

Table S1. Diet compositions

	C	HF
		g/kg diet
Corn starch ¹	397.5	167.5
Casein ²	200.0	200.0
Dextrin ³	132.0	132.0
Sucrose ⁴	100.0	100.0
Soybean oil ⁵	70.0	70.0
Cellulose ⁶	50.0	50.0
Mineral mixture ⁷	35.0	35.0
Vitamin mixture ⁸	10.0	10.0
L-Cystine ⁹	3.0	3.0
Choline hydrogen tartrate ¹⁰	2.5	2.5
Lard ¹¹	-	230.0

¹ Amylalpha (Chuo Shokuryo Co., Ltd.)

² NZMP Acid Casein (Fonterra Co-Operative Group Limited.)

³ TK-16 (Matsutani Chemical Industry Co., Ltd.)

⁴ Nippon Beet Sugar Manufacturing Co., Ltd.

⁵ J-Oil Mills, Inc.

⁶ Microcrystalline cellulose (Ceolus PH-102, Asahi Kasei Corporation)

⁷ AIN-93G mineral mixture (MP Biomedicals)

⁸ AIN-93 vitamin mixture (CLEA Japan, Inc.)

⁹ L-Cystine (Fujifilm Wako Pure Chemical Corporation)

¹⁰ Choline Hydrogen Tartrate (Fujifilm Wako Pure Chemical Corporation)

¹¹ Bell Shokuhin Co., Ltd.

Table S2. BAs analyzed in this study

12 α -hydroxylated BAs

Primary BAs

- 5 β -cholanic acid-3 α ,7 α ,12 α -triol (cholic acid, CA)
- 5 β -cholanic acid-3 α ,7 α ,12 α -triol-*N*-(2-sulfoethyl)-amide (taurocholic acid, TCA)
- 5 β -cholanic acid-3 α ,7 α ,12 α -triol-*N*-(carboxymethyl)-amide (glycocholic acid, GCA)

Secondary BAs

- 5 β -cholanic acid-3 α ,12 α -diol (deoxycholic acid, DCA)
 - 5 β -cholanic acid-3 α ,12 α -diol-*N*-(2-sulfoethyl)-amide (taurodeoxycholic acid, TDCA)
 - 5 β -cholanic acid-3 α ,12 α -diol-*N*-(carboxymethyl)-amide (glycodeoxycholic acid, GDCA)
 - 5 β -cholanic acid-3 α ,7 β ,12 α -triol (ursocholic acid, UCA)
 - 5 β -cholanic acid-3 α ,12 α -diol-7-one (7-oxo-deoxycholic acid, 7oDCA)
 - 5 β -cholanic acid-3 α -ol-12-one (12-oxo-lithocholic acid, 12oLCA)
 - 5 β -cholanic acid-12 α -ol-3-one (3-oxo-12 α -hydroxy-5 β -cholanic acid, 3o12 α)
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Non-12-hydroxylated BAs

Primary BAs

- 5 β -cholanic acid-3 α ,7 α -diol (chenodeoxycholic acid, CDCA)
- 5 β -cholanic acid-3 α ,6 α ,7 α -triol (hyocholic acid, HCA)
- 5 β -cholanic acid-3 α ,6 β ,7 α -triol (α -muricholic acid, α MCA)
- 5 β -cholanic acid-3 α ,6 β ,7 β -triol (β -muricholic acid, β MCA)
- 5 β -cholanic acid-3 α ,7 α -diol-*N*-(2-sulfoethyl)-amide (taurochenodeoxycholic acid, TCDCA)
- 5 β -cholanic acid-3 α ,7 α -diol-*N*-(carboxymethyl)-amide (glycochenodeoxycholic acid, GCDCA)
- 5 β -cholanic acid-3 α ,6 β ,7 α -triol-*N*-(2-sulfoethyl)-amide (tauro- α -muricholic acid, T α MCA)
- 5 β -cholanic acid-3 α ,6 β ,7 β -triol-*N*-(2-sulfoethyl)-amide (tauro- β -muricholic acid, T β MCA)

