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Predicting stereo-selective disposition of carvedilol in adult and pediatric chronic heart failure patients by incorporating pathophysiological changes in organ blood flows–A physiologically based pharmacokinetic approach

Muhammad Fawad Rasool, Feras Khalil and Stephanie Läer

Department of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine University, 40225 Düsseldorf, Germany, (M.F.R, F.K., S.L.)

Faculty of Pharmacy, Bahauddin Zakariya University 60800 Multan, Pakistan, (M.F.R.)

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Running Title: Predicting stereo-selective PK of carvedilol–A PBPK approach

Corresponding author and contact information:

Muhammad Fawad Rasool[1,2]

Telephone: +49 211 8113840

Fax: +49 211 8110741

E-mail: fawadrasool@bzu.edu.pk; fawad.rasool@uni-duesseldorf.de

[1]Department of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine University, 40225 Düsseldorf, Germany

[2]Faculty of Pharmacy, Bahauddin Zakariya University 60800 Multan, Pakistan

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List of abbreviations:

CHF, chronic heart failure; PK, pharmacokinetics; PD, pharmacodynamcs; PBPK, physiologically based pharmacokinetics; NYHA, New York Heart Association; EM, extensive metabolizer; PM, poor metabolizer; ADME, absorption, distribution, metabolism and eliminition; ADAM, advanced, dissolution, absorption and metabolism; $P_{eff,man}$, human jejunum permeability, P_{app}, apparent permeability, f_a, fraction

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absorbed; F_g, fraction of drug escaping metabolism in the gastrointestinal tract; F_h, fraction of drug escaping the hepatic metabolism; CL_H, hepatic clearance; Q_H, hepatic blood flow; $f_{u,Gut}$, unbound fraction of the drug in the enterocyte; $CL_{int,Gut}$, intrinsic clearance in the gut; Q_{villi}, villous blood flow; CL_{perm}, permeability clearance; Q_{Gut}, a hybrid term predicted by using villous blood flow and permeability clearance; WHO, the World Health Organization; NCA, non-compartmental analysis; AUC_{last}, area under the systemic drug concentration-time curve from time 0 to the last measured concentration; $AUC_{0-\infty}$ area under the systemic drug concentration-time curve from time 0 to infinity; C_{max}, maximal systemic drug concentration; iv, intravenous; CL_{iv}, clearance after iv application; CL/F, clearance after the oral application; V_{ss}, steady state volume of distribution; BCS, biopharmaceutics classification system; $ratio_{(Obs/Pred)}$, ratio of observed/predicted pharmacokinetic parameter; SA, sensitivity analysis.

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Abstract

Chronic heart failure (CHF) is a systemic low perfusion syndrome resulting from impairment in the pumping function of the heart. The decrease in blood supply to body organs can potentially affect the pharmacokinetics of the drugs being administered. Carvedilol is administered as a racemic mixture and undergoes extensive stereo-selective first pass metabolism. For such a drug, the pathophysiological changes occuring in CHF can have a profound impact on the pharmacokinetics (PK), and thus the resulting pharmacodynamic response, of both enantiomers. The aim of the current work was to predict stereo-selective disposition of carvedilol after incorporating the pathophysiological changes in CHF into a whole body physiologically based pharmacokinetic model using Simcyp®, and to scale that model to pediatric CHF patients on physiological basis to investigate if the same changes in the adult model can also be adopted for children. The developed model has successfully described PK of carvedilol enantiomers in healthy adults and in patients after the incorporation of reduced organ blood flows as seen by the visual predictive checks and the calculated ratios$_{(observed/predicted)}$ for all PK-parameters of interest. In contrast to adults, pediatric patients up to 12 years of age were better described without the reductions in organ blood flow, whereas older pediatric patients were better described after incorporating organ blood flow reductions. These findings indicate that the incorporated blood flow reductions in the adult model cannot be directly adopted in pediatrics, at least for the young ones; however, in order to draw definite conclusions, more data is still needed.

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Introduction

Chronic heart failure (CHF) is a systemic low perfusion syndrome resulting from impairment in the pumping function of the heart, leading to a decrease in the blood supply to various body organs and having a potential to affect the pharmacokinetics (PK) of administered drugs (Ogawa et al., 2013; Yancy et al., 2013). In CHF, the reduced blood flows to the gastrointestinal tract, the peripheral tissues, as well as to the liver and the kidneys can affect the drug absorption, distribution, metabolism and elimination (ADME) (Berkowitz et al., 1963; Zelis et al., 1975; Sica, 2003; Ogawa et al., 2013). These reductions in the organ blood flows are associated with the severity of disease (Leithe et al., 1984). The New York Heart Association functional classification of heart failure (NYHA class) is used for categorizing heart failure patients with respect to severity of disease, starting from compensated, mild (NYHA I) to decompensated, severe (NYHA IV) chronic heart failure (CHF) (Criteria Cimmittee NYHA, 1994). In compensated HF, there is no significant impact on PK of the administered drugs, whereas ~50 % reduction in clearance (CL) of drugs has been observed in decompensated CHF (NYHA III and IV) (Ogawa et al., 2013). The organ blood flow reductions in heart failure can be correlated with NYHA class of the patients and hence can be used to understand and predict the pharmacokinetics (PK) of drugs being administered in patients with heart failure.

Racemic drugs are composed of enantiomers that can differ greatly in their PK and pharmacodynamic (PD) properties (Birkett, 1989). The PK differences between the enantiomers are mainly due to differences in absorption and disposition which can lead to variations in their systemic concentrations and hence can influence the concentration-effect relationship (Tucker and Lennard, 1990). Since, some of the enantiomers show stereo-selective disposition, any pathophysiological condition that can affect their clearance can have a profound impact on their exposure and efficacy. Carvedilol is a racemic mixture of two enantiomers, with S-enantiomer having both α_1-receptor blocking and β-adrenoreceptor blocking activities while R-enantiomer is more selective towards α_1-receptor blocking activity (Neugebauer et al., 1990). Both enantiomers undergo extensive stereo-selective first pass

metabolism through CYP enzymes (CYP2D6, CYP1A2, CYP2C9, CYP3A4 and CYP2E1) and UGT enzymes (UGT1A1, UGT2B4 and UGT2B7) (Oldham and Clarke, 1997; Ohno et al., 2004; Takekuma et al., 2012), with reported absolute bioavailability (*F*) of 31.1 % for R-carvedilol and 15.1 % for S-carvedilol (Neugebauer et al., 1990). Because, CYP2D6 is the main metabolic enzyme that is involved in the metabolism of both enantiomers and is more selective towards the overall disposition of R-carvedilol, the decrease activity of this enzyme in poor metabolizers (PM's) may result in higher systemic concentration of R-carvedilol and hence an increase in α-blockade, which can cause acute blood pressure reduction and increased incidence of orthostatic hypotension in comparison with extensive metabolizers (EM's) of CYP2D6 (Zhou and Wood, 1995). Since, carvedilol is used in the management of CHF and it undergoes extensive stereo-selective first pass metabolism, the organ blood flow reductions occurring in CHF can significantly affect its ADME.

A physiologically based pharmacokinetic (PBPK) model incorporating reduced hepatic and renal blood flows has been used previously to predict PK of racemic carvedilol in adult and pediatric CHF patients (Rasool et al., 2015). However, the reductions in blood flow to limbs, adipose, skin and muscle tissues, which can additionally affect the drug distribution and hence the plasma concentration of the drug, were not yet incorporated in the previously reported carvedilol-CHF model (Rasool et al., 2015). Keeping in mind that carvedilol is administered as a racemic mixture of R and S enantiomers, which have ~2-fold difference in their F, the organ blood flow reductions occurring in CHF can affect the disposition of both in a stereo-selective fashion. The differences in the exposures of R and S carvedilol will influence the expected PD response and may potentially lead to adverse drug reactions. A PBPK model that incorporates all the reported relevant blood flow reductions occurring in CHF can be used to predict stereo-selective disposition of carvedilol in CHF patients. Furthermore, a developed and evaluated PBPK model with clinical data in adult CHF patients can be scaled to pediatrics on physiological basis by using a population based ADME simulator.

The main objective of this work was to develop a PBPK drug-disease model capable of predicting stereo-selective disposition of carvedilol in CHF patients after incorporating the relevant organ/tissue blood flow changes and to evaluate it with the available clinical data in adult and pediatric CHF patients.

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Materials and Methods

Modelling platform

The population based PBPK simulator, Simcyp® version 14.1 (Simcyp Ltd, Sheffield, UK) was used in developing a whole body PBPK model.

Modelling strategy

A PBPK model was developed by adopting a systematic model building strategy (Khalil and Laer, 2014), starting with the literature search for screening of drug specific input parameters and clinical pharmacokinetic data to be used in model development. This was followed by incorporation of these data into the simulator and selection of system parameters for running predictions in virtual populations and the final evaluation of the developed model with the comparison of predicted results with the observed clinical trial data. To avoid the complexity associated with the oral drug absorption, initially predictions were performed after iv drug application and all the drug-specific parameters that can potentially influence drug disposition such as, in-vivo clearance and contributions of various metabolic enzymes (CYP's and UGT's) were optimized. After successful evaluation of the iv predictions with the observed data, the previously selected parameters are kept constant and other additional drug-specific parameters which can affect drug absorption process, such as permeability and fraction unbound of the drug in the enterocyte are selected or optimized. Amongst the seven PK data sets (2 iv and 5 oral) in healthy adults, three data sets (1 iv and 2 oral) were used for model building and remaining data sets were used for model verification and all the data sets were used for model evaluation. After evaluation of developed model in healthy adults, pathophysiological changes in organ blood flows occurring in CHF were incorporated to predict ADME of carvedilol enantiomers in adult CHF patients. After successful evaluation of developed CHF model with the observed data, it was scaled to pediatrics on physiological basis by using the pediatric module of Simcyp®.In order to see impact of reduced organ blood flows on the model

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predictions in pediatric CHF patients, simulations were performed in duplicate i.e., with and without incorporating reductions in organ blood flows.

All the predictions were performed by creating a virtual population with same demographics as in the original trial by keeping the age range, proportion of females, fluid intake, fasting/fed states and where applicable same genotypic frequencies. In adults, all the predictions were performed by creating a virtual population of 100 individuals for every PK data set. In pediatrics, the initial simulations were performed in the entire age range including the young adult, without stratifying them in different age groups, by creating a virtual population of 1000 individuals within the age range of 0.12–19.3 years. This was followed by simulating pediatric patients in different age groups by creating a virtual population of 100 individuals for every age group. The workflow for the development of PBPK model can be seen in Figure 1.

PBPK Model parameterization

After undergoing through an extensive literature search, relevant in-vivo and in-vitro drug and population-specific data was selected for completing the model parameterization. The final model input parameters are summarized in Table 1. The detailed parameterization of various drug and disease-specific components used in the final PBPK model is given below.

Absorption

In order to predict oral drug absorption, the advanced, dissolution, absorption and metabolism (ADAM) model was used (Jamei et al., 2009). The human jejunum permeability ($P_{eff,man}$) of R-carvedilol was predicted using in vitro Caco-2 permeability (P_{app}) input data after calibrating it with reference value of atenolol within Simcyp® (Tian et al., 2012). For S-carvedilol, the $P_{eff,man}$ was optimized and adjusted manually after sensitivity analysis to get a good visual fit with the observed clinical data. The model $P_{eff,man}$ values R for S-carvedilol were 3.9×10^{-4} (cm/s) and 1.6×10^{-4} (cm/s) respectively. The predicted absorbed fractions (f_a) of R and S-carvedilol were 0.98 and 0.85 respectively, which are in accordance

with carvedilol having a high permeability and belonging to Biopharmaceutics Classification System (BCS) class II. Additionally, the unbound fractions of R and S-carvedilol within the enterocytes ($f_{u,Gut}$) were predicted using Simcyp®. Although, some reports suggest a possible role of P-glycoprotein (P-gp) in carvedilol disposition (Kaijser et al., 1997; Giessmann et al., 2004), but active transport process is considered to be significant only when carvedilol is given concomitantly with other P-gp substrates (Aiba et al., 2005). Furthermore, carvedilol is considered to be a strong inhibitor and not a good substrate to P-gp (Wessler et al., 2013), taking this information into consideration, no active transport data was incorporated in the developed PBPK model.

Distribution

A perfusion limited whole body full PBPK model was used for predicting enantiomeric distribution of carvedilol. The volumes of distribution at steady state (V_{ss}) and the tissue to plasma partition coefficients (K_p) for R and S-carvedilol were predicted by using Poulin and Theil method with the Bierezhkovskiy correction (Berezhkovskiy, 2004).

