Pharmacokinetics and Pharmacodynamics of Enalapril in ACE Inhibitor Naïve Children with Heart Failure

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Melina Theresa Christina Steichert
aus Hamm

Düsseldorf, Januar 2026

aus dem Institut für Klinische Pharmazie und Pharmakotherapie
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Berichterstatter:

1. Prof. Dr. Stephanie Läer

2. Prof. Dr. Georg Hempel

Tag der mündlichen Prüfung: 20. April 2026

‘Know what’s weird? Day by day nothing seems to change, but pretty soon, everything is different.’

Bill Watterson

I. Erklärung zur Dissertation

Ich versichere an Eides statt, dass die vorliegende Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keinen erfolglosen Promotionsversuch unternommen.

Düsseldorf, den _____ _____

(Melina Steichert)

II. Danksagung

An erster Stelle möchte ich mich bei Frau Prof. Dr. Stephanie Läer dafür bedanken, dass sie mir die Möglichkeit gegeben hat, dieses Promotionsprojekt zu bearbeiten. Ebenso danke ich ihr herzlich für die verständnisvolle Betreuung und die Förderung meiner fachlichen und persönlichen Entwicklung.

Bei Herrn Prof. Dr. Georg Hempel möchte ich mich herzlichst für die Übernahme des Korreferates bedanken.

Mein besonderer Dank gilt Herrn Dr. Willi Cawello, der diese Arbeit mit großem Engagement begleitet hat. Seine stetige Unterstützung sowie die Weitergabe seines Wissens und seiner langjährigen Erfahrung haben meine Arbeit wesentlich bereichert. Zudem danke ich ihm für die vielen hilfreichen Besprechungen sowie für die sorgfältige Durchsicht meiner Programme, Manuskripte, Poster und Präsentationen.

Herrn Prof. Dr. Holger Schwender möchte ich für die Übernahme der Mentorschaft und die fachliche Unterstützung bei statistischen Fragestellungen danken.

Herrn Prof. Dr. Björn Burckhardt und Herrn Dr. Fabian Süßenbach danke ich für ihre hilfsbereite Unterstützung bei Fragen.

Mein Dank gilt außerdem den Kindern, die an den LENA-Studien teilgenommen haben, und ihren Eltern. Durch ihre Bereitschaft zur Teilnahme an den LENA-Studien haben sie die Voraussetzung für diese Arbeit geschaffen. Des Weiteren möchte ich allen Mitgliedern des LENA-Konsortiums meinen Dank für ihren engagierten Einsatz im Rahmen des LENA-Projektes aussprechen.

Ein herzlicher Dank geht zudem an meine aktuellen sowie ehemaligen Kolleginnen und Kollegen: Ahmed, Alena, Anke, Armin, Bushra, Claire, Florian, Haidara, Imaneh, Jutta, Marah, Marwa, Mohammad, Petra, Rebecca, Sabina, Samieh, Shahzad, Tanja und Yousra. Neben dem fachlichen Austausch und euren Ermutigungen werden mir vor allem die vielen lustigen und schönen Momente mit euch stets in guter Erinnerung bleiben. Zusätzlich möchte ich mich bei Anke Bartel und Jutta Tins für ihre tatkräftige Unterstützung bei der Erstellung der Inputdatei, die Beantwortung meiner Fragen zum LENA-Projekt sowie für die sorgfältige Durchsicht zahlreicher Unterlagen bedanken.

Meiner Familie sowie meinen Freunden danke ich von Herzen für ihr Verständnis, ihren Zuspruch und die Ablenkung vom Arbeitsalltag. Darüber hinaus danke ich Julia Högemann für ihr wertvolles Feedback zu meinem Kongressvortrag und meinen Publikationen. Abschließend und ganz besonders möchte ich mich bei meinen Eltern und meinen Brüdern Tobias und Steffen bedanken, auf deren Unterstützung und Rückhalt ich mich immer verlassen kann.

III. Zusammenfassung

Die Therapie der pädiatrischen Herzinsuffizienz mit Angiotensin-Converting-Enzyme (ACE)-Hemmern ist weit verbreitet, basiert jedoch hauptsächlich auf der Extrapolation von Erkenntnissen, die bei Erwachsenen gewonnen wurden. Im Rahmen des europäischen Projekts „Labeling of Enalapril from Neonates up to Adolescents" (LENA) wurden altersgerechte, orodispersible Minitabletten mit Enalapril entwickelt und klinisch evaluiert, sodass sie 2023 für Kinder mit Herzinsuffizienz ab der Geburt zugelassen wurden. Die erwartete Zunahme der Anwendung von Enalapril bei sehr jungen und ACE-Hemmer naïven Kindern mit Herzinsuffizienz verstärkt die Notwendigkeit, die Pharmakokinetik und Pharmakodynamik von Enalapril sowie den Einfluss von alters- und krankheitsbedingten Unterschieden auf diese zu untersuchen. Zu diesem Zweck wurden Daten von ACE-Hemmer naïven Kindern mit Herzinsuffizienz aus den LENA-Studien modellunabhängig und modellabhängig analysiert.

Zunächst wurde ein kombiniertes pharmakokinetisches Populationsmodell für Enalapril und seinen aktiven Metaboliten Enalaprilat entwickelt. Die populationspharmakokinetische Analyse ergab, dass die Clearance von Enalaprilat mit zunehmendem Alter zunimmt und mit steigendem Serumkreatinin abnimmt. Außerdem nimmt das Verteilungsvolumen von Enalaprilat mit steigendem Ross-Score, einem Maß für den Schweregrad der Herzinsuffizienz, ab. Um die klinische Relevanz des Gewichts und der identifizierten Kovariaten zu bewerten, wurden Simulationen durchgeführt. Dabei wurde der Ross-Score als klinisch relevante Kovariate für die erste Dosis von Enalapril identifiziert. Darüber hinaus waren Alter, Gewicht und Nierenfunktion klinisch relevante Kovariaten sowohl für die erste Dosis als auch für die Steady-State-Dosis von Enalapril.

Als Zweites wurde eine systematische Literaturrecherche durchgeführt und Daten zur Plasma-Renin-Aktivität aus den LENA-Studien ausgewertet, um den Einfluss von Alter, Herzinsuffizienz und ACE-Hemmer-Behandlung auf den pharmakodynamischen Parameter Plasma-Renin-Aktivität zu beurteilen. Aus den Literaturdaten ging ein altersbedingter Rückgang der Plasma-Renin-Aktivität bei gesunden Kindern hervor, wobei die Werte bei Neugeborenen bis zu siebenmal höher waren als bei älteren Kindern. Im Vergleich zu gesunden Gleichaltrigen wiesen Kinder mit Herzinsuffizienz unter sechs Monaten eine drei- bis viermal höhere Plasma-Renin-Aktivität auf. Darüber hinaus hatten Kinder mit symptomatischer Herzinsuffizienz vor der Behandlung mit Enalapril eine signifikant höhere

Plasma-Renin-Aktivität als Kinder mit asymptomatischer Herzinsuffizienz. Nach wiederholter Verabreichung von Enalapril stieg die Plasma-Renin-Aktivität bei Kindern mit Herzinsuffizienz signifikant um den Faktor 4,5 an.

Als Drittes wurde ein pharmakokinetisches/pharmakodynamisches Populationsmodell für gesunde Erwachsene und ein pharmakodynamisches Populationsmodell für Kinder mit Herzinsuffizienz entwickelt, um die Wirkung von Enalaprilat auf das Verhältnis von Angiotensin II zu Angiotensin I zu beschreiben. Vor der ersten Dosis von Enalapril zeigte sich bei Kindern mit Herzinsuffizienz ein höheres Verhältnis von Angiotensin II zu Angiotensin I als bei gesunden Erwachsenen. Außerdem war die Populationsschätzung der halbmaximalen Hemmkonzentration bei Kindern mit Herzinsuffizienz niedriger als bei gesunden Erwachsenen, was darauf hindeutet, dass Kinder mit Herzinsuffizienz möglicherweise empfindlicher auf Enalaprilat reagieren als gesunde Erwachsene. Angesichts der Veränderung des Verhältnisses von Angiotensin II zu Angiotensin I kann angenommen werden, dass mit der verabreichten Dosis von Enalapril bei Kindern mit Herzinsuffizienz eine wirksame ACE-Hemmung erreicht wurde.

IV. Summary

The therapy of paediatric heart failure with angiotensin-converting enzyme (ACE) inhibitors is widespread, although it is primarily based on the extrapolation of findings obtained in adults. In the context of the European project ‘Labeling of Enalapril from Neonates up to Adolescents’ (LENA), age-appropriate, orodispersible minitablets with enalapril were developed and clinically evaluated, which resulted in their approval in 2023 for children with heart failure from birth. The expected increase in the use of enalapril in very young and ACE inhibitor naïve children with heart failure reinforces the need to investigate the pharmacokinetics and pharmacodynamics of enalapril as well as the influence of age- and disease-related differences on these. To this end, data from ACE inhibitor naïve children with heart failure from the LENA studies were analysed in a model-independent and model-dependent manner.

Firstly, a combined population pharmacokinetic model was developed for enalapril and its active metabolite enalaprilat. Population pharmacokinetic analysis revealed that the clearance of enalaprilat increases with increasing age and decreases with increasing serum creatinine. In addition, the volume of distribution of enalaprilat decreases with increasing Ross score, a measure of the severity of heart failure. To assess the clinical relevance of weight and the identified covariates, simulations were performed. Thereby, the Ross score was identified as a clinically relevant covariate for the first dose of enalapril. Furthermore, age, weight, and renal function were clinically relevant covariates for both the first dose and the steady state dose of enalapril.

Secondly, a systematic literature review was conducted and data on plasma renin activity from the LENA studies were evaluated to assess the influence of age, heart failure, and ACE inhibitor treatment on the pharmacodynamic parameter plasma renin activity. An age-related decline in plasma renin activity in healthy children was apparent from the literature data, with levels in neonates up to seven times higher than in older children. Compared with healthy peers, children with heart failure younger than six months had three to four times higher plasma renin activity. In addition, children with symptomatic heart failure had a significantly higher plasma renin activity than children with asymptomatic heart failure prior to treatment with enalapril. After repeated administration of enalapril, plasma renin activity increased significantly by a factor of 4.5 in children with heart failure.

Thirdly, a population pharmacokinetic/pharmacodynamic model for healthy adults and a population pharmacodynamic model for children with heart failure were developed to describe the effect of enalaprilat on the angiotensin II/angiotensin I ratio. Prior to the first dose of enalapril, children with heart failure had a higher angiotensin II/angiotensin I ratio than healthy adults. Moreover, the population estimate of the half-maximal inhibitory concentration was lower in children with heart failure than in healthy adults, suggesting that children with heart failure may be more sensitive to enalaprilat than healthy adults. In view of the change in the angiotensin II/angiotensin I ratio, it can be assumed that effective ACE inhibition was achieved with the administered dose of enalapril in children with heart failure.

