

Honokiol affects the composition of gut microbiota and the metabolism of lipid and bile acid in methionine-choline deficiency diet-induced NASH mice

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Table S1: Primer sequences used for RT-qPCR

Name	Accession number	Sequences	
<i>β-actin</i>	NM_007393	Forward	5'-AGAGGGAAATCGTGCGTGAC-3'
		Reverse	5'-CAATAGTGATGACCTGGCCGT-3'
<i>CYP7A1</i>	NM_007824	Forward	5'-GGGAATGCCATTTACTTGGA-3'
		Reverse	5'-GTCCGGATATTCAAGGATGC-3'
<i>CYP27A1</i>	NM_024264	Forward	5'-CTATGTGCTGCACTTGCCC-3'
		Reverse	5'-GGGCACTAGCCAGATTCACA-3'
<i>Bsep</i>	NM_021022	Forward	5'-CCAGAACATGACAAACGGAA-3'
		Reverse	5'-AAGGACAGCCACACCAACTC-3'
<i>Mrp2</i>	NM_013806	Forward	5'-TCCAGGACCAAGAGATTTGC-3'
		Reverse	5'-TCTGTGAGTGCAAGAGACAGGT-3'
<i>Ntcp</i>	NM_011387	Forward	5'-AGGGGGACATGAACCTCAG-3'
		Reverse	5'-TCCGTCTAGATTCCTTTGC-3'
<i>Oatp1b2</i>	NM_020495	Forward	5'-ACCAAACCTCAGCATCCAAGC-3'
		Reverse	5'-TAGCTGAATGAGAGGGCTGC-3'

Table S2 Levels of bile acids in serum of the tested mice

BAs (ng/ml)	MCS	MCD	HNK
7-KDCA	717.72±609.34	2875.19±1749.38*	1924.01±1137.45
DCA	223.43±110.20	540.44±301.67*	271.33±96.50
TDCA	197.29±229.70	308.34±70.54	147.28±29.11 ^{##}
23-DCA	27.22±17.66	277.30±55.15**	97.73±33.74 ^{##}
HDCA	22.94±10.95	44.70±11.27**	24.53±3.13 ^{##}
UCA	19.27±9.60	91.55±58.73*	35.31±23.46

3-oxo-DCA	13.85±8.02	44.12±29.71*	23.08±17.75
HCA	13.50±10.40	57.77±41.79*	47.82±26.85
GCA	6.43±10.42	11.91±1.42	6.18±1.54 ^{##}
3β-UDCA	3.30±1.19	6.33±2.97*	5.71±2.72
DHCA	1.35±0.55	2.46±0.66**	2.07±0.33
CDCA	174.90±115.66	492.11±368.43	562.00±156.60
ω -MCA	1718.36±1049.35	4055.40±2399.02	2516.06±1136.71
β -MCA	665.49±874.10	2287.85±1722.11	1551.97±721.77
α -MCA	82.50±113.35	205.27±123.40	117.16±52.36
UDCA	58.77±22.91	139.24±102.53	76.76±53.00
MDCA	11.80±11.65	16.70±13.75	7.97±3.64
LCA	16.49±4.40	25.18±3.01**	26.86±5.52
CA	997.81±1088.62	1947.89±789.86	1689±718.98
3β-DCA	5.02±1.54	26.40±22.64	8.62±5.13
3-oxo-CA	3.18±3.86	5.43±3.46	3.89±2.18
GCDCA	0.25±0.15	0.35±0.05	0.36±0.12
GLCA	1.80±0.16	1.93±0.21	1.77±0.62
Tβ-MCA	6483.13±11251.70	8271.16±1923.95	9959.98±4334.27
TUDCA	183.87±266.98	256.93±51.08	246.91±76.15
TLCA	10.87±8.55	16.35±2.71	12.38±4.92
TCDCA	386.30±637.60	429.11±104.93	590.60±175.49
TCA	6918.00±12057.00	5687.88±862.85	5795.15±1901.50

12-KLCA	—	—	—
NCA	—	—	—
DLCA	—	—	—
3 β -CA	—	—	—
6,7-DKLCA	—	—	—
12-oxo-CDCA	—	—	—
3 β -HDCA	—	—	—
IALCA	—	—	—
IDCA	—	—	—
ILCA	—	—	—
LCA-3S	—	—	—
7-KLCA	—	—	—
7,12-DKLCA	—	—	—
CDCA-3Gln	—	—	—
GLCA-3S	—	—	—
GHCA	—	—	—
GDHCA	—	—	—
GDCA	—	—	—
GUDCA	—	—	—
THCA	—	—	—
TDHCA	—	—	—
TLCA-3S	—	—	—

n=7. **p < 0.01, *p < 0.05 vs MCS group; ##p < 0.01, #p < 0.05 vs MCD group. MCD, methionine- and choline-deficient diet; MCS, methionine- and choline-sufficient diet; HNK, honokiol. __: not quantified.

Table S3 Effects of HNK on the Alpha diversity of GM in the tested mice

	Chao1	ACE	Shannon	Simpson
MCS	201.01±11.93	205.26±13.14	2.95±0.24	0.74±0.04
MCD	183.66±10.90	187.49±13.73	2.65±0.13	0.69±0.03
HNK	189.29±32.37	197.66±16.69	2.31±0.15	0.60±0.04

Data were expressed as mean±SD (n=7). MCD, methionine- and choline-deficient diet; MCS, methionine- and choline-sufficient diet; HNK, honokiol.