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>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>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>
</tr>
<tr>
<td>Glycodeoxycholic acid (GDCA)</td>
<td>MRM</td>
<td>448.3</td>
<td>74</td>
<td>9.452</td>
<td>190</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>
</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; $\text{Clint}_{\text{PDc}}$, passive diffusion intrinsic clearance; $\text{BP}$, blood:plasma ratio; $\text{KmNTCP}$, Michaelis-Menten constant for NTCP-mediated hepatic uptake; $\text{VmaxASBTc}$, 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; $\text{QPVc}$, fraction of blood flow to liver through the portal vein; $\text{VmaxNTCPc}$, maximal NTCP-mediated hepatic BA uptake; Hep, hepatocellularity; $\text{SF}_{\text{OATP}}$, scaling factor to adjust hepatic uptake for OATP-mediated uptake; $\text{BW}$, 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; $Km_BSEP$, Michaelis-Menten constant for BSEP-mediated BA efflux from the liver; $V_{max,ASBTc}$, maximal ASBT-mediated GCA absorption rate over the ileal epithelium; $V_{max,BSEPc}$, maximal BSEP-mediated BA efflux rate; $aBSEP$, BSEP protein abundance; $MW_{BSEP}$, 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; ktl_fed, ileal transit time in fed state; ktl_fed, jejunum 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$GCDCA,
  ALEW_GCDCA=0,
  ALIW_GCDCA=0,
  Alu_GCDCA=0,
  Aup_GCDCA=0,
  Alien_GCDCA=0,
  Acol_GCDCA=0,
  Al_GCDCA=0,
  AP_GCDCA=0,
  AF_GCDCA=0,
  AR_GCDCA=0,
  AS_GCDCA=0,
  AB_GCDCA=0,
  dose_GDCA=Gdose$GDCA,
  ALEW_GDCA=0,
  ALIW_GDCA=0,
ALu_GDCA=0,  
Aup_GDCA=0,  
Acol_GDCA=0, 
AP_GDCA=0,  
AI_GDCA=0,  
AF_GDCA=0,  
AR_GDCA=0,  
AS_GDCA=0,  
AB_GDCA=0, 
dose_GCA=Gdose$GCA, 
ALEW_GCA=0,  
ALIW_GCA=0,  
ALu_GCA=0,  
AP_GCA=0,  
AR_GCA=0,  
AS_GCA=0, 
ALEW_DCA=0,  
ALIW_DCA=0,  
Acol_DCA=0,  
AB_DCA=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) %>%
et(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)
VLW = 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 = VL+VLW;  
VR = VRc_all*BW_all;  # (L or Kg); volume of richly perfused tissue (calculated)
VS = VSsc_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 = VIc_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 = QRe_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 GDCA
SF_ASBT=surface_all*10^-6*SA_all; # {cm²/entire ileum}; fitted
SF_NTCP=Hep_all*SF_OATP_all*WL*10^-6; #{10^6 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 GCDCA 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)
ODOSEmg_all=ODOSEmg_all;

## 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)
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)
\[
\frac{d}{dt}(\text{dose}_{\text{GCDCA}}) = -ge*\text{dose}_{\text{GCDCA}} + Vmax_{\text{BSEP}_{\text{GCDCA}}} * CLIW_{\text{GCDCA}}/(Km_{\text{BSEP}_{\text{GCDCA}}} + CLIW_{\text{GCDCA}})^*QGb_{\text{all}}, \quad \text{# change in amount in the gall bladder, umol}
\]

\[
\frac{d}{dt}(\text{ALEW}_{\text{GCDCA}}) = QAV * (CB_{\text{GCDCA}} - CVLEW_{\text{GCDCA}}) + QPV *(CP_{\text{GCDCA}} - CVLEW_{\text{GCDCA}}) - Vmax_{\text{NTCP}_{\text{GCDCA}}} * CVLEW_{\text{GCDCA}}/(Km_{\text{NTCP}_{\text{GCDCA}}} + CVLEW_{\text{GCDCA}}); \quad \text{# change in amount in extracellular water (liver), umol}
\]