Elimination

Due to absence of relevant metabolic enzyme specific data which can support and predict the reported enantiomer specific carvedilol clearances, the intrinsic clearances of metabolic enzymes involved in R and S-carvedilol clearance were back calculated from their respective iv clearances (CL_{iv}) using the retrograde model for enzyme kinetics in Simcyp® (Neugebauer et al., 1990; Cubitt et al., 2011; Salem et al., 2014). In order to calculate the total hepatic intrinsic clearance (CL_{int}), the adult CL_{iv}, known fractions of hepatic and renal clearance, the fraction of unbound drug (f_u), the blood to plasma drug ratio and the hepatic blood flow were used as input parameters. The predicted hepatic CL_{int} was further divided and assigned to different CYP-enzymes, on the basis of available evidence regarding fractional contributions of these enzymes. The CL_{int} not being assigned to any CYP-enzyme was used as additional drug clearance in the program. The hepatic intrinsic clearance was predicted using the well-stirred liver model:

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$$CL_{int} = \frac{Q_H \times CL_H}{fu_B \times (Q_H - CL_H)} \quad (1)$$

The fractional contributions of CYP-enzymes involved in metabolism of R and S-carvedilol were obtained from available evidences in the published reports (Oldham and Clarke, 1997; Giessmann et al., 2004; Sehrt et al., 2011). It is stated that CYP2D6 is the major metabolic enzyme involved in carvedilol clearance with some minor contributions from CYP1A2, CYP2C9, CYP2E1, and CYP3A4. The 74 % of total R-carvedilol clearance is dependent on CYP2D6 while other CYP-enzymes have a minor role in its disposition, while 50 % of total S-carvedilol clearance is attributed to CYP2D6 and other metabolic enzymes may have an important role in its overall disposition (Zhou and Wood, 1995; Oldham and Clarke, 1997; Sehrt et al., 2011). In healthy adults, glucuronidation accounts for 20–23 % of total carvedilol clearance (Neugebauer and Neubert, 1991) and three UGT isoforms, UGT1A1, UGT2B4 and UGT2B7 are involved in its metabolism (Ohno et al., 2004). The contributions of UGT1A1, UGT2B4 and UGT2B7 are reported to be around 30 %, 25–40 %, and 30–45 % for R-carvedilol and 12–20 %, 15–26 % and 60–65 % for S-carvedilol respectively (Takekuma et al., 2012).

Taking into account the above mentioned information, 80 % of total carvedilol clearance was assigned to the CYP-enzymes (R-carvedilol: 74 % CYP2D6, 2 % CYP1A2, 2 % CYP2C9, 1 % CYP3A4 and 1 % CYP2E1 and S-carvedilol: 50 % CYP2D6, 10 % CYP1A2, 10 % CYP2C9, 5 % CYP3A4 and 5 % CYP2E1) using retrograde model and remaining 20 % was assigned to UGT-enzymes, which was predicted as additional clearance in the program. The UGT-enzyme contributions were optimized manually to achieve good agreement with the observed clinical data. The final values of different clearance parameters used in the developed PBPK model are shown in Table 1.

The hepatic clearance (CL_H) was predicted by using well stirred liver model using equation: 2 (Wilkinson and Shand, 1975),

$$CL_H = \frac{Q_H \times fu_B \times CLu_{H,int}}{Q_H + fu_B \times CLu_{H,int}} \quad (2)$$

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The reductions in hepatic blood flow (Q_H) occurring in CHF were incorporated into the model for predicting clearance of carvedilol enantiomers in CHF patients.

The fraction escaping the gut wall metabolism (F_G) was predicted using equation: 3,

$$F_G = \frac{Q_{Gut}}{Q_{Gut}+f_{u,Gut}\times CL_{u,int,Gut}} \quad (3)$$

Where $f_{u,Gut}$ is the unbound fraction of the drug in the enterocyte, $CL_{u,int,Gut}$ intrinsic clearance in the gut, and Q_{Gut} is a hybrid term predicted by using villous blood flow (Q_{villi}) and the permeability clearance (CL_{perm}), which is measured from the effective permeability of the compound. Q_{Gut} is calculated by using equation: 4,

$$Q_{Gut} = \frac{Q_{villi}\times CL_{perm}}{Q_{villi}+CL_{perm}} \quad (4)$$

The oral bioavailability was predicted by using equation: 5,

$$F = f_a \times F_g \times F_h \ (5)$$

Where, f_a is the fraction of drug absorbed, F_g is the fraction of drug that escapes metabolism in the gastrointestinal tract and F_h is the fraction of drug that escapes the hepatic metabolism.

Pediatric PBPK model

When the developed PBPK was able to predict ADME of both R and S-carvedilol in adult healthy and CHF patients, it was scaled to pediatrics on physiological basis using the pediatric module of Simcyp®. This module includes a wide variety of relevant age-specific physiological and anatomical parameters which facilitates the pediatric scaling of drug clearance on physiological basis. These parameters include the age related changes in, total body composition, plasma protein binding, blood volume, organ blood flows and abundance of different metabolic enzymes (Johnson and Rostami-Hodjegan, 2011). In pediatric module, the renal function is described on the basis of glomerular filtration rate (GFR), which is linked with BSA of the simulated individuals (Johnson et al., 2006). In order to simulate the oral drug absorption process in pediatrics, the β-version of the pediatric ADAM model was used with the similar input value of mean gastric emptying time as in the adult model (0.4 hour).

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In order to assess the uncertainty associated with some pediatric model input parameters, which were adopted from adult PBPK model, sensitivity analysis (SA) was performed. The details of the SA are described in the supplemental file (Supplemental Methods).

Because, all the pediatric patients included in model evaluation were diagnosed with CHF, the organ blood flow reductions were incorporated in the pediatric model to see impact of blood flow reductions on ADME of R and S-carvedilol.

Blood flow changes to different organs/tissues in heart failure

The blood flow to liver and kidney decreases with increasing severity of heart failure and it has been quantified previously (Leithe et al., 1984). The quantified fractional reduction in blood flow was, 0.76, 0.54 and 0.46 of normal hepatic flow in mild, moderate and severe CHF patients, while the reduction in renal blood flow was not linear when moving from moderate to severe CHF as the reported fractional decrease was, 0.78, 0.55 and 0.63 of normal blood flow in mild, moderate and severe CHF patients (Leithe et al., 1984). Furthermore, the changes in blood flow to limbs can affect the drug distribution, as the blood flow to the limbs also supplies skin, adipose, muscle and bone (Lee et al., 1993). The quantified fractional reduction in limb blood flow was, 0.57, 0.44 and 0.28 of normal limb blood flow in mild, moderate and severe CHF patients (Leithe et al., 1984).

The NYHA functional classification system for CHF can be directly correlated with the reported reductions in different organ blood flows, by categorizing mild CHF patient in NYHA class II, moderate CHF patient in NYHA class III and severe CHF patient in NYHA class IV (Leithe et al., 1984; NYHA, 1994). All these organ/tissue blood flow reductions were incorporated within the simulated virtual populations by decreasing the cardiac output to these organs within Simcyp®.

In CHF patients there is hepato-splanchic congestion, affecting the passive drug diffusion that results in decreased migration of drug from the intestinal lumen into systemic circulation, which is depicted as decrease in f_a of the drug (Sica, 2003). Furthermore, the gastrointestinal absorption of drugs having low solubility like carvedilol (0.01 mg/mL, BCS II) are more sensitive to CHF associated changes

occurring in gut blood flow (Ogawa et al., 2014). Since, in the developed model the reduction in blood flow to gut was accounted by reducing the Q_H (both arterial and portal) and in order to account for decrease in Q_{villi} with severity of CHF, the predicted Q_{Gut} (equation: 4) value due to its dependence on Q_{villi} was reduced in accordance with the reduction in hepatic blood flow.

Keeping in mind the reliance of NYHA functional classification system on assessment of physical activity in CHF patients and difficulty of assessing physical activity in pediatrics, NYHA functional classification of CHF is not generally used in pediatric patients and as an alternative Ross scoring method is used to assess the severity of CHF in pediatric patients (Ross et al., 1992). In Ross score system, a score of 0–2 categorizes the patient as asymptomatic, 3–6 as with mild CHF, 7–9 as with moderate CHF, and 10–12 as a patient with severe CHF (Ross et al., 1992; Laer et al., 2002). Since, there is no report of a clinical study quantifying the changes in the organ/tissue blood flow in pediatric patients, therefore, the organ blood flow reductions in pediatrics were assumed to be similar as in adult CHF patients (Leithe et al., 1984). The incorporated organ blood flow reductions with respect to severity of CHF in adults (NYHA class) and in pediatric patients (Ross score) are shown in Figure 1.

Pharmacokinetic/Clinical data

Healthy and Adult patients with CHF

MEDLINE database was searched for screening and identification of pharmacokinetic studies of R and S-carvedilol in healthy adults and CHF patients with known demographic information and reported systemic drug concentration-time profiles. As a result of the search, systemic drug concentration-time data from five different clinical studies in healthy adults (4 studies, and 36 subjects) and CHF patients (one study, 10 patients with NYHA III and 10 patients with NYHA IV, 4 PK data sets) were used in the adult model development and evaluation (Neugebauer et al., 1990; Spahn et al., 1990; Zhou and Wood, 1995; Tenero et al., 2000; Behn, 2001). These studies provided a total of 11 data sets (7 data sets in healthy and 4 data sets in CHF patients) (Table 2). Each PK data set used for model development and

evaluation represents a mean or median observed concentration-time profile after iv or oral doses of R and S-carvedilol. Amongst the data sets used, one was provided by the author (Behn, 2001) and rest were scanned from the publications' figures (Neugebauer et al., 1990; Spahn et al., 1990; Zhou and Wood, 1995; Tenero et al., 2000) using the "digitizer" tool in software OriginPro® version 9.0 (OriginLab. Northampton, MA). CYP2D6 specific genotype data was available in two clinical studies (Zhou and Wood, 1995; Behn, 2001).

Pediatric patients with CHF

One clinical PK data set, including 15 pediatric CHF patients and one young adult with known age, gender, height, weight, CYP2D6 genotype, dose, Ross score, and measured systemic drug concentration-time profiles was used (Table 3) (Behn, 2001). The age of the patients ranged from 43 days to 19.3 years (average: 6.7 years) and they received a 0.09 mg/kg dose of oral R and S-carvedilol. The pediatric patients were divided in different age groups i.e., infant (1 month–1 year), young child (2–6 year), children (6–12 year) and adolescents (12–18 year) according to guidelines set by World Health Organization (WHO).

Model evaluation

The evaluation of PBPK model was performed by visual predictive checks and comparison of observed and predicted PK parameters. The visual predictive checks were performed by overlaying the observed systemic drug concentration-time profile on the median predicted values along with the minimum/maximum, 5^{th} and 95^{th} percentiles of the predictions.

The PK parameters were compared by performing a non-compartmental analysis (NCA) for each observed PK profile and its corresponding predicted value using Phoenix WinNonLin® version 6.4 (Pharsight Corporation, CA, USA). The area under the systemic drug concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated via the linear trapezoidal rule by using best fit method with minimum of three systemic concentration vs time points for estimation of elimination rate constant (k_e).

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The maximal systemic concentration in a profile was defined as (C_{max}) and the clearance (CL for the IV application, CL/F for the oral application) was calculated by dividing the given dose by the calculated $AUC_{0-\infty}$. The results of NCA were presented as the observed/predicted ratios ($ratio_{Obs/Pred}$) of the PK parameters. Moreover, the calculated values of AUC_{last} and $AUC_{0-\infty}$ were compared in order to see if there is any significance difference that can impair the results. The $ratio_{Obs/Pred}$ for AUC_{last} and $AUC_{0-\infty}$ for all the clinical data sets were comparable (Supplemental Table 1).

Because, the developed model was used to simulate PK of R and S-carvedilol in both adult and pediatric populations and as reported in most PBPK model base studies (Johnson et al., 2006; De Buck et al., 2007; Li et al., 2012; Khalil and Laer, 2014), a 2-fold error range was used for evaluation of observed and predicted PK parameters.

Moreover, in order to identify any systemic error associated with predictions of R and S-carvedilol, population predicted vs. population observed plots with a 2-fold error range were used.

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Results

Healthy Adults

The model predictions after iv and oral application in healthy adults were in a good agreement with the observed data at all administered dosages of 12.5 mg iv and 6.4—50 mg oral racemic carvedilol (Figure 2). The ratios$_{(Obs/Pred)}$ for $AUC_{0-\infty}$, C_{max} and CL after iv and oral administration of R and S-carvedilol were within 2-fold error range (Figure 3). After iv administration, the systemic concentration of R-carvedilol was slightly higher than that of S-carvedilol which was evident from a mean R/S $AUC_{0-\infty}$ ratio of 1.2 and 1.4 for observed and predicted data, respectively. An increase in the mean observed and predicted R/S $AUC_{0-\infty}$ ratios was seen after oral administration of carvedilol as it was increased to 2.5 and 2.4 respectively, suggesting that stereo-selective disposition is more pronounced after oral administration.

