

## Supplemental Information

**Caecal butyrate (not propionate) was connected with metabolism related chemicals of mice, based on the different effects and mechanisms of the two *Inonotus obliquus* extracts on obesity**

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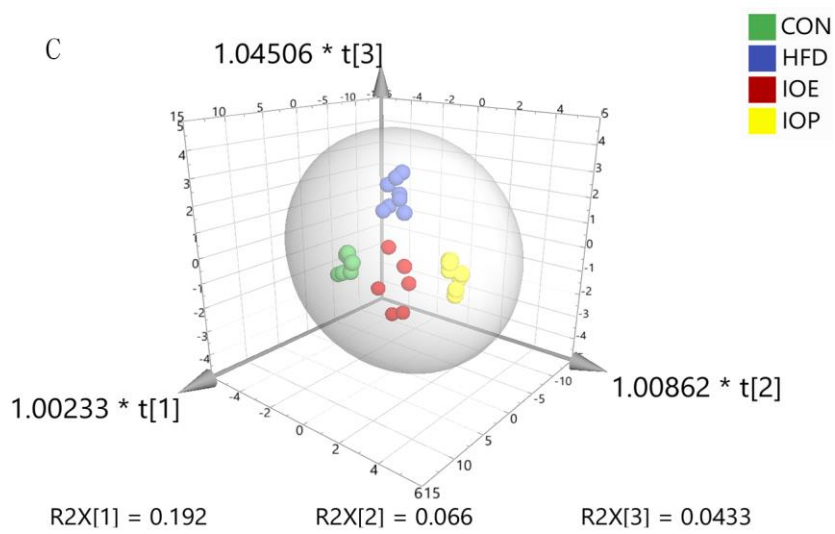
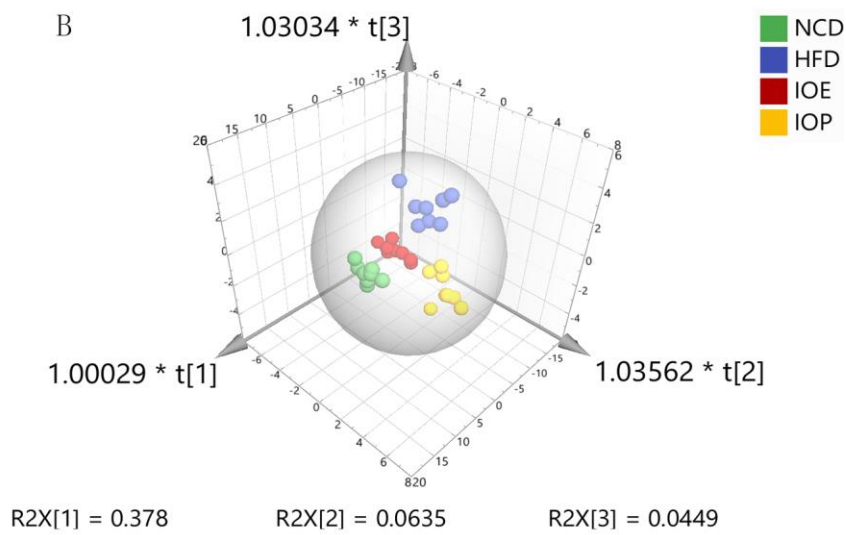
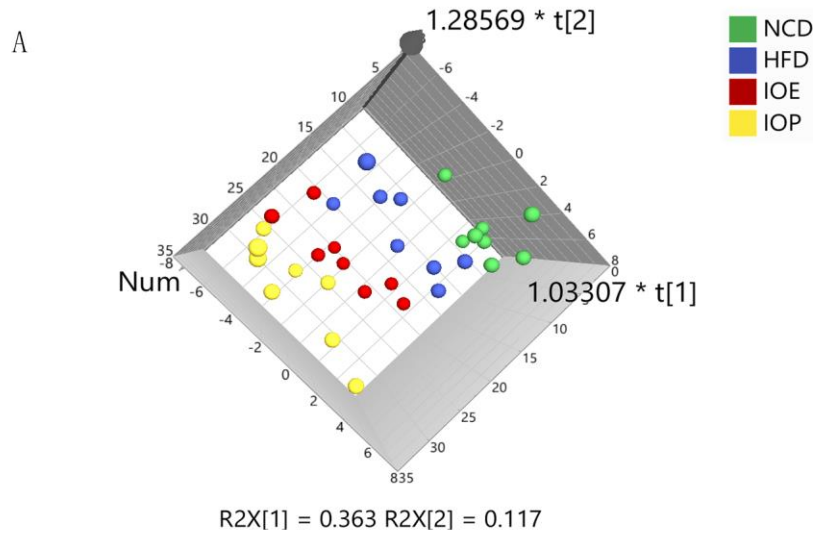