\[
\frac{d}{dt}(\text{ALIW}_{\text{GCDCA}}) = -Vmax_{\text{BSEP}_{\text{GCDCA}}} * CLIW_{\text{GCDCA}}/(Km_{\text{BSEP}_{\text{GCDCA}}} + CLIW_{\text{GCDCA}}) + \text{kam}_{\text{all}} * ALIW_{\text{DCA}} * Gdosel_{\text{DCA}} + Acol_{\text{DCA}} * 0.05 * Gdosel_{\text{DCA}}; \quad \text{# change in amount in intracellular water (liver), umol}
\]

\[
\frac{d}{dt}(\text{Aup}_{\text{GCDCA}}) = ge*\text{dose}_{\text{GCDCA}} + Vmax_{\text{BSEP}_{\text{GCDCA}}} * CLIW_{\text{GCDCA}}/(Km_{\text{BSEP}_{\text{GCDCA}}} + CLIW_{\text{GCDCA}}) * QIb_{\text{all}} - ka_{\text{GCDCA}} * Aup_{\text{GCDCA}} - ktj * Aup_{\text{GCDCA}}; \quad \text{# change in amount in upper intestinal lumen, umol}
\]

\[
\frac{d}{dt}(\text{AI}_{\text{GCDCA}}) = kq * Aup_{\text{GCDCA}} - kti * AI_{\text{GCDCA}} - Vmax_{\text{ASBT}_{\text{GCDCA}}} * CIl_{\text{GCDCA}}/(Km_{\text{ASBT}_{\text{GCDCA}}} * \text{modf}_Km + CIl_{\text{GCDCA}}); \quad \text{# change in amount in ileum, umol}
\]

\[
\frac{d}{dt}(\text{Acol}_{\text{GCDCA}}) = kti * AI_{\text{GCDCA}} - ka_{\text{GCDCA}} * Acol_{\text{GCDCA}} - kdec_{\text{all}} * Acol_{\text{GCDCA}}; \quad \text{# change in amount in colon, umol}
\]

\[
\frac{d}{dt}(\text{AB}_{\text{GCDCA}}) = QF *(CB_{\text{GCDCA}} - CVF_{\text{GCDCA}}) + (QAV + QPV) * CVLEW_{\text{GCDCA}} + QPV * CP_{\text{GCDCA}}; \quad \text{# amount in portal blood, umol}
\]

\[
\frac{d}{dt}(\text{AP}_{\text{GCDCA}}) = ka_{\text{GCDCA}} * Aup_{\text{GCDCA}} + Vmax_{\text{ASBT}_{\text{GCDCA}}} * CIl_{\text{GCDCA}}/(Km_{\text{ASBT}_{\text{GCDCA}}} * \text{modf}_Km + CIl_{\text{GCDCA}}) + ka_{\text{GCDCA}} * Acol_{\text{GCDCA}} - QPV * CP_{\text{GCDCA}}; \quad \text{# amount in portal blood, umol}
\]
\[
\frac{d}{dt}(\text{AB}_GCA) = QF \cdot \text{CVF}_GCA + (QAV + QPV) \cdot \text{CVLEW}_GCA + QS \cdot \text{CVS}_GCA + \text{QR} \cdot \text{CVR}_GCA + QI \cdot \text{CVI}_GCA - (QF + QAV + QS + QR + QI) \cdot \text{CB}_GCA; \quad \text{# change in amount in blood, umol}
\]

\[
\text{conc}_{\text{plasma}}_GCA = (\text{CB}_GCA / \text{BP}_{\text{all}}) + \text{CBfs}_GCA; \quad \text{# concentration in **plasma**, umol}
\]

