**Fig. S1**: Membrane integrity and cell viability in new cell culture inserts (N=5) vs recycled cell culture inserts (N=50) (A) Mean TEER values ± SD 12 days after seeding; (B) WST-1 assay; mean relative absorbances ± SD 17 days after seeding. Ns, no significant differences (unpaired t-test).
Tab. S1: MS parameters, limit of detection (LOD) and limit of quantification (LOQ) of the BAs studied

<table>
<thead>
<tr>
<th></th>
<th>Mode</th>
<th>Q1</th>
<th>Q3</th>
<th>Retention time (min)</th>
<th>LOD (nM)</th>
<th>LOQ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxycholic acid (DCA)</td>
<td>SIM</td>
<td>391.3</td>
<td></td>
<td>11.073</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid (GCDCA)</td>
<td>MRM</td>
<td>448.3</td>
<td>74</td>
<td>9.051</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Glycodeoxycholic acid (GDCA)</td>
<td>MRM</td>
<td>448.3</td>
<td>74</td>
<td>9.452</td>
<td>190</td>
<td>200</td>
</tr>
<tr>
<td>Glycocholic acid (GCA)</td>
<td>MRM</td>
<td>464.3</td>
<td>74</td>
<td>7.310</td>
<td>122</td>
<td>140</td>
</tr>
</tbody>
</table>

Fig. S2: The effect of odevixibat (ODE) on Caco-2 cells
(A) The effect of 24-h exposure to ODE on cell viability of undifferentiated Caco-2 cells assessed using the WST-1 assay. Absorbance is normalized to the solvent control. Values represent the mean ± SD of triplicate measurements in 3 independent experiments. Significance was assessed with a one-way ANOVA followed by post hoc tests using Bonferroni’s correction. *, p<0.05. POS, positive control (0.5 µM potassium dichromate). (B) The effects of 3-h co-exposure to 5 µM GCA and an ODE concentration range on TEER values of Caco-2 cells cultured on culture inserts on day 19-21. Values represent the mean ± SD, N = 3.
Fig. S3: Observed and predicted postprandial GCA kinetics using different values for $K_m$-values for active intestinal uptake
Black solid line = prediction. Left column: Meals were simulated at 8:00, 12:00, and 16:00. Orange circles = in vivo data obtained from Hepner and Demers (1977). Right column: Meal was simulated at 8:00. Blue circles = in vivo data obtained from Lamaziere (2020). (A) $K_m = 39$ µM (experimentally derived, no correction for the aqueous boundary layer). (B) $K_m = 22.5$ µM (experimentally derived, corrected for the aqueous boundary layer). (C) $K_m = 16.8$ µM (average of experimental ABL-corrected and literature). (D) $K_m = 11$ µM (literature). GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; uBA, unconjugated bile acids
Fig. S4: Sensitivity analysis of the influence of the PBK model parameters on the predicted maximal systemic BA concentration in plasma ($C_{\text{max}}$).

Only parameters with an absolute normalized sensitivity coefficient > 0.5 are shown. ka, first order rate absorption constant; Clint_PDc, passive diffusion intrinsic clearance; BP, blood:plasma ratio; KmNTCP, Michaelis-Menten constant for NTCP-mediated hepatic uptake; VmaxASBTc, maximal ASBT-mediated GCA absorption rate over the ileal epithelium; ES, empirical scalar for in vitro-in vivo extrapolation of Caco-2-derived kinetic parameters; surface, cylindrical surface of ileum; QPVc, fraction of blood flow to liver through the portal vein; VmaxNTCPC, maximal NTCP-mediated hepatic BA uptake; Hep, hepatocellularity; SF_OATP, scaling factor to adjust hepatic uptake for OATP-mediated uptake; BW, body weight; get, gall bladder ejection time. _GCA, _GCDCA or _GDCA indicates a parameter specifically for the GCA, GCDCA or GDCA submodel, respectively. _all indicates a parameter that is shared for all BA submodels.
Fig. S5: Sensitivity analysis of the influence of the PBK model parameters on the predicted maximal bile acid concentrations in the intracellular liver
Only parameters with an absolute normalized sensitivity coefficient > 0.5 are shown. ka, first order rate absorption constant; ge, first order rate constant for gall bladder ejection rate; kam, first order rate constant for amidation; BW, body weight; KmBSEP, Michaelis-Menten constant for BSEP-mediated BA efflux from the liver; VmaxASBTc, maximal ASBT-mediated GCA absorption rate over the ileal epithelium; VmaxBSEPc, maximal BSEP-mediated BA efflux rate; aBSEP, BSEP protein abundance; MWBSEP, molecular weight of BSEP; Hep, hepatocellularity; ES, empirical scalar for in vitro-in vivo extrapolation of Caco-2 derived kinetic parameters; surface, ileal surface; get, gall bladder ejection time; Qib, fraction of bile flow transported directly from liver to intestinal lumen via common bile duct. GCA, _GCDCA or _GDCA indicates a parameter specifically for the GCA, GCDCA or GDCA submodel, respectively. _all indicates a parameter that is shared for all BA submodels.
Fig. S6: Sensitivity analysis of the influence of the PBK model parameters on the predicted time it takes to reach maximum plasma BA concentration ($T_{\text{max}}$)

