

Supplementary file

Development and validation of a highly sensitive LC-MS/MS method for the analysis of bile acids in serum, plasma and liver tissue samples

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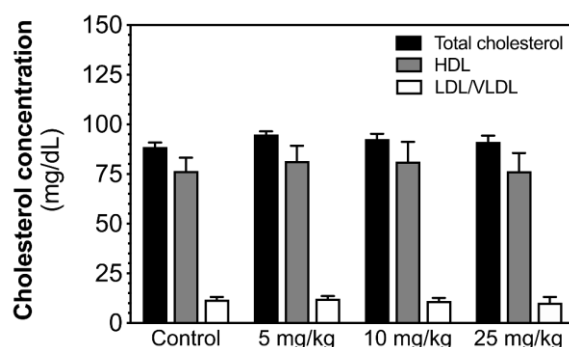
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Supplementary materials and methods

Lipid profile analysis

Total cholesterol, HDLc, and LDLc/VLDLc were measured by colorimetric assay according to the manufacturer's protocol using HDL and LDL/VLDL cholesterol assay kit (Abcam, ab 65390, Cambridge, England, UK).

Supplementary results



Supplementary figure 1: Lipid profile measured in simvastatin-treated and control mice. The graph shows the concentrations of total cholesterol, HDLc and LDLc/VLDLc in control and simvastatin-treated mice for three weeks at 5, 10, and 25 mg/kg. Data are expressed as mean $\pm$ SEM of six independent experiments.

Table S1: Concentrations of individual bile acids quantified in serum, plasma and liver tissue from mice and in human plasma. Values are expressed as mean $\pm$ SD (nM for serum and plasma, pg/mg tissue for liver samples). ND, not detected.

Name	Mouse serum n=9 (nM)	Mouse plasma n=6 (nM)	Mouse liver n= 20 (pg/mg tissue)	Human plasma n=18 (nM)
CA	289 $\pm$ 187	712 $\pm$ 368	1264 $\pm$ 1918	27 $\pm$ 77
CDCA	17 $\pm$ 12	58 $\pm$ 12	105 $\pm$ 102	63 $\pm$ 99
ω MCA	208 $\pm$ 129	194 $\pm$ 70	891 $\pm$ 702	ND
α MCA	53 $\pm$ 30	57 $\pm$ 15	446 $\pm$ 681	ND
β MCA	281 $\pm$ 171	609 $\pm$ 388	5384 $\pm$ 6344	ND
γ MCA	ND	ND	ND	ND
UDCA	7 $\pm$ 8	193 $\pm$ 97	47 $\pm$ 56	1 $\pm$ 2
HDCA	ND	95 $\pm$ 57	49 $\pm$ 14	ND
DCA	220 $\pm$ 140	278 $\pm$ 78	70 $\pm$ 14	91 $\pm$ 80
LCA	ND	ND	ND	ND
3oxoLCA	ND	ND	ND	ND
7oxoLCA	2 $\pm$ 2	17 $\pm$ 19	4 $\pm$ 10	0,5 $\pm$ 0,8
12oxoLCA	6 $\pm$ 5	ND	20 $\pm$ 28	1,4 $\pm$ 1
6,7dioxoLCA	ND	ND	107 $\pm$ 89	ND
7oxoDCA	221 $\pm$ 141	149 $\pm$ 39	472 $\pm$ 1171	ND
Allo-CA	73 $\pm$ 46	40 $\pm$ 15	172 $\pm$ 180	ND

Allo-DCA	27 ± 20	24 ± 26	ND	0,04 ± 0,1
Allo-3βDCA	1 ± 2	ND	ND	6 ± 6
Allo-12βDCA	ND	ND	ND	ND
Allo-3βLCA	ND	ND	ND	ND
Allo-LCA	ND	ND	ND	ND
TCA	16160 ± 6702	12006 ± 6995	156284 ± 122604	14 ± 10
TCDCa	1104 ± 598	441 ± 37	7060 ± 14945	50 ± 41
TωMCA	5369 ± 2737	1902 ± 364	57347 ± 64084	ND
TαMCA	4538 ± 2336	2280 ± 447	33327 ± 56545	ND
TβMCA	9917 ± 4008	8705 ± 5520	138231 ± 137542	ND
TUDCA	1330 ± 657	645 ± 116	11643 ± 18165	6 ± 7
TDCA	839 ± 508	535 ± 49	7532 ± 12444	19 ± 13
T7oxoLCA	242 ± 141	83 ± 13	2268 ± 3185	0,03 ± 0,1
TLCA	18 ± 15	27 ± 29	469 ± 115	0,9 ± 1
GCA	81 ± 45	38 ± 8	885 ± 1868	51 ± 37
GCDCA	3 ± 1	9 ± 1	17 ± 13	226 ± 191
GUDCA	5 ± 2	3 ± 1	15 ± 15	65 ± 80
GDCA	ND	ND	13 ± 8	71 ± 48
GLCA	ND	ND	ND	9 ± 7
G7oxoLCA	ND	ND	ND	ND

Table S2: Concentrations of individual bile acids quantified in plasma and liver tissue in simvastatin-treated and control mice. Values are expressed as mean ± SD (nM for plasma, pg/mg tissue for liver samples). ND, not detected.

Name	Mouse plasma n=24 (nM)	Mouse liver n= 24 (pg/mg tissue)
CA	125 ± 77	284 ± 248
CDCA	21 ± 12	6 ± 4
ωMCA	314 ± 213	113 ± 91
α-MCA	15 ± 11	80 ± 74
βMCA	213 ± 136	668 ± 532
γMCA	ND	ND
UDCA	36 ± 23	13 ± 11
HDCA	15 ± 11	12 ± 7
DCA	247 ± 130	21 ± 13
LCA	ND	ND
3oxoLCA	ND	ND
7oxoLCA	ND	ND
12oxoLCA	4 ± 2	3 ± 4
6,7dioxoLCA	ND	ND
7oxoDCA	96 ± 100	292 ± 354
Allo-CA	15 ± 12	23 ± 16
Allo-DCA	16 ± 8	5 ± 2
Allo-3βDCA	ND	3 ± 1

Allo-12βDCA	ND	ND
Allo-3βLCA	ND	ND
Allo-LCA	ND	ND
TCA	528 $\pm$ 952	20521 $\pm$ 15487
TCDCA	11 $\pm$ 13	1195 $\pm$ 562
TωMCA	251 $\pm$ 388	10759 $\pm$ 8692
TαMCA	154 $\pm$ 263	4921 $\pm$ 3858
TβMCA	381 $\pm$ 713	20036 $\pm$ 13613
TUDCA	26 $\pm$ 29	1709 $\pm$ 793
TDCA	65 $\pm$ 48	4000 $\pm$ 1865
T7oxoLCA	4 $\pm$ 7	185 $\pm$ 123
TLCA	ND	191 $\pm$ 89
GCA	7 $\pm$ 4	59 $\pm$ 45
GCDCA	ND	ND
GUDCA	ND	ND
GDCA	ND	ND
GLCA	ND	ND
G7oxoLCA	ND	ND