Figure S1. 3D score plots of the OPLS-DA classification of (A) serum samples (B) urine samples and



Figure S2. VIP plot of OPLS-DA based on NMR data from (A) serum samples (B) urine samples and (C) caecum contents samples.

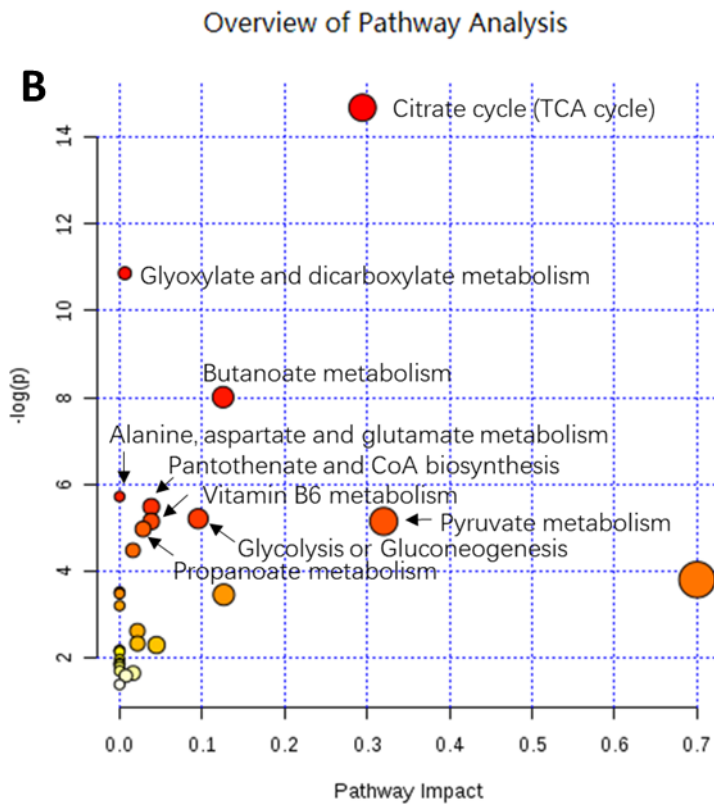
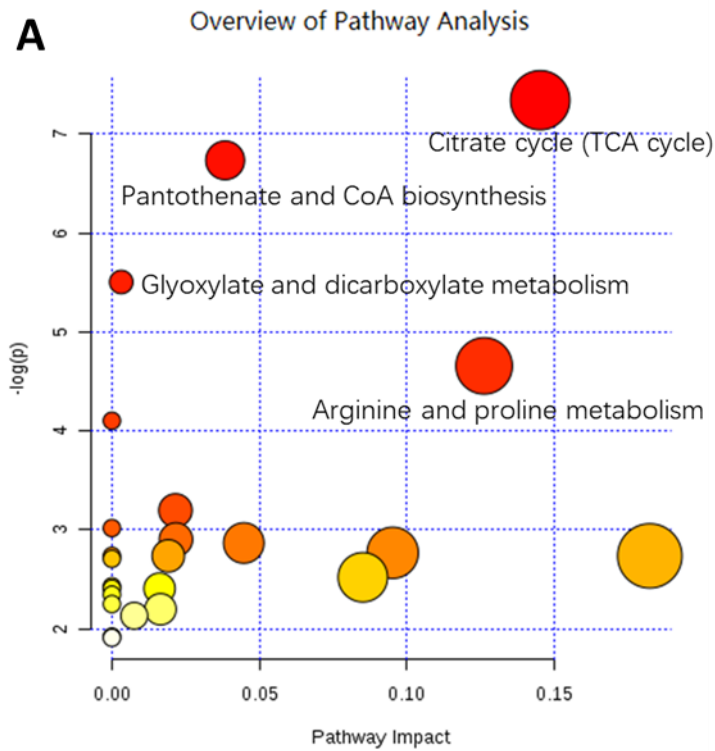