Secondary BAs

- 5 β -cholanic acid-3 α ,6 α ,7 β -triol (ω -muricholic acid, ω MCA)
 - 5 β -cholanic acid-3 α ,6 α ,7 β -triol-*N*-(2-sulfoethyl)-amide (tauro- ω -muricholic acid, T ω MCA)
 - 5 β -cholanic acid-3 α ,7 β -diol (ursodeoxycholic acid, UDCA)
 - 5 β -cholanic acid-3 α ,7 β -diol-*N*-(2-sulfoethyl)-amide (tauroursodeoxycholic acid, TUDCA)
 - 5 β -cholanic acid-3 α ,7 β -diol-*N*-(carboxymethyl)-amide (glycoursodeoxycholic acid, GUDCA)
 - 5 β -cholanic acid-3 α -ol (lithocholic acid, LCA)
 - 5 β -cholanic acid-3 α -ol-*N*-(2-sulfoethyl)-amide (tauroolithocholic acid, TLCA)
 - 5 β -cholanic acid-3 α -ol-*N*-(carboxymethyl)-amide (glycolithocholic acid, GLCA)
 - 5 β -cholanic acid-3 α -ol-7-one (7-oxo-lithocholic acid, 7oLCA)
 - 5 β -cholanic acid-3 α ,6 α -diol (hyodeoxycholic acid, HDCA)
 - 5 β -cholanic acid-3 α ,6 α -diol-*N*-(2-sulfoethyl)-amide (taurohyodeoxycholic acid, THDCA)
 - 5 β -cholanic acid-3 α ,6 α -diol-*N*-(carboxymethyl)-amide (glycohyodeoxycholic acid, GHDCa)
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Each name was listed as systemic name with synonym and abbreviation. All BA standards analyzed in this study were obtained from Steraloids, Inc., except for UCA, which was obtained from Toronto Research Chemicals.

Table S3. Primer sequences for qRT-PCR

Gene		sequence (5' - 3')	NCBI reference sequence
<i>Cyp7a1</i>	Forward	CCTGCAACCTTCTGGAGCTTA	NM_007824.3
	Reverse	AGCCTCCTTGATGATGCTATCTAGT	
<i>Cyp8b1</i>	Forward	ATGAGCTGTTCAGGAAGTTC	NM_010012.3
	Reverse	TGTCCTGCATGGATGAAGC	
<i>Cyp27a1</i>	Forward	CCAATGTGGACAACCTCCT	NM_024264.5
	Reverse	CTTGTGGTCTCGGTGGTC	
<i>Star</i>	Forward	GGAGCTCTCTGCTTGGTTCTC	NM_011485.5
	Reverse	ACCTCCAAGCGAAACACCTT	
<i>Gapdh</i>	Forward	TGACCTCAACTACATGGTCTACA	NM_001289726.1
	Reverse	CTTCCCATTCTCGGCCTTG	
<i>Bbox1</i>	Forward	TTCTCAACAGGCCAGAGCAA	NM_130452.1
	Reverse	TTCAGAGTTGGCAGCTGGAG	

Table S4. Oxysterols analyzed in this study

5-cholesten-3 β ,4 β -diol (4 β -hydroxycholesterol, 4 β OH)
5 α -cholestan-3 β -ol-6-one (6-ketocholestanol, 6keto)
5-cholesten-3 β -ol-7-one (7-ketocholesterol, 7keto)
5-cholesten-3 β ,7 α -diol (7 α -hydroxycholesterol, 7 α OH)
5-cholesten-3 β ,7 β -diol (7 β -hydroxycholesterol, 7 α OH)
cholestan-5 α ,6 α -epoxy-3 β -ol (5 α -epoxycholesterol, α -epoxy)
cholestan-5 β ,6 β -epoxy-3 β -ol (5 β -epoxycholesterol, β -epoxy)
cholestane-3 β ,5 α ,6 β -triol (β -triol)
5-cholesten-3 β ,25-diol (25-hydroxycholesterol, 25OH)
25*R*-cholest-5-en-3 β ,26-diol (27-hydroxycholesterol, 27OH)
5-cholestene-3 β ,22(*R*)-diol (22(*R*)-hydroxycholesterol)
5-cholesten-3 β ,7 α ,25-triol (7 α , 25(*R*)-dihydroxycholesterol)
5-cholesten-3 β ,24(*S*)-diol (24(*S*)-hydroxycholesterol)

Each name was listed as systemic name with synonym and abbreviation if necessary.

