Computational Modeling of Dynamical Liver Function Tests: LiMAx and Methacetin Breath Test (MBT)

Matthias König[1*], Tilo Wünsch[2], Daniel Lill[3], Johann F. Lock[4], Julia Mohr[2], Pouria Taheri[2], Jens Timmer[3,5,6], Martin Stockmann[2]

[1]Institute for Theoretical Biology, Institute for Biology, Humboldt Universität zu Berlin, Berlin, Germany

[2]Department of Surgery, Campus Charité Mitte and Campus Virchow Klinikum, Charité - Universitätsmedizin Berlin, Berlin, Germany

[3]Faculty of Mathematics and Physics, Institute of Physics, University of Freiburg, Freiburg, Germany

[4]Department of General, Visceral, Vascular and Pediatric Surgery, University Hospital of Würzburg, Würzburg, Germany

[5]Freiburg Center for Data Analysis and Modelling, University of Freiburg, Freiburg, Germany

[6]BIOSS, Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany

*** Correspondence:**
Dr. Matthias König
koenigmx@hu-berlin.de

Keywords: LiMAx, liver function test, breath test, methacetin, CYP1A2, smoking

ABSTRACT

Assessment of liver function is a key task in hepatology but accurate quantification of hepatic function has remained a clinical challenge. Dynamic liver function tests are a promising tool for the non-invasive evaluation of liver function *in vivo*. One class of such tests are breath tests based on the conversion of ^{13}C-labeled substrates by the liver to $^{13}CO_2$ subsequently measured in the breath. A commonly applied substrate is ^{13}C-methacetin, converted to paracetamol and $^{13}CO_2$ via cytochrome P450 1A2 (CYP1A2), used orally in the methacetin breath test (MBT) and intravenously in the LiMAx test. An important clinical question is which factors can affect MBT and LiMAx results. The aim of our study was to answer this question using computational modeling to derive basic information for a better understanding of the methacetin breath test and factors influencing its results. A physiological based pharmacokinetics (PBPK) model for ^{13}C-methacetin breath tests including absorption, distribution, metabolism and elimination of ^{13}C-methacetin, paracetamol, and ^{13}C-bicarbonate/$^{13}CO_2$ was developed. The model correctly predicts data from more than 20 clinical studies after oral and intravenous application under various dosing regimes of paracetamol, ^{13}C-bicarbonate, and ^{13}C-methacetin, based on retrospective analysis. Model predictions were validated based on data from multiple studies consisting of ^{13}C-bicarbonate kinetics, LiMAx dosing study and retrospective analysis of LiMAx data from healthy smokers and non-smokers. Sensitivity analysis was performed to identify key factors influencing MBT and LiMAx. Main factors are i) parameters of hepatic methacetin metabolization such as CYP1A2 content and functional liver volume (parenchymal liver fraction); ii) hepatic perfusion; and iii) parameters for $^{13}CO_2$ exhalation like CO_2 production, lung volume and lung perfusion. The MBT based on oral administration of methacetin is strongly affected by absorption kinetics of methacetin, which is not the case for LiMAx. The model was applied to study the effect of clinically relevant parameters like CYP1A2 content,

hepatic perfusion or lifestyle factors like smoking on LiMAx. In summary, we present a valuble tool for the evaluation of dynamical liver function tests based on ^{13}C-methacetin.

INTRODUCTION

Liver function tests

The liver fulfills a broad variety of physiological functions, such as metabolism of carbohydrates, fat, and proteins, storage of glycogen, synthesis of proteins like albumin, clotting and immune factors, detoxification of toxins and pharmacological agents, or excretion of bile {Sakka2007, Rubin2017}. Assessment of liver function is a key task in hepatology. It is important for detecting impaired liver function, the monitoring of patient status and progression in primary liver diseases, or the evaluation of liver capacity before and after liver resection and transplantation. Accurate quantification of the function of the liver has remained a clinical challenge. Most commonly, the diagnostics is based on measurements of static biochemical parameters of synthesis (prothrombin, cholesterol, albumin), hepatocellular integrity (transaminases), detoxification (ammonium), excretion and cholestasis (bilirubin, alkaline phosphatase, γGT), in combination with imaging techniques or scores such as the Child-Turcotte-Pugh classification, which combines clinical parameters (ascites and degree of encephalopathy) with serum parameters {Bonfrate2014}. Even if these conventional static biochemical liver tests can be useful to value a mixture of injury and function, none is regarded to be a reliable marker to quantify either functional hepatic reserve or liver dysfunction in critically ill patients {Nista2004, Bonfrate2014}. Liver biopsy, often considered the gold standard, is an invasive procedure and has its limitations including inter-observer variability, sampling error and the risk of complications {Gorowska-Kowolik2017}. Importantly, biopsies only provide a static histological picture, but no information about functionality of the liver *in vivo*.

Dynamic liver function tests (D-LFT)

In contrast to these static methods, dynamic liver function tests (D-LFT) enable quantitative assessment of the organ's functional reserve for selected functions by analyzing the kinetics of the metabolization of a specific test substrate (hepatic elimination) {Herold2000, Rubin2017}. D-LFTs using various test substances have been investigated in the past providing information about *in vivo* hepatic clearance, e.g., indocyanine green {DeGasperi2016}, caffeine {Renner1984}, galactose {Bernstein1960} or methacetin {Gorowska-Kowolik2017, Rubin2017}. Most of these tests are based on repeated blood sampling with subsequent analysis of the concentration time curves.

An important subclass of D-LFTs are non-invasive breath tests measuring the exhaled $^{13}CO_2$ by the lungs after the conversion of ^{13}C-labeled substrates by the liver. The amount and rate of appearance of $^{13}CO_2$ in the breath is hereby a proxy for hepatic clearance via the respective pathway. Breath samples are collected after predetermined time intervals to measure changes in the $^{13}CO_2/^{12}CO_2$ ratio R(t) from the baseline R(t_0). The relative change in the ratio is called delta over baseline (DOB) and given in per mil (‰) normalized by the reference value for the $^{13}CO_2/^{12}CO_2$ ratio (R_{PDB} = 0.01123) {Rubin2017}.

$$DOB = \frac{R(t) - R(t_0)}{R_{PDB}}$$

Based on the obtained DOB time courses, various parameters describing the kinetics of the ^{13}C-labeled substrate can be calculated: time from administration of ^{13}C-labeled substrate to the peak elimination of $^{13}CO_2$ (T_{max}); peak value reached (DOB_{max}); area under the DOB curve (AUC);

momentary or cumulative dose of ^{13}C-substrate recovered from the exhaled air (recovery) {Gorowska-Kowolik2017}.

Methacetin breath test (MBT) & LiMAx

Various substrates specific for particular hepatic functions have been applied in breath tests: ^{13}C-phenylalanine (mitochondrial metabolism) {Bozek2018}, ^{13}C-caffeine (P450 1A2 detoxification) {Park2003}, ^{13}C-galactose (central carbon metabolism), ^{13}C-aminopyrine {Minon1995}, or the often applied ^{13}C-methacetin (P450 1A2 detoxification) {Lalazar2009}. In most test protocols for the methacetin breath test (MBT), a constant dose of 75–150 [mg] ^{13}C-methacetin is ingested, absorbed into the blood and transported to the liver, where it is specifically metabolized by the microsomal cytochrome P450 1A2 (CYP1A2) {Rubin2017}. The oral administration of methacetin can hereby result in high individual variability of the MBT due to the absorption of methacetin from the gastrointestinal tract {Gorowska-Kowolik2017}. An alternative avoiding this limitation is the LiMAx test (liver maximum function capacity, see Figure 1A) using a bolus injection of a body-weight adjusted dose of ^{13}C-methacetin and continuous online breath sampling at the bedside for a maximum of 1h after injection {Rubin2017}.

Open question

The test with ^{13}C-methacetin belongs to the best described and most widely applied methods in noninvasive liver function assessment. Due to the rising availability of this method, knowledge concerning its limitations, as well as its usefulness in sub-groups of patients, is important {Gorowska-Kowolik2017}. An important clinical and research question is which factors can influence the results of MBT and LiMAx, consequently affecting accuracy, specificity and sensitivity of these tests.

The rate of $^{13}CO_2$ expiration after administration of ^{13}C-labeled substrates is a function of the metabolism of the given substance, as well as the kinetics of the CO_2 bicarbonate pools which the labeled CO_2 traverses. {Winchell1970, Winchell1970a}. Factors influencing bicarbonate kinetics in the body or $^{13}CO_2$ exhalation could be important parameters. Furthermore, the hepatic clearance of a test substance is determined by the extraction of these substances by the liver as well as the blood flow of the liver {Herold2000}. Based on the extraction ratio E for a given test substance, i.e. the difference between inflow and outflow substrate concentrations and inflow concentrations, tests can provide information about liver blood flow (flow-limited, E=0.7-1.0) or about hepatic metabolic capacity (enzyme-limited, E<0.3) {Nista2004}. Hepatic perfusion and factors influencing perfusion (e.g. heart rate or cardiac output) could play an important role for ^{13}C-methacetin D-LFTs due to the high extraction ratio of methacetin. Other possible factors are the amount of CYP1A2 in the liver, and factors resulting in altered expression of CYP1A2 e.g. induction via smoking {Dobrinas2011, Dobrinas2013} or suppression via oral contraceptives {Granfors2005}. Furthermore, it remains unclear to which extent the absorption of methacetin impacts MBT test results.

The aim of our study was to use computational modeling to derive basic information for a better understanding of the ^{13}C-methacetin breath test and factors influencing its results. Within this study a physiologically based pharmacokinetics (PBPK) model for the evaluation of ^{13}C-methacetin breath tests including absorption, distribution, metabolism and elimination of ^{13}C-methacetin, paracetamol, and $^{13}CO_2$ was developed. The model was applied to systematically analyze MBT and LiMAx to provide clinically relevant insights about factors affecting dynamical liver function tests.

MATERIALS AND METHODS

Computational model

The presented PBPK model is an ordinary differential equation (ODE) model encoded in the Systems Biology Markup Language (SBML) {Hucka2003}. The model is provided in Supplementary Material 1 with a human-readable model description containing information on species, parameters, reactions and rate equations annotated to biomedical ontologies available in Supplementary Material 2. The model was developed using sbmlutils {Koenig2018_sbmlutils}, tellurium {Medley2018} and cy3sbml {Koenig2012}.

If not stated otherwise, all tissue partition coefficients for ^{13}C-methacetin,^{13}C-bicarbonate and paracetamol for heart (Kphe), gut (Kpgu), kidney (Kpki), liver (Kpli), lung (Kplu), spleen (Kpsp), bone (Kpbo), rest of body (Kpre), the fraction absorbed after oral administration (F), fraction unbound in plasma (fup), blood to plasma ratio (BP), and fraction unbound in microsomes (fumic) were set to 1.0. Reference values for organ volumes and tissue blood flows were taken from literature {Jones2013}.

Numerical integration

Simulations were performed by numerical integration of the ODE system with the high-performance simulator libroadrunner {Somogy2015} (CVODE integrator for stiff problems with relative tolerance of 1E-6, absolute tolerance of 1E-12). For the individual simulations, the doses of ^{13}C-methacetin, ^{13}C-bicarbonate and paracetamol and the respective route of application were set to the values provided in Table 1. MBT simulations were performed if not stated otherwise with a oral dose of 75 [mg] of ^{13}C-methacetin, LiMAx simulations with a intravenous bolus dose of 2 [mg/kg].

Parameter fitting

The following parameters were optimized based on parameter fitting: APAPD_HLM_CL, APAPD_Km_apap, CO2FIX_HLM_CL, CYP1A2MET_CL, CYP1A2MET_Km_met, Ka_apap, Ka_co2c13, Ka_metc13, KBO_FIXCO2, KBO_RELCO2, KBO_MAXCO2, KLU_EXCO2 (for description of parameters and optimal values see Supplementary Material 1 and 2). The parameters were estimated from published data (Table 1) minimizing the log-likelihood using a trust-region algorithm {Write1999}. The optimization was initialized 1000 times to avoid being stuck to a local optimum and to ensure that the optimizer works reliably {Raue2013}. The fitting was carried out in the R framework dMod {Kaschek2016}.

Variability analysis

To analyze variability of results due to parameters all simulations were performed with parameters changed by ±10% of the reference value. In detail, every simulation was performed with the reference parameter set and with every parameter changed individually by ±10%. Parameters for physical constants (molecular weights and the Pee Dee Belemnite (PDB) ratio (R_{PDB})), and parameters for the doses of ^{13}C-methacetin,^{13}C-bicarbonate and paracetamol (respective intravenous dose IVDOSE, oral dose PODOSE and injection time ti), as well as reference parameters (resting heart rate HRrest) were excluded from changes. In the figures the mean (solid line), range between standard deviation (mean±SD, dark shaded area) and range between minimum and maximum simulation results (outer shaded area) for all parameter changes are depicted.

