Synthesis of non-radioactive *N*-methyl-taurine conjugated bile acids – Reference materials

Chemicals and materials

Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), *N*-methyl-taurine sodium salt, diethyl phosphoryl cyanide (DEPC), triethylamine (Et_3N), dimethylformamide (DMF; dry) were obtained from Sigma-Aldrich Ltd. and used as received. Concentrated HCl (diluted to approximately 10% with water), ethanol (EtOH), methanol (MeOH), diethyl ester (Et_2O) and ethylacetate (EtOAc) were obtained as analytical grade from VWR International Ltd. 1 N aqueous NaOH (sterile) was prepared by the pharmacy at Aarhus University Hospital. Sep-Pak® C18 Plus Short Cartridges (360 mg sorbent per cartridge, 55–105 μm particle size) were obtained from Waters® and preconditioned before use with ethanol (10 mL) followed by water (10 mL).

Synthesis

Analytical amounts of non-radioactive *N*-methyl-taurine conjugated bile acids, used as reference materials in the radiosyntheses of their respective ^{11}C-derivatives, were prepared by a method similar to one reported for preparation of the corresponding taurine conjugates (*1*). In brief, to a solution of the unconjugated bile acid in dry DMF (0.25 M) at 0 °C were added successively *N*-methyl-taurine sodium salt (1 eq.), DEPC (1.1 eq.) and Et_3N (2.9 eq.). The solution was stirred at 0 °C for 45 min, then overnight at room temperature. The reaction mixture was quenched by adding 1 N NaOH until pH >12. The pH was then adjusted to approximately 7 with aqueous 10% HCl. The neutralized mixture was diluted with water (20 mL) and then put on a preconditioned C18-cartridge. The cartridge was successively washed with water (20 mL) and 25% aqueous ethanol (20 mL) and then eluted with 100% ethanol (20 mL). The last fraction was collected and evaporated to dryness under a stream of nitrogen at room temperature. The remaining solid residue was recrystallized from EtOH/EtOAc (MTCA, MTCDCA, MTUDCA), aqueous MeOH/EtOAc (MTDCA) or MeOH/Et_2O (MTLCA) to

give the final *N*-methyl-taurine conjugated bile acid as its sodium salt. All conjugated bile acids were obtained as white solids and characterized by ESI-MS.

Determination of lipophilicity by reverse phase thin layer chromatography (RP-TLC)

The lipophilicity (= hydrophobicity – polarity) of CSar (MGCA), MTCA, MTCDCA, MTDCA, MTUDCA, MTLCA as well as GCA, TCA and CA was determined by RP-TLC (*2*): Samples of non-radioactive bile acids in methanol (approximately 2 μM) were spotted on pre-coated C18 RP-TLC (5x10x0.15) plates (Alugram® RP-18W/UV_{254}, Macherey-Nagel) and the solvent was subsequently evaporated gently by heating with warm air (50 °C). The plates were eluted in a closed chamber at room temperature using mixtures of methanol and aqueous ammonium acetate (15 mM) adjusted to pH 7.40 with 25% aqueous ammonia. The plates were eluted with five different concentrations of methanol (50%, 60%, 70%, 80%, and 90%). The eluted spots were visualized with 5% aqueous H_2SO_4 and heating with hot air (300 °C). The measured retention factor R_f (i.e. travelled distance of spot over travelled distance of solvent front) was used to calculate R_M according to equation 1. Over the five concentration levels of methanol, ϕ, the relationship with R_M was linear ($r^2 > 0.95$) for all compounds investigated. This allowed for determination of R_{Mw} and S, where R_{Mw} reflects the lipophilicity of the compound, i.e. the higher the value of R_{Mw}, the more lipophilic, and S the degree of responsiveness to changes in mobile phase composition.

$$R_M = R_{Mw} - S\phi \quad \text{where } R_M = \log(1/R_f - 1) \qquad \text{(eq. 1)}$$

Tracer specific details for LC-MS analysis

For all tracers, the chromatographic column used was a Phenomenex® Synergi™ 4 μm MAX-RP 80A (2.0x100 mm) and the flow was 0.9 mL/min. Tracer specific details for LC-MS analysis are given in Supplemental Tables 1 and 2.