V. Table of Contents

VI. List of Abbreviations

ACE	Angiotensin-converting enzyme
ADH	Antidiuretic hormone
ANG I	Angiotensin I
ANG II	Angiotensin II
AT_1 receptor	Angiotensin II type 1 receptor
AUC	Area under the concentration–time curve
AUC_∞	Area under the concentration–time curve from time zero to infinity
AUC_τ	Area under the concentration–time curve during a dosage interval
CES1	Carboxylesterase 1
CHD	Congenital heart disease
CI	Confidence interval
CL/F	Apparent clearance
CL_ENA/F	Apparent clearance of enalapril
CL_ENAAT/F	Apparent clearance of enalaprilat
C_{max}	Maximum serum drug concentration
$C_{max,1}$	Maximum serum drug concentration after the first dose
$C_{max,ss}$	Maximum steady state serum drug concentration during a dosage interval
COSSAC	Conditional sampling use for stepwise approach based on correlation tests
CV	Coefficient of variation
DCM	Dilated cardiomyopathy
E_0	Baseline effect
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
E_{max}	Maximum effect

f	Female
f_m	Metabolised fraction of enalapril
γ	Sigmoidicity factor
IC_{50}	Half-maximal inhibitory concentration
IIV	Interindividual variability
I_{max}	Maximum inhibition
k_{20}	Transfer rate constant from the central compartment of enalapril to the urine
k_{23}	Transfer rate constant from the central compartment of enalapril to the central compartment of enalaprilat
k_{30}	Transfer rate constant from the central compartment of enalaprilat to the urine
k_a	Absorption rate constant
k_{e0}	Effect compartment transfer rate constant
k_{tr}	Transit rate constant
LENA	Labeling of Enalapril from Neonates up to Adolescents
LLOQ	Lower limit of quantification
m	Male
Max	Maximum
Min	Minimum
Mtt	Mean transit time
na	Data not available
NS	Not significant
NT-proBNP	N-terminal pro-B-type natriuretic peptide
ODMT	Orodispersible minitablets
PD	Pharmacodynamic
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/pharmacodynamic
PRA	Plasma renin activity

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
Q/F	Apparent intercompartmental clearance
RIA	Radioimmunoassay
RSE	Relative standard error
SD	Standard deviation
SE	Standard error
t_{lag}	Lag time
V_1/F	Apparent central volume of distribution
V_2/F	Apparent peripheral volume of distribution
V_d_ENA/F	Apparent volume of distribution of enalapril
V_d_ENAAT/F	Apparent volume of distribution of enalaprilat
WHO	World Health Organization

VII. List of Figures

VIII. List of Tables

1 Introduction

1.1 Paediatric Heart Failure

The International Society for Heart and Lung Transplantation defines paediatric heart failure as ‘a complex clinical syndrome resulting from impaired ventricular function (ejection or relaxation), volume or pressure overload, either alone or in combination’ [1, p. e23]. As with heart failure in adults, the consequence is that the oxygen demand of the organs cannot be adequately met [2]. However, the aetiology differs, as the main causes of heart failure in children are congenital heart diseases and cardiomyopathies [3]. The most common congenital heart disease is the ventricular septal defect, while dilated cardiomyopathy is the most frequent type of cardiomyopathy in childhood [4, 5].

The symptoms of heart failure differ particularly in infants and young children compared with adults. Common symptoms in infants and young children include tachypnoea, feeding difficulty, diaphoresis and pallor [6]. To account for these differences in symptoms when assessing the severity of heart failure in infants, the Ross score was developed [7]. Furthermore, the Ross score was modified to extend its application from infants to older children. The modified Ross score assesses six criteria: diaphoresis, tachypnoea, breathing, respiratory rate, heart rate and hepatomegaly [8]. For each criterion, the investigator awards zero to two points depending on the severity, so that the maximum achievable score is 12.

In contrast to paediatric heart failure caused by cardiomyopathy, patients with paediatric heart failure due to congenital heart disease can often undergo corrective catheter- or surgery-based intervention [9]. Nevertheless, pharmacotherapy also plays a role in these patients. On the one hand, for the period until corrective intervention to improve their nutritional and clinical status prior to the intervention [10]. On the other hand, for cases in which heart failure persists despite catheter- or surgery-based intervention [11].

In both adult and paediatric heart failure, the renin–angiotensin–aldosterone system is activated as a compensatory mechanism to maintain the cardiac output [3, 12]. As with heart failure in adults, angiotensin-converting enzyme (ACE) inhibitors are used to treat heart failure in children. In the CONSENSUS study, the ACE inhibitor enalapril significantly reduced mortality compared with placebo in adults with severe congestive heart failure [13].

The international guideline for paediatric heart failure from 2025 recommends that ACE inhibitors should be used routinely in children with biventricular circulation and a systemic left ventricle if left ventricular systolic dysfunction is present [1]. However, the guideline also states that this recommendation is based on extrapolation from adult studies due to the lack of randomised controlled trials with ACE inhibitors in paediatric heart failure.

1.2 Enalapril and the Renin–Angiotensin–Aldosterone System

The above-mentioned ACE inhibitor enalapril has been used for more than 40 years to treat hypertension and heart failure in adults. Enalaprilat, the active metabolite of enalapril, is a potent ACE inhibitor but is poorly absorbed after oral administration [14]. Therefore, the prodrug enalapril as maleate salt is used for oral administration [15]. Enalapril is absorbed by 60 to 70% and is then hydrolysed in the liver by carboxylesterase 1 to enalaprilat (Figure 1.1) [16, 17]. Both enalapril and enalaprilat are mainly excreted renally [15]. Enalaprilat interferes with the renin–angiotensin–aldosterone system by inhibiting ACE.

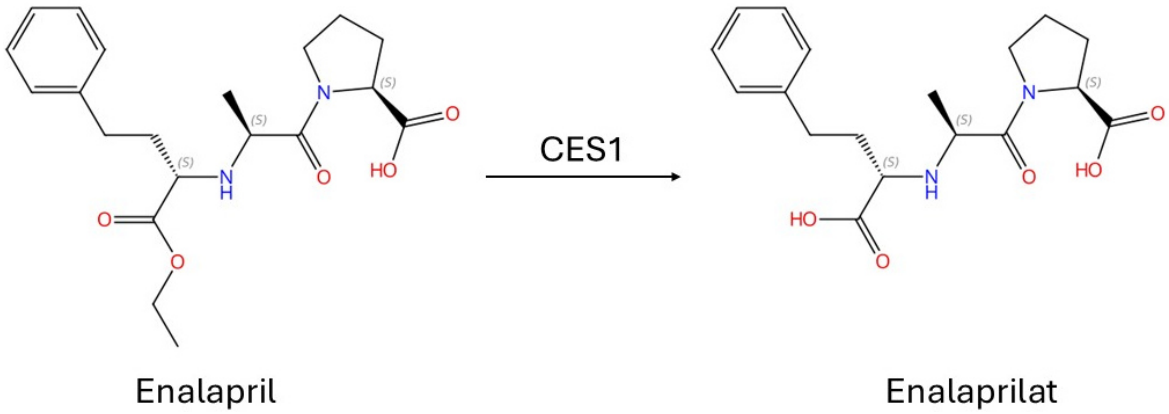

Figure 1.1 **Enzymatic hydrolysis of enalapril to enalaprilat.** Hepatic carboxylesterase 1 cleaves the ethyl ester of enalapril, resulting in enalaprilat. For simplicity, other reactants and products of the reaction have been omitted in the illustration. CES1, carboxylesterase 1.

The renin–angiotensin–aldosterone system regulates the fluid and electrolyte balance of the body. The endopeptidase renin cleaves angiotensinogen to angiotensin I (Figure 1.2). The release of renin from the juxtaglomerular cells of the kidney is regulated by the renal perfusion pressure, the sodium chloride concentration at the macula densa, the sympathetic activity via beta-1 receptors, and the negative feedback mediated by angiotensin II [18]. The generated angiotensin I is cleaved by ACE into angiotensin II. The binding of angiotensin II to the angiotensin II type 1 receptor triggers, among other things, vasoconstriction, increased release of aldosterone from the adrenal cortex and increased release of antidiuretic hormone from the posterior pituitary gland [19].

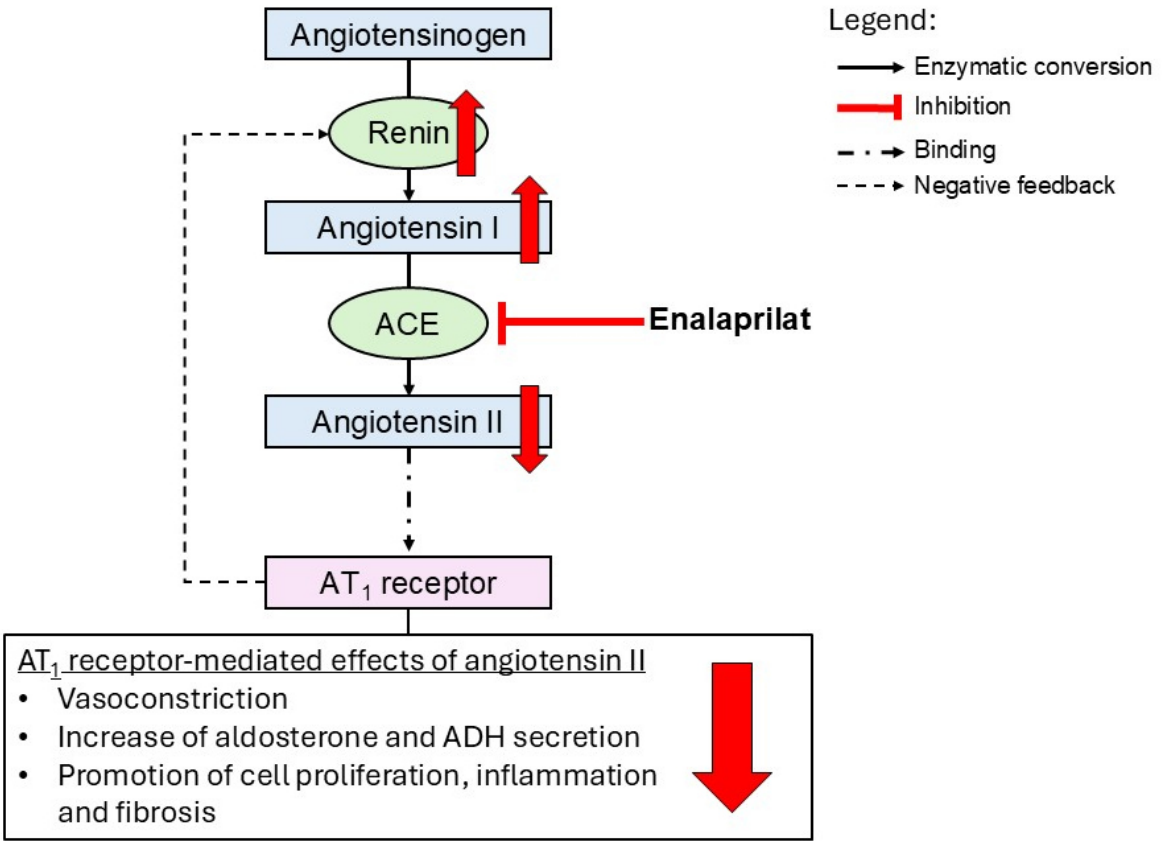

Figure 1.2 **Schematic diagram of the renin–angiotensin–aldosterone system and illustration of the effects of enalaprilat.** The red arrows indicate the changes caused by the inhibition of ACE by enalaprilat. ACE, angiotensin-converting enzyme; ADH, antidiuretic hormone; AT_1 receptor, angiotensin II type 1 receptor.