Pharmacokinetic parameters

Single-dose pharmacokinetic parameters of ^{13}C-methacetin, paracetamol, $^{13}CO_2$ and DOB curves were calculated by standard non-compartmental methods. The maximum observed value and the corresponding sampling time were defined as C_{max} and T_{max}, respectively. The elimination rate constant (k_{el}) was determined from the slope of the regression line that best fit the terminal portion of the log-linear concentration-time curve for ^{13}C-methacetin and paracetamol (first order kinetics). The terminal half-life ($t_{1/2}$), was calculated as log(2)/k_{el}. The $^{13}CO_2$ and DOB curves did not show first order kinetics and pharmacokinetic parameters depending on log-linear regression were not calculated. The area under the time curve up to the last sampling time, AUC=$AUC_{(0–t)}$, was calculated by the log-trapezoidal rule. The total area under the curve extrapolated to infinity, AUC_{inf}=$AUC_{(0–inf)}$, was determined for ^{13}C-methacetin and paracetamol by summing $AUC_{(0–t)}$ + C_t/K_{el}, where C_t was the last observed value. Apparent clearance (CL) of ^{13}C-methacetin and paracetamol were calculated as $dose_{[13C]methacetin}/AUC_{inf}$, the apparent volume of distribution V_d as CL/K_{el} {Amchin1999}.

LiMAx calculation

The LiMAx value in [µg/kg/h] was calculated from the maximum DOB (DOB_{max}) via

$$LiMAx = \frac{DOB_{max} \cdot R_{PDB} \cdot P_{CO2BSA} \cdot BSA \cdot M_R}{BW}$$

with P_{CO2BSA} the CO_2 production according to body surface area (BSA), equal to 300 [mmol/h/m^2], M_R the molar mass of ^{13}C-methacetin (166 [g/mol]), and BW the body weight in [kg] {Rubin2017}. BSA in [m^2] is calculated using the height H in [cm] and the formula by Haycock {Haycock1978}:

$$BSA = 0.024265 \cdot BW^{0.5378} \cdot H^{0.3964}$$

Sensitivity analysis

Sensitivity analysis of the pharmacokinetic parameters was performed by increasing and decreasing all model parameters individually by 10%. The sensitivity of a pharmacokinetic parameter q (AUC, T_{max}, C_{max}, Kel, Vd and CL) for a substance i ([13C]methacetin, paracetamol, $^{13}CO_2$ and DOB) due to changes in a parameter k was calculated as a two sided sensitivity based on increased parameter $p_k^+ = 1.1 \cdot p_k^{ref}$ and decreased parameter $p_k^- = 0.9 \cdot p_k^{ref}$ with the sensitivity calculated as

$$q_{i,k} = \frac{1}{2} \frac{(q_{i,k}^+ - q_{i,k}^-)}{q_{i,k}^{ref}}$$

Parameters excluded from sensitivity analysis are the same parameters excluded from variability analysis.

Smoking & Cirrhosis

The cytochrome CYP1A2 variability in the population was modeled based on a lognormal distribution {Achour2014}. A baseline CYP1A2 content of 0.4 was assumed for smokers and non-smokers. In case of smoking an induction of CYP1A2 by the factor 1.4 was assumed relative to nonsmokers (see resulting distributions in Figure S2).

Cirrhosis was modeled by assuming reduced parenchymal liver fraction compared to healthy subjects ($F_{PAR}^{healthy} = 0.85$). An exponential distribution of parenchymal liver fraction $p(F_{PAR}^{cir})$ in cirrhosis was assumed (see Figure S2)

$$p(F_{PAR}^{cir}) = \frac{1}{\beta} e^{-\frac{1}{\beta} F_{PAR}^{cir}} \text{ with } \beta = 0.12$$

For healthy and cirrhotic nonsmokers and smokers each N=1000 simulations were performed sampling from the respective CYP1A2 and F_PAR distributions.

Experimental/clinical data

Experimental/clinical data were digitized from published figures and tables. All error measurements were converted to standard deviation (SD) from standard error (SE) using the number of subjects (n) of the respective studies ($SD = \sqrt{n} \cdot SE$). References, dosing and number of subjects underlying all curves are listed in Table 1. Data depicted in figures are means±SD.

Validation data

The PBPK model was validated using clinical study data not used in model building and parameter fitting.

Bicarbonate study (Mohr2018)

The ^{13}C-bicarbonate breath test was performed in 44 healthy control subjects after an fasting period of at least 6 hours. ^{13}C-bicarbonate ($NaH^{13}CO_3$) was obtained from dan pharma (Dannenberg, Germany) and the injection solution prepared at a concentration of 2.0343 mg/ml in 0.9% saline by the in-house pharmacy of Charité – Universitätsmedizin Berlin. The ^{13}C-bicarbonate solution was administered intravenously in a final amount of 0.20343 [mg/kg] body weight and the $^{13}CO_2/^{12}CO_2$ ratio was recorded continuously in the exhaled breath for 30 minutes using the Fast Liver Investigation Packet [FLIP®] 2.0 device (Humedics GmbH, Berlin, Germany). Prior to the ^{13}C-bicarbonate injection, the baseline $^{13}CO_2/^{12}CO_2$ ratio was recorded for 10 minutes and the changes of this ratio is expressed as Delta Over Baseline (DOB). The participants remained in a lying position during the whole test period.

Dosing study (Taheri2017)

Plasma methacetin and paracetamol and LiMAx were measured in 19 healthy subjects (15 male / 4 female, mean age 30±7 [y], range 21-47 [y], mean body weight 75±13kg, mean body mass index 23.2±2.9 kg/m^2, mean body surface area 1.9±0.2 [m^2]) under 2 [mg/kg] and 4 [mg/kg] doses {Holzhuetter2013, Taheri2017}. The 13CO2/12CO2 ratio in the exhaled air was tracked for more than 60 minutes as described above. 13C-methacetin as well as paracetamol blood levels were determined by HPLC at 0.5, 1, 2, 5, 10, 30, and 60 minutes after iv 13C-methacetin administration. Blood samples were drawn in a standardized manner as reported previously {Holzhuetter2013}. Five ml were discarded and a sample of 5 ml was drawn into a serum tube. Samples were centrifuged at 1,500 g for 4 minutes and the serum aliquot was separated. Blinded samples were analyzed for 13C-methacetin and paracetamol by high performance liquid chromatography (HPLC). HPLC analysis was performed using a Ultrashere ODS with a LC-6B system (Shimadzu) at a flow rate of 1.5 ml/min, with UV-detection at 260 nm. Samples of 50 µl serum were mixed with 100 µl of acetronitrile/methanol solution (1:1) and centrifuged at 10,000 g for 8 minutes before HPLC. Samples of 10 µl each were loaded into the analyzer. A commercial HPLC-Test-Kit for measurement of levetiracetam was used for the analysis. The Kit-conditions were modified for estimation of methacetin and paracetamol. Chromatography was performed with the LC-6B system. The sensitivity was 0.5 µg/ml with proven test linearity up to a concentration of 100 µg/ml. The mean interassay variability was 6.8% for methacetin and 6.9% for paracetamol.

Smoking effect on LiMAX (Wuensch2018)

A retrospective analysis of clinical database at Charité - Universitätsmedizin Berlin was performed for LiMAx in healthy patients and with cirrhosis (the subjects from the bicarbonate and exercise study were included in the analysis). Only subjects with smoking status, age, body weight, height, gender and limax values (two subjects were excluded due to inconsistent data). The final data set consisted of n=115 subjects (91 healthy non-smokers, 24 healthy smokers).

RESULTS

Main result of this study is the first PBPK model for the evaluation of ^{13}C-methacetin breath tests, i.e., the LiMAx and MBT dynamical liver function tests (Figure 1). The model describes correctly data from more than 20 clinical studies (Table 1) after oral and intravenous application under various dosing regimes of paracetamol (Figure 2), ^{13}C-bicarbonate (Figure 3), ^{13}C-methacetin in MBT (Figure 4) and ^{13}C-methacetin in LiMAx (Figure 5). Sensitivity analysis was performed to analyze factors affecting MBT and LiMAx (Figure 6). The effect of key factors on the test results, i.e., parenchymal liver tissue fraction, CYP1A2, or liver perfusion on LiMAx were studied in detail (Figure 7). Finally, the model was applied to analyze the effect of impaired liver function as well as smoking on LiMAx results (Figure 8).

Physiologically based pharmacokinetics model (PBPK)

The main result of this study is a detailed PBPK model for the evaluation of ^{13}C-methacetin breath tests (MBT and LiMAx) including absorption, distribution, metabolism and elimination of ^{13}C-methacetin, paracetamol, and $^{13}CO_2$/$^{13}CO_2$ bicarbonate (Figure 1B). The model contains all organs relevant for the methacetin breath tests, i.e. lung (exhalation), heart (cardiac output), bone (slow $^{13}CO_2$), kidney (renal clearance), spleen (venous blood for liver), gut (absorption and venous blood for liver), liver (hepatic clearance) and the remaining body (rest). The liver is perfused via hepatic artery and portal vein (gut and spleen) as input and hepatic vein as output. The metabolic model of the liver (Figure 1C) includes the conversion of ^{13}C-methacetin to $^{13}CO_2$ and paracetamol by CYP1A2 and the detoxification of paracetamol (APAPD). The resulting model allows the *in silico* application of paracetamol, ^{13}C-bicarbonate and ^{13}C-methacetin via either oral or intravenous route. The applied substances are distributed in the systemic circulation based on organ volumes, blood flows and partitioning coefficients for the different tissues.

Paracetamol kinetics

To describe the kinetics of the paracetamol product in MBT and LiMAx correctly, the model includes the processes involved in paracetamol clearance. The resulting model is in good agreement with paracetamol kinetics after oral and intravenous paracetamol application under varying doses (Figure 2, Table 1) {Chiew2010; Critchley2005; Albert1974; Baraka1990; Rawlins1977}. Paracetamol is rapidly and completely absorbed and subsequently mainly cleared by the liver, with minor renal clearance.

Bicarbonate kinetics

The $^{13}CO_2$ expiration in the breath following administration of ^{13}C-methacetin is not only a function of the *in vivo* hepatic metabolism by CYP1A2 for methacetin but also of the kinetics of the bicarbonate pools of the body in which the labeled CO_2 is distributed before its expiration in the breath. {Winchell1970; Winchell1970a}. An important part of the computational model is hence a correct description of the ^{13}C-bicarbonate distribution in the body. The resulting model is in good agreement

with bicarbonate kinetics after oral and intravenous ^{13}C-bicarbonate application under varying doses (Figure 3, Table 1) {Meineke1993; Irving1983; Barstow1990}. The resulting bicarbonate kinetics measured in the breath as DOB is hereby a combination of multiple processes, i.e. exhalation of $^{13}CO_2$ depending on the amount of CO_2 produced in the lungs, distribution of bicarbonate in the body, and storage in slow pools {Irving1983}. Importantly not only the very fast initial distribution kinetics of bicarbonate in the first minutes, but also the slower kinetics due to bicarbonate in internal slow pools over hours {Irving1993; Barstow1990} as well as the overall recovery over very long time frames (up to 12 hours) {Fuller2000; Leijssen1996} could be reproduced by the model.

To validate that the model can reproduce bicarbonate kinetics measured in a typical LiMAx protocol model predictions were compared with clinical data for bicarbonate in 44 subjects recorded with the FLIP device (Mohr2018 in Figure 3 and Table 1).

Methacetin breath test (MBT)

The resulting model is in good agreement with momentary and cumulative ^{13}C recovery after oral ^{13}C-methacetin application under varying doses (Figure 4, Table 1) {Ciccocioppo2003; Holtmeier2006; Kasicka-Jonderko2011; Kasicka-Jonderko2008; Kasicka-Jonderko2013a; Lalazar2008}. Within the normal test duration of the MBT of around 2-3h depending on dose around 20-40% of the ^{13}C-label are recovered. Importantly also the time-course of long-term recovery reaching around 60% after 10h are correctly described by our model {Krumbiegel1985}.

LiMAx

In a next step, the LiMAx test under varying doses of intravenous ^{13}C-methacetin (2 [mg/kg] and 4 [mg/kg]) was simulated. Resulting model predictions are in good agreement with ^{13}C-methacetin and paracetamol time courses and DOB curves after different doses of ^{13}C-methacetin in the LiMAx test (Figure 5, Table 1) {Taheri2017}. Methacetin is completely and rapidly metabolized after administration, paracetamol appears accordingly and reaching a plateau due to the slow paracetamol kinetics in the liver compared to methacetin. DOB curves rise in line with the appearance of paracetamol, but decrease faster than paracetamol due to the bicarbonate kinetics in the body.

Factors affecting LiMAx and MBT

To analyze which factors could affect the results of ^{13}C-breath test sensitivity analysis of the model parameters for LiMAx (Figure 6A) and MBT (Figure 6B) was performed. In the analysis, model parameters were changed individually and the effect of the given parameter change on the resulting pharmacokinetic parameters of ^{13}C-methacetin, paracetamol and DOB curves was quantified. Changes on area under the curve (AUC), time of peak (T_{max}), peak value (C_{max}), elimination constant (K_{el}) and volume of distribution (Vd) were calculated. The parameters having the strongest effect on the DOB_{max} are listed in Table 2.

Parameters affecting the amount of produced CO_2 and availability of CO_2 in the lungs have a strong effect on DOB_{max}. Both for MBT and LiMAx the strongest effect is a negative effect of the CO_2 production rate per body surface area on (P_CO2BSA) on DOB_{max}. In addition, perfusion of the lungs has a strong negative effect (FQlu), i.e. with increasing perfusion and CO2 production the DOB_{max} is decreased. A strong positive effect on DOB_{max} is observed by the rate with which CO2 is exhaled depending on blood CO_2 concentration (KLU_EXCO2), i.e. the more effective the release of

CO2 the higher the observed DOB_{max}. An increase in relative lung volume (FVlu) increases DOB_{max}, as does the partitioning coefficient of CO_2 in the lungs (Kplu_co2c13).