Figure S3. Pathway analysis was based on different metabolites (A) between HFD group and IOE group

and (B) between HFD group and IOP group

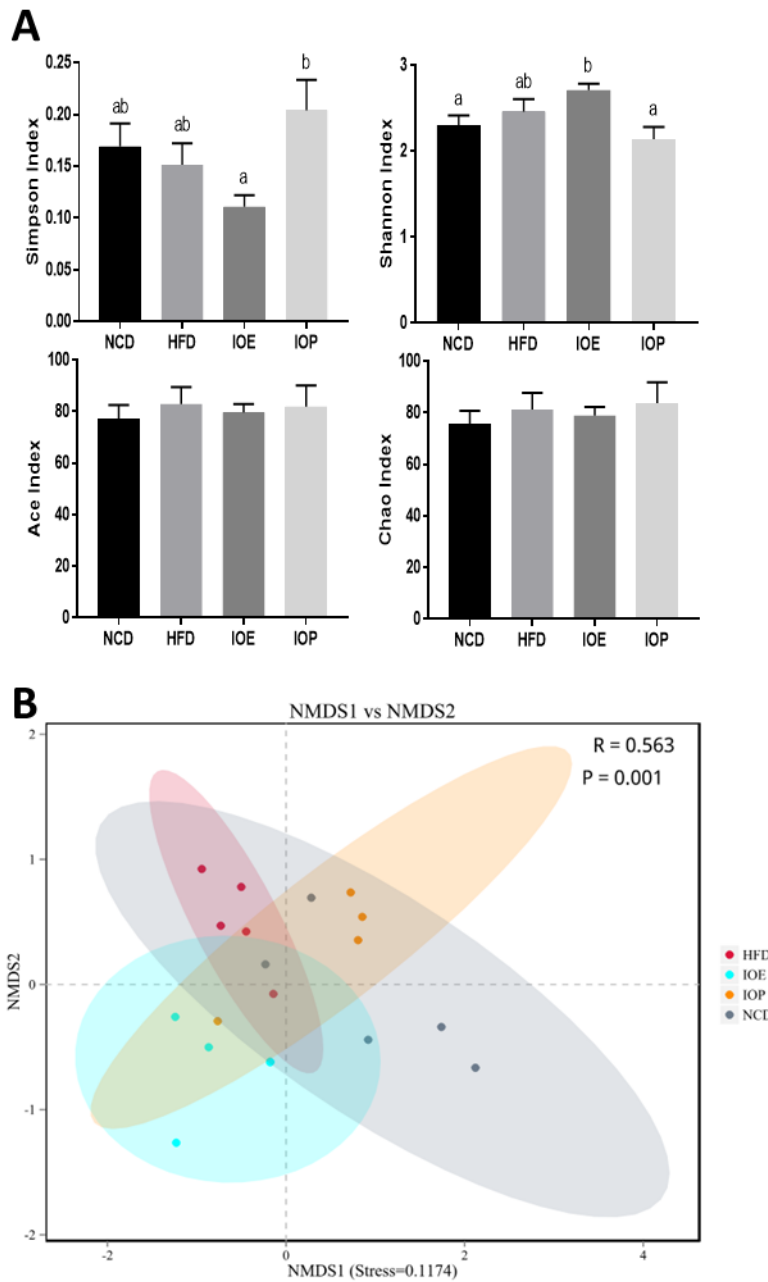


Figure S4. Effects of IOE/IOP on gut microbial diversity. (A) Alpha diversity and (B) beta diversity of caecal microbiota. The data were expressed as the mean  $\pm$  SEM. Significance was determined by one-way ANOVA statistical analysis ( $P < 0.05$ ).