Supplemental Table 1. Tracer specific details for LC-MS analysis

Tracer	Eluent		R.T.	R.T.	R.T.
	Acetonitrile	Aq. NH_4OAc*	(tracer)†	(*N*-desmethyl tracer)†	(parent bile acid)†
^{11}C-MTCA	30 %	70 %	7.5 min	5.8 min	10.9 min
			(528.3 m/z)	(514.3 m/z)	(407.3 m/z)
^{11}C-MTUDCA	31 %	69 %	6.2 min	4.6 min	11.3 min
			(512.3 m/z)	(498.3 m/z)	(391.3 m/z)
^{11}C-MTCDCA	36 %	64 %	6.8 min	5.5 min	12.1 min
			(512.3 m/z)	(498.3 m/z)	(391.3 m/z)
^{11}C-MTDCA	37 %	63 %	6.8 min	5.6 min	11.0 min
			(512.3 m/z)	(498.3 m/z)	(391.3 m/z)
^{11}C-MTLCA	46 %	54 %	5.8 min	4.9 min	12.7 min
			(496.3 m/z)	(482.3 m/z)	(375.3 m/z)

* 1 mM aqueous ammonium acetate adjusted to pH 3.0 with glacial acetic acid,

† Retention times (R.T.) for the tracer, its non-^{11}C-methylated conjugate and its parent unconjugated bile acid. Values in parentheses are the target masses. For all tracers, the mass spectrometer is running in negative ionization mode ($M-H^+$).

Supplemental Table 2. Mass Spectrometer Instrument Settings

Trap settings		ESI inlet setting		Smart Parameter Setting (SPS)	
Ion charge control (ICC):	On	Capillary voltage:	+4,500 V	Target mass:	(reported in Supplemental Table 1)
SmartTarget:	40,000	Nebulizer pressure:	70.0 psi	Compound stability:	100 %
Max. accu. Time:	100.00 ms	Dry gas:	12.0 L/min	Trap drive level:	60 %

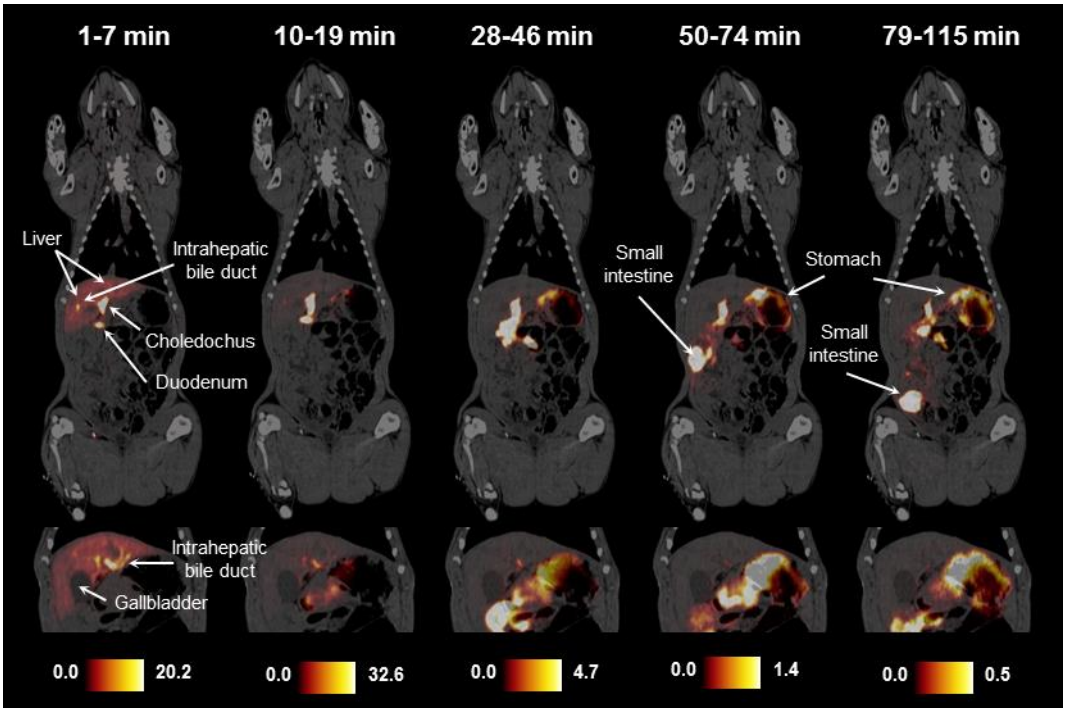
Scan window:	400–600 m/z	Dry temperature:	365 °C