A strong positive factor on DOB_{max} is the hepatic perfusion (FQh), resulting in higher LiMAx values with increased liver perfusion. In LiMAx the hepatic perfusion has the second highest sensitivity, whereas in the MBT the effect is not so strong, but still hepatic perfusion is found in the top sensitivities of the MBT.

Among the top sensitivities for DOB_{max} are parameters affecting CYP1A2 and the availability of methacetin in the liver. An Increase in CYP1A2 methacetin clearance per microsomes (CYP1A2MET_CL) increases the DOB_{max}, as does the amount of microsomes (MPPGL), the parenchymal liver fraction (F_PAR) and the free methacetin concentration in the liver (fumic_metc13). The CYP1A2 content (CYP1A2MET_CL) and parenchymal liver fraction (F_PAR) are among the top sensitivities in LiMAx whereas in the MBT these are much lower on the list. Both MBT and LiMAx are sensitive to changes in actual hepatic clearance of methacetin by CYP1A2 and the parenchymal liver fraction corresponding to functional liver tissue. LiMAx outperforms MBT in detecting changes in CYP1A2 and parenchymal liver function due to its higher sensitivity to these values using the DOB_{max} as evaluation criterion.

An important difference between MBT and LiMAx is that MBT is very sensitive towards the parameters for absorption of the oral dose of methacetin, i.e., the rate of absorption (Ka_metc13) and the fraction of methacetin absorbed (F_metc13), whereas LiMAx is not affected by these parameters due to the intravenous route of application.

Body weight has a strong effect, but this could be indirect because many quantities in the model are calculated based on body weight, e.g., the total cardiac output, organ volumes, organ perfusion or the total CO2 production.

For the MBT test (Figure 6B) the overall pattern of sensitivities on the pharmacokinetic parameters for plasma paracetamol and bicarbonate/^{13}CO2 as well as DOB are similar to the LiMAx test. The main difference is the ^{13}C-methacetin rate of absorption (Ka_metc13) which has a very strong effect on the pharmacokinetic parameters of methacetin, paracetamol and DOB with an increase in methacetin absorption rate resulting in an increase in DOB_{max} and a decrease in T_{max}. An interesting observation is that many of the pharmacokinetics parameters of ^{13}C-methacetin and paracetamol in plasma are much stronger affected by changes in parameters in MBT than in LiMAx, with the overall effect of parameters being similar.

The different pharmacokinetics parameter calculated on the DOB curves (AUC, Tmax and DOB_{max}) are influenced by parameter changes in a heterogeneous manner, i.e. certain parameters affect AUC, others T_{max}, others DOB_{max}.

Effect of CYP1A2, parenchymal liver fraction and hepatic perfusion on LiMAx

Subsequently, we analysed selected parameters relevant for the clinical application of LiMAx in more detail, i.e., parenchymal liver fraction, hepatic perfusion (Figure 7) and CYP1A2 (Supplementary Figure S1). For the calculation of the pharmacokinetic parameters of the DOB curve either the first 60 minutes of the simulation, corresponding to data typically available in a LiMAx test, or the full simulated time course was used (10h).

LiMAx (DOB_{max}) values are monotonously decreasing with decreasing parenchymal liver volume, indicating that LiMAx is a good proxy for measuring parenchymal liver fraction. The decrease is nonlinear. AUC_{0-60} calculated on the DOB curve of the first 60 minutes is different from AUC calculated on the full time course so are time of maximum value (T_{max}) and time to half maximal value ($T_{max0.5}$). The relationship between decreasing CYP1A2 and pharmacokinetic parameters is very similar to a decrease in parenchymal liver fraction, i.e., changes in functional liver volume and enzyme have very similar effect on measured liver function.

Hepatic perfusion has a strong effect on LiMAx test results with an almost perfect linear relationship. $DOB_{max,}$ AUC, T_{max} and $T_{max0.5}$ are all affected by changes in hepatic perfusion.

Additional key results:

- LiMAx based on DOB_{max} is very robust (Figure 7). No difference when calculated on 60 min compared to full time course, and even sufficient only to use the first 12 min with minimal changes at very low LiMAx values. This suggests that the LiMAx test protocol can be substantially shortened (to about 15min) without any loss in accuracy of the test. This has to be evaluated on real patient data (and how the difference would perform!).
- The Tmax 0.5 seems a very valuable second dimension to the LiMAX which could indicacte reduced hepatic perfusion and would than allow to, i.e. relatively high limax with long Tmax0.5 above 3 minutes could warrant subsequent measurements of hepatic perfusion via doppler sonography.

LiMAx distributions

The prediction of the model based on a continuous decrease in parenchymal liver volume were compared to a recent study analyzing 10000 LiMAx tests {Rubins2017} (Figure 8). The model predictions are in very good agreement with the clinical data, not only the change in curve form with decreasing parenchymal liver volume, but also the shift in $T_{max0.5}$ to larger times.

Effect of smoking on LiMAx

The effect of smoking on LiMAx test results was simulated (Figure 8). The observed shift in LiMAx distribution in smokers could be explained solely on the bases of an induction of CYP1A2 in smokers. The same simulations were performed for cirrhosis assuming a decrease in parenchymal liver fraction. Interestingly, the missing shift in distribution in cirrhosis could be correctly reproduced.

DISCUSSION AND SUMMARY

Within this study, computational modeling of the dynamical liver function tests (D-LFT) was used to derive basic information for a better understanding of the ^{13}C-methacetin breath test and factors influencing its results. The first PBPK model of ^{13}C-methacetin based breath tests including absorption, distribution, metabolism and elimination of ^{13}C-methacetin, paracetamol, and $^{13}CO_2$ was developed and applied to analyse MBT and LiMAx.

To our knowledge this is the first detailed physiological model for a $^{13}CO_2$ breath test for liver function. Previous work is limited to partial aspects, i.e., i) pharmacokinetics models of paracetamol metabolism {Zurlinden2016; Sluka2016; Jiang2013; Geenen2013}; ii) compartment models for ^{13}C-bicarbonate distribution {Irving1983; Barstow1990} iii) compartment model for LiMAx

{Holzhuetter2013}. In contrast, this work provides a comprehensive physiological model of the ^{13}C-methacetin test in line with a wide range of clinical data.

“The first consideration is the precision or within-subject variability. Few studies report the within-subject variability for the principle outcome measures, for example cumulative recovery of the label in the breath over the duration of the test. For example, reported within-subject variability for the 13C-MBT [37] tests is 11%. This variability is much larger than that which can be attributed to analytical imprecision, and reflects true biological differences in the rate of metabolism. The factors contributing to the within-subject variance must be more completely defined and, where possible, must be considered in the design and conduct of the test.”{Afolabi2013}

This allowed us for the first time to analyze key factors influencing methacetin breath tests. LiMAx and MBT are most sensitive to parameters affecting the exhalation of $^{13}CO_2$, hepatic blood flow, CYP1A2 and parenchymal liver fraction. In addition MBT was strongly affected by absorption kinetics of methacetin compared to LiMAx. In the following these factors and their clinical implications are discussed.

Effect of CO_2 production

Our model predicts that MBT and LiMAx are influenced by all factors that increase the total amount of CO_2 production. Examples of such factors are age, physical activity, consumed meal or sparkling beverage, diseases of the respiratory tract, fever, and thyroid disorders {Gorowska-Kowolik2017}. Variations in movement or physical activity during the test, or preceding food intake, can affect the rate of CO2 production, which can, in turn, affect the appearance of the label in the breath {Afolabi2013}. Most of these factors have no clinical relevance, because the LiMAx is performed after an overnight fast with the subject in a resting position avoiding changes due to physical activity as well as meals and beverages. Bicarbonate kinetics are strongly altered in exercise {Barstow1989, Barstow1990, Slanger1970}, as are LiMAx test results {Taheri2017}.

Diseases of the lungs should be considered in the context of LiMAx, and can easily be ruled out via testing lung function.

Effect of hepatic blood flow

Our model predicts that MBT and LiMAx are influenced by hepatic blood flow. Methacetin has a high hepatic extraction, which results in clearance of methacetin which depends on hepatic perfusion {Bonfrate2014}. Therefore, is crucial to study the effect of altered blood flow on its pharmacokinetics {Nista2004}. Factors and diseases altering hepatic blood flow will alter LiMAx and MBT test results. The reduced $^{13}CO_2$ in MBT observed in elderly could be partly explained by the reduced perfusion with increasing age {Scheider2004}. For instance, Ciccocioppo et al. showed that MBT values are influenced by age, and peak 13C excretion in elderly subjects was inversely related to the intra-hepatic resistance index (assessed by Doppler pulsed wave analysis) {Nista2004, Ciccocioppo2003}.

Hepatic blood flow is directly related to cardiac output. Liver function abnormalities are commonly observed in patients with low cardiac index and are not associated with clinically apparent hepatic disease, resolving with compensation of heart failure {Naschitz2000; Malek2008}. In the MBT the cumulative dose of ^{13}C methacetin after 120 minutes was related to the degree of heart failure assessed by NYHA staging system as well as the dimensions of the left atrium in patients with chronic heart failure {Malek2008; {Gorowska-Kowolik2017}.

Exercise is an example with significant changes in cardiac output (in addition to changes in CO_2 production), and therefore also in hepatic perfusion resulting in altered LiMAx results {Taheri2017}.

Effect of gastric emptying

As predicted by our model, in case of oral methacetin administration, the $^{13}CO_2$ kinetics is influenced to a great degree by the rate of the gastric emptying and the absorption of the substrate from the gastrointestinal tract {Afolabi2013; Gorowska-Kowolik2013}. The strong effect of absorption kinetics can for instance be seen when applying 13C-methacetin with a standardized meal, resulting in peak DOB values at around 2h compared to 20-30min when applied with a drink (mainly due to the much slower absorption under standard meal conditions compared to fluid) {Wutzke2008}.

Such confounding, based on absorption kinetics, can be avoided by intravenous administration of methacetin as performed in the LiMAx test.

Effect of CYP1A2 changes

Our model predicts that LiMAx is influenced by changes in CYP1A2 and factors resulting in the induction or suppression of CYP1A2. Habitual smoking stimulates the activity of the cytochrome CYP1A2 {Dobrinas2011; Dobrinas2013}, and resulting in increased LiMAX, as confirmed by our retrospective analysis. Oral contraceptives reduce CYP1A2 activity {Granfors2005}, resulting in decreased methacetin clearance, as shown for instance via lower momentary and cumulative $^{13}CO_2$ recovery during medication with oral contraceptives {Jonderko2013}.

In chronic liver disease, decreased cytochrome P450 activity can be due to down regulation of gene expression by interferons, tumour necrosis factors and other cytokines {Nishta2004}. Patho-biochemical investigations of human livers have shown that specifically CYP1A2 is reduced in different stages of chronic liver diseases {Palmer1992; George1995; Goetze2007}. Other factors inducing CYP1A2 are substrates of CYP1A2, for instance the effect of caffeine consuption {REF}, or even the repeated testing via methacetin {Kasicka-Jonderko2011}.

Consequently, it is important for the interpretation of methacetin breath tests to check for factors like smoking, oral contraceptives or other factors affecting CYP1A2 expression. An important result is the correct prediction of smoking effect on LiMAX, which provides an opportunity for improved evaluation of the LiMAx test by accounting for individual smoking status.

Other lifestyle factors (grapefruite juice, …)

All factors affecting CYP1A2 activity are crucial. This includes for instance also sustances and medications which are CYP1A2 substrates, consequently functioning as competitive inhibitor on methacetin -> paracetamol conversion, e.g., coffeine consumption (see studies). A standardized questionaire could help here and information than certain food should not be eaten before the test. Especially no coffee consumption for 24h.

Clinical relevance

It is important to note, that many of the predicted factors have only limited clinical relevance, especially in the context of follow-up or repeated measurements within an individual person. Examples are height or bodyweight, which affect LiMax and MBT, but are not relative constant within a single individuum. Other factors like influence of meals, CO2 containing beverages, exercise or absorption kinetics are ruled out by the used protocol, i.e., overnight fast with intravenous injection of

methacetin in the LiMAx test. Factors like heart or lung diseases can easily be ruled out in clinical routine. Consequently, despite many factors having a theoretical influence on LiMAx test results, of clinical relevance are mainly factors influencing CYP1A2 content which have high prevalence in people with liver disease, e.g. smoking.

Our modeling approach has imacts which could directly be translated in the clinics (after validation in cohort studies, namely reduced LiMAx protocols of ~15 min, accounting for Tmax0.5 as second dimension of the test outcome, put special efforts in normalizing the injection protocol which could improve evalutation, take lifestyle factors influencing CYP1A2 into account in patient history and evaluation of LiMAx test protocols.

Accounting for changes in body composition, liver volume and liver perfusion with age, i.e. building a more specific model representing the actual patient. Currently only mean parameters for most parameters. Putting age-adjusted paramters in such a model will further improve predictions.

Advances

The search for a noninvasive method that would enable optimal functional diagnostics of the liver is indisputably a challenge of modern hepatology. In the light of the presented research, LiMAx appears to be a useful tool for assessing the capacity of the organ, and as a prognostic marker in many diseases. Methacetin is a very promising test substance because of its rapid metabolism in healthy subjects allowing a rapid non-invasive quantification of liver function. Our computational model allowed to identify important parameters for the interpretation of ^{13}C D-LFTs which can have clinical implications. For the first time, our model allows to quantify effects of clinically and medically relevant parameters on LiMAx test results, providing a tool which could be applied for an improved interpretation LiMAx tests in the future, e.g., by adjusting for lifestyle factors like smoking.