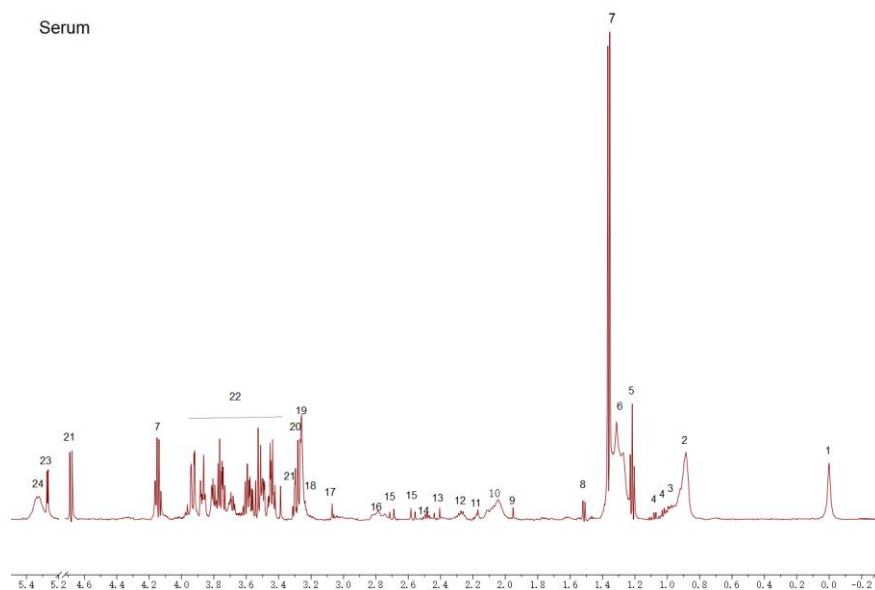


Figure S5. 600 MHz  $^1\text{H}$  NMR spectra of mice serum. Keys: 1, TSP; 2, Lipid( $\text{CH}_3$ -); 3, leucine; 4, valine; 5, Ether; 6, Lipid( $-\text{CH}_2$ -); 7, Lactate; 8, Alanine; 9, Acetate; 10, Lipid(  $-\text{CH}_2\text{-C=O}$ ); 11, glutamine; 12, lipid(  $-\text{CH}_2\text{-CH=CH-}$ ); 13, Succinate; 14, 2-Oxoglutarate; 15, Citrate; 16, Lipid(  $=\text{CH-CH}_3\text{-CH=}$ ); 17, Creatine; 18, choline; 19, glycerophosphocholine (GPC); 20, trimethylamine N-oxide (TMAO); 21,  $\beta$ -glucose; 22, glucose; 23,  $\alpha$ -Glucose; 24, Unsaturated fatty acid.