The inhibition of ACE by enalaprilat affects the parameters of the renin–angiotensin–aldosterone system (Figure 1.2). In healthy men, it has been shown that after administration of enalapril, angiotensin II and aldosterone decreased significantly, while active renin and angiotensin I increased significantly [20]. In addition, a significant increase in plasma renin activity, which reflects renin activity ex vivo, was observed in these subjects. The increase in active renin and plasma renin activity can be explained by the fact that the decrease in angiotensin II leads to reduced negative feedback on renin secretion. The increase in active renin in turn leads to increased formation of angiotensin I.

A significant decrease in angiotensin II and a significant increase in plasma renin activity were also observed in a group of 18 infants and children with congestive heart failure after administration of enalapril [21]. Moreover, a significant decrease in aldosterone levels was found in seven infants with congestive heart failure during oral enalapril therapy [22]. However, another study found no significant difference in plasma renin activity and aldosterone levels before and during enalapril therapy in 10 infants with congestive heart

failure [23]. Although further studies with enalapril have been conducted in children with heart failure, no parameters of the renin–angiotensin–aldosterone system were reported in these studies [24–29]. Overall, only limited data are available on the effects of enalapril administration on the parameters of the renin–angiotensin–aldosterone system in children with heart failure. Thus, further investigations are needed to better understand the effects of enalapril administration on the renin–angiotensin–aldosterone system in children with heart failure.

1.3 Age- and Disease-Related Differences in Children with Heart Failure

As mentioned above, the use of ACE inhibitors in paediatric heart failure is primarily based on the extrapolation of data from adults. However, there are age- and disease-related differences in children with heart failure that could influence the pharmacokinetics and pharmacodynamics of drugs.

Absorption might be influenced by changes in gastric pH and gastric emptying during maturation, but studies on these topics are rather contradictory [30]. Changes in body composition and plasma protein levels might influence the volume of distribution. Enalapril and enalaprilat can be classified as hydrophilic based on their log *P* values [31]. Extracellular water and total body water decrease with age, so that a decrease in volume of distribution has been observed for hydrophilic drugs with increasing age [30]. In addition, the amount of plasma proteins is lower in newborns and young infants [32]. A resulting increase in the unbound fraction might also influence pharmacodynamics, as more free substance would be available for action [32]. However, it remains unclear to what extent this is relevant for enalaprilat, which has moderate plasma protein binding of less than 50% [33]. Changes in enzyme expression and renal function might influence the clearance. The expression of carboxylesterase 1, the enzyme responsible for the hydrolysis of enalapril to enalaprilat, increases with age [34]. The glomerular filtration rate per 1.73 m^2 also increases with age after birth, reaching adult levels towards the end of the first year of life [32]. This could be relevant for enalapril as well as enalaprilat, as both are mainly excreted renally. [15]. So far, the age-related dependence of pharmacodynamic responses has been studied less than the age-related dependence of pharmacokinetic processes [35]. It is known that the renin–angiotensin–aldosterone system is influenced by age. Systematic reviews have shown that active renin, angiotensin I, angiotensin II and aldosterone decrease in healthy children during childhood [36–38]. Since enalaprilat affects the renin–angiotensin–aldosterone system, age-related changes could potentially also affect the pharmacodynamics of enalaprilat.

Pathophysiological differences caused by heart failure might influence pharmacokinetics and pharmacodynamics. In adults with heart failure, it is known that peripheral hypoperfusion can affect absorption, volume of distribution, hepatic metabolism and renal elimination [39]. The distribution of water-soluble drugs can also be effected by the presence of ascites, for example [40]. Based on findings from cachectic patients with other diseases

in whom a reduced volume of distribution was observed, it is assumed that cardiac cachexia might also influence pharmacokinetics [40]. Since peripheral hypoperfusion also occurs in children with heart failure, and ascites as well as poor weight gain are possible symptoms, the pharmacokinetic changes mentioned above might also occur in children with heart failure [6]. A systematic review of population pharmacokinetic analyses of digoxin in the paediatric population showed that congestive heart failure was associated with reduced clearance in three studies and increased volume of distribution in one study [41]. There are indications of altered pharmacokinetics of enalapril and enalaprilat from studies in adults with heart failure. In adults with congestive heart failure, a reduced apparent oral clearance of enalapril and a slightly longer elimination half-life of enalaprilat were observed compared with adults with hypertension [42]. Another study found that the severity of chronic heart failure in adults significantly influenced enalaprilat trough levels [43]. Due to the disease, the renin–angiotensin–aldosterone system is activated in children with heart failure [3]. The disease-related activation of the renin–angiotensin–aldosterone system could potentially influence the pharmacodynamics of enalaprilat, as enalaprilat affects the renin–angiotensin–aldosterone system.

The differences in children with heart failure highlight the need for clinical studies in children with heart failure to collect pharmacokinetic and pharmacodynamic data in this population. The evaluation of pharmacokinetic and pharmacodynamic data from children with heart failure treated with enalapril is necessary to assess whether age- and disease-related differences have a clinically significant impact on the pharmacokinetics and pharmacodynamics of enalapril and enalaprilat.

1.4 The LENA Project

1.4.1 Background

The Paediatric Regulation of the European Union from 2007 introduced paediatric-use marketing authorisation to promote the development of products with off-patent drugs for use in children [44]. Enalapril was one of the drugs on the priority list for studies into off-patent paediatric medicinal products of the Paediatric Committee of the European Medicines Agency from 2009 [45]. It was stated that data on the pharmacokinetics, efficacy and safety of enalapril, as well as an age-appropriate formulation, are needed. Furthermore, at the European Medicines Agency Expert Group Meeting on Paediatric Heart Failure in 2010, it was declared that ACE inhibitors are recommended as first-line treatment for chronic heart failure [46]. At that time, enalapril was only available in tablet form in Europe and was only approved for children aged 6 years and older with arterial hypertension. However, the off-label use of enalapril in children with heart failure was widespread [47]. Due to the lack of a paediatric dosage form, extemporaneous preparations of enalapril, such as a suspension of crushed tablets in water, were used [48]. Nevertheless, such extemporaneous preparations have the disadvantage that their bioavailability is unknown and their dosing may be inaccurate [48].

For these reasons, the collaborative project ‘Labeling of Enalapril from Neonates up to Adolescents’ (LENA) was initiated, which was funded by Seventh Framework Programme of the European Union (grant agreement no. 602295). For the LENA project, an academic consortium was established (Appendix 9.1). The aim of the LENA project was the development and clinical evaluation of a new age-appropriate formulation of enalapril for children with heart failure. As part of the LENA project, two pharmacokinetic bridging studies and a safety follow-up study were conducted to collect the data required for devising a paediatric-use marketing authorisation.

1.4.2 Investigational Medicinal Product and Dosing Regimen

In the context of the LENA project, orodispersible minitablets with 0.25 mg or 1 mg of enalapril maleate were developed as an age-appropriate dosage form that can be used from birth [49]. These were used as investigational medicinal products in the LENA studies. The orodispersible minitablets are round, biconvex and have a diameter of only 2 mm (Figure 1.3). After placing the orodispersible minitablets in the patient’s cheek pouch, they rapidly disintegrate into small, easily swallowable particles. The administration of the

orodispersible minitablets does not require any liquid, but a drink of the patient's/parents' choice can be given to facilitate swallowing. Even the administration of a single dose of 0.025 mg or 0.125 mg enalapril maleate is possible by dispersing the 0.25 mg orodispersible minitablet directly in tap water in a syringe and administering the corresponding volume of the resulting dispersion [50].

Before the orodispersible minitablets were used in children with heart failure, a relative bioavailability study was conducted in healthy adults, using a standard tablet formulation as a reference [51]. The non-compartmental pharmacokinetic analysis indicated that no dose adjustment is necessary for reasons of bioavailability. Furthermore, the results of the relative bioavailability study showed that there is no significant difference in the bioavailability of enalapril whether the orodispersible minitablets are swallowed with water or dispersed in the mouth.

Figure 1.3 **Size comparison of orodispersible minitablets with normal enalapril tablets.** The orodispersible minitablets on the left have a diameter of 2 mm and the normal tablets on the right have a diameter of 8 mm. The orodispersible minitablets contain 0.25 mg enalapril maleate (top left) and 1 mg enalapril maleate (bottom left). The normal tablets contain 10 mg enalapril maleate (top right) and 20 mg enalapril maleate (bottom right). From 'Acceptability and Palatability of Novel Orodispersible Minitablets of Enalapril in Children up to the Age of 6 with Heart Failure', by M. Lazic, M. Djukic, V. Vukomanovic, M. Bijelic, E. Obarcanin and M. Bajcetic, 2025, *JCM*, *14*(3), p. 4 (doi:10.3390/jcm14030915). CC BY 4.0.

For the LENA studies, a dosing regimen was developed using a physiologically based pharmacokinetic simulation [52]. The basic idea behind the development was to achieve a similar exposure to enalapril and enalaprilat in children with heart failure as in adults. The dosing regimen specifies recommended titration doses, target doses and maximum doses depending on age and weight (Appendix 9.2).

1.4.3 Pharmacokinetic Bridging Studies

The developed enalapril orodispersible minitablets were administered to children with heart failure for eight weeks in two multicentre, prospective, open-label, phase II/III pharmacokinetic bridging studies. The studies were conducted from 2016 to 2018 at 7 sites in 5 European countries. The primary objective of the studies was the collection of pharmacokinetic data to characterise the dose exposure of enalapril and its active metabolite enalaprilat in children with dilated cardiomyopathy or congenital heart disease [53]. For this purpose, the first pharmacokinetic bridging study included children aged 1 month to under 12 years with heart failure due to dilated cardiomyopathy, while the second pharmacokinetic bridging study included children from birth to under 6 years of age with heart failure due to congenital heart disease. A total of 102 children were included in both studies, comprising 35 ACE inhibitor naïve subjects and 67 subjects with ACE inhibitor pretreatment. This work focuses on ACE inhibitor naïve subjects, as the pharmacodynamic effects can be better assessed when no ACE inhibition has taken place previously. Unless otherwise stated, all information in this work therefore refers to ACE inhibitor naïve subjects.

Part of the pharmacokinetic bridging studies was an exploratory pharmacodynamic assessment and the collection of data on clinical parameters, acceptability, palatability and safety. The pharmacodynamic parameters were plasma renin activity, renin, angiotensin I, and aldosterone. The sampling regimen comprised a full pharmacokinetic/pharmacodynamic (PK/PD) profile, which was usually collected during the initial dose visit, as well as single pharmacokinetic and pharmacodynamic samples during the remaining study visits. Further details on the study course as well as pharmacokinetic and pharmacodynamic sampling can be found in Figure 1.4.