The visual predictive checks in extensive and poor metabolizers (EM's and PM's) of CYP2D6 show that the model has slightly over-predicted the absorption phase (C_{max}) for S-carvedilol but for R-carvedilol, the C_{max} predictions were in agreement with the observed data (Figure 2). The ratios$_{(Obs/Pred)}$ for all the PK-parameters in EM's and PM's of CYP2D6 were within 2-fold error range (Figure 3). Furthermore, the predicted vs. observed systemic drug concentration plots after iv and oral application of R and S-carvedilol showed that the model has successfully predicted the observed data at high and low systemic drug concentrations (Supplemental Figure 3).

The predicted V_{ss} were 1.57 and 1.95 L/kg for R and S-carvedilol, which are in line with reported values (range) of 1.39–3.40 and 1.42–3.84 L/kg respectively. Additionally, the predicted bioavailability of R and S-carvedilol in healthy adults was 0.34 and 0.17 respectively, which is in agreement with the reported absolute bioavailability of these enantiomers (Table 4).

Adult CHF patients

The developed adult CHF model was successful in predicting stereo-selective disposition of R and S-carvedilol after administering steady state oral doses of racemic carvedilol (6.25–50 mg) in CHF

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patients (Figure 4). The mean ratios$_{(Obs/Pred)}$ of the PK parameters for both enantiomers were within 2-fold error range and close to unity. The mean ratios$_{(Obs/Pred)}$ for $AUC_{0-\infty}$ and CL/F were 1.2 and 0.8 for R-carvedilol and 1.1 and 0.9 for S-carvedilol (Figure 5). Moreover, the predicted vs. observed systemic drug concentration plots in CHF showed that the model has successfully predicted steady state systemic concentrations of R and S-carvedilol at all dosage levels (Supplemental Figure 3). In adult with CHF, the mean R/S $AUC_{0-\infty}$ ratios were reduced to 1.8 and 1.6 for observed and predicted data, respectively, showing a relative increase in S-carvedilol concentration in CHF patients.

A decrease in predicted bioavailability (F) of both R and S-carvedilol was seen in CHF patients, which was associated with decrease in f_a and F_h. The predicted f_a, F_h and F in adult CHF patients were reduced to 0.74, 0.19 and 0.14 for R-carvedilol and to 0.55, 0.11 and 0.05 for S-carvedilol (Table 4).

Pediatric CHF patients

The systemic concentration-time profiles of R and S-carvedilol after administering an oral dose of 0.09 mg/kg racemic carvedilol in the entire age range (0.12–19.3 years) without incorporating any pathophysiological changes show that the developed model was capable of predicting the age specific changes in systemic concentrations of both enantiomers, since, most of the observed systemic concentration-time were within predicted 5th and 95th percentiles (Supplemental Figure 4). Moreover, the age related changes occurring in CL/F of R and S-carvedilol was captured by the model, as the observed values were within the predicted CL/F range, except in two patients with age of 17.5 and 19.3 years, where the observed CL/F was lower than the predicted values (Supplemental Figure 4).

The predicted systemic concentration-time profiles and the ratios$_{(Obs/Pred)}$ of the PK parameters in different pediatric age groups after administering an oral dose of 0.09 mg/kg racemic carvedilol are shown in Figures 6 and 7. The infants, young children and children who were classified with respect to Ross score were better described without incorporating pathophysiological changes in the model, as the $AUC_{0-\infty}$ and CL/F ratios$_{(Obs/Pred)}$ were always within 2-fold error range and the results in these age groups

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are as follows: In infants, the model has slightly over-predicted systemic concentration of both enantiomers, that can be seen in the ratios$_{(Obs/Pred)}$ for C_{max} and $AUC_{0-\infty}$ which were 0.8 for R-carvedilol and 0.7 for S-carvedilol. The predictions in young children for R-carvedilol were in close agreement with the observed data but the C_{max} for S-carvedilol was over predicted in this age group and the $AUC_{0-\infty}$ ratios$_{(Obs/Pred)}$ for R and S-carvedilol were 1.2 and 1.7 respectively. In children, the predictions for both enantiomers were in agreement with the observed data and the CL/F and C_{max} ratios$_{(Obs/Pred)}$ for R and S carvedilol were, 1.1 and 1.2 respectively. (Figures 6 and 7).

Amongst the three patients (2 adolescents and 1 young adult) who were classified as adults, according to NYHA functional classification, two (17.5 and 19.3 years) were better described with incorporation of the pathophysiological changes, as in adults with CHF and are presented individually in the visual predictive checks and comparison of PK parameters (Figures 6and 7). The 17.5-year-old patient classified as NYHA class II was better described with organ blood flow reductions as the ratios$_{(Obs/Pred)}$ for $AUC_{0-\infty}$ and CL/F without reduction in organ blood flows were outside the 2-fold error range, but with incorporation of adult organ blood flow reductions they were improved and were within 2-fold error range (Figure7). The 17.8-year patient classified as NYHA class III was better described without reductions in organ blood flows. The organ blood flow reductions in 19.3-year young adult classified as NYHA class III significantly improved the predictions as the ratios$_{(Obs/Pred)}$ for CL/F and C_{max} without reductions in blood flow were, 0.6 and 3.2 for R-carvedilol and 0.3 and 4.7 for S-carvedilol and were improved to 1.7 for R-carvedilol and 1.5 and 1.4 for S-carvedilol respectively (Figure 7).

Lastly, the predicted vs. observed systemic drug concentration plots in pediatrics show that with few exceptions, particularly with R-carvedilol, where model has under-predicted the systemic concentrations, in general the model was capable of predicting the individual concentrations of both enantiomers, as most of the concentrations were within 2-fold error range (Supplemental Figure 5).

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Discussion

In the presented work, the pathophysiological organ blood flow changes occurring in CHF were incorporated into whole body PBPK model to predict stereo-selective disposition of carvedilol in CHF patients. When the developed PBPK model has successfully described PK of R and S-carvedilol in healthy adults and after incorporation of reduced organ blood flows in adult CHF patients, it was scaled to pediatric CHF patients. The scaling of adult model to children helped in exploring whether the same pathophysiological changes seen in adult CHF patients could be adopted for the pediatric CHF patients.

The model development was initiated by parameterization of various drug-specific parameters after iv application in healthy adults, which was followed by predictions of R and S-carvedilol after oral administration. The predicted bioavailability of R and S-carvedilol was in very close agreement with the reported absolute bioavailability of these enantiomers (Neugebauer et al., 1990) (Table 4). The additional success in predicting the disposition of R and S-carvedilol in EM's and PM's of CYP2D6 provided additional confidence in the CYP2D6 CL_{int} values used in the developed model, as this enzyme is the most relevant for the drug metabolism. Moreover, in comparison to R-carvedilol, the slight over-prediction of C_{max} with S-carvedilol highlights the equally important role of other cyp-enzymes involved in its metabolism, as in the developed model only 50 % clearance of S-carvedilol is attributed to CYP2D6 and remaining 30 % to other cyp-enzymes while with R-carvedilol 74 % clearance is associated with CYP2D6 and remaining 6 % is attributed to other cyp-enzymes. Therefore, suggesting that, in addition to CYP2D6-gentotype, the incorporation of genotype-specific data for other cyp-enzymes involved in clearance of S-carvedilol is equally important for predicting its ADME.

The developed model was successful in predicting the ~2-fold difference in *F* of both enantiomers (Table 4). It was seen that carvedilol undergoes extensive stereo-selective first pass metabolism which is more sensitive towards S-carvedilol. Furthermore, the resulted R/S $AUC_{0-\infty\ ratios}$ suggest that stereo-selective clearance of carvedilol is more distinct after oral administration, since the predicted R/S $AUC_{0-\infty}$ ratio in healthy adults after iv administration was 1.4 and it was increased to 2.4 after oral administration

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of carvedilol. The predicted R/S $AUC_{0-\infty}$ ratio was decreased to 1.6 in adult CHF patients after administering steady state oral application of carvedilol. This decrease in R/S $AUC_{0-\infty}$ ratio was associated with differences in CL_{int} of both enantiomers, as the reduction of Q_H in CHF resulted in a differential effect on clearance of both enantiomers. Therefore, in CHF compared to R-carvedilol there will be a relative increase in S-carvedilol systemic concentration and hence its $AUC_{0-\infty}$. This relative increase in S-carvedilol exposure is expected to expand with increased severity of disease.

The incorporation of reduced blood flows to liver and kidney in adult CHF patients resulted in decrease CL/F of R and S-carvedilol, because, both carvedilol enantiomers undergo extensive first pass metabolism (Neugebauer et al., 1990), this decrease in CL/F was primarily attributed to reduction in Q_H. The reduced Q_H lead to an increased first-pass metabolism (decrease in F_h) which in turn, resulted in reduced F of both enantiomers. The decrease in carvedilol CL/F and F was successfully predicted by the developed model in CHF patients (Figures 4, 5 and Table 4). Furthermore, the predicted f_a of both enantiomers was reduced in adult CHF patients (Table 4), which is consistent with the reports stating reduction in passive drug diffusion due to reduction in Q_{villi} in CHF (Berkowitz et al., 1963; Sica, 2003). Moreover, in CHF, the absorption of drugs with low solubility are more susceptible to changes in intestinal blood flow, therefore, for drug like carvedilol (BCS class II) having low solubility and high permeability, any change in blood flow to intestine can have an impact on its f_a (Ogawa et al., 2014). In the developed model due to absence of any clear information on the intestinal blood flow in relation to severity of CHF, intestinal blood flow was not reduced with severity of CHF, instead reduction in Q_H was used as a surrogate, therefore, to account decrease in Q_{villi} and its impact on absorption of both enantiomers, the Q_{Gut} was reduced in relation to reduction in Q_H.

The pediatric simulations showed that in contrast to the adults, the patients up to 12 years of age, all categorized with Ross scoring system, were better described without the reductions in organ blood flow. On the other hand, one from the two adolescent' patients as well as the young adult patient (17.5 and 19.3 years, all classified according to NYHA classification), were better described after incorporating organ

blood flow reductions. One of the possible reasons for such a difference may be the use of the same organ blood flow reductions in pediatric population as in adults. Since, the incorporated blood flow reductions in pediatrics simulations were based on adult values, it is likely that these values might be close to what is happening in the late adolescence but not be true for young children, as improvement in predictions with incorporation of reduced organ blood flows was only seen in old adolescents (the young adults). Moreover, the pathophysiology of CHF is different between adult and pediatric patients, with congenital heart disease being the main cause of CHF in the vast majority of pediatric patients (Hsu and Pearson, 2009). When compared with adults, children have higher frequency of heart rate (Tanaka et al., 2001; Fleming et al., 2011) and a higher drug CL due to higher percentage of liver weight in relation to body weight (Noda et al., 1997). This can lead to differences in the total impact of these changes on drug CL between both populations. In addition to that, it is not clear if the different grading system that was used is related, in any way, to this finding, as both grading systems are based on different criteria. In order to draw conclusions about the validity of this finding as well as the possible reasons for it, more data is needed specially to confirm if this difference is true. However, the presented findings indicate that the incorporated blood flow reductions in the adult model cannot be directly adopted in pediatrics, at least for the young ones.

The ontogeny of the metabolic cyp-enzymes seems to have a minor impact on the overall disposition of carvedilol enantiomers in the pediatric CHF patients that were included in the model evaluation (Behn, 2001). This is, because, all of these pediatric patients were above one month of age and the two major cyp-enzymes for carvedilol metabolism (i.e. CYP2D6 and CYP2C9) have a fast ontogeny profile, as they achieve more than ~50 % of adult activity by the age of 0.1 year (Salem et al., 2013). Nevertheless, in the developed model about 20 % of the total assigned metabolism of S-carvedilol is due to cyp-enzymes with slow enzyme-ontogeny and a later maturation time point, i.e. CYP1A2: 10 %, CYP2E1: 5 % and CYP3A4: 5 %. The latter enzymes contribute only to about 4 % in the case of R-carvedilol, i.e. CYP1A2: 2 %, CYP2E1: 1 % and CYP3A4: 1 %. As a result, the impact of the slow maturation of these enzymes will be more profound on the clearance of S rather than the R-carvedilol. Moreover, if pediatric patients

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less than one month of age would have been included, the effect of enzyme ontogeny on the predicted drug clearance would have been more pronounced.