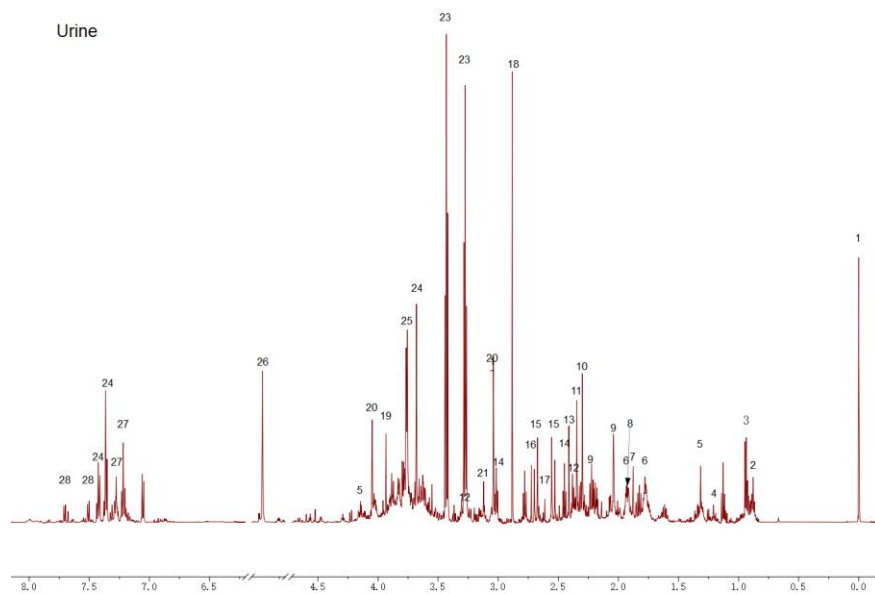


Figure S6. 600 MHz  $^1\text{H}$  NMR spectra of mice urine. Keys: 1, TSP; 2, Butyrate; 3, Isoleucine; 4, 3-hydroxybutyrate; 5, Lactate; 6, Arginine; 7, Thymine; 8, Acetate; 9, N-acetylglutamate (NAG); 10, Acetoacetate; 11, Pyruvate; 12, 3-Ureidopropionate; 13, Succinate; 14, 2-Oxoglutarate; 15, Citrate; 16, Dimethylamine (TMA); 17, Methylamine; 18, Trimethylamine (TMA); 19, Creatine; 20, Creatinine; 21, Cis-aconitate; 22, Choline; 23, Taurine; 24, PhenylAcetyl Glycine (PAG); 25, Amino acid; 26, Allatoin; 27, 3-Indoxylsulfate; 28, N-methylnicotinamide (NMND).

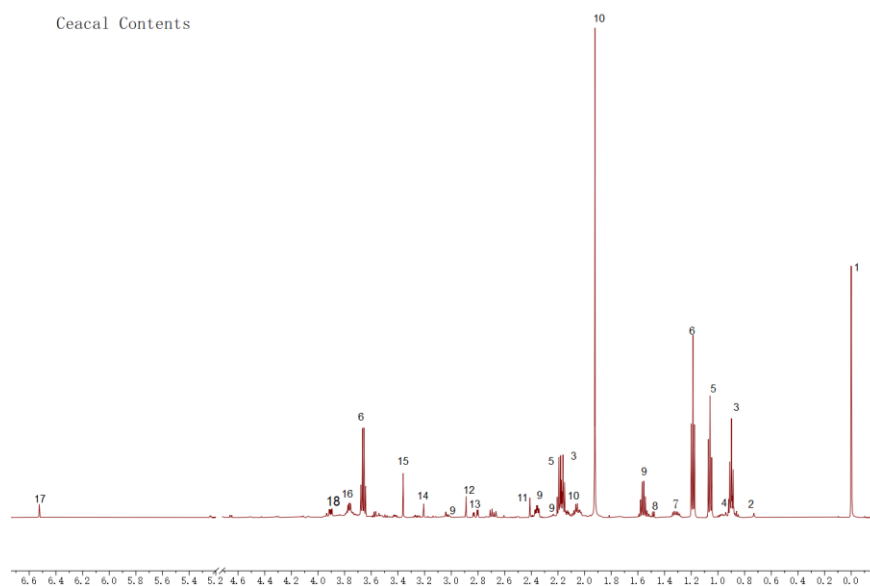


Figure S7. 600 MHz <sup>1</sup>H NMR spectra of mice caecal contents. Keys:1, TSP; 2, Bile acids; 3, Butyrate; 4, Branched chain amino acids (BCAA); 5, Propionate; 6, Ethanol; 7, Lactate; 8, Alanine; 9, 5-aminovalerate; 10, Glutamine; 11, Succinate; 12, Trimethylamine (TMA); 13, Aspartate; 14, Choline; 15, Glycerylphosphocholine (GPC) ; 16, Amino acid; 17, Fumarate; 18,  $\beta$ -Glucose..