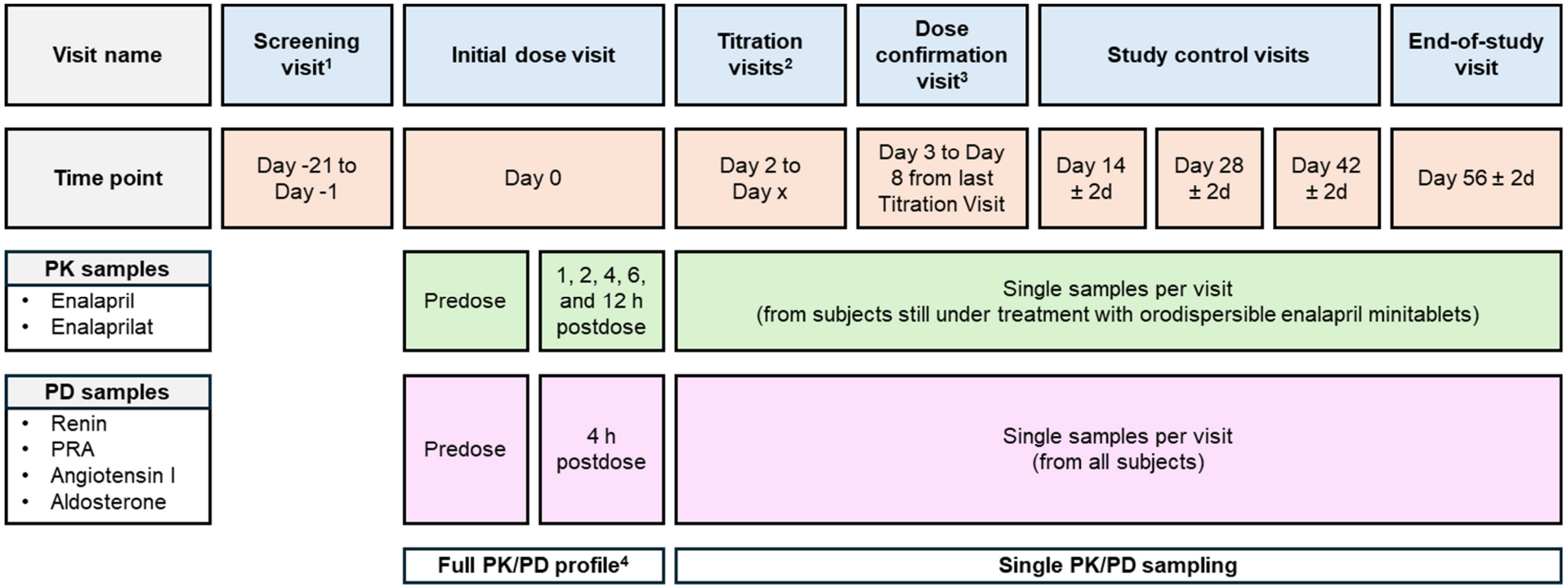

Figure 1.4 **Typical study course regarding pharmacokinetic and pharmacodynamic sampling for an ACE inhibitor naïve subject in the pharmacokinetic bridging studies.** [1]The screening visit and the initial dose visit could be conducted on the same day if the children weighed more than 4.2 kg. [2]The number of titration visits depended on the age of the patient and the investigator's judgement. [3]The dose confirmation visit and the first study control visit could be combined into one visit. [4]As an alternative, the full pharmacokinetic/pharmacodynamic profile could also be collected after reaching steady state at the optimal dose. In this case, no pharmacokinetic sample was taken 12 hours postdose. ACE, angiotensin-converting enzyme; PD, pharmacodynamic; PK, pharmacokinetic; PK/PD, pharmacokinetic/pharmacodynamic; PRA, plasma renin activity.

1.4.4 Safety Follow-up Study

Following the pharmacokinetic bridging studies, a joint, multicentre, prospective, open-label, phase II/III safety follow-up study with a study period of 10 months was conducted. Subjects from both pharmacokinetic bridging studies were included if they were still being treated with enalapril orodispersible minitablets or had been treated with enalapril orodispersible minitablets for at least three days. The primary objective was to prove the safety of enalapril orodispersible minitablets [53]. In addition, data on pharmacokinetics, pharmacodynamics, clinical parameters, acceptability and palatability during long-term therapy with enalapril orodispersible minitablets were collected. The study visits of the safety follow-up study took place 3, 6, 9 and 12 months after the first administration of the enalapril orodispersible minitablets. During the study visits, single pharmacokinetic samples for enalapril and enalaprilat were taken from subjects who were still being treated with enalapril orodispersible minitablets, and single pharmacodynamic samples for plasma renin activity, renin, angiotensin I and aldosterone were taken from all subjects.

1.4.5 Additional Investigation

In addition to the pharmacodynamic parameters specified in the study protocols, various angiotensin peptides, including angiotensin II, were simultaneously determined as part of a doctoral thesis [54]. The existing samples were used for this investigation, and no additional samples were taken.

1.4.6 Regulatory Status of Enalapril Orodispersible Minitablets and Therapeutic Alternatives

Based on data from the LENA studies, the orodispersible minitablets with 0.25 mg enalapril maleate received a paediatric-use marketing authorisation from the European Medicines Agency in 2023 for the treatment of heart failure in children from birth to 17 years of age (Aqumeldi®, Proveca Pharma Limited, Dublin, Ireland) [55]. In 2025, the marketing authorisation was extended so that the orodispersible minitablets with 1 mg enalapril maleate are meanwhile also authorised in Europe. This now enables simple and age-appropriate administration of enalapril from birth onwards.

Apart from enalapril, the only drugs approved in Europe for children with heart failure that act on the renin–angiotensin–aldosterone system are the ACE inhibitor captopril and the angiotensin receptor-neprilysin inhibitor sacubitril/valsartan.

Captopril has long been approved for the treatment of chronic heart failure with reduced systolic ventricular function in children, and an oral solution of captopril has been commercially available in Germany since 2023. Due to the longer half-life of enalapril, the enalapril orodispersible minitablets have the advantage that they only need to be administered once or twice a day, whereas the captopril solution usually needs to be administered three times a day [56, 57].

Sacubitril/valsartan was approved in Europe in 2023 for the treatment of symptomatic chronic heart failure with left ventricular dysfunction in children aged one year and older [58]. Since then, sacubitril/valsartan granules in capsules for opening have been commercially available as a paediatric dosage form. In comparison, however, enalapril orodispersible minitablets have the advantage of being approved for use from birth. Furthermore, no superiority of sacubitril/valsartan over enalapril could be shown in the 52-week randomised, double-blind clinical efficacy study of sacubitril/valsartan in children from 1 month to under 18 years of age with heart failure due to systemic left ventricular systolic dysfunction [59]. In the study, a clinically meaningful reduction in the heart failure severity score and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels was observed with both enalapril and sacubitril/valsartan. In line with this, an analysis of clinical outcomes in children with congenital heart disease after eight weeks of therapy with enalapril orodispersible minitablets found a significant reduction in the heart failure severity score, the left ventricular diastolic dimension z-score, and NT-proBNP levels [60].

Considering the positive study results and the advantages mentioned above compared with the therapeutic alternatives, it is likely that the use of enalapril will increase, particularly in very young and ACE inhibitor naïve children with heart failure. Therefore, analyses of pharmacokinetic and pharmacodynamic data are highly relevant to gain further insights into the optimal dosage and effects of enalapril in this population. In the LENA studies, pharmacokinetic and pharmacodynamic data were generated before the first dose, after the first dose, and during long-term therapy. So far, the pharmacokinetic data have only been analysed non-compartmental, and the pharmacodynamic data have not yet been analysed [52]. Further analyses are essential to fully exploit the potential of the data. A suitable method for analysing pharmacokinetic and pharmacodynamic data is the population approach using nonlinear mixed effects modelling.

1.5 Nonlinear Mixed Effects Modelling

Nonlinear mixed effects modelling is an approach that was introduced in the late 1970s to estimate population characteristics of pharmacokinetic parameters based on data from clinical routine [61]. This approach is now also used in the field of pharmacodynamics and in the context of drug development [62]. In addition to the approach, Lewis B. Sheiner and Stuart L. Beal also developed NONMEM®, the first computer programme for population analysis using nonlinear mixed effects modelling [63].

The population approach 'aims at describing the typical drug behaviour in the population as well as the variability observed from individual to individual' [64, p. 198]. A population model typically comprises a structural model, a statistical model, and a covariate model. The structural model consists of one or multiple functions that describe the typical time course of a dependent variable, such as a concentration or an effect within the population [65]. The statistical model takes into account the variability in the observed data [62]. A population model considers at least two types of variability: the interindividual variability and the residual variability. The interindividual variability is the variability between different individuals of the population. The residual variability represents the variability between the individual prediction and the observation, which can arise from errors in dosage documentation, sampling and analytics, as well as model misspecification. The covariate model is used to explain the variability attributable to characteristics of the subjects such as age, sex and renal function [65].

Mixed-effects models contain fixed-effect parameters and random-effect parameters. Fixed-effect parameters can be defined as 'structural parameters that take on a single value that represents the population typical value of the parameter' [66, pp. 10-11]. Examples of fixed effect parameters are the population typical value of the clearance for pharmacokinetics and the population typical value of the half-maximal inhibitory concentration for pharmacodynamics. Fixed effects parameters also include the population typical value for the relationships between a covariate and a pharmacokinetic or pharmacodynamic parameter. Random effects are the interindividual variability and residual variability mentioned above.

The population approach using nonlinear mixed effects modelling is widely used for paediatric populations. One of the advantages is that even sparse and unbalanced datasets, which are common in children, can be analysed through the simultaneous analysis of all data

[67]. Another advantage is that covariates such as age can be identified to explain part of the interindividual variability of pharmacokinetic and pharmacodynamic parameters [68]. In addition, simulations can be carried out with the developed population model to test what-if scenarios, such as the administration of a different dose [67]. Through the identification of covariates and insights gained from simulations, dosages can be optimised to ensure safe and effective treatment. Although studies with enalapril have been conducted in children with heart failure, in which pharmacokinetic data or pharmacokinetic and pharmacodynamic data were collected, these data have not been analysed using population modelling [21, 23, 28].

1.6 Aims of this Thesis

The general aim is to investigate the pharmacokinetics and pharmacodynamics in ACE inhibitor naïve children with heart failure who were treated with enalapril orodispersible minitablets. A key aspect that will be examined in this context is whether age- and disease-related differences in children with heart failure have an influence on the pharmacokinetics and pharmacodynamics. The investigations concerning the pharmacokinetics of enalapril and enalaprilat aim to provide deeper insights for the dosage of enalapril in this vulnerable population of very young and diseased children. In addition, a better understanding of the effects of enalapril on the renin–angiotensin–aldosterone system in children with heart failure shall be achieved through the pharmacodynamic analyses. Overall, the findings shall contribute to the safe and effective treatment of children with heart failure with enalapril.

The thesis is divided into three parts, which pursue the following aims:

1. In the first part, one aim is to develop a combined pharmacokinetic model for enalapril and enalaprilat in very young and ACE inhibitor naïve children with heart failure. Furthermore, clinically relevant covariates for the dosage of enalapril in this population shall be identified through the population pharmacokinetic analysis and subsequent simulations.