Dosimetry data

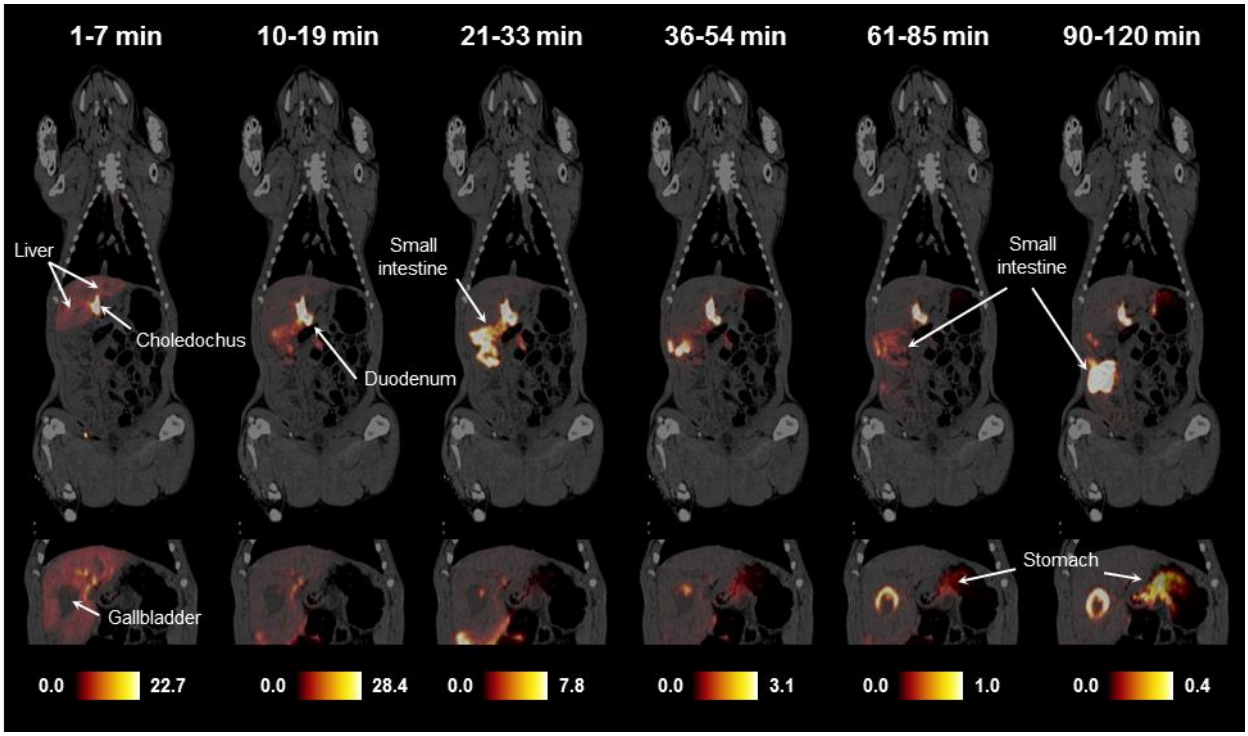
For each tracer, the data was obtained from PET/CT biodistribution of the tracers in pigs (36–41 kg) and extrapolated to 74-kg human data as described in the main paper. Data for ^{11}C-MGCA (^{11}C-CSar) is from reference (*3*).

Supplemental Table 3. Absorbed Dose Estimates for ^{11}C-MTCA, ^{11}C-MTUDCA, ^{11}C-MTLCA, and ^{11}C-MGCA