The predicted systemic drug concentration profiles for R and S-carvedilol in different pediatric age groups have successfully captured the observed data, with few exceptions, where model has over-predicted the systemic concentrations of R and S-carvedilol, particularly in infants. These over-predictions in infants may be associated with the knowledge gaps with respect to intestinal permeability and perfusion within CHF patients of this age group, as low drug absorption in comparison to adults has been previously reported in pediatric CHF due to congenital heart defects (Nakamura et al., 1994). Since, changes in intestinal morphology, permeability and absorption are affected in adult CHF patients, the possibility of such changes in pediatric CHF patients cannot be completely ruled out (Sica, 2003; Sandek et al., 2007).

The age related changes in CL/F for R and S-carvedilol have been successfully captured by the developed PBPK model (Supplemental Figure 5). The observed CL/F values were within the predicted values, except in 17.5 and 19.3 year patients, where the observed CL/F for both enantiomers were low, which can be attributed to reduced blood supply to eliminating organs in these patients as only in these 2 patients, the predicted PK parameters were improved with incorporation of reduced organ blood flows (Figure 7). Additionally, due to the higher hepatic extraction of S-carvedilol, the impact of reduction in Q_H on its CL/F was more significant when compared to R-carvedilol. However, it seems that the role of reduced organ blood flows becomes important only in adolescents, who were categorized according to NYHA classification of CHF. Since, the number of participants in the clinical study used for model evaluation in pediatrics was small, therefore, these results cannot be generalized for all the pediatric CHF patients.

Since, the developed model has successfully predicted the stereo-selective disposition of carvedilol in healthy and diseased populations, it can be used to predict genotype-specific CL/F in special populations (pediatrics, geriatrics and cirrhosis) and can assist in improving the safety profile of

carvedilol by reducing the ADR's associated with it, particularly, the ones associated with higher systemic concentrations of R-carvedilol (orthostatic hypotension), that can lead to serious consequences in geriatric population.

We will end by quoting G. T Tucker and M. S. Lennard *"When looking glass drugs are given their pharmacokinetics should, whenever possible, be viewed from both sides of the mirror"* (Tucker and Lennard, 1990).

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Author Contributions

Participated in research design: Rasool, Khalil and Läer

Conducted experiments: Rasool

Performed data analysis: Rasool and Khalil

Wrote or contributed to the writing of the manuscript: Rasool, Khalil and Läer

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Correspondence

Muhammad Fawad Rasool, Department of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine University, 40225 Düsseldorf, Germany. e-mail: fawadrasool@bzu.edu.pk; fawad.rasool@uni-duesseldorf.de

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Figure Legends:

Figure1 Workflow for the development of adult and pediatric PBPK heart failure model. The white area shows model development in healthy adults and the grey shaded area shows stages of model development in chronic heart failure patients. (A) Full PBPK model with different body compartments. The grey compartments show the organs in which blood flow reductions are incorporated. (B) Incorporated organ blood flow reductions in chronic heart failure patients with respect to severity of disease. *CHF* chronic heart failure, *NYHA* New York Heart Association functional classification of heart failure *PBPK model* physiologically based pharmacokinetic model

Figure 2 Comparison of observed and predicted systemic R and S-carvedilol concentration-time profiles in healthy adults after intravenous or oral drug dosing. *Healthy adults, iv application* (A, F) 12.5 mg, ● (Neugebauer et al., 1990), ○ (Spahn et al., 1990). *Oral application*, (B, G) 50 mg, ● (Neugebauer et al., 1990), ○ (Spahn et al., 1990), 25 mg, (Zhou and Wood, 1995), (C, H) extensive metabolizers, (D, I) poor metabolizers, (E, J) 0.09 mg/kg, n = 7, (Behn, 2001). Prediction results are shown as median (lines) and5th and 95th percentiles (dotted lines). The observed data is shown as filled and empty circles

Figure 3 Comparison between the observed and predicted values of the area under the plasma concentration-time curve ($AUC_{0-\infty}$), the maximum concentration (C_{max}), and drug clearance in healthy adults. Results are presented as ratios$_{(observed/predicted)}$ (A, B) R-carvedilol and (D, F) S-carvedilol ● oral application, ■ intravenous application. *EM*: extensive metabolizers and *PM*: poor metabolizers. The shadowed gray area indicates a 2-fold error range. When more than one clinical observed data was available at the same dose level, a line was used to show the mean of the ratio$_{(observed/predicted)}$. Clearance is the calculated oral clearance (CL/F) if the dose is given orally.

Figure 4 Comparison of observed and predicted systemic R and S-carvedilol concentration-time profiles after steady state oral drug dosing in *Heart failure patients:* (A, E) 6.25 mg, (B, F) 12.5 mg, (C, G) 25 mg, and (D, H) 50 mg oral carvedilol. Observed data are shown as dark circles (Tenero et al., 2000).

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Prediction results are shown as median (lines), 5th and 95th percentiles (dotted lines), and minimum/maximum (dashed lines).

Figure 5 Comparison between the observed and predicted values of the area under the plasma concentration-time curve ($AUC_{0-\infty}$) ■, the maximum concentration (C_{max})▲, and drug clearance (CL/F) ● in adult heart failure population. Results are presented as mean ratios$_{(observed/predicted)}$ for R-carvedilol (A) and S-carvedilol (B).

Figure 6 Model predictions in different pediatric age groups for R and S-carvedilol (A, B) infants, (C, D) young children, (E, F) children and (G, H) adolescents. Model predictions in individual patients (I–P) after administering 0.09 mg/kg oral dose racemic carvedilol, without (○) and with (●) adjusting the organ blood flows, — median prediction, ---- minimum and maximum prediction, ···· 5th and 95th percentiles and ●, ○ observed data (Behn, 2001)

Figure 7 Comparison between the observed and predicted values of the maximum concentration (C_{max})and drug clearance (CL/F) in pediatric chronic heart failure patients. Results are presented as individual and ratios$_{(observed/predicted)}$ (A, B) R-carvedilol and (C, D) S-carvedilol ● predictions without organ blood flow reductions and ■ predictions with incorporation of organ blood flow reductions. The arrow head of the line points from ratio$_{(observed/predicted)}$ without reduction in organ blood flow to ratio$_{(observed/predicted)}$ with reduction in organ blood flow in the same patient. The shadowed gray area indicates a 2-fold error range.

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Tables:

Table 1 The drug dependent parameters and characteristics of the presented PBPK model

Parameter	R-Carvedilol	S-Carvedilol	Source/ Reference (R, S)
Molecular weight (g/mol)	406.47	406.47	PubChem.
Log$P_{o:w}$	4.19	4.19	PubChem.
pK_a	7.97	7.97	(Caron et al., 1999)
Absorption			
Model	ADAM		
Solubility (mg/mL)[a]	0.01	0.01	(Benet et al., 2011)
$P_{eff,man}$ (cm/s)	3.9×10^{-4}[b]	1.6×10^{-4}	(Tian et al., 2012), Sensitivity analysis and manual optimization
$f_{u,Gut}$	0.00138	0.00124	Simcyp predicted
Q_{Gut} (L/h)[c]	12.2	8.1	Simcyp predicted
Distribution			
Model	Full PBPK		
V_{ss} (L/kg)—predicted	1.57	1.95	Poulin and Theil method
V_{ss} (L/kg)— observed	1.39–3.40	1.42–3.84	(Neugebauer et al., 1990)
Blood to plasma (*B:P*) ratio	0.67	0.74	(Fujimaki et al., 1990)
fu_P	0.0045	0.0063	(Fujimaki et al., 1990)
Elimination			
CL_{iv} (L/h)—used as input in retrograde model	41	54	(Neugebauer et al., 1990), Optimized

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CYP2D6 CL_{int} (µL/min/mg/pmol of isoform)[d]	656.5	702.2	
CYP1A2 CL_{int} (µL/min/mg/pmol of isoform)[d]	2.7	21.6	
CYP2C9 CL_{int} (µL/min/mg/pmol of isoform)[d]	1.9	15.3	
CYP3A4 CL_{int} (µL/min/mg/pmol of isoform)[d]	0.5	4.1	[d,e] Simcyp retrograde model of enzyme kinetics
CYP2E1 CL_{int} (µL/min/mg/pmol of isoform)[d]	1.1	9.2	
UGT1A1 CL_{int} (µL/min/mg/pmol of isoform)[e]	8.8	9.1	
UGT2B4 CL_{int} (µL/min/mg/pmol of isoform)[e]	10.5	10.4	
UGT2B7 CL_{int} (µL/min/mg/pmol of isoform)[e]	8.9	19.6	
CL_R (L/h)[f]	0.25	0.25	(Gehr et al., 1999)

$LogP_{o:w}$ octonal-water partition coefficient, fu_P fraction of unbound drug in plasma, pK_a acid dissociation constant, *ADAM* Advanced, Dissolution, Absorption and Metabolism, $f_{u,Gut}$ fraction unbound drug in enterocytes, Q_{Gut} hybrid term derived from villous blood flow and drug permeability through the enterocyte membrane, CL_{iv} intravenous clearance, CL_R renal clearance, CL_{int} intrinsic clearance,

[a]Assumed to be similar for both enantiomers

[b]Human jejunum permeability calculated from P_{app} value of a Caco-2 assay by calibrating with atenolol and using Simcyp®

[c]Q_{gut} value was adjusted according to decrease in hepatic blood flow in chronic heart failure patients, see method section for details

[d]Values calculated by using retrograde model in Simcyp®

[e]Values calculated manually by predicted additional clearance using retrograde model in Simcyp®

[f]Assumed to be similar in both enantiomers

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Table 2 Characteristics of the adult data sets used for carvedilol model development

No.	Population	Nr. of subjects	Dose (mg)	Application	Age (years)		Body Weight (kg)		Ref.
					Mean	Range	Mean	Range	
1	Healthy	10[a]	12.5	iv infusion[b]	29.5	21-39	73.9	56.5-98	(Neugebauer et al., 1990)
2	Healthy	3	12.5	iv infusion[b]	-	-	-	-	(Spahn et al., 1990)
3	Healthy	10[a]	50	oral	29.5	21-39	73.9	56.5-98	(Neugebauer et al., 1990)
4	Healthy	3	50	oral	-	-	-	-	(Spahn et al., 1990)
5	Healthy	9	25	oral	28.4[c]	-	82.1[c]	-	(Zhou and Wood, 1995)
6	Healthy	7	25	oral	32[d]	-	89.0[d]	-	(Zhou and Wood, 1995)
7	Healthy	7	6.4[e]	oral	29.7	24-37	71	56-100	(Behn, 2001)
8	Heart failure[f,g]	20	6.25	oral	55	39-64	89.5	60.8-113.1	

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9	Heart failure[f,g]	20	12.5	oral	55	39-64	89.5	60.8-113.1	(Tenero et al., 2000)
10	Heart failure[f,g]	20	25	oral	55	39-64	89.5	60.8-113.1	
11	Heart failure[f,g]	20	50	oral	55	39-64	89.5	60.8-113.1	

[a] The number of patients included in pharmacokinetic analysis of S-carvedilol after iv and oral application were 6 and 7 respectively

[b] Intravenous infusion was given over 1 hour

[c] SEM for age ± 1.3 years and for weight ± 3.2 kg

[d] SEM for age ± 2.4 years and for weight ± 6.9 kg

[e] Dose administered as 0.09 mg/kg but normalized to total dose by multiplying with the average weight of the participants in the clinical trial

[f] 20 patients completed the study (10 patients with NYHA III and 10 with NYHA IV heart failure)

[g] The presented values for age and weight are the reported values for the initial study population (n=22)

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Table 3 Characteristics of pediatric data used for model development

No.	Age (years)	Gender	Body Weight (kg)	Dose (mg/kg)	Ross score/ NYHA Class
1	0.12	Female	3.1	0.09	3
2	0.13	Male	4	0.09	6
3	0.15	Male	3.9	0.09	3
4	0.5	Female	5.2	0.09	8
5	0.75	Male	8	0.09	3
6	1.25	Male	10.1	0.09	3
7	1.5	Male	9.5	0.09	10
8	3.5	Female	13.1	0.09	3
9	5.5	Male	20.2	0.09	3
10	7.5	Male	24.3	0.09	5
11	8.25	Male	25.8	0.09	7
12	11.6	Female	34.3	0.09	4
13	11.8	Male	39	0.09	2
14	17.5	Male	56	0.09	NYHA II
15	17.8	Male	61	0.09	NYHA III
16	19.3[a]	Male	98.2	0.09	NYHA III
Mean	6.7		26	-	-
SD	6.72		25.6	-	-

NYHA New York heart association classification of heart failure, *SD* standard deviation

All patients were diagnosed with heart failure and were participants in the same clinical trial (Behn, 2001)

[a] Patient out of the pediatric age range according to guidelines set by World Health Organization (WHO).

