

Supplemental Material

Urinary Bile Acids Profile of Cesarean Sectioned Newborns is Characterized by Oxidative Metabolism of Primary Bile Acids: Limited Roles of Fetal-specific CYP3A7 in Cholate Oxidations.

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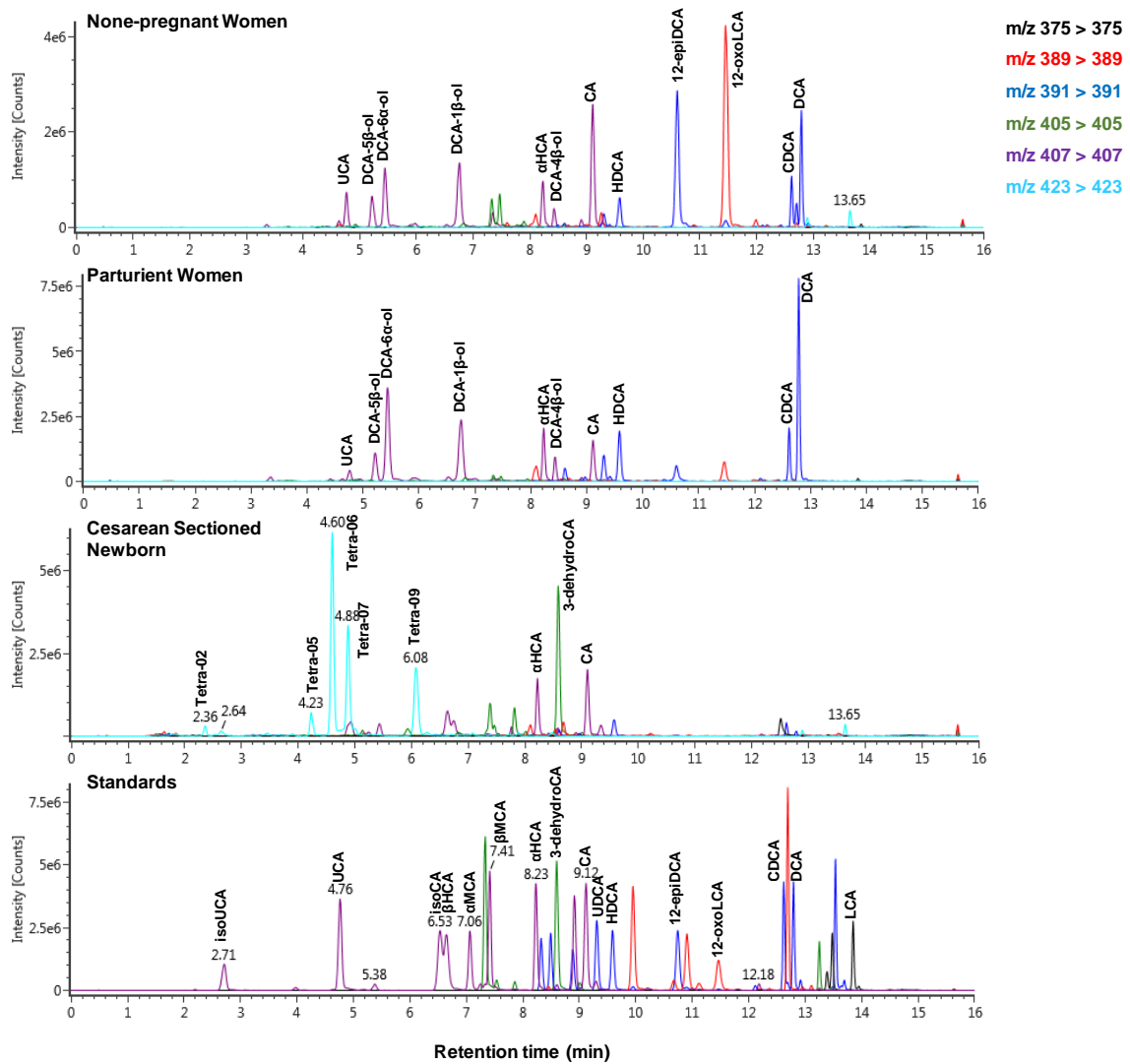


Figure S1. The representative ion chromatograms of the digested urine samples from a non-pregnant women, a parturient women and a cesarean sectioned newborn in comparison to that of the mixed standard samples.

Table S1. Donor information of human liver microsomes used in this work.

