Enhancing Our Understanding of Enalapril's Pharmacokinetics: A Physiologically Based Modeling Approach

PROJECT REPORT

Submitted To
Faculty of Science, Savitribai Phule Pune University
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IBB-2019-13
under the guidance of
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Pune, India

May 2024

Humboldt-Universität zu Berlin

LEBENSWISSENSCHAFTLICHE FAKULTÄT
INSTITUT FÜR BIOLOGIE

MASTERARBEIT
ZUM ERWERB DES AKADEMISCHEN GRADES MASTER OF SCIENCE

Enhancing Our Understanding of Enalapril's Pharmacokinetics: A Physiologically Based Modeling Approach

Verbesserung des Verständnisses der Pharmakokinetik von Enalapril: Ein physiologisch basierter Modellierungsansatz

vorgelegt von
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Geburtsdatum und -ort: 06.11.2000, Pune, Indien

angefertigt in der Arbeitsgruppe Systems Medicine of the Liver
am Institut für Theoretische Biologie
Berlin, im Mai 2024
Erstprüfer: **Dr. Matthias König**

Student Declaration

This is to certify that I, the undersigned, have completed my Master's thesis project titled, **"Enhancing Our Understanding of Enalapril's Pharmacokinetics and Dynamics: A Physiologically Based Modeling Approach"** under the guidance of Dr. Matthias König, during the period November 2023 to April 2024 at the Institut für Theoretische Biologie at Humboldt-Universität zu Berlin, in fulfilment of the course IBT 723 (P), during the period November 2023 to April 2024 for my Master of Science degree in Biotechnology at the Institute of Bioinformatics and Biotechnology (Jointly merged with Department of Biotechnology) at the Savitribai Phule University. I hereby confirm that I have written this work independently and have not used resources or tools other than those specified herein. The parts of the work that are referenced from other works (including internet sources) in terms of wording or meaning have been cited with due credit to the respective authors and appropriate sources.

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AUC	area-under-the-curve
CES1	carboxylesterase 1
C_{max}	maximal concentration
K_{el}	elimination rate
MRP2	multi-drug resistance-associated protein 2
MRP4	multi-drug resistance-associated protein 4
OATP1B1	organic anion transporting polypeptide 1B1
OATP1B3	organic anion transporting polypeptide 1B3
PBPK	physiologically based pharmacokinetic model
$t_{1/2}$	time to half maximal concentration
t_{max}	time to maximal concentration

Abstract

Enhancing Our Understanding of Enalapril's Pharmacokinetics and Dynamics: A Physiologically Based Modeling Approach

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Duration: 6 months

Enalapril is a medication used to treat high blood pressure and a number of other cardiovascular conditions. The renin-angiotensin-aldosterone system (RAAS) is the main component involved in the regulation of blood pressure in the body. One of the key steps in this process is the conversion of angiotensin I to angiotensin II by angiotensin converting enzyme (ACE). Enalapril is an ACE inhibitor and therefore helps to lower blood pressure. Pharmacokinetics is the branch of pharmacology that studies how the body absorbs, distributes, metabolizes, and eliminates drugs over time. After administration as enalapril maleate, it is absorbed in the intestine and converted by carboxylesterase 1 (CES1) in the liver to the active metabolite, enalaprilat. Enalapril and enalaprilat are eliminated from the body by renal clearance. A physiologically based pharmacokinetic (PBPK) model was developed to investigate the pharmacokinetics of enalapril and the factors influencing it. As part of the work, an extensive database of enalapril pharmacokinetics consisting of data from 49 clinical trials was established and used to parameterize and validate the computational model. The model was used to investigate the effect of renal impairment, hepatic impairment and changes in CES1 activity on the pharmacokinetics of enalapril and enalaprilat, as these three factors are known to have a major influence on the pharmacokinetics of enalapril. The model shows good agreement with a wide range of enalapril and enalaprilat data in serum, plasma and urine under different conditions (healthy, renal impairment, hepatic impairment, CES1 mutations). The model is available in SBML under a CC-BY 4.0 license

with all data freely available from the PK-DB pharmacokinetics database.

Keywords: hypertension, enalapril, pharmacokinetics, ACE inhibitor, renal impairment, hepatic impairment, CES1

1 Introduction

Hypertension, commonly known as high blood pressure, is a prevalent cardiovascular condition in which the force of the blood against the artery walls is consistently too high. This chronic condition often develops over many years and can go undetected because it may have few or no symptoms. However, uncontrolled high blood pressure significantly increases the risk of serious health problems, including heart attack, stroke and kidney disease. It is often referred to as the 'silent killer' because of its subtle nature but serious impact on health if left untreated.

The renin-angiotensin-aldosterone system (RAAS) is the main mechanism that regulates blood pressure, homeostasis and electrolyte balance in the body (see Fig. 1). This system works through the coordinated actions of the hormones renin, angiotensin and aldosterone. Triggered by reduced renal blood flow, juxtaglomerular cells convert prorenin to renin. Renin then catalyses the conversion of angiotensinogen to angiotensin I, which is then converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II, a potent vasoconstrictor, not only constricts blood vessels to increase blood pressure, but also stimulates the adrenal cortex to secrete aldosterone. Aldosterone increases sodium reabsorption, which in turn promotes water retention, thereby helping to maintain vascular volume and systemic vascular resistance.

Dysfunction of the renin-angiotensin-aldosterone system (RAAS) can lead to significantly increased blood pressure, a condition known as hypertension. A number of drugs that target different components of the RAAS are used to manage and treat this condition. These include ACE inhibitors, which block the conversion of angiotensin I to angiotensin II, thereby reducing blood pressure. Renin inhibitors directly block the activity of renin, preventing the initial production of angiotensin I. In addition, angiotensin II receptor blockers (ARBs) block the action of angiotensin II on its receptors, which helps to dilate blood vessels and lower blood pressure [27, 57]. These pharmacological interventions are essential for controlling high blood pressure and minimising the risk of cardiovascular complications.

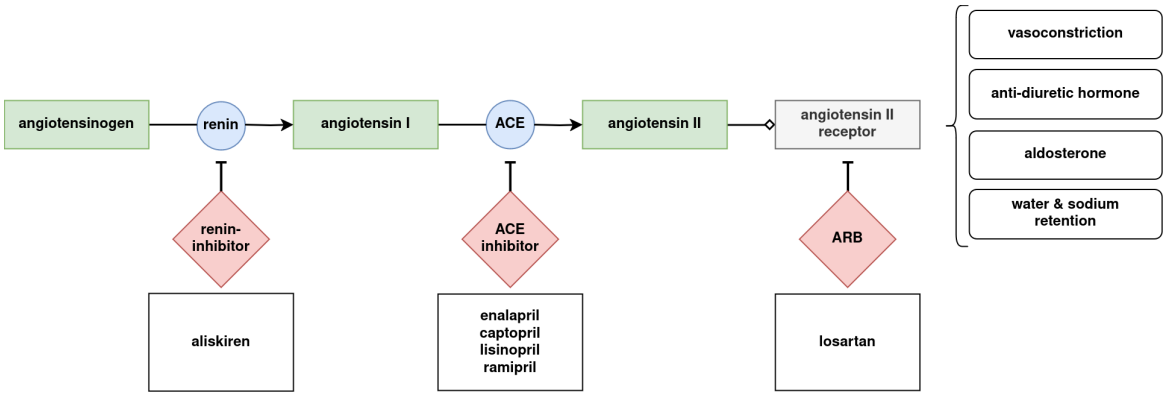

Figure 1: **Overview of the renin-angiotensin-aldosterone system (RAAS).** Key steps in the RAAS are the conversion of angiotensinogen to angiotensin I via renin and the conversion of angiotensin I to angiotensin II via ACE. Angiotensin II binds to its receptor, resulting in vasoconstriction, antidiuretic hormone effects, aldosterone effects, and changes in water and ion balance. Several classes of drugs are used to regulate blood pressure: the direct renin inhibitors inhibit renin (e.g., aliskiren), the ACE inhibitors inhibit ACE (e.g., enalapril), and the ARBs inhibit angiotensin II receptors (e.g., losartan). ACE: angiotensin converting enzyme; ARB: angiotensin receptor blocker.

1.1 ACE inhibitors

ACE inhibitors are a class of medications widely used to treat a range of cardiac conditions, including hypertension and heart failure. These drugs function by blocking the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, effectively lowering blood pressure. Additionally, ACE inhibitors prevent the breakdown of bradykinin, a vasodilator, which further contributes to the reduction of blood pressure. This dual action enhances their effectiveness in managing blood pressure and alleviating strain on the heart. Some of the most commonly prescribed ACE inhibitors include enalapril, lisinopril, ramipril, captopril, and benazepril, each playing a vital role in cardiovascular disease management [77].

1.2 Enalapril

Enalapril is an FDA-approved ACE inhibitor drug commonly used to treat high blood pressure (see Fig. 2). It is sold as enalapril maleate, chemically known as [(S)-1-(N-(1-(ethoxycarbonyl)-3-phenylpropyl)-L-alanyl)-L-proline(Z)-2-butenedioate salt] [7]. Enalapril is the esterified prodrug, which is hydrolysed in the liver (*in vivo* de-esterification) to enalaprilat, the active metabolite involved in ACE inhibition. Enalaprilat acts as a com-

petitive inhibitor of the converting enzyme, preventing angiotensin I from interacting with ACE. The resulting enzyme-inhibitor complex has a slower dissociation rate, which effectively causes ACE inhibition. Enalapril is usually given orally as enalapril maleate.

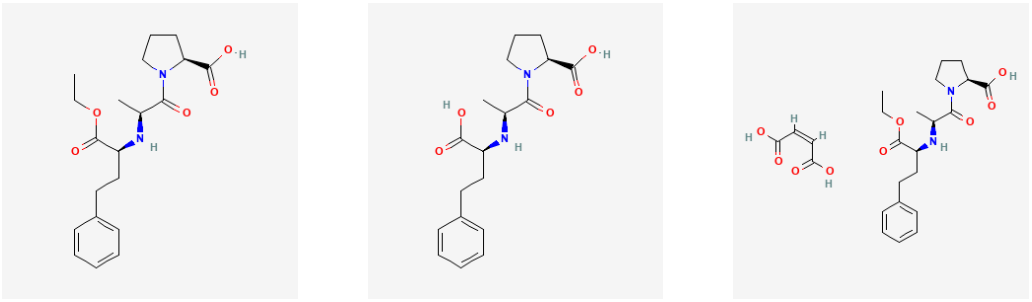

Figure 2: **Chemical formulas of enalapril maleate, enalapril, and enalaprilat.** Enalapril maleate, enalapril, and enalaprilat.

The effects of enalapril treatment may vary depending on a number of factors, including the characteristics of the patient, concomitant medication, renal, cardiac or hepatic impairment, and genetic variations, particularly mutations in the genes that code for the enzymes responsible for the metabolism of enalapril.

1.3 Pharmacokinetics of enalapril

Pharmacokinetics is the study of how a drug moves through the body from the moment it is administered until it is completely eliminated. The key processes are absorption, distribution, metabolism and excretion (ADME) of a given drug. This field studies various parameters that describe the drug's journey, including the maximum concentration of the drug in the bloodstream (Cmaxmax), the time it takes to reach this peak concentration (Tmaxmax), the half-life (T1/21/2), which indicates how long it takes for half of the drug to be eliminated from the body, and the rate of elimination (kelel). Understanding these metrics is critical to optimising drug dosage, timing and administration methods to achieve maximum therapeutic efficacy with minimal side effects.

A good review of the pharmacokinetics of enalapril is given in [41]. Enalapril is known to dissolve and be absorbed in the intestine. After an oral dose, the plasma concentration of enalapril peaks between 1 and 2 hours [8,

74, 76]. After absorption, enalapril is transported via the systemic circulation to the liver where it is hydrolysed or de-esterified by the enzyme carboxylesterase 1 (CES1) to form enalaprilat. As a result, the plasma concentration of enalapril is almost negligible around the 4-hour mark, whereas the concentration of enalaprilat is usually observed to be maximal at this time.

The elimination half-life of enalapril is approximately 2-3 hours. Untransformed enalapril is excreted mainly in the urine but also in the faeces. The oral bioavailability of enalapril is approximately 60% and is not affected by food intake [67]. It should be noted that the time of day at which enalapril is administered has a delaying effect on the time to maximum concentration t_{max}. Administration of enalapril in the evening leads to a delay in t_{max} [80].

For enalaprilat, the time to maximum concentration ($t_{max)}$ is 3-4 hours after biotransformation [6]. Only one metabolite, enalaprilat, is known for enalapril in humans. The bioavailability of enalaprilat is about 40% [6, 71].

1.4 Pharmacodynamics of enalapril

Pharmacodynamics explores the effects that drugs have on the body, encompassing both their therapeutic benefits and potential side effects. This field of study focuses on understanding how drugs act at their target sites, the mechanisms by which they achieve their medical benefits, and how they can inadvertently cause adverse effects.

For an ACE inhibitor such as enalapril, the main pharmacodynamic parameters of interest are systolic and diastolic blood pressure, mean arterial pressure, ACE activity, heart rate, renin activity and concentrations of renin, aldosterone, angiotensin I and angiotensin II. A large number of studies have carried out enalapril trials with particular emphasis on the pharmacodynamic characteristics of patients [9, 35, 55, 59]. Donnelly et al. 1990 [10] have shown a significant reduction in blood pressure after a dose of enalapril. This study involved a daily dose of enalapril for 6 weeks and the maximum effect (E_{max}) was found to be 46.1 ± 16.5 and 19.7 ± 3.8 for systolic and diastolic blood pressure respectively. In addition, the E_{max} for inhibition of ACE activity was found to be $102.4 \pm 5\%$ on the first day, $103 \pm 5\%$ after 1 week and $101.3 \pm 2.2\%$ at the end of 6 weeks. Ribeiro et al. 1996 [55]

is another such important study, reporting ACE inhibition of 90% 3-5 hours after enalapril administration and about 50% at the 24-hour mark.

Lees et al. 1987 [35] compared the pharmacodynamics between young and elderly patients and found that ACE inhibition was similar in both groups. They also reported that older patients had a greater fall in blood pressure than younger patients. However, they attributed this to the higher baseline blood pressure, as the fall in blood pressure was not apparent when normalised and expressed as a percentage.

1.5 Factors affecting enalapril pharmacokinetics & pharmacodynamics

Understanding the factors that influence the pharmacokinetics and pharmacodynamics of enalapril is essential to optimise dosing and therapy, thereby increasing treatment efficacy and avoiding adverse effects.

There are studies which have shown that hypertension does not affect the pharmacokinetics of enalapril or enalaprilat [72], but other factors such as age [20, 35, 40] and diseases such as renal impairment [12, 26, 38] or hepatic impairment [2, 51] do have a marked effect.

Hockings et al. 1986 [20], Lees et al. 1987 [35], MacDonald et al. 1993 [40] studied the age-related effects on the pharmacokinetics and dynamics of enalapril and enalaprilat. Hockings et al. 1986 reported no differences in pharmacokinetics or ACE inhibition between young and elderly groups. Lees et al. 1987 report a similar finding to that mentioned above. MacDonald et al. 1993 reported a higher area under the curve (AUC) for enalaprilat.

1.5.1 Renal impairment

Renal dysfunction can have a marked effect on the pharmacokinetics of drugs, especially if they are eliminated by renal excretion, as is the case with enalapril and enalaprilat.

Fruncillo et al. 1987 [12] studied the pharmacokinetics of enalaprilat in 4 groups of patients with varying degrees of renal impairment. They found a gradual increase in peak serum enalapril concentrations with progressive

reduction in renal activity, which was attributed to reduced urinary elimination of enalapril. This in turn leads to a higher proportion of enalapril being converted to enalaprilat. Kelly et al. 1986 [26] also included 4 groups of patients with varying degrees of renal impairment and found that renal impairment correlated with high serum enalaprilat concentrations, longer times to maximum concentration and lower rates of urinary elimination. Lowenthal et al. 1985 [37] had patients with 3 degrees of renal impairment and their results also report an increase in the metabolism of enalapril to enalaprilat, with higher plasma enalaprilat concentrations and lower excretion rates of enalapril and enalaprilat.

1.5.2 Hepatic impairment

Hepatic dysfunction can have a marked effect on the pharmacokinetics of drugs, especially if they are metabolised by the liver, as is the case with the hepatic conversion of enalapril to enalaprilat via CES1.

Ohnishi et al. 1989 [51] studied the pharmacokinetics and dynamics of enalapril and enalaprilat in 7 cirrhotic and 7 healthy patients. They found that the C_{max}, AUC, and urinary excretion of enalapril and enalaprilat were higher and lower, respectively, in cirrhotic patients than in healthy patients. Baba et al. 1990 [2] included 7 patients with cirrhosis and 10 healthy patients and they tried to confirm the possibility of reduced hepatic de-esterification of enalapril. However, they found that the pharmacokinetics (C_{max}, t_{max}) and ACE inhibition were not significantly different between the two groups of patients. They concluded that cirrhosis does not affect the hepatic metabolism of enalapril.

1.5.3 CES1 genotypes

Genotypes of key enzymes involved in the ADME of drugs can have a marked effect on pharmacokinetics, e.g. drug transporters or key metabolising enzymes such as CES1 in the case of enalapril. There are a few studies that have investigated the effect of CES1 variants on the pharmacokinetics of enalapril.

Her et al 2021 [19] focused on the G143E nonsynonymous mutation,

which is a loss-of-function mutation that causes reduced activity of CES1. They found that enalapril metabolism was significantly impaired. The pharmacokinetics of enalapril also showed an effect of the mutation and the C_{max} and $AUC_{0-\infty}$ were found to be 30.5% and 27.5% lower, respectively, than in the case of wild-type CES1. T_{max} was also found to be 30.7% higher in the G143E mutation than in the wild type. They also found a significantly greater decrease in systolic blood pressure in the wild-type (14.6 ± 13.13 mmHg) than in the G143E patients (-1.0 ± 10.68 mmHg).

Tarkiainen et al. 2015 [68] also studied the G143E mutation and its effect on enalapril pharmacokinetics. They found reduced urinary enalaprilat concentrations and a 20% decrease in $AUC_{0-\infty}$ in the G143E mutation compared to the wild type.

Stage et al. 2017 [64] was a more comprehensive study that analysed 6 variants: wild type with two copies of CES1 without non-synonymous SNPs; a variant with four copies of CES1; a variant with the G143E mutation; a variant with three copies of CES1 (CES1A2); the CES1A1c variant; a variant with three copies of CES1 and the common promoter with low transcriptional activity of the duplication. The 4-copy variant, the G143E variant and the 3-copy variant with low transcriptional activity all show reduced enzymatic activity. The CES1A2 variant is known to have low transcriptional activity, but in some cases the enzymatic activity is the same or even increased. The CES1A1c variant produces mRNA with a variation in the Kozak sequence that affects translation, and it also has non-synonymous SNPs. They reported that the median AUC for enalaprilat for the wild-type group was not significantly different from any of the variants. The $T_{1/2}$ was found to be longer for the 3-copy variant with low transcriptional activity than for the wild-type gene.

1.6 Questions, scope and hypotheses

This project aims to address several important questions regarding the pharmacokinetics and pharmacodynamics of enalapril, in particular how they are influenced by a variety of patient-specific factors. Key questions include: How do individual characteristics such as age, sex and body weight affect

the behaviour and efficacy of the drug? How do general health and organ-specific diseases affect the metabolism and effectiveness of enalapril? How do genetic variations affect the metabolism of enalapril in the liver?

Given the scope of these questions, this study aims to develop a physiologically based pharmacokinetic model for enalapril using existing data from healthy subjects and patients with hypertension, renal impairment and hepatic impairment. This comprehensive approach will allow a comparative analysis across health states to understand how hepato-renal impairment and CES1 activity affect the distribution, metabolism and excretion of the drug.

The hypotheses driving this research are:

- Variations in health status significantly alter the pharmacokinetics of enalapril.
- Impairments in renal and hepatic function lead to reduced metabolism and excretion of enalapril and alterations in its active metabolite enalaprilat, potentially increasing drug toxicity.
- The developed model will accurately simulate the pharmacokinetics of enalapril in different patient groups, providing insights that could lead to more personalised treatment strategies.

In addition to filling gaps in current knowledge, the model will provide a robust tool for predicting drug behaviour in different clinical scenarios, thereby improving the efficacy and safety of enalapril treatment.

2 Methods

The methods used in this work consisted of a systematic literature search for enalapril (Sec. 2.1), followed by data curation (Sec. 2.2), simulation of data for each study (Sec. 2.5), parameter optimisation by fitting simulated data to reference data (Sec. 2.4), parameter scans for renal function, liver function and CES1 activity (Sec. 2.6) and calculation of pharmacokinetic parameters (Sec. 2.7).

2.1 Systematic literature research

A systematic literature search was conducted to select studies that included data on the pharmacokinetics of enalapril under different conditions. PubMed was searched using the keywords `enalapril` `AND` `pharmacokinetics` and PKPDAI [14] using the keyword `enalapril` to retrieve an initial corpus of literature on the pharmacokinetics of enalapril. Available PDFs were retrieved for the literature. Articles on clinical trials of enalapril were selected based on pre-specified inclusion criteria. In addition to healthy subjects, trials in patients with hypertension, renal, cardiac and hepatic impairment were included. Studies in paediatric patients and animal studies were excluded. Fig. 3 provides an overview of the literature review process.

In addition, the literature was searched for *in vitro* studies to determine kinetic parameters and enzyme kinetic information.

2.2 Data curation

Data from the selected literature were curated and uploaded to the open pharmacokinetics database PK-DB [16]. The articles were screened for patient information such as age, sex, specific diseases, drugs and genotypes, enalapril dosing protocol and enalapril pharmacokinetic profiles. These data were then curated using standard protocols for pharmacokinetic information [16]. Data from figures were digitised using WebPlotDigitizer [56]. Data from tables and textual descriptions were also curated in a specific format as described in [16].

Data were systematically curated according to the following structure: (i)

Groups: Patient groups were entered into the database with relevant group characteristics such as age, height, weight, sex and ethnicity. Information on the degree of cardiac, renal or hepatic impairment and other diseases was coded. (ii) Individuals: For some trials, individual patient characteristics were reported. Information on individual subject characteristics was recorded in the same way as for the groups. (iii) Interventions: In each trial, enalapril was given to patients either as an oral dose in the form of a tablet or capsule, or as an intravenous dose in the form of a solution. Information on dose, time of administration (if multiple doses were administered), and route of administration were recorded. (iv) Time course data: In each study, the authors reported a number of different parameters. The main data of interest were the time courses of concentrations and amounts of enalapril or its metabolite enalaprilat in serum, plasma and urine. (v) Pharmacokinetic/pharmacodynamic data: In addition, many studies reported pharmacokinetic parameters such as C_{max}, T_{max} and $T_{1/2}$ and pharmacodynamic parameters such as systolic and diastolic blood pressure, heart rate, mean arterial pressure and substances such as renin, angiotensin I and II, and aldosterone.

The extensive heterogeneous data set provided the database for model development and validation. All data are available in PK-DB (`https://pk-db.com`) [16] with an overview of the curated studies in Tab. 3.1.

2.3 Model

The PBPK and tissue models were developed in the Systems Biology Markup Language (SBML) [22, 25]. Software such as sbmlutils [32] and cy3sbml [33] were used for the programmatic manipulation and visualisation of the models. The models are ordinary differential equation (ODE) models solved numerically using sbmlsim [31] based on the high performance SBML simulator libroadrunner [63, 81]. The model is made available in SBML under a CC-BY 4.0 licence from `https://github.com/matthiaskoenig/enalapril-model` with model equations available from the repository. The version of the model used in this thesis is 0.9.5 [52].

The PBPK model developed consists of a whole-body model linking different organs via the systemic circulation (Fig. 4). The PBPK model fol-

lows a hierarchical structure, with the whole-body model linking sub-models for the intestine, kidney and liver. The model describes the metabolic and biochemical pathways in the tissue consisting of the transport of enalapril through the intestine, liver and kidney. Transport processes and biochemical reactions were modelled using reversible and irreversible Michaelis-Menten equations (e.g. Eq. 1) or mass action kinetics (e.g. Eq. 2).

$$v_{mmk} = \frac{V_{max} \cdot [S]}{K_m + [S]} \tag{1}$$

$$v_{ma} = k \cdot [S] \tag{2}$$

v_{mmk} and v_{ma} are the respective reaction or transport rates, where $[S]$ is the substrate concentration. V_{max} is the maximum reaction rate, K_m is the Michaelis-Menten constant, k is the forward rate constant.

Hepatic functional impairment Liver impairment was modelled as a gradual increase in cirrhosis by scaling liver function with the parameter `f_cirrhosis` from 0.0 (no cirrhosis) to 0.95 (critical cirrhosis). The cirrhosis implementation is based on a recent indocyanine green model of cirrhosis [30, 29] with parameters for mild (0.40), moderate (0.70) and severe cirrhosis (0.81) corresponding to the Child-Pugh-Turette classes CPT A, CPT B and CPT C respectively. The Child-Turcotte-Pugh (CTP) score is a quantifier used to estimate the likelihood of death in cirrhotic patients. The original system given by Child and Turcotte had five criteria to categorise patients: serum bilirubin, serum albumin, ascites, neurological disorder and clinical nutritional status [5]. It was later modified by Pugh, who replaced clinical nutritional status with prothrombin time [54]. Cirrhosis is modelled as a combination of a reduction in functional liver volume and shunting of blood around the liver, both of which lead to a reduction in blood flow.

Renal functional impairment Renal impairment was modelled as a progressive decline in renal function by scaling all renal processes with the factor `f_renal_function`, where 1.0 represents normal function and 0.0 represents no renal function. The cut-offs for the different stages of renal im-

pairment were based on the international KDIGO guidelines [65] with mild renal impairment (0.69), moderate renal impairment (0.32) and severe renal impairment (0.19) [42].

Cardiac functional impairment For the cardiac impairment simulations the cardiac output was reduced via the factor `f_cardiac_output` = 0.70, i.e. the total blood flow was reduced via 30%.

CES genotypes Changes in CES1 activity were modelled by scaling the CES1 metabolic conversion rate with the factor `f_ces1`, where 1.0 represents normal function corresponding to wild type, values > 0 correspond to increased function, values < 0 to reduced CES1 function. The following alleles were modelled: wild type (1.0), G143E (0.1), active allele (1.2), CES1A1C (1.0). The total CES1 function was then calculated as the combination of the existing alleles. For example, for WT/G143E as (1.0 + 0.1)/2.

2.4 Parameter optimization

Parameter fitting was used to minimise the distance between experimental data and model predictions by optimising a subset of ten parameters of the model. For this purpose, a subset of curated time curves from healthy and hypertensive subjects after single dose application was used, as listed in Tab. 3.1. Parameters were optimised in a multistep process based on the route of administration. First, parameters relevant to intravenous enalaprilat were optimised using the subset of intravenous data. Next, parameters for oral enalapril were optimised using a subset of the oral enalapril data. Finally, metabolic conversion and liver transport parameters were optimised. This strategy allowed the parameters of enalaprilat and enalapril to be determined separately with subsequent optimisation of the coupling step.

The cost function, which depends on the parameter $\vec{p}$, minimised the sum of the quadratic weighted residuals $r_{i,k}$ for all time courses k and data points i. Time courses were weighted by the number of participants in each study n_k and individual time points with the standard deviation $\sigma_{i,k}$ associated with the measurement, resulting in weights $w_{i,k} = n_k/\sigma_{i,k}$.

$$F(\vec{p}) = 0.5 \sum_{i,k} (w_{i,k} \cdot r_{i,k}(\vec{p}))^2$$

Multiple optimisation runs (n=100) were performed with different initial parameters based on a local optimiser, with the optimal parameters used in the final model. The fitted parameters are shown in Tab. 3. For validation, all data after multiple applications, disease states, CES1 activity other than wild-type were used.

2.5 Simulations

For all curated clinical trials (Tab. 3.1), *in silico* simulation experiments were created. For the simulations, the parameters corresponding to the the genetic variants (`f_ces1`), pathophysiology (`f_renal_function`, `f_cirrhosis`, `f_cardiac_output`), and the dosing (`PODOSE_ena`, `IVDOSE_ena`, `PODOSE_eat`, `IVDOSE_eat`) were modified according to the study. In the case of multiple dosing, the appropriate doses were given at the specified times. The model was then used to simulate the time courses of enalapril and enalaprilat in plasma and urine, and clinical data were compared with the model predictions.

2.6 Parameter scans

Parameter scans were performed for three main cases: liver impairment, kidney impairment and CES1 gene activity. The parameters were scanned in the following ranges: `f_cirrhosis` in `linspace(0, 0.9, num=19)` with normal liver function corresponding to 0.0; `f_renal_function` in `logspace(-1, 1, num=19` with normal kidney function corresponding to 1. 0; `f_ces1` in `logspace(-1, 1, num=19` with normal CES1 function corresponding to 1.0; Simulations were performed for a single oral dose of enalapril of 20 mg.

2.7 Pharmacokinetic parameters

Pharmacokinetic parameters of enalapril and enalaprilat were calculated from plasma concentration time curves and urinary excretion using standard

non-compartmental methods. The elimination rate k_{el}[1/min] was calculated by linear regression in logarithmic space in the decay phase. The area under the curve AUC [mmole·min/L] was calculated using the trapezoidal rule and extrapolated to infinity by linear interpolation. Apparent clearance Cl [ml/min] was calculated as $Cl/F = k_{\mathrm{el}} \cdot V_{\mathrm{d}}$ with apparent volume of distribution $V_{\mathrm{d}}/F = D/(AUC_{\infty} \cdot k_{el})$. D is the applied dose of enalapril maleate.

3 Results

3.1 Database of enalapril pharmacokinetics

In this work, an extensive database of enalapril pharmacokinetics was established and used to develop and validate a physiologically based pharmacokinetic (PBPK) model of enalapril.

A comprehensive literature search on the pharmacokinetics of enalapril was performed in the literature databasesPKPDAI [14] and PubMed. From over 600 trials assessed, 91 were prioritised according to the following criteria: (1) the study had to be conducted in humans; (2) the study had to be conducted in adult subjects; (3) the study had to be conducted *in vivo*; (4) the study had to report time-course data on plasma or serum and urine concentrations of enalapril and/or its metabolite enalaprilat. Particular attention was paid to studies in subjects with hypertension and renal, hepatic and cardiac impairment. Based on these criteria, 49 studies were selected for data curation, which formed the database for the development and evaluation of the PKDB model. Fig. 3 provides an overview of the study selection process.

Data was curated from Arafat et al. 2005 [1], Baba et al. 1990 [2], Biollaz et al 1982 [4], Dayyih et al. 2017 [7], Dickstein et al. 1987 [8], Donnelly et al. 1990 [10], Fruncillo et al. 1987 [12], Ghosh et al. 2012 [13], Gu et al. 2004 [17], He et al. 2014 [18], Her et al. 2021 [19], Hockings et al. 1986 [20], Howes et al. 1991 [21], Ishizaki et al. 1988 [23], Johnston et al. 1992 [24], Kelly et al. 1986 [26], Lee et al. 2003 [34], Lees et al. 1987 [35], Lowenthal et al. 1985 [37], Lu et al. 2009 [39], MacDonald et al. 1993 [40], Marzo et al. 2002 [43], Matalka et al. 2002 [44], Moffett et al. 2014 [45], Mujais et al. 1992 [46], Najib et al. 2003 [47], Niopas et al. 2004 [49], Oguchi et al. 1993 [50], Ohnishi et al. 1989 [51], Portoles et al. 2004 [53], Ribeiro et al. 1996 [55], Schwartz et al. 1985 [58], Shionoiri et al. 1985 [59], Shioya et al. 1992 [60], Stage et al. 2017 [64], Sunaga et al. 1995 [66], Swanson et al. 1984 [67], Tarkiainen et al. 2015 [68], Thongnopnua et al. 2005 [70], Till et al. 1984 [71], Tsuruoka et al. 2007 [73], Ulm et al. 1982 [75], VanHecken et al. 2020 [76], Wade et al. 1992 [78], Weisser et al. 1991 [80], Weisser et al. 1992 [79], and Witte et al. 1993 [82].

Tab. 3.1 provides an overview of the curated studies and key information such as number of subjects, dosing protocol, route of administration, diseases, physiological impairments and genetic variants. All data have been uploaded to the pharmacokinetics database PK-DB [16] and are freely accessible via the PBPK identifier.