Limitations

Despite the usefulness of the current study there are potential shortcomings and limitations: i) the model was developed for healthy subjects, with most of the clinical data sets from healthy controls under resting conditions. For the transfer of the results to patient groups with certain liver diseases the applicability of the model must be shown in such cohorts; ii) The heterogeneity of the liver was not taken into account and no spatially resolved model of the liver was developed. Heterogeneity of function in the liver lobulus, e.g., zonation of CYP1A2, as well as on the organ, e.g., perfusion heterogeneity being important for liver function were not considered; iii) Model simulations are limited to mean model simulations, and no stratified or personalized evaluation of liver function was performed; iv) Parameter variability was analysed in a simplified manner, no realistic parameter distributions were used; v) Modeling of cirrhosis was based on the assumption of reduction of parenchymal liver volume. Hereby, no structural or perfusion changes or alterations like hepatic shunts were included which accompany cirrhosis.

Outlook

This work provides the foundation for many future applications. Understanding the factors influencing D-LFTs based on computational modeling will allow to improve the interpretation of liver function tests. By correcting the test results for factors which can easily be recorded like hepatic perfusion with doppler methods, smoking behavior in the patient history, or lung parameters via lung function tests it should be possible to further increase the sensitivity and specificity of LiMAx. An

interesting future approach could be combination tests using a substance with a high extraction ratio like methacetin (E=0.8-1.0) and low extraction ratio like caffeine (E=0.1-0.2) for the same enzyme CYP1A2. Such a combination would allow to non-invasively decouple liver perfusion and hepatic metabolism based on a non-invasive test, thereby providing a more complete picture of hepatic function and hepatic perfusion.

Future work will extend the presented model in two directions, towards modeling the spatial heterogeneity of the liver on organ and tissue-scale (zonation) and towards an individualized interpretation of D-LFTs. Personalization of model simulations based on anthropometric data will allow the calculation of individual reference ranges of liver function and subsequently an improved sensitivity and specificity using these individual cutoffs.

By providing a reproducible model encoding in SBML large parts of the developed modeling infrastructure and methods can be easily transferred to other D-LFTs like caffeine, galactose or indocyanogreen and other breath tests relevant for the liver and easily be personalized.

SUPPLEMENTARY MATERIAL

S1 SBML model
S2 SBML model report
S3 Sensitivities of pharmacokinetics parameters

AUTHOR CONTRIBUTIONS

MK designed the study, developed the computational model, performed the analysis and wrote the initial draft of the manuscript.
TW performed the retrospective analysis of the LiMAx data and prepared the clinic data sets.
DL and **JT** implemented the parameter fitting of the model.
JL and **PT** performed the clinical study on dosing effect of LiMAx.
JM, TW, JL and PT performed the clinical breath test studies
MS and **JL** designed the clinical breath test studies
All authors contributed and revised the manuscript critically.

ACKNOWLEDGEMENTS

MK is supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM, grant number 031L0054). DL is supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM, grant number 031L0048). TW is supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM, grant number 031L0057).

CONFLICT OF INTEREST

Martin Stockmann has capital interest in Humedics, providing the FLIP device and LiMAx test. All other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

{Afolabi2013} Afolabi, P.; Wright, M.; Wootton, S. A. & Jackson, A. A.; Clinical utility of 13C-liver-function breath tests for assessment of hepatic function.; Digestive diseases and sciences, 10.1007/s10620-012-2340-z, 2013, 58, 33-41

{Albert1974} Albert, K. S.; Sedman, A. J.; Wilkinson, P.; Stoll, R. G.; Murray, W. J. & Wagner, J. G. Bioavailability studies of acetaminophen and nitrofurantoin. J Clin Pharmacol, 1974, 14, 264-270

{Amchin1999} Amchin, J.; Zarycranski, W.; Taylor, K. P.; Albano, D. & Klockowski, P. M. Effect of venlafaxine on CYP1A2-dependent pharmacokinetics and metabolism of caffeine. Journal of clinical pharmacology, 1999, 39, 252-259

{Archour2014} Achour, B.; Rostami-Hodjegan, A. & Barber, J. Protein expression of various hepatic uridine 5'-diphosphate glucuronosyltransferase (UGT) enzymes and their inter-correlations: a meta-analysis. Biopharmaceutics & drug disposition, 10.1002/bdd.1906, 2014, 35, 353-361

{Baraka1990} Baraka, O. Z.; Truman, C. A.; Ford, J. M. & Roberts, C. J. The effect of propranolol on paracetamol metabolism in man. Br J Clin Pharmacol, Department of Medicine, Bristol Royal Infirmary., Department of Medicine, Bristol Royal Infirmary., 1990, 29, 261-264

{Barstow1989} Barstow, T. J.; Cooper, D. M.; Epstein, S. & Wasserman, K. Changes in breath 13CO2/12CO2 consequent to exercise and hypoxia. Journal of applied physiology (Bethesda, Md. : 1985), 1989, 66, 936-942

{Barstow1990} Barstow, T. J.; Cooper, D. M.; Sobel, E. M.; Landaw, E. M. & Epstein, S. Influence of increased metabolic rate on [13C]bicarbonate washout kinetics. The American journal of physiology, 1990, 259, R163-R171

(Bernstein1960) Bernstein, L. M.; Wheeler, J. X.; Bond, E. E.; Rohmsdahl, M. & Dougherty, N. The blood galactose disappearance curve as a test of liver function. Gastroenterology, 1960, 39, 293-304

{Bozek2018} Bożek, M.; Kamińska, M.; Kasicka-Jonderko, A.; Krusiec-Świdergoł, B.; Ptaszek, K.; Juszczyk, M. & Jonderko, K. Scrutiny of , javax.xml.bind.JAXBElement@6d9692a2, C-phenylalanine breath test reproducibility. Isotopes in environmental and health studies, 10.1080/10256016.2018.1431627, 2018, 54, 312-323

{Chiew2010} Chiew, A.; Day, P.; Salonikas, C.; Naidoo, D.; Graudins, A. & Thomas, R. The comparative pharmacokinetics of modified-release and immediate-release paracetamol in a simulated overdose model. 2010, 22, 548-555

{Ciccocioppo2003} Ciccocioppo, R.; Candelli, M.; Di Francesco, D.; Ciocca, F.; Taglieri, G.; Armuzzi, A.; Gasbarrini, G. & Gasbarrini, A. Study of liver function in healthy elderly subjects using the 13C-methacetin breath test. Alimentary pharmacology & therapeutics, 2003, 17, 271-277

{Critchley2005} Critchley, J. A. J. H.; Critchley, L. A. H.; Anderson, P. J. & Tomlinson, B. Differences in the single-oral-dose pharmacokinetics and urinary excretion of paracetamol and its conjugates between Hong Kong Chinese and Caucasian subjects. J Clin Pharm Ther, 2005, 30, 179-184

{DeGasperi2016} De Gasperi, A.; Mazza, E. & Prosperi, M. Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery? World journal of hepatology, 10.4254/wjh.v8.i7.355, 2016, 8, 355-367

{Dobrinas2011} Dobrinas, M.; Cornuz, J.; Oneda, B.; Kohler Serra, M.; Puhl, M. & Eap, C. B. Impact of smoking, smoking cessation, and genetic polymorphisms on CYP1A2 activity and inducibility. Clinical pharmacology and therapeutics, 10.1038/clpt.2011.70, 2011, 90, 117-125

{Dobrinas2013} Dobrinas, M.; Cornuz, J. & Eap, C. B. Pharmacogenetics of CYP1A2 activity and inducibility in smokers and exsmokers. Pharmacogenetics and genomics, 10.1097/FPC.0b013e3283602e75, 2013, 23, 286-292

{Fuller2000} Fuller, N. J.; Harding, M.; McDevitt, R.; Jennings, G.; Coward, W. A. & Elia, M. Comparison of recoveries in breath carbon dioxide of H13CO-3 and H14CO-3 administered simultaneously by single 6 h constant unprimed intravenous infusion. The British journal of nutrition, 2000, 84, 269-274

{Geenen2013} Geenen, S.; Yates, J. W.; Kenna, J. G.; Bois, F. Y.; Wilson, I. D. & Westerhoff, H. V.; Multiscale modelling approach combining a kinetic model of glutathione metabolism with PBPK models of paracetamol and the potential glutathione-depletion biomarkers ophthalmic acid and 5-oxoproline in humans and rats; Integrative Biology, Royal Society of Chemistry, 2013, 5, 877-888

{Gorowska-Kowolik2017} Gorowska-Kowolik, K.; Chobot, A. & Kwiecien, J. 13C Methacetin Breath Test for Assessment of Microsomal Liver Function: Methodology and Clinical Application. Gastroenterology research and practice, 2017, 2017, 7397840

{Goetze2007} Goetze, O.; Selzner, N.; Fruehauf, H.; Fried, M.; Gerlach, T. & Mullhaupt, B. 13C-methacetin breath test as a quantitative liver function test in patients with chronic hepatitis C infection: continuous automatic molecular correlation spectroscopy compared to isotopic ratio mass spectrometry. Alimentary pharmacology & therapeutics, 2007, 26, 305-311

{Granfors2005} Granfors, M. T.; Backman, J. T.; Laitila, J. & Neuvonen, P. J. Oral contraceptives containing ethinyl estradiol and gestodene markedly increase plasma concentrations and effects of tizanidine by inhibiting cytochrome P450 1A2. Clinical pharmacology and therapeutics, 10.1016/j.clpt.2005.06.009, 2005, 78, 400-411

{Haycock1978} Haycock, G. B.; Schwartz, G. J. & Wisotsky, D. H. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. The Journal of pediatrics, 1978, 93, 62-66

{Holtmeier2006} Holtmeier, J.; Leuschner, M.; Schneider, A.; Leuschner, U.; Caspary, W. F. & Braden, B. 13C-methacetin and 13C-galactose breath tests can assess restricted liver function even in early stages of primary biliary cirrhosis. Scandinavian journal of gastroenterology, 2006, 41, 1336-1341

{Holzhuetter2013} Holzhütter, H.-G.; Lock, J. F.; Taheri, P.; Bulik, S.; Goede, A. & Stockmann, M. Assessment of hepatic detoxification activity: proposal of an improved variant of the (13)c-methacetin breath test. PloS one, 2013, 8, e70780

{Hucka2003} Hucka, M.; Finney, A.; Sauro, H. M.; Bolouri, H.; Doyle, J. C.; Kitano, H.; Arkin, A. P.; Bornstein, B. J.; Bray, D.; Cornish-Bowden, A.; Cuellar, A. A.; Dronov, S.; Gilles, E. D.; Ginkel, M.; Gor, V.; Goryanin, I. I.; Hedley, W. J.; Hodgman, T. C.; Hofmeyr, J.-H.; Hunter, P. J.; Juty, N. S.; Kasberger, J. L.; Kremling, A.; Kummer, U.; Le Novère, N.; Loew, L. M.; Lucio, D.; Mendes, P.; Minch, E.; Mjolsness, E. D.; Nakayama, Y.; Nelson, M. R.; Nielsen, P. F.; Sakurada, T.; Schaff, J. C.; Shapiro, B. E.; Shimizu, T. S.; Spence, H. D.; Stelling, J.; Takahashi, K.; Tomita, M.; Wagner, J.; Wang, J. & Forum, S. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics (Oxford, England), 2003, 19, 524-531

{Irving1983} Irving, C. S.; Wong, W. W.; Shulman, R. J.; Smith, E. O. & Klein, P. D. [13C]bicarbonate kinetics in humans: intra- vs. interindividual variations. The American journal of physiology, 1983, 245, R190-R202

{Jiang2013} Jiang, X.-L.; Zhao, P.; Barrett, J. S.; Lesko, L. J. & Schmidt, S.; Application of physiologically based pharmacokinetic modeling to predict acetaminophen metabolism and pharmacokinetics in children.; CPT Pharmacometrics Syst Pharmacol, 10.1038/psp.2013.55, 2013, 2, e80

{Jonderko2013} Jonderko, K.; Skałba, P.; Kasicka-Jonderko, A.; Kamińska, M.; Bizior-Frymus, D. & Dyja, R.; Impact of combined oral contraceptives containing ethinylestradiol on the liver microsomal metabolism. The European journal of contraception & reproductive health care : the official journal of the European Society of Contraception, 10.3109/13625187.2013.785515, 2013, 18, 284-292

{Jones2013} Jones, H.M. and Rowland-Yeo, K., 2013. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT: pharmacometrics & systems pharmacology*, *2*(8), pp.1-12.

