

# **Body distribution of stable copper isotopes during the progression of cholestatic liver disease induced by common bile duct ligation in mice**

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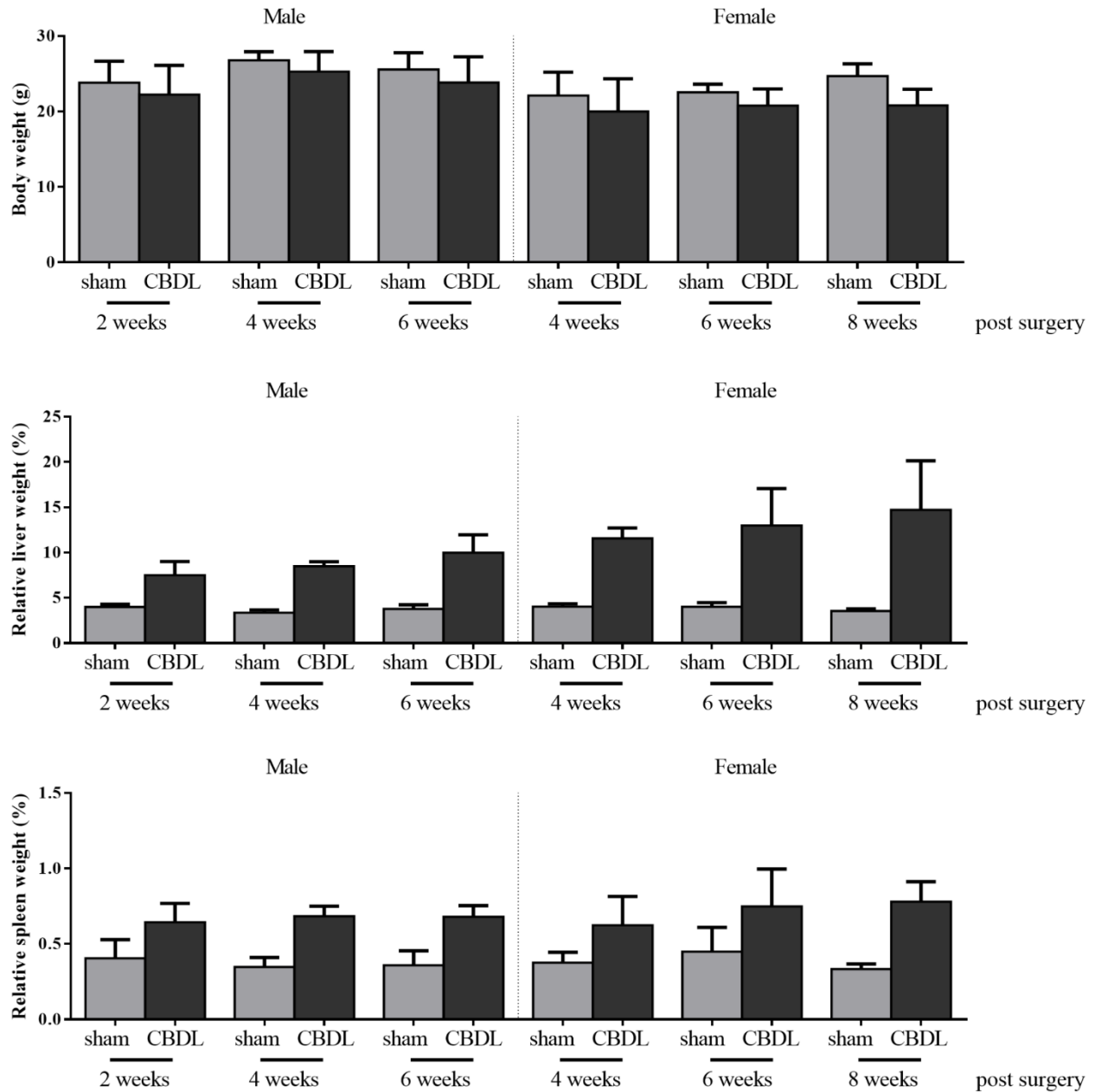
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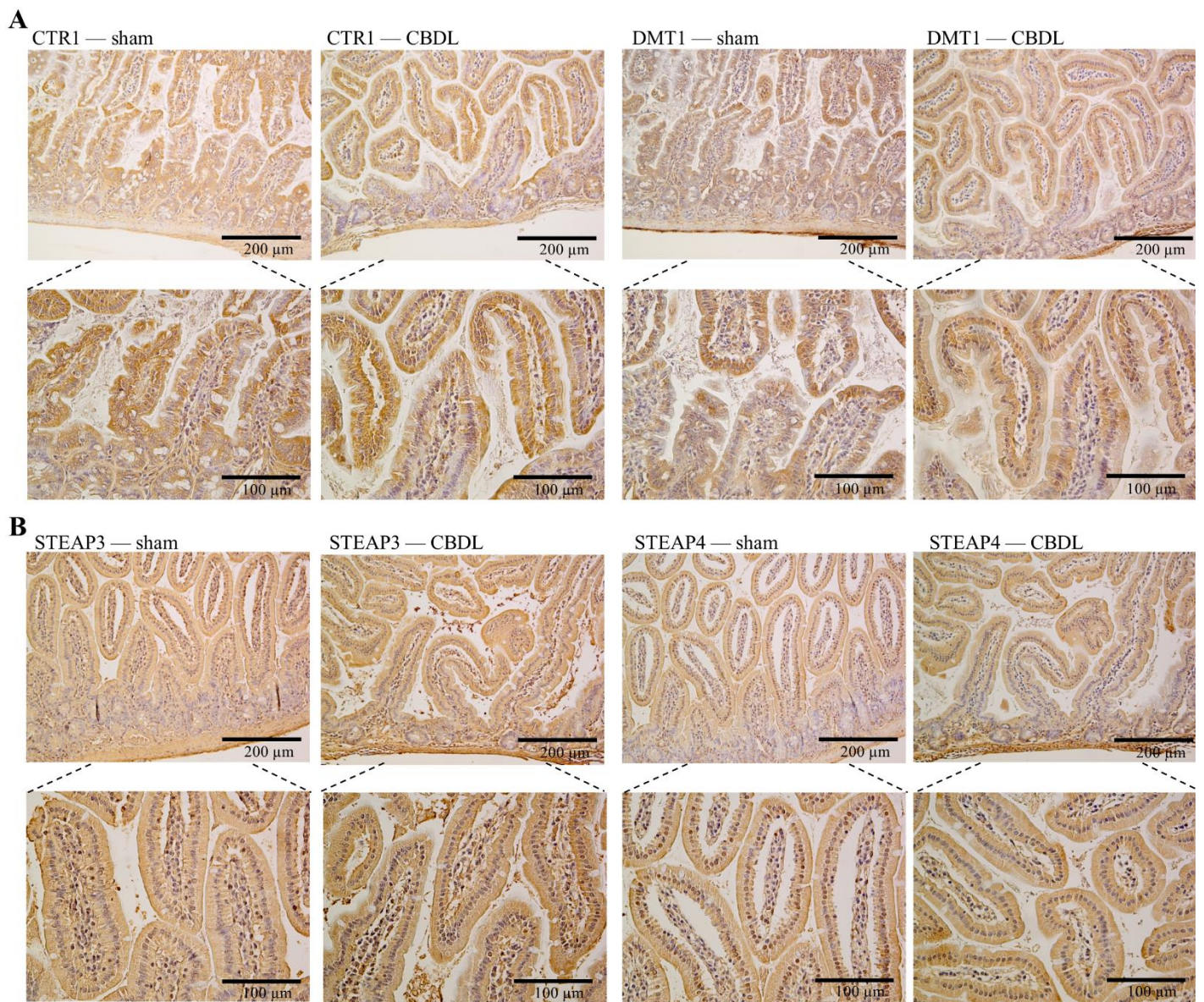
## **Supplementary material**