Only parameters with an absolute normalized sensitivity coefficient $> 0.03$ are shown. BW, body weight; kti$_{\text{fed}}$, ileal transit time in fed state; kti$_{\text{fed}}$, jejunal transit time in fed state; QAVc, fraction of blood flow through the hepatic arterial vein; BP, blood:plasma ratio; get, gall bladder ejection time; QC, cardiac output; PS, slowly perfused tissue/plasma partition coefficient; VBc, fraction of blood (excluding portal vein); VSc, fraction of slowly perfused tissue; ka, first-order rate constant for absorption. GCA, _GCDCA or _GDCA indicates a parameter specifically for the GCA, GCDCA or GDCA submodel, respectively. _all indicates a parameter that is shared for all BA submodels.
Model code and input parameters

#PBK model 4 bile acids
#GCDCA, GCA, GDCA + DCA
#species: human
#author: Véronique de Bruijn
#date: June 2023

## The input parameters, as well as their units, explanation and a reference is described in the supplementary Excel file "parms_4BA_compLiver.xlsx". Save this file in the working directory and load it in in step 1.

#load required packages
library(RxODE)
library(tidyverse)
library(readxl)
library(data.table)
library(pmxTools)

#set working directory
setwd()

#Simulations
amount.units="umol" #unit for the amount
time.units="h" #unit for time
nbr.doses=1 #number of doses
time.0=8 #time start dosing
time.end=32 #time end of simulation
time.frame=0.01 #time steps of simulation
N=1 #Number of individuals

#############################################################
#step 1: read in parameters
parms <- read_excel("parms_4BA_compLiver.xlsx") %>%
dplyr::select(1:6)

parms_long <- melt(setDT(parms), id.vars=1, variable.name="parm") %>%
mutate(name=paste(parm, parm.1, sep="_")) %>%
dplyr::select(3,4) %>%
drop_na()

my_names <- parms_long$name
var <- parms_long$value

parameters <- setNames(var, my_names)

Gdose <- parms %>% filter(parm="Gdose")

#############################################################
#step 2: initial values compartment
inits <- c(dose_GCDCA=Gdose$GCDCA,
ALEW_GCDCA=0,
ALIW_GCDCA=0,
ALu_GCDCA=0,
Aup_GCDCA=0,
Alow_GCDCA=0,
Acol_GCDCA=0,
AI_GCDCA=0,
AP_GCDCA=0,
AF_GCDCA=0,
AR_GCDCA=0,
AS_GCDCA=0,
AB_GCDCA=0,
dose_GDCA=Gdose$GDCA,
ALEW_GDCA=0,
ALIW_GDCA=0,
### Step 3: Exposure

qd <- eventTable(amount.units = amount.units, time.units = time.units) %>%
  add.dosing(dose = 0.00001, cmt = "ALEW_GCDCA", nbr.doses = 1, do.sampling = FALSE) %>%
eq(seq(from = time.0, to = time.end, by = time.frame))