Table S1. Main metabolites of serum (VIP &gt; 2)

Compartments	Metabolite	<sup>1</sup> H(ppm)	Multiplicity	Metabolic pathway	Remarks
Serum	LDL/VLDL	0.805-0.95	m	Lipid metabolism	CH <sub>3</sub> -
Serum	LDL/VLDL	1.245-1.33	m	Lipid metabolism	-(CH <sub>2</sub> ) <sub>n</sub> -
Serum	Lactate	1.36	d	Carbohydrate metabolism	
Serum	Glucose + Choline	3.20-3.32	m		
Serum	Glucose	3.39-3.95	m	Carbohydrate metabolism	
Serum	Lactate	4.16	q	Carbohydrate metabolism	
Serum	β-Glucose	4.68	d	Carbohydrate metabolism	
Serum	α-Glucose	5.26	d	Carbohydrate metabolism	

Table S2. Main metabolites of urine (VIP &gt; 2)

Compartments	Metabolite	<sup>1</sup> H(ppm)	Multiplicity	Metabolic pathway	Remarks
Urine	2-Oxoglutarate	2.45	t	Citrate cycle (TCA cycle)	
Urine	2-Oxoglutarate	3.01	t	Citrate cycle (TCA cycle)	
Urine	3-Ureidopropionate	2.38	t	Pyrimidine metabolism	Substrates for the synthesis of CoA
Urine	3-Ureidopropionate	3.2	q	Pyrimidine metabolism	Substrates for the synthesis of CoA
Urine	Acetoacetate	2.3	s	Fatty acid degradation	
Urine	Allatoxin	5.38	s	Purine metabolism	Metabolites of ATP/GTP
Urine	Amino acid	3.77	t	Amino acid metabolism	α-H
Urine	Arginine	1.78	m	Amino acid metabolism	Important Compounds of Urea cycle
Urine	Arginine + Acetate	1.91-1.96	m + s		
Urine	Cis-aconitate	3.12	s	Citrate cycle (TCA cycle)	
Urine	Citrate	2.56	d	Citrate cycle (TCA cycle)	
Urine	Citrate	2.68	d	Citrate cycle (TCA cycle)	
Urine	Creatine + Creatinine	3.04-3.055	s + s		Substrate level phosphorylation
Urine	Creatinine	4.06	s		Substrate level phosphorylation
Urine	Dimethylamine	2.72	s	Microbial metabolism	
Urine	N-acetylglutamate (NAG)	2.04	s	Amino acid metabolism	
Urine	N-acetylglutamate (NAG)	2.22	t	Amino acid metabolism	
Urine	PAG + 3-Indoxylsulfate	7.35-7.385	m		
Urine	phenylacetyl glycine (PAG)	3.68	s	Amino acid metabolism	
Urine	Pyruvate	2.35	s	Glycolysis / Gluconeogenesis	

Urine	Succinate	2.41	s	Citrate cycle (TCA cycle)
Urine	Taurine	3.27	t	Primary bile acid biosynthesis
Urine	Taurine	3.43	t	Primary bile acid biosynthesis
Urine	Thymine	1.88	s	Pyrimidine metabolism
Urine	trimethylamine	2.88	s	Microbial metabolism
Urine	Unknown Metabolite	2.78	t	

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Table S3. Main metabolites of Caecum contents (VIP &gt; 2)