**Figures S1-S2**

**Tables S1-S2**



**Figure S1: Body, liver and spleen weights of sham- and CBDL-operated mice 2, 4, 6 and 8 weeks post-surgery.** Results are presented as the mean  $\pm$  95% confidence interval (n=5-7). Liver and spleen weights are given relative to total body weight. Statistical analysis was performed using a univariate three-factorial analysis of variance. A significant effect of surgery type (p=0.001), gender (p=0.000) and the time post-surgery (p=0.033) on total body weight was observed without interaction effects. Significant effects of operation type were also observed for relative liver and spleen weight (both p=0.000) with an interaction between gender and the time post-surgery for relative liver weight (p=0.047).



**Figure S2: Immunohistochemical stainings on duodenum tissue of sham- and CBDL-operated mice 4 weeks post-surgery. A. Tissue stained for metal receptors CTR1 and DMT1. B. Tissue stained for copper reductases STEAP3 and STEAP4.**

**Table S1: Quantitative RT-PCR Primers.**

<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>
<i>Gapdh</i>	CATGGCCTTCCGTGTTCCCTA	GCGGCACGTCAGATCCA
<i>Hmbs</i>	AAGGGCTTTTCTGAGGCACC	AGTTGCCCATCTTTCATCACTG
<i>Hprt</i>	GTTAAGCAGTACAGCCCCAAA	AGGGCATATCCAACAACAAACTT
<i>Sdha</i>	CTTGAATGAGGCTGACTGTG	ATCACATAAGCTGGTCCTGT
<i>Slc31a1(CTR1)</i>	GCCTTCGTGGCAGTGTTTTTA	GCGAATGCTGACTTGAGACTTTC
<i>Slc31a2(CTR2)</i>	GGCTTTACTGTTGATGCACTCC	CTGGATGCCCAAGTAGTAGTTTC
<i>Slc11a2 (DMT1)</i>	GTA CTCAGGGGCATGTTCGT	GTTGTGCGGCATGATCACAG
<i>Steap2</i>	CCAGAATCCAATGCAGAGTACC	GCCCAAGCTGAGATCACATTA AAA
<i>Steap3</i>	CAGGGGCTGGGAGAAAGATG	CCCACAAGCCATCTCCACTT
<i>Steap4</i>	ACCTCCCTGGTATTCTCGCT	AGCATCCAATGGTCAAGCCA
<i>Cybrd1</i>	ACAGTGATTGCGACGGTTCT	GGTACGAGGGGTGTTTCAGG
<i>Atox1</i>	CGAGTTCTCCGTGGACATGA	CACTCCTCCCAGCTTGTTGA
<i>Atp7a</i>	ACCTCTCCAGAAACCTTGCG	TCCAGTGAGGGCTGAGCTAT
<i>Ccs</i>	TGGCATCATTGCACGCTCT	GAGTCCTTTCGGCCTTGACC
<i>Sod1</i>	TATGGGGACAATACACAAGGCT	CCACCATGTTTCTTAGAGTGAGG

**Table S2. Copper isotopic composition and concentrations in the sham- and CBDL-operated mice 2, 4, 6 and 8 weeks post-surgery.** Cu concentrations in organs are expressed in  $\mu\text{g/g}$  wet weight. The relative standard deviation of the Cu concentrations was  $\leq 3\%$ . Delta values are expressed as average  $\pm$  standard deviation (for n=1-3 measurement replicates).

