoate (n3 DPA; 22:5n3)	0.0013
Polyunsaturated Fatty Acid (n3 and n6)	arachidonate (20:4n6)	0.0014
Lysophospholipid	1-oleoyl-GPE (18:1)	0.0014
Polyunsaturated Fatty Acid (n3 and n6)	heneicosapentaenoate (21:5n3)	0.0016
Glycogen Metabolism	maltopentaose	0.0018
Pyrimidine Metabolism, Thymine containing	thymine	0.0018
Pantothenate and CoA Metabolism	3'-dephosphocoenzyme A	0.0023
Phosphatidylethanolamine (PE)	1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)*	0.0026
Glutamate Metabolism	glutamate, gamma-methyl ester	0.0029
Long Chain Fatty Acid	palmitate (16:0)	0.0029
Phosphatidylcholine (PC)	1,2-dilinoleoyl-GPC (18:2/18:2)	0.0031
Methionine, Cysteine, SAM and Taurine Metabolism	N-formylmethionine	0.0033
Lysophospholipid	1-arachidonoyl-GPI (20:4)*	0.0033
Lysine Metabolism	N-trimethyl 5-aminovalerate	0.0034
Phosphatidylcholine (PC)	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	0.0035
Ceramides	N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.0035
Endocannabinoid	N-oleoyltaurine	0.0036
Lysophospholipid	1-palmitoyl-GPE (16:0)	0.0036
Fatty Acid Metabolism (also BCAA Metabolism)	methylmalonate (MMA)	0.0039
Pantothenate and CoA Metabolism	pantetheine	0.0039
Secondary Bile Acid Metabolism	taurolithocholate	0.0044
Long Chain Fatty Acid	stearate (18:0)	0.0045
Glycogen Metabolism	maltohexaose	0.0054
Folate Metabolism	7,8-dihydrofolate	0.0054
Lysophospholipid	1-arachidonoyl-GPE (20:4n6)*	0.0057
Long Chain Fatty Acid	oleate/vaccenate (18:1)	0.0058
Pentose Metabolism	ribulose/xylulose	0.0061
Glycogen Metabolism	maltotetraose	0.0066
Phosphatidylethanolamine (PE)	1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	0.0066
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	glucose	0.0067
Glutathione Metabolism	cysteinylglycine	0.0070
Polyunsaturated Fatty Acid (n3 and n6)	dihomo-linolenate (20:3n3 or n6)	0.0071
Fructose, Mannose and Galactose Metabolism	mannitol/sorbitol	0.0073
Fatty Acid, Monohydroxy	3-hydroxylaurate	0.0079
Phosphatidylserine (PS)	1-stearoyl-2-oleoyl-GPS (18:0/18:1)	0.0101
Vitamin B6 Metabolism	pyridoxamine	0.0101

Supplementary Table 13. Top 46 metabolites exhibiting the most significant genotype: diet interactions. Metabolites are listed with sub-pathway to which they belong.

Genotype:Diet interaction

p<0.01 24 metabolites

0.01<p<0.05 62 metabolites

Sub Pathway	Biochemical Name	p-value
Diacylglycerol	palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [2]*	0.0000196
Pentose Phosphate Pathway	ribose 1-phosphate	0.0010000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*	0.0014000
Nucleotide Sugar	cytidine 5'-monophospho-N-acetylneuraminic acid	0.0017000
Diacylglycerol	diacylglycerol (12:0/18:1, 14:0/16:1, 16:0/14:1) [1]*	0.0023000
Methionine, Cysteine, SAM and Taurine Metabolism	hypotaurine	0.0032000
Purine Metabolism, (Hypo)Xanthine/Inosine containing	inosine	0.0034000
Sphingolipid Metabolism	lignoceroyl sphingomyelin (d18:1/24:0)	0.0037000
Pyrimidine Metabolism, Cytidine containing	5-methylcytidine	0.0037000
Diacylglycerol	stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]*	0.0049000
Fatty Acid Metabolism (Acyl Choline)	palmitoloelycholine	0.0050000
Histidine Metabolism	imidazole lactate	0.0058000
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	0.0061000
Pyrimidine Metabolism, Cytidine containing	cytidine	0.0061000
Diacylglycerol	oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	0.0078000
Diacylglycerol	oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	0.0078000
Diacylglycerol	stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]*	0.0079000
Fatty Acid Metabolism (Acyl Choline)	oleoylcholine	0.0081000
Diacylglycerol	diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	0.0084000
Histidine Metabolism	N-acetylhistidine	0.0085000
Diacylglycerol	oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]*	0.0089000
Tetrahydrobiopterin Metabolism	dihydrobiopterin	0.0089000
Monoacylglycerol	1-palmitoylglycerol (16:0)	0.0092000
Food Component/Plant	cinnamoylglycine	0.0099000
Phosphatidylcholine (PC)	1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*	0.0112000
Chemical	3-hydroxypyridine sulfate	0.0122000
Purine Metabolism, (Hypo)Xanthine/Inosine containing	hypoxanthine	0.0124000
Food Component/Plant	tartarate	0.0131000
Long Chain Fatty Acid	myristoleate (14:1n5)	0.0138000
Purine Metabolism, (Hypo)Xanthine/Inosine containing	urate	0.0139000
Nucleotide Sugar	UDP-galactose	0.0143000
Sphingolipid Metabolism	sphinganine	0.0158000
Diacylglycerol	palmitoyl-oleoyl-glycerol (16:0/18:1) [1]*	0.0160000
Dipeptide	valylglutamine	0.0164000
Monoacylglycerol	1-docosahexaenoylglycerol (22:6)	0.0164000
Diacylglycerol	palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]*	0.0166000
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	fructose-6-phosphate	0.0167000
Phospholipid Metabolism	cytidine-5'-diphosphoethanolamine	0.0168000
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	0.0173000
Lysophospholipid	1-palmitoyl-GPS (16:0)*	0.0176000
Polyunsaturated Fatty Acid (n3 and n6)	docosadienoate (22:2n6)	0.0179000
Lysine Metabolism	pipecolate	0.0180000
Diacylglycerol	palmitoyl-oleoyl-glycerol (16:0/18:1) [2]*	0.0195000
Pentose Phosphate Pathway	sedoheptulose-7-phosphate	0.0202000
Phospholipid Metabolism	choline phosphate	0.0214000
Fatty Acid, Dicarboxylate	azelate (C9-DC)	0.0215000