2. The second part aims to assess the nature and extent of the influence of age, heart failure and ACE inhibitor treatment on the pharmacodynamic parameter plasma renin activity in children. To this end, a systematic literature review will be conducted and data from the LENA studies will be evaluated.

3. The third part is intended to investigate the effect of enalaprilat on the angiotensin II/angiotensin I ratio in children with heart failure. Moreover, possible differences compared with healthy adults shall be identified. To achieve these aims, a population PK/PD model for healthy adults and a pharmacodynamic model for children with heart failure will be developed.

2 Population Pharmacokinetic Analysis of Enalapril and Enalaprilat in Children with Heart Failure and Implications for Safe Dosing of Enalapril

2.1 Background

As enalapril orodispersible tablets are approved for use from birth, it is expected that more newborns and infants with heart failure who have not yet received an ACE inhibitor will be treated with enalapril orodispersible tablets. Therefore, it is important to investigate whether the differences described above due to age and disease have an influence on the pharmacokinetics of enalapril and enalaprilat, which must be considered in the dosage for ACE inhibitor naïve children with heart failure.

Non-compartmental analyses revealed indications of potential covariates that may influence the pharmacokinetics of enalapril and enalaprilat in children. Wells et al. [69] found a significantly higher area under the concentration–time curve (AUC) of enalaprilat in adolescents with hypertension compared with infants with hypertension when they normalised the AUC to a dose of 0.15 mg/kg, but not when they normalised the AUC to a dose of 1 mg/m^2. In contrast, Nakamura et al. [28] found an inverse correlation between age and the AUC of enalapril and enalaprilat, normalised to a dose of 1 mg/m^2, in paediatric patients with congenital heart disease aged between 10 days and 6.5 years. A previous non-compartmental analysis of the LENA studies firstly indicated a potential impact of the aetiology of heart failure on the pharmacokinetic of enalapril, as patients with dilated cardiomyopathy had a 50% lower enalapril exposure than patients with congenital heart disease [52]. Secondly, differences in the pharmacokinetic parameters of enalapril and enalaprilat were found in the different age groups, indicating a potential impact of age on the pharmacokinetics of enalapril and enalaprilat.

A population pharmacokinetic analysis of enalapril and enalaprilat in healthy adults receiving enalapril once as a tablet and once as orodispersible minitablets identified a covariate effect of normalised body weight on the volume of distribution of enalapril and a covariate effect of formulation on the mean transit time of enalapril absorption [70]. In an early population pharmacokinetic analysis of enalaprilat in healthy men from 1985, no covariates were investigated [71]. A population pharmacokinetic analysis of enalaprilat

based on literature data from children with hypertension identified a covariate effect of weight on the volume of distribution and clearance of enalaprilat [72].

As far as is known, no population pharmacokinetic analysis of enalapril and enalaprilat focussing on very young and ACE inhibitor naïve children with heart failure has been performed previously. Therefore, reliable information on covariates that may influence the pharmacokinetics of enalapril and enalaprilat in this vulnerable population is lacking. However, safe dosing requires consideration of clinically relevant covariates, especially in ACE inhibitor naïve subjects, as in these subjects no previous experience with the dosage of ACE inhibitor is available.

The aim is therefore to identify covariates that are clinically relevant for the dosing of enalapril in very young and ACE inhibitor naïve children with heart failure. To this purpose, a simultaneous population pharmacokinetic analysis of enalapril and enalaprilat in ACE inhibitor naïve children with heart failure was conducted on the basis of data from the LENA studies.

2.2 Methods

2.2.1 Study Design and Investigated Population

The data used originate from the two pharmacokinetic bridging studies of the LENA project, which are described in Chapter 1.4.3. The studies were conducted in hospitals in Austria, Germany, Hungary, the Netherlands (two sites) and Serbia (two sites). The ethics committees of the participating institutions had given their approval and informed parental consent was obtained before each subject was enrolled in the study. Assent of participating children was obtained in accordance with national requirements. The studies were registered on the EU Clinical Trials Register (EudraCT 2015-002335-17, EudraCT 2015-002396-18).

Male and female patients with a weight greater than 2.5 kg and heart failure due to congenital heart disease or dilated cardiomyopathy were included in the studies. Subjects with congenital heart disease were eligible if they required after load reduction by drug therapy and were between birth to under 6 years of age. Subjects with dilated cardiomyopathy were eligible if they had left ventricular end-diastolic dimension > P95 and/or left ventricular shortening fraction < 25%. In addition, the subjects with dilated cardiomyopathy had to be between 1 month and 12 years old.

The exclusion criteria were:

- Severe heart failure and/or end stage heart failure precluding introduction of ACE inhibitor.
- Too low blood pressure, e.g. less than P5 for age.
- Restrictive and hypertrophic cardiomyopathies.
- Obstructive valvular disease (peak echocardiographic gradient more than 30 mmHg).
- Uncorrected severe peripheral stenosis of large arteries including severe coarctation of the aorta.
- Severe renal impairment with serum creatinine above two times the upper limit of normal according to the hospital's test methodology.
- History of angioedema.
- Concomitant medication: renin inhibitors, angiotensin II antagonists or non-steroidal anti-inflammatory drugs except acetylsalicylic acid only for antiplatelet therapy.
- Already enrolled in an interventional trial with an investigational drug, unless no interference with the current study can be shown.

Both studies together involved 35 ACE inhibitor naïve subjects. The serum concentrations of enalapril and enalaprilat used in this analysis were collected between January 2016 and April 2018.

2.2.2 Dosing

The dosing regimen developed for the LENA studies with age- and weight-dependent recommended titration doses, target doses and maximum doses was used (Appendix 9.2). The dosage for the subjects was selected according to the investigator's judgement.

For dosing, the appropriate number of orodispersible minitablets with 0.25 mg enalapril maleate or 1 mg enalapril maleate (now approved as Aqumeldi®, Proveca Pharma Limited, Dublin, Ireland) were administered orally. The orodispersible minitablets were placed in the patient's cheek pouch, where they rapidly disintegrate into small particles that could be easily swallowed. A drink of the patient's/parent's choice (e.g. breast milk, formula milk, cow milk, and water) could be taken to facilitate swallowing. If the investigator considered an initial dose of 0.25 mg to be too high for the patient, the 0.25 mg orodispersible minitablet was dissolved in tap water in a syringe and the appropriate volume of the resulting dispersion was administered. According to the dosing regimen, daily doses ≤ 0.25 mg were administered once daily in the morning and daily doses > 0.25 mg were divided into two equal doses, each administered in the morning and evening.

The dosing times at the study visits and 7 days before were recorded by the investigator or study nurse in electronic case report forms. The dosing times 7 days before were taken from the patient diary in which the parents noted the dosing times.

2.2.3 Sampling

The sampling regimen consisted of a pharmacokinetic full profile day with blood sampling at five or six predefined time points and single pharmacokinetic samples on the remaining study visits. For the investigated ACE inhibitor naïve subjects, it was recommended to obtain the pharmacokinetic full profile at the initial dose visit. The predefined time points for pharmacokinetic sampling were before as well as 1, 2, 4, 6, and 12 hours after administration of enalapril orodispersible minitablets.Alternatively, the pharmacokinetic full profile could also be obtained after reaching steady state at the optimal dose, that is, after at least 7 days at this dose. In this case, the predefined time points for pharmacokinetic sampling were before as well as 1, 2, 4, and 6 hours after administration of enalapril orodispersible

minitablets. A deviation of ± 15 minutes from the predefined time point was permitted. The exact time point of each pharmacokinetic sampling was noted.

Single pharmacokinetic samples were collected during titration, dose confirmation, study control visits and at the end of the study. The predefined time points of the study visits are listed in Appendix 9.3. During titration and at the end of the study, the pharmacokinetic sample was collected predose and the sampling time was recorded. The time point of the single pharmacokinetic sample at the dose confirmation visit or at the study control visits could be determined by the investigator if the exact time of sampling and the time of the last dose intake were specified in the electronic case report form.

A total of 200 µL of whole blood was taken per pharmacokinetic sample. If possible, an additional back-up sample was taken for reanalysis, which also comprised up to 200 µL of whole blood. The sampling regimen was designed using modelling and simulation techniques. The total collected blood volume during the studies did not exceed the recommended limits for blood loss in paediatrics in clinical studies according to the European Medicines Agency guideline [73].

2.2.4 Analytical Methods

Serum samples were analysed after solid phase extraction using liquid chromatography–triple quadrupole tandem mass spectrometry [Shimadzu HPLC 10 (Shimadzu, Duisburg, Germany) coupled with AB Sciex API 2000 mass spectrometer (Sciex, Darmstadt, Germany)]. The lower limit of quantification (LLOQ) was 0.195 µg/L for enalapril and 0.180 µg/L for enalaprilat. Further information can be found elsewhere [52].

2.2.5 Software

Population pharmacokinetic analysis was performed by nonlinear mixed effects modelling using NONMEM® version 7.2.0 (ICON plc, Dublin, Ireland) and Perl-speaks-NONMEM version 4.9.0 via the graphical user interface Pirana® version 2.9.6 (Certara, Radnor, PA, USA) [74, 75]. The first-order conditional estimation method with interaction was used to estimate pharmacokinetic parameters and their variability. The dataset for the population pharmacokinetic analysis was created with Excel® version 2406 (Microsoft, Redmond, WA, USA) and R version 4.2.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Statistical analyses and graphics were realized with R version 4.2.2 (The R Foundation for Statistical Computing, Vienna, Austria). The R packages used included nonmem2R for the goodness-of-fit plots, mrgsolve for the simulations and coveffectsplot for the creation of the forest plot [76, 77].

2.2.6 Base Model Development

The starting point for the model development was a combined model in which a one-compartment model of enalapril is coupled with a one-compartment model of enalaprilat with first-order absorption and elimination. This starting point was chosen because a combined model with one-compartment models for enalapril and enalaprilat had previously been used for an initial analysis of all LENA subjects [78]. As no urine data are available for enalapril and enalaprilat, a fixed value of 0.7 was used for the metabolised fraction of enalapril in this model based on literature data [69]. Due to the small number of samples in the absorption phase, the absorption rate constant was fixed to the estimated value of the aforementioned final model (0.6 h^{-1}) [78]. Since bioavailability could not be determined on the basis of the available data, the apparent clearance and apparent volume of distribution for enalapril and enalaprilat are given. It was assumed that the interindividual variability of the parameters was lognormally distributed. The interindividual variability of the apparent clearance and apparent volume of distribution of enalapril and enalaprilat was therefore built in exponentially. For enalapril, a proportional error model and a combined error model with an additive and a proportional error were tested as a residual error model. For enalaprilat, the residual error was described by a proportional error model. Allometric scaling was applied to the apparent clearance and apparent volume of distribution of enalapril and enalaprilat as follows:

$$TV = \theta \cdot \left(\frac{BW_{ind}}{BW_{median}}\right)^{n} \qquad \textbf{Equation 2.1}$$

where TV represents the typical value of the pharmacokinetic parameter at given weight, θ represents the population mean of the pharmacokinetic parameter at median body weight of the analysed population (BW_{median}) and BW_{ind} represents the individual body weight. The exponent, n, was fixed to 0.75 for apparent clearances and fixed to 1 for apparent volumes of distribution.