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Liver	Sham, 2 w	1M	3.31	$0.10 \pm 0.03$
		2M	3.61	$0.27 \pm 0.02$
		3M	3.43	$0.13 \pm 0.02$
	CBDL, 2 w	4M	10.7	$-0.67 \pm 0.01$
		5M	17.8	$-0.62 \pm 0.01$
		6M	14.1	$-0.84 \pm 0.01$
		7M	13.8	$-0.58 \pm 0.06$
	Sham, 4 w	8M	4.52	$0.20 \pm 0.01$
		9M	4.46	$0.41 \pm 0.01$
		10M	3.37	$0.22 \pm 0.03$
	CBDL, 4 w	11M	29.2	$-0.59 \pm 0.01$
		12M	27.0	$-0.83 \pm 0.01$
		13M	35.6	$-0.81 \pm 0.04$
		14M	16.1	$-0.55 \pm 0.02$
	Sham, 6 w	15M	4.96	$0.35 \pm 0.01$
		16M	3.67	$0.18 \pm 0.03$
		17M	3.33	$0.24 \pm 0.01$
	CBDL, 6 w	18M	42.0	$-0.70 \pm 0.01$
		19M	62.4	$-0.83 \pm 0.01$
		20M	49.8	$-0.46 \pm 0.02$
		21M	20.4	$-0.73 \pm 0.01$
	Sham, 4 w	22F	2.87	$0.31 \pm 0.01$
		23F	2.50	$0.25 \pm 0.01$
		24F	2.65	$0.32 \pm 0.01$
	CBDL, 4 w	25F	15.0	$-0.71 \pm 0.01$
		26F	8.74	$-0.90 \pm 0.01$
		27F	10.1	$-0.68 \pm 0.03$
		28F	14.2	$-0.80 \pm 0.02$
	Sham, 6 w	29F	3.03	$0.24 \pm 0.03$
		30F	3.09	$0.33 \pm 0.02$
		31F	2.40	$0.33 \pm 0.02$
	CBDL, 6 w	32F	23.1	$-0.65 \pm 0.01$
		33F	13.9	$-0.83 \pm 0.01$
		34F	34.8	$-0.51 \pm 0.06$
		35F	22.1	$-0.90 \pm 0.01$

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Liver	Sham, 8 w	36F	2.97	$0.34 \pm 0.02$
		37F	3.30	$0.42 \pm 0.01$
		38F	3.40	$0.31 \pm 0.05$
	CBDL, 8 w	39F	18.8	$-0.90 \pm 0.06$
		40F	13.7	$-0.94 \pm 0.01$
		41F	14.1	$-0.97 \pm 0.02$
		42F	17.2	$-1.18 \pm 0.05$
Kidney	Sham, 2 w	2M	4.29	$1.35 \pm 0.02$
		3M	3.86	$1.65 \pm 0.06$
	CBDL, 2 w	4M	3.87	$1.02 \pm 0.02$
		7M	3.65	$1.15 \pm 0.01$
	Sham, 4 w	8M	3.75	$1.92 \pm 0.02$
		9M	2.81	$1.91 \pm 0.04$
	CBDL, 4 w	11M	3.49	$0.55 \pm 0.02$
		12M	4.20	$0.98 \pm 0.01$
	Sham, 6 w	15M	3.09	$1.87 \pm 0.06$
		16M	3.46	$1.72 \pm 0.08$
	CBDL, 6 w	18M	4.16	$0.85 \pm 0.01$
		19M	4.04	$0.72 \pm 0.04$
	Sham, 4 w	22F	4.29	$1.35 \pm 0.02$
		23F	3.86	$1.65 \pm 0.06$
	CBDL, 4 w	25F	3.87	$1.02 \pm 0.02$
		26F	3.65	$1.15 \pm 0.01$
	Sham, 6 w	30F	3.75	$1.92 \pm 0.02$
		31F	2.81	$1.91 \pm 0.04$
	CBDL, 6 w	32M	3.49	$0.55 \pm 0.02$
		33F	4.20	$0.98 \pm 0.01$
Sham, 8 w	36F	3.09	$1.87 \pm 0.06$	
	37F	3.46	$1.72 \pm 0.08$	
CBDL, 8 w	39F	4.16	$0.85 \pm 0.01$	
	40F	4.04	$0.72 \pm 0.04$	
Brain	Sham, 2 w	2M	4.32	$0.58 \pm 0.07$
		3M	2.96	$0.86 \pm 0.01$
	CBDL, 2 w	4M	2.93	$0.90 \pm 0.08$
		7M	3.09	$0.95 \pm 0.03$
	Sham, 4 w	8M	2.37	$0.80 \pm 0.05$
		9M	3.26	$0.98 \pm 0.04$
	CBDL, 4 w	11M	3.51	$0.58 \pm 0.08$
		12M	3.38	$0.72 \pm 0.06$
	Sham, 6 w	15M	3.01	$0.85 \pm 0.08$
		16M	3.38	$0.91 \pm 0.01$