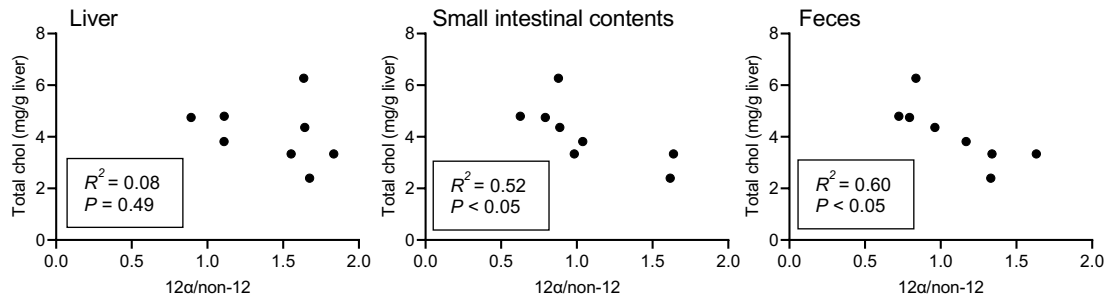


Figure S1. Correlation between the ratio of 12 α BA/non-12 BA and hepatic total chol (Total chol) concentration. Correlation in the liver, small intestine contents, feces, aortic plasma, and portal plasma. Filled bars represent HF (n = 8). P-values are shown in the inset.

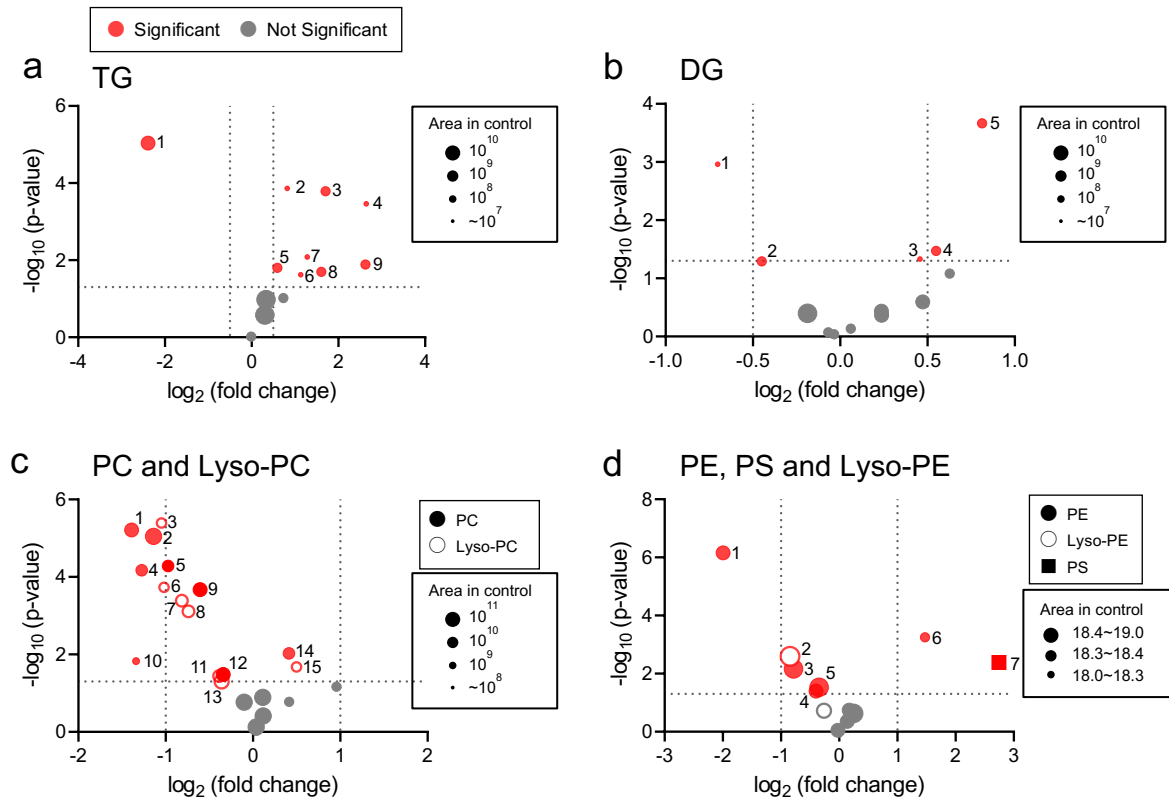


Figure S2. Composition of various metabolites in liver of the mice fed control and HF diet.