Category	Lot No.	Gender	Age	Race
Adult	HFC205	Female	47 years	Caucasian
Adult	HFC208	Female	60 years	Caucasian
Adult	HFH617	Female	62 years	Hispanic
Adult	HFH705	Female	42 years	Hispanic
Adult	HG18	Male	41 years	Caucasian
Adult	HG43	Female	23 years	Caucasian
Adult	HG43-1	Female	23 years	Caucasian
Adult	HG64	Male	63 years	Caucasian
Adult	HH13-2	Male	55 years	Asian
Adult	HH37	Male	54 years	Caucasian
Adult	HH519	Male	70 years	Caucasian
Adult	HH581	Male	58 years	Caucasian
Adult	HH741	Male	68 years	Caucasian
Adult	HH837	Female	52 years	Asian
Infant	H0845	Male	1 month	Caucasian
Infant	H0671	Male	3 months	Hispanic
Infant	H0238	Male	3 months	Asian
Infant	H0825	Male	11 months	Caucasian
Infant	H0322	Male	1 year	Hispanic
Infant	H0551	Male	2 years	Caucasian
Infant	H0852	Male	2 years	Hispanic
Infant	H0866	Female	3 years	Caucasian

Table S2. Demographic information of healthy man, none-pregnant healthy women and parturient pregnant women enrolled in this work.

Population	Healthy man	Healthy women (none-pregnant)	Pregnant women (parturient)
n	30	28	29
age (year)	26.07±4.70	24.46±2.07	31.52±4.16
Height (m)	1.71±0.05	1.60±0.06	1.58±4.81
Weight (Kg)	62.53±7.31	53.02±5.87	64.48±6.68
BMI (Kg/m ²)	21.35±2.11	20.59±1.61	25.69±2.18

Data were shown as mean±SD.

Table S3. Demographic information of cesarean sectioned newborns enrolled in this work.

Case	Gender	Birth weight (g)	Gestational age (week)	Diaper collection time after birth (hour)	Feeding patterns
1	Female	3150	38	24	breast-fed
2	Male	2910	38	25	breast-fed
3	Male	2950	36	24	breast-fed
4	Male	3150	38	22	breast-fed
5	Male	3020	37	22	breast-fed
6	Male	3070	38	43	breast-fed
7	Female	3300	38	25	breast-fed
8	Male	3240	38	5	breast-fed
9	Female	3380	39	23	formula-fed
10	Male	3102	38	23	mixed feeding
11	male	3270	38	16	formula-fed
12	Female	3430	39	21	breast-fed
13	Female	2640	38	21	mixed feeding
14	Female	3100	38	26	breast-fed
15	Female	3530	39	22	breast-fed
16	Female	3080	39	18	mixed feeding
17	Female	2900	38	25	breast-fed
18	Female	2850	37	20	breast-fed
19	Female	3010	39	41	breast-fed
20	Female	3250	39	20	breast-fed
21	Female	2800	38	22	breast-fed
Mean±SD		3102±226	38±1	23±8	

Table S4. Extraction recoveries of urinary BAs absorbed in diaper gel in comparison to the direct analysis of a pooled urine sample of in-lab volunteers.

BA analytes	Extraction recovery (%)	
	The methanol method (n=3)	The CaCl ₂ method (n=4)
LCA	44±7	64±9
6-oxoLCA	59±9	86±6
12-oxoLCA	38±8	102±6
isoUDCA	41±6	91±7
UDCA	44±7	103±8
HDCA	35±8	85±4
12epiDCA	31±10	83±4
CDCA	53±12	90±6
DCA	49±12	86±4
7-oxoDCA	43±6	91±4
isoUCA	38±16	96±1
DCA-5b-ol	40±9	100±5
DCA-6α-ol	40±12	100±4
DCA-1β-ol	40±8	91±3
HCA	41±7	90±3
DCA-4β-ol	47±9	96±8
CA	49±8	94±4

BAs were determined with the enzyme digestion method described in the article. Data were shown as mean±SD.

Table S5. Hill coefficients for regioselective oxidations of DCA in human liver microsomes within the substrate range of 1-300 μM .