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Table 4 Predicted bioavailability of carvedilol enantiomers in different populations

Simulated population	R-Carvedilol				S-Carvedilol			
	F_g	F_h	f_a	F	F_g	F_h	f_a	F
Healthy adults	0.98	0.35	0.99	0.34	0.96	0.21	0.88	0.17
Extensive metabolizers	0.99	0.35	0.99	0.34	0.96	0.20	0.89	0.17
Poor metabolizers	1.00	0.65	0.99	0.64	0.98	0.34	0.89	0.30
Adults with heart failure	0.97	0.19	0.74	0.14	0.92	0.11	0.55	0.05
Pediatrics with heart failure[a]	0.98	0.42	0.99	0.41	0.96	0.27	0.93	0.23

f_a fraction of drug absorbed, F_g fraction of drug escaping metabolism in the gut, F_h fraction of drug escaping the hepatic metabolism and F is the bioavailability. [a] simulation performed without reducing organ blood flows

Figure 1

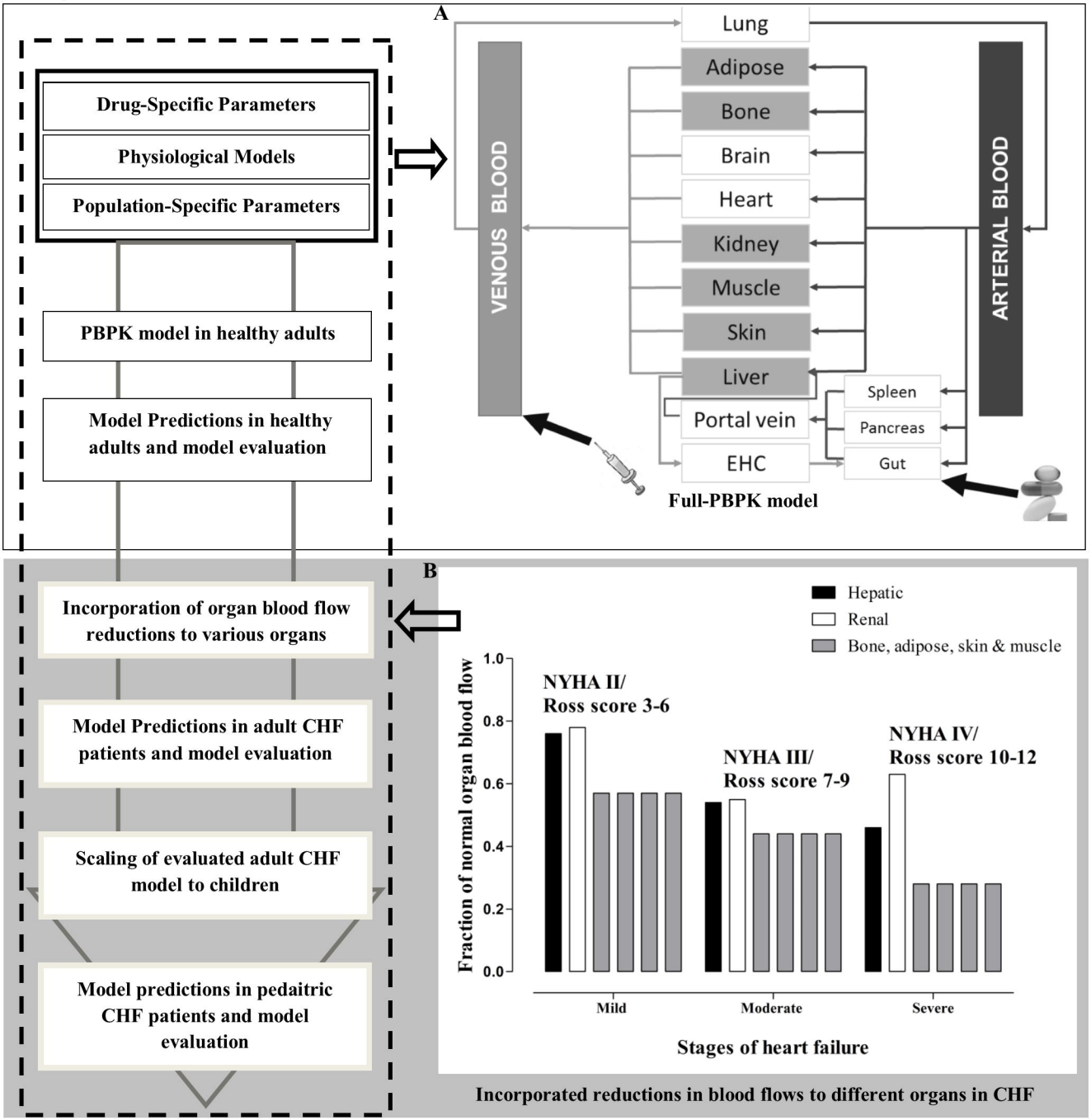

Drug-Specific Parameters
Physiological Models
Population-Specific Parameters
PBPK model in healthy adults
Model Predictions in healthy adults and model evaluation
Incorporation of organ blood flow reductions to various organs
Model Predictions in adult CHF patients and model evaluation
Scaling of evaluated adult CHF model to children
Model predictions in pedaitric CHF patients and model evaluation
A
Lung
Adipose
Bone
Brain
Heart
Kidney
Muscle
Skin
Liver
Spleen
Pancreas
Portal vein
EHC
Gut
VENOUS BLOOD
ARTERIAL BLOOD
Full-PBPK model
B
Hepatic
Renal
Bone, adipose, skin & muscle
NYHA II/
Ross score 3-6
NYHA III/
Ross score 7-9
NYHA IV/
Ross score 10-12
Fraction of normal organ blood flow
1.0
0.8
0.6
0.4
0.2
0.0
Mild
Moderate
Severe
Stages of heart failure
Incorporated reductions in blood flows to different organs in CHF

Figure 2

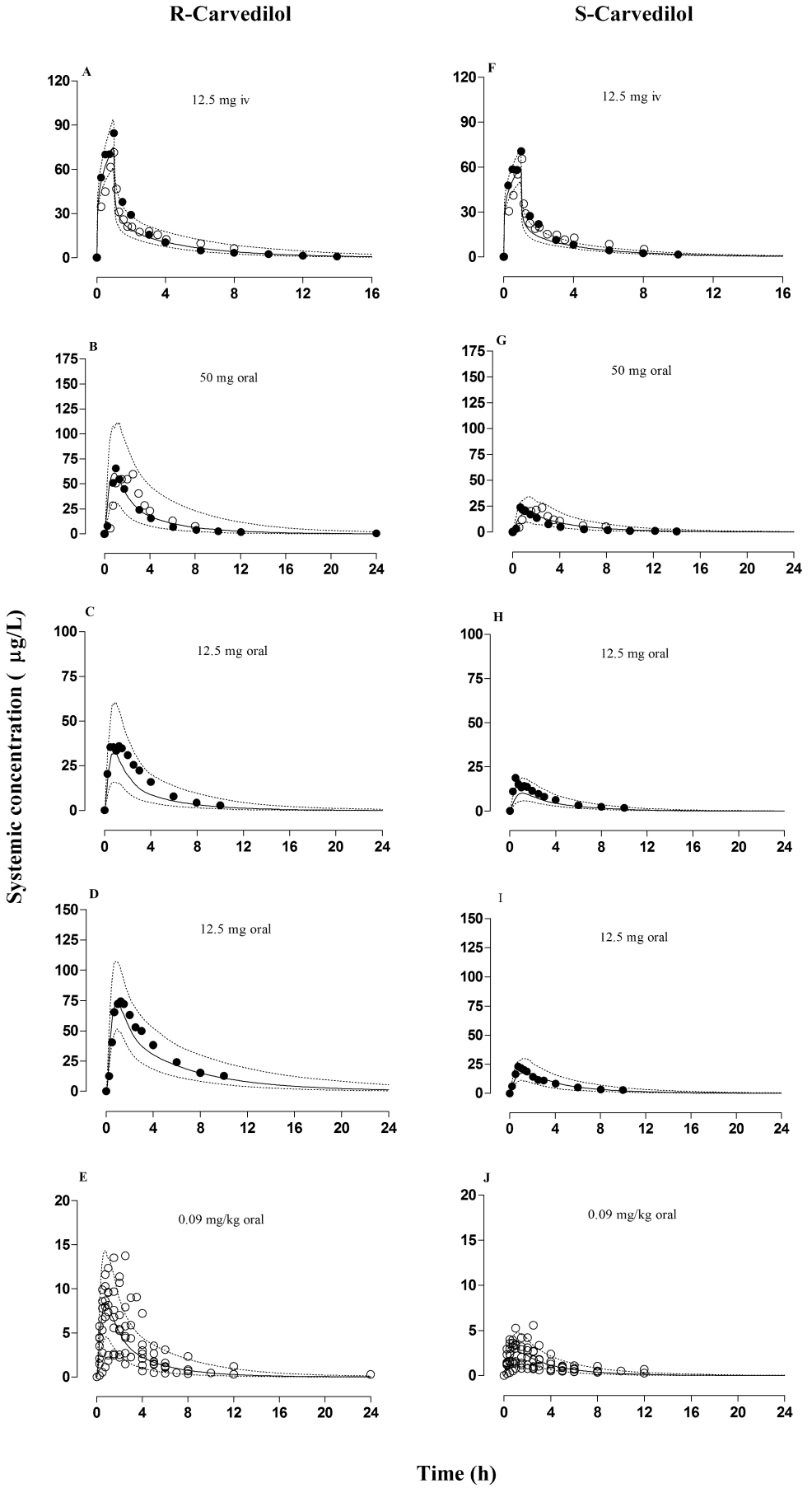

R-Carvedilol
S-Carvedilol
A
12.5 mg iv
F
12.5 mg iv
B
50 mg oral
G
50 mg oral
C
12.5 mg oral
H
12.5 mg oral
D
12.5 mg oral
I
12.5 mg oral
E
0.09 mg/kg oral
J
0.09 mg/kg oral
Systemic concentration (µg/L)
Time (h)

Figure 3

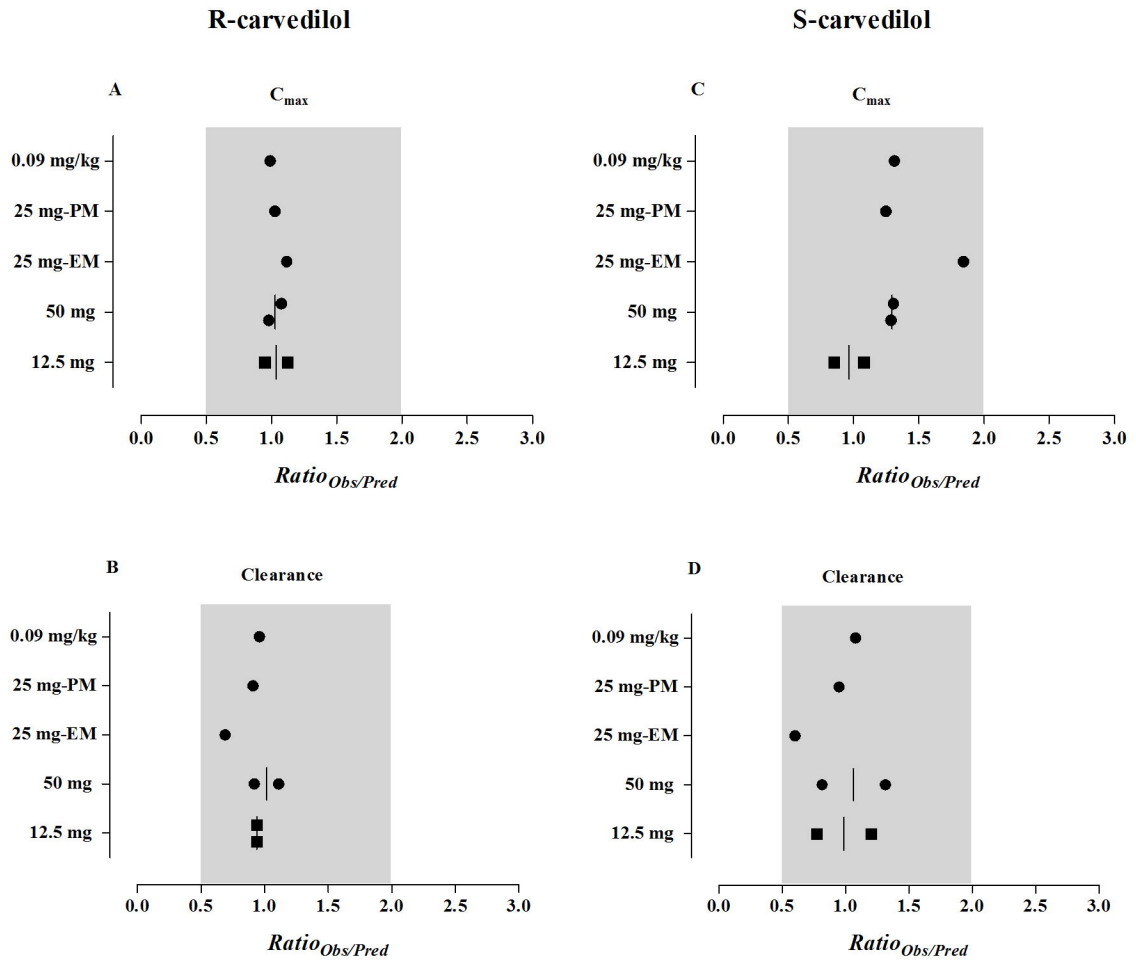

R-carvedilol
S-carvedilol
A
C_{max}
C
C_{max}
0.09 mg/kg
25 mg-PM
25 mg-EM
50 mg
12.5 mg
0.0 0.5 1.0 1.5 2.0 2.5 3.0
$Ratio_{Obs/Pred}$
B
Clearance
D
Clearance