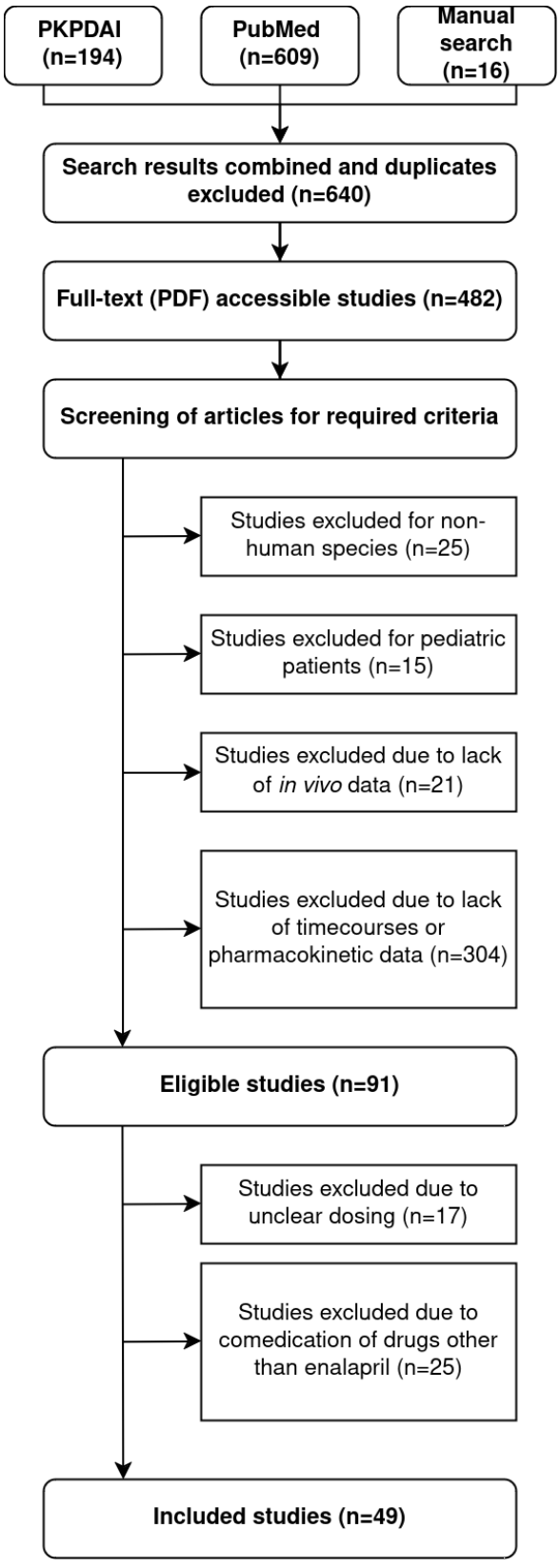

Figure 3: **Overview of the literature search and selection of studies for data curation.** Publications related to enalapril pharmacokinetics were retrieved using PKPDAI and PubMed searches. Studies with full text available in PDF format were screened. Eligible studies included in vivo pharmacokinetic data in adult humans. Studies with unclear dosing and coadministration of other drugs were excluded from data curation.

Table 2: **Overview of curated clinical studies.** po: oral, iv: intravenous

Study	PK-DB	PMID	route	substance	dosing	dose [mg]	healthy	hyper-tension	renal im-pair-ment	hepatic im-pair-ment	cardiac im-pair-ment	CES1
Arafat2005 [1]	PKDB00741	15985045	po	enamal	single	10	✓					
Baba1990 [2]	PKDB00740	2165799	po	enamal	single	10	✓			✓		
Biollaz1982 [4]	PKDB00768	6289859	po	enamal	single	10	✓					
Dayyih2017 [7]	PKDB00769	28894622	po	enamal	single	20	✓					
Dickstein1987 [8]	PKDB00770	3034316	po, iv	enamal, eat	single	5, 10	✓				✓	
Donnelly1990 [10]	PKDB00771	2154407	po	enamal	single, multi	20		✓				
Fruncillo1987 [12]	PKDB00773	3037160	po	enamal	single, multi	5		✓	✓			
Ghosh2012 [13]	PKDB00746	21341376	po	enamal	single	20	✓					
Gu2004 [17]	PKDB00747	15556550	po	enamal	single	10	✓					
He2014 [18]	PKDB00774	24721587	po	enamal	single	40	✓					
Her2021 [19]	PKDB00775	33963573	po	enamal	multi	2.5	✓					wt, G143E
Hockings1986 [20]	PKDB00776	3011046	po, iv	enamal, eat	single	10	✓		✓			
Howes1991 [21]	PKDB00777	1932608	po	enamal	single	10	✓					
Ishizaki1988 [23]	PKDB00750	2468049	po	enamal	single	10	✓					
Johnston1992 [24]	PKDB00778	1329474	po	enamal	single, multi	2.5					✓	
Kelly1986 [26]	PKDB00779	3004546	po	enamal	single, multi	10	✓		✓			
Lee2003 [34]	PKDB00780	12772271	po	enamal	single	20	✓					
Lees1987a [35]	PKDB00781	3034468	po	enamal	multi	10	✓		✓			
Lowenthal1985 [37]	PKDB00782	2998676	po	enamal	single	10	✓		✓			
Lu2009 [39]	PKDB00745	19046843	po	enamal	single	10	✓					
MacDonald1993 [40]	PKDB00783	9114905	po	enamal	single	10	✓		✓			
Marzo2002 [43]	PKDB00784	12040965	po	enamal	multi	10, 20	✓					
Matalka2002 [44]	PKDB00785	12165071	po	enamal	single	10, 20	✓					
Moffett2014 [45]	PKDB00786	27129124	po	enamal	single	10	✓					
Mujais1992 [46]	PKDB00787	1310827	iv	eat	multi	1.5	✓					
Najib2003a [47]	PKDB00788	14520685	po	enamal	single	20	✓					
Niopas2004 [49]	PKDB00789	15112862	po	enamal	single	20	✓					
Oguchi1993 [50]	PKDB00790	8504625	po	enamal	single	5	✓	✓	✓			
Ohnishi1989 [51]	PKDB00791	2543535	po	enamal	single	10	✓			✓		
Portoles2004 [53]	PKDB00792	24936102	po	enamal	single	20	✓					
Ribeiro1996 [55]	PKDB00793	8839663	po	enamal	single	20	✓					
Schwartz1985 [58]	PKDB00794	2410720	po	enamal	single	2.5, 5, 10, 20, 40		✓			✓	
Shionoiri1985 [59]	PKDB00749	2982051	po	enamal	single	10		✓				
Shioya1992 [60]	PKDB00795	1322206	po	enamal	single	5	✓					
Stage2017 [64]	PKDB00797	28639420	po	enamal	single	10	✓					wt, c4, c3, ca3, G143E, CES1A1c
Sunaga1995 [66]	PKDB00798	8582461	po	enamal	single	5		✓				
Swanson1984 [67]	PKDB00799	6097665	po	enamal	single	40	✓					
Tarkiainen2015 [68]	PKDB00800	25919042	po	enamal	single	10	✓					wt, G143E
Thongnopnua2005 [70]	PKDB00801	15797799	po	enamal	single	5	✓					
Till1984 [71]	PKDB00802	6091806	po	enamal	single, multi	10	✓					
Ulm1982 [75]	PKDB00804	6289858	po	enamal	single	9.8	✓					
VanHecken2020 [76]	PKDB00805	31411383	po	enamal	single	10						
Wade1992 [78]	PKDB00806	1312853	po	enamal	single	10	✓					
Weisser1991 [80]	PKDB00807	1647954	po	enamal	single	10	✓					
Weisser1992 [79]	PKDB00808	1330574	po	enamal	single	10		✓	✓			
Witte1993 [82]	PKDB00809	8394796	po	enamal	single	10		✓				

3.2 PBPK model of enalapril

Using the curated dataset, a physiologically based pharmacokinetic (PBPK) model was developed for enalapril (Fig. 4). The model is hierarchical, with the top layer representing the whole body, including lung, liver, kidney, intestine and finally the rest of the body. The transport of enalapril and enalaprilat is modelled via the systemic circulation. Enalapril can be given orally as enalapril maleate tablets or capsules, or intravenously as enalapril solution. Enalaprilat may be given orally or intravenously. Enalaprilat can be given as a single dose or as multiple doses given at regular intervals, e.g. every day for a week.

After oral administration, the tablet dissolves in the stomach and the drug is released into the intestine. Enalapril is absorbed in the small intestine by passive diffusion without the use of transport proteins. The absorbed drug enters the body through the portal vein. The unabsorbed fraction of enalapril in the intestine is excreted in the faeces (Fig. 4B) [28, 61, 62].

Enalapril is then taken up by the liver with the help of the transport proteins OATP1B1 and OATP1B3 [36]. The metabolism of enalapril to enalaprilat is catalysed by the enzyme carboxylesterase 1 (CES1) [69]. The enalaprilat formed is exported into the systemic circulation by the multi-drug resistance-associated protein 4 (MRP4) [11] (Fig. 4C).

Enalapril and enalaprilat are eliminated by glomerular filtration and tubular secretion via the kidneys (Fig. 4D).

The model is made available in SBML under a CC-BY 4.0 licence from `https://github.com/matthiaskoenig/enalapril-model` with model equations available from the repository. The version of the model used in this thesis is 0.9.5 [52]. An overview of the SBML graphs of the whole body is shown in Fig. 5, the intestine in Fig. 6, the liver in Fig. 7, and the kidneys in Fig. 8.

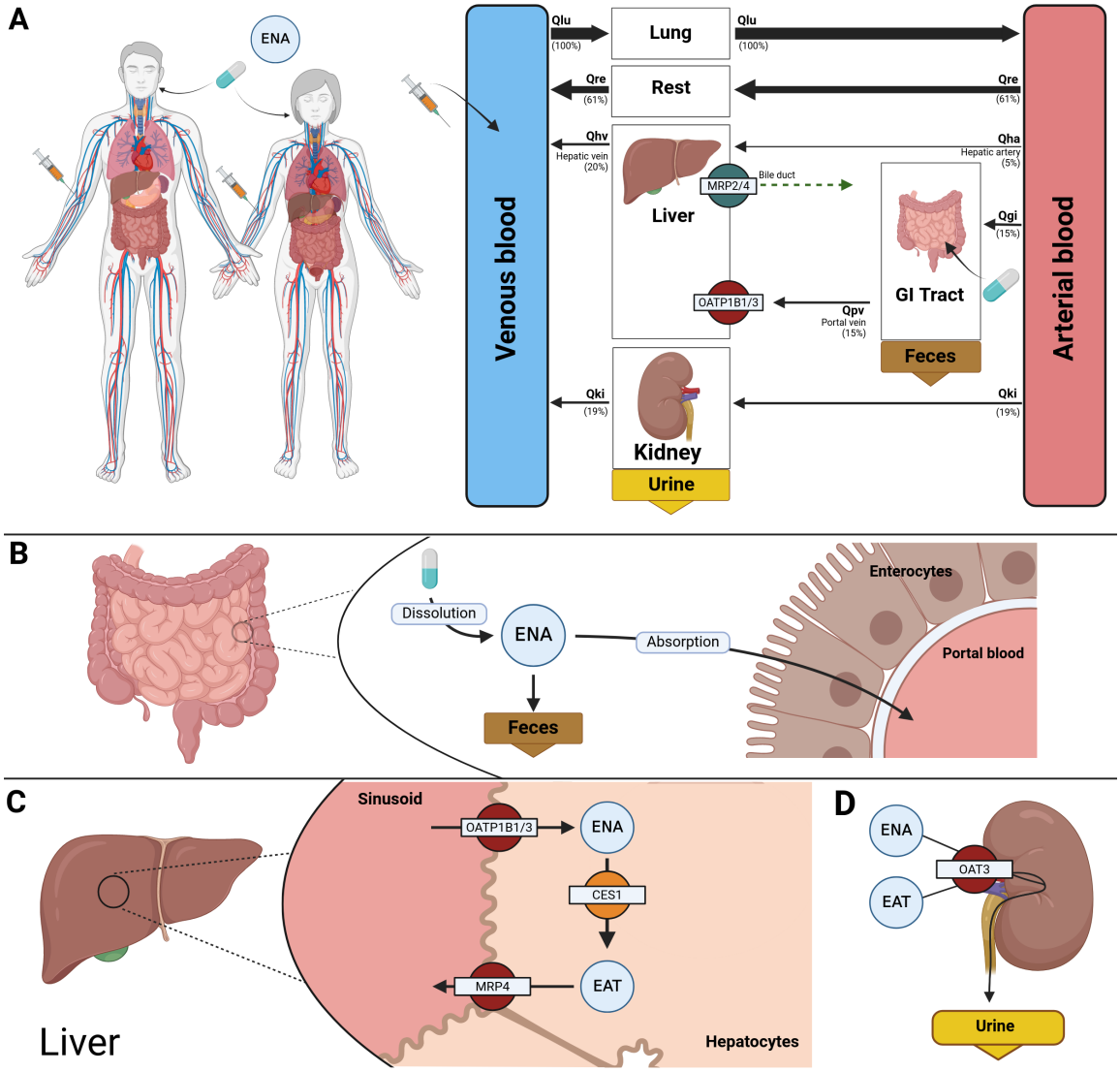

Figure 4: **Overview of the physiologically-based model of enalapril.** **A)** Whole body model showing cardio-pulomonary circulation via the blood. **B)** Intestine model showing dissolution and absorption of enalapril by enterocytes. **C)** Liver model with the uptake of enalapril by hepatocytes and its conversion to enalaprilat. **D)** Kidney model showing transport of enalapril and enalaprilat into the kidney and their subsequent excretion via urine.

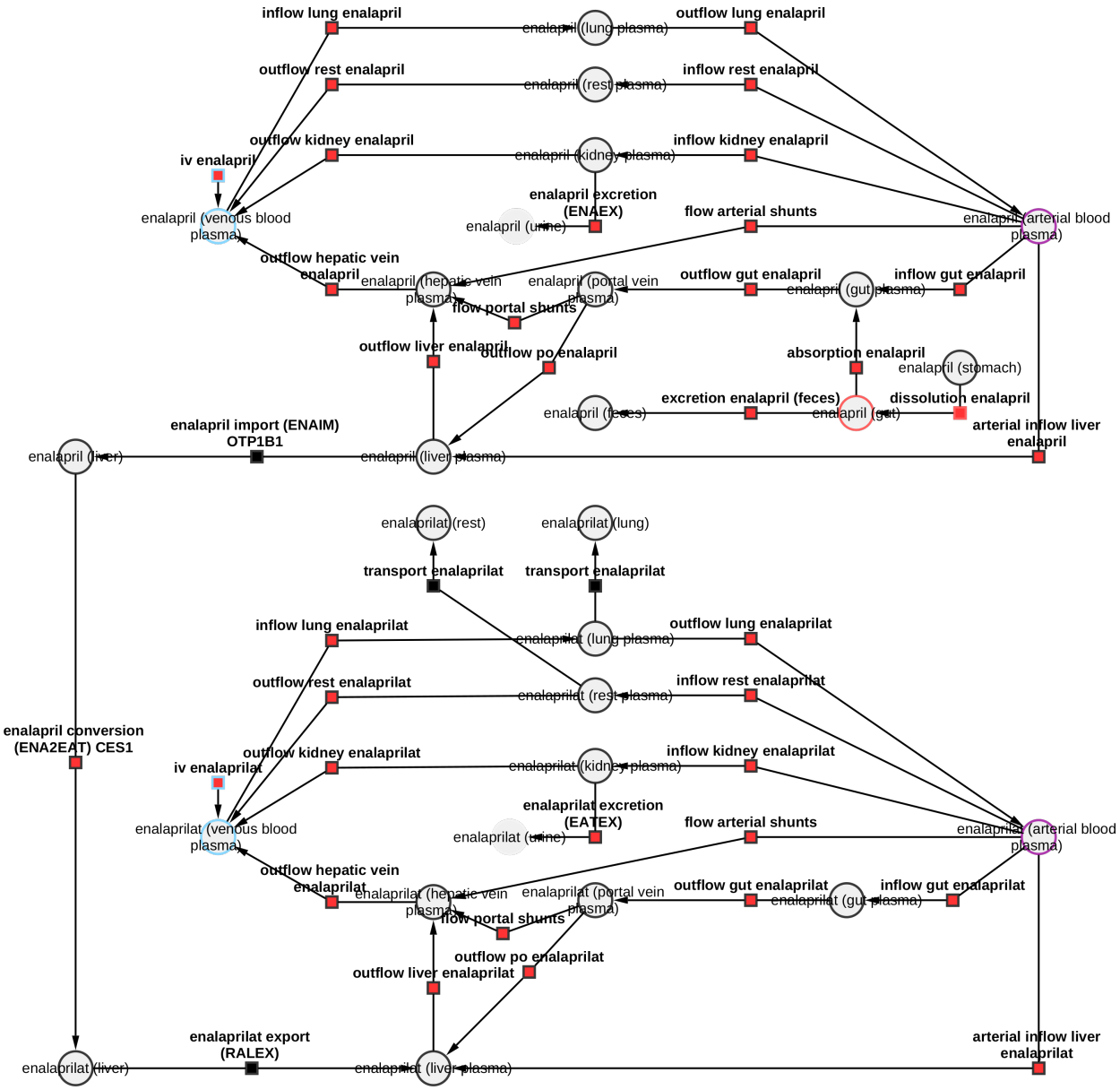

Figure 5: **SBML species-reaction graph of the whole-body model of enalapril.**

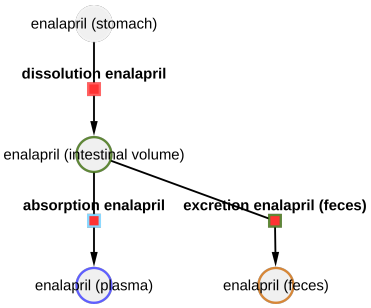

Figure 6: **SBML graph of the intestine model.**

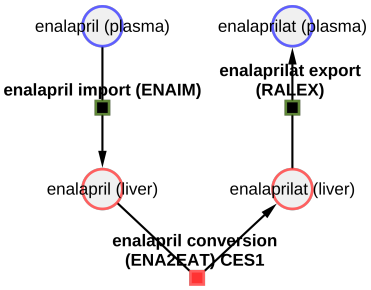

Figure 7: **SBML graph of the liver model.**

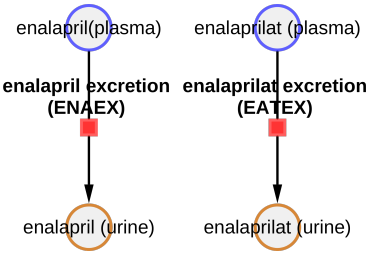

Figure 8: **SBML graph of the kidney model.**

3.2.1 Hepatic and renal functional impairment

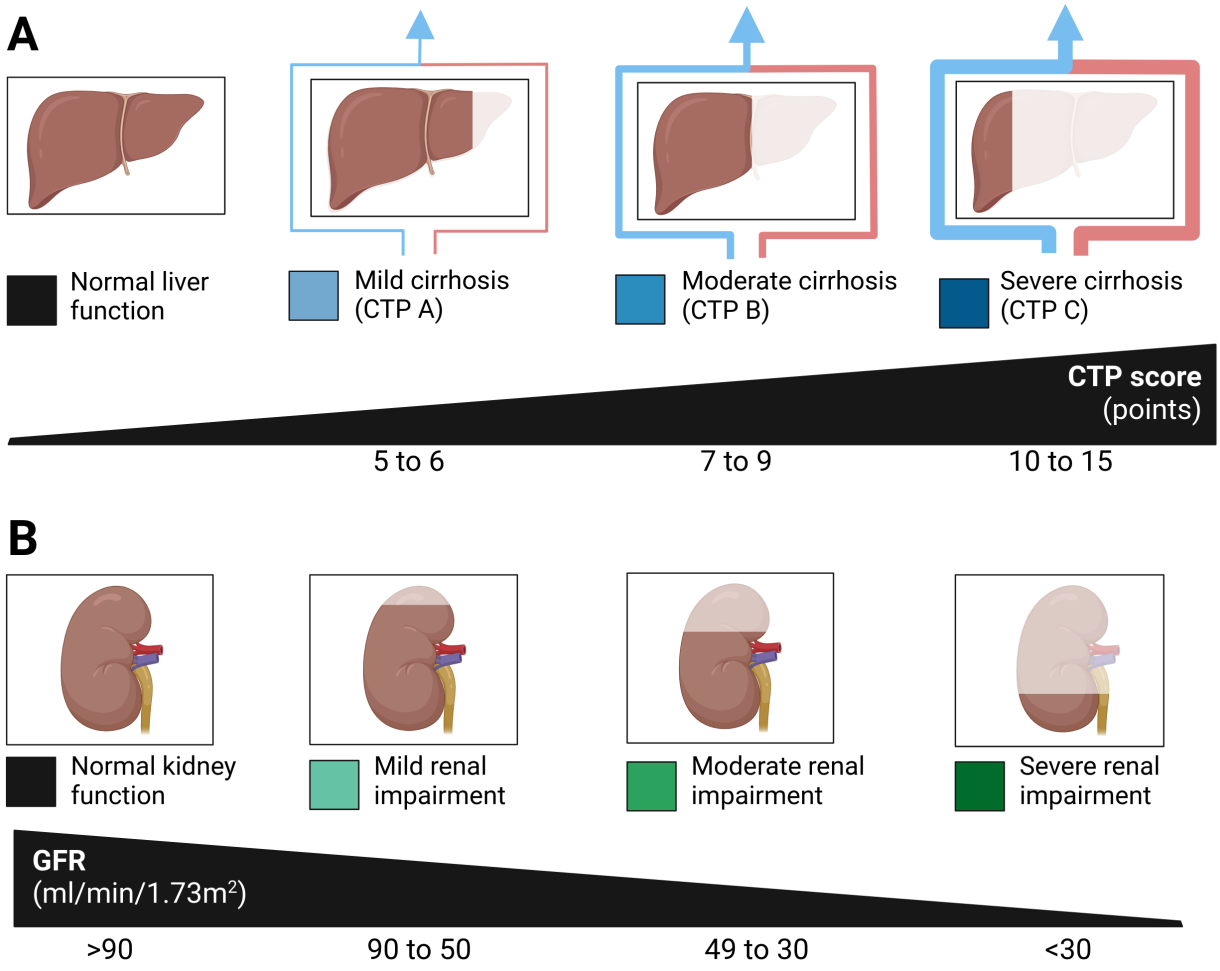

Figure 9: **Overview of hepatic and renal impairment.** **A)** Liver impairment was modelled as a gradual increase in cirrhosis by scaling liver function with the parameter `f_cirrhosis` from 0.0 (no cirrhosis) to 0.95 (critical cirrhosis). Normal liver function: black (1.0), mild cirrhosis: light blue (0.40), moderate cirrhosis: blue (0.7) and severe cirrhosis: dark blue (0.81), corresponding to Child Pugh Turette classes CPT A, CPT B and CPT C respectively. Cirrhosis is modelled as a combination of reduction in functional liver volume and shunting of blood around the liver, both of which result in reduced liver function. **B)** Renal impairment has been modelled as a progressive decline in renal function by scaling all renal processes with the factor `f_renal_function`, where 1.0 represents normal renal function and 0.0 represents no renal function. Normal renal function: black (1.0), mild renal impairment: light green (0.69), moderate renal impairment: green (0.32) and severe renal impairment: dark green (0.19).

Renal and hepatic impairment have far-reaching effects on the pharmacokinetics of drugs. The model has been adapted to simulate different scenarios of hepatic, renal and hepato-renal impairment on the pharmacokinetics of enalapril and enalaprilat (Fig. 9). The figure provides an overview of the different degrees of hepatic and renal impairment considered in our

model.

Accounting for hepatic impairment in a pharmacokinetic model requires quantification of the degree of hepatic impairment. Indocyanine green is a compound that is routinely used in clinical settings to test liver function in subjects. Köller et al 2021 established a pharmacokinetic model of indocyanine green in patients with hepatic impairment in two studies [30, 29]. Thus, the ICG levels reported in the enalapril studies [3, 51] helped us to determine the respective degrees of hepatic impairment.

Similarly, in renal impairment, creatinine clearance or glomerular filtration rate are reliable parameters to assess renal function and hence the degree of renal impairment [20]. The reported values of these renal markers were used to determine the degree of renal impairment.

The resulting PBPK model allows the pharmacokinetics of enalapril and enalaprilat to be simulated under various degrees of hepatic and renal impairment. Similarly, the effect of cardiac impairment and the activity of CES1 on the pharmacokinetics of enalapril and enalaprilat were included in the model.

3.3 Parameter Fitting

Plasma and urine enalapril and enalaprilat time course data from the curated studies [1, 13, 23] were used to fit a subset of model parameters (Tab. 3). Only data from healthy and hypertensive subjects after single application were used for fitting. Tab. 3.1 provides information on individual study protocols with additional information on application and doses. Fitting was performed in three steps to fit different subsets of parameters: i) intravenous administration of enalaprilat; ii) oral administration of enalapril; iii) metabolism of enalapril to enalaprilat.

Table 3: **Parameters fitted in the model.**

Parameter	Description	Optimal value	Lower bound	Upper bound	Unit
Intravenous administration of enalaprilat					
KI__EATEX_k	rate of urinary excretion of enalaprilat	0.527922	0.1	10	$\frac{1}{min}$
ftissue_eat	tissue distribution of enalaprilat	9.995439	0.01	10	$\frac{l}{min}$
K_p_eat	partition coefficient between tissue and plasma for enalaprilat	0.101726	0.01	1	dimensionless
Oral administration of enalapril					
GU__ENAABS_k	rate of absorption of enalapril in the intestine	0.100000	0.1	100	$\frac{1}{min}$
ftissue_ena	tissue distribution of enalapril	9.999987	0.01	10	$\frac{l}{min}$
K_p_ena	partition coefficient between tissue and plasma for enalapril	0.304908	0.1	10	dimensionless
LI__ENAIM_Vmax	V_{max} for uptake of enalapril in the liver	0.748446	0.001	100	$\frac{mmol \cdot l}{min}$
KI__ENAEX_k	rate of urinary excretion of enalapril	0.910320	0.001	10	$\frac{1}{min}$
cMetabolism of enalapril to enalaprilat					
LI__ENA2EAT_V_{max}	rate of conversion of enalapril to enalaprilat in the liver	0.640305	0.001	100	$\frac{mmol \cdot l}{min}$
LI__EATEX_V_{max}	rate of export of enalaprilat out of the liver	0.001480	0.001	100	$\frac{mmol \cdot l}{min}$
LI__EATEX_K_m_eat	Michaelis constant for export of enalaprilat out of the liver	0.430425	0.001	1	mM

3.3.1 Intravenous administration of enalaprilat

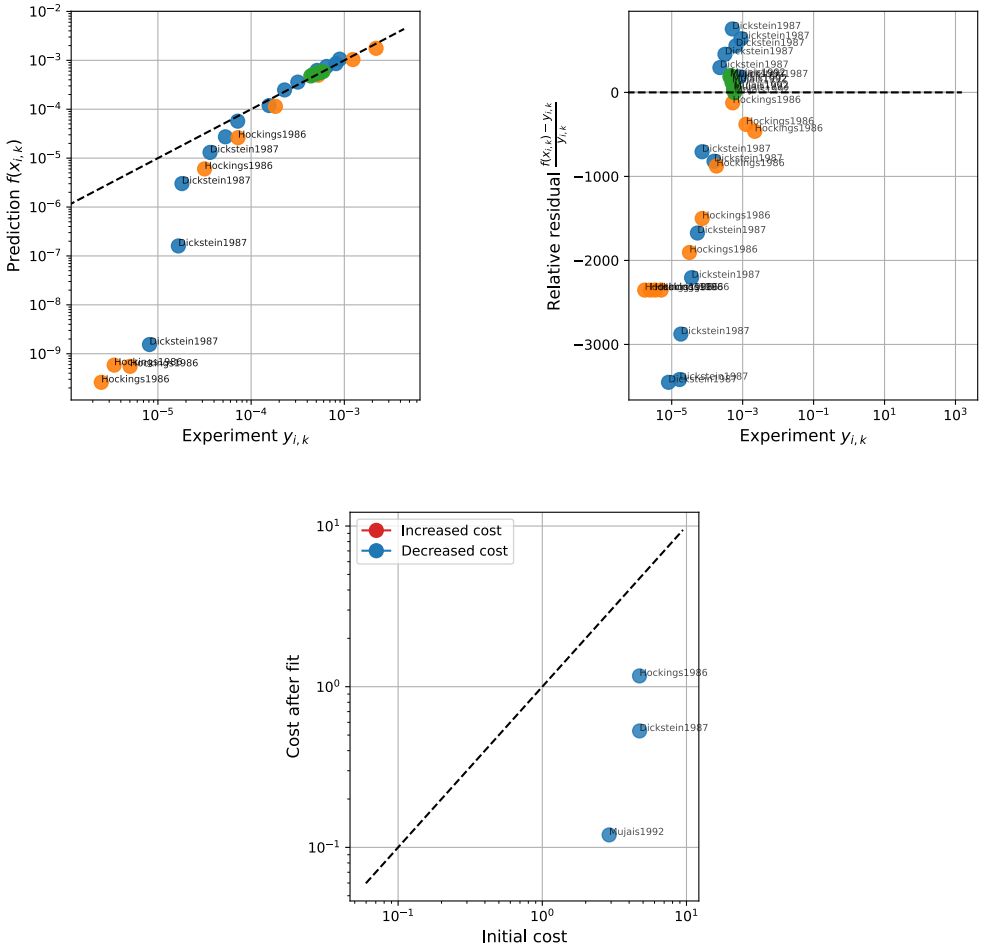

Figure 10: **Parameter fitting results for intravenous dose of enalaprilat.** The top left panel shows the goodness of fit plot between model prediction and experimental data for the resulting optimal parameters. The top right panel shows the weighted residuals of the optimal parameter fit for the data used in the parameter fitting (table 3). The bottom panel shows the initial vs. final cost of fitting, and all three studies showed a decrease in cost for all three studies.

The first round of fitting was done for intravenous enalaprilat using only data from such subjects and only data on enalaprilat concentrations in plasma and urine. Fig. 10 shows an overview of the results of the parameter fitting consisting of a goodness-of-fit plot and residuals. Parameter fitting improved the agreement between data and model predictions, with the final model showing good agreement between experimental data and model predictions as can be seen from the cost function.

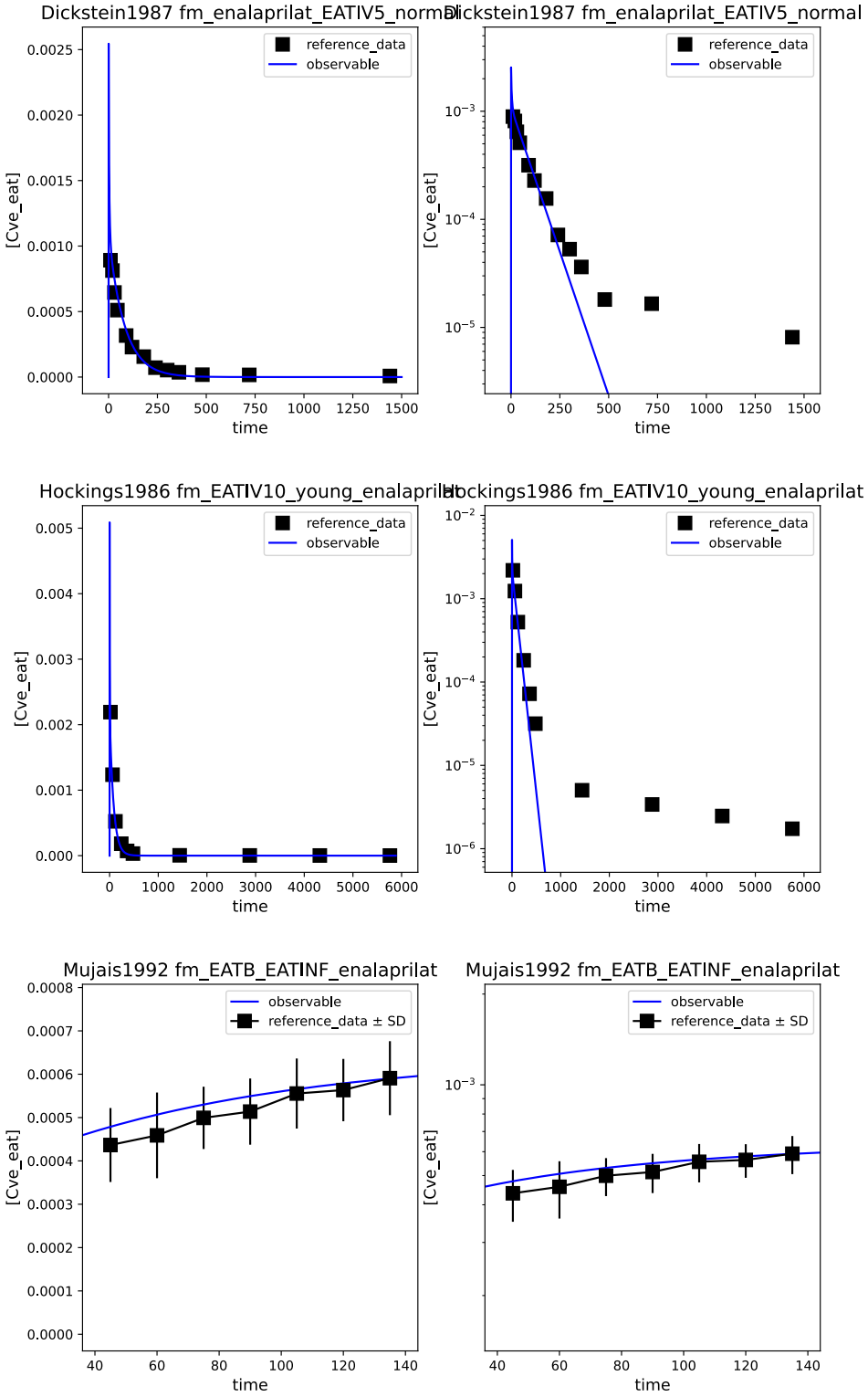

Figure 11: **Parameter fitting results for intravenous dose of enalaprilat.**

Fig. 11 shows plots for each of the fitted studies with the simulated observed data and the reference data. All three studies use an intravenous dose of enalaprilat. They could therefore be used together to perform the fitting

and validate the optimal parameters for enalaprilat. It can be seen that after fitting, the observed data were in agreement with the reference data. For Mujais et al 1992 [46], the authors also reported the standard deviations and we can see that the observed data may not be exactly in line with the reference data points, but they are within the error bars.