### GCA submodel ###

\[
\text{CLEW}_GCA = \text{ALEW}_GCA / \text{VLEW}; \quad \text{# concentration in the extracellular water (liver), (umol/L)}
\]

\[
\text{CVLEW}_GCA = \text{CLEW}_GCA / (\text{PEWP}_GCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the extracellular water (liver) (umol/L)}
\]

\[
\text{CLIW}_GCA = \text{ALIW}_GCA / \text{VLIW}; \quad \text{# concentration in intracellular water (liver)}
\]

\[
\text{CI}_GCA = \text{AI}_GCA / \text{VI}; \quad \text{# concentration in the ileum (umol/L)}
\]

\[
\text{CIV}_GCA = \text{CI}_GCA / (\text{PG}_GCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the intestinal tissue(umol/L)}
\]

\[
\text{CF}_GCA = \text{AF}_GCA / \text{VF}; \quad \text{# concentration in the fat tissue (umol/L)}
\]

\[
\text{CVF}_GCA = \text{CF}_GCA / (\text{PF}_GCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the fat tissue (umol/L)}
\]

\[
\text{CR}_GCA = \text{AR}_GCA / \text{VR}; \quad \text{# concentration in the rapidly perfused tissue (umol/L)}
\]

\[
\text{CVR}_GCA = \text{CR}_GCA / (\text{PR}_GCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the rapidly perfused tissue (umol/L)}
\]

\[
\text{CS}_GCA = \text{AS}_GCA / \text{VS}; \quad \text{# concentration in the slowly perfused tissue (umol/L)}
\]

\[
\text{CVS}_GCA = \text{CS}_GCA / (\text{PS}_GCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving slowly perfused tissue (umol/L)}
\]

\[
\text{CB}_GCA = \text{AB}_GCA / \text{VB}; \quad \text{# concentration in blood excluding portal vein (umol/L)}
\]

\[
\text{CP}_GCA = \text{AP}_GCA / \text{VP}; \quad \text{# concentration in portal blood (umol/L)}
\]

\[
\frac{d}{dt}(\text{dose}_GCA) = -\text{ge} \cdot \text{dose}_GCA + \text{VmaxBSEP}_GCA \cdot \text{CLIW}_GCA / (\text{KmBSEP}_GCA + \text{CLIW}_GCA) \cdot \text{QGb}_{\text{all}}; \quad \text{# change in amount in the gall bladder, umol}
\]

\[
\frac{d}{dt}(\text{dose}_GCA) = \text{QAV} \cdot (\text{CB}_GCA - \text{CVLEW}_GCA) + \text{QPV} \cdot (\text{CP}_GCA - \text{CVLEW}_GCA) - \text{VmaxNTCP}_GCA \cdot \text{CVLEW}_GCA / (\text{KmNTCP}_GDA + \text{CVLEW}_GDA); \quad \text{# change in amount in extracellular liver compartment, umol}
\]

\[
\frac{d}{dt}(\text{dose}_GCA) = -\text{VmaxBSEP}_GDA \cdot \text{CLIW}_GDA / (\text{KmBSEP}_GDA + \text{CLIW}_GDA) + \text{VmaxNTCP}_GDA \cdot \text{CVLEW}_GDA / (\text{KmNTCP}_GDA + \text{CVLEW}_GDA); \quad \text{# change in amount in intracellular liver compartment, umol}
\]

\[
\frac{d}{dt}(\text{dose}_GDA) = \text{QIb}_{\text{all}} \cdot \text{Aup}_GDA; \quad \text{# change in amount in upper intestinal lumen, umol}
\]

\[
\frac{d}{dt}(\text{dose}_GDA) = \text{ka}_{\text{all}} \cdot \text{Alow}_GDA; \quad \text{# change in amount in colon, umol}
\]

\[
\frac{d}{dt}(\text{Aup}_GDA) = \text{ka}_{\text{all}} \cdot \text{Alow}_GDA; \quad \text{# change in amount in upper intestinal lumen, umol}
\]