Figure 4

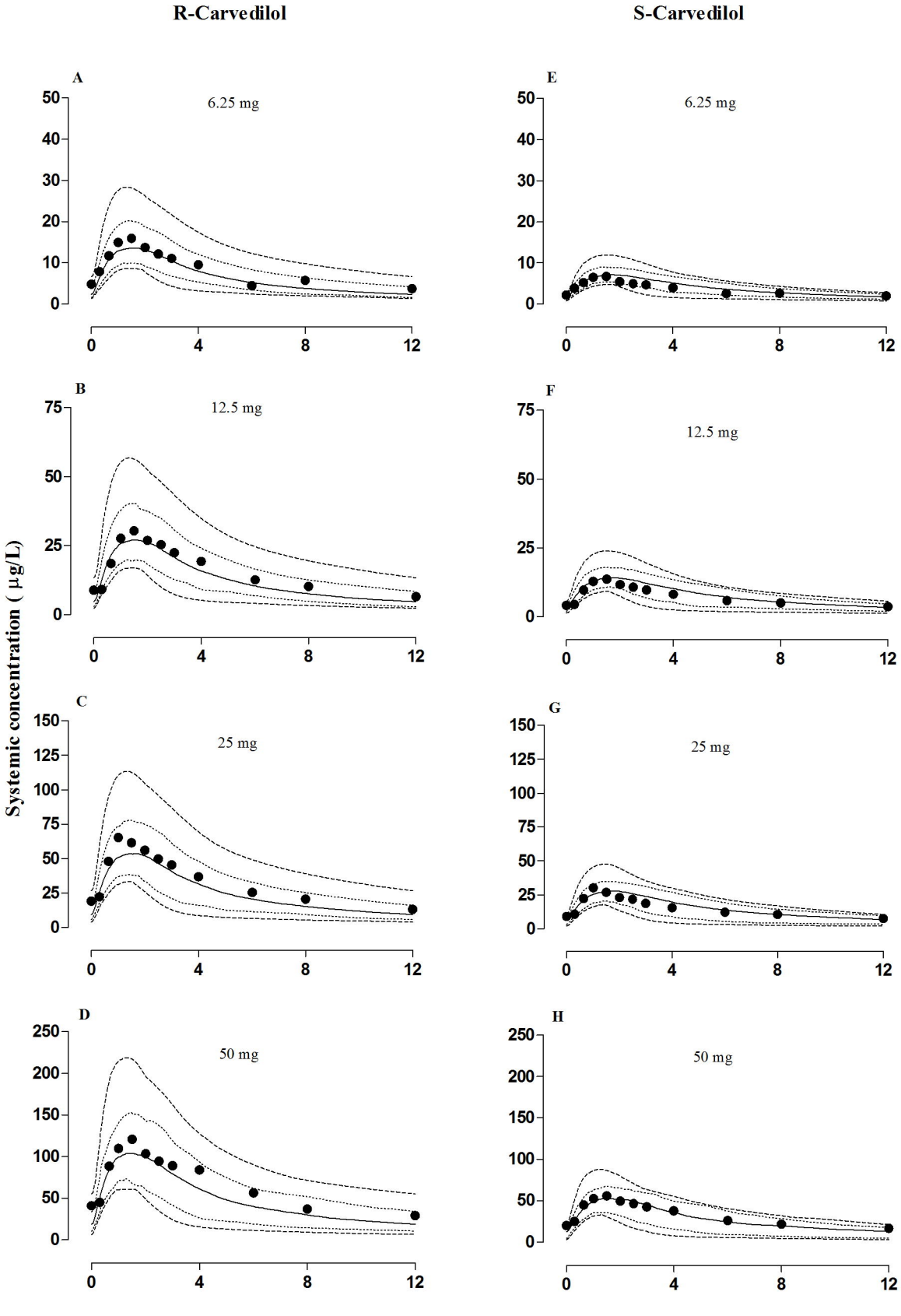

R-Carvedilol
S-Carvedilol
A
6.25 mg
E
6.25 mg
B
12.5 mg
F
12.5 mg
C
25 mg
G
25 mg
D
50 mg
H
50 mg
Systemic concentration (μg/L)
Time (h)

Figure 5

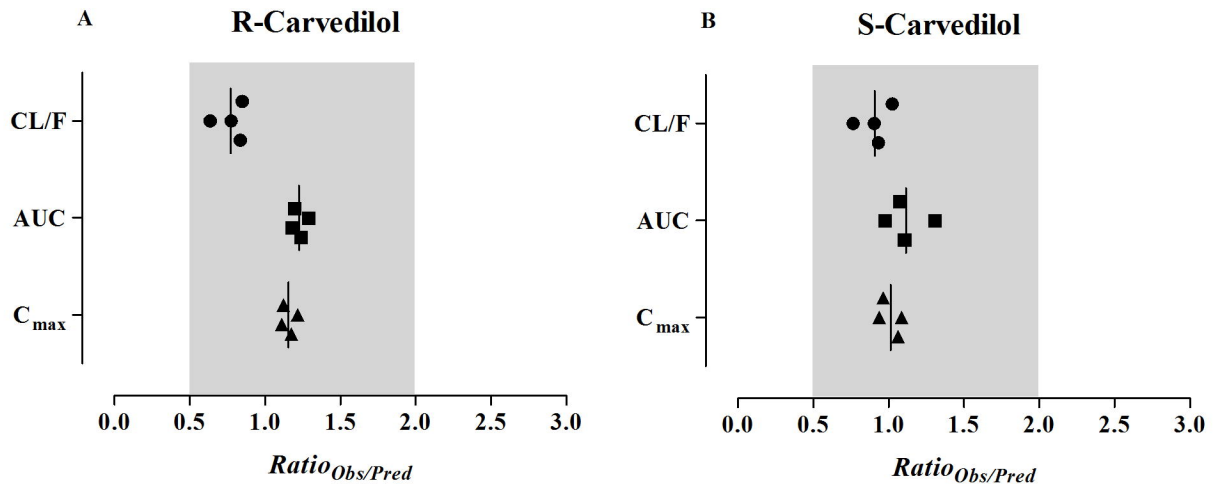

A
R-Carvedilol
CL/F
AUC
C max
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Ratio Obs/Pred
B
S-Carvedilol
CL/F
AUC
C max
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Ratio Obs/Pred

Figure 6

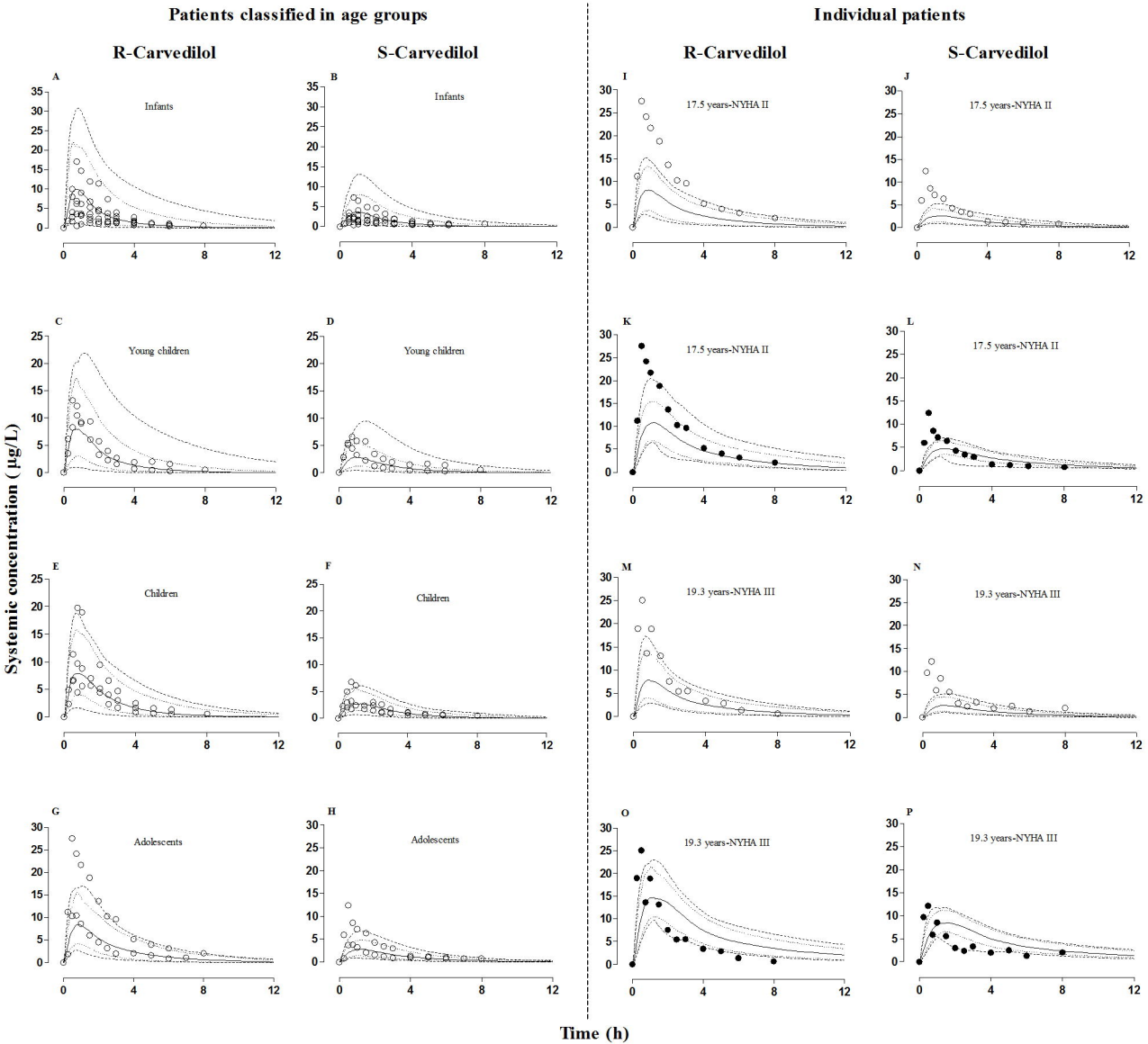
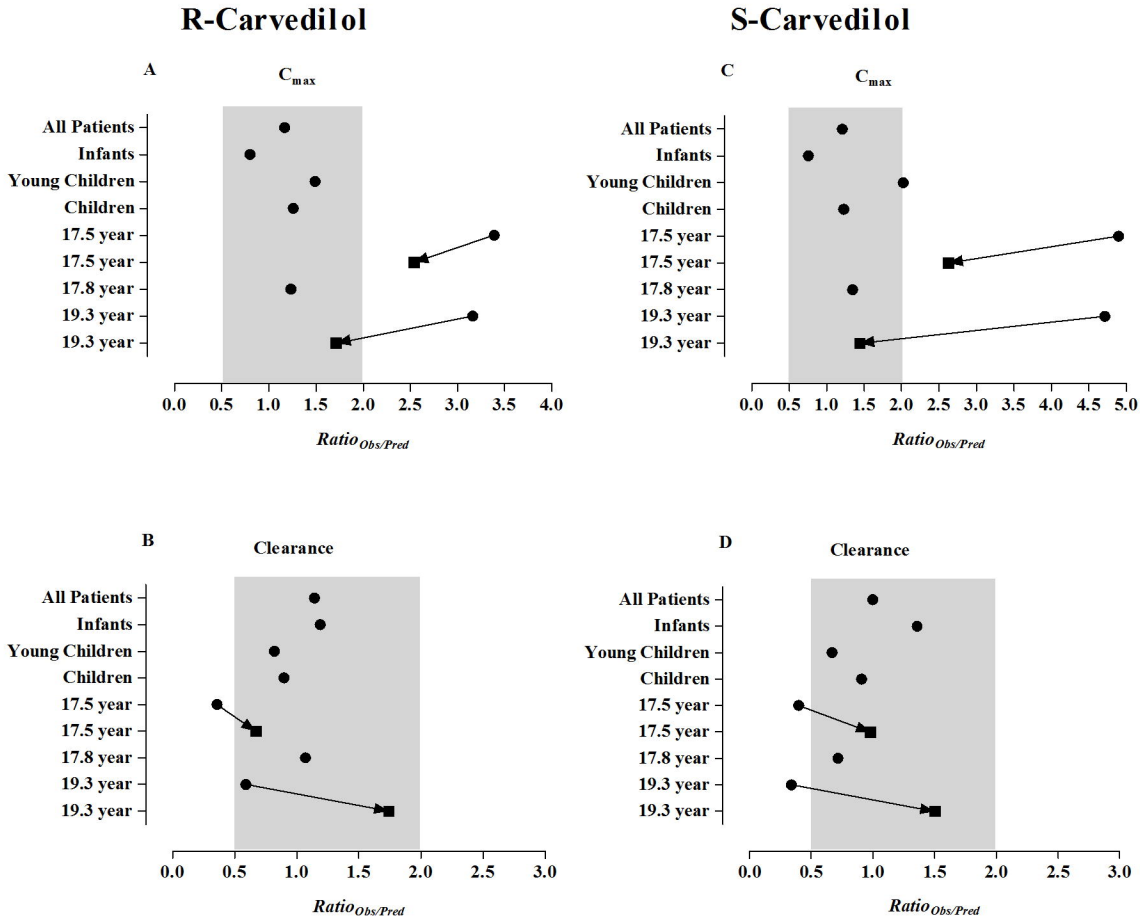