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Brain	CBDL, 6 w	18M	3.60	$0.67 \pm 0.07$
		19M	3.47	$0.71 \pm 0.06$
	Sham, 4 w	22F	3.24	$0.95 \pm 0.01$
		23F	3.09	$0.92 \pm 0.04$
	CBDL, 4 w	25F	3.45	$0.84 \pm 0.01$
		26F	3.46	$0.81 \pm 0.02$
	Sham, 6 w	30F	3.08	$0.93 \pm 0.04$
		31F	3.07	$0.95 \pm 0.03$
	CBDL, 6 w	32M	3.81	$0.72 \pm 0.01$
		33F	3.80	$0.66 \pm 0.04$
	Sham, 8 w	36F	3.29	$0.89 \pm 0.06$
		37F	3.54	$0.81 \pm 0.02$
	CBDL, 8 w	39F	3.98	$0.53 \pm 0.03$
		40F	3.63	$0.67 \pm 0.01$
Heart	Sham, 2 w	2M	5.75	$0.08 \pm 0.04$
		3M	6.33	$0.34 \pm 0.01$
	CBDL, 2 w	4M	4.90	$0.11 \pm 0.03$
		7M	5.22	$0.22 \pm 0.04$
	Sham, 4 w	8M	4.33	$0.27 \pm 0.03$
		9M	6.23	$0.48 \pm 0.03$
	CBDL, 4 w	11M	5.79	$-0.14 \pm 0.04$
		12M	5.05	$-0.04 \pm 0.02$
	Sham, 6 w	15M	5.50	$0.36 \pm 0.01$
		16M	5.11	$0.27 \pm 0.07$
	CBDL, 6 w	18M	6.15	$-0.09 \pm 0.04$
		19M	5.07	$-0.15 \pm 0.01$
	Sham, 4 w	22F	6.03	$0.28 \pm 0.03$
		23F	5.91	$0.40 \pm 0.04$
	CBDL, 4 w	25F	5.59	$-0.01 \pm 0.04$
		26F	6.74	$-0.05 \pm 0.04$
	Sham, 6 w	30F	4.72	$0.26 \pm 0.04$
		31F	4.21	$0.29 \pm 0.01$
	CBDL, 6 w	32M	5.73	$0.01 \pm 0.04$
		33F	5.68	$-0.11 \pm 0.04$
Sham, 8 w	36F	5.09	$0.26 \pm 0.03$	
	37F	4.80	$0.29 \pm 0.03$	
CBDL, 8 w	39F	5.35	$-0.34 \pm 0.05$	
	40F	5.64	$-0.34 \pm 0.06$	
Spleen	Sham, 2 w	2M	1.50	$-0.08 \pm 0.00$
		3M	1.10	$0.00 \pm 0.02$