For missing weight values, the last observation carried forward approach was used, in which the missing value is replaced by the last observed value of the subject. For enalapril, both the addition of an absorption lag time and the use of a two-compartment model were tested. For the apparent clearance of enalapril and enalaprilat, the addition of an exponential function and a (sigmoid) maximum effect (E_{max}) function with postmenstrual or postnatal age was tested as a maturation function.

Model selection was based on the change in the objective function, the change in the residual and interindividual variability, the change in the relative standard errors of the parameter estimates and the visual inspection of the goodness-of-fit plots. Samples collected prior to the first administration of enalapril were excluded from the analysis, as no concentration was to be expected at that time in the ACE inhibitor naïve subjects. Furthermore, samples with insufficient information on dosing were excluded from the analysis. Samples with concentrations below the limit of quantification were either excluded (M1 method) or treated as censored (M3 method), and the respective estimates of the pharmacokinetic parameters were compared [79].

2.2.7 Covariate Model Development

The stepwise covariate modelling approach was used to test potential covariates. Physiologically plausible covariate relationships were previously defined. Thus, age, sex, serum creatinine and Ross score were tested as covariates for the apparent clearance of enalapril and enalaprilat. In addition, age, sex and Ross score were tested as covariates for the apparent volume of distribution of enalapril and enalaprilat. The covariate Ross score represents the modified Ross score determined by the investigator [8]. In the forward step, a reduction in the objective function value of at least 3.84 ($p < 0.05$, one degree of freedom) led to the inclusion of the covariate in the model. To retain the covariate in the model, the increase in the objective function value after removal of the covariate had to be at least 6.63 ($p < 0.01$, one degree of freedom) in the backward step. A linear function model was tested for continuous and categorical covariates. For age and serum creatinine, a power function model was initially tested. If this led to the inclusion of the covariate, in the next step it was tested whether an exponential function model was superior to the power function model. For the Ross score, the exponential function model was tested directly, as the Ross score can also be zero and therefore the power function model cannot be used. As the continuous covariates age, serum creatinine and Ross score were time-varying covariates, this was considered in the stepwise covariate modelling.

The only categorical covariate, sex, was included in the model as follows for the covariate search:

$$TV = \theta_1 \cdot (1 + \theta_2 \cdot sex) \qquad \textbf{Equation 2.2}$$

where TV represents the typical value of the pharmacokinetic parameter for the respective sex (female = 0, male = 1), θ_1 represents the population mean of the pharmacokinetic parameter for female subjects and θ_2 represents the proportional change in the population mean of the pharmacokinetic parameter in male subjects compared with female subjects.

2.2.8 Model Evaluation

The final model was evaluated by visual inspection of the goodness-of-fit plots and the prediction- and variability-corrected visual predictive checks [80, 81]. The prediction- and variability-corrected visual predictive checks were generated based on 2000 simulated replicates of the original dataset design. In addition, a nonparametric bootstrap was performed. Therefore, a total of 1000 bootstrap datasets were generated by resampling with replacement from the original dataset. The calculation of the median and the 95% confidence interval (CI) of the model parameters was carried out once with and once without the runs with rounding errors. Furthermore, the condition number was calculated for the final model to check for overparameterisation, where a value above 1000 may indicate overparameterisation [80].

2.2.9 Simulations

Simulations were performed with the final model to assess the impact of weight and the included covariates. A subject with the weighted median weight of the population and the weighted medians of the included covariates (calculated by Perl-speaks-NONMEM) served as the reference subject. For the variation of the covariates, the 5th, 25th, 75th and 95th percentiles of the weight and the included covariates of the analysed population were calculated. One covariate was varied at a time and serum concentrations of enalaprilat were simulated for the different scenarios. As the subjects were children, it was not sensible to vary the age and weight completely independently. Therefore, a suitable weight was selected for the above-mentioned percentiles of age using the World Health Organization (WHO) weight percentiles [82]. Similarly, a suitable age for the above-mentioned percentiles of weight was selected using the WHO weight percentiles. The population analysed included both underweight and normal weight subjects. Therefore, when selecting the appropriate

weights and ages, the first WHO weight percentile was used to represent underweight children and the 50th WHO weight percentile was used to represent normal weight children. As there are separate WHO weight percentiles for girls and boys, the mean of the values determined for girls and boys was used.

The parameter uncertainty was considered by using the estimates of the fixed-effect parameters of the bootstrap runs, while the interindividual variability was omitted. For each bootstrap dataset, AUC and the maximum serum drug concentration (C_{max}) after the first dose and at steady state were determined for each covariate scenario. The AUC and C_{max} were dose normalised by dividing by the simulated dose and standardised by dividing by the respective AUC and C_{max} of the reference person. Finally, the 5th, 50th and 95th percentiles for the dose normalised and standardised AUC and C_{max} were calculated for each covariate scenario investigated and presented graphically in a forest plot.

Simulated serum concentrations of enalaprilat over 240 hours after a single dose of 0.25 mg enalapril maleate were used to obtain an approximation of the area under the concentration–time curve from time zero to infinity (AUC_{∞}) after the first dose. The dose of 0.25 mg enalapril maleate was chosen according to the dosing regimen (Appendix 9.2). For the steady state simulation, it was assumed that the age-appropriate target dose according to the dosing regimen was administered every 12 hours. The serum concentration of enalaprilat was simulated for 252 hours and then the AUC from 240 to 252 hours was calculated to obtain the area under the concentration–time curve during a dosage interval (AUC_{τ}) at steady state. To obtain the maximum serum drug concentration after the first dose ($C_{max,1}$) and the maximum steady state serum drug concentration during a dosage interval ($C_{max,ss}$), the highest concentration reached after the first dose and at steady state was determined.

In addition, analogue simulations after the first dose were performed solely with the variation of the Ross score. Every possible expression of the Ross score between the minimum and maximum Ross score of the population was tested. As only the single dose of 0.25 mg enalapril maleate was used in the simulations, no dose normalisation was performed. The $C_{max,1}$ at different Ross scores was illustrated with boxplots.

2.3 Results

2.3.1 Data

Of the 35 ACE inhibitor naïve subjects, 34 subjects could be included in the population pharmacokinetic analysis. One subject was excluded because, for unknown reasons, no enalapril or enalaprilat concentration was measurable during the full profile. A total of 173 quantifiable serum concentrations of enalapril and 268 quantifiable serum concentrations of enalaprilat were included in the analysis. Per subject, a median of 4.5 quantifiable observations (range 3–9) were available for enalapril and a median of 8 quantifiable observations (range 3–10) were available for enalaprilat. For the M3 method, the samples below the quantification limit (46.3% of the enalapril samples and 17.0% of the enalaprilat samples) were included and treated as censored. For enalapril and enalaprilat, eight measured concentrations had to be excluded due to insufficient information about the dosage or insufficient information about the time of treatment discontinuation. As a result, for one subject only the full profile measurements were included in the analysis. In this subject, however, enalapril therapy was prematurely discontinued anyway after 9 days due to hypotension. In two subjects, enalapril therapy was terminated prematurely at the third study control visit, as the subjects' condition had improved to such an extent that the therapy was no longer required. A total of 34 enalapril and 34 enalaprilat measurements prior to administration of the first dose were excluded, as no concentration was expected in the ACE inhibitor naïve subjects at this time. In addition, an enalapril measurement more than 1 month after the last enalapril administration was excluded, as no concentration was expected at this time either. The observed enalapril and enalaprilat serum concentrations over time are shown in Figure 2.1. The majority of enalapril values below the limit of quantification (90%) were more than 10 hours since the last dose. The majority of enalaprilat values below the limit of quantification (82%) were less than 2.5 hours since the last dose.

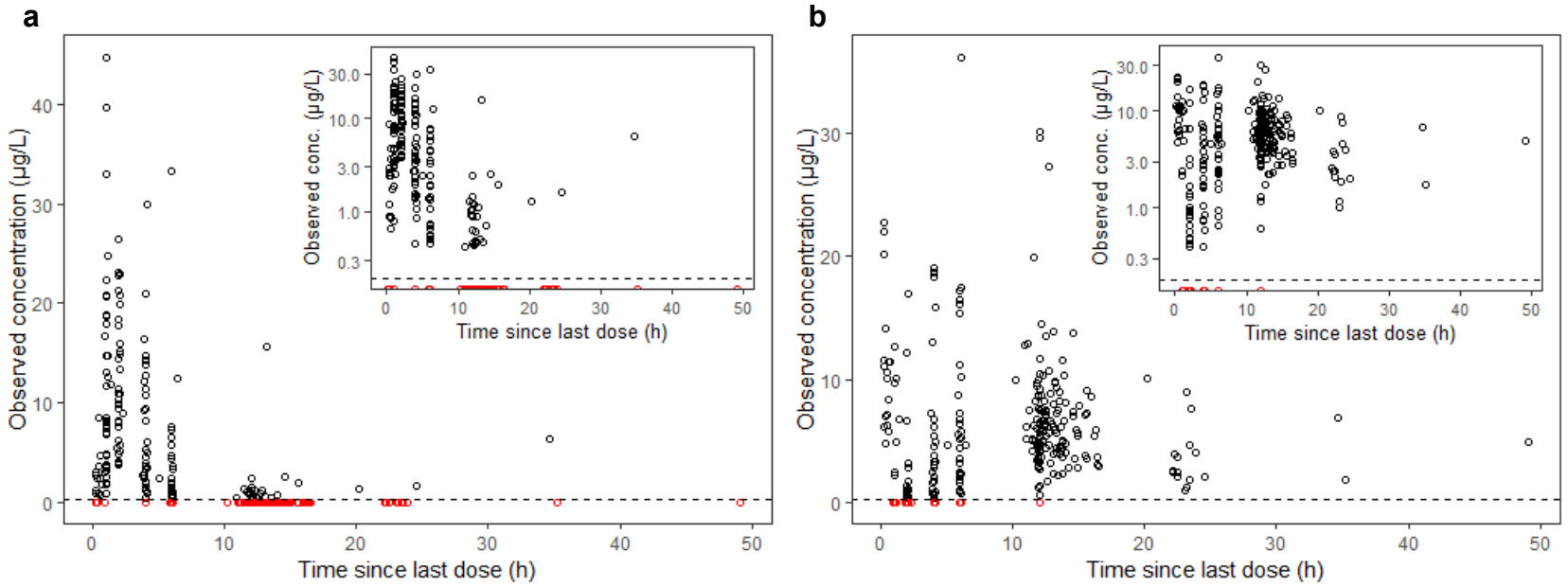

Figure 2.1 **Observed concentration of enalapril (a) and enalaprilat (b) plotted against time since last dose.** The main plots show the data on a linear scale and the inset plots on a logarithmic–linear scale. The dashed line indicates the lower limit of quantification. Observations above the lower limit of quantification are shown as black circles and observations below the lower limit of quantification are shown as red circles. Darker circles indicate overlapping observations.