(a) Triacylglycerol (TG); (1) 20:0/20:1 (n-9)/20:4 (n-6), (2) 18:2 (n-6)/18:2 (n-6)/18:2 (n-6), (3) 16:1 (n-7)/18:1 (n-9)/18:1 (n-9), (4) 16:0/18:2 (n-6)/18:2 (n-6), (5) 17:1 (n-8)/20:2 (n-6)/20:2 (n-6), (6) 18:4 (n-3)/19:4 (n-6)/18:4 (n-3), (7) 18:3 (n-3)/14:0/18:3 (n-3), (8) 18:1 (n-9)/18:2 (n-6)/18:1 (n-9), (9) 18:2 (n-6)/18:2 (n-6)/20:2 (n-6). (b) Diacylglycerol (DG); (1) 18:2 (n-6)/18:2 (n-6), (2) 18:3 (n-3)/20:3 (n-6), (3) 14:0/14:1 (n-5), (4) 15:0/16:1 (n-7), (5) 16:1 (n-7)/18:1 (n-9). (c) Phosphatidyl choline (PC) and lyso-PC; (1) 18:3 (n-3)/18:2 (n-6) PC, (2) 16:0/18:3 (n-3) PC, (3) 16:1 (n-7) lyso-PC, (4) 18:3 (n-6)/p-18:1 (n-7) PC, (5) p-16:0/16:0 PC, (6) 22:5 (n-3) lyso-PC, (7) 20:4 (n-6) lyso-PC, (8) 18:2 (n-6) lyso-PC, (9) 14:0/18:1 (n-9) PC, (10) 20:5 (n-3)/24:1 (n-9) PC, (11) 18:1 (n-9) lyso-PC, (12) 16:0/18:0 PC, (13) 16:0 lyso-PC, (14) 18:3 (n-3)/18:3 (n-3) PC, (15) 17:0 lyso-PC. (d) Phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), and lyso-PE (1) 20:4 (n-6)/22:6 (n-3) PE, (2) 22:6 (n-3) lyso-PE, (3) 18:1 (n-7)/20:4 (n-6) PE, (4) 18:1 (n-9)/18:2 (n-6) PE, (5) 18:4 (n-3)/24:1 (n-9) PE, (6) 18:2 (n-6)/24:0 PE, (7) 24:0/24:0 PS. Each marker size indicates range of area values shown in inset. Markers in red indicate a significant difference between the dietary groups ($n = 8$, $P < 0.05$).

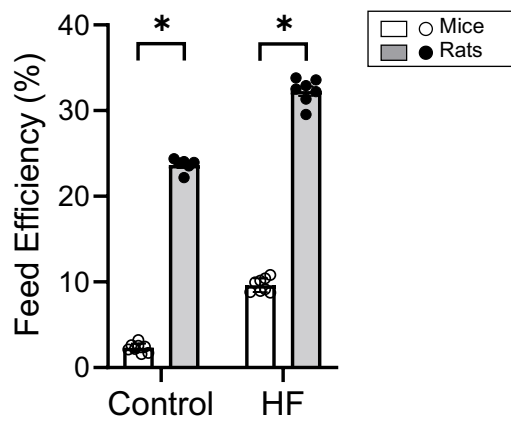


Figure S3. Difference in feed efficiency between mice and rats

The ratio of weight gain to food intake over 8 weeks was calculated. The values in rats were calculated with the data in our previous study¹⁴.

$$\text{Feed Efficiency (\%)} = \frac{\text{Weight gain (g)}}{\text{Total food intake (g)}} \times 100$$

Open bars for mice (n = 8) and filled bars for rats (n = 6-7). Values were shown as the mean with the SEM. Asterisks indicate a significant difference compared to control ($P < 0.05$).