Compartments	Metabolite	<sup>1</sup> H(ppm)	Multiplicity	Metabolic pathway	Remarks
Caerum	Butyrate	0.89	t	Carbohydrate metabolism	Short-chain fatty acids
Caerum	Leucine + Isoleucine	0.92-1.03	m	Amino acid metabolism	
Caerum	Propionate	1.06	t	Carbohydrate metabolism	Short-chain fatty acids Metabolites
Caerum	Ethanol	1.19	t	Carbohydrate metabolism	of Short-chain fatty acids
Caerum	Lactate	1.34	d	Carbohydrate metabolism	
Caerum	Alanine	1.48	d	Amino acid metabolism	
Caerum	5-aminovalerate	1.65	m	Amino acid metabolism	
Caerum	Acetate	1.92	s	Carbohydrate metabolism	Short-chain fatty acids
Caerum	Butyrate + Propionate	2.14-2.20	m	Carbohydrate metabolism	Short-chain fatty acids
Caerum	5-aminovalerate	2.24	t	Amino acid metabolism	
Caerum	Succinate	2.41	s	Carbohydrate metabolism	
Caerum	5-aminovalerate	3.02	t	Amino acid metabolism	
Caerum	Choline	3.21	s	Lipid metabolism	
Caerum	Taurine	3.27	t	Metabolism of other amino acids	
Caerum	Methanol	3.36	s	Methane metabolism	Metabolites of Short-chain fatty acids
Caerum	Taurine	3.43	t	Metabolism of other amino acids	

Caerum	Ethanol	3.67	q	Carbohydrate metabolism	Metabolites of Short-chain fatty acids
Caerum	Amino acid ( $\alpha$ -H)	3.77	t	Amino acid metabolism	

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Table S4. The compositions of NCD

	gm	kcal	Kcal/gm	gm%	kcal%
H <sub>2</sub> O	100	0			
Crude Protein	180	720			
Crude Fat	40	360			
Crude Fibre	50	0			
Vitamin	0.1	0.4			
Choline	1.25	0			
Inorganic Salt	40	0			
Crude Starch	450	1800			
Total	861.4	2880.4	3.34		
Protein				21	25
Carbohydrate				52	62.5
Fat				5	12.5

Table S5. The compositions of HFD

	gm	kcal	Kcal/gm	gm%	kcal%
Casein, 30 Mesh	200	800			
L-Cystine	3	12			
Corn Starch	72.8	291			
Maltodextrin 10	100	400			
Sucrose	172.8	691			
Cellulose, BW200	50	0			
Soybean Oil	25	225			
Lard	177.5	1598			
Mineral Mix S10026	10	0			
DiCalcium Phosphate	13	0			
Calcium Carbonate	5.5	0			
Potassium Citrate, 1 H <sub>2</sub> O	16.5	0			
Vitamin Mix V10001	10	40			
Choline Bitartrate	2	0			
FD&C Red Dye #40	0.05	0			
Total	858.15	4057	4.73		
Protein				24	20
Carbohydrate				41	35
Fat				24	45

Table S6. Primer sequence for amplification of the fragments

Primer	Forward sequence	Reverse sequence
Chrebp	CCCTCAGACACCCACATCTT	CAGAGCTCAGAAAGGGGTTG
GK	GGCCACCAAGAAGGAAAAGGT	CCTCTCCCACCTTGACCAGCA
PK	CAACAGGAAGGGTGTGAACTTG	ACAAAGGAGGCAAAGATGATGT
PEPCK	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCCGTACTCC
PC	CTGAAGTTCCAAACAGTTCGAGG	CGCACGAAACACTCGGATG
FBPase	CACCGCGATCAAAGCCATCT	AGGTAGCGTAGGACGACTTCA
G6Pase	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Ppar- $\gamma$	TTCGCTGATGCACTGCCTATG	CGAAGTTGGTGGGCCAGAA
Srebp1c	TATGGAGGGCATGAAACCCGAAGT	TTGACCTGGCTATCCTCAAAGGCT
Fas	GCAGCAAGTGTCCACCAACAA	CTCATCGGAGCGCAGGATAGA
Acl	AGGAAGTGCCACCTCCAACAGT	CGCTCATCACAGATGCTGGTCA
Scd1	TCCCTCCGGAAATGAACGAGAGAA	AGTGCAGCAGGACCATGAGAATGA
Acc	TAACAGAATCGACACTGGCTGGCT	ATGCTGTTCCCTCAGGCTCACATCT
Acox	GCCCAACTGTGACTTCCATT	GGCATGTAACCCGTAGCACT
Ppar- $\alpha$	CCTCAGGGTACCACTACGGAGT	GCCGAATAGTTCGCCGAA
Cpt1	CGCACGGAAGGAAAATGG	TGTGCCCAATATTCCTGG
Mcad	GAGCCTGGGAACCTCGGCTTGA	GCCAAGGCCACCGCAACTTT
Dgat1	GACGGCTACTGGGATCTGA	TCACCACACACCAATTCAGG
Dgat2	CGCAGCGAAAACAAGAATAA	GAAGATGTCTTGGAGGGCTG
Gpat	CAACACCATCCCCGACATC	GTGACCT TCGATTATGCGATCA
18S	ACGGAAGGGCACCACCAGGA	CACCACCACCCACGGAATCG