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Spleen	CBDL, 2 w	4M	2.23	$-0.40 \pm 0.03$
		7M	3.72	$-0.25 \pm 0.05$
	Sham, 4 w	8M	2.17	$0.08 \pm 0.04$
		9M	1.83	$0.34 \pm 0.04$
	CBDL, 4 w	11M	3.40	$-0.47 \pm 0.12$
		12M	2.82	$-0.31 \pm 0.05$
	Sham, 6 w	15M	2.26	$0.13 \pm 0.04$
		16M	1.35	$0.00 \pm 0.05$
	CBDL, 6 w	18M	2.82	$-0.25 \pm 0.05$
		19M	2.51	$-0.46 \pm 0.07$
	Sham, 4 w	22F	1.71	$-0.15 \pm 0.05$
		23F	3.01	$0.08 \pm 0.05$
	CBDL, 4 w	25F	4.38	$-0.50 \pm 0.01$
		26F	3.95	$-0.65 \pm 0.02$
	Sham, 6 w	30F	1.73	$0.16 \pm 0.02$
		31F	1.65	$0.19 \pm 0.02$
	CBDL, 6 w	32M	3.88	$-0.09 \pm 0.02$
		33F	3.55	$-0.46 \pm 0.02$
	Sham, 8 w	36F	1.97	$0.25 \pm 0.02$
		37F	1.73	$0.09 \pm 0.02$
CBDL, 8 w	39F	3.16	$-0.57 \pm 0.02$	
	40F	2.55	$-0.59 \pm 0.02$	
Pancreas	Sham, 2 w	2M	1.26	$-0.04 \pm 0.02$
		3M	1.12	$0.09 \pm 0.02$
	CBDL, 2 w	4M	2.02	$-0.06 \pm 0.02$
		7M	1.43	$-0.06 \pm 0.02$
	Sham, 4 w	8M	1.01	$0.25 \pm 0.02$
		9M	1.15	$0.42 \pm 0.02$
	CBDL, 4 w	11M	1.46	$-0.41 \pm 0.01$
		12M	1.12	$-0.31 \pm 0.02$
	Sham, 6 w	15M	1.26	$0.32 \pm 0.02$
		16M	0.90	$0.09 \pm 0.02$
	CBDL, 6 w	18M	1.07	$-0.45 \pm 0.02$
		19M	1.52	$-0.26 \pm 0.02$
	Sham, 4 w	22F	0.93	$0.12 \pm 0.02$
		23F	1.25	$0.00 \pm 0.02$
	CBDL, 4 w	25F	1.19	$-0.45 \pm 0.02$
		26F	1.64	$-0.52 \pm 0.02$
	Sham, 6 w	30F	0.96	$0.15 \pm 0.02$
		31F	1.18	$0.20 \pm 0.02$



Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Pancreas	CBDL, 6 w	32M	1.59	$-0.23 \pm 0.02$
		33F	1.41	$-0.45 \pm 0.02$
	Sham, 8 w	37F	1.22	$0.21 \pm 0.02$
		38F	1.46	$0.22 \pm 0.02$
	CBDL, 8 w	40F	2.53	$-0.17 \pm 0.02$
41F		1.43	$-0.53 \pm 0.03$	
Bone	Sham, 2 w	1M	1.05	$0.11 \pm 0.03$
		2M	0.99	$-0.13 \pm 0.04$
	CBDL, 2 w	4M	1.17	$-0.14 \pm 0.05$
		7M	1.34	$-0.09 \pm 0.07$
	Sham, 4 w	8M	1.47	$-0.01 \pm 0.07$
		10M	1.16	$0.14 \pm 0.04$
	CBDL, 4 w	11M	1.66	$-0.35 \pm 0.05$
		12M	1.30	$-0.33 \pm 0.08$
	Sham, 6 w	15M	1.26	$0.02 \pm 0.05$
		16M	1.18	$-0.03 \pm 0.3$
	CBDL, 6 w	18M	1.26	$-0.23 \pm 0.03$
		19M	1.48	$-0.30 \pm 0.13$
	Sham, 4 w	22F	1.28	$0.00 \pm 0.05$
		23F	0.83	$-0.04 \pm 0.04$
	CBDL, 4 w	25F	1.86	$-0.30 \pm 0.03$
		26F	1.31	$-0.48 \pm 0.02$
	Sham, 6 w	30F	0.99	$0.18 \pm 0.04$
		31F	1.11	$-0.02 \pm 0.04$
	CBDL, 6 w	32M	2.04	$-0.33 \pm 0.04$
		33F	1.57	$-0.49 \pm 0.04$
Sham, 8 w	36F	1.15	$0.04 \pm 0.05$	
	37F	1.19	$0.03 \pm 0.03$	
CBDL, 8 w	39F	2.46	$-0.48 \pm 0.04$	
	40F	1.62	$-0.44 \pm 0.05$	
Muscle	Sham, 4 w	8M	0.83	$0.04 \pm 0.05$
		9M	0.79	$0.03 \pm 0.04$
	CBDL, 4 w	11M	0.70	$-0.41 \pm 0.05$
		12M	0.74	$-0.51 \pm 0.05$
Lung	Sham, 4 w	8M	2.07	$0.61 \pm 0.05$
	CBDL, 4 w	11M	2.74	$0.03 \pm 0.05$
Gallbladder and bile	Sham, 2 w	43M	1.25	$-0.31 \pm 0.02$
		44M	2.31	$-0.18 \pm 0.03$
	CBDL, 2 w	45M	2.07	$-0.02 \pm 0.03$
		46M	0.94	$-0.15 \pm 0.05$
		47M	1.09	$-0.01 \pm 0.01$

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Gallbladder and bile	Sham, 4 w	48M	4.64	$-0.02 \pm 0.03$
		49M	5.00	$0.01 \pm 0.03$
	CBDL, 4 w	50M	3.90	$-0.39 \pm 0.04$
		51M	4.00	$-0.10 \pm 0.03$
		52M	1.81	$-0.24 \pm 0.03$
Duodenum	Sham, 4 w	8M	2.11	$0.50 \pm 0.05$
		9M	1.77	$0.57 \pm 0.05$
		53M	1.71	$0.34 \pm 0.03$
		54M	1.14	$0.48 \pm 0.05$
		55M	1.37	$0.44 \pm 0.07$
	CBDL, 4 w	11M	2.35	$0.01 \pm 0.05$
		12M	2.34	$-0.22 \pm 0.05$
		56M	2.23	$0.11 \pm 0.07$
		57M	3.07	$0.05 \pm 0.10$
		58M	2.41	$0.02 \pm 0.03$
	Sham, 6 w	15M	2.51	$0.55 \pm 0.04$
		16M	1.59	$0.52 \pm 0.04$
	CBDL, 6 w	18M	2.42	$0.00 \pm 0.04$
		19M	2.25	$-0.12 \pm 0.03$
	Caecum	Sham, 2 w	1M	3.02
CBDL, 2 w		4M	6.14	$-0.19 \pm 0.03$
		5M	4.56	$0.02 \pm 0.05$
Sham, 6 w		15M	3.52	$0.19 \pm 0.08$
		16M	3.44	$0.45 \pm 0.03$
CBDL, 6 w		18M	5.27	$-0.01 \pm 0.01$
		19M	5.48	$-0.04 \pm 0.03$
Faeces	Sham, 2 w	2M	22.1	$0.06 \pm 0.03$
		3M	43.5	$0.09 \pm 0.02$
	CBDL, 2 w	4M	42.3	$0.07 \pm 0.03$
		7M	39.9	$0.10 \pm 0.02$
	Sham, 4 w	9M	46.8	$0.13 \pm 0.04$
		10M	58.1	$0.01 \pm 0.01$
	CBDL, 4 w	11M	39.6	$0.08 \pm 0.02$
		12M	43.9	$0.08 \pm 0.03$
	Sham, 6 w	15M	44.1	$0.12 \pm 0.01$
		16M	48.2	$-0.03 \pm 0.05$
	CBDL, 6 w	18M	35.8	$0.03 \pm 0.02$
		20M	47.4	$0.15 \pm 0.05$
	Sham, 4 w	22F	28.3	$0.04 \pm 0.01$
		23F	63.6	$0.10 \pm 0.01$