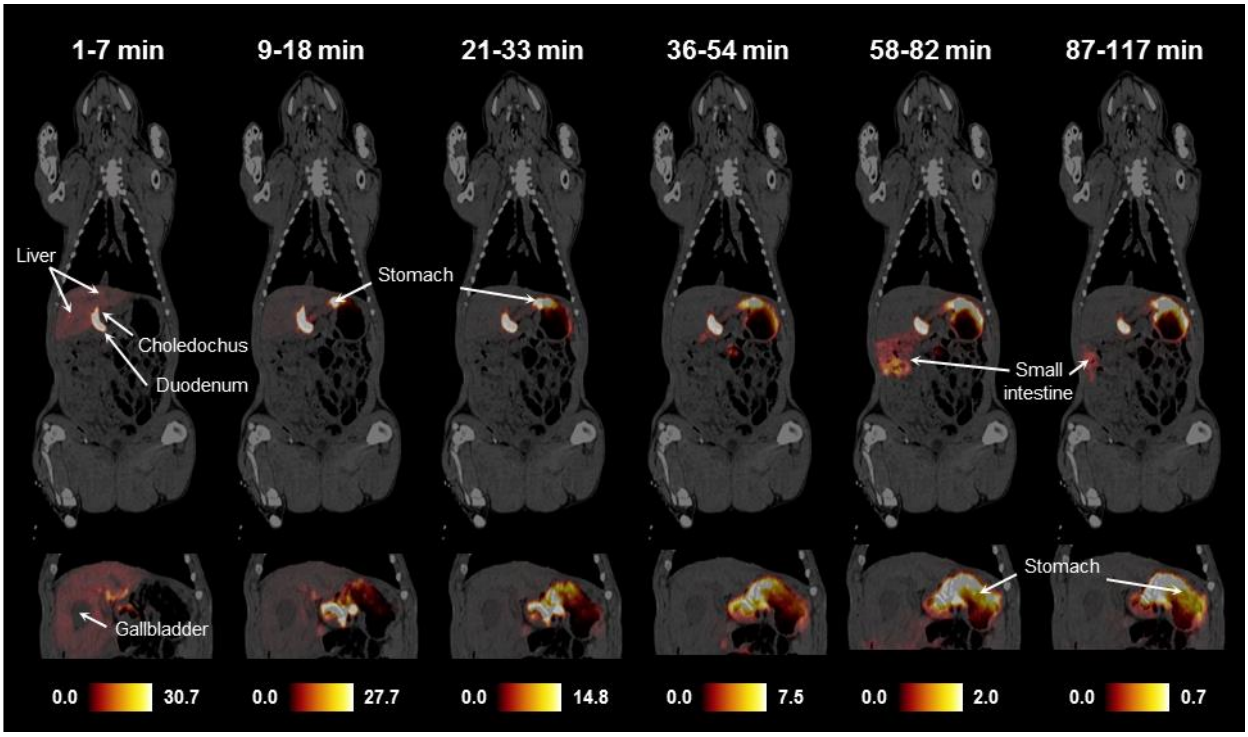
Target organ	**^{11}C-MTCA (µGy/MBq)**		**^{11}C-MTUDCA (µGy/MBq)**		**^{11}C-MTLCA (µGy/MBq)**		**^{11}C-MGCA (µGy/MBq)**
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	From (*3*)
Adrenals	3.2	3.0	3.0	3.0	3.4	3.3	2.9
Brain	1.4	1.5	1.5	1.8	1.4	1.8	1.6
Breasts	1.6	1.7	1.6	1.8	1.7	1.9	1.7
Gallbladder wall	5.4	31.1	22.0	17.3	5.1	4.5	59.4
Lower large intestine wall	3.5	3.7	3.9	3.8	2.9	3.0	3.9
Small intestine	34.3	36.4	41.1	32.1	19.6	15.0	39.3
Stomach wall	13.0	11.8	3.5	3.4	53.2	38.0	3.9
Upper large intestine wall	6.2	6.6	7.0	6.1	4.8	4.3	7.1
Heart wall	2.5	2.4	2.3	2.5	2.8	2.9	2.3
Kidneys	3.1	3.1	3.1	3.1	3.1	3.1	2.3
Liver	19.5	13.4	15.7	11.8	13.7	11.2	10.8
Lungs	2.1	2.1	2.1	2.3	2.2	2.4	2.1
Muscle	2.1	2.2	2.1	2.3	2.1	2.3	2.2
Ovaries	4.4	4.7	5.0	4.6	3.4	3.4	5.0
Pancreas	3.7	3.7	3.2	3.2	5.6	4.9	3.4
Red marrow	2.2	2.3	2.3	2.4	2.1	2.2	2.3
Bone surface	2.6	2.8	2.7	3.1	2.7	3.1	2.9
Skin	1.5	1.6	1.5	1.8	1.5	1.8	1.6
Spleen	2.5	2.6	2.3	2.5	3.8	3.6	2.4
Testes	1.6	1.7	1.7	2.0	1.6	2.0	1.8
Thymus	1.8	1.9	1.8	2.1	1.9	2.2	1.9
Thyroid	1.6	1.7	1.7	2.0	1.7	2.0	1.8
Urinary bladder wall	2.4	2.5	2.5	2.8	2.2	2.5	2.7
Uterus	4.1	4.4	4.6	4.3	3.2	3.2	4.6
Total body	2.9	2.8	2.9	2.9	2.7	2.7	2.8
Effective dose (µSv/MBq)	**5.6**	**5.3**	**4.6**	**4.2**	**9.2**	**7.3**	**4.4**