Supplementary Table 14. The fourty-four metabolites exhibiting the most significant genotype: sex: diet interactions. Metabolites are listed with sub-pathway to which they belong.

Genotype:Sex:Diet interaction

p<0.01 11 metabolites

0.01<p<0.05 33 metabolites

Sub Pathway	Biochemical Name	p-value
Eicosanoid	12-HETE	0.0007000
Ceramides	N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.0012000
Diacylglycerol	oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	0.0029000
Diacylglycerol	stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [2]*	0.0037000
Phosphatidylserine (PS)	1-stearoyl-2-oleoyl-GPS (18:0/18:1)	0.0042000
Glutathione Metabolism	5-oxoproline	0.0057000
Glutathione Metabolism	glutathione, oxidized (GSSG)	0.0066000
Carnitine Metabolism	carnitine	0.0067000
Guanidino and Acetamido Metabolism	4-guanidinobutanoate	0.0074000
Lysophospholipid	1-stearoyl-GPI (18:0)	0.0087000
Pyrimidine Metabolism, Uracil containing	3-ureidopropionate	0.0097000
Phosphatidylethanolamine (PE)	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.0103000
Glutamate Metabolism	pyroglutamine*	0.0107000
Eicosanoid	12-HHTrE	0.0112000
Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	0.0125000
Tryptophan Metabolism	kynurenate	0.0137000
Histidine Metabolism	formiminoglutamate	0.0142000
Glycine, Serine and Threonine Metabolism	betaine aldehyde	0.0144000
Phospholipid Metabolism	glycerophosphoinositol*	0.0154000
Diacylglycerol	oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	0.0157000
Fatty Acid, Dihydroxy	12,13-DiHOME	0.0183000
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.0187000
Fatty Acid Synthesis	2-methylmalonylcarnitine (C4-DC)	0.0197000
Eicosanoid	prostaglandin F2alpha	0.0234000
Phosphatidylinositol (PI)	1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	0.0248000
Food Component/Plant	N-glycolylneuraminate	0.0249000
Aminosugar Metabolism	N-acetylglucosamine/N-acetylgalactosamine	0.0273000
Diacylglycerol	stearoyl-linoleoyl-glycerol (18:0/18:2) [2]*	0.0275000
Fatty Acid Metabolism (Acyl Choline)	palmitoloelycholine	0.0284000
Glutathione Metabolism	cysteine-glutathione disulfide	0.0293000
Phospholipid Metabolism	choline	0.0303000
Histidine Metabolism	N-acetyl-3-methylhistidine*	0.0312000
Leucine, Isoleucine and Valine Metabolism	tiglylcarnitine (C5:1-DC)	0.0336000
Tryptophan Metabolism	picolinate	0.0367000
Ceramides	N-erucoyl-sphingosine (d18:1/22:1)*	0.0371000
Phosphatidylethanolamine (PE)	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	0.0385000
Sphingolipid Metabolism	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	0.0386000
Pyrimidine Metabolism, Cytidine containing	5-methyl-2'-deoxycytidine	0.0404000
Leucine, Isoleucine and Valine Metabolism	ethylmalonate	0.0408000
Gamma-glutamyl Amino Acid	gamma-glutamyltryptophan	0.0421000
Aminosugar Metabolism	N-acetylneuraminate	0.0424000
Lysine Metabolism	glutarylcarnitine (C5-DC)	0.0457000
Lysophospholipid	1-stearoyl-GPC (18:0)	0.0461000
Plasmalogen	1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	0.0500000
Histidine Metabolism	4-imidazoleacetate	0.0509000
Phosphatidylethanolamine (PE)	1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	0.0525000