The patient characteristics on the day of the first administration of enalapril are shown in Table 2.1. The subjects received a median first dose of 0.06 mg/kg (range 0.03–0.08 mg/kg) enalapril maleate. In one subject, 0.125 mg enalapril maleate was administered as a dispersion in the first week of treatment. One subject received 0.75 mg enalapril maleate twice daily during the dose increase. This intermediate step in the dose increase was not normally provided for in the dosing regimen (Appendix 9.2). Of the 34 subjects, 27 received a higher starting dose than recommended in the dosing regimen, based on the investigator's judgement. In three subjects, titration was carried out up to the recommended target dose of the dosing regimen. The highest daily dose administered to the subjects was a median of 0.12 mg/kg (range 0.07–0.29 mg/kg, n = 33) enalapril maleate, determined at the time of the first administration of this daily dose.

A total of eight missing weights were replaced by the last observed weight of the subject according to the last observation carried forward approach. In three cases, the time deviation from the predefined time points of the full profile was greater than 15 minutes. In five cases, the pharmacokinetic sample was taken during titration or at the end of the study in deviation from the specifications after dosing. In all cases, the exact dosing and sampling times were used. In two cases, the planned 12-hour pharmacokinetic sample of the full profile could not be collected. For one subject, the 4-hour value of the full profile was not available.

Table 2.1 Patient characteristics of the children with heart failure on the day of the first administration of enalapril

Characteristic	Number (%)	Mean (SD)	Median (range)
Age (years)	34 (100)	0.38 (0.39)	0.3 (0.07–2.09)
Weight (kg)	34 (100)	5.08 (1.89)	4.47 (2.52–11.3)
Ross score	34 (100)	4.91 (2.6)	5 (0–9)
Serum creatinine (µmol/L)	34 (100)	28.03 (11.77)	27 (12–68)
Sex			
Male	16 (47.1)	-	-
Female	18 (52.9)	-	-
Aetiology of heart failure			
Dilated cardiomyopathy	3 (8.8)	-	-
Congenital heart disease	31 (91.2)	-	-

SD, standard deviation.

2.3.2 Base Model

A combined model with a one-compartment model of enalapril coupled with a one-compartment model of enalaprilat with first-order absorption and absorption lag was selected as structural model on the basis of the predefined model selection criteria (Figure 2.2). In the base model, a combined error model with an additive and a proportional error for enalapril and a proportional error model for enalaprilat were used. For enalapril and enalaprilat, an allometric scaling with an exponent of 0.75 for the apparent clearance and an exponent of 1 for the apparent volume of distribution was applied. The inclusion of a maturation function for the apparent clearance of enalapril or enalaprilat resulted in high relative standard errors for the parameters of the maturation function and was therefore not considered further. The described base model was run once without the data below the limit of quantification (M1 method) and once with the data below the limit of quantification, treated as censored (M3 method). When data below the limit of quantification were included and treated as censored, the estimates of the pharmacokinetic parameters (clearances and volumes of distribution) were only slightly lower (between 2.2 and 23.9% lower) compared with the estimates of the pharmacokinetic parameters obtained when data below the limit of quantification were excluded. Therefore, data below the limit of quantification were excluded in the further steps of model development.

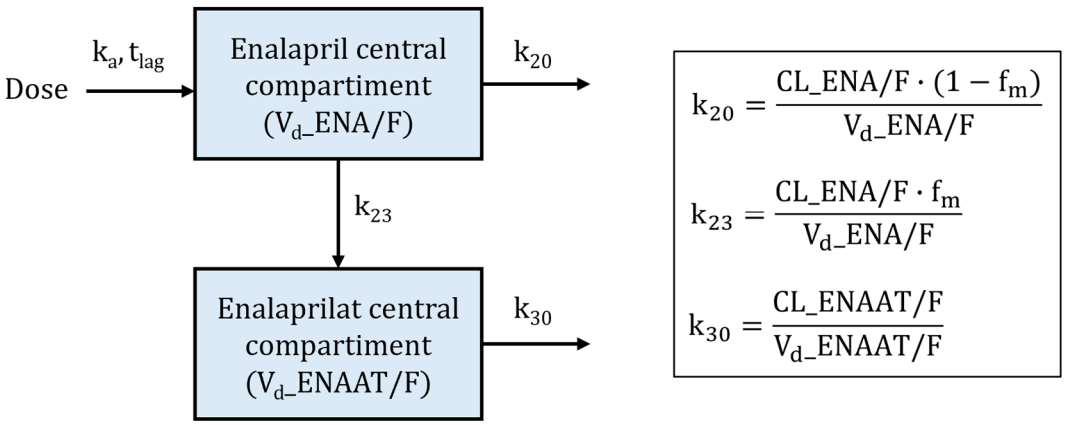

Figure 2.2 **Schematic illustration of the structural model used in the final combined pharmacokinetic model.** The box on the right contains the underlying equations for the transfer rate constants. CL_ENA/F, apparent clearance of enalapril; CL_ENAAT/F, apparent clearance of enalaprilat; f_m, metabolised fraction of enalapril (fixed to 0.7); k_{20}, transfer rate constant (first-order) from the central compartment of enalapril to the urine; k_{23}, transfer rate constant (first-order) from the central compartment of enalapril to the central compartment of enalaprilat; k_{30}, transfer rate constant (first-order) from the central compartment of enalaprilat to the urine; k_a, absorption rate constant (fixed to 0.6 h^{-1}); t_{lag}, lag time; V_d_ENA/F, apparent volume of distribution of enalapril; V_d_ENAAT/F, apparent volume of distribution of enalaprilat.

2.3.3 Covariate Analysis

The base model with allometric scaling was used for the covariate analysis. Age and serum creatinine were identified as covariates for the apparent clearance of enalaprilat and the Ross score as a covariate for the apparent volume of distribution of enalaprilat in the forward step of the stepwise covariate modelling. The backward deletion of the covariates mentioned led to a significant increase in the objective function. Therefore, the above covariates were included in the final model. In the final model, the disposition parameters of enalapril and enalaprilat of the individual patient are expressed as follows:

$$CL\_ENA/F = 4.61 \cdot \left(\frac{Weight}{5}\right)^{0.75} \cdot e^{\eta_1} \quad \textbf{Equation 2.3}$$

$$CL\_ENAAT/F = 1.55 \cdot \left(\frac{Weight}{5}\right)^{0.75} \cdot \left(\frac{Age}{0.34}\right)^{0.311} \cdot e^{-0.0141 \cdot (Serum\ creatinine - 23.37)} \cdot e^{\eta_2} \quad \textbf{Equation 2.4}$$

$$V_d\_ENA/F = 4.98 \cdot \left(\frac{Weight}{5}\right)^{1} \cdot e^{\eta_3} \quad \textbf{Equation 2.5}$$

$$V_d\_ENAAT/F = 34.1 \cdot \left(\frac{Weight}{5}\right)^{1} \cdot e^{-0.15 \cdot (Ross\ score - 4)} \cdot e^{\eta_4} \quad \textbf{Equation 2.6}$$

where CL_ENA/F represents the apparent clearance of enalapril, CL_ENAAT/F represents the apparent clearance of enalaprilat, V_d_ENA/F represents the apparent volume of distribution of enalapril, V_d_ENAAT/F represents the apparent volume of distribution of enalaprilat and η_i represents the deviation of the individual patient from the population value. In the population studied, the weighted median calculated by Perl-speaks-NONMEM is for weight 5 kg, for age 0.34 years, for serum creatinine 23.37 µmol/L and for Ross score 4.

The weight-adjusted apparent clearance of enalaprilat increases with increasing age and decreases with increasing serum creatinine. The weight-adjusted apparent volume of distribution of enalaprilat decreases with increasing Ross score. For a subject weighing 5 kg, the apparent volume of distribution of enalaprilat is 62.1 L for a Ross score of 0, and 11.9 L for a Ross score of 11.

The NONMEM® code for the final combined population pharmacokinetic model for enalapril and enalaprilat is given in Appendix 9.4.

2.3.4 Model Evaluation

The goodness-of-fit plots (Figure 2.3) and the prediction- and variability-corrected visual predictive checks (Figure 2.4) indicate a good model performance of the final model for enalapril and enalaprilat. The plots of the observed versus the individual predicted concentration show an even distribution around the unity line. Although the scatter around the unity line is greater for enalapril than for enalaprilat. For enalapril and enalaprilat, the conditional weighted residuals versus the population predicted concentration and versus the time since last dose were evenly distributed around zero, and the majority of the conditional weighted residuals ranged between −2 and 2.

Since 43% of the 1000 performed bootstrap runs resulted in rounding errors, the medians and CI of the model parameters were calculated once with and once without the runs with rounding errors. The results of the two calculations were similar (maximum 7.5% deviation), so the bootstrap results were unaffected by the minimisation status, as already shown by others [83]. Of the runs with rounding errors, 75% had significant digits greater than 2 and 72% had significant digits greater than 3. A total of 1.3% of the runs were not included in the calculation because the estimates were near a boundary, or the hessian of posterior density was non-positive-definite during the search. The condition number of the final model was 2.9 and therefore does not indicate overparameterisation. The results of the final model and the bootstrap results, including the runs with rounding errors, are shown in Table 2.2. The parameter estimates of the final model were similar to the medians of the bootstrap results and were within the 95% CI of the bootstrap results, indicating that the parameters were well estimated.