3.3.2 Oral administration of enalapril

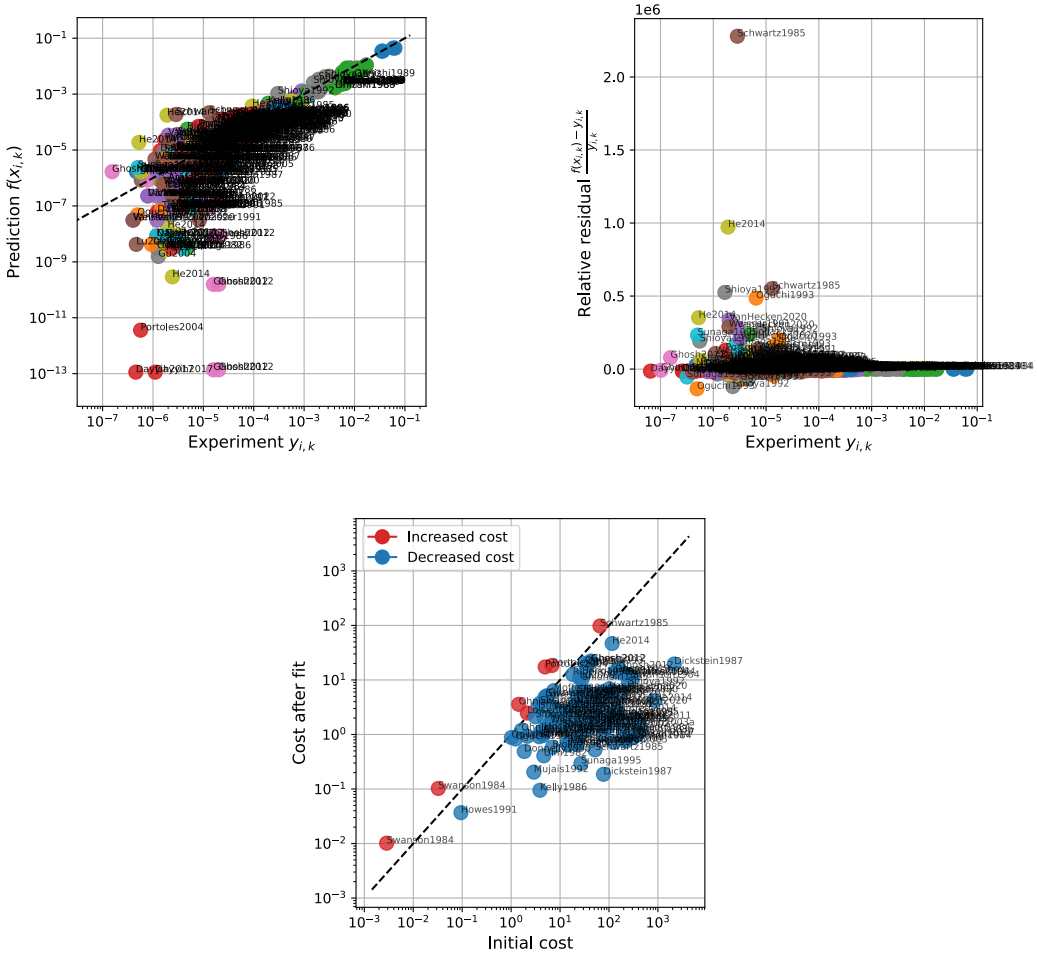

Figure 12: **Parameter fitting results for oral dose of enalapril.**

The second round of fitting was done for oral enalapril using data on enalapril concentrations in plasma and urine. Fig. 12 shows the residual plots for this fitting. Most of the reference data points are in close agreement with the predicted data. The fitting runs show a decrease in cost for most studies, with a few studies showing slightly more than optimal cost values. Fig. 11 shows the simulations for two studies (representative) for the

optimal parameters. Oguchi et al. 1993 [50] show a very good fit between the reference data and the simulated data. Even for Biollaz et al. 1982 [4], where the fit is not optimal, the simulated data show the same trend as the reference data and the simulation is within the standard deviation of the reference data. The parameters for the oral dose of enalapril have thus been optimised.

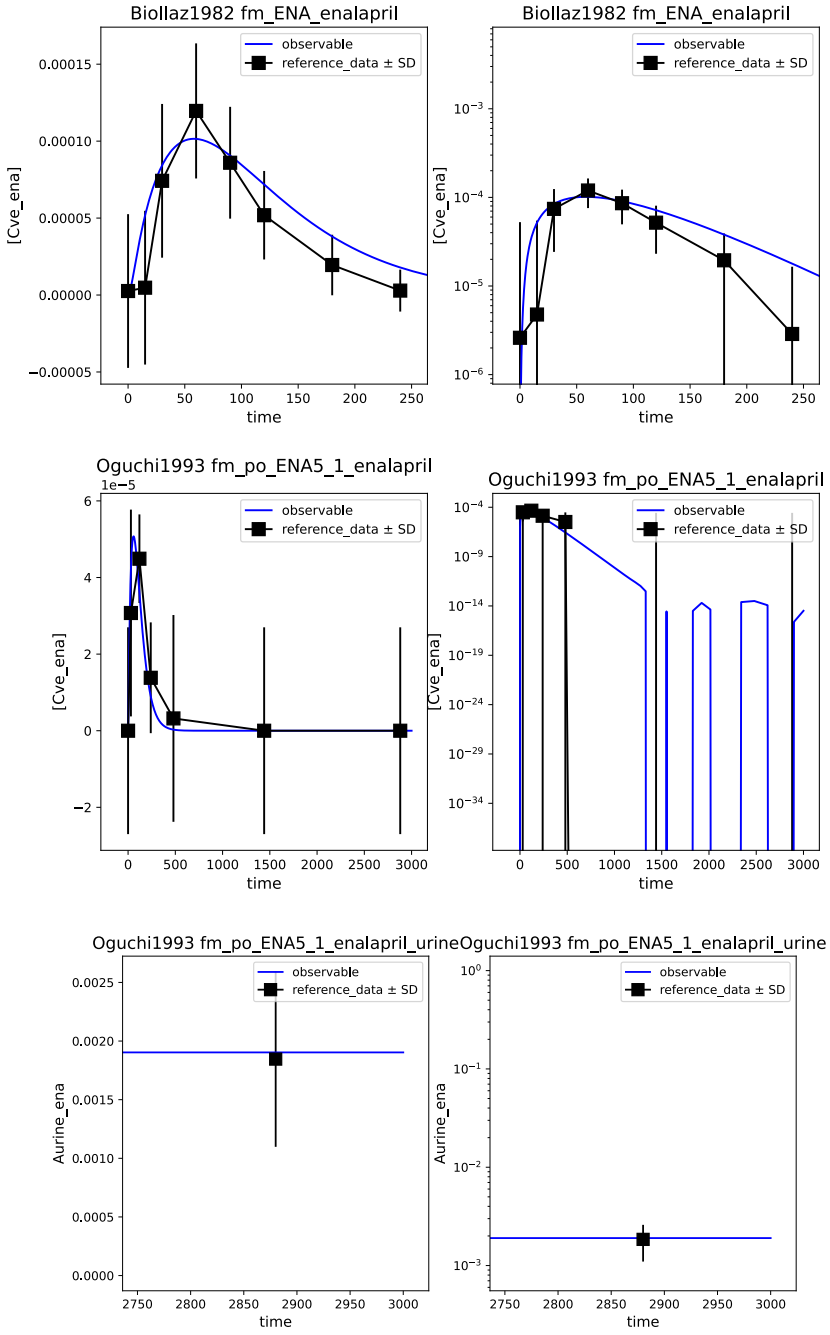

Figure 13: **Parameter fitting results for oral dose of enalapril.**

3.3.3 Metabolism of enalapril to enalaprilat

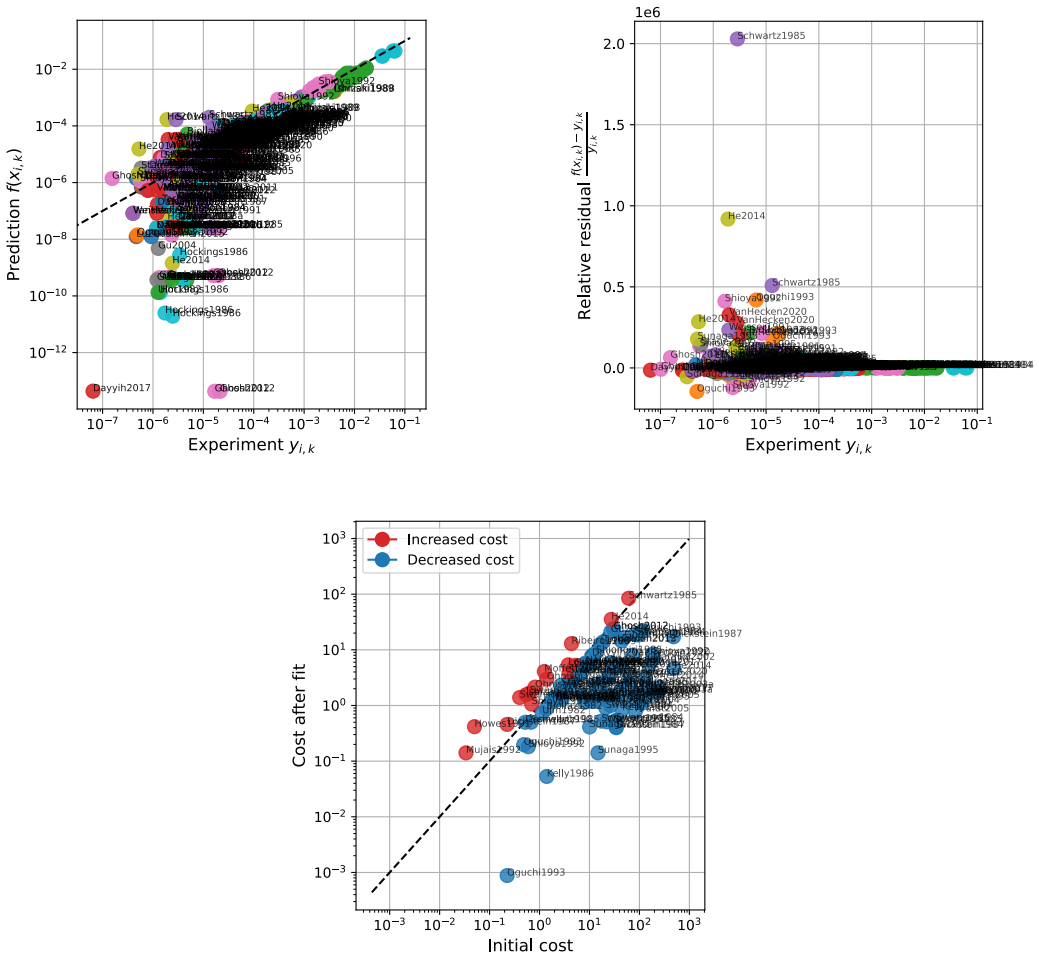

Figure 14: **Parameter fitting results for enalapril to enalaprilat metabolism.**

The final round of fitting focused on the conversion of enalapril to enalaprilat. The predicted data agree with the reference data except for a few points 14. This adjustment resulted in reduced costs in most cases, but also increased costs in some cases. Fig. 11 shows the representative plots, Ohnishi et al. 1989 [51] and Kelly et al. 1986 [26], for enalaprilat concentrations. For Ohnishi et al. 1989, the simulations are not in complete agreement with the reference data, but the general trend is followed and the simulation is within the standard deviation. The simulation of plasma and urine enalaprilat concentrations in Kelly et al. 1986 is in almost perfect agreement with the reference data, demonstrating that all rounds of fitting have contributed to a significant improvement in the model.

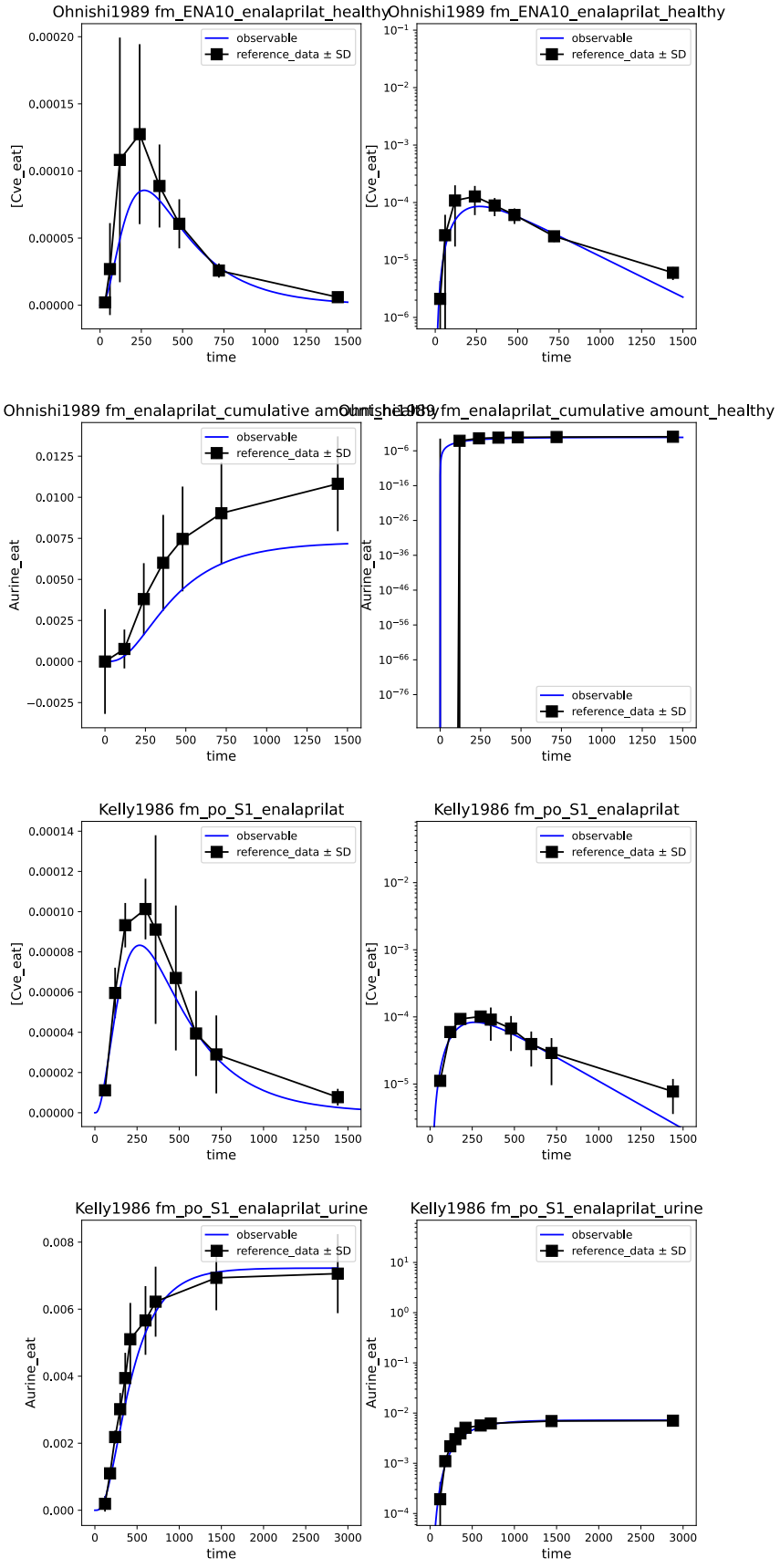

Figure 15: **Parameter fitting results for enalapril to enalaprilat metabolism.**

In summary, the parameters have been optimised, resulting in much better agreement between model predictions and clinical data. The optimal parameters (Tab. 3) have been incorporated into the model version 0.9.5 [52].

3.4 Simulations

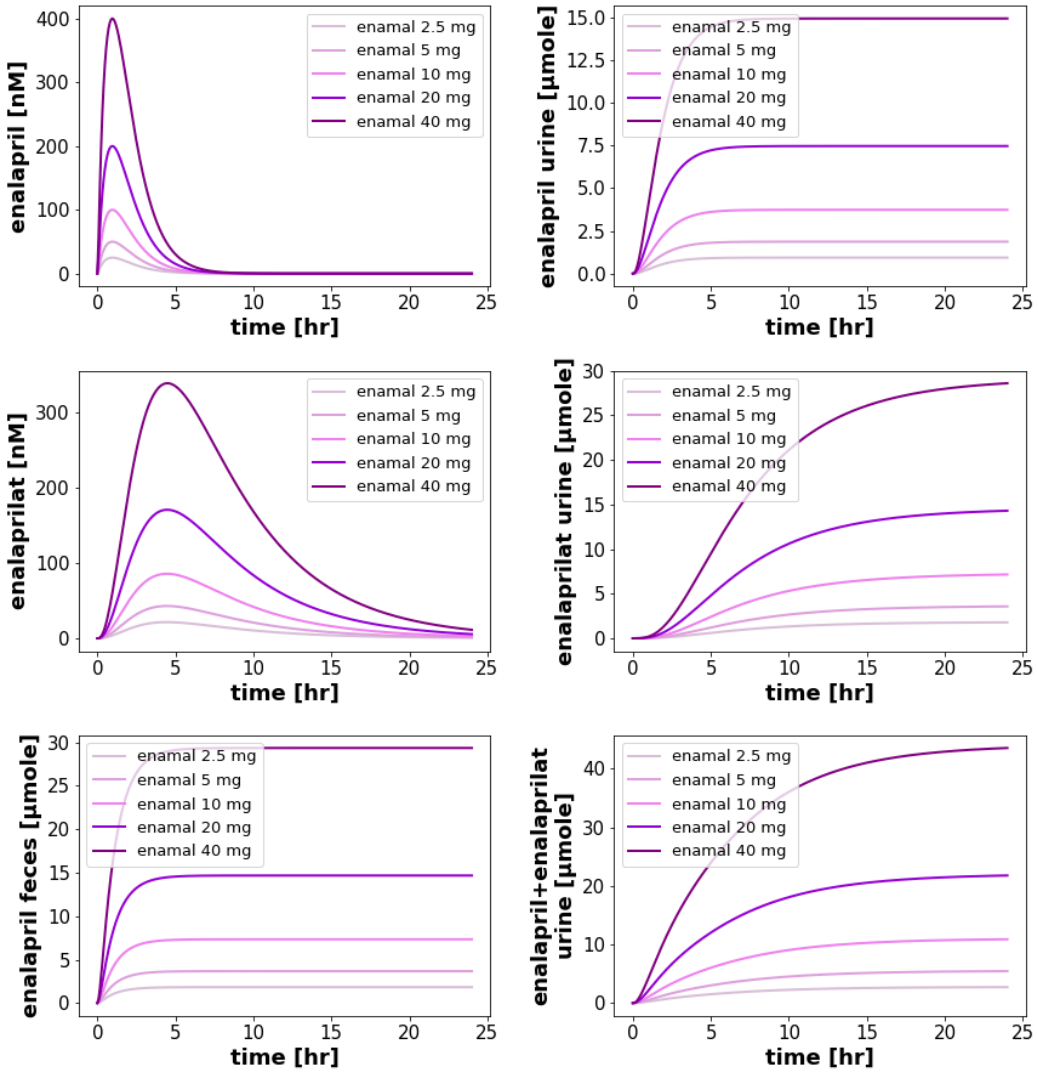

Figure 16: **Dose dependency of enalapril pharmacokinetics.** A representative simulation showing the enalapril and enalaprilat plasma concentrations and urinary and faecal amounts for varying doses of enalapril maleate namely, 2.5mg, 5mg, 10mg, 20mg, 40mg.

Several simulations were performed using the optimised model. Fig. 16 shows a representative simulation carried out for the most common dosages of enalapril found in our literature search. With increasing dose, the plasma concentrations of enalapril and enalaprilat increase, as do the amounts excreted in urine and faeces. The pharmacokinetics of enalapril is much faster than that of enalaprilat, with plasma concentrations returning to baseline after approximately 5 hours. In contrast, the elimination of enalapril is much slower, with larger doses being eliminated after approximately 24 hours. Enalapril and enalaprilat are excreted in the urine, with the unabsorbed frac-

tion of enalapril being excreted in the faeces. The total amount of drug is recovered as enalapril and enalaprilat in urine and faeces.

We then run simulations for all the curated studies, which allows us to compare the simulation with the reference data in the study. For example, there was a study by Schwartz et al 1985 [58] (Fig. 17) in which hypertensive patients were given different doses of enalapril.

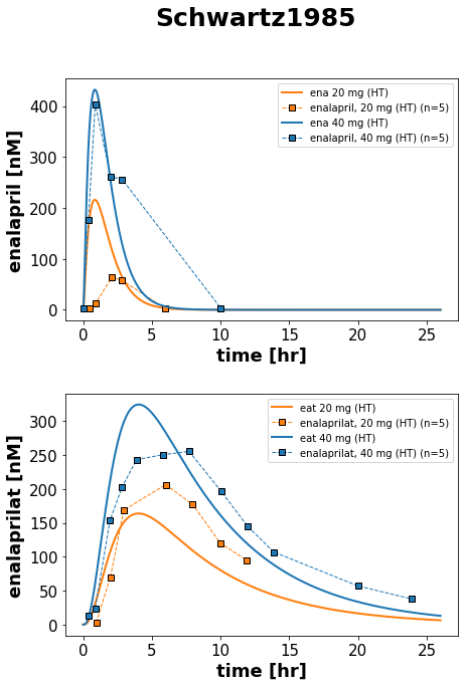

Figure 17: **Dose dependency healthy subjects.** Timecourse data for Schwartz1985 for hypertensive patients with its respective simulations [58].

Simulations for all datasets in the curated studies were carried out and the respective graphs were plotted with the reference data as reported in each study. The results are reported in the Supplementary Data as Arafat2005 Fig. 48 [1], Baba1990 Fig. 49 [2], Biollaz1982 Fig. 50 [4], Dayyih2017 Fig. 51 [7], Dickstain1987 Fig. 52, 53, 54, 55, 56, 57 [8], Donelly1990 Fig. 58 [10], Fruncillo1987 Fig. 59 [12], Ghosh2012 Fig. 60 [13], Gu2004 Fig. 61 [17], He2014 Fig. 62 [18], Her2021 Fig. 63 [19], Hockings1986 Fig. 64 [20], Howes1981 Fig. 65 [21], Ishizaki1988 Fig. 66 [23], Johnston1992 Fig. 67 [24], Kelly1986 Fig. 69 [26], Lee2023 Fig. 71 [34], Lees-1987a Fig. 72 [35], Lowenthal1985 Fig. 73 [37], Lu2009 Fig. 74 [39], MacDonald1993 Fig. 75 [40], Marzo2002 Fig. 76 [43], Matalka2002 Fig. 77 [44], Moffett2014 Fig. 78 [45], Mujais1992 Fig. 79 [46], Najib2003a Fig. 80

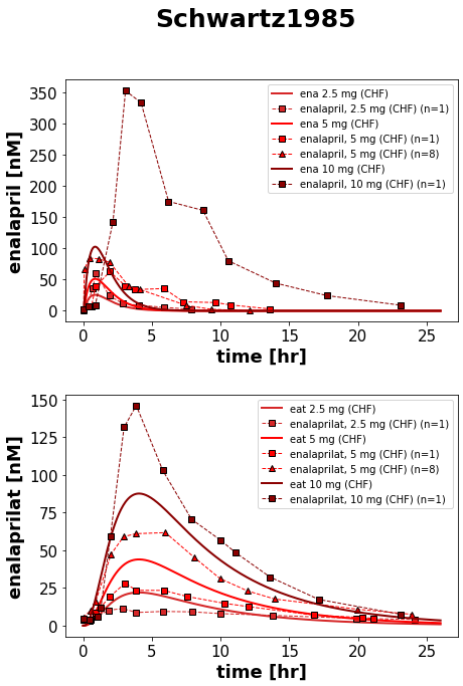

Figure 18: **Dose dependency chronic heart failure subjects.** Timecourse data for Schwartz1985 for patients having chronic heart failure with its respective simulations [58].

[47], Niopas2004 Fig. 81 [49], Oguchi1993 Fig. 82 [50], Ohnishi1989 Fig. 83 [51], Portales2004 Fig. 84 [53], Ribeiro1996 Fig. 85 [55], Schwartz1985 Fig. 86, 87 [58], Shionoiri1985 Fig. 88 [59], Shioya1992 Fig. 89 [60], Stage-2017 Fig. 90, 91 [64], Sunaga1995 Fig. 92 [66], Swanson1984 Fig. 93 [67], Tarkiainen2015 Fig. 94 [68], Thonnopnua2005 Fig. 95 [70], Till1984 Fig. 96 [71], Tsuruoaka2007 Fig. 97 [73], Ulm1982 Fig. 98 [75], Van-Hecken2020 Fig. 99 [76], Wade1992 Fig. 100 [78], Weisser1991 Fig. 101 [80], Weisser1992 Fig. 102 [79], Witte1993 Fig. 103 [82].

Two example simulations are shown below Fig. 19 and Fig. 20.

Biollaz et al. 1982 [4] (Fig. 19) studied the pharmacokinetics of enalapril in 12 normotensive subjects aged between 22 and 33 years. They analysed the plasma concentrations of enalapril and enalaprilat after a single dose of 10 mg enalapril maleate. Our model was able to simulate the time courses of enalapril and enalaprilat accurately. Dayyih et al.2017 [7] (Fig. 20) took a slightly different approach and attempted to compare the pharmacokinetics between two different formulations of enalapril maleate. Their results showed that there was no significant difference in the pharmacokinetics of the two formulations. Our model was also able to simulate a similar time

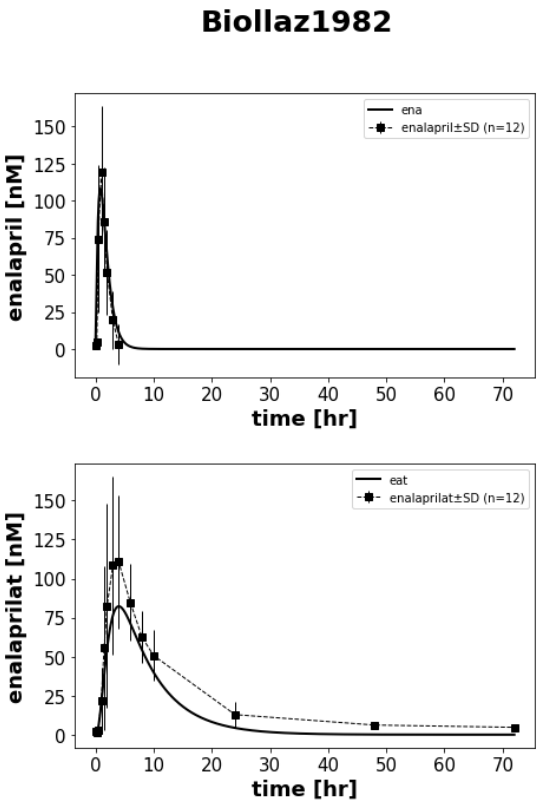

Figure 19: **Example simulation Biollaz1982.** Biollaz1982 studied the pharmacokinetics of enalapril/-at in healthy, normotensive subjects with 10mg administration of enalapril maleate [4].

course that is in good agreement with the data for both formulations.

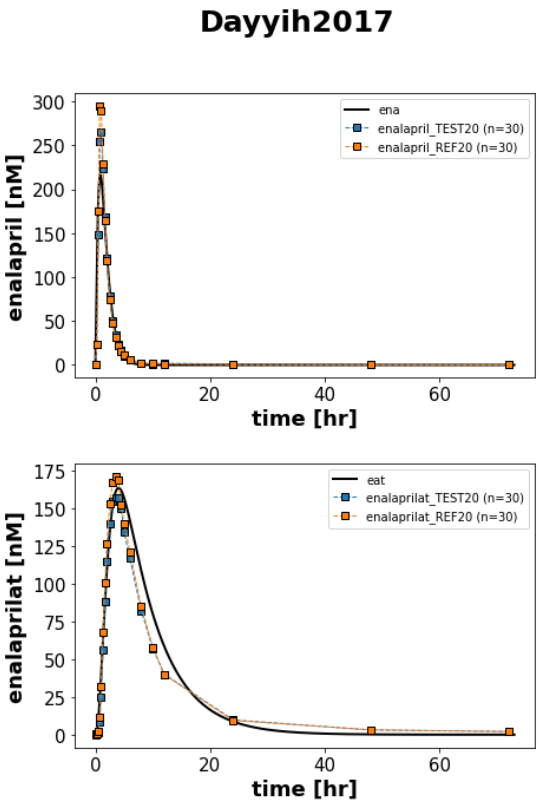

Figure 20: **Example simulation Dayyih2017.** Dayyih2017 compared the pharmacokinetics of enalapril/-at in healthy subjects with 20mg administration of two different formulations of enalapril maleate [7].

3.5 Effect of renal impairment

We used the model to investigate the effect of renal impairment on the pharmacokinetics of enalapril. As shown in Fig. 21, the model was able to simulate the time courses for the plasma concentrations of enalapril and enalaprilat under the given conditions of hepatic impairment. It was observed that the plasma concentrations of enalapril and enalaprilat decreased more rapidly with increasing renal activity, i.e. less renal impairment. The opposite effect was observed for enalapril and enalaprilat recovered from urine, where higher renal activity led to higher urinary concentrations of both substances. In the absence of cirrhosis, enalapril levels fell more quickly because enalapril metabolism to enalaprilat was working efficiently. In severe cirrhosis, however, enalapril stayed in the blood longer and the peak concentration was higher than in the control group. It was also observed that as the degree of cirrhosis increased, enalaprilat concentrations didn't reach the maxima seen in the control group. In cirrhotic conditions, urinary enalapril was found to be much higher and enalaprilat much lower than in the control group.

For the pharmacokinetic parameters (Fig 22, Fig. 23), cirrhotic conditions also increased the AUC and decreased the elimination rate ($t_{1/2}$) of enalapril, which was consistent with the above facts that enalapril remained longer in plasma when it could not be converted to enalaprilat due to impaired liver function. We also found that the AUC and $t_{1/2}$ showed a difference between the cirrhotic groups under a condition of reduced renal activity, but for baseline and higher renal activity there was no difference between the cirrhotic groups for these two parameters.The renal and hepatic clearance for enalapril showed an increase with increasing renal function, but the hepatic clearance decreased with progressive stages of cirrhosis, which is justified. For enalaprilat, the AUC and $t_{1/2}$ showed a steady decrease with increasing renal function and with increasing degree of cirrhosis.