Patients classified in age groups
Individual patients
R-Carvedilol
S-Carvedilol
R-Carvedilol
S-Carvedilol
A
Infants
B
Infants
I
17.5 years-NYHA II
J
17.5 years-NYHA II
C
Young children
D
Young children
K
17.5 years-NYHA II
L
17.5 years-NYHA II
E
Children
F
Children
M
19.3 years-NYHA III
N
19.3 years-NYHA III
G
Adolescents
H
Adolescents
O
19.3 years-NYHA III
P
19.3 years-NYHA III
Systemic concentration (μg/L)
Time (h)

Figure 7


R-Carvedilol
S-Carvedilol
A
C_{max}
B
Clearance
C
C_{max}
D
Clearance
All Patients
Infants
Young Children
Children
17.5 year
17.5 year
17.8 year
19.3 year
19.3 year
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0
$Ratio_{Obs/Pred}$

Supplemental Data

Journal Title

Drug Metabolism and Disposition

Article Title

Predicting stereo-selective disposition of carvedilol in adult and pediatric chronic heart failure patients by incorporating pathophysiological changes in organ blood flows–A physiologically based pharmacokinetic approach

Authors

Muhammad Fawad Rasool, Feras Khalil and Stephanie Läer

Supplemental Methods

Sensitivity Analysis

In order to assess the uncertainty associated with some pediatric model input parameters, which were adopted from adult PBPK model, sensitivity analysis (SA) was performed. The SA was performed on parameters that have the highest potential effect on the clearance (as oral clearance *CL/F*) and area under the curve ($AUC_{0-\infty}$) of both enantiomers, i.e., hepatic (portal and arterial), P_{eff} and $f_{u,Gut}$.

Sensitivity index (SI) was used to determine the magnitude of change in output of the parameter (PK-parameters) per unit change in the magnitude of input parameter from its initial value.

The SI is determined by using the following relation **(Supplemental equation 1)** implemented within Simcyp® version 14.1,

$$SI_n = \left(Q(P_{n+1}) - Q(P_n)\right) \Big/ (P_{n+1} - P_n) \qquad (1)$$

Where SI is the sensitive index for the output variable which is function of parameter *Q(P)* with respect to change in the value of input parameter *P* from the initial value P_n.

The SA showed that the predicted *CL/F* and $AUC_{0-\infty}$ in pediatric CHF patients were most sensitive to changes in $f_{u,Gut}$, hepatic portal blood flow, and P_{eff} **(Supplemental Figure 1).** However, the PK parameters of interest were only sensitive to P_{eff} at low values, which is not expected in the case of carvedilol as it is a BCS II drug with high permeability and a P_{eff} value of <1 is not reasonable, which leaves the $f_{u,Gut}$ and hepatic portal blood flow as the most sensitive parameters. This finding was further confirmed from the simulated plasma concentration-time profiles of R and S-carvedilol after periodically changing the values of input parameters in 10 consecutive runs **(Supplemental Figure 2).**

Due to the lack of measured/reported data for these parameters, it was not plausible to use different values of input parameters in adult and pediatric CHF populations. Therefore, as mentioned previously in

the modelling strategy, the evaluated adult CHF model was scaled to pediatric CHF patients by keeping all the input parameters consistent with the evaluated adult PBPK model.

Supplemental Table 1 Comparison of area under the systemic drug concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) and area under the systemic drug concentration-time curve from time zero to last measured concentration (AUC_{last})

Population	R-Carvedilol		S-Carvedilol		Reference
	$Ratio_{(observed/predicted)}$				
	AUC_{last}	$AUC_{0-\infty}$	AUC_{last}	$AUC_{0-\infty}$	
Adults					
Healthy adults (iv)	1.00	1.06	0.99	1.06	(Neugebauer et al., 1990);
Healthy adults (50 mg oral)	0.93	0.99	0.88	0.99	(Spahn et al., 1990)
Healthy adults (0.09 mg/kg oral)	0.99	1.04	0.76	0.92	(Behn, 2001)
Healthy adults (EM)	1.47	1.44	1.52	1.66	(Zhou and Wood, 1995)
Healthy adults (PM)	1.12	1.09	1.00	1.05	
Adults with CHF	1.18	1.23	0.94	1.11	(Tenero et al., 2000)
Pediatrics					
All patients	0.84	0.83	0.83	1.00	(Behn, 2001)
Infants	0.71	0.84	0.62	0.74	
Young children	1.21	1.24	1.63	1.72	
Children	1.08	1.10	0.91	1.11	
17.8 year	0.89	0.93	0.93	1.28	
17.5 year	2.65	2.73	2.25	2.50	
17.5 year[a]	1.63	1.49	1.08	1.04	
19.3 year	1.84	1.67	2.52	3.01	
19.3 year[a]	0.74	0.57	0.67	0.68	

[a] predictions with organ blood flow reductions, *iv*: intravenous, *EM*: extensive metabolizers, *PM*: poor metabolizers and *CHF*: chronic heart failure

Figure Legends

Supplemental Figure 1 Sensitivity analysis on various model input parameters and their effect on predicted oral clearance (CL/F) and area under the curve ($AUC_{0-\infty}$) of R and S carvedilol in pediatric chronic heart failure patients. *Liver hepatic blood flow* (% of cardiac output) (A, B, C, D), *liver arterial blood flow* (% of cardiac output) (E, F, G, H), *human jejunum permeability* (P_{eff}) (I, J, K, L) and *fraction of unbound drug in gut* ($f_{u,Gut}$) (M, N, O, P). Y-axis on the right side shows sensitivity index and on the left side shows *CL/F* and $AUC_{0-\infty}$. Solid lines indicate *CL/F* and $AUC_{0-\infty}$. The dotted line indicates sensitivity index. The solid circles (●) show the value of the parameter used in healthy male population. The grey shaded area shows the range of reduced organ blood flow used for performing simulations in chronic heart failure patients.

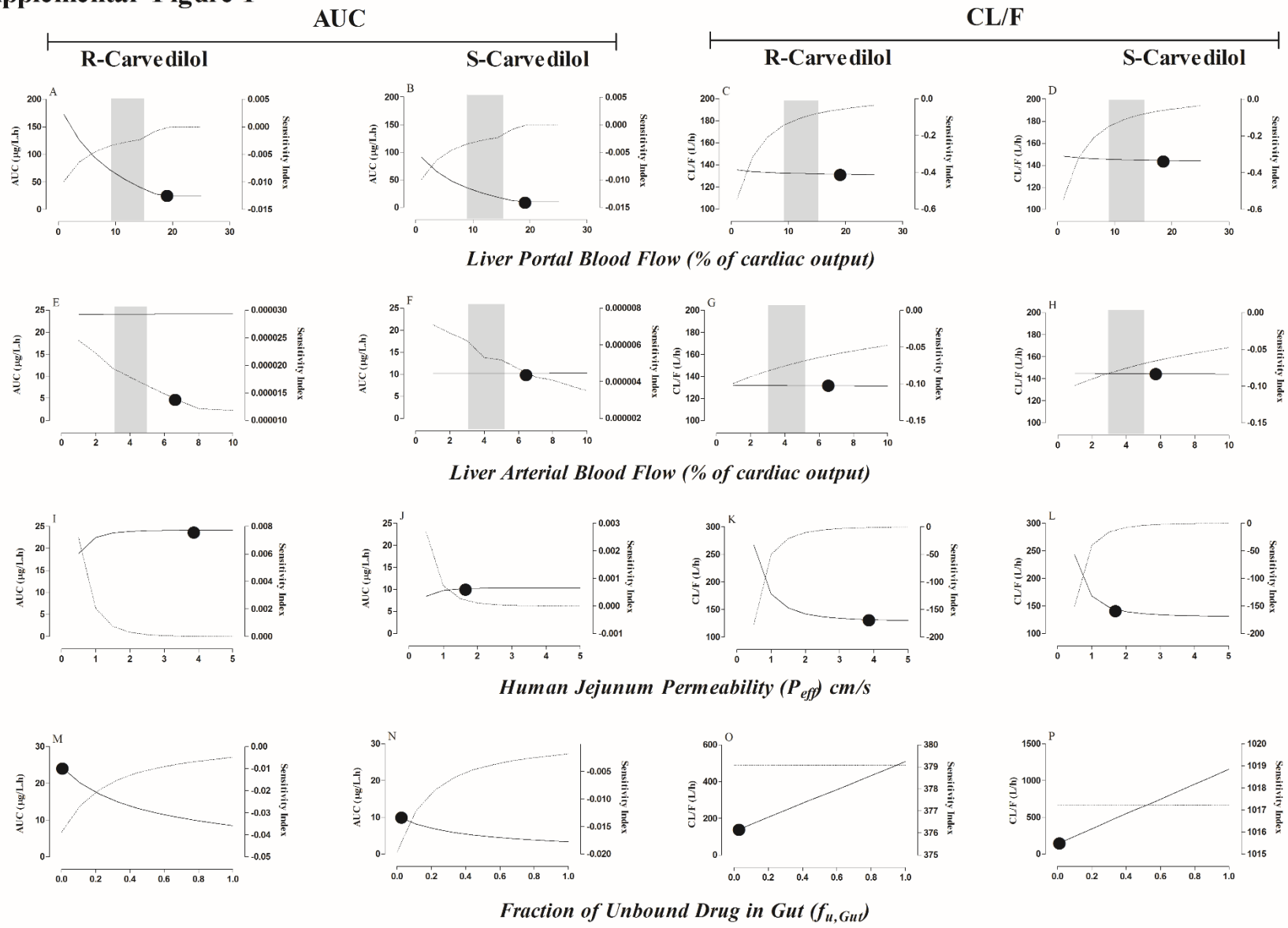
Supplemental Figure 2 Predicted R and S-carvedilol systemic concentration vs. time profiles from the sensitivity analysis by changing the values of input parameters in 10 runs. *Liver hepatic blood flow* (% of cardiac output), simulated range: 1–25 % (A, B), *liver arterial blood flow* (% of cardiac output), simulated range: 1–10 % (C, D), *human jejunum permeability* (P_{eff}), simulated range: 0.5–5 cm/s (E, F) and *fraction of unbound drug in gut* ($f_{u,Gut}$), simulated range: 0–1 (G, H).

Supplemental Figure 3 Observed vs. predicted concentrations plots in healthy adults after intravenous (A, B), and oral (C, D) racemic carvedilol application, and in chronic heart failure patients after oral application racemic carvedilol (E, F). The solid line indicates line of identity and the dashed line show a 2-fold error range.