### Step 4: Differential Equations

PBK <- RxODE(
  
  ## Physiological parameters
  # Tissue volumes
  VF = VFc_all*BW_all; # (L or Kg); volume of fat tissue (calculated)
  VLIW = VLIWc_all*BW_all; # (L or Kg); volume of liver intracellular water (calculated)
  VLEW = VLEWc_all*BW_all; # (L or Kg); volume of liver extracellular water (calculated)
  VL = VLIW + VLEW;
  VR = VRc_all*BW_all; # (L or Kg); volume of richly perfused tissue (calculated)
  VS = VSc_all*BW_all; # (L or Kg); volume of slowly perfused tissue (calculated)
  VB = VBc_all*BW_all; # (L or Kg); volume of blood excluding portal vein (calculated)
  VI = Vlc_all*BW_all; # (L or Kg); volume of intestinal tissue (calculated)
  VG = VGc_all*BW_all; # (L or Kg); volume of gall bladder tissue (calculated)
  VLu = VLuc_all*BW_all; # (L or Kg); volume of intestinal lumen (calculated)
  VP = VBPc_all*BW_all; # (L or Kg); volume of portal blood

  # Blood flow rates
  QF = QFc_all*QC_all; # (L/hr); blood flow to fat tissue (calculated)
  QAV = QAVc_all*QC_all; # (L/hr); arterial blood flow to liver tissue (calculated)
  QPV = QPVc_all*QC_all; # (L/hr); portal blood flow to liver tissue (calculated)
  QS = QSc_all*QC_all; # (L/hr); blood flow to slowly perfused tissue (calculated)
  QR = Q Rc_all*QC_all; # (L/hr); blood flow to richly perfused tissue (calculated)
  QI = QIc_all*QC_all; # (L/hr); blood flow to intestines (calculated)

  ## Scaling of maximal transport rate (Vmax) for BSEP, ASBT and NTCP mediated BA transport
  WL = 20*BW_all; # weight of liver, g
SF_BSEP=aBSEP_all*MWBSEP_all*Hep_all*WL*10^-9; # mg BSEP/entire liver; scaling factor for BSEP mediated hepatic efflux for GCA and GCDCA
SF_ASBT=surface_all*10^-6*ES_all; # cm²/entire ileum; fitted
SF_NTCP=Hep_all*SF_OATP_all*WL*10^-6; # 10⁶ hepatocytes/entire liver; scaling factor for hepatic transport

VmaxBSEP_GCDCA=VmaxBSEPc_GCDCA*SF_BSEP*60; # umol/h/entire liver) maximum speed for BSEP-mediated GCDCA efflux (calculated)
VmaxASBT_GCDCA=VmaxASBTc_GCDCA*SF_ASBT*60; # (umol/hr/ileum) maximum speed for ASBT-mediated GCDCA uptake (calculated)
VmaxNTCP_GCDCA=VmaxNTCPc_GCDCA*SF_NTCP*60; # (umol/hr/entire liver) maximum speed for hepatic GCDCA uptake (calculated)

VmaxBSEP_GCA=VmaxBSEPc_GCA*SF_BSEP*60; # (umol/h/entire liver) maximum speed for BSEP-mediated GCA efflux (calculated)
VmaxASBT_GCA=VmaxASBTc_GCA*SF_ASBT*60; # (umol/hr/ileum) maximum speed for ASBT-mediated GCA uptake (calculated)
VmaxNTCP_GCA=VmaxNTCPc_GCA*SF_NTCP*60; # (umol/hr/entire liver) maximum speed for hepatic GCA uptake (calculated)

VmaxBSEP_GDCA=VmaxBSEPc_GDCA*SF_BSEP*60; # (umol/h/entire liver) maximum speed for BSEP-mediated GDCA efflux (calculated)
VmaxASBT_GDCA=VmaxASBTc_GDCA*SF_ASBT*60; # (umol/hr/ileum) maximum speed for ASBT-mediated GDCA uptake (calculated)
VmaxNTCP_GDCA=VmaxNTCPc_GDCA*SF_NTCP*60; # (umol/hr/entire liver) maximum speed for hepatic GDCA uptake (calculated)

## Fast/fed state

ge=ifelse((ctime>8 & ctime<(8+get_all)), ge_all,0); # specifying the duration of gallbladder emptying (1 meal)
ge=ifelse(((ctime >8 & ctime<(8+get_all)) | (ctime>12 & ctime<(12+get_all)) | (ctime>16 & ctime<(16+get_all))), ge_all,0); # specifying the duration of gallbladder emptying (3 meals)