{Kaschek2016} Dynamic Modeling, Parameter Estimation and Uncertainty Analysis in R; Daniel Kaschek, Wolfgang Mader, Mirjam Fehling-Kaschek, Marcus Rosenblatt, Jens Timmer; bioRxiv 085001; doi: https://doi.org/10.1101/085001

{Kasicka-Jonderko2008} Kasicka-Jonderko, A.; Jonderko, K.; Chabior, E. & Błonska-Fajfrowska, B. Exact profiles of (13)CO(2) recovery in breath air after per oral administration of [(13)C]methacetin in two groups of different ages. Isotopes in environmental and health studies, 2008, 44, 295-303

{Kasicka-Jonderko2011} Kasicka-Jonderko, A.; Loska, D.; Jonderko, K.; Kaminska, M. & Błonska-Fajfrowska, B. Interference of acute cigarette smoking with [^{13}C]methacetin breath test. Isotopes in environmental and health studies, 2011, 47, 34-41

{Kasicka-Jonderko2013a} Kasicka-Jonderko, A.; Jonderko, K.; Budniok, M.; Krusiec-Świdergo, B. & Błońska-Fajfrowska, B. Comparison of two dosage regimens of the substrate for the [13C]methacetin breath test. Isotopes in environmental and health studies, 2013, 49, 109-121

{Koenig2012} König, M., Dräger, A., & Holzhütter, H. G. (2012). CySBML: a Cytoscape plugin for SBML. *Bioinformatics*, *28*(18), 2402-2403.

{Koenig2018_sbmlutils} König M. (2017, November 12). matthiaskoenig/sbmlutils: sbmlutils-v0.1.8 DFBA release (Version v0.1.8). Zenodo. http://doi.org/10.5281/zenodo.1045519

{Krumbiegel1985} Krumbiegel, P.; Günther, K.; Faust, H.; Möbius, G.; Hirschberg, K. & Schneider, G. Nuclear medicine liver function tests for pregnant women and children. 1. Breath tests with 14C-methacetin and 13C-methacetin. European journal of nuclear medicine, 1985, 10, 129-133

{Lalazar2008} Lalazar, G.; Pappo, O.; Hershcovici, T.; Hadjaj, T.; Shubi, M.; Ohana, H.; Hemed, N. & Ilan, Y. A continuous 13C methacetin breath test for noninvasive assessment of intrahepatic inflammation and fibrosis in patients with chronic HCV infection and normal ALT. Journal of viral hepatitis, 2008, 15, 716-728

{Lalazar2009} Lalazar, G. & Ilan, Y. Assessment of liver function in acute or chronic liver disease by the methacetin breath test: a tool for decision making in clinical hepatology. Journal of breath research, 2009, 3, 047001

{Leijssen1996} Leijssen, D. P. & Elia, M. Recovery of 13CO2 and 14CO2 in human bicarbonate studies: a critical review with original data. Clinical science (London, England : 1979), 1996, 91, 665-677

{Malek2008} Málek, F.; Hendrichová, M.; Krátká, K.; Sedlaková, M.; Vránová, J. & Horák, J.; Correlation of the functional liver mass with left ventricular ejection fraction and left atrial diameter in patients with congestive heart failure.; International journal of cardiology, 10.1016/j.ijcard.2007.04.100, 2008, 127, 271-273

{Medley2018} Medley, J.K., Choi, K., König, M., Smith, L., Gu, S., Hellerstein, J., Sealfon, S.C. and Sauro, H.M., 2018. Tellurium notebooks—An environment for reproducible dynamical modeling in systems biology. *PLoS computational biology*, *14*(6), p.e1006220.

{Meineke1993} Meineke, I.; De Mey, C.; Eggers, R. & Bauer, F. E. Evaluation of the 13CO2 kinetics in humans after oral application of sodium bicarbonate as a model for breath testing. European journal of clinical investigation, 1993, 23, 91-96

{Minon1995} Mion, F.; Queneau, P. E.; Rousseau, M.; Brazier, J. L.; Paliard, P. & Minaire, Y. Aminopyrine breath test: development of a 13C-breath test for quantitative assessment of liver function in humans. Hepato-gastroenterology, 1995, 42, 931-938

{Naschitz2000} Naschitz, J. E.; Slobodin, G.; Lewis, R. J.; Zuckerman, E. & Yeshurun, D.; Heart diseases affecting the liver and liver diseases affecting the heart.; American heart journal, 10.1067/mhj.2000.107177, 2000, 140, 111-120

{Nista2004} Nista, E. C.; Fini, L.; Armuzzi, A.; Candelli, M.; Zocco, M. A.; Cazzato, I. A.; Merra, G.; Finizio, R.; Miele, L.; Grieco, A.; Gasbarrini, G. & Gasbarrini, A. 13C-breath tests in the study of microsomal liver function. European review for medical and pharmacological sciences, 2004, 8, 33-46

{Park2003} Park, G. J.-H.; Katelaris, P. H.; Jones, D. B.; Seow, F.; Le Couteur, D. G. & Ngu, M. C. Validity of the 13C-caffeine breath test as a noninvasive, quantitative test of liver function. Hepatology, 10.1053/jhep.2003.50475, 2003, 38, 1227-1236

{Renner1984} Renner, E.; Wietholtz, H.; Huguenin, P.; Arnaud, M. J. & Preisig, R. Caffeine: a model compound for measuring liver function. Hepatology, 1984, 4, 38-46

{Raue2013} Raue, A., Schilling, M., Bachmann, J., Matteson, A., Schelke, M., Kaschek, D., Hug, S., Kreutz, C., Harms, B.D., Theis, F.J. and Klingmüller, U., 2013. Lessons learned from quantitative dynamical modeling in systems biology. *PloS one*, *8*(9), p.e74335.

{Rawlins1977} Rawlins, M. D.; Henderson, D. B. & Hijab, A. R. Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration. Eur J Clin Pharmacol, 1977, 11, 283-286

{Rowland2011} Rowland, M. & Tozer, T. N. Troy, D. B. (Ed.) Clinical Pharmacokinetics and pharmacodynamics - Concepts and Applications (Fourth Edition) Wolters Kluwer, 2011

{Sakka2007} Sakka, S. G. Assessing liver function. Current opinion in critical care, 2007, 13, 207-214

{Slanger1970} Slanger, B. H.; Kusubov, N. & Winchell, H. S. Effect of exercise on human CO2-HCO3 minus kinetics. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1970, 11, 716-718

{Sluka2016} Sluka, J. P.; Fu, X.; Swat, M.; Belmonte, J. M.; Cosmanescu, A.; Clendenon, S. G.; Wambaugh, J. F. & Glazier, J. A. A Liver-Centric Multiscale Modeling Framework for Xenobiotics; PloS one, Public Library of Science, 2016, 11, e0162428

{Somogyi2015} Somogyi, E. T.; Bouteiller, J.-M.; Glazier, J. A.; König, M.; Medley, J. K.; Swat, M. H. & Sauro, H. M. libRoadRunner: a high performance SBML simulation and analysis library. Bioinformatics (Oxford, England), 2015, 31, 3315-3321

{Taheri2017} Taheri, P. Untersuchung von 13CO2 Entstehung, Verteilung und Ausscheidung zur verbesserten Interpretation der Ergebnisse beim 13C-Methacetin Atemtest zur Messung der enzymatischen Leberfunktion Medizinische Fakultät Charité - Universitätsmedizin Berlin, Medizinische Fakultät Charité - Universitätsmedizin Berlin, 2017

{Vranova2013} Vranova, J.; Hendrichova, M.; Kolarova, H.; Kratka, K.; Rosina, J. & Horak, J.; ^{13}C-methacetin breath test in the evaluation of disease severity in patients with liver cirrhosis.; Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia, 10.5507/bp.2012.114, 2013, 157, 392-400

{Winchell1970} Winchell, H. S.; Stahelin, H.; Kusubov, N.; Slanger, B.; Fish, M.; Pollycove, M. & Lawrence, J. H. Kinetics of CO2-HCO3 minus in normal adult males. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1970, 11, 711-715

{Winchell1970a} Winchell, H. S. & Wiley, K. Considerations in analysis of breath 14CO2 data. Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1970, 11, 708-710

{Wright1999} Wright, Stephen, and Jorge Nocedal. "Numerical optimization." *Springer Science* 35, no. 67-68 (1999): 7.

{Zurlinden2016} Zurlinden, T. J. & Reisfeld, B. Physiologically based modeling of the pharmacokinetics of acetaminophen and its major metabolites in humans using a Bayesian population approach. Eur J Drug Metab Pharmacokinet, 10.1007/s13318-015-0253-x, 2016, 41, 267-280

FIGURES

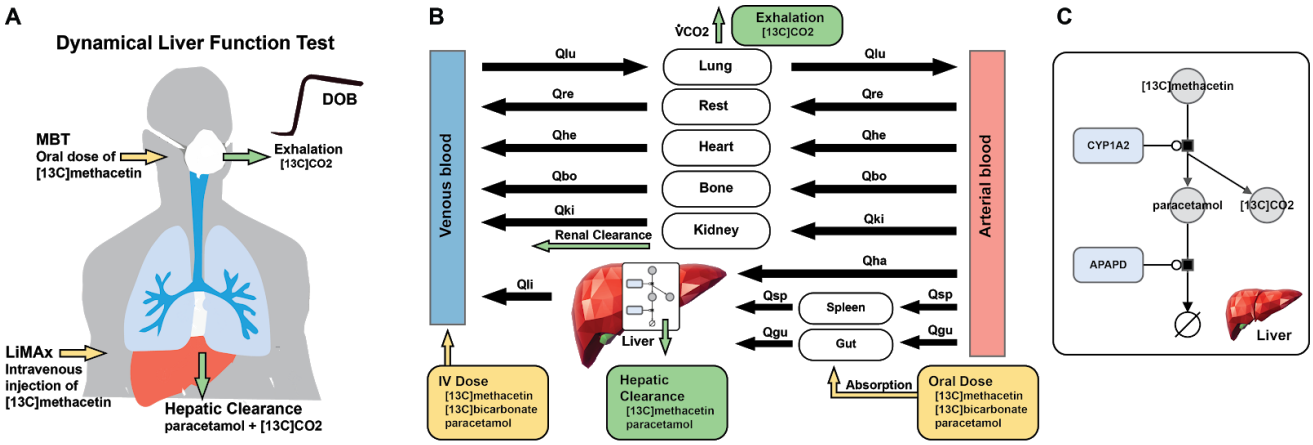


Figure 1 - Computational model. A) Overview of [13C]methacetin breath test. [13C]methacetin is given either orally in the MBT or intravenously in LiMAx. The liver converts [13C]methacetin to paracetamol and [13C]-labelled CO_2, which is detected in the breath. B) Physiological based pharmacokinetics model for the description of MBT and LiMAx liver function tests. The model includes the absorption, distribution, metabolization and elimination of [13C]methacetin, [13C]bicarbonate, [13C]CO_2, and paracetamol. Application routes are shown in orange, routes of elimination in green. C) Overview of metabolic model included in the liver. Metabolism of methacetin and paracetamol is modeled via two reactions: i) conversion of [13C]methacetin to paracetamol and [13C]CO_2 via CYP1A2 and ii) paracetamol detoxification reaction (APAPD).

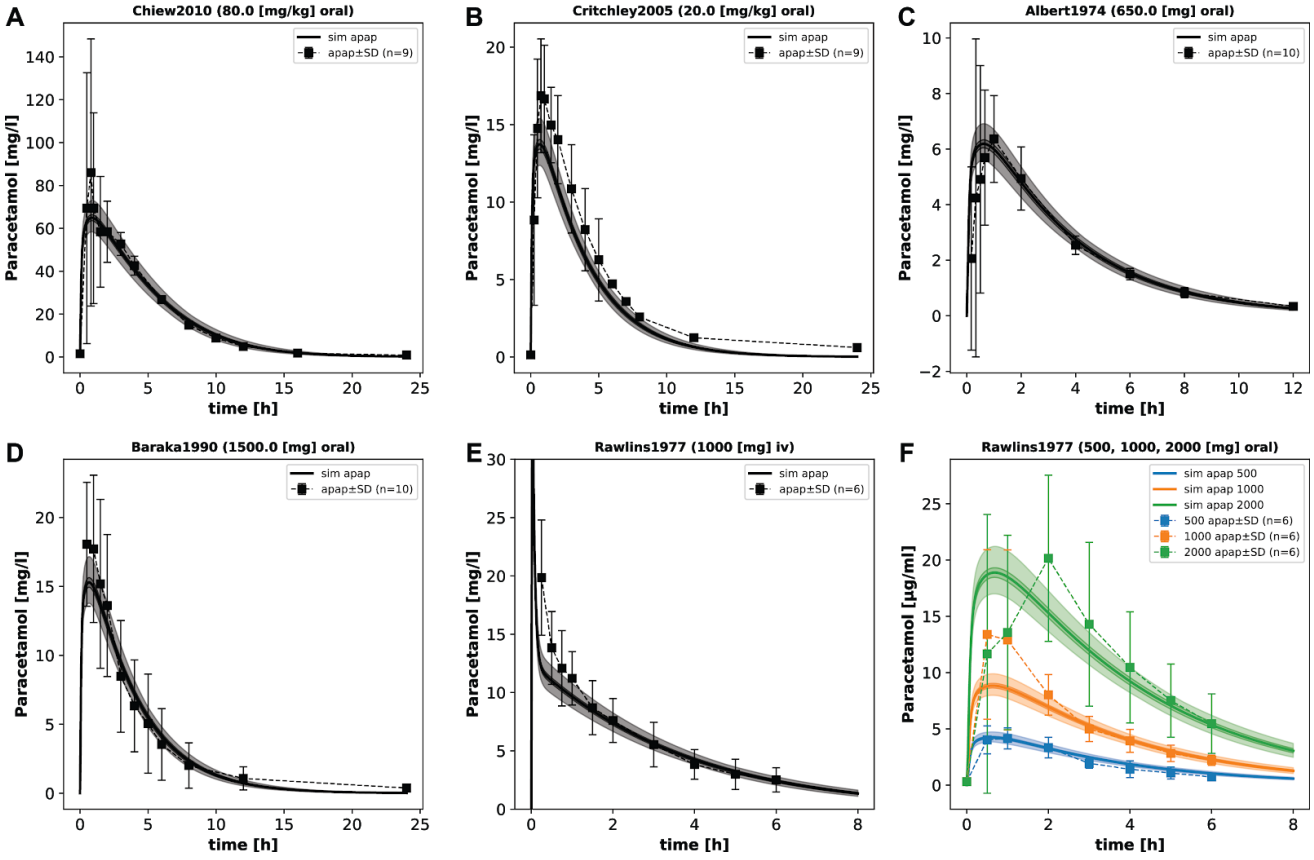


Figure 2 - Paracetamol kinetics. Paracetamol clearance after oral and intravenous application of different doses. Depicted are the venous paracetamol concentrations in [mg/l] over time. Respective studies, doses, application route, and number of subjects are stated in figure (A {Chiew2010}, B {Critchley2005}, C {Albert1974}, D {Baraka1990}, E {Rawlins1977}, F {Rawlins1977}). All experimental data is depicted as mean+-SD. Simulation curves are mean simulation curves, standard deviation of +-10% parameter changes (dark shaded area) and minimum and maximum of +-10% parameter changes (light shaded area). Details and references for experimental data is provided in Table 1.

