Supporting information

Supplemental Tables

Table S1 Compositions of the experimental diets (g/100 g of diet)

Ingredients	CON	HFD	LMD
L-arginine	1.12	1.12	1.12
L-histidine-HCl-H_2O	0.33	0.33	0.33
L-isoleucine	0.82	0.82	0.82
L-leucine	1.11	1.11	1.11
L-lysine	1.44	1.44	1.44
L-methionine[1]	0.86	0.86	0.17
L-phenylalanine	1.16	1.16	1.16
L-threonine	0.82	0.82	0.82
L-tryptophan	0.18	0.18	0.18
L-valine	0.82	0.82	0.82
L-glutamic acid[1]	2.70	2.70	3.39
L-glycine	2.33	2.33	2.33
Corn starch	47.25	31.25	31.25
Maltodextrin	5.00	5.00	5.00
Dextrose	20.00	20.00	20.00
Cellulose	5.00	5.00	5.00
Lard	4.00	20.00	20.00
Mineral mix[2]	3.50	3.50	3.50

Vitamin mix[2]	1.00	1.00	1.00
Choline bitartrate	0.20	0.20	0.20

[1]When the methionine content in the diet was decreased, the glutamic acid content was increased on an equal mass.

[2]Mineral mix and Vitamin mix were prepared based on the AIN-93 diet.

Table S2 Sequences of primers used in quantitative real-time reverse transcription PCR

Gene symbol	Forward primer (5′–3′)	Reverse primer (5′–3′)
MyoG	GTAAGGTGTGTAAGAGGAAG	GCTCAATGTACTGGATGG
PI3K	TGCTCCGTAGTGGTAGAC	GTATGCTAGTGTGACATTGAG
Akt	CGGGCACATCAAGATAACG	CGTGGTCCTGGTTGTAGAAG
S6K1	GGCAATGATAGTGAGGAATG	CGGTCTGAAAGGCATAAATC
4EBP1	AGCCGTAGGACGCAATGATG	GGTATAGACAGAGGCACAAGGAGG
FOXO3a	TAAGTGTCGTCTTGTGTTTGTTTCC	CTTCTGCTTTTAAGTGTGCTAGGGA
MAFbx	AGCATCCAACTCAAGTCACCCT	ATCTCCTTCTCCTTTCTTCCACA
MuRF1	CCTGGACGAGAAGAAGAGCGAG	TTGGCACTTGAGAGGAAGGTAGC
TPO	CACTGGTCCTCTGTTTGCATGTAT C	TAGTTCCTGCCTCTGAGCTCTG
NIS	CTTGCTCTTCTTGCCGATCTTCTAC	TGCGTAGATCACGATGCCAGTATA
TGB	ATCCTGCCCACCCAGAATCAA	GGGGAAAACTGCTCTGATGGC
TSHR	GCTTCTCATTGCCTCTGTAGACCT C	AGGGTGATGACCGTCAGTGTGT

DIO2	CTTCCTCCTAGATGCCTACAAAC	GGCATAATTGTTACCTGATTCAGG
TRα1	GGTGCTGCATGGAGATCATG	GGAATGTTGTGTTTGCGGTG
β-actin	GGGTCAGAAGGACTCCTATG	GTAACAATGCCATGTTCAAT

9

10 **Table S3** Systemic endogenous metabolites of mice in urine and plasma

Keys	Metabolites	Moieties	δ^1H (ppm) and multiplicity	Samples[a]
1	Bile acids	CH_3	0.64(m), 0.75(m)	U
2	α-Hydroxy-iso-valerate	δCH_3	0.83(d), 0.97(d)	U
3	Valerate	δCH_3, γCH_2, βCH_2, αCH_2	0.88(t), 1.31(m), 1.61(m), 2.28(t)	U
4	α-Hydroxybutyrate	CH_3	0.89(t)	U
5	Butyrate	CH_3	0.92(t)	U
6	2-Ketoisocaproate	CH_3, CH, CH_2	0.93(dd), 2.12(m), 2.59(m)	U
7	Propionate	CH_3, CH_2	1.06(t), 2.18(q)	U, P
8	2-Keto-isovalerate	CH_3, CH_2	1.11(d), 3.03(m)	U
9	Isobutyrate	CH_3	1.14(d)	U, P
10	Ethanol	CH_3, CH_2	1.18(t), 3.66(q)	U, P
11	3-Hydroxybutyrate	γCH_3, αCH_2, βCH	1.20(d), 2.28(dd), 2.42(dd), 4.16(m)	U, P
12	Methylmalonate	CH_3, CH	1.24(d), 3.75(m)	U
13	Lactate	βCH_3, αCH	1.33(d), 4.13(q)	U, P
14	2-Hydroxyisobutyrate	CH_3	1.36(s)	U
15	Alanine	βCH_3, αCH	1.48(d), 3.77(q)	U, P
16	Citrulline	γCH_2, βCH_2	1.56(m), 1.82(m)	U, P