Table S7. Metabolite identifications

Metabolites	<sup>1</sup> H (ppm) & Multiplicity
Bile acids	0.72(m)
Lipid(CH <sub>3</sub> -)	0.86(Br)
Butyrate	0.89 (t),1.55 (m),2.16 (t)
Branched chain amino acids (BCAA)	0.9–1.05 (m)
Isoleucine	0.94 (t)
Valine	0.97 (d), 1.02 (d)
Propionate	1.05 (t),2.19 (q)
Ether	1.19(t)
Ethanol	1.19(t), 3.63(q)
3-hydroxybutyrate	1.20(d)
Lipid(-CH <sub>2</sub> -)	1.28 (Br)
Lactate	1.32 (dd), 4.10 (q)
Alanine	1.46 (d), 3.78 (q)
5-aminovalerate	1.65 (m),2.24 (t), 3.02 (t)
Arginine	1.72(m); 1.90(m); 3.25(t); 3.76(t)
Thymine	1.88 (s)
N-acetylglutamate (NAG)	1.89 (m), 2.06 (m), 2.1 (m)
Acetate	1.91 (s)
Lipid( -CH <sub>2</sub> -CH=CH-)	2.02 (br)
Glutamine	2.14(m); 2.45(m); 3.77(t)
Lipid( -CH <sub>2</sub> -C=O)	2.22(br)
Acetoacetate	2.28(s)
Pyruvate	2.37(s)
3-Ureidopropionate	2.38 (t), 3.20 (q)
Succinate	2.41(s)
2-Oxoglutarate	2.43 (t), 2.99 (t)
Citrate	2.53 (d), 2.67 (d)
Methylamine	2.60(s)
Dimethylamine	2.72 (s)
Lipid( =CH-CH <sub>3</sub> -CH=)	2.79 (br)
Aspartate	2.82 (d)
Trimethylamine (TMA)	2.88 (s)
Creatine	3.04(s), 3.93(s)
Creatinine	3.06(s), 4.06(s)
Cis-aconitate	3.12(s)
Choline	3.20(s)
Glycerophosphocholine (GPC)	3.23(s)
Taurine	3.25 (t), 3.42 (t)
Trimethylamine N-oxide (TMAO)	3.27(s)
α-Glucose	3.42 (t), 5.24 (d)
Glucose	3.4-3.95 (m)
Phenylacetyl glycine (PAG)	3.66 (s), 3.74 (d)

Amino acid	3.77 (m)
N-methylnicotinamide (NMND)	3.96 (d), 7.54, 7.62 (tt), 7.82 (d)
$\beta$ -Glucose	4.63 (d)
Unsaturated fatty acid	5.37 (br)
Allatoin	5.39(s)
Fumarate	6.53(s)
3-Indoxylsulfate	7.18(t), 7.26(t), 7.36(s), 7.49(d), 7.69(d)

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