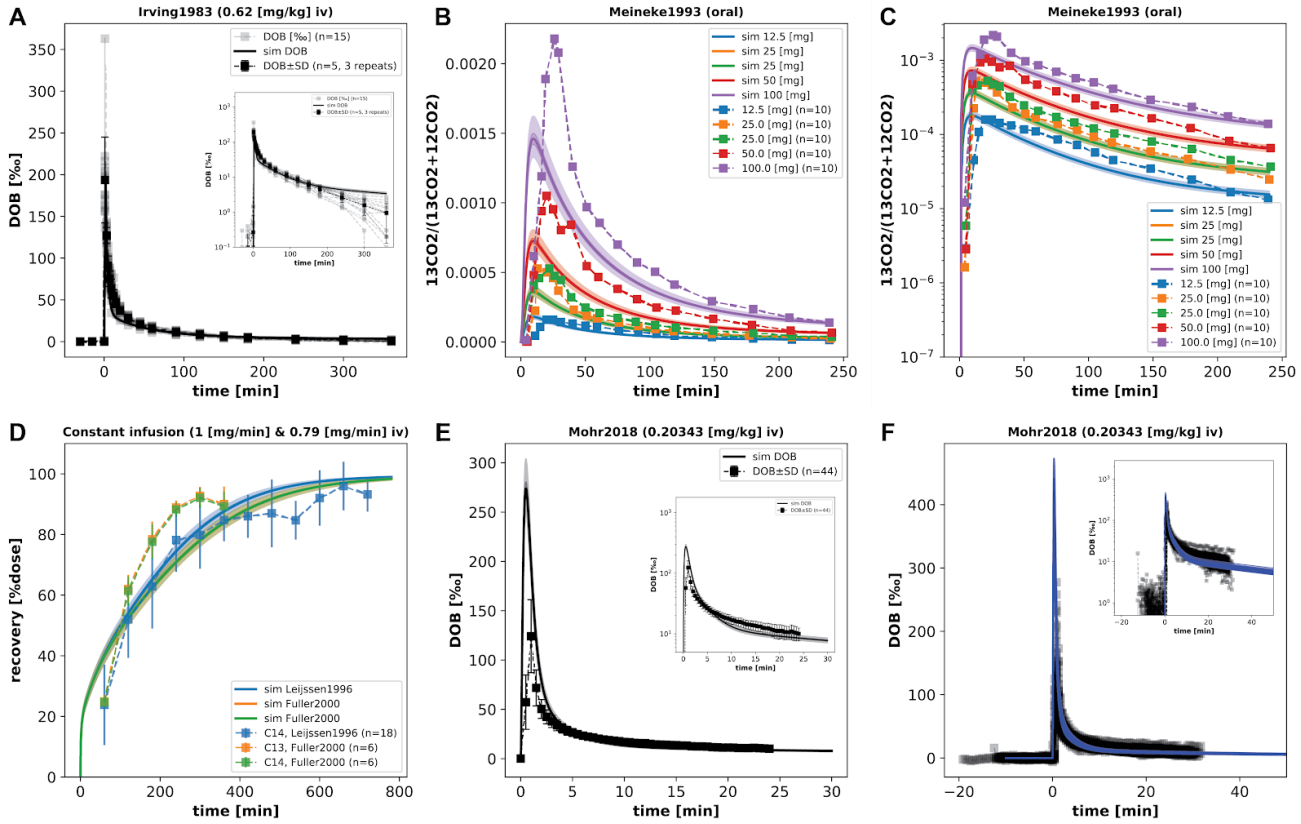


Figure 3 - Bicarbonate kinetics. Bicarbonate exhalation after oral and intravenous application of different doses of [13C]bicarbonate or [14C]bicarbonate. Respective studies, doses, application route, and number of subjects are stated in figure (A {Irving1983}, B {Meineke1993}, C {Meineke1993}, D {Leijssen1996; Fuller2000}, E {Mohr2018}, F {Mohr2018}). All experimental data is depicted as mean+-SD. Simulation curves are mean simulation curves, standard deviation of +-10% parameter changes (dark shaded area) and minimum and maximum of +-10% parameter changes (light shaded area). Details and references for experimental data is provided in Table 1.

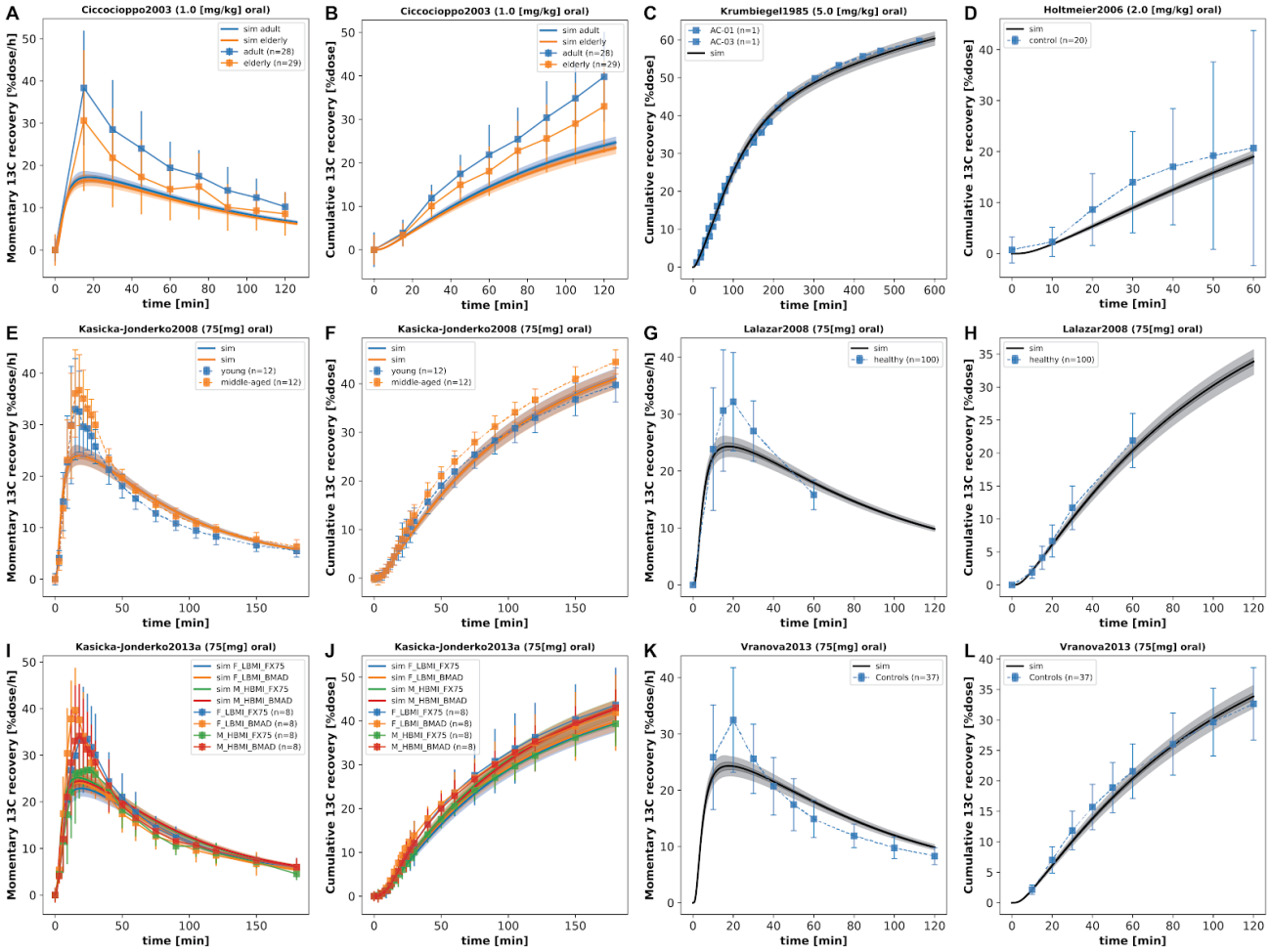


Figure 4 - Methacetin breath test (MBT). Methacetin breath tests after oral and intravenous application of different doses of [13C]methacetin. Respective studies, doses and application route are stated in the title, number of subjects in the figure legend (A {Ciccocioppo2003}, B {Ciccocioppo2003}, C {Krumbiegel1985}, D {Holtmeier2006}, E {Kasicka-Jonderko2008}, F {Kasicka-Jonderko2008}, G {Lalazar2008}, H {Lalazar2008}, I {Kasicka-Jonderko2013a}, J {Kasicka-Jonderko2013a}, K {Vranova2013}, L {Vranova2013}). All experimental data is depicted as mean+-SD. Simulation curves are mean simulation curves, standard deviation of +-10% parameter changes (dark shaded area) and minimum and maximum of +-10% parameter changes (light shaded area). Details and references for experimental data is provided in Table 1.

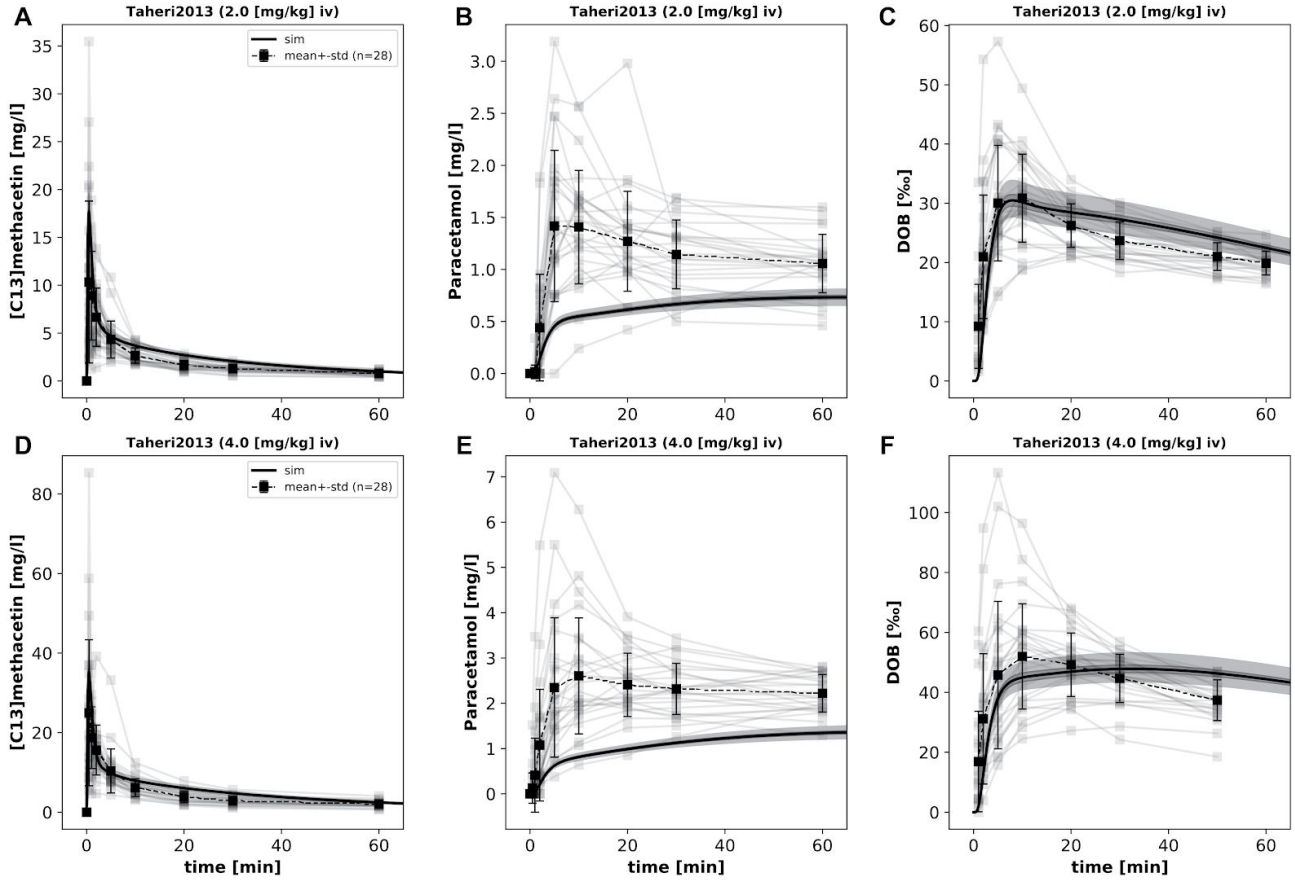
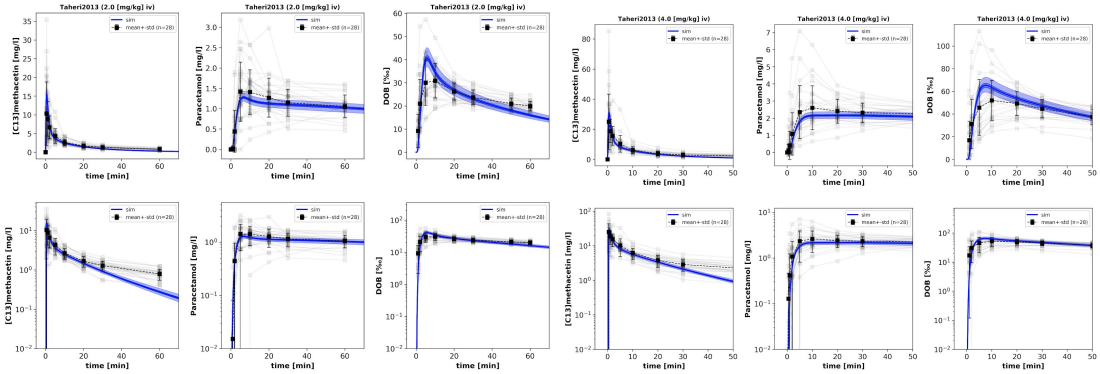