17	Putrescine	CH_2, CH_2-NH_2	1.78(m), 3.06(t)	U
18	N-Acetylglutamate	γCH_2, CH_3, βCH_2	1.88(m), 2.04(s), 2.07(m)	U
19	Acetate	CH_3	1.92(s)	U, P
20	Acetamide	CH_3	2.00(s)	U
21	Acetone	CH_3	2.24(s)	U, P
22	Acetoacetate	CH_3	2.28(s)	U, P
23	Pyruvate	CH_3	2.37(s)	U, P
24	Succinate	CH_2	2.41(s)	U
25	α-Ketoglutarate	βCH_2, γCH_2	2.45(t), 3.01(t)	U
26	Citrate	CH_2	2.55(d), 2.68(d)	U, P
27	Methylamine	CH_3	2.60(s)	U, P
28	Dimethylamine	CH_3	2.71(s)	U, P
29	Sarcosine	CH_3, CH_2	2.76(s), 3.65(s)	U
30	Succinimide	CH_2	2.78(s)	U
31	Methylguanidine	CH_3	2.84(s)	U
32	Trimethylamine	CH_3	2.88(s)	U, P
33	Dimethylglycine	CH_3	2.93(s)	U
34	Creatine	CH_3, CH_2	3.04(s), 3.93(s)	U, P
35	Creatinine	CH_3, CH_2	3.05(s), 4.05(s)	U, P
36	Ethanolamine	CH_2	3.13(t)	U
37	Malonate	CH_2	3.16(s)	U
38	Choline	OCH_2, NCH_2, $N(CH_3)_3$	4.07(t), 3.53(t), 3.20(s)	U, P

39	Phosphocholine	$N(CH_3)_3$, OCH_2, NCH_2	3.23(s), 4.21(t), 3.61(t)	U
40	Taurine	$-CH_2-S$, $-CH_2-NH_2$	3.27(t), 3.43(t)	U, P
41	Trimethylamine-N-oxide	CH_3	3.30(s)	U, P
42	Phenylacetate	CH_2, 2,6-CH, 4-CH, 3,5-CH	3.55(s), 7.28(m), 7.29(m), 7.32(m)	U
43	Glycine	CH_2	3.57(s)	U, P
44	p-Hydroxyphenylacetate	6-CH, 2-CH, 3,5-CH	3.6(s), 6.87(d), 7.15(d)	U
45	Phenylacetyglycine	2,6-CH, 3,5-CH, 7-CH, 10-CH	7.31(t), 7.37(m), 7.42(m), 3.68(s)	U
46	Guanidoacetate	CH_2	3.81(s)	U
47	Hippurate	CH_2, 3,5-CH, 4-CH, 2,6-CH	3.97(d), 7.57(t), 7.65(t), 7.84(d)	U
48	Glycolate	CH_2	3.96(s)	U
49	Inosine	8-CH, 2-CH, 1−CH, 2−CH, 3-CH, 4-CH, CH_2	8.24(s), 8.35(s), 6.05(d), 4.79(m), 4.46(dd), 4.28(m), 3.87(dd)	U
50	N-Methylnicotinamide	CH_3, 5-CH, 4-CH, 6-CH, 2-CH	4.51(s), 8.19(m), 8.91(m), 8.97(d), 9.28(s)	U
51	β-Glucose	1-CH, 2-CH, 3-CH, 4-CH, 5-CH, 6-CH	4.65(d), 3.25(dd), 3.49(t), 3.41(dd), 3.46(m), 3.73(dd), 3.90(dd)	U, P
52	α-Glucose	1-CH, 2-CH, 3-CH, 4-CH, 5-CH, 6-CH	5.24(d), 3.54(dd), 3.71(dd), 3.42(dd), 3.84(m), 3.78(m)	U, P
53	4-Cresol glucuronide	CH_3, 2, 6-CH, 3, 5-CH, 5-CH, 4-CH, 3-CH	2.30(s), 7.05(d), 7.23(d), 5.07(d), 3.61(m), 3.89(m)	U
54	Allantoin	CH	5.40(s), 6.05(s)	U