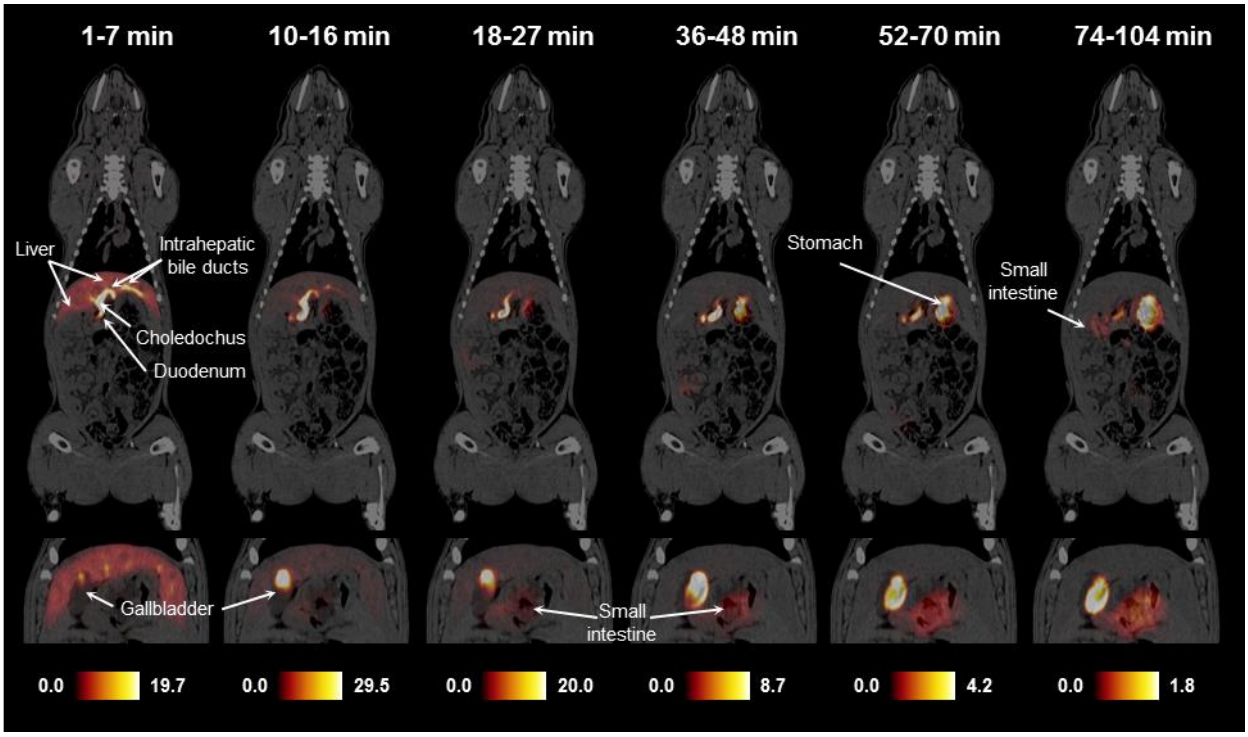
Supplemental Figure 1. Whole-body PET/CT images (coronal view) recorded successively after intravenous bolus administration of ^{11}C-MTCA (511 MBq) in pig no. 1. The insets are for the same respective time intervals, but a different slice to illustrate the content of the gallbladder. The scans were performed with 3, 9, 4 and 5 min between scans and with a progressive increase in scan duration per bed position of 1, 1.5, 3, 4, and 6 min. The color scales are in MBq/ml.