The model was used to simulate the pharmacokinetics of enalapril and enalaprilat in patients with varying degrees of renal impairment [12, 20, 26, 35, 37, 79]. The simulated data showed varying degrees of goodness of fit to the reference data and the fit improved with more iterations of the parame-

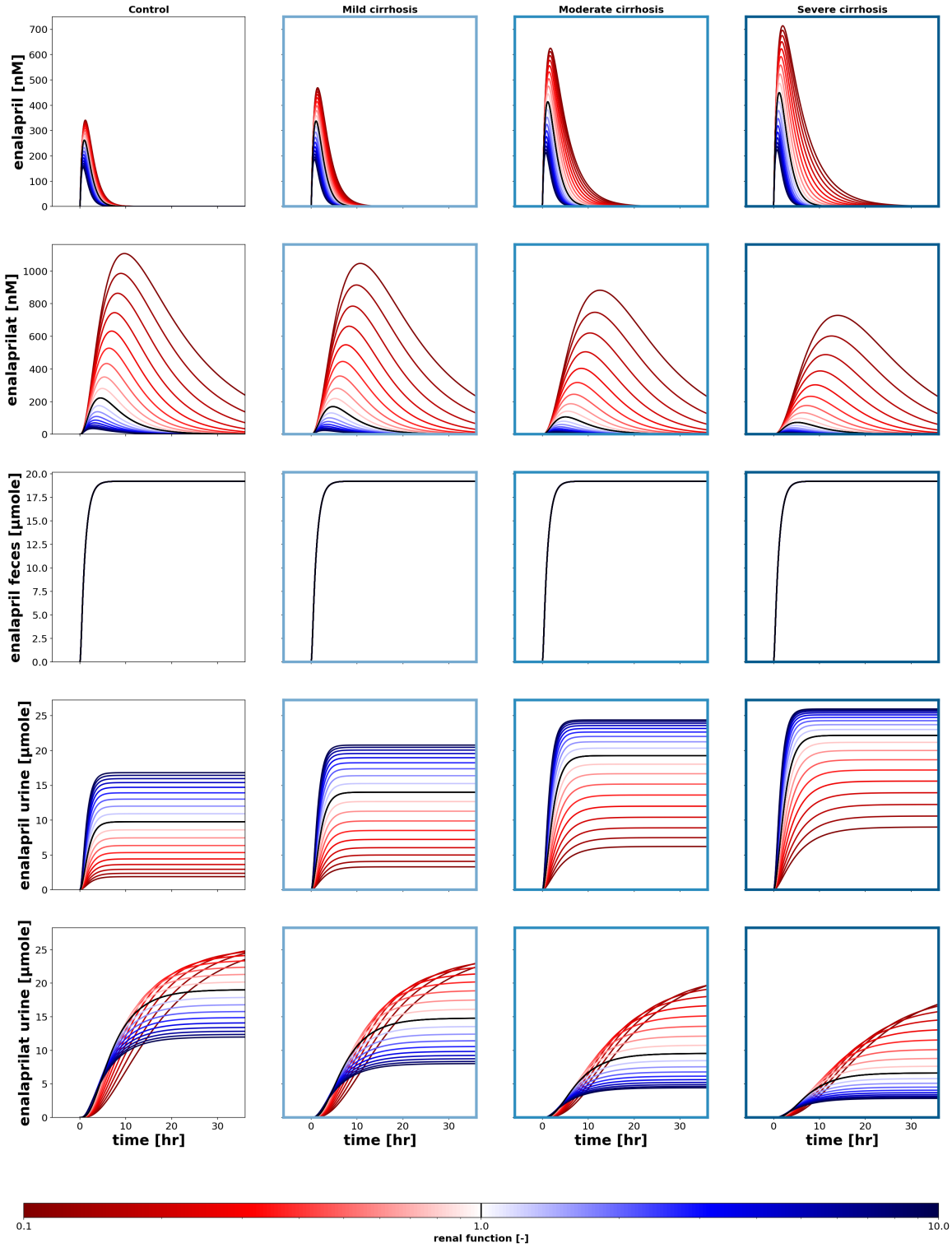

Figure 21: **Timecourse for renal function scan.** Time courses for different degrees of renal function. Decreased renal function in red, increased renal function in blue, normal function in black. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

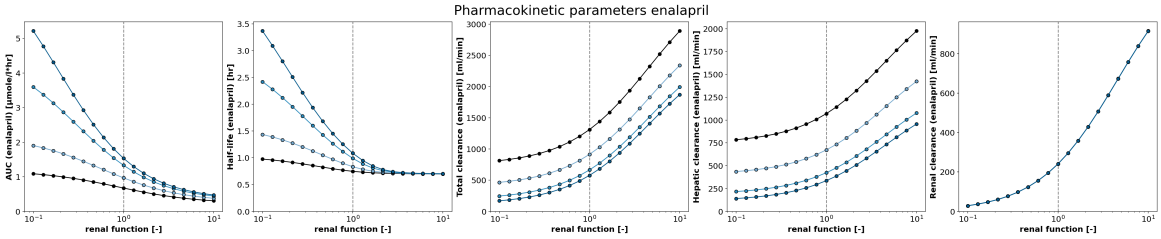

Figure 22: **Pharmacokinetic parameters of enalapril for renal function scan.** Pharmacokinetic parameters of enalapril for varying degrees of renal function. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

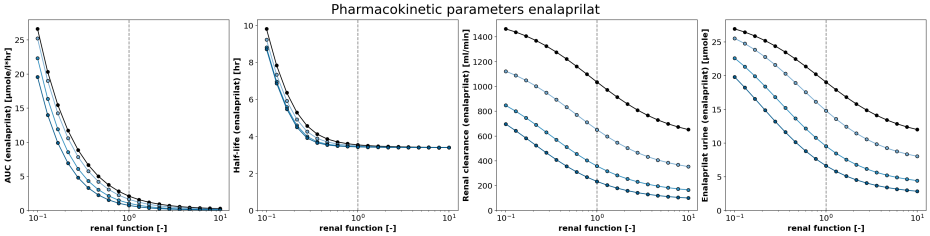

Figure 23: **Pharmacokinetic parameters of enalaprilat for renal function scan.** Pharmacokinetic parameters of enalaprilat for varying degrees of renal function. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

terisation. The figures below show plots of the simulated and reference data for the renal impairment studies.

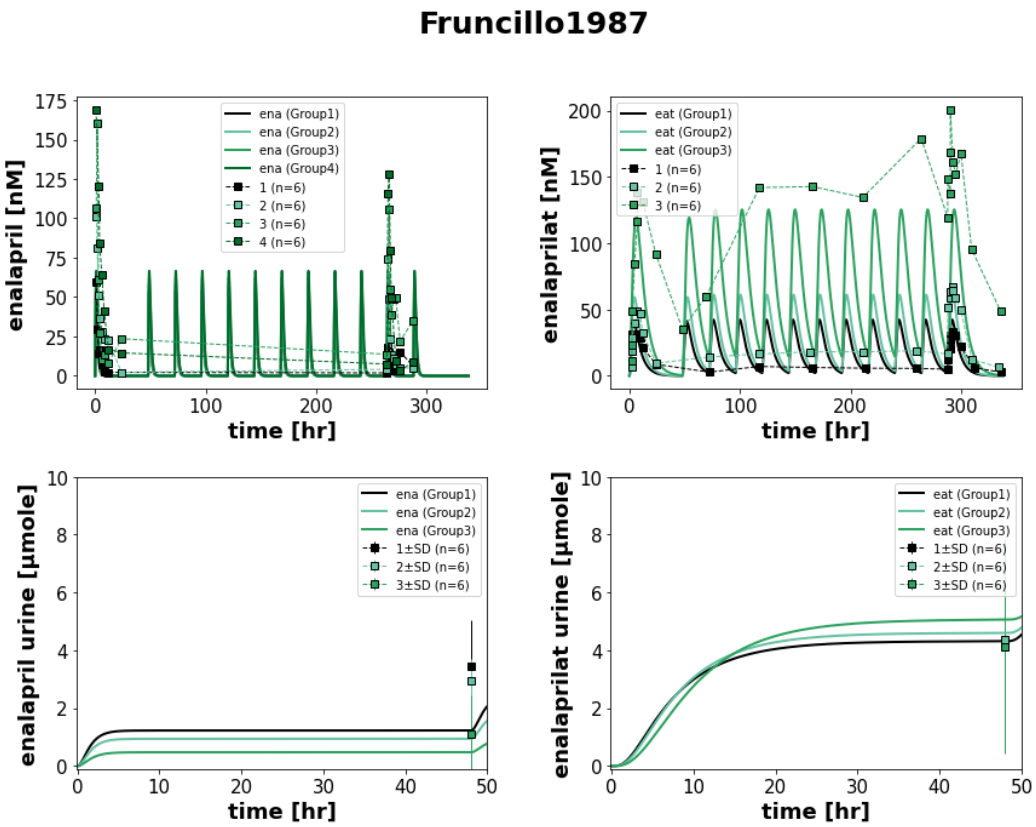

Figure 24: **Simulation of renal impairment for Fruncillo1987.** Normal renal function: black, renal impairment: green. [12]

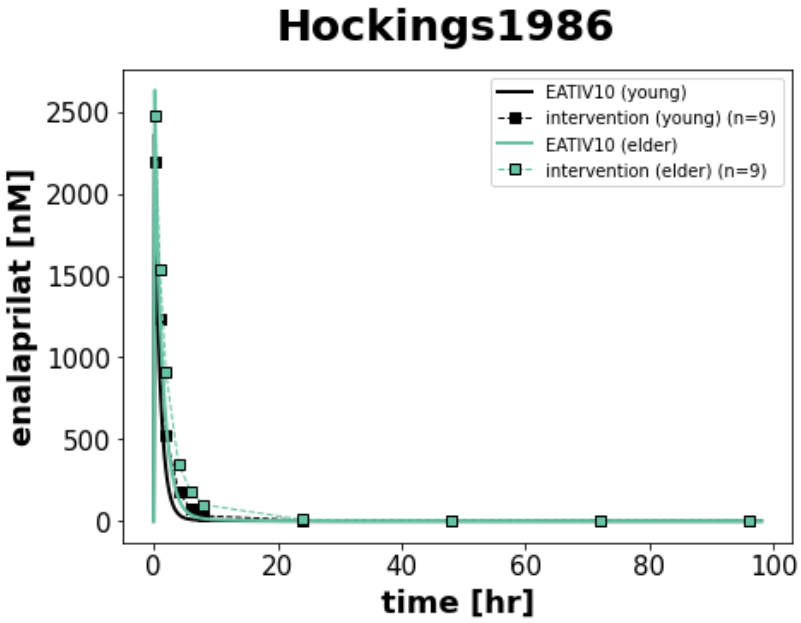

Figure 25: **Simulation of renal impairment for Hockings1986.** Normal renal function: black, renal impairment: green. [20]

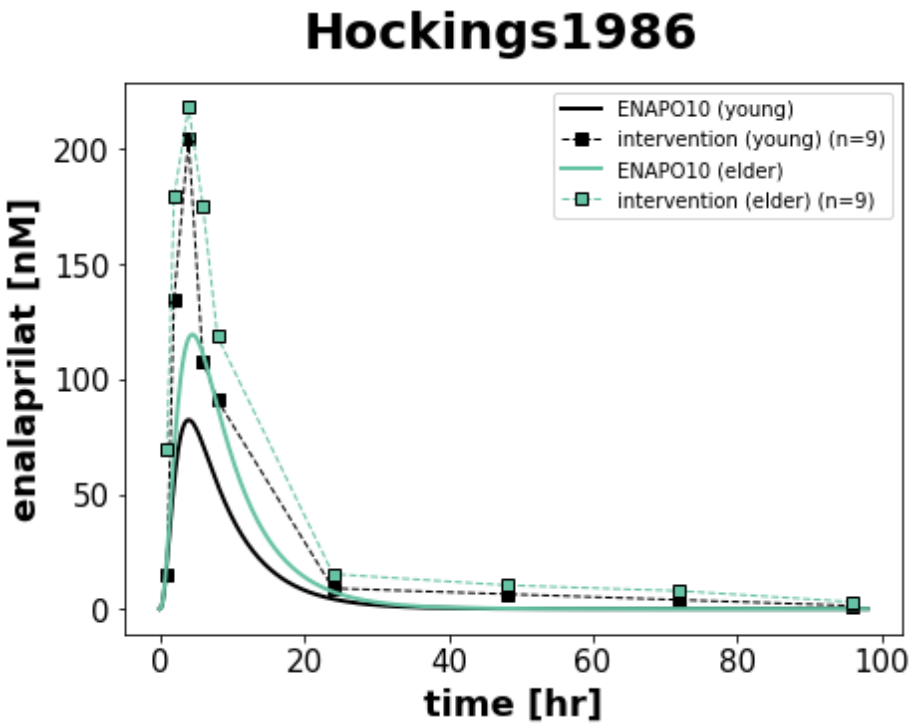

Figure 26: **Simulation of renal impairment for Hockings1986.** Normal renal function: black, renal impairment: green. [20]

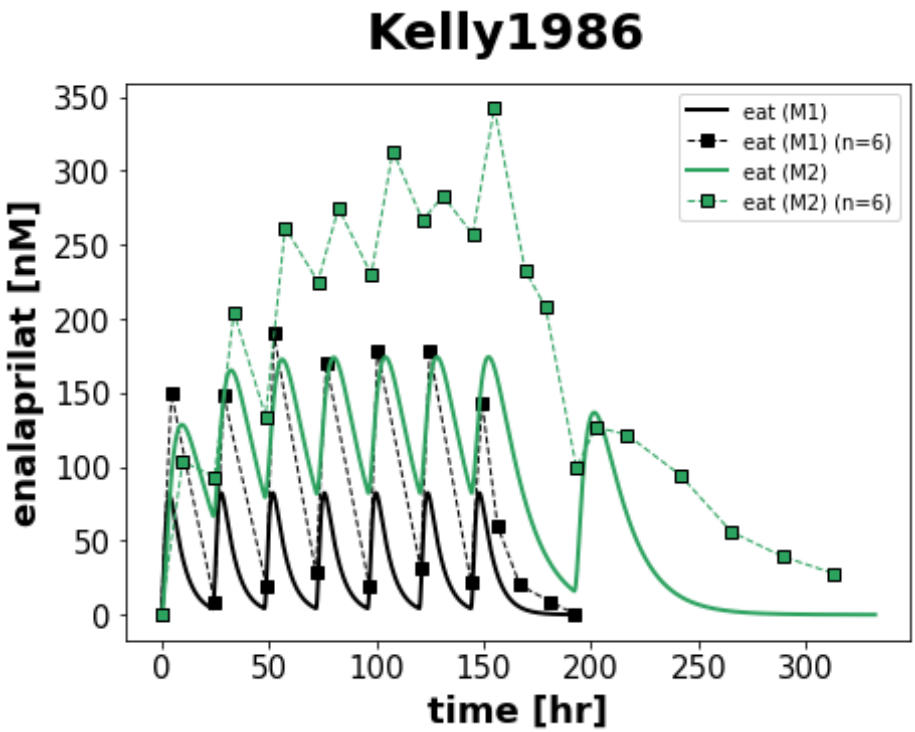

Figure 27: **Simulation of renal impairment for Kelly1986.** Normal renal function: black, renal impairment: green. [26]

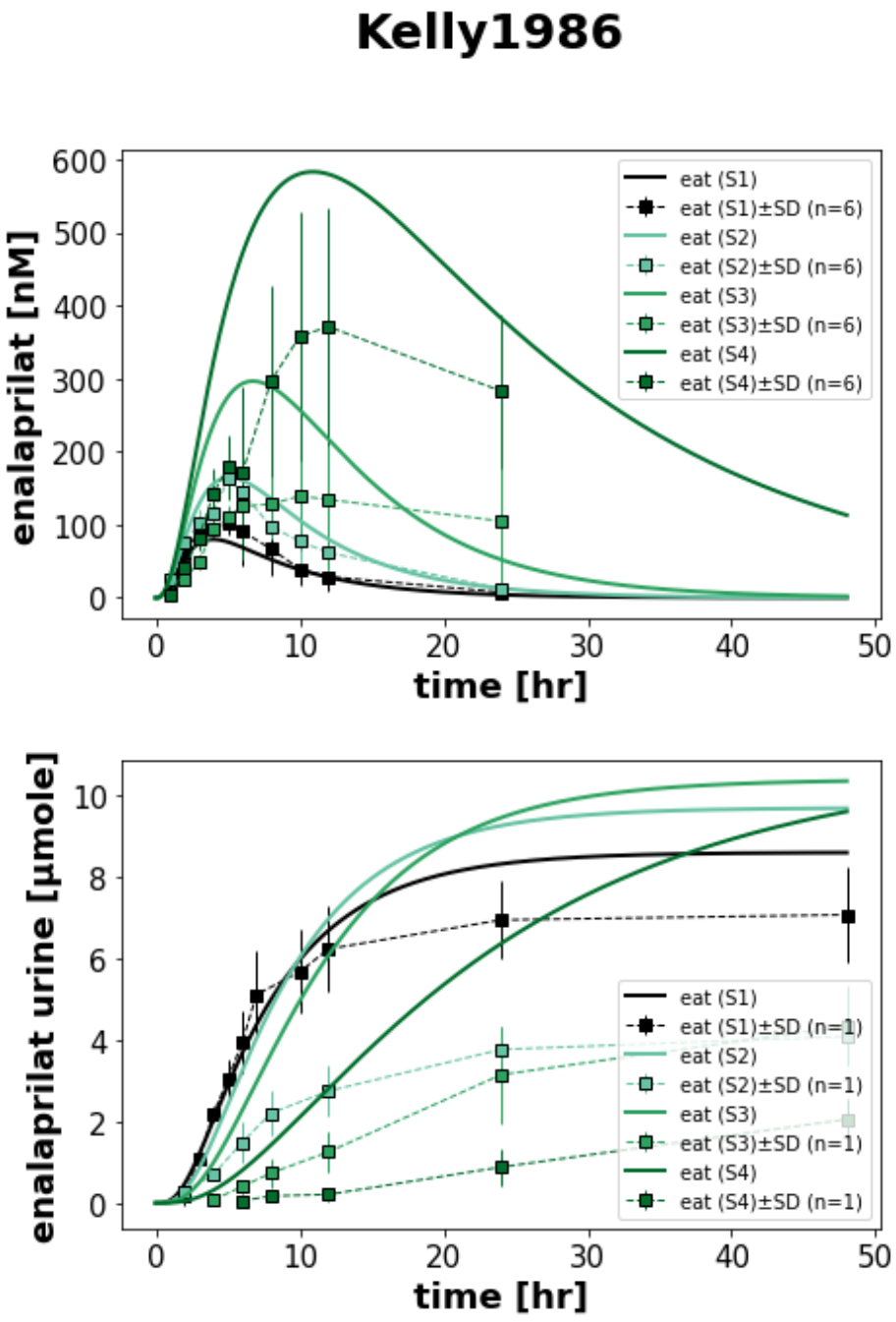

Figure 28: **Simulation of renal impairment for Kelly1986.** Normal renal function: black, renal impairment: green. [26]

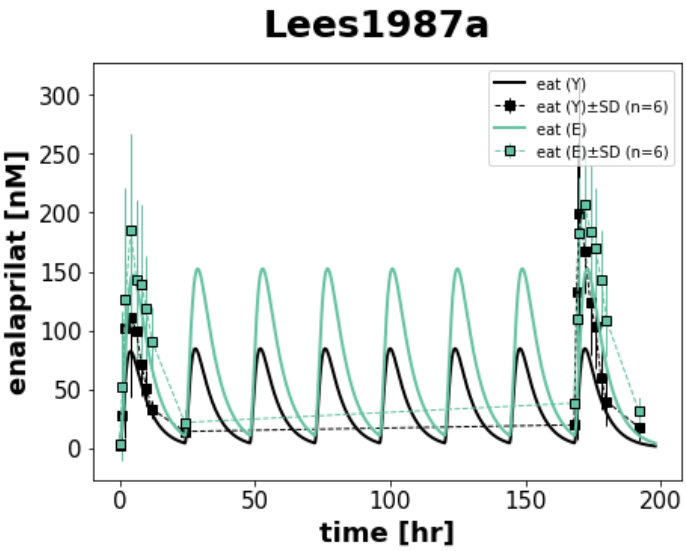

Figure 29: **Simulation of renal impairment for Lees1987a.** Normal renal function: black, renal impairment: green. [35]

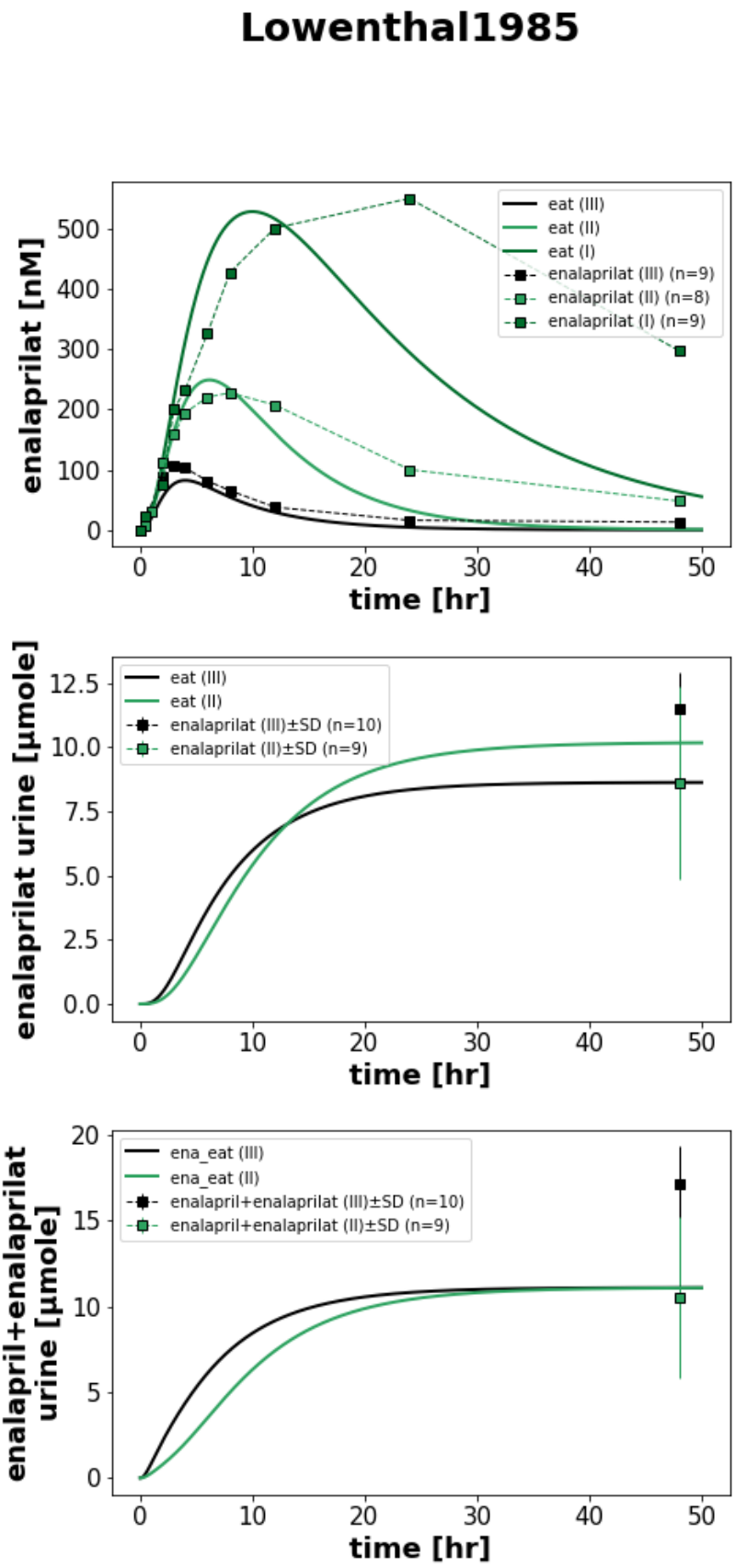

Figure 30: **Simulation of renal impairment for Lowenthal1985.** Normal renal function: black, renal impairment: green. [37]

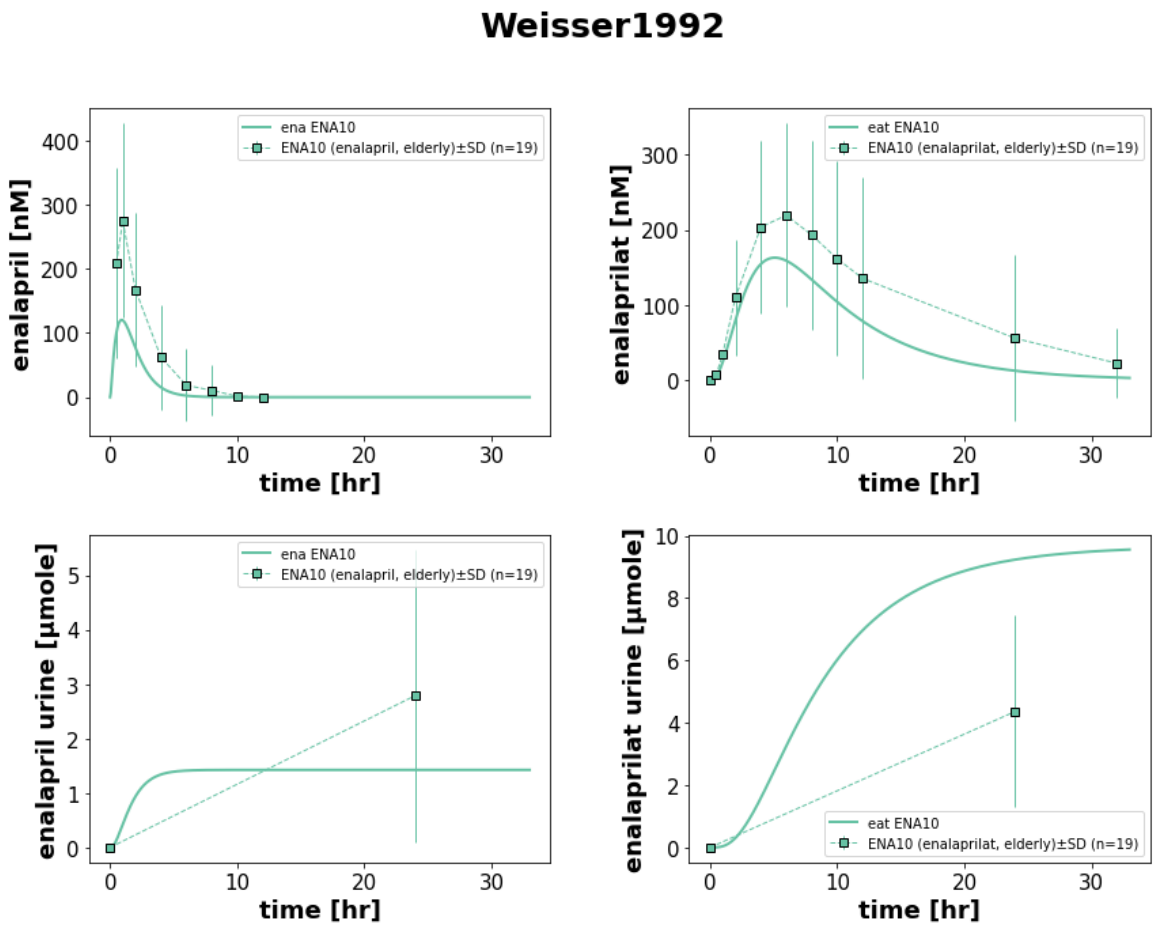

Figure 31: **Simulation of renal impairment for Weisser1992.** Normal renal function: black, renal impairment: green. [79]

3.6 Effect of hepatic impairment

We used the model to investigate the effect of hepatic impairment on enalapril pharmacokinetics. Similarly to renal impairment, simulations were also performed for two studies investigating enalapril pharmacokinetics in cirrhotic patients. The simulated data agreed with the reference data for the general trend of rise and fall of enalapril and enalaprilat concentrations.

Time courses were simulated for enalapril pharmacokinetics as shown in Fig. 32. Plasma enalapril concentrations increased with increasing hepatic and renal impairment. Decreased efficiency of the liver to convert enalapril to enalaprilat and decreased ability of the kidney to excrete enalapril from the blood lead to increased concentrations. The opposite effect is observed with enalaprilat, where dramatic increases in plasma concentrations are observed. With enalapril, urinary concentrations increase with progressive stages of cirrhosis, but decrease with increasing renal impairment. With enalaprilat, urine concentrations decrease with increasing cirrhosis.

For the pharmacokinetic parameters of enalapril, AUC and $t_{1/2}$ increased with increasing degree of cirrhosis, whereas hepatic clearance decreased. No effect of degree of cirrhosis on renal clearance was observed. The AUC for enalaprilat decreased slightly with increasing cirrhosis, but showed an increase with increasing renal impairment. The $t_{1/2}$ hardly changed in these scenarios. The renal clearance of enalaprilat decreased significantly with increasing cirrhosis and renal impairment.

The model was used to simulate the pharmacokinetics of enalapril and enalaprilat in patients with varying degrees of hepatic impairment [2, 51]. The figures below are the graphs for the simulations of the two studies that focused on patients with hepatic impairment or patients with different degrees of cirrhosis.

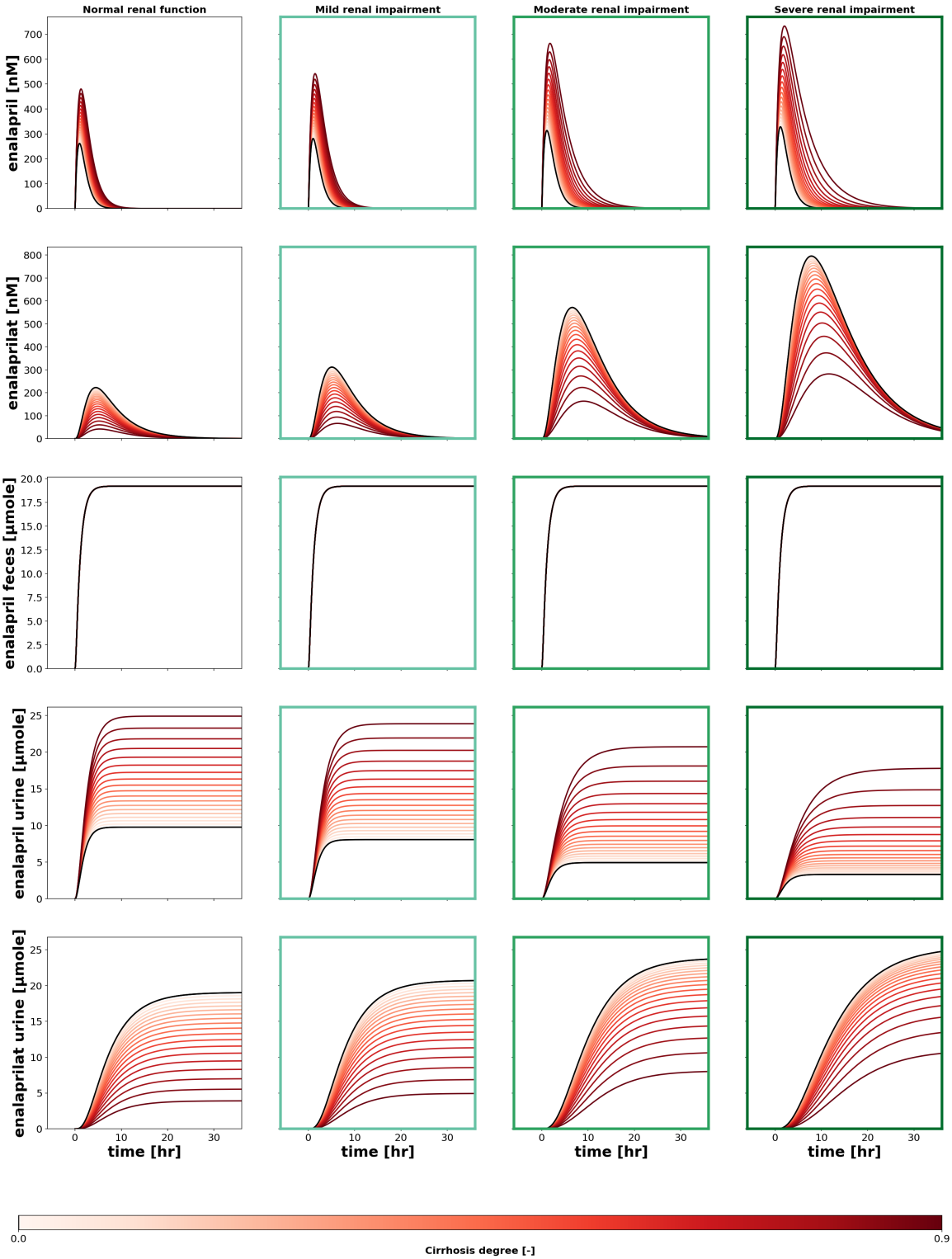

Figure 32: **Timecourse for hepatic function scan.** Time courses for different degrees of cirrhosis. Cirrhosis in red, normal function in black. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

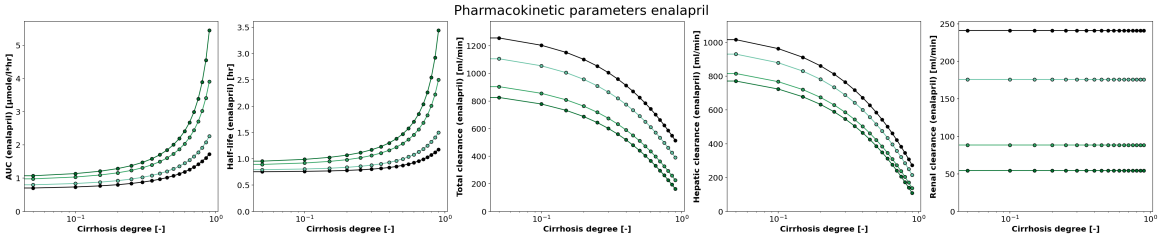

Figure 33: **Pharmacokinetic parameters of enalapril for renal function scan.** Pharmacokinetic parameters of enalapril for varying degrees of hepatic function. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

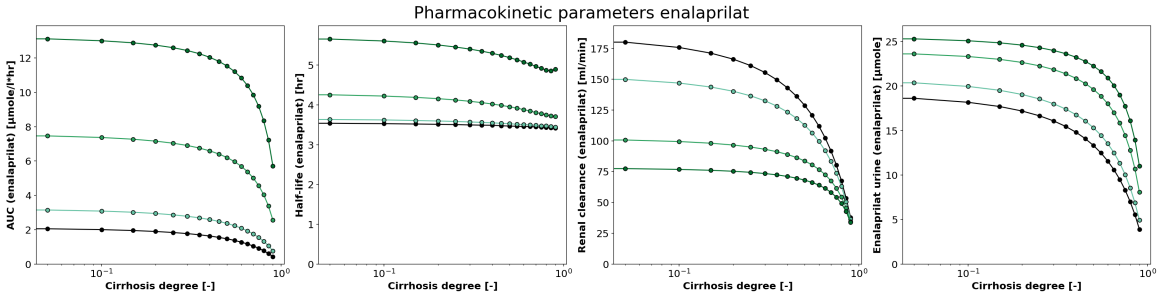

Figure 34: **Pharmacokinetic parameters of enalaprilat for renal function scan.** Pharmacokinetic parameters of enalaprilat for varying degrees of hepatic function. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