Figure 5 - LiMax. Predicted time courses compared with model validation (bicarbonate kinetics in LiMAx tests and methacetin, paracetamol and DOB predictions). LiMAx simulations for healthy subjects (DOB and recoveries, also long term recoveries). Prediction of model validation based on independent LiMAX test data under different concentrations.



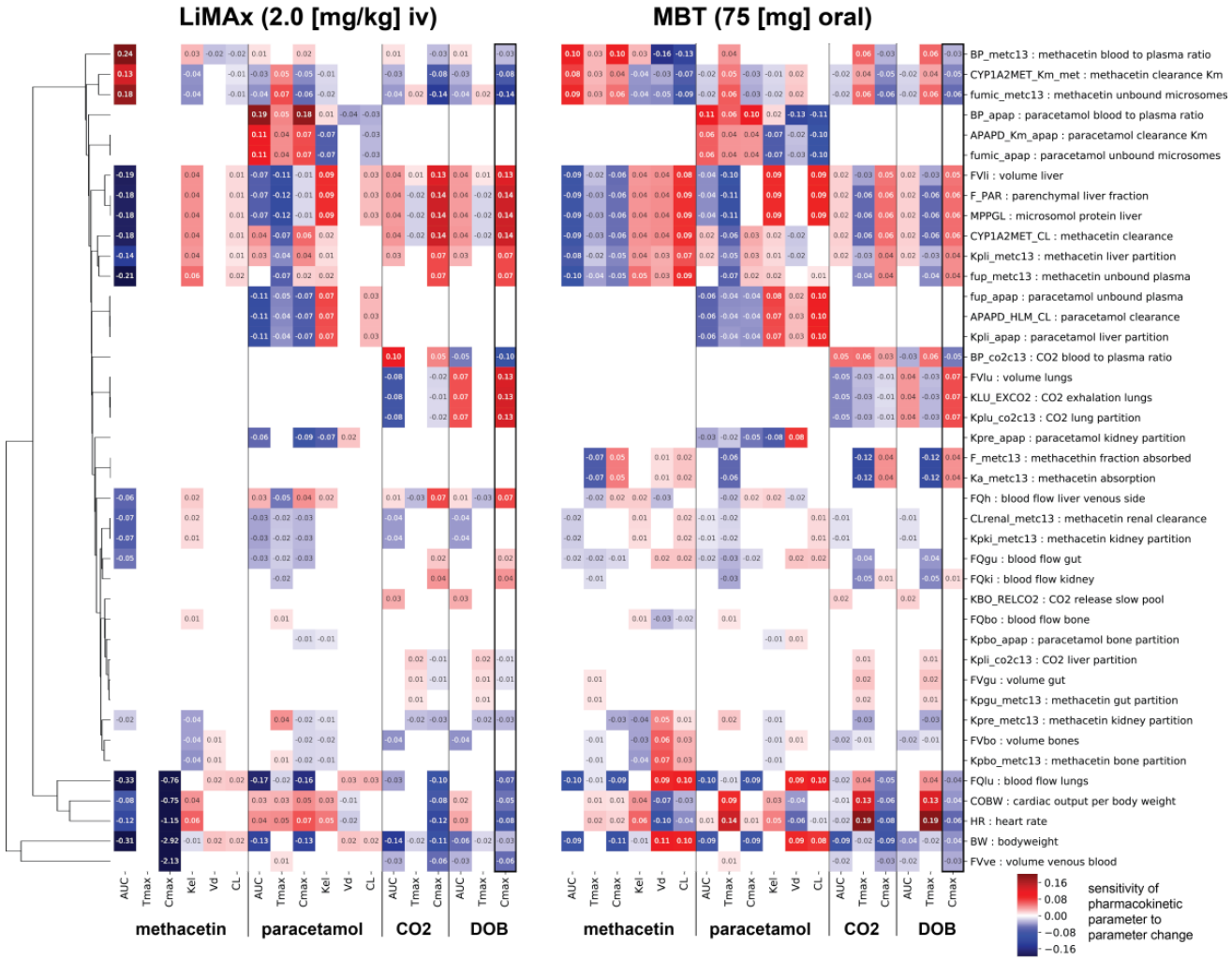


Figure 6. Sensitivity analysis of pharmacokinetic parameters. Effect of changes in model parameters (rows) on pharmacokinetic parameters (columns) of methacetin, paracetamol, CO_2 and DOB curves for LiMAx (left) and MBT (right). The following pharmacokinetic parameters were analysed: Area under the curve (AUC), Time to peak (Tmax), peak value (Cmax), elimination rate constant (Kel) and volume of distribution (Vd) and clearance (CL). For paracetamol kel and Vd were calculated using the respective dose of ^{13}C-methacetin. Positive effects marked in red, negative effects in blue linear colormap ranging from -0.2 to 0.2. Only sensitivities larger ≥0.01 are shown. Model parameters which resulted in changes <0.01 for all pharmacokinetic parameters are not displayed. The complete set of numerical values are provided in Supplementary Material S3. The maximum value of the DOB curve, used for the evaluation LiMAx and MBT is highlighted. Rows are ordered based on hierarchical clustering of parameters (MBT and LiMAx combined) using Nearest Point Algorithm (single) with euclidian metric (without Z scores).

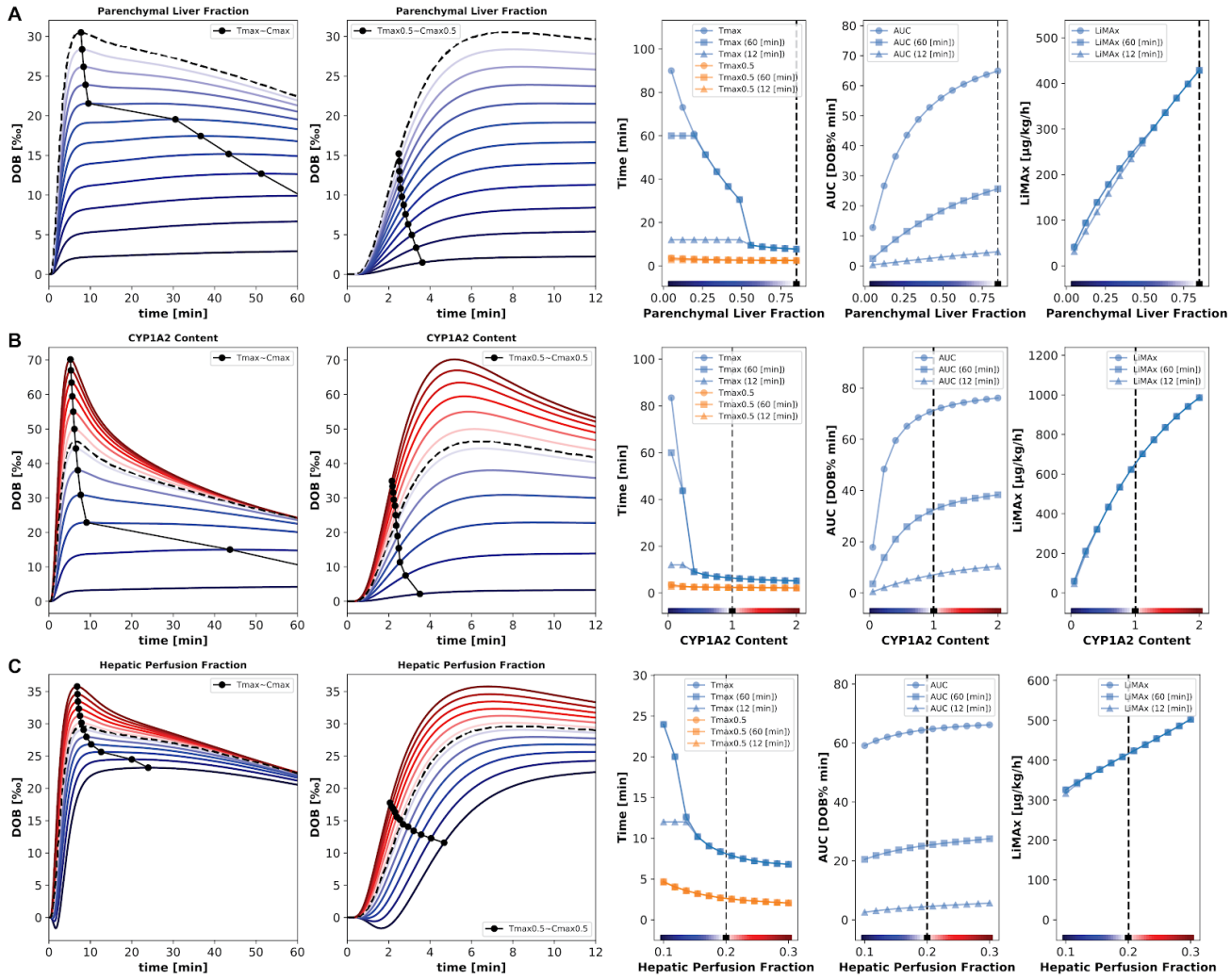
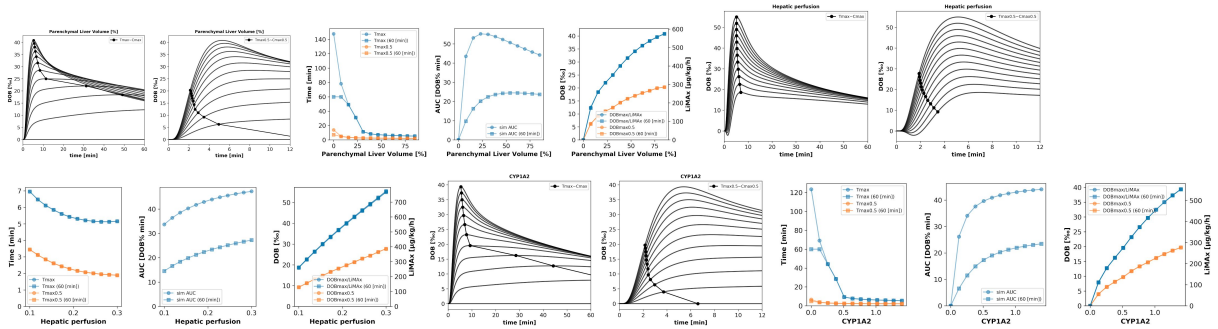


Figure 7. Key factors affecting LiMAx.

A) Effect of reduced parenchymal liver fraction.

B) Effect of CYP1A2 content/activity. C) Effect of hepatic perfusion.

Analysis via reduction in actual clearance of the liver with subsequent classification in classes analogue to {Rubin2017}.