55	Cis-aconitate	CH_2, CH	3.12(s), 5.68(s)	U
56	Urea	NH_2	5.80(s)	U
57	Orotate	CH	6.19(s)	U
58	Fumarate	CH, CH_3	6.53(s)	U, P
59	Trans-aconitate	CH_2, CH	3.44(s), 6.60(s)	U
60	N1-methyl-4-pyridone-5-carboxamide	N-CH_3, 3-CH, 2-CH, 6-CH	3.89(s), 6.71(d), 7.83(dd), 8.55(d)	U
61	N1-methyl-2-pyridone-5-carboxamide	N-CH_3, 3-CH, 4-CH, 6-CH	3.64(s), 6.67(d), 7.96(dd), 8.33(d)	U
62	4-Hydroxyphenylpyruvate	CH_2, 3, 5-CH, 2, 6-CH	4.01(s), 6.81(m), 7.18(m)	U
63	2-(4-Hydroxyphenyl)propanoate	CH_3, CH, 3, 5-CH, 2, 6-CH	1.37(d), 3.58(q), 6.83(m), 7.18(m)	U
64	3-Methylhistidine	4-CH, 2-CH	7.12(s), 7.67(s)	U
65	Indoxyl sulfate	4-CH, 5-CH, 6-CH, 7-CH, CH_3	7.51 (m), 7.22 (m), 7.28 (m), 7.71 (m), 7.37 (s)	U
66	m-Hydroxyphenylacetate	6-CH, 4-CH, 3-CH	6.92(m), 7.05(d), 7.27(t)	U
67	Nicotinate	2,6-CH, 4-CH, 5-CH	8.62(d), 8.25(d), 7.50(dd)	U
68	Guanine	CH	7.68(s)	U
69	4-Aminohippurate	CH_2	7.71(d)	U
70	Nicotinamide N-oxide	5-CH, 6-CH, 2-CH, 4-CH	7.74(m), 8.12(m), 8.75(m), 8.49(m)	U
71	Formate	CH	8.46(s)	U, P

72	Trigonelline	2-CH, 4-CH, 6-CH, 5-CH, CH_3	9.12(s), 8.85(m), 8.83(dd), 8.19(m), 4.44(s)	U
73	LDL/ VLDL	$CH_3(CH_2)_n$, $CH_3CH_2CH_2C=$	0.88(m), 0.90(t)	P
74	Leucine	αCH, βCH_2, γCH, δCH_3	3.73(t), 1.72(m), 0.96(d), 0.91(d)	P
75	Isoleucine	αCH, βCH, βCH_3, γCH_2, δCH_3	3.68(d), 1.99(m), 1.01(d), 1.26(m), 1.47(m), 0.94(t)	P
76	Valine	αCH_3, βCH, γCH_3	3.62(d), 2.28(m), 0.99(d), 1.04(d)	P
77	Lipids	CH_2*CH_2CO, CH_2-C=C CH_2-C=O, CH-O-CO	1.29(m), 1.58(m), 2.02(m) 2.25(m), 2.77(m)	P
78	Threonine	αCH, βCH, γCH_3	1.32(d), 4.25(m), 3.58(d)	P
79	Lysine	αCH, βCH_2, γCH_2, δCH_2	3.77(t), 1.89(m), 1.73(m)	P
80	N-Acetyl glycoprotein	CH_3	2.05(s)	P
81	O-Acetyl glycoprotein	CH_3	2.09(s)	P
82	Glutamate	αCH, βCH_2, γCH_2	3.75(m), 2.12(m), 2.35(m)	P
83	Methionine	αCH, βCH_2, γCH_2, S-CH_3	3.87(t), 2.16(m), 2.65(t), 2.14(s)	P
84	Glutamine	αCH, βCH_2, γCH_2	3.68(t), 2.15(m), 2.45(m)	P
85	Albumin	Lysyl-CH_2	3.02(s)	P
86	Glycerophosphorylcholine	N-$(CH_3)_3$, OCH_2, NCH_2	3.22(s), 4.33(t), 3.51(t)	P
87	Betaine	CH_3, CH_2	3.28(s), 3.90(s)	P
88	Proline	βCH_2, γCH_2, δCH_2	2.02-2.33(m), 2.00(m), 3.35(t)	P
89	Ornithine	CH_2, αCH	3.80(s), 3.79(t)	P
90	Myo-Inositol	5-CH, 4,6-CH, 2-CH	3.30(t), 3.63(t), 4.06(t)	P