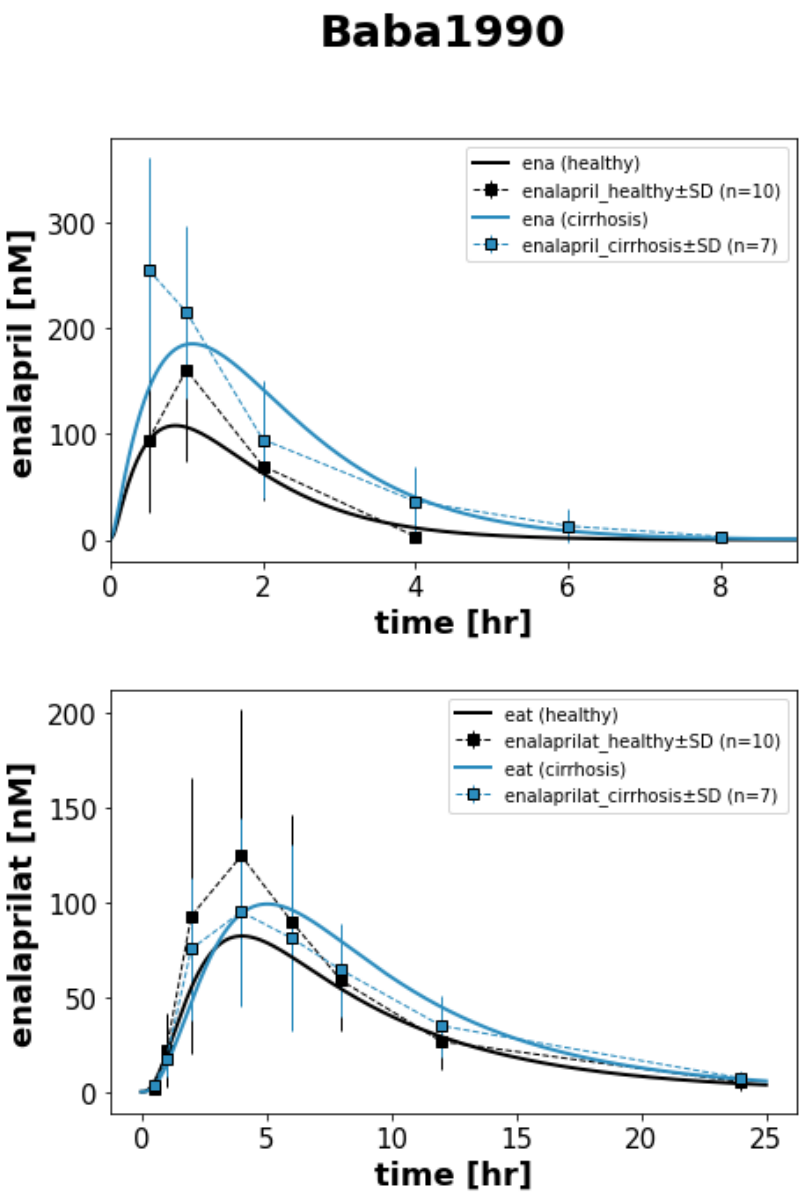

Figure 35: **Simulation of hepatic impairment for Baba1990.** Normal hepatic function: black, cirrhosis: blue. [2]

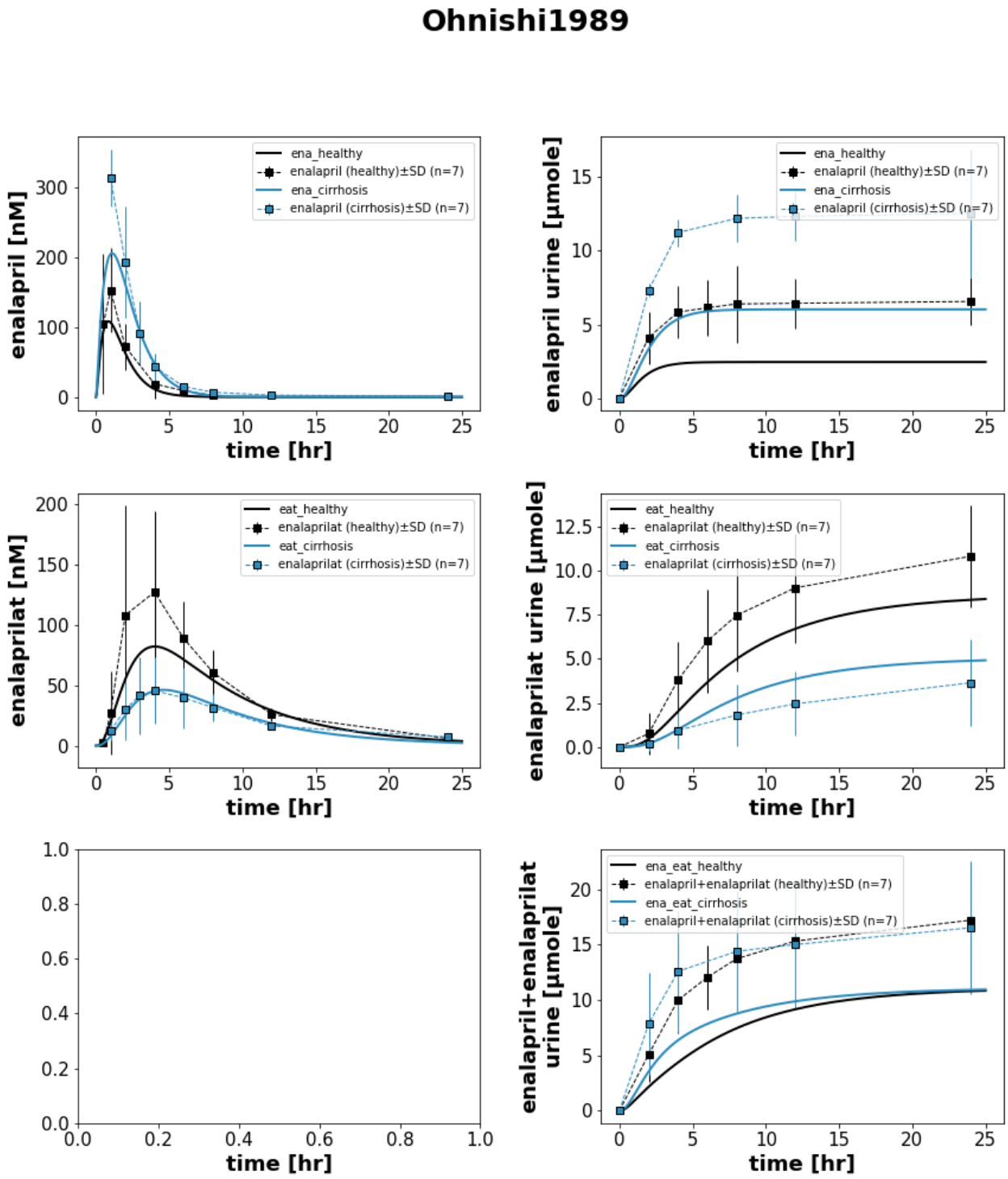

Figure 36: **Simulation of hepatic impairment for Ohnishi1989.** Normal hepatic function: black, cirrhosis: blue. [51]

3.7 CES1 genotype

The model was also used to investigate the effect of CES1 genotype on enalapril pharmacokinetics. Simulations were performed for a range of CES1 activity against 4 degrees of cirrhosis. 37 It was observed that CES1 and hepatic impairment have similar and synergistic effects on the pharmacokinetics of enalapril. In both cases, the conversion of enalapril to enalaprilat is inhibited. Therefore, under non-mutated conditions, enalapril concentrations tend to be lower than in the case of reduced CES1 activity and higher than in the case of increased CES1 activity. On the other hand, enalaprilat concentrations tend to be higher under conditions of higher CES1 activity and they tend to be lower under conditions of lower CES1 activity. Urinary enalapril concentrations tend to increase when CES1 activity decreases and vice versa. The opposite trend is observed for urinary enalaprilat concentrations. The degree of cirrhosis does not seem to have any effect other than to increase the total enalapril concentration and decrease that of enalaprilat in all the above cases.

Pharmacokinetic parameters were also estimated with the simulations using our model. The AUC and total clearance of enalapril were unaffected between the 4 degrees of cirrhosis at subnormal CES1 activity, but at higher CES1 activity the AUC was increased and total clearance decreased compared to normal conditions.

The pharmacokinetic parameters for enalapril and enalaprilat were estimated as shown in Fig. 38 and Fig. 39. The AUC and $t_{1/2}$ for enalapril are seen to decrease with higher levels of CES1 activity. Hepatic clearance also decreases, but no effect on renal clearance is observed. For enalaprilat, the AUC increases with increasing CES1 activity, but the $t_{1/2}$ remains the same; renal clearance also increases.

CES1 activity was also investigated in relation to renal impairment. The plasma concentrations of enalapril and enalaprilat were dramatically higher in patients with renal impairment than in healthy patients. This is because renal impairment impairs renal filtration and reduces the excretion of both substances, resulting in a higher presence of these substances in the blood-/plasma. In terms of CES1 activity, enalapril levels decreased as CES1 ac-

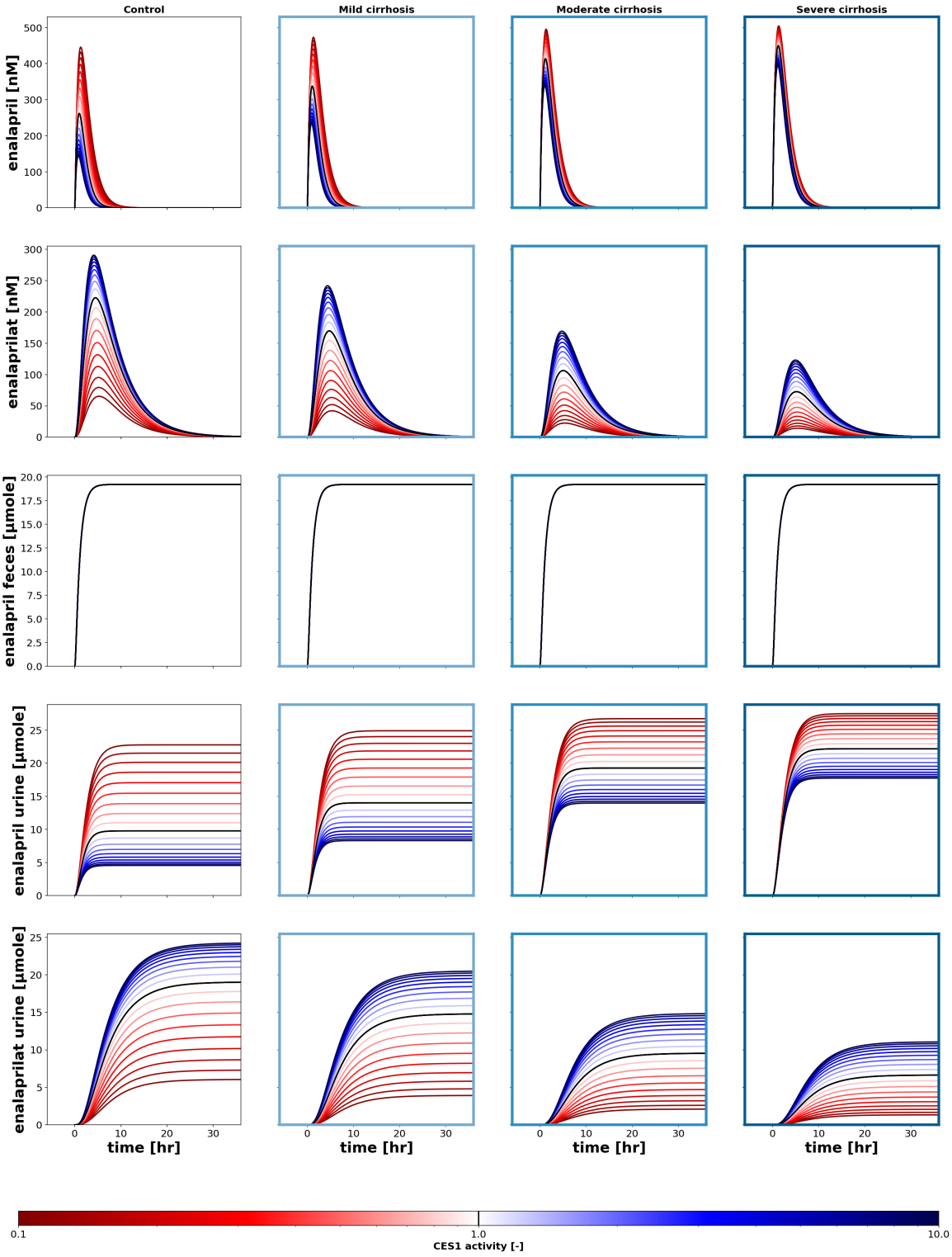

Figure 37: **Timecourse for CES1 function scan.** Time courses for different degrees of CES1 function. Decreased CES1 function in red, increased CES1 function in blue, normal function in black. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

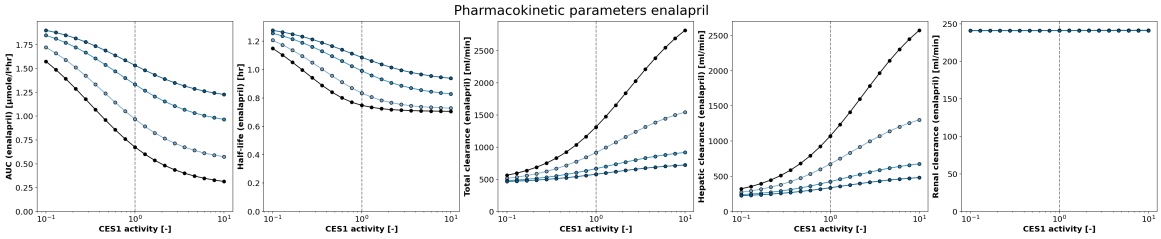

Figure 38: **Pharmacokinetic parameters of enalapril for CES1 function scan.** Pharmacokinetic parameters of enalapril for varying degrees of CES1 function. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

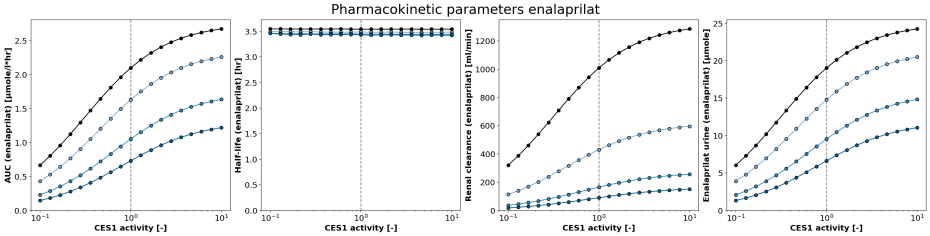

Figure 39: **Pharmacokinetic parameters of enalaprilat for CES1 function scan.** Pharmacokinetic parameters of enalaprilat for varying degrees of CES1 function. The scan was repeated for different degrees of cirrhosis (control: black, mild cirrhosis: light blue (CPT A), moderate cirrhosis: blue (CPT B) and severe cirrhosis: dark blue (CPT C).

tivity increased. Conversely, enalaprilat levels increased as CES1 activity increased.

Renal impairment only increased the concentration of enalapril and decreased that of enalaprilat. The urinary concentrations of enalapril and enalaprilat showed opposite trends. Thus, a combined effect of higher CES1 activity and severe renal impairment was that the urinary concentration of enalapril decreased dramatically, whereas the urinary concentration of enalaprilat did not decrease, but was certainly excreted more rapidly. On the other hand, with reduced CES1 activity and no renal impairment, the urinary concentrations of enalapril and enalaprilat increased and decreased, respectively. Only a change in CES1 activity was associated with a change in enalapril-at concentration and the trend was the same, i.e. as above, enalapril concentration increased with higher CES1 activity and enalaprilat concentration increased with higher CES1 activity.

Pharmacokinetic parameters were also estimated for CES1 activity as shown in Fig. 42 and Fig. 43. For enalapril, AUC and $t_{1/2}$ both increase with decreasing CES1 activity, but remain the same at higher than normal

CES1 activity. In addition, the greater the degree of renal impairment, the higher the AUC at lower CES1 activity; the same is true for $t_{1/2}$. Hepatic clearance of enalapril has been shown to increase with increasing CES1 activity, while renal clearance remains the same. Only renal impairment has been shown to affect the renal clearance of enalapril.

For enalaprilat, the AUC increases with CES1 activity, but this change is more apparent with higher degrees of renal impairment. The $t_{1/2}$ remains the same for different CES activities and only increases with higher degrees of renal impairment. The renal clearance of enalaprilat is interesting because at lower levels of CES1 activity it is lower with less renal impairment; however, beyond normal CES1 activity, the renal clearance of enalaprilat decreases with higher degrees of renal impairment.

The model was used to simulate the pharmacokinetics of enalapril and enalaprilat in patients with different degrees of CES1 function [19, 64, 68]. Simulations were also performed with a special focus on studies comparing the pharmacokinetics between variants of the CES1 gene, which gives rise to the CES1 enzyme responsible for the conversion of enalapril to enalaprilat (see Fig. 44, Fig. **??**, Fig. **??** and Fig. 47).

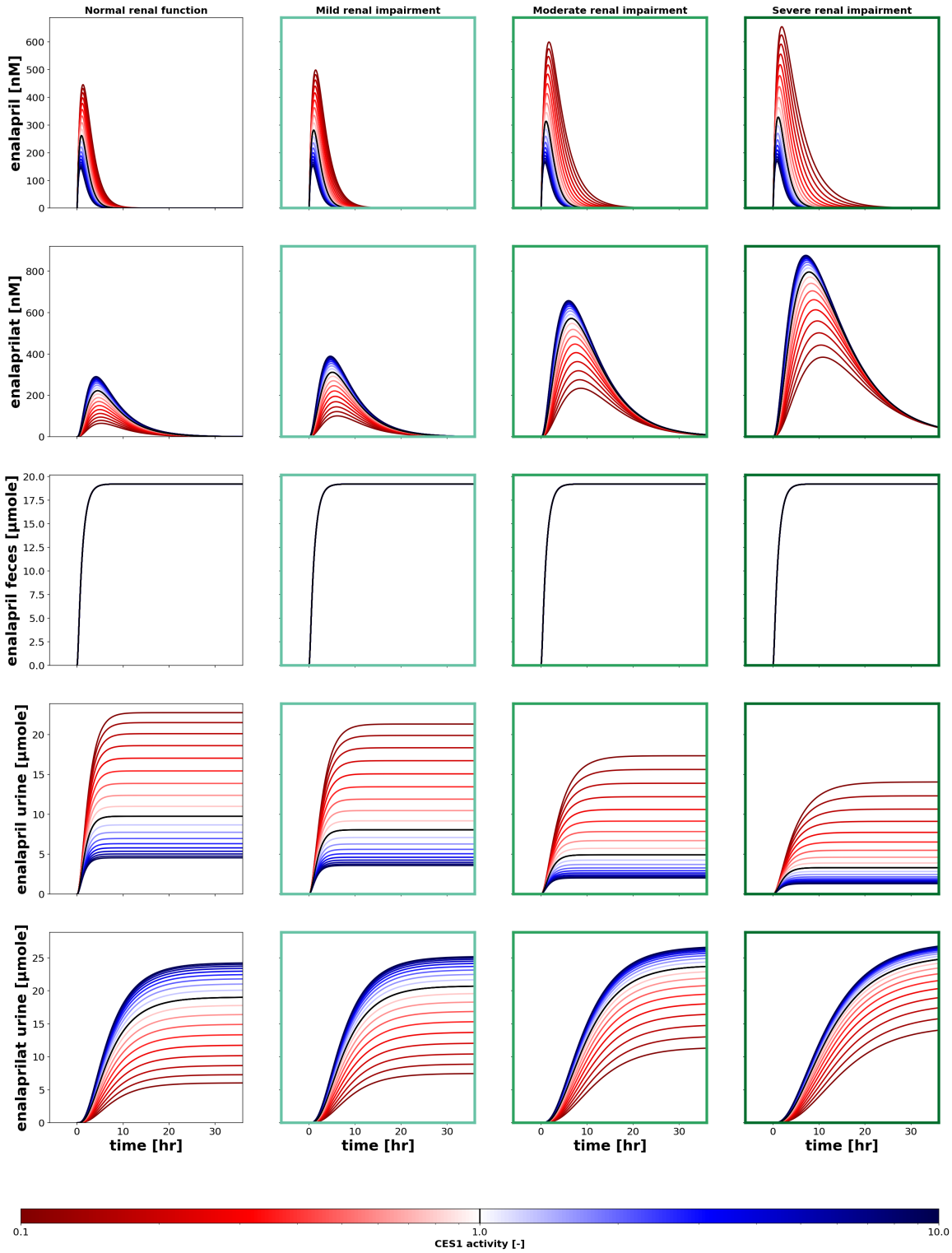

Figure 40: Timecourses for enalapril with varying degrees of renal impairment against CES1 activity

Figure 41: **Timecourse for CES1 function scan.** Time courses for different degrees of CES1 function. Decreased CES1 function in blue, increased CES1 function in red, normal function in black. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

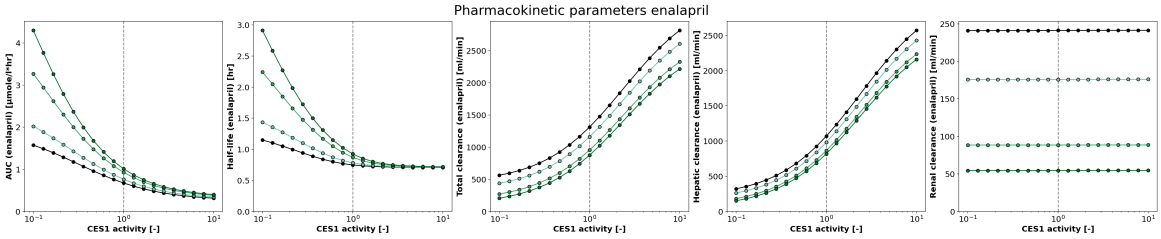

Figure 42: **Pharmacokinetic parameters of enalapril for CES1 function scan.** Pharmacokinetic parameters of enalapril for varying degrees of CES1 function. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

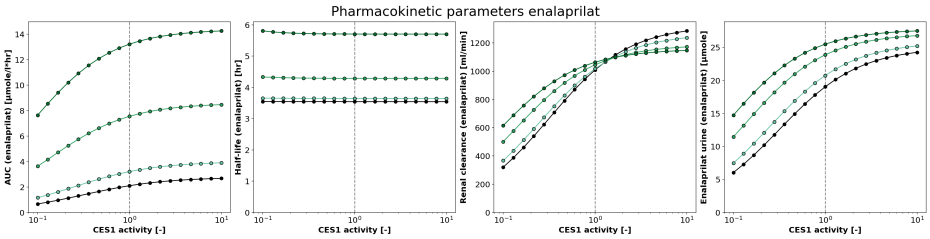

Figure 43: **Pharmacokinetic parameters of enalaprilat for CES1 function scan.** Pharmacokinetic parameters of enalaprilat for varying degrees of CES1 function. The scan was repeated for different degrees of renal function (control: black, mild renal impairment: light green, moderate renal impairment: green, and severe renal impairment: dark green.

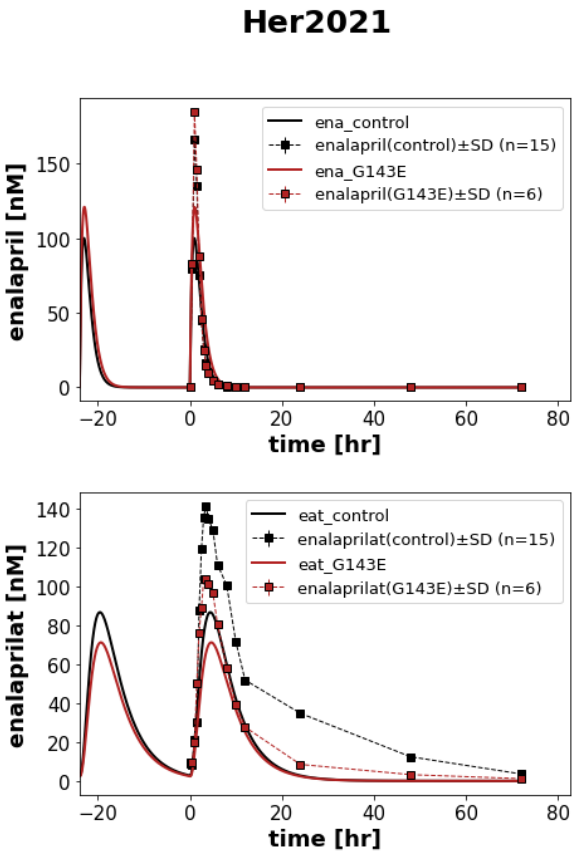

Figure 44: **Simulation for CES1 activity Her2021.** Normal CES1 function: black, increased CES1 function: blue, decreased CES1 function: red. [19]

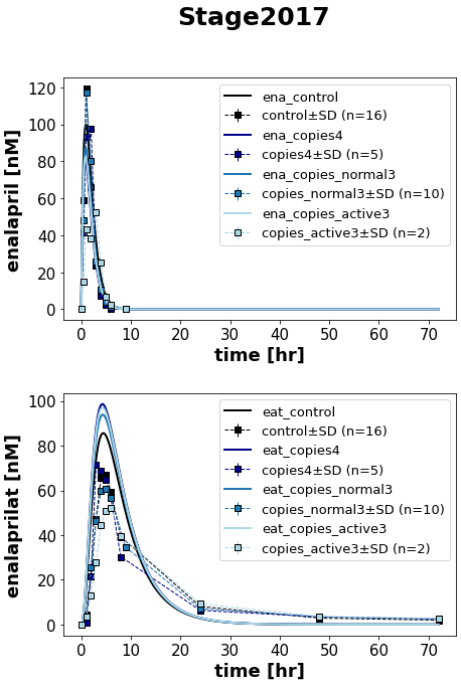

Figure 45: **Simulation for CES1 activity Stage2017.** Normal CES1 function: black, increased CES1 function: blue, decreased CES1 function: red. [64]

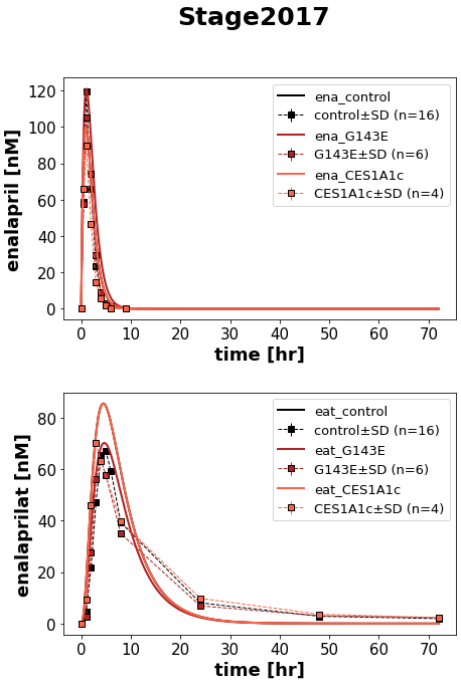

Figure 46: **Simulation for CES1 activity Stage2017.** Normal CES1 function: black, increased CES1 function: blue, decreased CES1 function: red. [64]

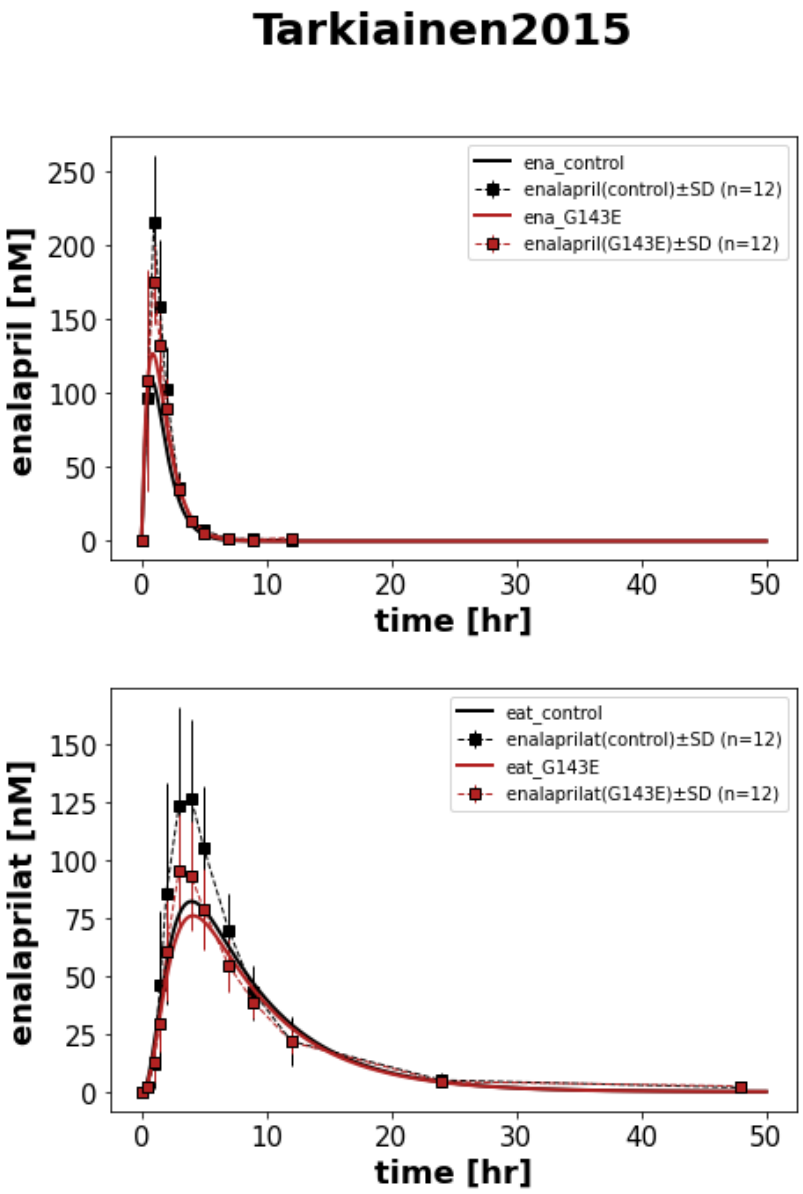

Figure 47: **Simulation for CES1 activity Tarkiainen2015.** Normal CES1 function: black, increased CES1 function: blue, decreased CES1 function: red. [68]

3.8 Summary

In this study, a comprehensive and detailed model for the pharmacokinetics of enalapril and its active metabolite, enalaprilat, was successfully constructed using the Systems Biology Markup Language (SBML). This model incorporates all relevant pharmacokinetic parameters and was designed to simulate the drug's behavior under various dosing conditions and patient characteristics.

Pharmacokinetic simulations conducted as part of this study facilitated the iterative refinement of the model parameters, ensuring they accurately reflect observed data. Comparative analysis between the simulated outcomes and empirical data from the literature was instrumental in fine-tuning the model to enhance its predictive accuracy and reliability.

The final, refined model has demonstrated its effectiveness in replicating the pharmacokinetics of enalapril and enalaprilat, providing a robust tool for future research and application in clinical pharmacology. This model is now publicly accessible and can be downloaded from `https://github.com/matthiaskoenig/enalapril-model`.

4 Discussion

In this thesis, a physiologically based pharmacokinetic (PBPK) model for enalapril was developed and applied under various physiological and pathological conditions. Using a comprehensive database derived from 49 clinical trials, this model reliably predicts the pharmacokinetics of enalapril and its active metabolite, enalaprilat, in a diverse patient population. The focus on renal and hepatic impairment is particularly important given their impact on drug clearance and metabolism, which can significantly affect therapeutic outcomes.

4.1 Enalapril Database

This work has created the first openly available comprehensive database of enalapril pharmacokinetics and pharmacodynamics. To our knowledge, this is the first time that systematic data curation of enalapril pharmacokinetic data has been undertaken. All data are freely available for re-use and can be downloaded from https://pk-db.com.

4.2 PBPK Model

Despite the developed PBPK model being in good agreement with the experimental data certain limitations exist. (i) The focus of the model was on the description of the pharmacokinetics of enalapril and enalaprilat. Pharmacodynamic data was curated on systolic and diastolic blood pressure, heart rate, mean arterial pressure and substances such as renin, angiotensin I and II and aldosterone from the 49 studies, but is not part of the computational model. (ii) The model of renal and hepatic functional impairment was based on existing validated models for indocyanine green [30, 29] and talinolol [42]. In contrast the cardiac functional impairment model was very simplified and only assumed reduced cardiac output. A more detailed model of the pathophysiological changes in cardiac impairment could substantially improve the agreement between predictions and clinical data. (iii) The tissue models for renal excretion, hepatic metabolism and intestinal uptake are simplifications, mainly due to the limited availability of data. I.e. transport and metabolism

were modeled as simple mass-action or Michaelis-Menten kinetics often lumping multiple steps in a single overall reaction rate. With the availability of more detailed experimental information these models could be extended.

The model is made available in SBML under a CC-BY 4.0 licence from `https://github.com/matthiaskoenig/enalapril-model`, providing an important resource for re-use and further study.

4.3 Parameter optimization

In this work, a multi-step approach was used to optimise the parameters. I.e. first the parameters specific for enalaprilat were optimised, then the parameters for enalapril and finally the parameters for the hepatic conversion of enalapril to enalaprilat. All three optimisations significantly improved the agreement between the model and the experimental data, and the resulting model is in good agreement with most of the training data. I.e. the optimal parameters given in Tab. 3 provide a good model fit as shown in Fig. 13, Fig. 11 and Fig. 15.