# (umol/hr/entire liver), calculated
ktj=ifelse(ctime>17.5 | ctime <8, ktj_fasted_all, ktj_fed_all); # specifying different jejunum transit rates for fast/fed state (1/hr)
kti=ifelse(ctime>17.5 | ctime <8, kti_fasted_all, kti_fed_all); # specifying different ileum transit rates for fast/fed state (1/hr)

QGb_all=1-QIb_all; # fraction of bile flow from liver stored in gall bladder, calculated

## Competitive inhibition by ODE on ASBT-mediated BA transport

IDOSE=ODOSEmg_all/740.29/9*10^-6*ODE_fub_all; # free ODE concentration in ileum, calculated (nM), MW=740.29, volume intestinal tract=9L
modf_Km=1+(IDOSE/0.02); # modulation factor for Km based on competitive inhibition, Ki=0.02 nM (experimental)

## Model calculations

### GCDCA submodel ###

CLEW_GCDCA=ALEW_GCDCA/VLEW; # concentration in the extracellular water (liver)
CVLEW_GCDCA=CLEW_GCDCA/(PEWP_GCDCA*BP_all); # concentration leaving the extracellular water (liver)
CLIW_GCDCA=ALIW_GCDCA/VLIW; # concentration in intracellular water (liver)
CI_GCDCA=Alow_GCDCA/VIl_all; # concentration in the ileum (umol/L)
CI_GCDCA=AI_GCDCA/VI; # concentration in the intestinal tissue (umol/L)
CVI_GCDCA=CI_GCDCA/(PG_GCDCA*BP_all); # concentration in venous blood leaving the intestinal tissue (umol/L)
CF_GCDCA=AF_GCDCA/VF; # concentration in the fat tissue (umol/L)
CVF_GCDCA=CF_GCDCA/(PF_GCDCA*BP_all); # concentration in venous blood leaving the fat tissue (umol/L)
CR_GCDCA=AR_GCDCA/VR; # concentration in the rapidly perfused tissue (umol/L)
CVR_GCDCA=CR_GCDCA/(PR_GCDCA*BP_all); # concentration in venous blood leaving the rapidly perfused tissue (umol/L)
CS_GCDCA=AS_GCDCA/VS; # concentration in the slowly perfused tissue (umol/L)
CVS_GCDCA=CS_GCDCA/(PS_GCDCA*BP_all); # concentration in venous blood leaving slowly perfused tissue (umol/L)
CB_GCDCA=AB_GCDCA/VB; # concentration in blood excluding portal vein (umol/L)
CP_GCDCA=AP_GCDCA/VP; # concentration in portal blood (umol/L)
\[
d\frac{dt}{dt}(dose_{GCDCA}) = -ge*dose_{GCDCA} + VmaxBSEP_{GCDCA} * CLIW_{GCDCA} / (KmBSEP_{GCDCA} + CLIW_{GCDCA}) * QGb\text{ all} \\
\text{# change in amount in the gall bladder, umol}
\]

\[
d\frac{dt}{dt}(ALEW_{GCDCA}) = -VmaxBSEP_{GCDCA} * CLIW_{GCDCA} / (KmBSEP_{GCDCA} + CLIW_{GCDCA}) \\
\text{+ Cam_all} * ALIW_{DCA} * Gdose_{GCDCA} + Acol_{DCA} * 0.05 * Gdose_{GCDCA} \ ; \text{# change in amount in intracellular water (liver), umol}
\]

\[
d\frac{dt}{dt}(Aup_{GCDCA}) = ge*dose_{GCDCA} + VmaxBSEP_{GCDCA} * CLIW_{GCDCA} / (KmBSEP_{GCDCA} + CLIW_{GCDCA}) * QIb\text{ all} \\
\text{# change in amount in upper intestinal lumen, umol}
\]

\[
d\frac{dt}{dt}(Alow_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in ileum, umol}
\]

\[
d\frac{dt}{dt}(Acol_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) + ka_{GCDCA} * Aup_{GCDCA} \\
\text{# change in amount in portal blood, umol}
\]

\[
d\frac{dt}{dt}(AF_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in fat tissue, umol}
\]

\[
d\frac{dt}{dt}(AR_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in slowly perfused tissue, umol}
\]