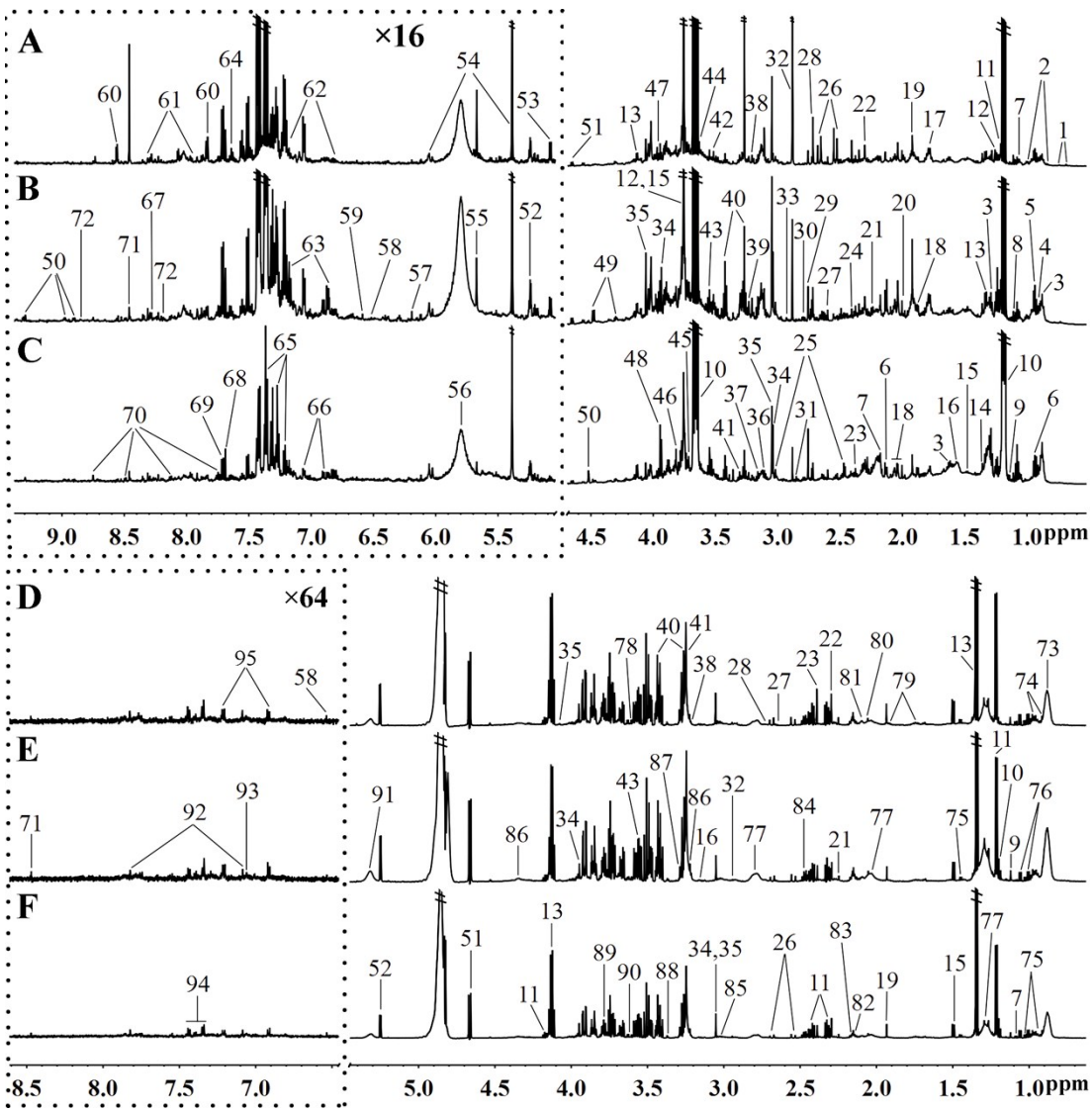
91	Unsaturated lipids	=C-CH2-C=, -CH=CH-	5.19(m), 5.31(m)	P
92	1-Methylhistidine	4-CH, 2-CH	7.05(s), 7.75(s)	P
93	3-Methylhistidine	4-CH, 2-CH	7.07(s), 7.67(s)	P
94	Phenylalanine	2,6-CH, 3,5-CH, 4-CH	7.32 (m), 7.42 (m), 7.37 (m)	P
95	Tyrosine	2,6-CH, 3,5-CH	7.19(dd), 6.90(d)	P

11 U, urine; P, plasma; s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet;

12 LDL, low density lipoprotein; VLDL, low density lipoprotein.

13

14 **Supplemental Figures**



15

Fig. S1 Representative 600 MHz ^{1}H NMR spectra of urine (A, CON; B, HFD; C, MRD) and plasma (D, CON; E, HFD; F, MRD) samples. CON, the control group; HFD, the high fat diet group; MRD, the high fat + methionine-restricted diet group. The dashed boxes were perpendicularly enlarged 16 times in the spectra of urine samples, 64 times in the plasma samples. The keys for metabolites are given in Table S3.