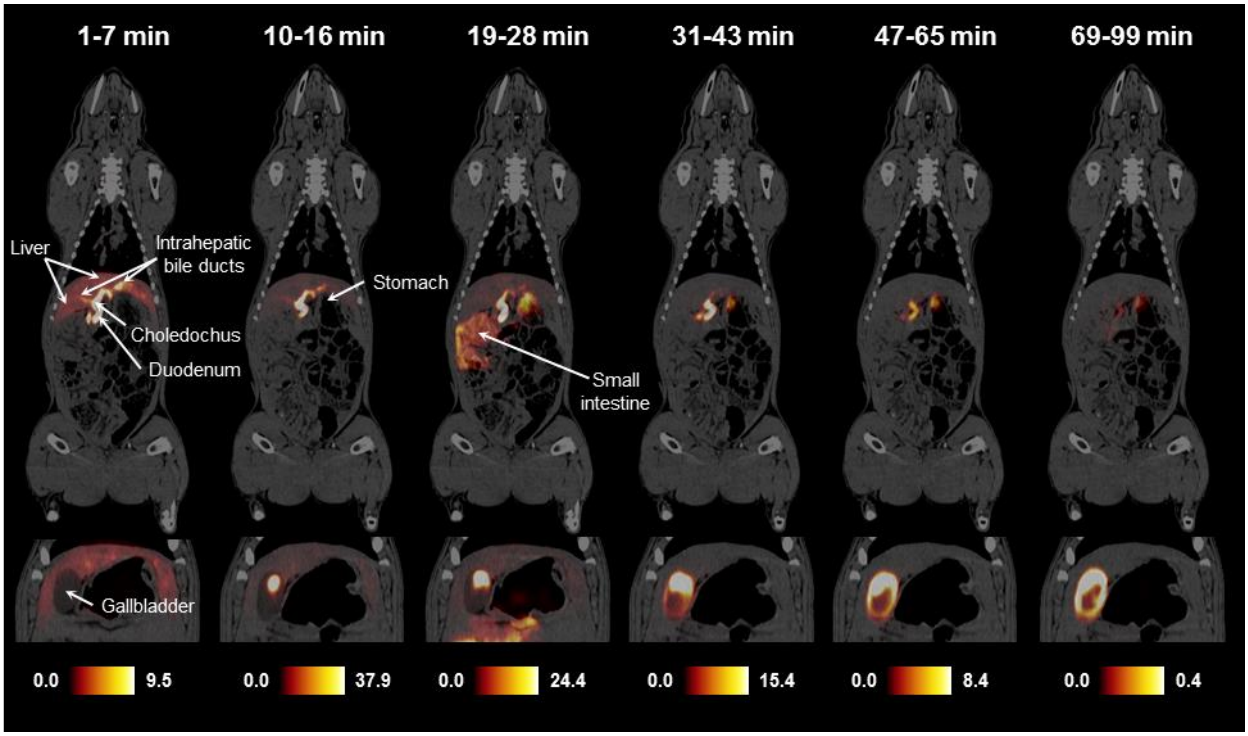
Supplemental Figure 2. Whole-body PET/CT images (coronal view) recorded successively after intravenous bolus administration of ^{11}C-MTUDCA (508 MBq) in pig no. 1. The insets are for the same respective time intervals, but a different slice to illustrate the content of the gallbladder. The scans were performed with 3, 2, 3, 7 and 5 min between scans and with a progressive increase in scan duration per bed position of 1, 1.5, 2, 3, 4, and 5 min. The color scales are in MBq/ml.