There are some limitations in the parameter optimisation: (i) The optimisation used a local gradient descent combined with a multistart method (n=100). We did not use a global optimisation approach such as differential evolution, which could give better results. Most of the optimisation runs resulted in identical optimal parameters (as seen in the waterfall plots), with some runs resulting in two alternative local optima. From our experience with previous models, the local multi-start methods with a large number of runs and the single global optimisations gave very similar results, although the global optimisations were much more computationally intensive. Therefore, we restricted the parameter optimisation to a local optimiser. (ii) A multi-step method was used instead of a single-step method that fits all parameters with all training data at once. The main reason for this is the heterogeneity of the data. Most studies report plasma or serum time courses of enalaprilat, enalapril is reported much less frequently, and data on urinary levels of enalapril and enalaprilat are much more limited. As a result of this imbalance in exercise data, certain parameters are difficult to determine. It is necessary to weight the data to account for the imbalance in the exercise

data. Instead of implementing a weighting scheme, we decided to perform a multi-step optimisation, which results in much less bias in the training data, rather than pooling all the data in a single step method. A single global optimisation of all parameters at once could provide a better overall fit because the parameters in the multi-step methods could compensate for each other. (iv) Classical single point optimisation was used instead of the Bayesian approach. This could provide a better fit and an estimate of the variability (posteriors) of the parameters, but at the cost of a much higher computational cost.

Despite these limitations and potential for improvement, we observed very good parameter optimisation results, resulting in very good model performance (agreement with data). There is a slight discrepancy between simulation and data for some studies, notably Dickstein et al. 1987 [8] and Hockings et al. 1986 [20]. While the model can certainly be improved to simulate these data points, a major problem is that the reported data itself is sometimes unreliable. One problem is that assays often have limited accuracy at very low concentrations of enalapril and enalaprilat. In some cases, the assays are also not specific enough to distinguish between enalapril and enalaprilat and can give false positives due to cross-reactivity. Other problems include systematic errors in data reporting. Therefore, even if the graph shows disagreement between the simulation and the reference, the model can still be said to provide a robust estimate. For a discussion and example of these problems see Grzegorzewski et al [15].

4.4 Simulations

The developed PBPK model accurately simulates a wide range of clinical studies of enalapril in different patient populations, single and multiple dose, oral and intravenous routes, and different pathophysiologies (renal and hepatic impairment). As shown in Fig. 16, Fig. 17 and Fig. 18, the model could adapt to a range of dosing variations. It could also adapt to renal and hepatic impairment and give a good estimate of the effect of these two conditions on the pharmacokinetics of enalapril.

The model was able to capture the reduced elimination of enalapril in

patients with renal impairment with a good degree of accuracy. The simulation could not match the observed reference data perfectly, but it captured the overall trend very well. (Fig. 27, Fig. 28, Fig. 24). Importantly, these simulations are model validations and none of the disease data were used for parameter optimisation. Including some of the renal impairment data in the model fitting would improve the fit.

In the case of hepatic impairment, the model was able to account for the reduced metabolic capacity of the liver and simulate the pharmacokinetics of enalapril and enalaprilat in plasma and urine with very good agreement with reference data (Fig. 35, Fig. 36).

Mutations in the CES1 genotype are an important factor affecting the pharmacokinetics of enalapril and the model was able to reproduce the effects through simulations (Fig. 44, Fig. **??**, Fig. **??**, Fig. 47). The overall effect of reduced and increased activity of CES1 due to variants was captured by the model. Due to the large variability between different studies, the predicted curves have a systematic bias. Importantly, none of the CES1 variant data were used in parameter optimisation, i.e. all predictions are model checks. With appropriate parameterisation, the model will be able to simulate the known variants even better, and in the future it could simulate additional CES1 variants as activity data of the variants become available.

4.5 Parameter scans

The predicted changes in enalapril and enalaprilat pharmacokinetics with changes in renal function, hepatic function and CES1 activity are in good agreement with cinical data.

The plot of renal activity versus degree of hepatic impairment (Fig. 21) shows that the plasma concentration of enalapril decreases with increasing renal activity and decreasing degree of hepatic impairment. This is because reduced renal activity means that enalapril is not excreted efficiently and reduced hepatic activity results in less conversion of enalapril to enalaprilat. This is also the case for enalaprilat, which is found in higher concentrations in the urine of patients with less severe cirrhosis (healthier patients). We can see that enalapril concentrations increase and enalaprilat concentrations

decrease as liver damage increases. This shows that our model can correctly describe the liver metabolism of enalapril to enalaprilat. With increasing renal impairment, the concentrations of enalapril and enalaprilat show a general increasing trend, which is justified by the impaired elimination of both substances by the kidneys. With increasing or decreasing CES1 activity due to different mutations, enalapril and enalaprilat concentrations show different trends. With increased CES1 activity, enalapril levels fall faster due to higher metabolism and enalaprilat levels rise earlier due to higher formation rates. When CES1 activity is reduced, the opposite occurs, as shown by the parameter scans from our model.

Our analysis focused on the effect of renal function, liver function and CES1 activity. No systematic parameter sensitivity analysis was performed, but the focus was on the effect of selected parameters corresponding to typical functional changes, i.e. hepatic impairment, renal impairment, changes in CES1 activity. A more detailed analysis of the effect of different parameters on the pharmacokinetics of enalapril may provide additional information.

4.6 Conclusion

In conclusion, this thesis has successfully developed and validated a physiologically based pharmacokinetic (PBPK) model for enalapril, which has significantly improved our understanding of its pharmacokinetics under various physiological and pathological conditions. Using data from 49 clinical trials, the model provides robust predictions of the behaviour of enalapril and enalaprilat in a wide range of patient populations. Its accuracy in simulating plasma and urine pharmacokinetic parameters in healthy individuals, as well as its ability to reflect changes due to renal and hepatic impairment and CES1 genetic variants, demonstrates its practical relevance and scientific validity. The predictive performance of this model is not only consistent with the existing literature, but also provides a reliable framework for further research and application in clinical settings. Thus, this work represents a substantial contribution to pharmacokinetic modelling, paving the way for more personalised and effective therapeutic strategies in the treatment of car-

diovascular disease and beyond.

5 Future prospects

The extensive research into the pharmacokinetics of enalapril and its active metabolite, enalaprilat, facilitated by the development of a physiologically based pharmacokinetic (PBPK) model, opens up several possibilities for future investigation and application. The robust development and validation of the model using data from 49 clinical trials provides a reliable basis for its application in further studies. Here we outline potential areas for future research and the wider implications of this work.

Personalized medicine The validated PBPK model for enalapril offers the potential to tailor dosing regimens to individual patients based on specific physiological and genetic profiles. Future research could focus on integrating genetic data on CES1 variants and other enzymes involved in the drug metabolism pathway. Combined with information on age, sex, body weight and liver, kidney and heart function, this could enable personalised treatment plans that optimise efficacy and minimise adverse effects, particularly in patients with renal or hepatic impairment.

The availability of the PBPK model under an open licence allows it to be used as a tool in clinical trial design. The model can help predict outcomes in populations that are not extensively studied in clinical trials, such as patients with extreme values of renal or hepatic function or those on polypharmacy regimens.

The availability of a detailed *in silico* model, which could act as a digital twin, could allow some clinical trials to be replaced by computer simulations. This is a much more ethical approach, as it avoids potential side effects, and is also much faster. New drugs are often invented or discovered, but their efficacy in treating the target disease is unpredictable and their unknown side effects are sometimes discovered too late. Computational modelling can give a fairly accurate idea of all these aspects and is therefore a highly desirable approach to drug design.

Expansion to pharmacodynamics This work focused on the pharmacokinetics of enalapril and enalaprilat. As part of the data curation effort, an extensive database on the pharmacodynamics of enalapril was established, consisting of information on systolic and diastolic blood pressure, heart rate,

mean arterial pressure and substances such as renin, angiotensin I and II and aldosterone in the RAAS. Extending the model to include pharmacodynamics would be an interesting next step.

Expansion to Other ACE Inhibitors Given the success of the PBPK model in simulating the pharmacokinetics of enalapril, similar models could be developed for other ACE inhibitors. This would improve the understanding of class effects of ACE inhibitors and support the rational design of combination therapies or new ACE inhibitors with improved pharmacokinetic profiles.

Advanced Computational Modeling The application of advanced computational techniques such as machine learning could further refine the PBPK model. These methods can be used to predict pharmacokinetic responses under a wider range of conditions not covered by the original model. In addition, simulations of drug-drug interactions could be explored to provide insights into the safe and effective use of enalapril with other drugs.

Educational use and training Finally, the model and its underlying database could serve as a valuable educational resource for pharmacology and pharmacokinetics training. They provide a practical case study in model-based drug development, demonstrating the integration of clinical data and computational modelling in understanding and predicting drug behaviour in humans.

In conclusion, the development and validation of the PBPK model for enalapril represents a significant step forward. Future research can use this model to improve the precision of hypertension therapy, extend its findings to other therapeutic areas and ultimately contribute to the development of more effective and safer approaches to pharmacotherapy.

6 Contributions

Shubhankar Palwankar (SP) carried out the data curation, simulations and data analysis. SP wrote the thesis and create the figures and tables.

Matthias König (MK) supervised the project. Matthias König (MK) supervised the project. MK acted as a second data curator to ensure the consistency of the curated data and simulations.

Acknowledgements

MK was supported by the Federal Ministry of Education and Research (BMBF, Germany) within ATLAS by grant number 031L0304B and by the German Research Foundation (DFG) within the Research Unit Program FOR 5151 "QuaLiPerF (Quantifying Liver Perfusion-Function Relationship in Complex Resection - A Systems Medicine Approach)" by grant number 436883643 and by grant number 465194077 (Priority Programme SPP 2311, Subproject SimLivA). This work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A537B, 031A533A, 031A538A, 031A533B, 031A535A, 031A537C, 031A534A, 031A532B).

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7 Supplementary Data

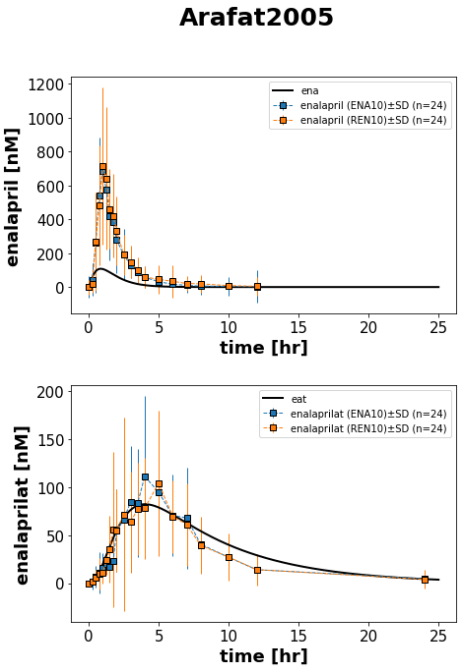


Figure 48: **Simulation for Arafat2005 [1].**

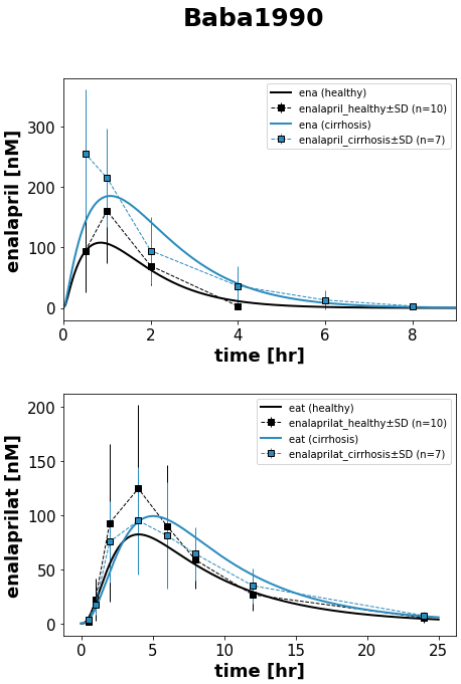


Figure 49: **Simulation for Baba1990 [2].**

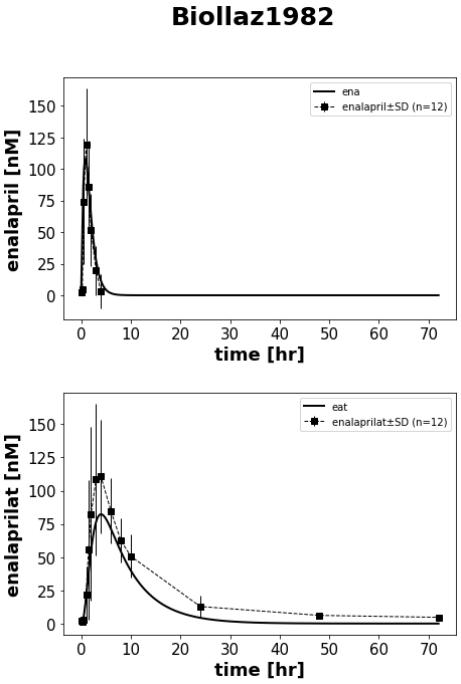


Figure 50: **Simulation for Biollaz1982 [4].**

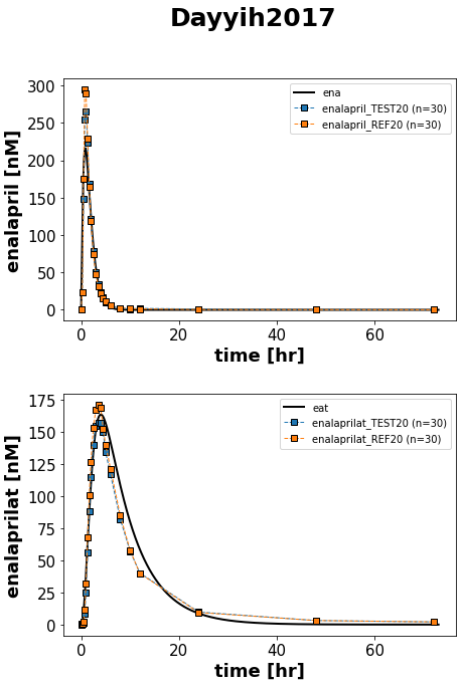


Figure 51: **Simulation for Dayyih2017 [7].**

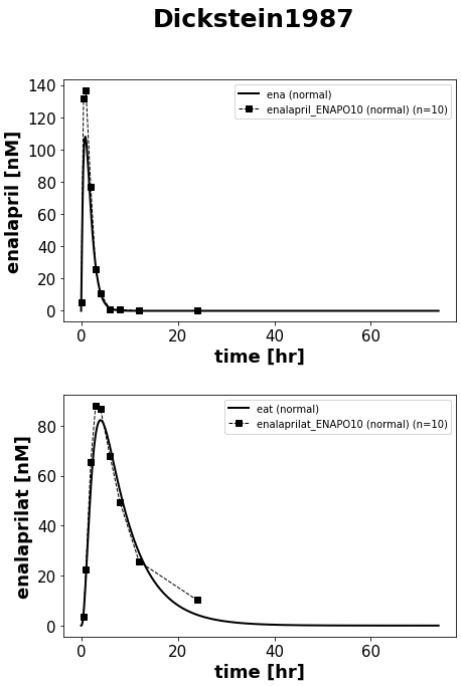


Figure 52: **Simulation for Dickstein1987_ENAPO10_normal [8].**

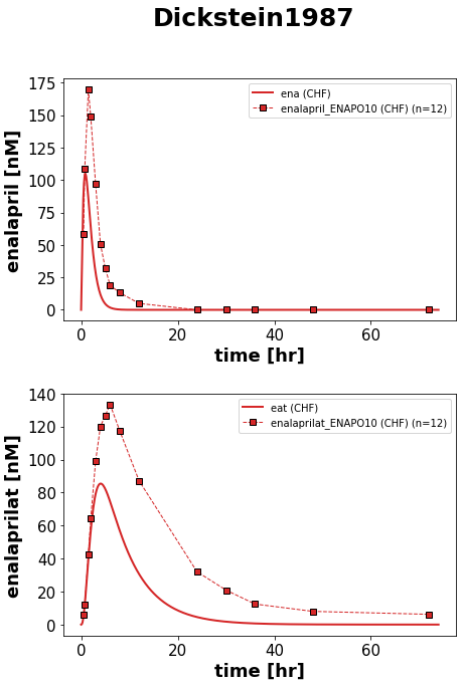


Figure 53: **Simulation for Dickstein1987_ENAPO10_CHF [8].**

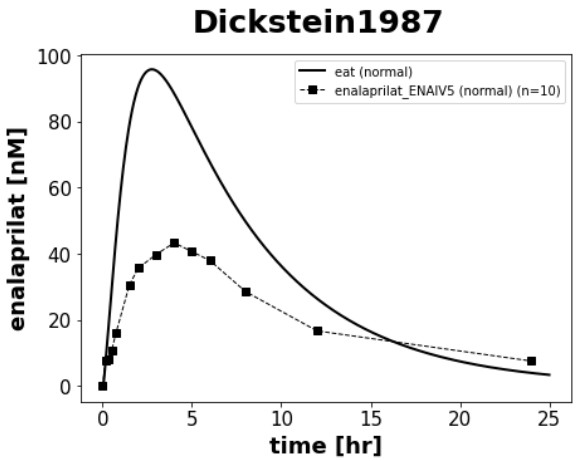
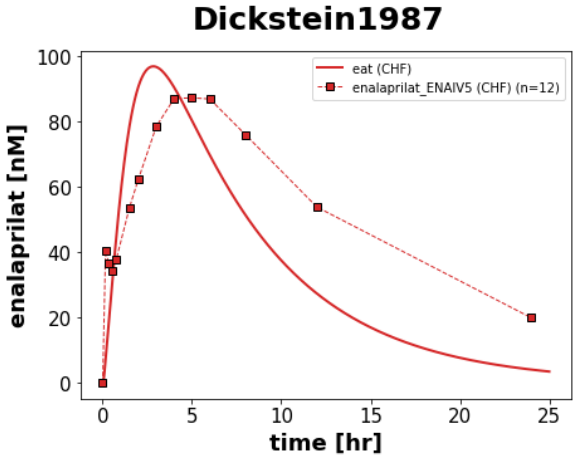


Figure 54: **Simulation for Dickstein1987_ENAIV5_normal [8].**



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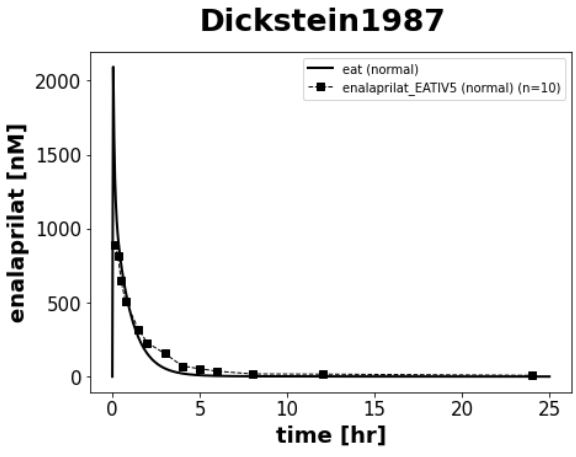
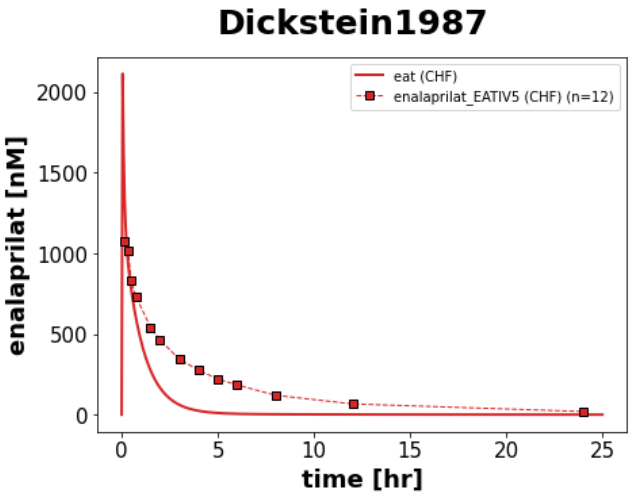


Figure 56: **Simulation for Dickstein1987_EATIV5_normal [8].**



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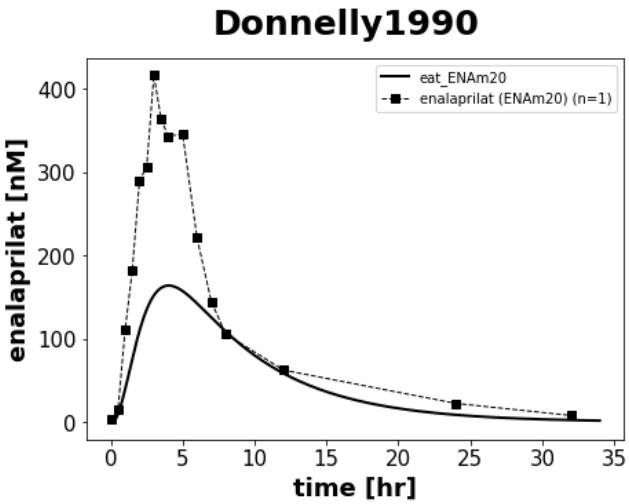