\[
d\frac{dt}{dt}(AS_GCDCA) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in slowly perfused tissue, umol}
\]

\[
d\frac{dt}{dt}(AB_{GCDCA}) = VmaxBSEP_{GCDCA} * CLIW_{GCDCA} / (KmBSEP_{GCDCA} + CLIW_{GCDCA}) \\
\text{# change in amount in extracellular water (liver), umol}
\]

\[
d\frac{dt}{dt}(ALIW_{GCDCA}) = VmaxNTCP_{GCDCA} * CVLEW_{GCDCA}/ (KmNTCP_{GCDCA} + CVLEW_{GCDCA}) \\
\text{# change in amount in intracellular water (liver), umol}
\]

\[
d\frac{dt}{dt}(Alow_{GCDCA}) = VmaxNTCP_{GCDCA} * CVLEW_{GCDCA}/ (KmNTCP_{GCDCA} + CVLEW_{GCDCA}) \\
\text{+ Cam_all} * ALIW_{DCA} * Gdose_{GCDCA} + Acol_{DCA} * 0.05 * Gdose_{GCDCA} \\
\text{# change in amount in intracellular water (liver), umol}
\]

\[
d\frac{dt}{dt}(Aup_{GCDCA}) = ge*dose_{GCDCA} + VmaxBSEP_{GCDCA} * CLIW_{GCDCA} / (KmBSEP_{GCDCA} + CLIW_{GCDCA}) * QIb\text{ all} \\
\text{# change in amount in upper intestinal lumen, umol}
\]

\[
d\frac{dt}{dt}(Alow_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in ileum, umol}
\]

\[
d\frac{dt}{dt}(Acol_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in portal blood, umol}
\]

\[
d\frac{dt}{dt}(AF_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in fat tissue, umol}
\]

\[
d\frac{dt}{dt}(AR_{GCDCA}) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in slowly perfused tissue, umol}
\]

\[
d\frac{dt}{dt}(AS_GCDCA) = VmaxASBT_{GCDCA} * CIl_GCDCA / (KmASBT_{GCDCA} * modf_Km + CIl_GCDCA) \\
\text{# change in amount in slowly perfused tissue, umol}
\]
\[
d\frac{d}{dt}(AB_{GCA}) = Q_F \cdot CVF_{GCA} + (Q_{AV}+Q_{PV}) \cdot CVLEW_{GCA} + Q_S \cdot CVS_{GCA} + Q_R \cdot CVR_{GCA} + Q_I \cdot CVI_{GCA} - (Q_F + Q_{AV} + Q_S + Q_R + Q_I) \cdot CB_{GCA}; \quad \text{# change in amount in blood, umol}
\]

\[
\text{conc}_{\text{plasma}}_{GCA} = \frac{CB_{GCA}}{BP_{all}} + CB_{fs} ; \quad \text{#concentration in **plasma**, umol}
\]

### GCA submodel ###

\[
CLEW_{GCA} = ALEW_{GCA} / VLEW;
\]

\[
CVLEW_{GCA} = CLEW_{GCA} / (PEWP_{GCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the extracellular water (liver) (umol/L)}
\]

\[
CLIW_{GCA} = ALIW_{GCA} / VLIW;
\]

\[
CI_{GCA} = AI_{GCA} / VI;
\]

\[
CVP_{GCA} = CP_{GCA} - CVLEW_{GCA}; \quad \text{#concentration in venous blood leaving the fat tissue (umol/L)}
\]

\[
CF_{GCA} = AF_{GCA} / VF;
\]

\[
CF_{GCA} = CF_{GCA} / (PF_{GCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the fat tissue (umol/L)}
\]

\[
CR_{GCA} = AR_{GCA} / VR;
\]

\[
CVR_{GCA} = CR_{GCA} / (PR_{GCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the rapidly perfused tissue (umol/L)}
\]

\[
CS_{GCA} = AS_{GCA} / VS;
\]

\[
CVS_{GCA} = CS_{GCA} / (PS_{GCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the slowly perfused tissue (umol/L)}
\]

\[
CB_{GCA} = AB_{GCA} / VB;
\]

\[
CP_{GCA} = AP_{GCA} / VP;
\]