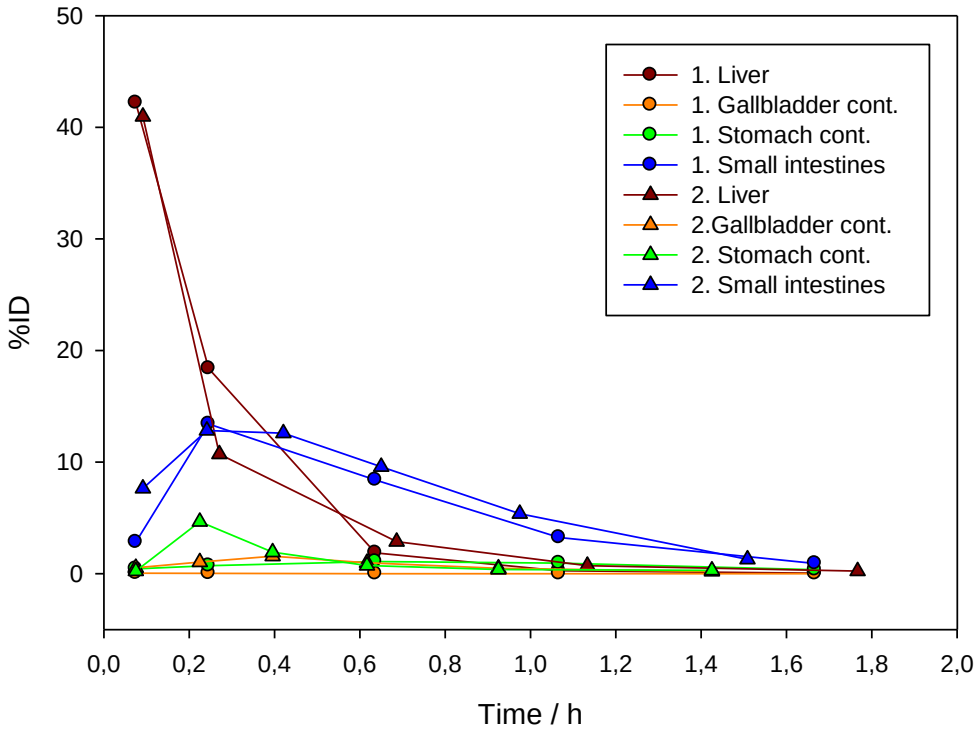
Supplemental Figure 3. Whole-body PET/CT images (coronal view) recorded successively after intravenous bolus administration of ^{11}C-MTLCA (486 MBq) in pig no. 1. The insets are for the same respective time intervals, but a different slice to illustrate the content of the gallbladder. The scans were performed with 2, 3, 3, 4 and 5 min between scans and with a progressive increase in scan duration per bed position of 1, 1.5, 2, 3, 4, and 5 min. The color scales are in MBq/ml.



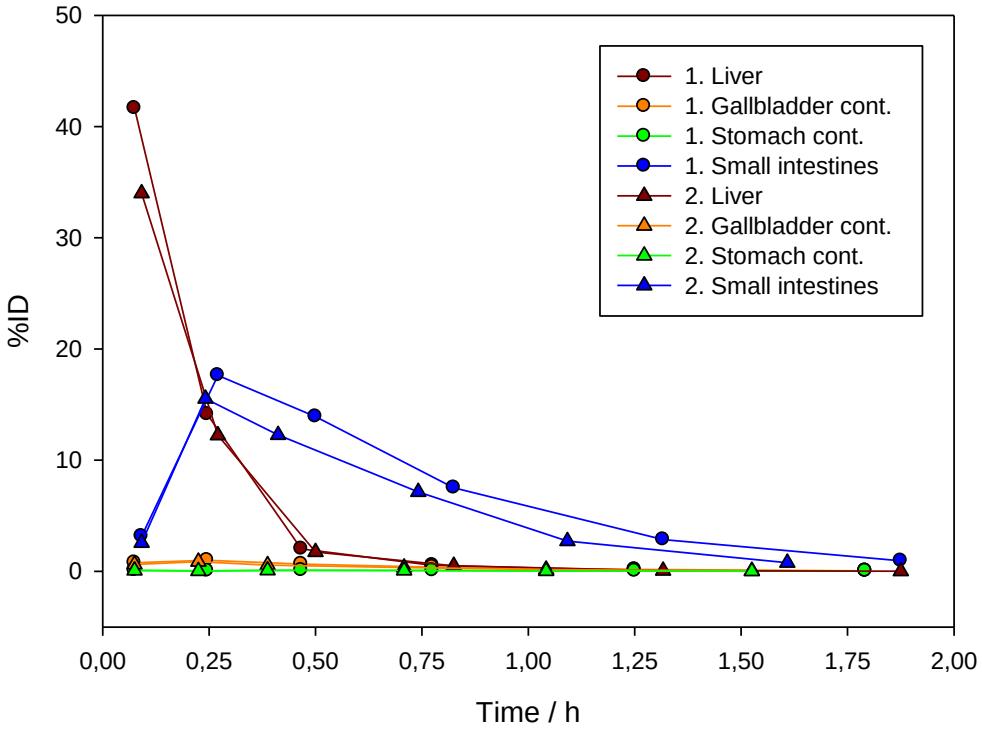
Supplemental Figure 4. Whole-body PET/CT images (coronal view) recorded successively after intravenous bolus administration of ^{11}C-MUDCA (553 MBq) in pig no. 2. The insets are for the same respective time intervals, but a different slice to illustrate the content of the gallbladder. The scans were performed with 3, 2, 9, 5 and 4 min between scans and with a progressive increase in scan duration per bed position of 1, 1, 1.5, 2, 3, and 5 min. The color scales are in MBq/ml.