Lot No.	Age	V_{\max} (pmol/min/mg protein)						S_{50} (μM)						CL_{int} (mL/min/ μg protein)					
		DCA-1 β -ol	DCA-4 β -ol	DCA-5 β -ol	DCA-6 α -ol	DCA-6 β -ol	DCA-19-ol	DCA-1 β -ol	DCA-4 β -ol	DCA-5 β -ol	DCA-6 α -ol	DCA-6 β -ol	DCA-19-ol	DCA-1 β -ol	DCA-4 β -ol	DCA-5 β -ol	DCA-6 α -ol	DCA-6 β -ol	DCA-19-ol
HFC205	47 years	453.2	6.127	108.7	81.73	42.21	4.076	155.8	874.0	423.8	571.0	1422	208.6	2.909	0.007	0.256	0.143	0.030	0.020
HFC208	60 years	569.0	4.471	122.1	143.2	37.67	5.200	146.9	259.6	276.8	807.5	716.2	541.7	3.873	0.017	0.441	0.177	0.053	0.010
HFH617	62 years	209.4	2.939	35.59	20.21	11.98	6.988	137.3	133.4	255.9	215.0	758.8	91.52	1.525	0.022	0.139	0.094	0.016	0.076
HFH705	42 years	55.23	0.624	7.728	4.498	1.331	ND	123.1	356.7	136.4	179.1	169.4	NA	0.449	0.002	0.057	0.025	0.008	NA
HG18	41 years	80.21	1.067	19.62	20.55	2.643	0.4019	252.2	454.8	705.4	1198	372.2	132.4	0.318	0.002	0.028	0.017	0.007	0.003
HG43	23 years	139.1	1.668	21.41	17.35	8.567	ND	165.6	488.3	183.6	349.4	612.9	NA	0.840	0.003	0.117	0.050	0.014	NA
HG43-1	23 years	137.6	0.766	22.44	19.10	6.962	0.4798	179.4	183.2	230.5	437.0	434.1	106.8	0.767	0.004	0.097	0.044	0.016	0.004
HG64	63 years	137.1	3.028	17.11	16.34	2.024	14.11	118.7	65.90	176.7	242.6	161.7	79.7	1.155	0.046	0.097	0.067	0.013	0.177
HH13-2	55 years	36.20	1.067	4.557	6.071	ND	3.170	122.3	139.1	176.1	480.5	NA	88.41	0.296	0.008	0.026	0.013	NA	0.036
HH37	54 years	218.2	1.857	39.42	29.51	7.407	4.616	113.1	105.7	189.2	295.8	222.9	79.8	1.929	0.018	0.208	0.100	0.033	0.058
HH519	70 years	345.9	14.39	39.92	39.74	3.161	46.30	110.7	132.4	200.0	196.8	156.0	111.6	3.125	0.109	0.200	0.202	0.020	0.415
HH581	58 years	131.4	1.664	17.42	14.29	4.464	1.105	159.9	598.3	192.6	364.0	409.1	432.6	0.822	0.003	0.090	0.039	0.011	0.003
HH741	68 years	115.6	0.898	15.56	10.61	2.484	1.046	103.4	112.8	132.6	211.2	122.4	59.32	1.118	0.008	0.117	0.050	0.020	0.018
HH837	52 years	319.4	6.308	53.49	39.80	11.41	1.860	127.5	1960	228.5	399.0	474.1	107.6	2.505	0.003	0.234	0.100	0.024	0.017
H0845	1 month	668.2	59.0	96.5	106.8	25.9	243.3	96.1	147.0	189.3	118.4	1547.0	129.0	7.0	0.40	0.51	0.90	0.02	1.89
H0671	3 months	201.4	9.5	24.4	24.2	1.9	46.1	141.7	102.3	384.9	157.5	326.2	107.2	1.4	0.09	0.06	0.15	0.01	0.43
H0238	3 months	282.7	18.5	27.8	38.8	2.2	73.4	72.1	79.9	87.8	89.3	72.0	61.3	3.9	0.23	0.32	0.43	0.03	1.20
H0825	11 months	636.4	55.7	347.8	364.9	/	221.8	103.8	316.9	1631.0	1952.0	/	253.9	6.1	0.18	0.21	0.19	/	0.87
H0322	1 year	37.4	1.7	3.7	2.9	/	4.7	134.7	118.3	128.1	83.4	/	52.4	0.3	0.01	0.03	0.03	/	0.09
H0551	2 years	120.8	0.9	16.2	10.6	3.5	1.1	53.0	59.9	52.4	68.3	63.7	40.8	2.3	0.01	0.31	0.15	0.06	0.03
H0852	2 years	178.8	5.1	36.0	28.1	3.3	19.2	160.7	77.3	558.8	369.8	205.2	61.1	1.1	0.07	0.06	0.08	0.02	0.31
H0866	3 years	379.0	3.9	92.7	/	/	9.7	280.2	92.8	755.2	/	/	43.4	1.4	0.04	0.12	/	/	0.22

Table S6. Retention time of tetrahydroxy-cholan-24-oic acids (Tetra-BA) detected *in vivo* (urine samples of newborns) and *in vitro* (incubation samples of CA, TCA and GCA in infant liver microsomes).

BA metabolites	Retention time (min, mean±SD)	
	<i>in vivo</i>	<i>in vitro</i>
3DHCA	8.594±0.009	8.590±0.002
Tetra01	1.824±0.016	ND
Tetra02	2.354±0.008	2.352±0.005
Tetra03	2.648±0.011	2.651±0.015
Tetra04	3.454±0.009	ND
Tetra05	4.240±0.007	4.239±0.009
Tetra06	4.603±0.008	ND
Tetra07	4.883±0.008	4.838±0.033
Tetra08	5.407±0.027	ND
Tetra09	6.087±0.008	ND