\[
\frac{d}{dt}(dose_{GCA}) = -ge \cdot dose_{GCA} + V_{maxBSEP_{GCA}} \cdot CLIW_{GCA} / (K_{mBSEP_{GCA}} + CLIW_{GCA}) \cdot QGb_{all}; \quad \text{# change in amount in the gall bladder, umol}
\]

\[
\frac{d}{dt}(ALEW_{GCA}) = QAV \cdot (CB_{GCA} - CVLEW_{GCA}) + QPV \cdot (CP_{GCA} - CVLEW_{GCA}) - V_{maxNTCP_{GCA}} \cdot CVLEW_{GCA} / (K_{mNTCP_{GCA}} + CVLEW_{GCA}); \quad \text{#change in amount in extracellular liver compartment, umol}
\]

\[
\frac{d}{dt}(ALIW_{GCA}) = V_{maxBSEP_{GCA}} \cdot CLIW_{GCA} / (K_{mBSEP_{GCA}} + CLIW_{GCA}) + V_{maxNTCP_{GCA}} \cdot CVLEW_{GCA} / (K_{mNTCP_{GCA}} + CVLEW_{GCA}) + k_{am_{all}} \cdot ALIW_{GCA} \cdot Gdosel_{GCA} + Acol_{GCA} \cdot 0.05 \cdot Gdosel_{GCA}; \quad \text{#change in amount in intracellular liver compartment, umol}
\]

\[
\frac{d}{dt}(Aup_{GCA}) = ge \cdot dose_{GCA} + V_{maxBSEP_{GCA}} \cdot CLIW_{GCA} / (K_{mBSEP_{GCA}} + CLIW_{GCA}) \cdot Qlb_{all} - \text{ka}_{GCA} \cdot Aup_{GCA} - \text{ktj} \cdot Aup_{GCA}; \quad \text{# change in amount in upper intestinal lumen, umol}
\]

\[
\frac{d}{dt}(Alow_{GCA}) = \text{ktj} \cdot Aup_{GCA} - \text{kti} \cdot Alow_{GCA} - V_{maxASBT_{GCA}} \cdot CI_{all} / (K_{mASBT_{GCA}} \cdot \text{modf}K_{m} + CI_{all}); \quad \text{#change in amount in ileum, umol}
\]

\[
\frac{d}{dt}(Acol_{GCA}) = \text{kti} \cdot Alow_{GCA} - \text{ka}_{GCA} \cdot Acol_{GCA} - k_{dec_{all}} \cdot Acol_{GCA}; \quad \text{#amount in colon, umol}
\]

\[
\frac{d}{dt}(AI_{GCA}) = Q_{I} \cdot (CB_{GCA} - CVI_{GCA}); \quad \text{#change in amount in portal blood, umol}
\]

\[
\frac{d}{dt}(AF_{GCA}) = Q_{F} \cdot (CB_{GCA} - CVF_{GCA}); \quad \text{#change in amount in fat tissue, umol}
\]

\[
\frac{d}{dt}(AR_{GCA}) = Q_{R} \cdot (CB_{GCA} - CVR_{GCA}); \quad \text{#change in amount in rapidly perfused tissue, umol}
\]

\[
\frac{d}{dt}(AS_{GCA}) = Q_{S} \cdot (CB_{GCA} - CVS_{GCA}); \quad \text{#change in amount in slowly perfused tissue, umol}
\]

\[
\frac{d}{dt}(AB_{GCA}) = QF \cdot CVF_{GCA} + (QAV+QPV) \cdot CVLEW_{GCA} + QS \cdot CVS_{GCA} + QR \cdot CVR_{GCA} + QI \cdot CVI_{GCA} - (QF + QAV + QS + QR + QI) \cdot CB_{GCA}; \quad \text{# change in amount in blood, umol}
\]

\[
\text{conc}_{plasma}_{GCA} = \frac{CB_{GCA}}{BP_{all}} + CB_{fs}_{GCA}; \quad \text{#concentration in **plasma**, umol}
\]