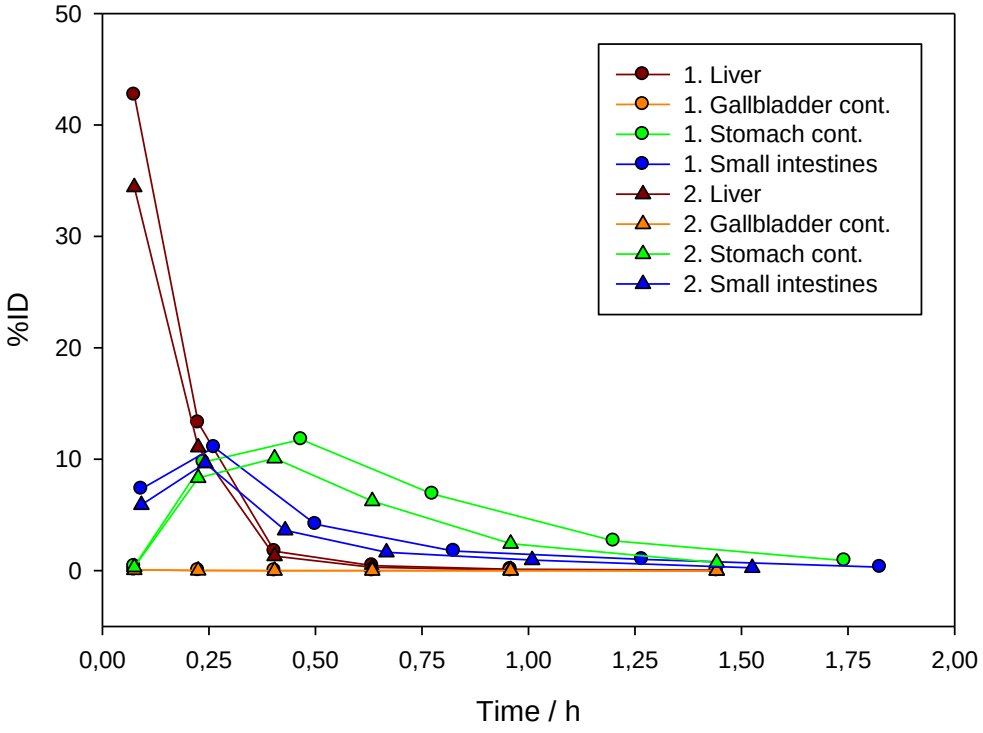
Supplemental Figure 5. Whole-body PET/CT images (coronal view) recorded successively after intravenous bolus administration of ^{11}C-MLCA (536 MBq) in pig no. 2. The insets are for the same respective time intervals, but a different slice to illustrate the content of the gallbladder. The scans were performed with 3, 3, 3, 4 and 4 min between scans and with a progressive increase in scan duration per bed position of 1, 1, 1.5, 2, 3, and 5 min. The color scales are in MBq/ml.



Supplemental Figure 6. Time-activity-curve (% of injected dose versus time; not decay-corrected) for ^{11}C-MTCA. Circles are for pig no. 1, triangles for pig no. 2.



Supplemental Figure 7. Time-activity-curve (% of injected dose versus time; not decay-corrected) for ^{11}C-MTUDCA. Circles are for pig no. 1, triangles for pig no. 2.



Supplemental Figure 8. Time-activity-curve (% of injected dose versus time; not decay-corrected) for ^{11}C-MTLCA. Circles are for pig no. 1, triangles for pig no. 2.

References

1. Momose T, Tsubaki T, Iida T, Nambara T. An improved synthesis of taurine- and glycine-conjugated bile acids. *Lipids.* 1997;32:775–778.

2. Sharma R, Majer F, Peta VK, et al. Bile acid toxicity structure–activity relationships: correlations between cell viability and lipophilicity in a panel of new and known bile acids using an oesophageal cell line (HET-1A). *Bioorg Med Chem.* 2010;18:6886–6895.

3. Frisch K, Jakobsen S, Sørensen M, et al. [*N*-Methyl-^{11}C]Cholylsarcosine, a novel bile acid tracer for PET/CT of hepatic excretory function: radiosynthesis and proof-of-concept studies in pigs. *J Nucl Med*. 2012;53:772–778.