Figure 58: **Simulation for Donnelly1990 [10].**

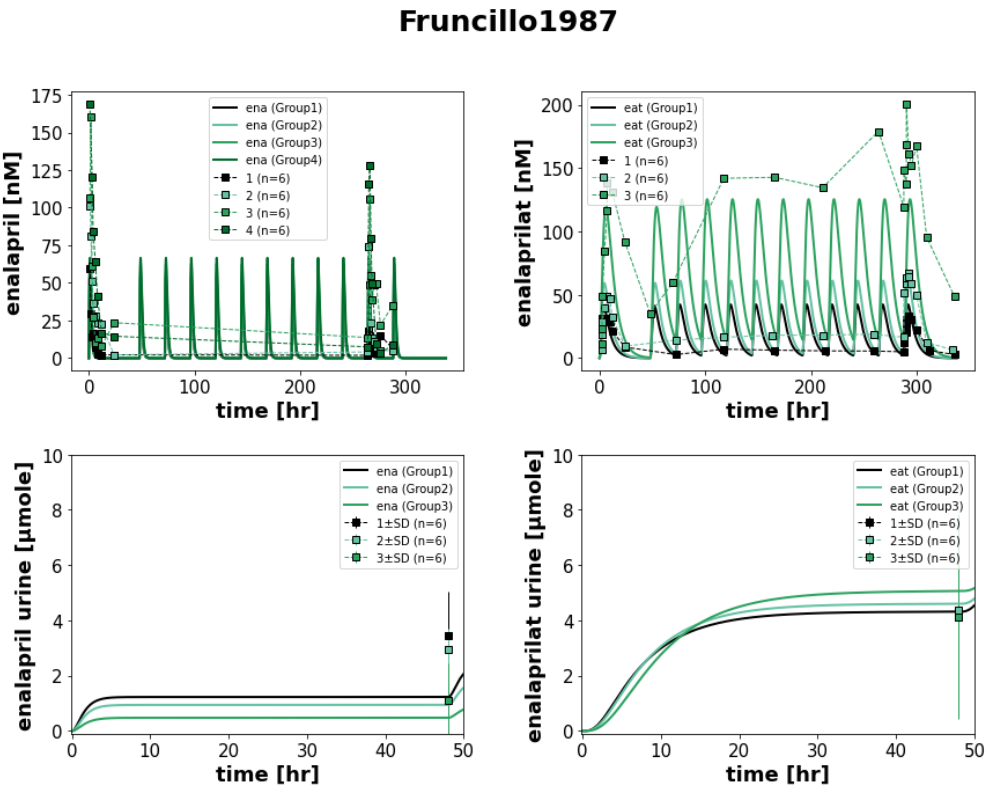

Figure 59: **Simulation for Fruncillo1987 [12].**

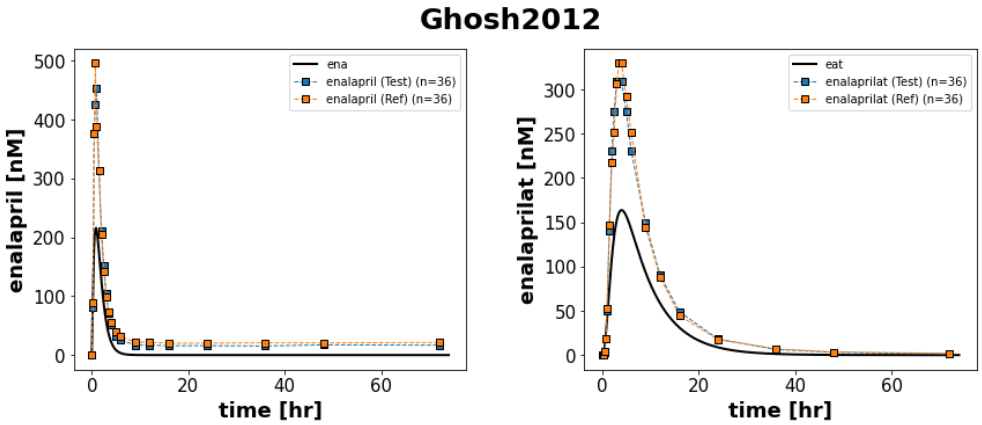

Figure 60: **Simulation for Ghosh2012 [13].**

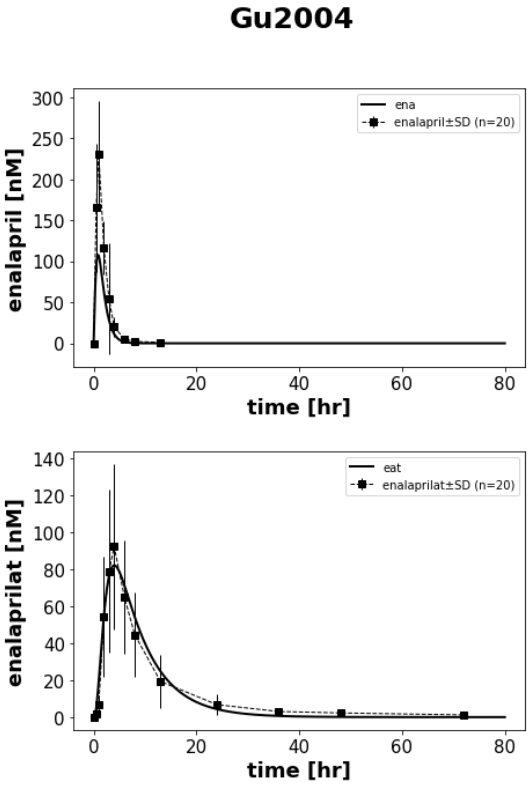

Figure 61: **Simulation for Gu2004 [17].**

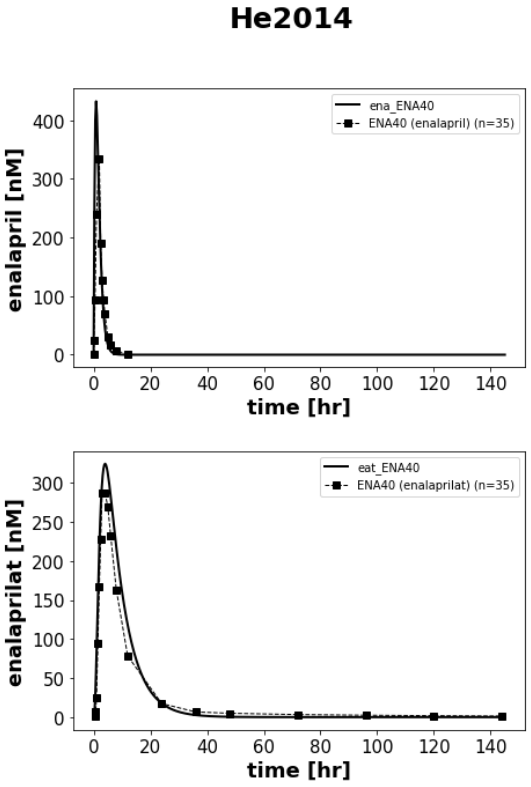

Figure 62: **Simulation for He2014 [18].**

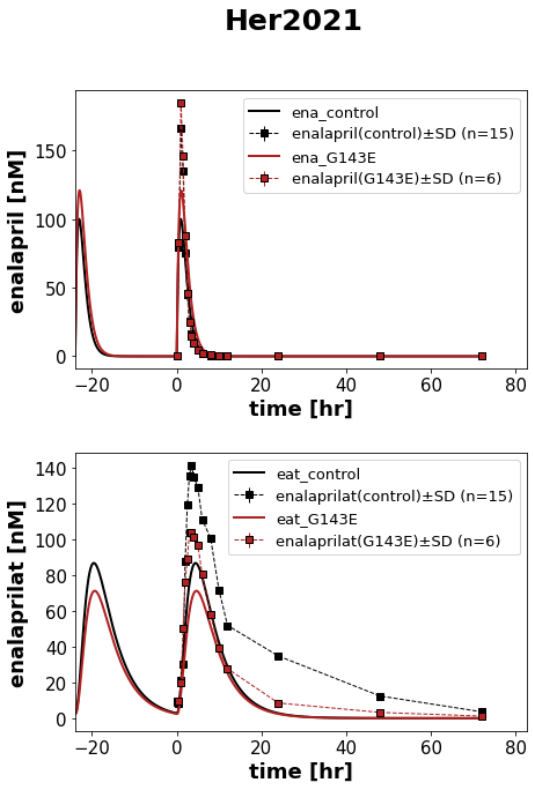

Figure 63: **Simulation for Her2021 [19].**

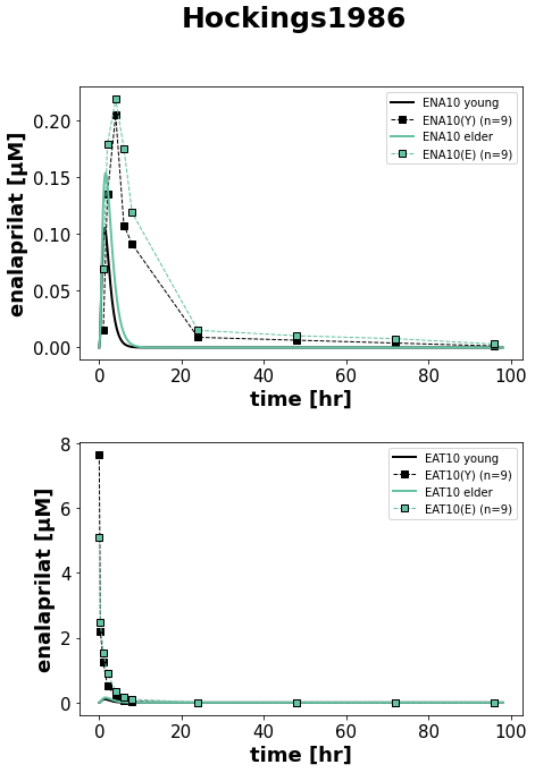

Figure 64: **Simulation for Hockings1986 [20].**

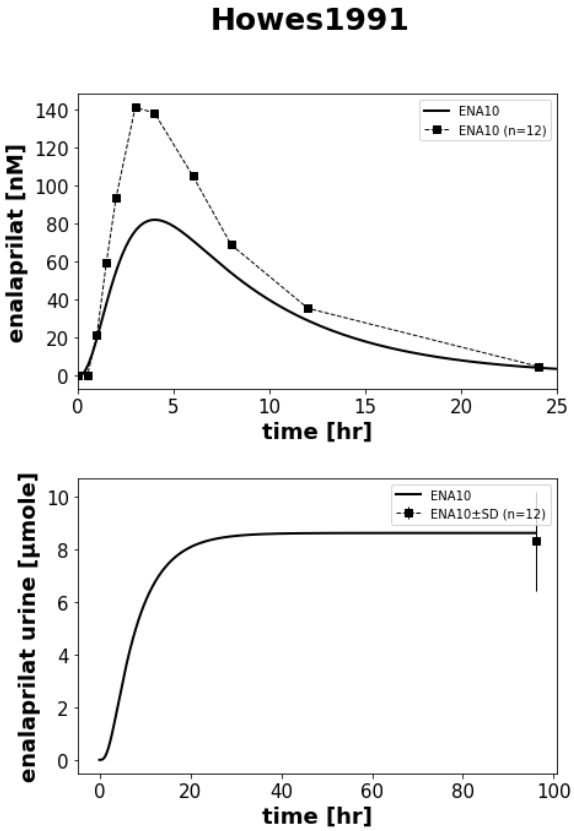

Figure 65: **Simulation for Howes1991 [21].**

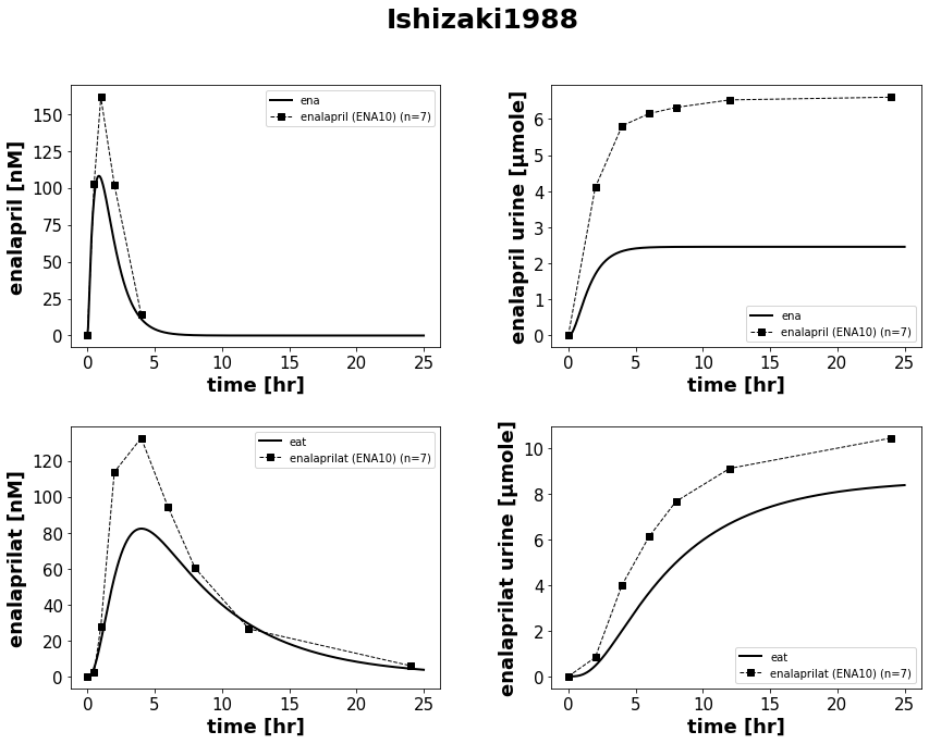

Figure 66: **Simulation for Ishizaki1988 [23].**

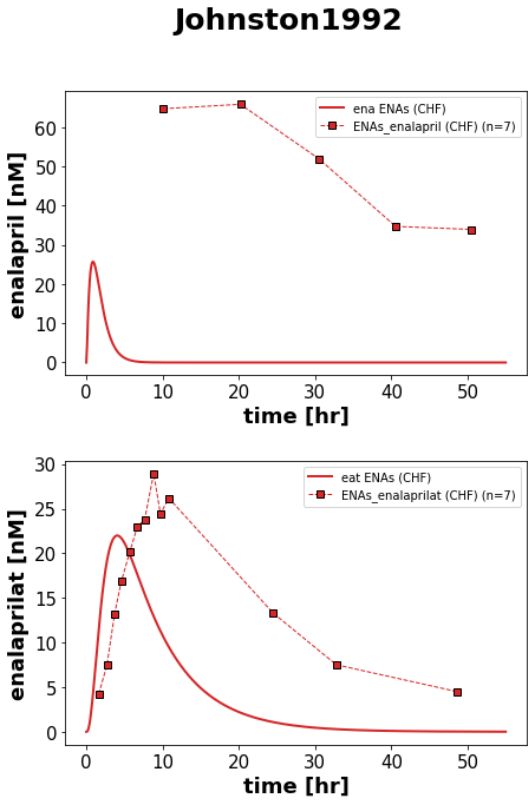

Figure 67: **Simulation for Johnston1992_single dose [24].**

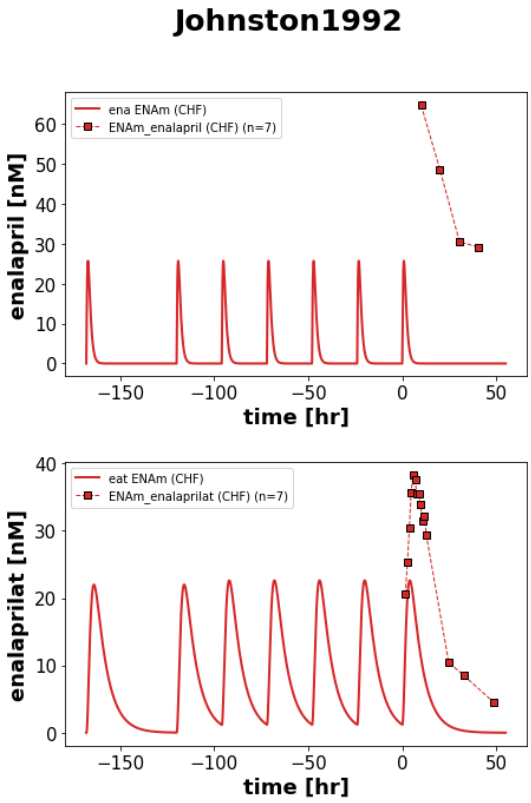

Figure 68: **Simulation for Johnston1992_multi dose [24].**

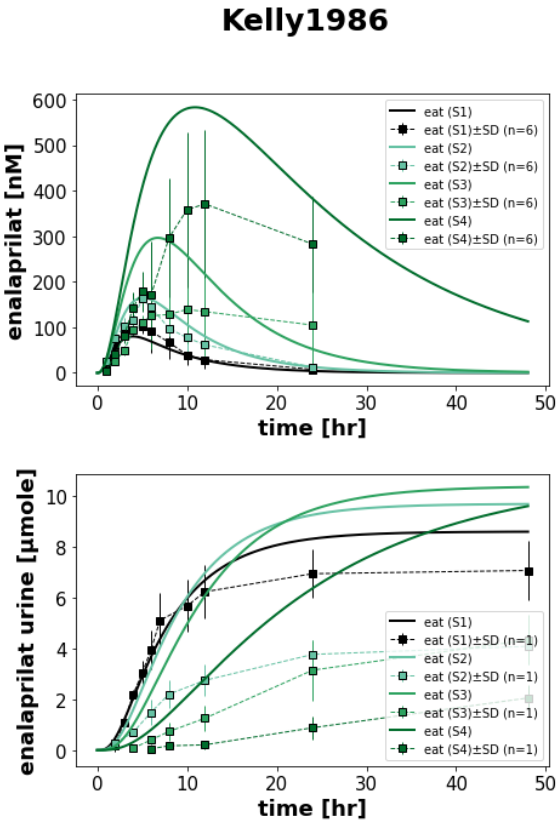

Figure 69: **Simulation for Kelly1986_single dose [26].**

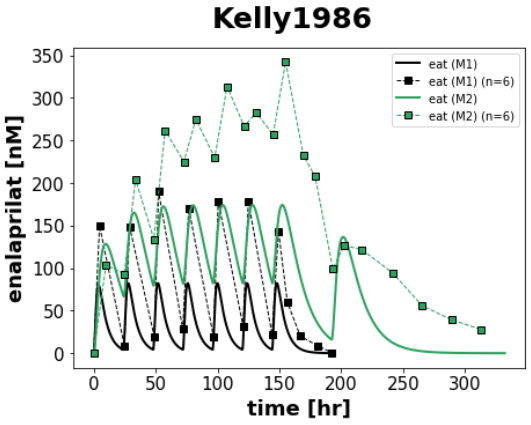

Figure 70: **Simulation for Kelly1986_multi dose [26].**

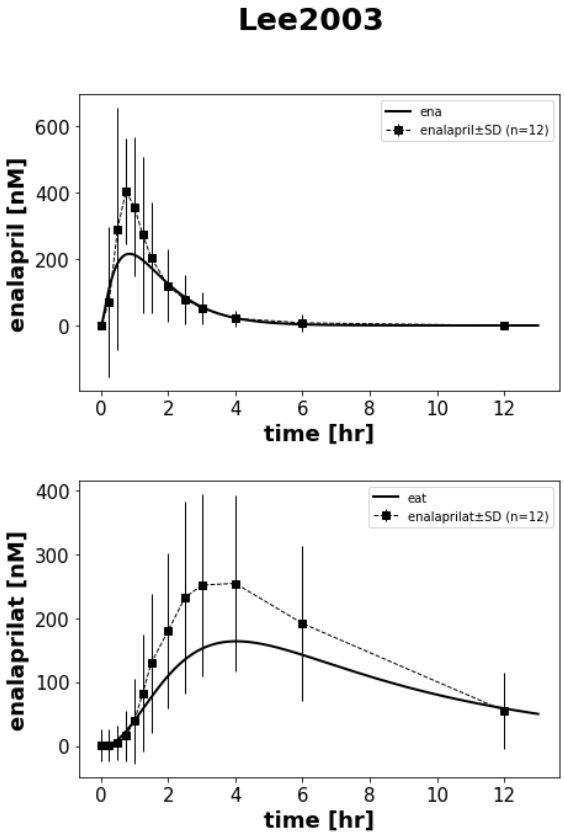

Figure 71: **Simulation for Lee2003 [34].**

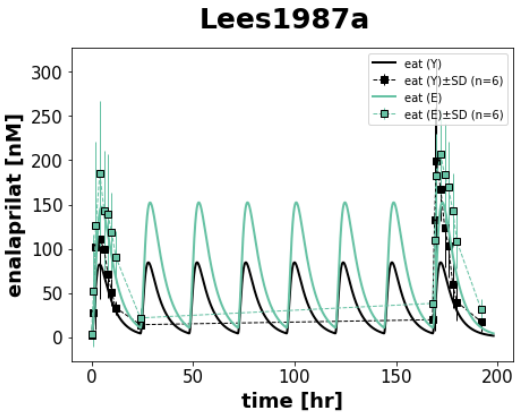

Figure 72: **Simulation for Lees1987a [35].**

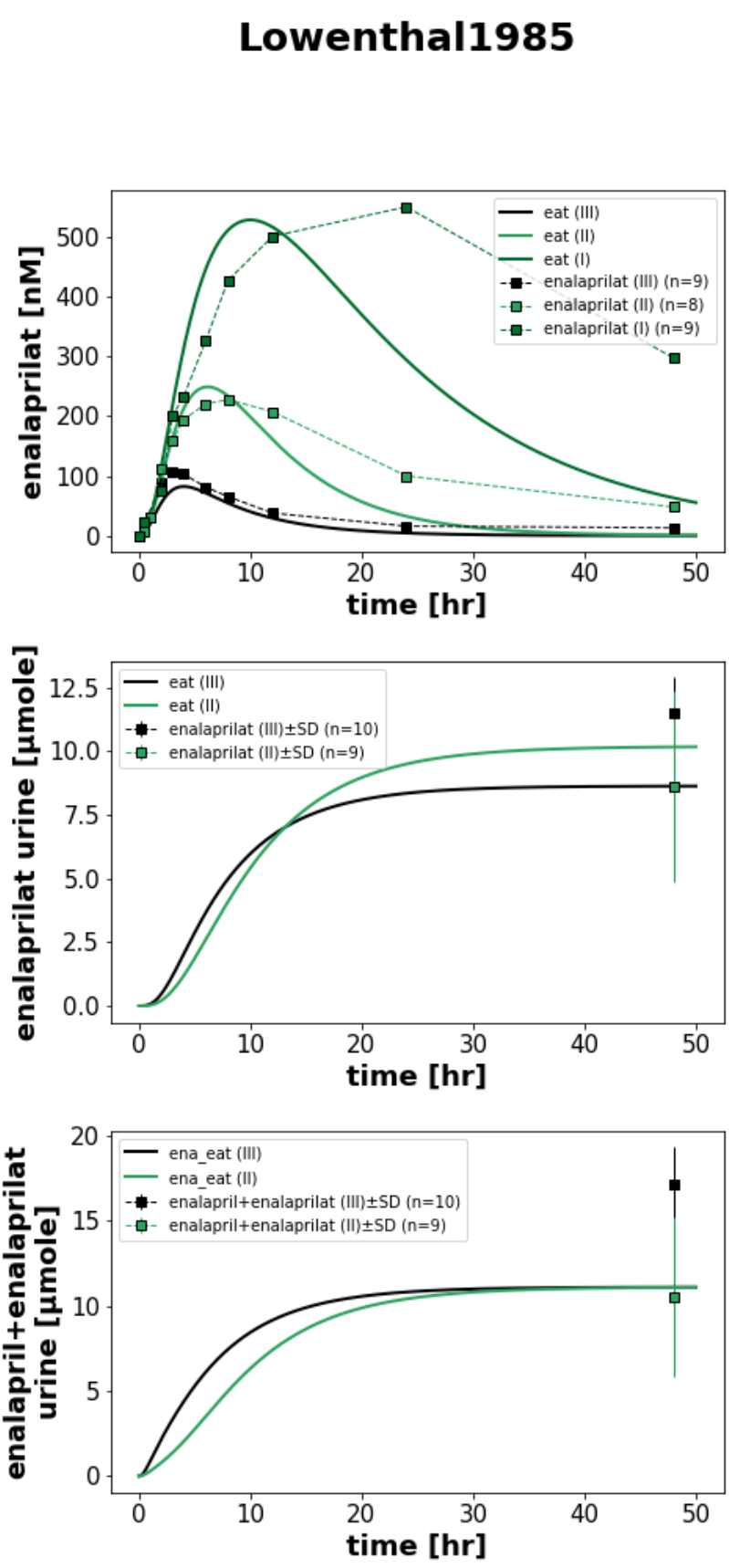

Figure 73: **Simulation for Lowenthal1985 [37].**

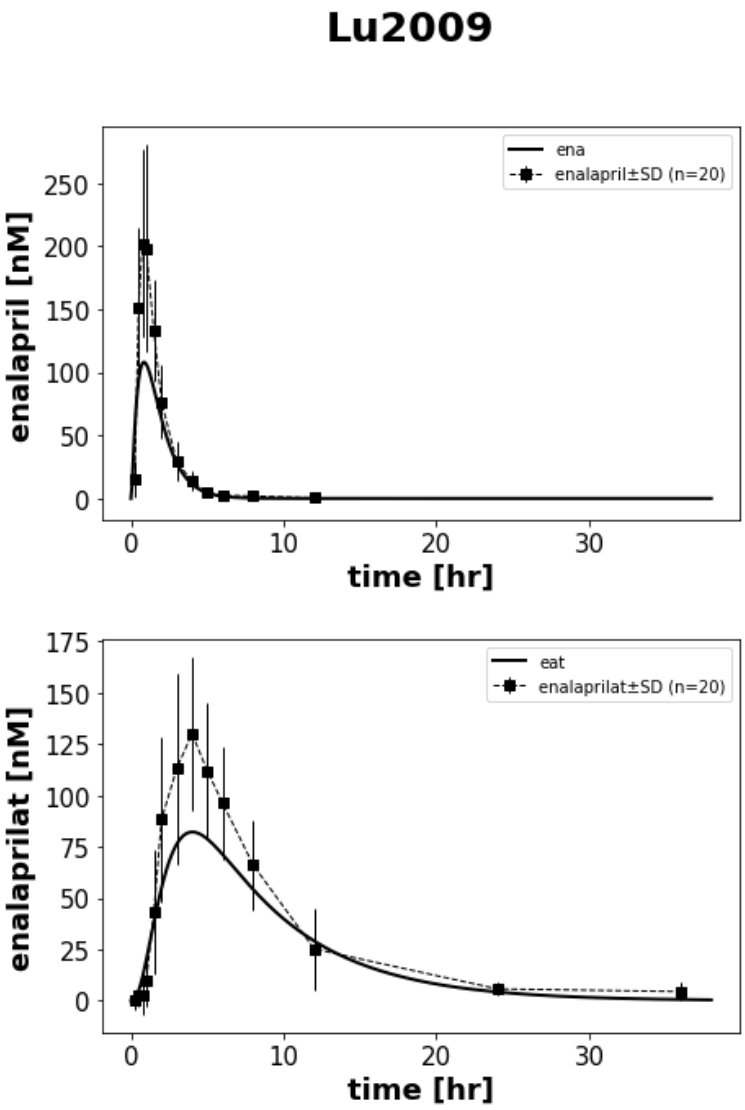

Figure 74: **Simulation for Lu2009 [39].**

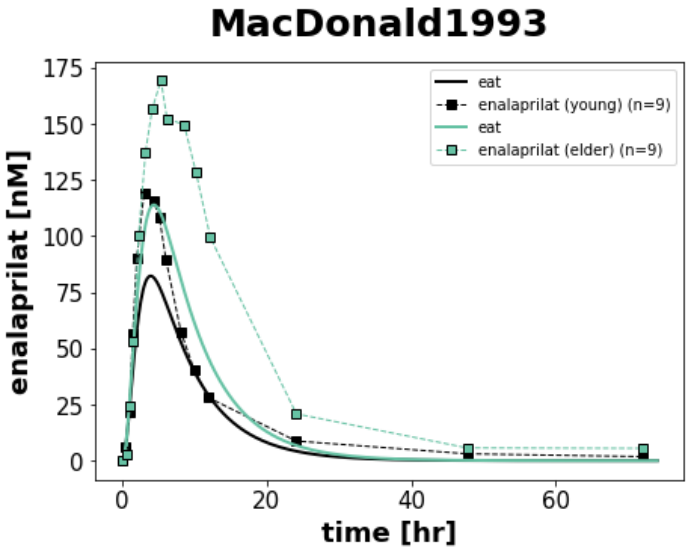

Figure 75: **Simulation for MacDonald1993 [40].**

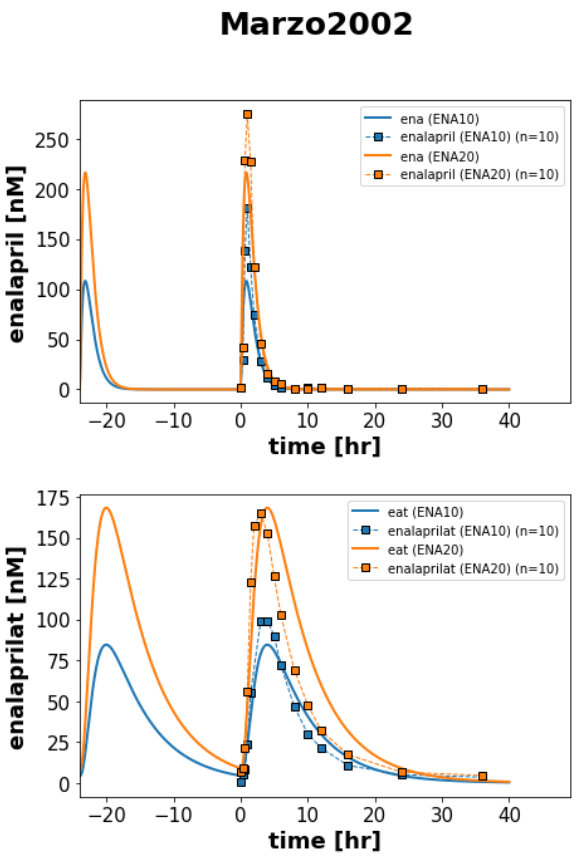

Figure 76: **Simulation for Marzo2002 [43].**

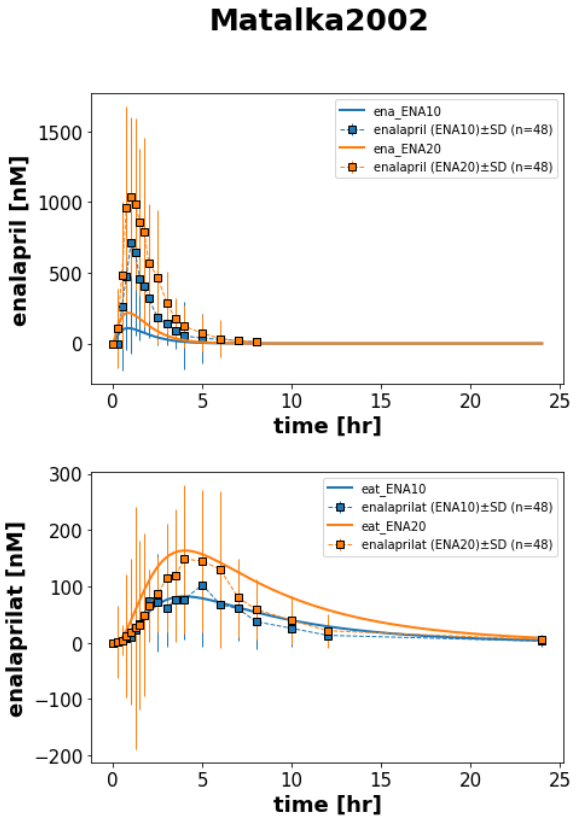

Figure 77: **Simulation for Matalka2002 [44].**

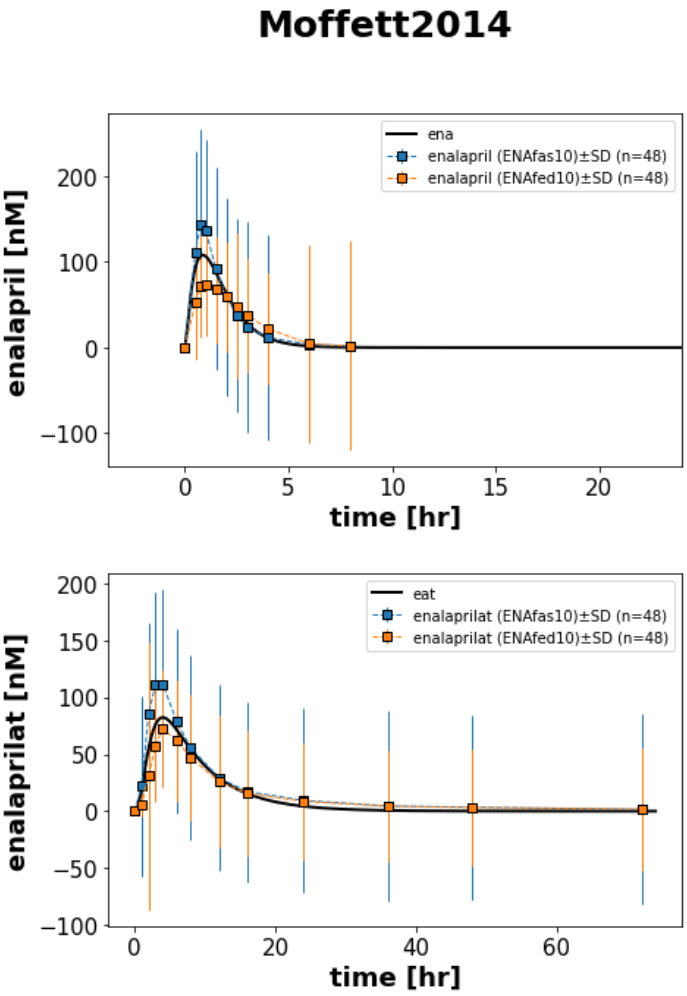

Figure 78: **Simulation for Moffett2014 [45].**

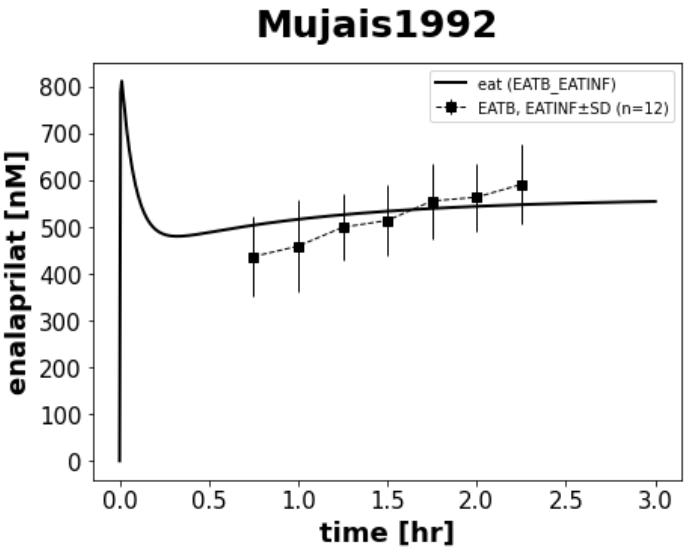

Figure 79: **Simulation for Mujais1992 [46].**

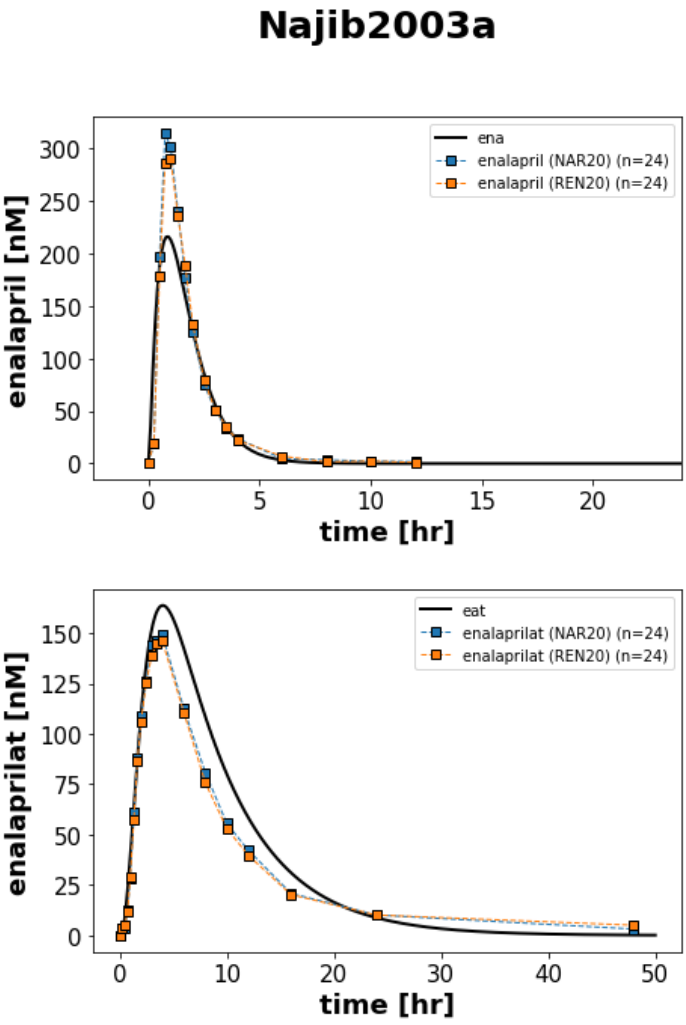

Figure 80: **Simulation for Najib2003a [47].**

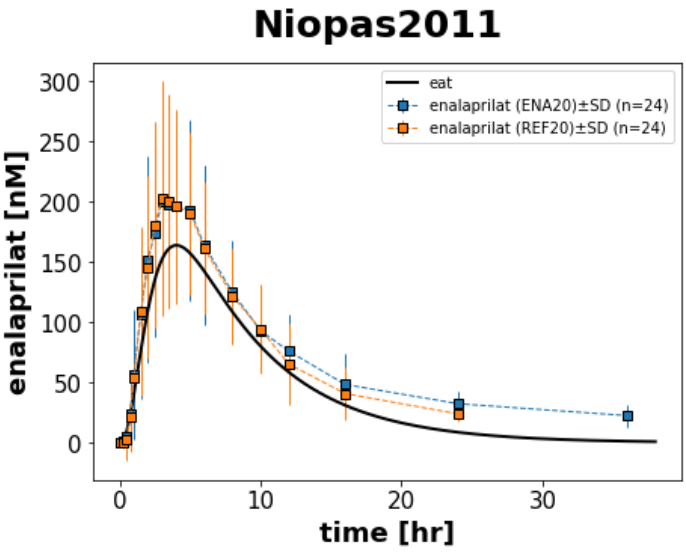