Sample	Description	Individual ID/ Gender	Cu concentration, $\mu\text{g g}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Faeces	CBDL, 4 w	25F	35.2	$0.08 \pm 0.01$
		26F	32.6	$0.11 \pm 0.03$
	Sham, 6 w	30F	47.6	$0.19 \pm 0.01$
		31F	86.3	$0.22 \pm 0.02$
	CBDL, 6 w	32M	27.0	$0.09 \pm 0.01$
		33F	18.4	$0.11 \pm 0.02$
	Sham, 8 w	36F	26.5	$0.04 \pm 0.01$
		37F	29.5	$0.11 \pm 0.01$
	CBDL, 8 w	39F	38.4	$0.10 \pm 0.02$
		40F	57.1	$0.08 \pm 0.02$
Food	2 w	M	16.4	$0.09 \pm 0.03$
		M	24.4	$0.12 \pm 0.01$
	4 w	F	20.7	$0.17 \pm 0.02$
		F	20.7	$0.10 \pm 0.02$
	4 w	M	17.8	$0.14 \pm 0.01$
		M	23.5	$0.04 \pm 0.02$
	6 w	F	15.8	$0.06 \pm 0.02$
		F	18.0	$0.18 \pm 0.01$
	6 w	M	16.5	$0.12 \pm 0.01$
		M	14.0	$0.19 \pm 0.02$
8 w	F	15.4	$0.10 \pm 0.02$	
	F	14.2	$0.15 \pm 0.05$	

Sample	Description	Gender	Cu concentration, $\mu\text{g L}^{-1}$	$\delta^{65}\text{Cu} \pm \text{sd} (\text{‰})$
Serum	Sham, 2 w	M	0.54	$-0.88 \pm 0.07$
	CBDL, 2 w	M	1.99	$-0.91 \pm 0.01$
		M	1.52	$-1.27 \pm 0.01$
	Sham, 4 w	M	0.53	$-0.66 \pm 0.02$
	CBDL, 4 w	M	1.63	$-1.20 \pm 0.01$
		M	1.81	$-1.08 \pm 0.04$
	Sham, 6 w	M	0.62	$-0.64 \pm 0.02$
	CBDL, 6 w	M	1.96	$-1.26 \pm 0.04$
	Sham, 4 w	F	0.59	$-0.71 \pm 0.02$
		F	0.52	$-0.49 \pm 0.02$
	CBDL, 4 w	F	1.69	$-1.43 \pm 0.02$
		F	2.02	$-1.28 \pm 0.02$
	Sham, 6 w	F	0.83	$-0.73 \pm 0.01$
	CBDL, 6 w	M	2.49	$-1.20 \pm 0.07$
	Sham, 8 w	F	0.44	$-0.55 \pm 0.02$
	CBDL, 8 w	F	2.36	$-1.54 \pm 0.06$
F		2.27	$-1.50 \pm 0.04$	
Whole blood	Sham, 6 w	M	0.53	$0.19 \pm 0.04$
		M	0.50	$-0.07 \pm 0.05$
	CBDL, 6 w	M	1.35	$-0.62 \pm 0.02$
		M	1.77	$-0.82 \pm 0.04$
Drinking water	2 w	M	6.03	$-0.30 \pm 0.02$
	4 w	M	5.68	$0.08 \pm 0.02$
	4 w	F	16.3	$-0.20 \pm 0.02$
	6 w	M	3.08	$0.03 \pm 0.02$
	6 w	F	6.27	$-0.05 \pm 0.03$
	8 w	F	12.1	$-0.19 \pm 0.01$

\* Serum samples from several individuals were pooled.