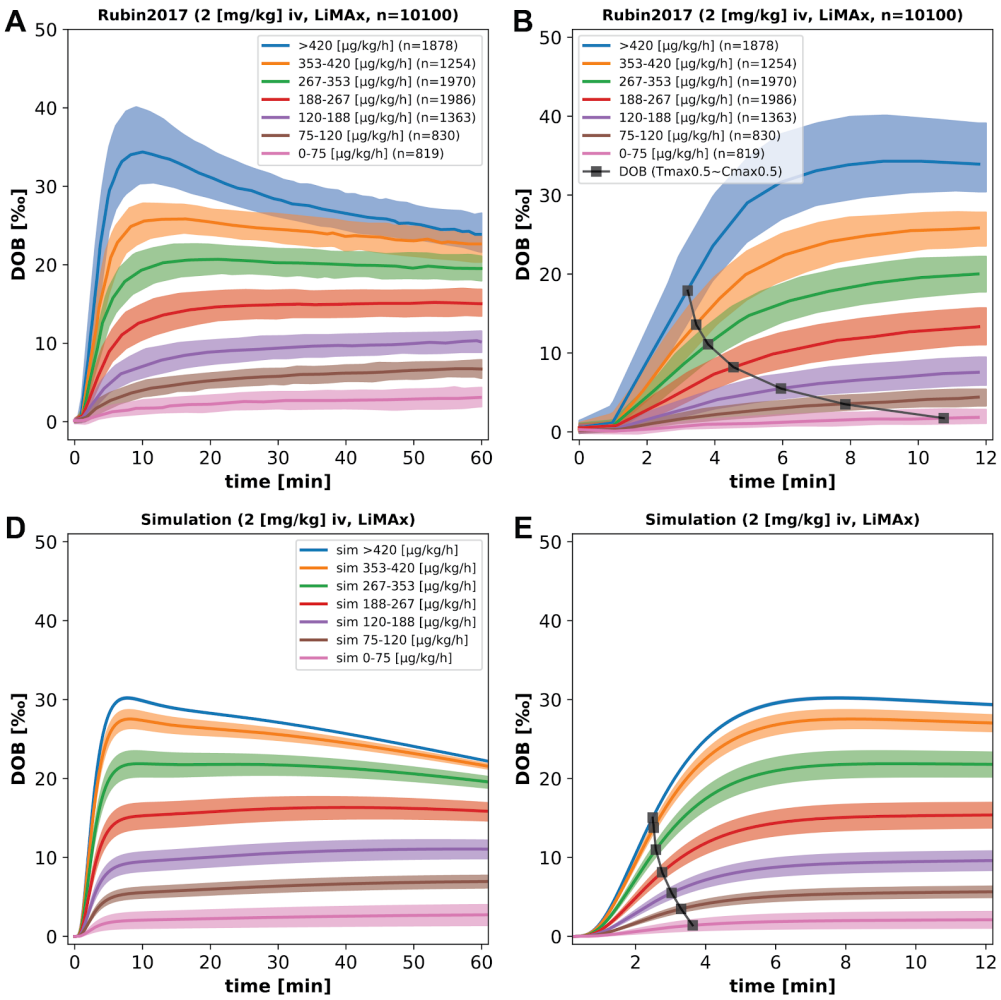
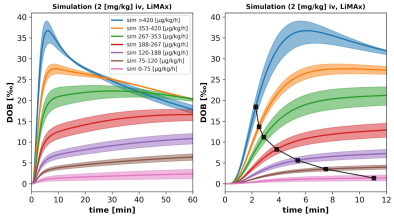


Figure 8. Prediction of LiMAx in liver disease. Clinical data in A and B, corresponding model simulations in B and C. Time courses on the left) Kinetics of LiMAx tests were assigned to groups given in the legend with median and quartiles are displayed (see {Rubin2017} for details). For the simulations only reduction in parenchymal liver fraction (F_PAR) was assumed and 500 simulations performed varying F_PAR in [1E-5, 0.85]. Curves were classified to the respective groups based on LiMAx values. Medians and quartiles are plotted analogue to experimental data.



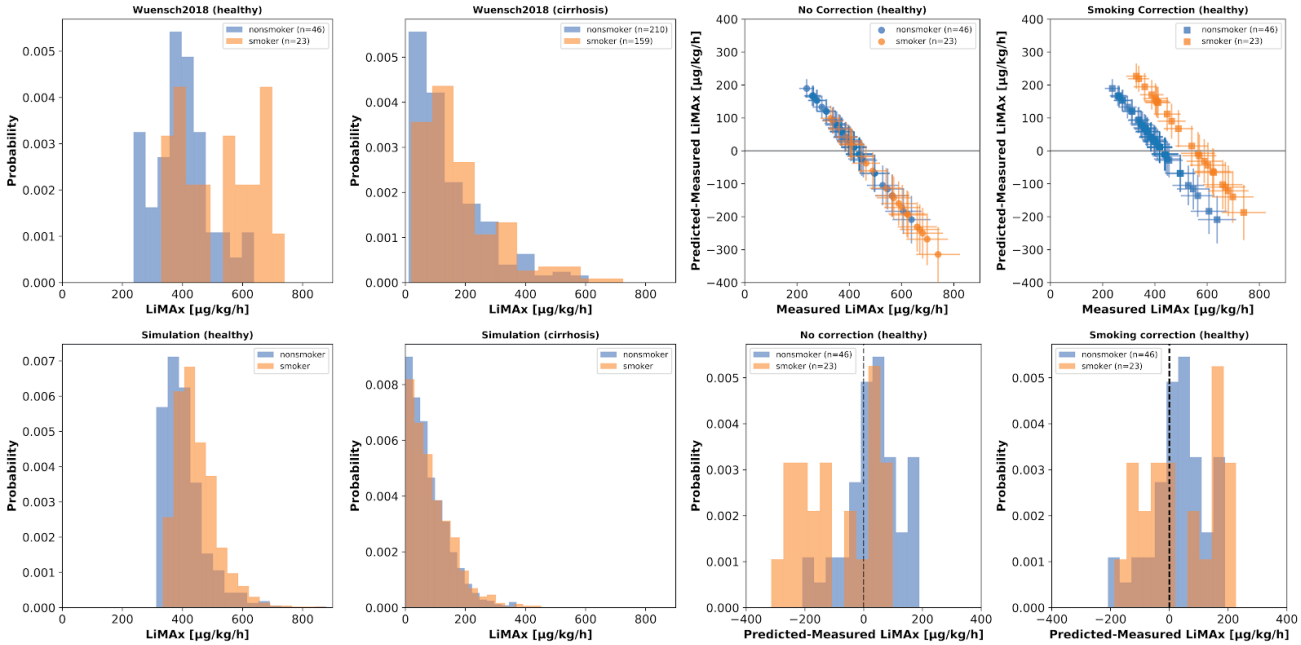
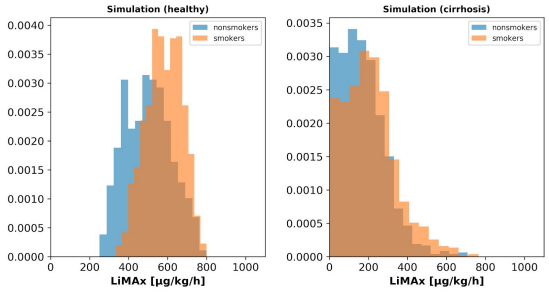


Figure 8. Prediction of LiMAx in smoking. Clinical data depicted in A and B, corresponding model simulations in B and C. Prediction of distribution of LiMAx results in smokers and nonsmokers, healthy and cirrhosis. CYP1A2 content was assumed lognormal distributed with distribution shifted to higher values in smokers (see methods for details).



SUPPLEMENTARY FIGURES

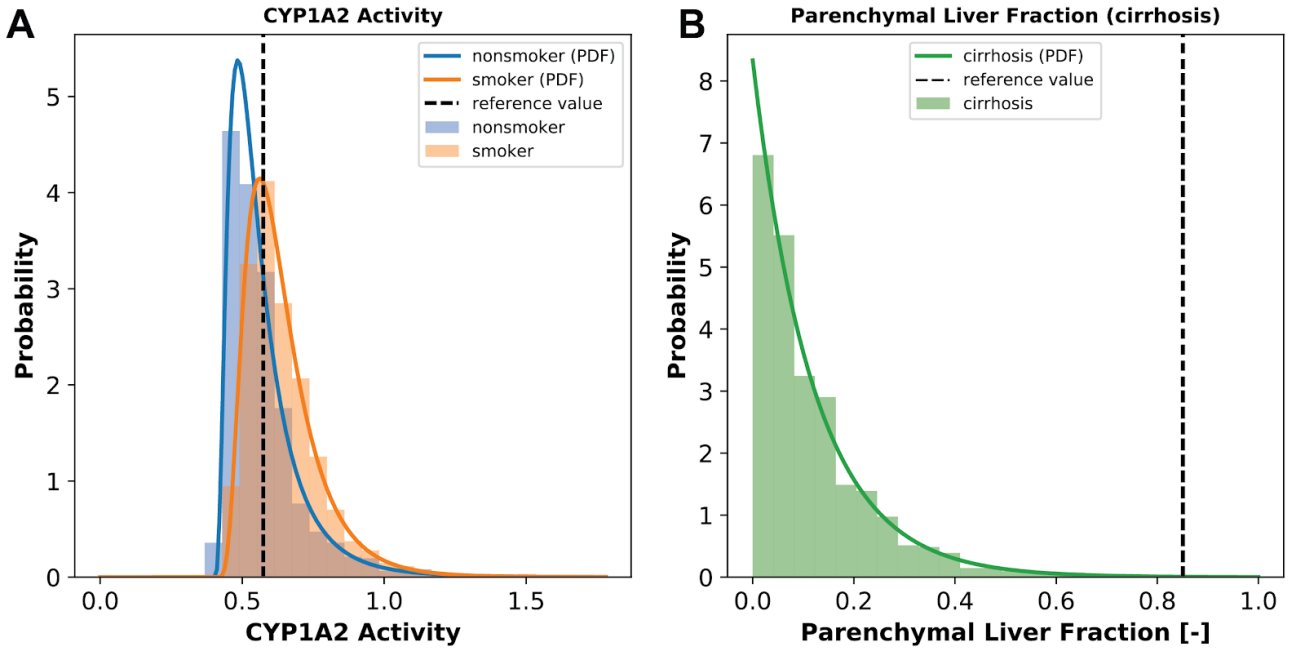
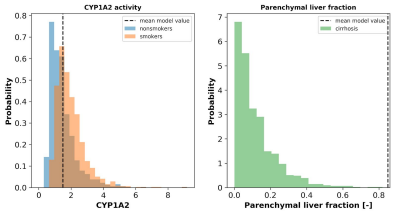


Figure S1 - CYP1A2 distributions and parenchymal liver fraction in cirrhosis. A) Distribution of CYP1A2 in healthy non-smokers (blue) and smokers (orange) based on n=1000 samples from distribution. Dashed line shows the mean reference parameter for CYP1A2. The CYP1A2 distribution is shifted to higher values in smokers {REF}. B) Distribution of parenchymal liver fraction in cirrhotic patients based on n=1000 samples from distribution. Dashed line shows the mean reference parameter for parenchymal liver fraction in healthy subjects (0.85).



TABLES

id	Study by authors	Subjects (n)	Substance	Dose	Application	Reference
Albert1974		10 (soft gelatine) 10 (tablet)	paracetamol	650 [mg]	oral, bolus	{Albert1974}
Baraka1990		10	paracetamol	1500 [mg]	oral, bolus	{Baraka1990}
Chiew2010		9	paracetamol	80 [mg/kg]	oral, bolus	{Chiew2010}
Critchley2005		9	paracetamol	20 [mg/kg]	oral, bolus	{Critchley2005}
Rawlins1977		6	paracetamol	500, 1000, 2000 [mg]	oral, bolus	{Rawlins1977}
Fuller2000		6	[13C]bicarbonate, [14C]bicarbonate	0.79 [mg/min]	IV, constant infusion	{Fuller2000}
Irving1993		5 (3 repeats)	[13C]bicarbonate	0.62 [mg/kg]	IV, bolus	{Irving1993}
Leijssen1996		18	[14C]bicarbonate	1 [mg/min]	IV, constant infusion	{Leijssen1996}
Meineke1993		10	[13C]bicarbonate	12.5, 25, 50, 100 [mg]	oral, bolus	{Meineke1993}
Mohr2018	✓	44	[13C]bicarbonate	0.2 [mg/kg]	IV, bolus	
Ciccocioppo2003		28 (adult) 29 (elderly)	[13C]methacetin	1.0 [mg/kg]	oral, bolus	{Ciccocioppo2003}
Holtmeier2006		20 (control)	[13C]methacetin	2.0 [mg/kg]	oral, bolus	{Holtmeier2006}
Kasicka-Jonderko2011a		12	[13C]methacetin	75 [mg]	oral, bolus	{Kasicka-Jonderko2011a}
Kasicka-Jonderko2011		12	[13C]methacetin	75 [mg]	oral, bolus	{Kasicka-Jonderko2011}
Kasicka-Jonderko2008		12	[13C]methacetin	75 [mg]	oral, bolus	{Kasicka-Jonderko2008}
Kasicka-Jonderko2013a		8 (LBMI) 8 (HBMI)	[13C]methacetin	75 [mg], 1 [mg/kg]	oral, bolus	{Kasicka-Jonderko2013a}
Lalazar2008		100 (healthy)	[13C]methacetin	75 [mg]	oral, bolus	{Lalazar2008}
Krumbiegel1985		2 (healthy)	[13C]methacetin	5.0 [mg/kg]	oral, bolus	{Krumbiegel1985}
Vranova2013		37 (healthy)	[13C]methacetin	75 [mg]	oral, bolus	{Vranova2013}
Rubens2017		10100 (LiMAx Tests)	[13C]methacetin (LiMAx)	2 [mg/kg]	iv, bolus	{Rubens2017}
Taheri2017	✓		[13C]methacetin (LiMAx)	2, 4 [mg/kg]	iv, bolus	{Taheri2017}
Wuensch2018	✓		[13C]methacetin (LiMAx)	2 [mg/kg]	iv, bolus	

Table 1 - Overview clinical studies. Simulations were run with the respective dosing and applications.