### DCA submodel ###

\[
CLEW_{DCA} = ALEW_{DCA} / VLEW;
\]

\[
CVLEW_{DCA} = CLEW_{DCA} / (PEWP_{DCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the liver (umol/L)}
\]

\[
CLIW_{DCA} = ALIW_{DCA} / VLIW;
\]

\[
CI_{DCA} = AI_{DCA} / VI;
\]

\[
CVP_{DCA} = CP_{DCA} - CVLEW_{DCA}; \quad \text{#concentration in venous blood leaving the rapidly perfused tissue (umol/L)}
\]

\[
CF_{DCA} = AF_{DCA} / VF;
\]

\[
CF_{DCA} = CF_{DCA} / (PF_{DCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the fat tissue (umol/L)}
\]

\[
CR_{DCA} = AR_{DCA} / VR;
\]

\[
CVR_{DCA} = CR_{DCA} / (PR_{DCA} \cdot BP_{all}); \quad \text{#concentration in venous blood leaving the slowly perfused tissue (umol/L)}
\]

\[
CS_{DCA} = AS_{DCA} / VS;
\]
CVS_DCA=CS_DCA/(PS_DCA*BP_all); # concentration in venous blood leaving slowly perfused tissue (umol/L)

CB_DCA=AB_DCA/VB; # concentration in blood excluding portal vein (umol/L)

CP_DCA=AP_DCA/VP; # concentration in portal vein (umol/L)

d/dt(ALEW_DCA)=QAV*(CB_DCA-CVEW_DCA)+QPV*(CP_DCA-CVEW_DCA)-Clint_PD_DCA*CVEW_DCA; # change in amount in extracellular water, umol/hr

d/dt(ALIW_DCA)=Clint_PD_DCA*CVEW_DCA-kam_all*ALIW_DCA; # change in amount in intracellular water liver, umol/hr

d/dt(Acol_DCA)=kdec_all*(Acol_GDCDA+Acol_GCA+Acol_GDCA)-ka_DCA*Acol_DCA-0.05*Acol_DCA; # change in amount in colon, umol/hr

d/dt(AI_DCA)=QI*(CB_DCA-CVI_DCA); # change in amount in intestinal tissue, umol/hr

d/dt(AP_DCA)=ka_DCA*Acol_DCA-OPV*CP_DCA; # change in amount in portal vein tissue, umol/hr

d/dt(AF_DCA)=QF*(CB_DCA-CVF_DCA); # change in amount in fat tissue, umol/hr

d/dt(AR_DCA)=QR*(CB_DCA-CVR_DCA); # change in amount in rapidly perfused tissue, umol/hr

d/dt(AS_DCA)=QS*(CB_DCA-CVS_DCA); # change in amount in slowly perfused tissue, umol/hr

d/dt(AB_DCA)=QF*CVF_DCA+(QAV+QPV)*CVEW_DCA+QS*CVS_DCA+QR*CVR_DCA+QI*CVI_DCA-(QF+QS+QR+QI+QAV)*CB_DCA; # change in amount in blood, umol/hr

c_conc_plasma_DCA=(CB_DCA/BP_all)+CBfs_DCA;#concentration in **plasma**, umol

######################################################
##Step 5: Solve the model
qd$time<-qd$time
print(PBK)

solve.pbk<-as_tibble(solve(PBK, params=parameters, events = qd, inits=inits, cores=4, covs_interpolation="nocb"))

## Mass balance calculations
solve.pbk

mutate(OUT=dose_GCDCA+ALEW_GCDCA+ALIW_GCDCA+Aup_GCDCA+Alow_GCDCA+Acol_GCDCA+AI_GCDCA+AP_GCDCA+AF_GCDCA+AS_GCDCA+AB_GCDCA+ALEW_DCA+ALIW_DCA+Acol_DCA+AI_DCA+AP_DCA+
AF_DCA+AR_DCA+AS_DCA+AB_DCA+dose_GCA+ALEW_GCA+ALIW_GCA+Aup_GCA+Alow_GCA+Acol_GCA+AP_GCA+AF_GCA+AI_GCA+AR_GCA+AS_GCA+AB_GCA+
dose_GDCA+ALEW_GDCA+ALIW_GDCA+Aup_GDCA+Alow_GDCA+Acol_GDCA+AI_GDCA+AP_GDCA+AF_GDCA+AR_GDCA+AS_GDCA+AB_GDCA,
MB=Gdose$GCDCA+Gdose$GDCA+Gdose$GCA-OUT)