Figure 81: **Simulation for Niopas2003 [48].**

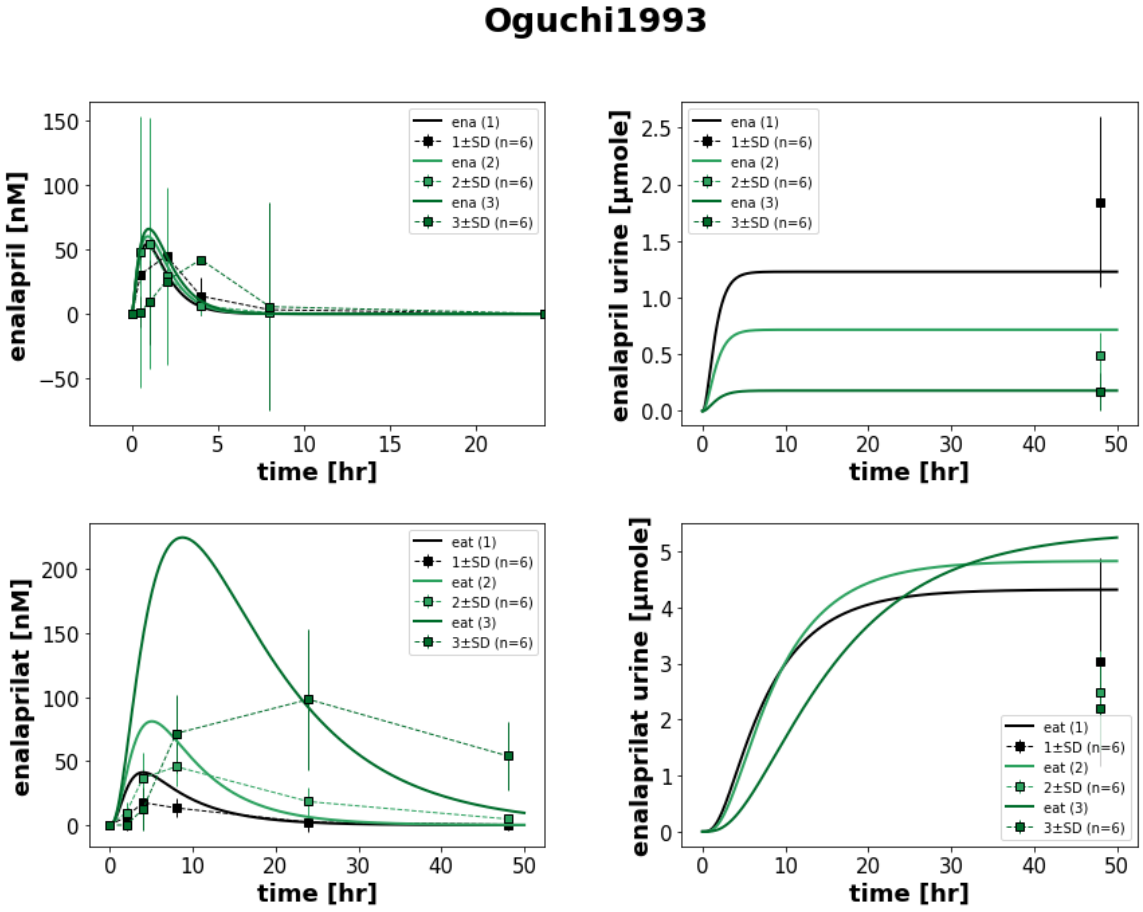

Figure 82: **Simulation for Oguchi1993 [50].**

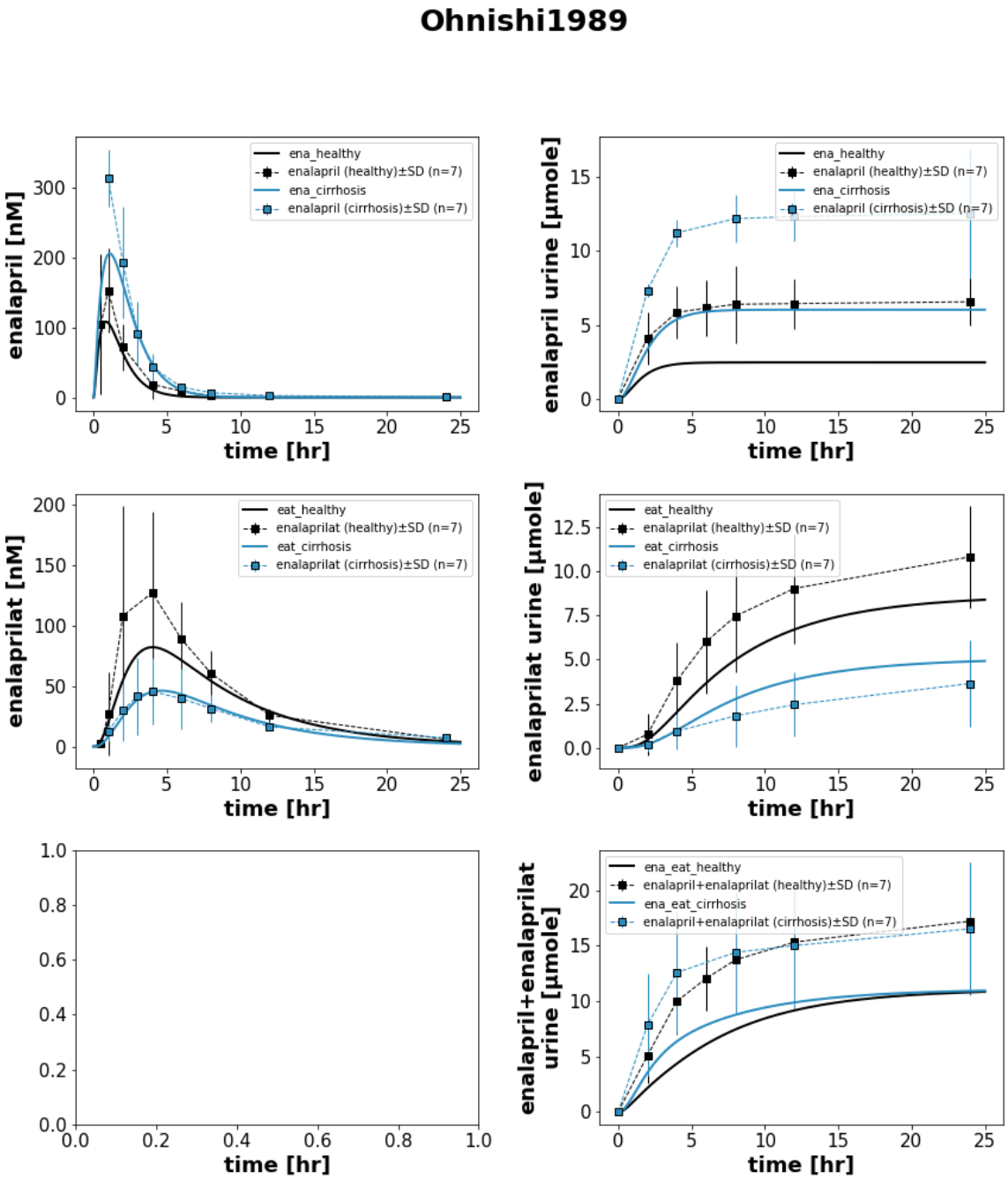

Figure 83: **Simulation for Ohnishi1989 [51].**

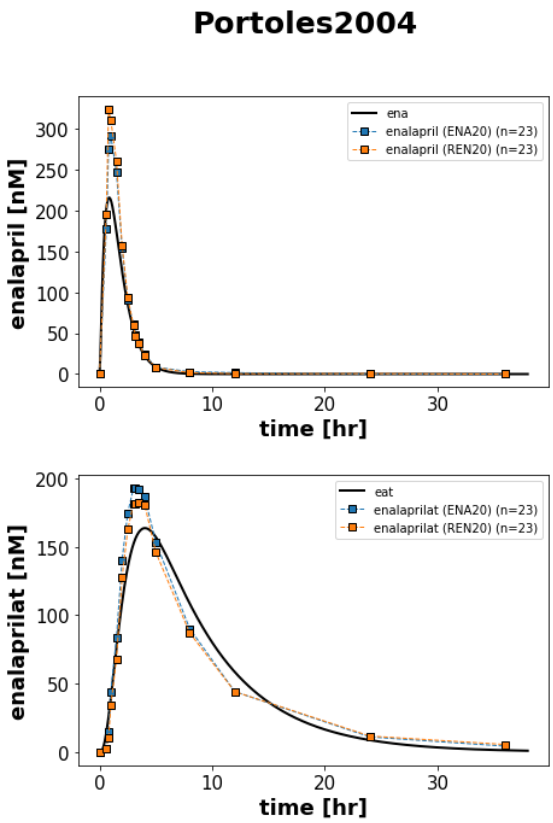

Figure 84: **Simulation for Portoles2004 [53].**

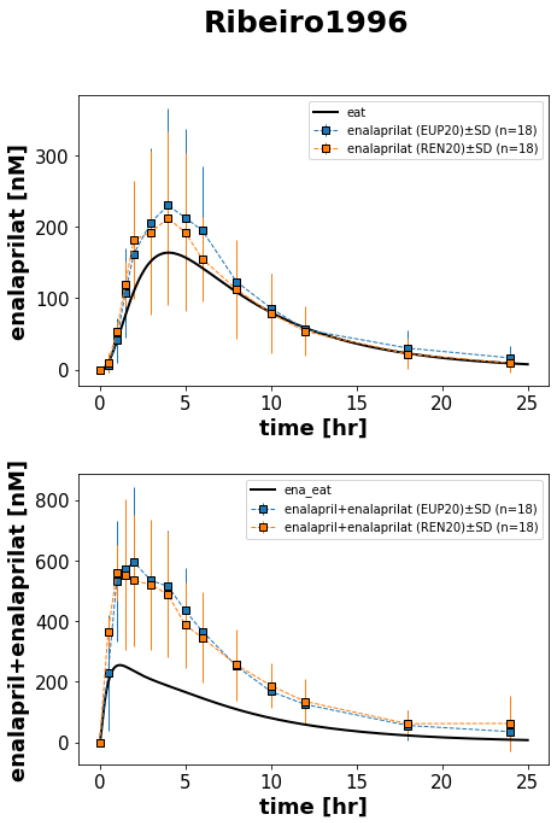

Figure 85: **Simulation for Ribeiro1996 [55].**

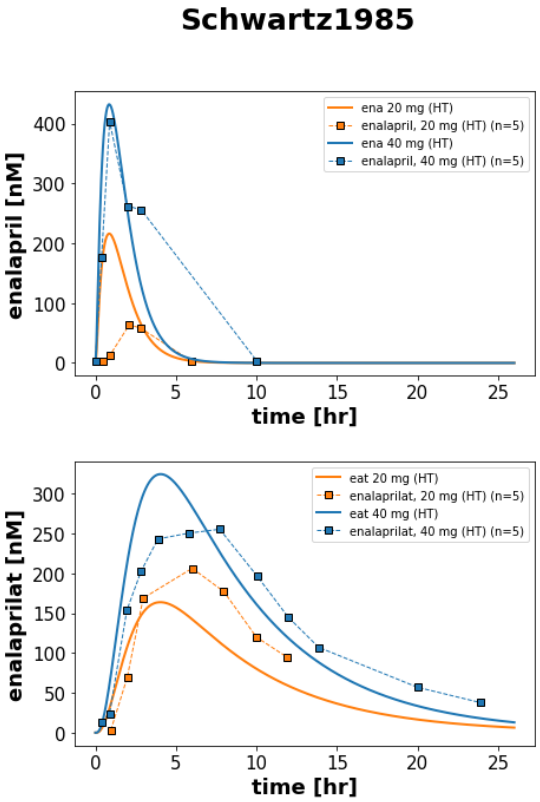

Figure 86: **Simulation for Schwartz1985_hypertension [58].**

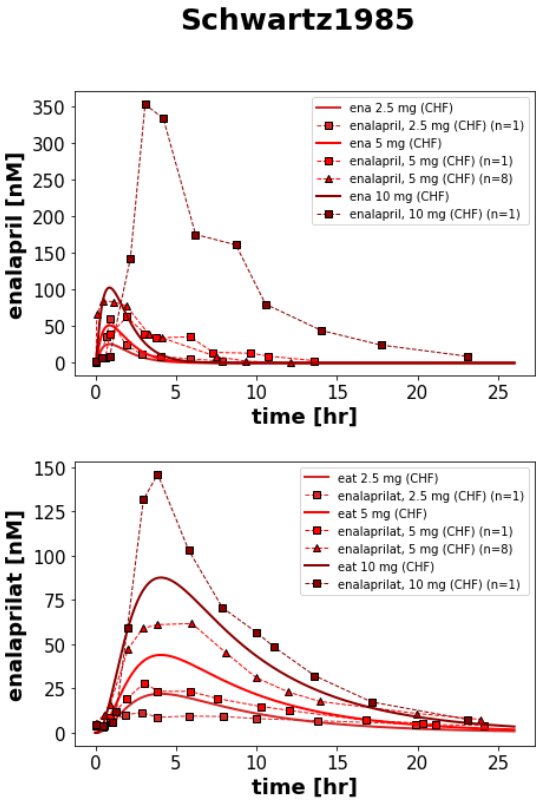

Figure 87: **Simulation for Schwartz1985_CHF [58].**

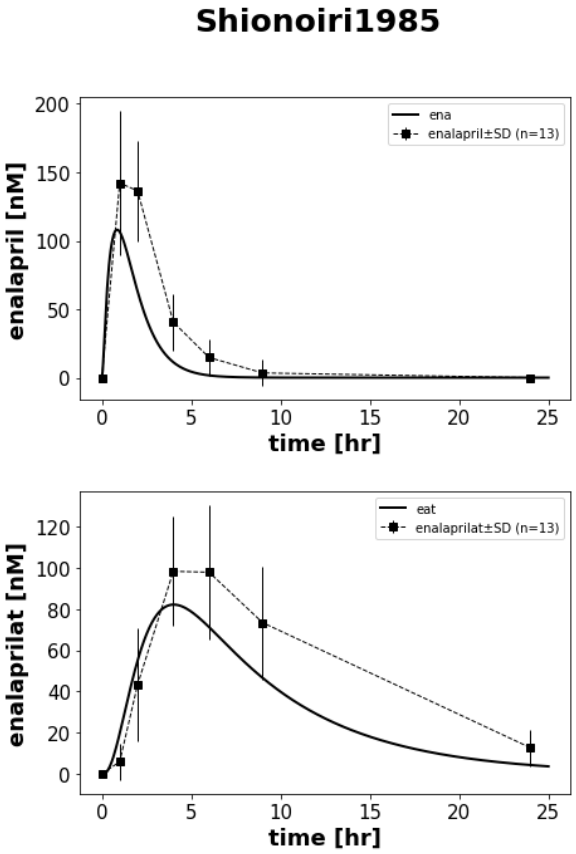

Figure 88: **Simulation for Shionoiri1985 [59].**

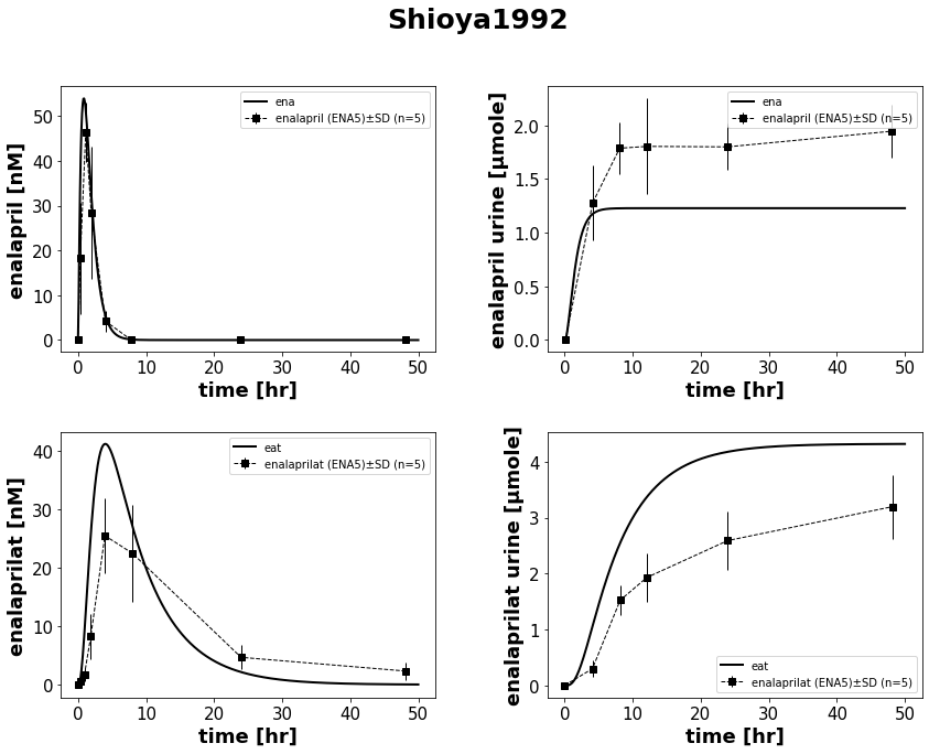

Figure 89: **Simulation for Shioya1992 [60].**

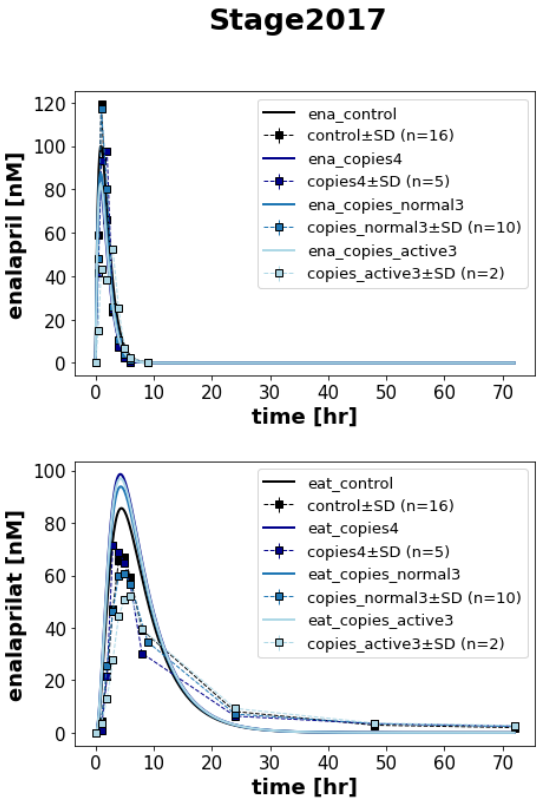

Figure 90: **Simulation for Stage2017_GAIN mutation [64].**

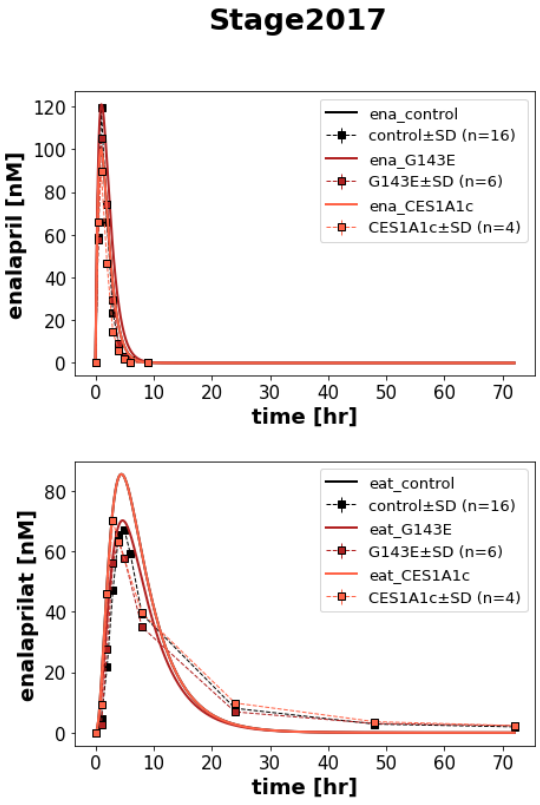

Figure 91: **Simulation for Stage2017_LOSS mutation [64].**

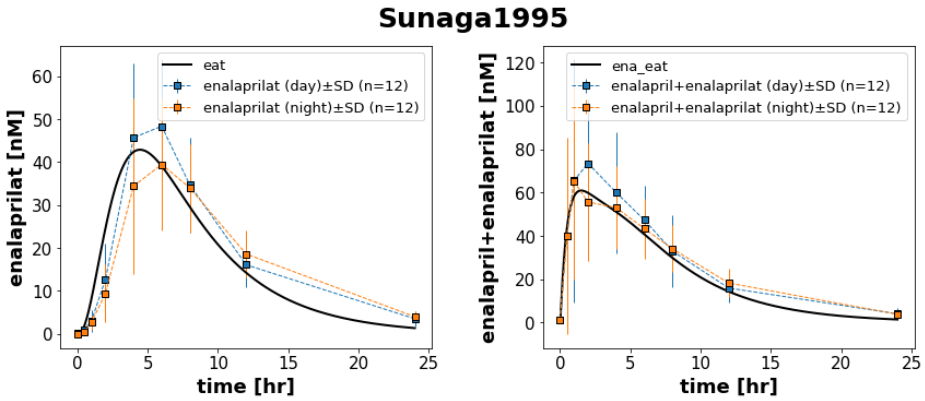

Figure 92: **Simulation for Sunaga1995 [66].**

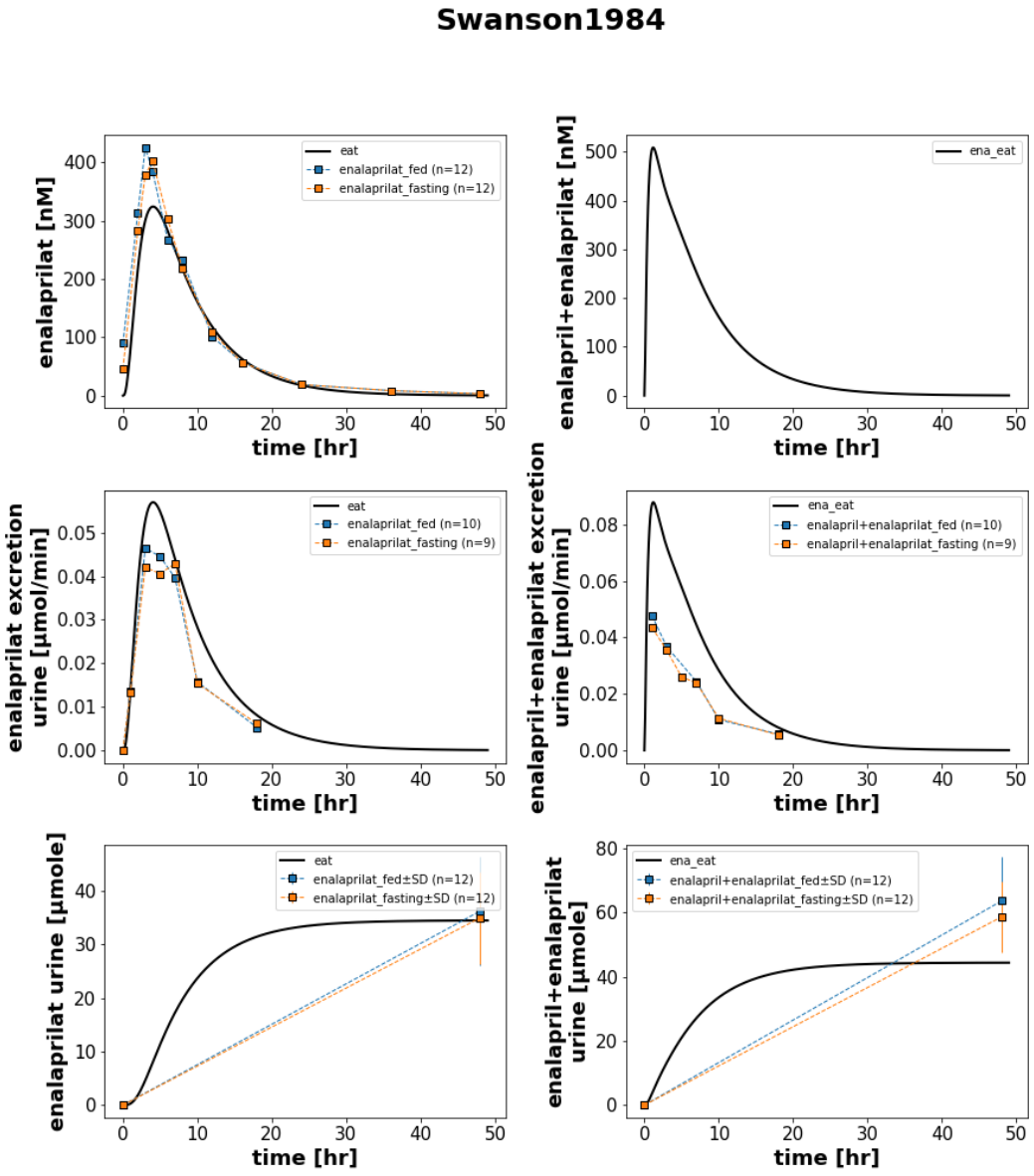

Figure 93: **Simulation for Swanson1984 [67].**

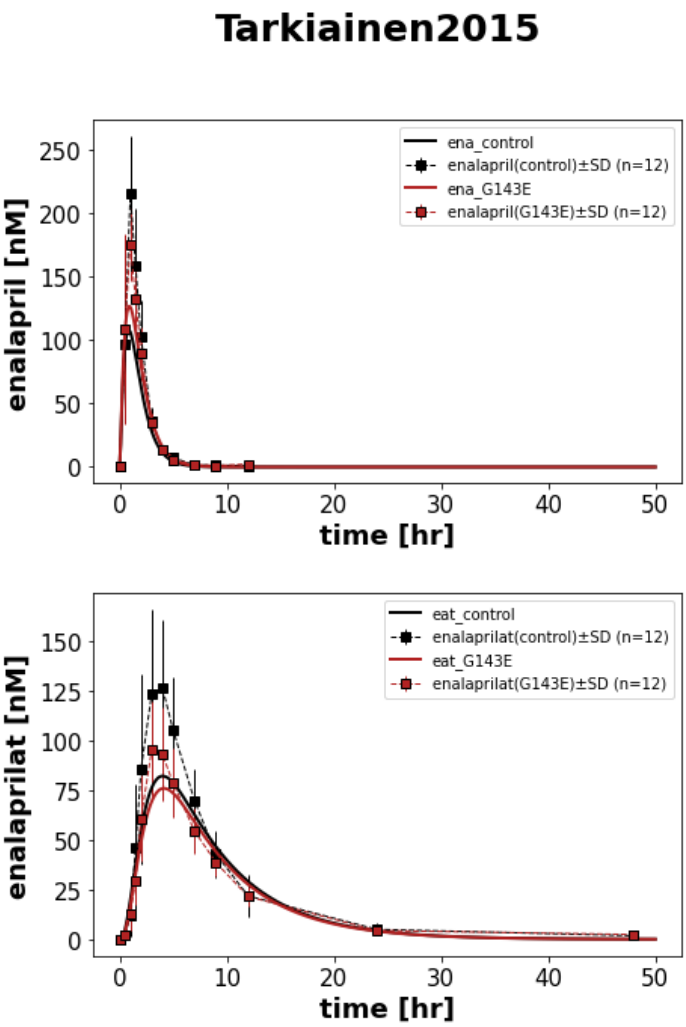

Figure 94: **Simulation for Tarkiainen2015 [68].**

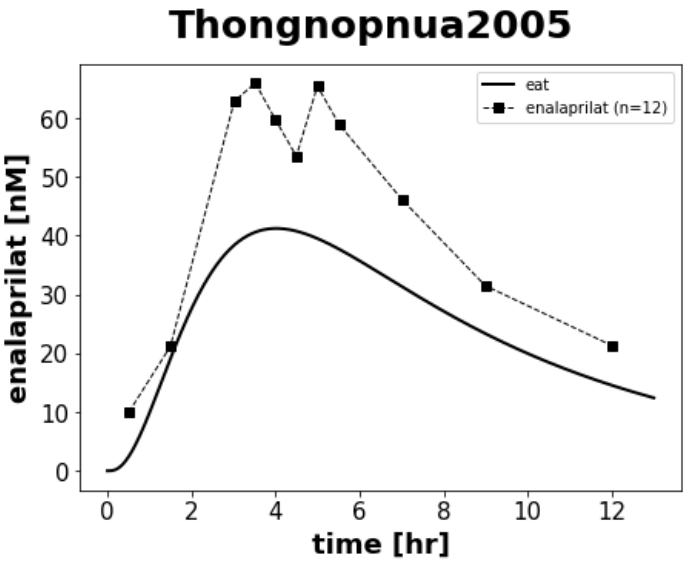

Figure 95: **Simulation for Thongnopnua2005 [70].**

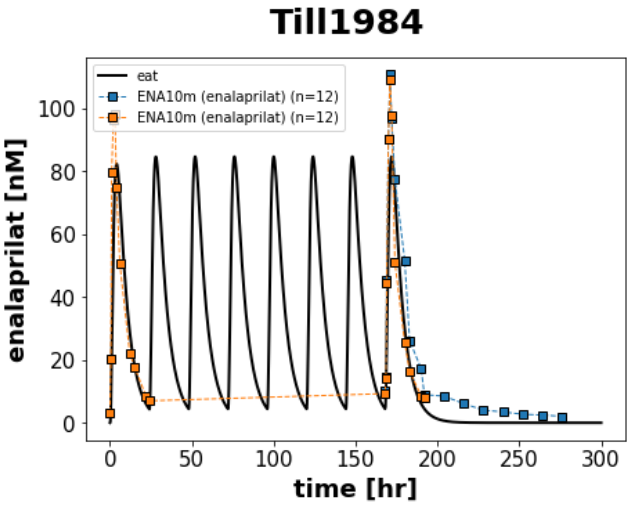

Figure 96: **Simulation for Till1984 [71].**

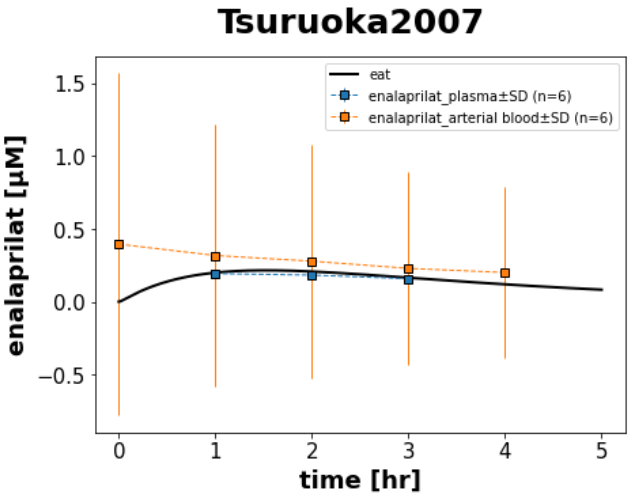

Figure 97: **Simulation for Tsuruoka2007 [73].**

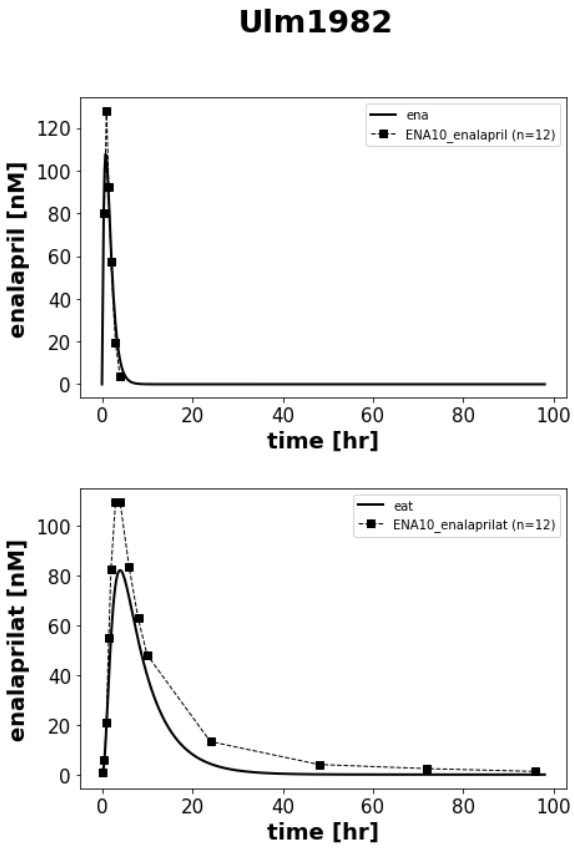

Figure 98: **Simulation for Ulm1982 [75].**

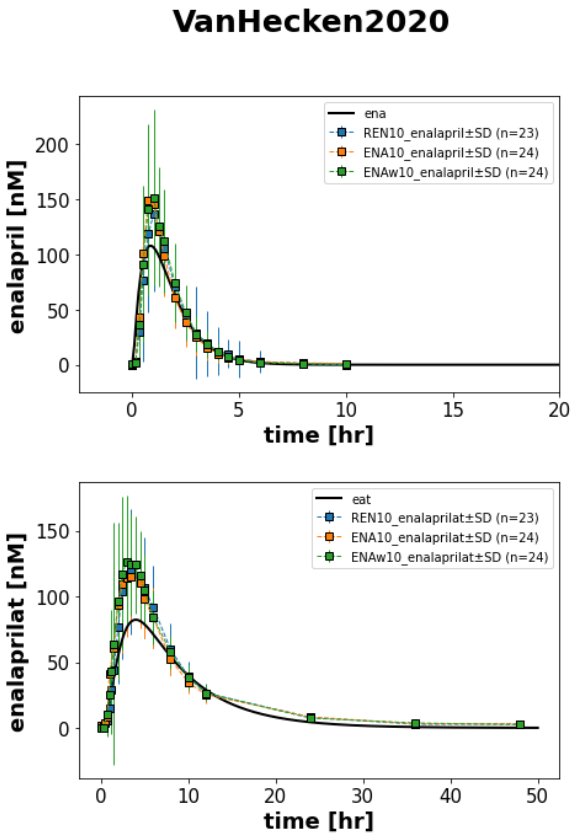

Figure 99: **Simulation for VanHecken2020 [76].**

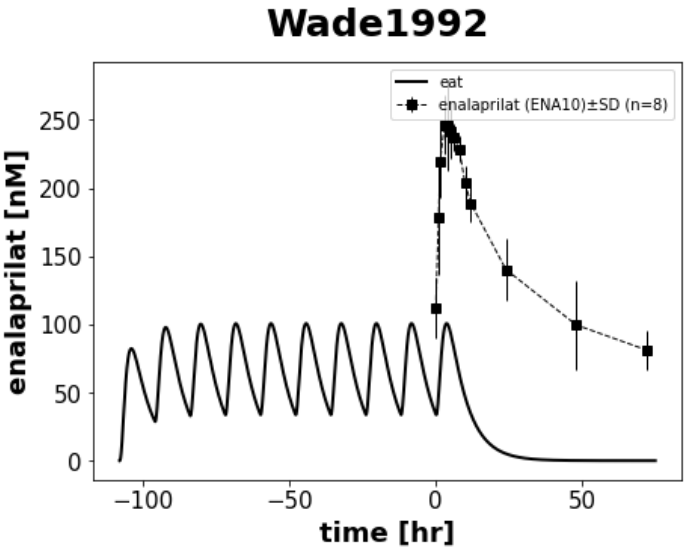

Figure 100: **Simulation for Wade1992 [78].**

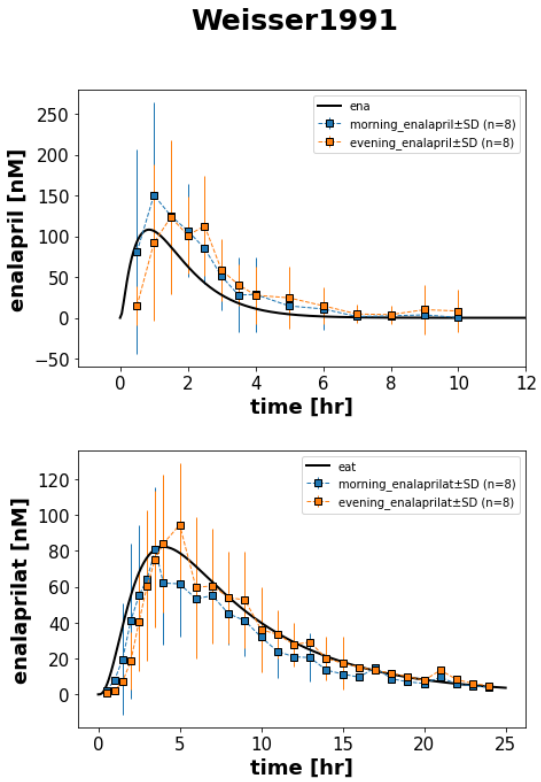

Figure 101: **Simulation for Weisser1991 [80].**

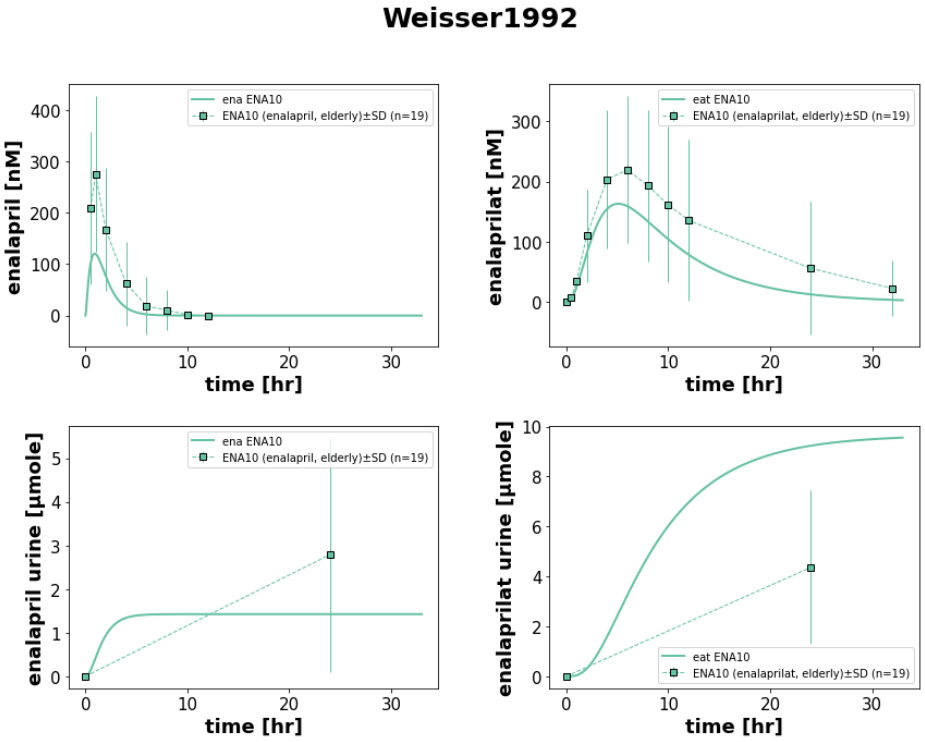

Figure 102: **Simulation for Weisser1992 [79].**

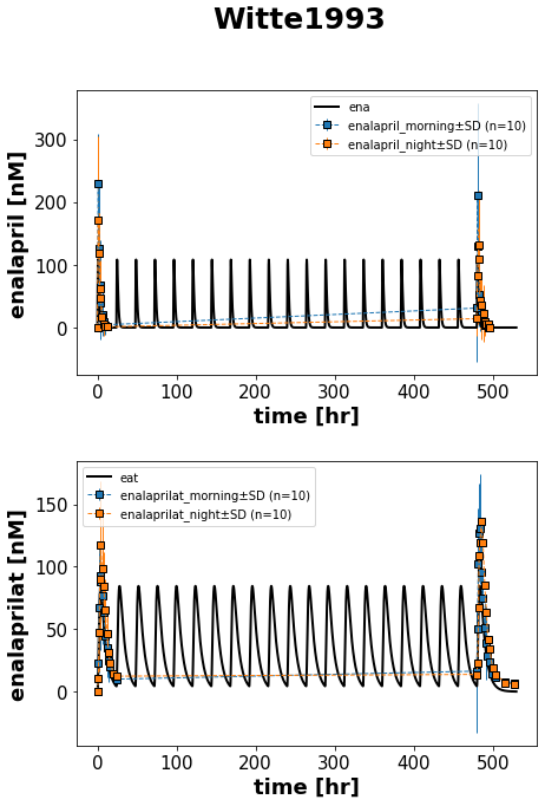

Figure 103: **Simulation for Witte1993 [82].**