\[
\frac{d}{dt}(\text{Aup}_GDA) = \text{QPV} \cdot \text{CP}_GDA; \quad \text{# amount in portal blood, umol}
\]

\[
\frac{d}{dt}(\text{Alow}_GDA) = \text{ktj} \cdot \text{Aup}_GDA; \quad \text{# change in amount in slowly perfused tissue, umol}
\]

\[
\frac{d}{dt}(\text{Acol}_GDA) = \text{ka}_{\text{all}} \cdot \text{Aup}_GDA; \quad \text{# amount in portal blood, umol}
\]

\[
\frac{d}{dt}(\text{AI}_GDA) = \text{QI} \cdot (\text{CB}_GDA - \text{CVI}_GDA); \quad \text{# change in amount in intestinal tissue, umol}
\]

\[
\text{conc}_{\text{plasma}}_GDA = (\text{CB}_GDA / \text{BP}_{\text{all}}) + \text{CBfs}_GDA; \quad \text{# concentration in **plasma**, umol}
\]

### DCA submodel ###

\[
\text{CLEW}_DCA = \text{ALEW}_DCA / \text{VLEW}; \quad \text{# concentration in the liver (umol/L)}
\]

\[
\text{CVLEW}_DCA = \text{CLEW}_DCA / (\text{PEWP}_DCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the liver (umol/L)}
\]

\[
\text{CLIW}_DCA = \text{ALIW}_DCA / \text{VLIW}; \quad \text{# concentration in intracellular water (liver)}
\]

\[
\text{CI}_DCA = \text{AI}_DCA / \text{VI}; \quad \text{# concentration in the intestinal tissue (umol/L)}
\]

\[
\text{CIV}_DCA = \text{CI}_DCA / (\text{PG}_DCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the intestinal tissue(umol/L)}
\]

\[
\text{CF}_DCA = \text{AF}_DCA / \text{VF}; \quad \text{# concentration in the fat tissue (umol/L)}
\]

\[
\text{CVF}_DCA = \text{CF}_DCA / (\text{PF}_DCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the fat tissue (umol/L)}
\]

\[
\text{CR}_DCA = \text{AR}_DCA / \text{VR}; \quad \text{# concentration in the rapidly perfused tissue (umol/L)}
\]

\[
\text{CVR}_DCA = \text{CR}_DCA / (\text{PR}_DCA \cdot \text{BP}_{\text{all}}); \quad \text{# concentration in venous blood leaving the rapidly perfused tissue (umol/L)}
\]

\[
\text{CS}_DCA = \text{AS}_DCA / \text{VS}; \quad \text{# concentration in the slowly perfused tissue (umol/L)}
\]
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-CVLEW_DCA)+QPV*(CP_DCA-CVLEW_DCA)-Clint_PD_DCA*CVLEW_DCA;  #
    change in amount in extracellular water liver, umol/hr

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

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

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

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

##########################################################################
##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_GCD
    CA+AP_GCDCA+AF_GCDCA+AR_GCDCA+AS_GCDCA+AB_GCDCA+ALEW_DCA+ALIW_DCA+Acol_DCA+AI_D
    CA+AP_DCA+AF_DCA+AR_DCA+AS_DCA+AB_DCA+dose_GCA+ALEW_GCA+ALIW_GCA+Aup_GCA+Alow_G
    CA+Acol_GCA+AP_GCA+AF_GCA+AR_GCA+AS_GCA+AB_GCA+dose_GDCA+ALEW_GDCA+ALIW_GDCA+Aup
    _GDCA+Alow_GDCA+Acol_GDCA+AP_GDCA+AF_GDCA+AR_GDCA+AS_GDCA+AB_GDCA+MB=Gdose$GC
    DCa+Gdose$GDca+Gdose$GCA-OUT)