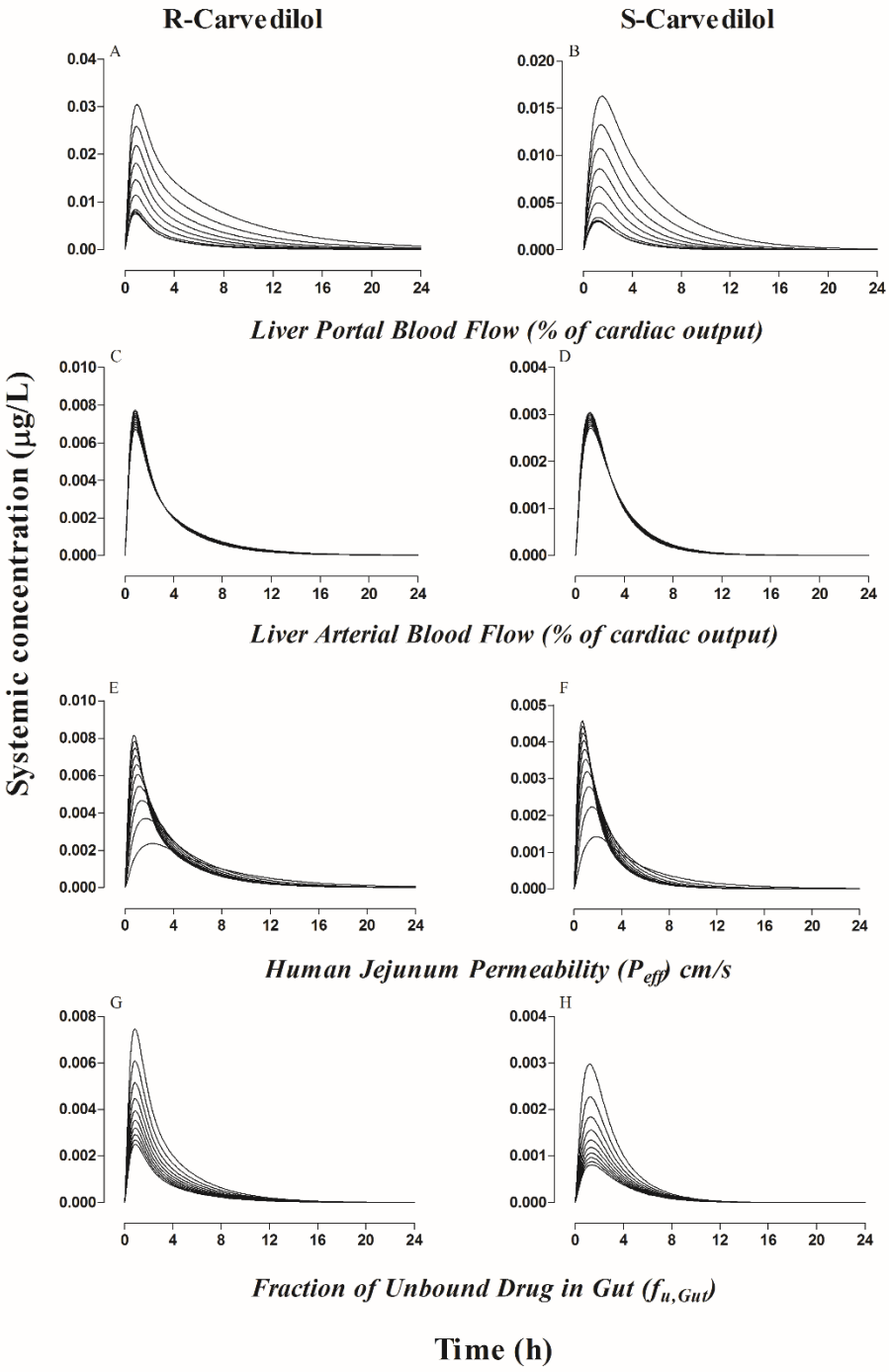
Supplemental Figure 4 Model predictions in the entire pediatric age range n=15 (0.12 to 17.8 year) including the young adult n=1 (19.3 year) after administering 0.09 mg/kg racemic carvedilol, (A, B) systemic concentration-time plots, ○ observed data — median prediction, ---- minimum and maximum prediction, ···· 5^{th} and 95^{th} percentiles and (C, D) Change in oral clearance of R and S-carvedilol with age ○ Predicted CL/F, ● observed CL/F (Behn, 2001). Simulations performed by creating a virtual population of 1000 individuals.

Supplemental Figure 5 Observed vs. predicted concentrations plots in pediatric CHF patients after administering 0.09 mg/kg racemic carvedilol (a, b). The solid line indicates line of identity and the dashed line show a 2-fold error range.

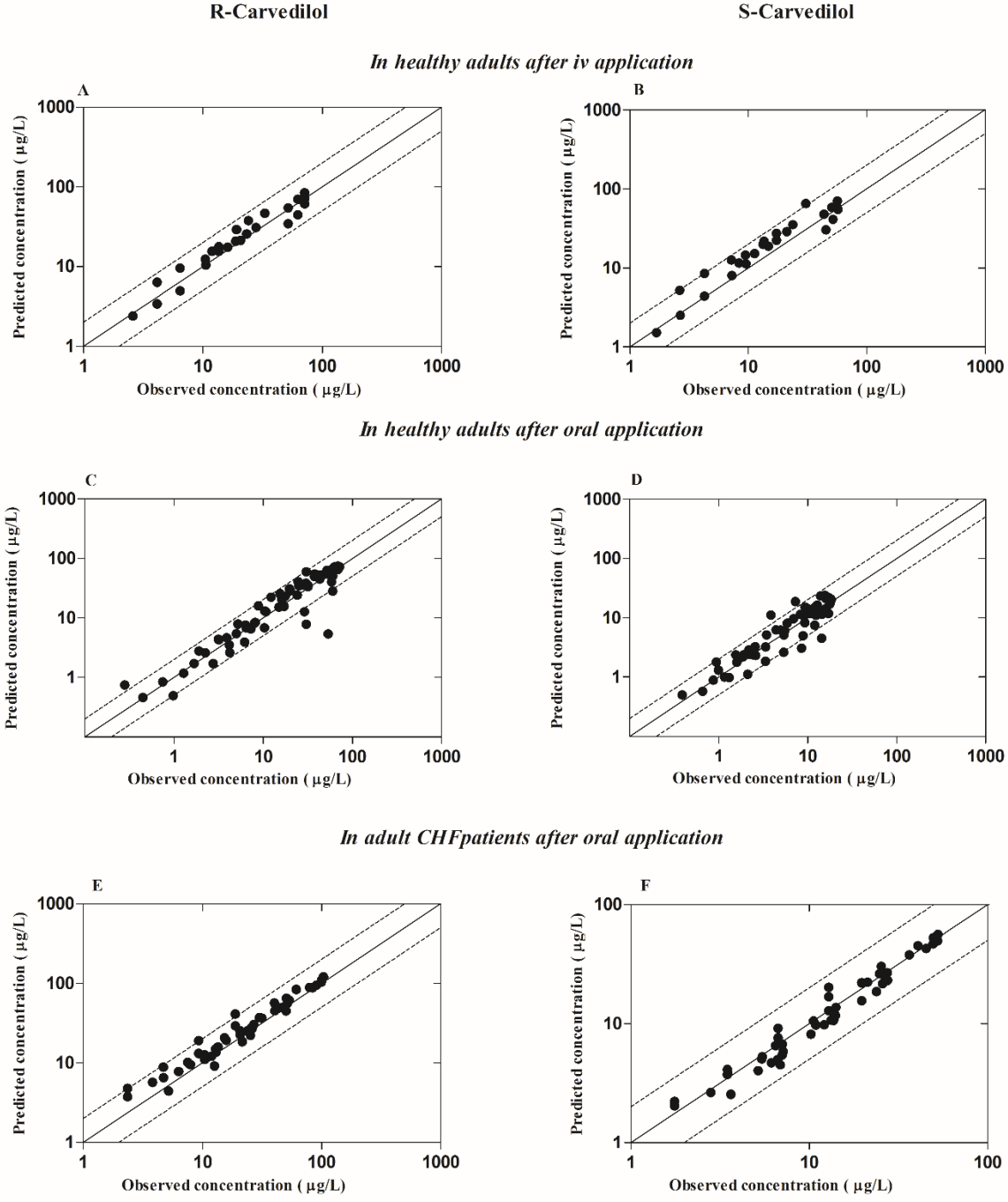
Supplemental Figure 1



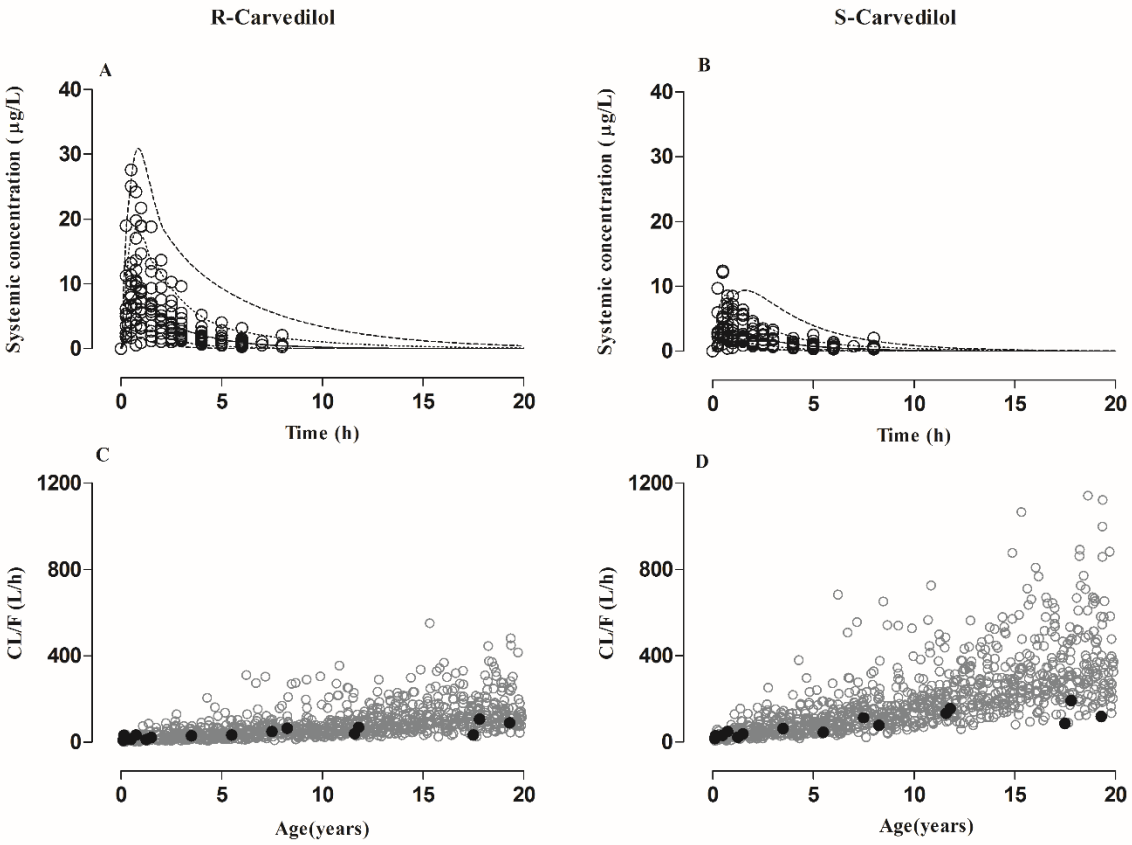
Supplemental Figure 2



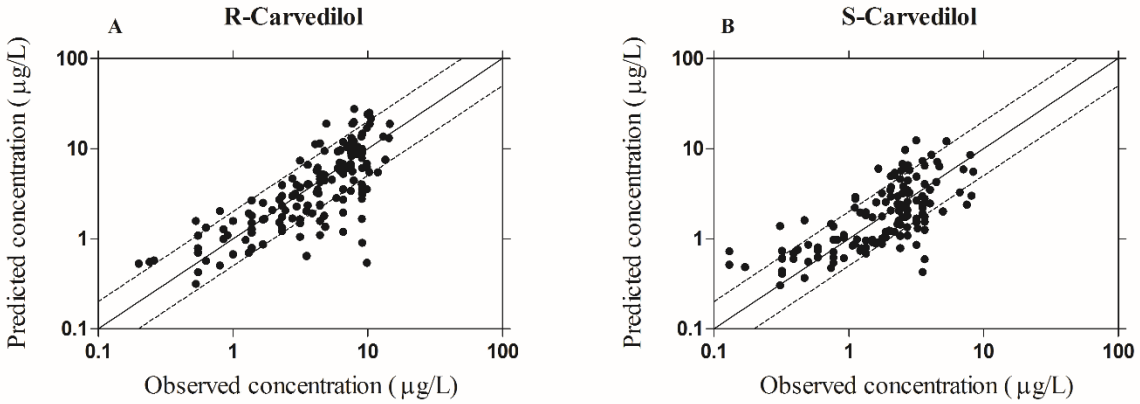
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



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Zhou HH and Wood AJ (1995) Stereoselective disposition of carvedilol is determined by CYP2D6. *Clin Pharmacol Ther* **57:**518-524.