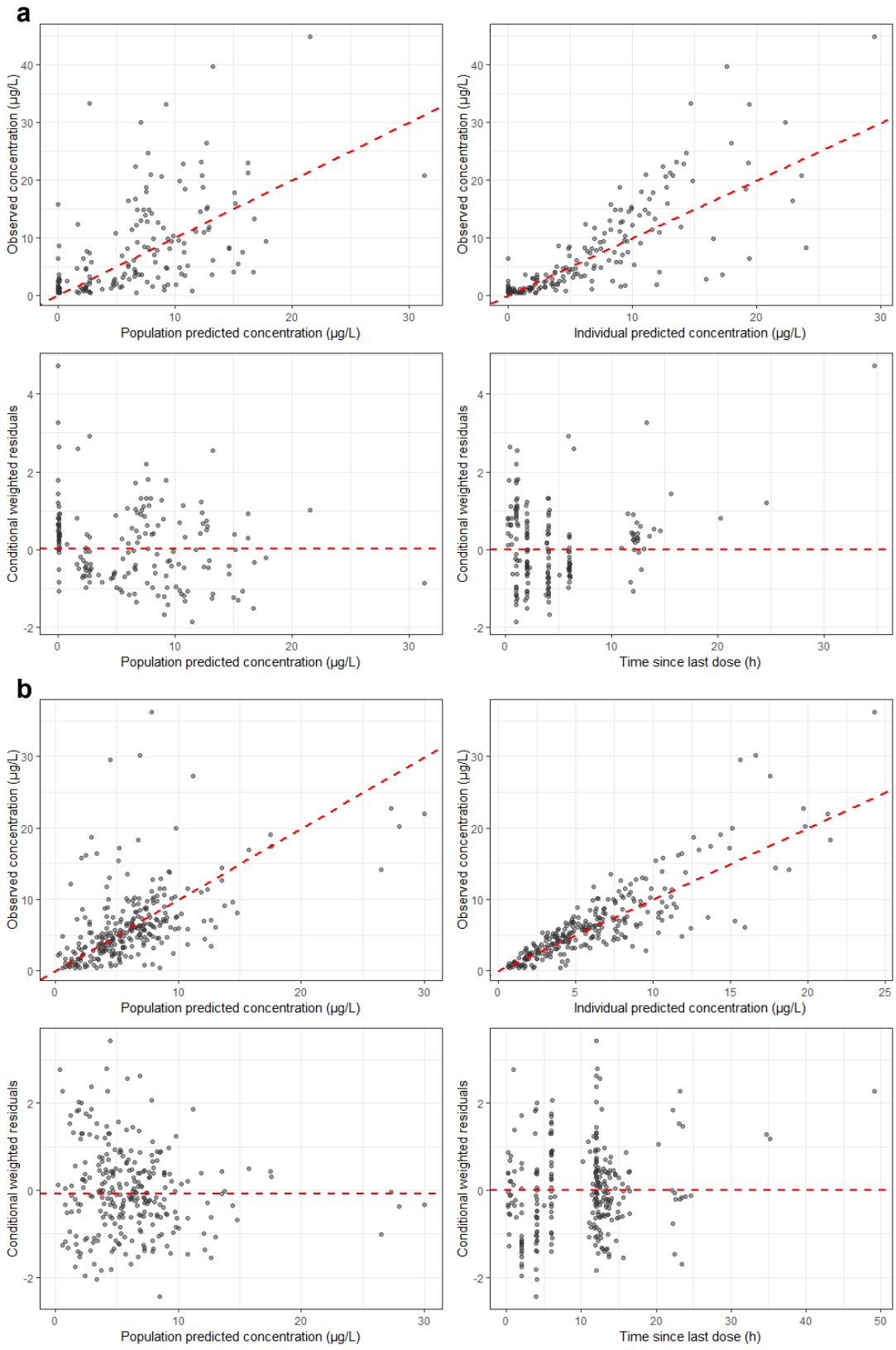

Figure 2.3 **Goodness-of-fit plots of the final combined pharmacokinetic model.** In (a), the goodness-of-fit plots for enalapril are shown, and in (b), those for enalaprilat. For (a) and (b), the red dashed line is the line of unity (top left and top right), the median of the conditional weighted residuals (bottom left) or the reference line of zero (bottom right). Darker circles indicate overlapping observations.

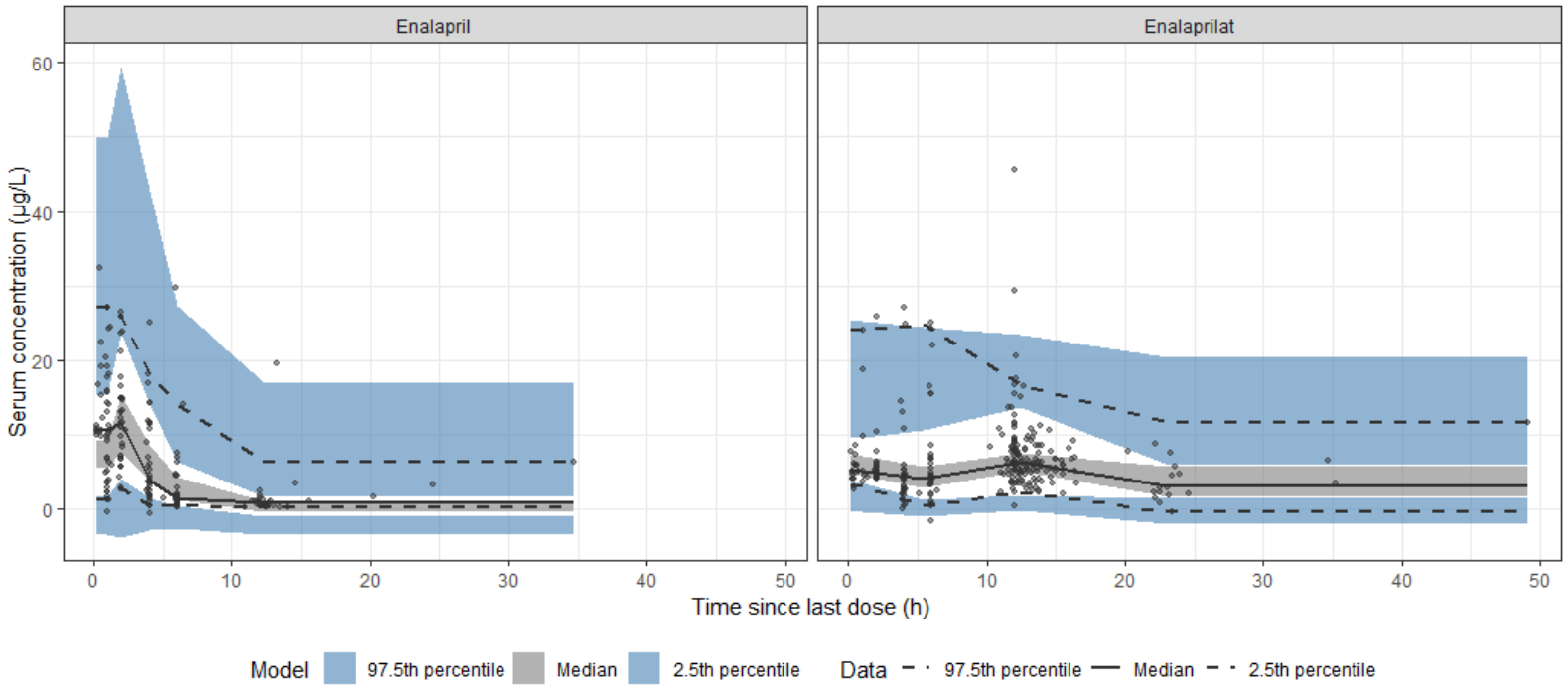

Figure 2.4 **Prediction- and variability-corrected visual predictive checks of enalapril and enalaprilat for the final combined pharmacokinetic model.** Observations are shown as circles. Darker circles indicate overlapping observations. The solid line represents the median of the observations. The dashed lines are the 2.5th and 97.5th percentiles of the observations. The shaded areas represent the 95% confidence intervals of the median (grey) and the 2.5th and 97.5th percentiles (blue) predicted by the model.

Table 2.2 Parameter estimates and bootstrap results of the final combined pharmacokinetic model.

Parameter	Unit	Final model			Bootstrap	
		Estimate	RSE (%)	Shrinkage (%)	Median	95% CI
k_a	h^{-1}	0.6	Fixed	-	0.6	Fixed
CL_ENA/F	L/h	4.61	12.6	-	4.53	3.43 to 5.67
CL_ENAAT/F	L/h	1.55	7.1	-	1.54	1.36 to 1.75
V_d_ENA/F	L	4.98	18.1	-	4.68	2.83 to 8.32
V_d_ENAAT/F	L	34.1	15.7	-	33.66	24.45 to 47.14
t_{lag}	h	0.515	2.8	-	0.515	0.195 to 0.731
Weight on CL_ENA/F (referenced to 5 kg)	-	0.75	Fixed	-	0.75	Fixed
Weight on CL_ENAAT/F (referenced to 5 kg)	-	0.75	Fixed	-	0.75	Fixed
Weight on V_d_ENA/F (referenced to 5 kg)	-	1	Fixed	-	1	Fixed
Weight on V_d_ENAAT/F (referenced to 5 kg)	-	1	Fixed	-	1	Fixed
Age on CL_ENAAT/F	-	0.311	29.3	-	0.316	0.092 to 0.553
Serum creatinine on CL_ENAAT/F	-	–0.0141	33.3	-	–0.0141	–0.0288 to –0.004
Ross score on V_d_ENAAT/F	-	–0.15	26.3	-	–0.149	–0.248 to –0.004
Interindividual variability[1]						
IIV CL_ENA/F	%	65.3	30	9.7	62.6	37.7 to 82.7
IIV CL_ENAAT/F	%	37.7	30.1	6.5	35.6	25.4 to 48.7
IIV V_d_ENA/F	%	80.2	41.5	17.8	79.8	41.0 to 114.3
IIV V_d_ENAAT/F	%	90.4	27.9	4.9	87.5	56.1 to 114.9
Residual variability enalapril						
Proportional error[2]	%	53.5	19.9	10	52.7	39.6 to 64.1
Additive error[3]	µg/L	1.34	23.1	10	1.27	0.56 to 2.09
Residual variability enalaprilat						
Proportional error[2]	%	39.5	11.3	9.9	39.2	33.9 to 44.2

CL_ENA/F, CL_ENAAT/F, V_d_ENA/F and V_d_ENAAT/F estimates are given for a patient with a body weight of 5 kg. RSE derived from the covariance matrix (R matrix).

[1]The interindividual variability is expressed as a coefficient of variation calculated according to the equation $\sqrt{\omega^2} \cdot 100\%$, where ω^2 is the NONMEM® output for the interindividual variability of the parameter estimate.

[2]The proportional error is expressed as a coefficient of variation calculated according to the equation $\sqrt{\sigma^2} \cdot 100\%$, where σ^2 is the NONMEM® output for the proportional error term.

[3]The additive error is expressed as standard deviation calculated according to the equation $\sqrt{\sigma^2}$, where σ^2 is the NONMEM® output for the additive error term.

CI, confidence interval; CL_ENA/F, apparent clearance of enalapril; CL_ENAAT/F, apparent clearance of enalaprilat; IIV, interindividual variability; k_a, absorption rate constant; RSE, relative standard error; t_{lag}, lag time; V_d_ENA/F, apparent volume of distribution of enalapril; V_d_ENAAT/F, apparent volume of distribution of enalaprilat.

2.3.5 Simulations

The impact of weight and the included covariates on the predicted pharmacokinetic parameters of enalaprilat after the first dose (AUC_{∞}, $C_{max,1}$) and at steady state (AUC_{τ}, $C_{max,ss}$) compared with the reference subject is shown in Figure 2.5. A subject with a weight of 5 kg, an age of 0.34 years, a Ross score of 4 and a serum creatinine of 23.37 µmol/L served as the reference subject. The ratio to the reference subject for the aforementioned pharmacokinetic parameters is presented for the 5th, 25th, 75th and 95th percentile of weight (underweight), age (underweight and normal weight), Ross score and serum creatinine of the analysed population. For weight (normal weight), the ratio to the reference subject for the 5th percentile of weight (3.32 kg) is not presented. To represent a normal weight, an age of 0.02 years was assigned based on the 50th WHO weight percentile. As this was an extrapolation, because the youngest child in the analysed population was 0.07 years old, this covariate scenario was excluded. The simulations were each carried out with 988 parameter datasets (dataset of the final model + 987 bootstrap datasets).

As expected, the simulations provided the same results for the AUC_{∞} after the first dose and the AUC_{τ} at steady state. Compared with the reference subject with a weight of 5 kg, an underweight subject weighing 3.32 kg was estimated to have a median AUC that was 100% [90% CI: 61 to 153%] higher and an underweight subject weighing 9.61 kg was estimated to have a median AUC that was 67% [90% CI: –77 to –53%] lower. In comparison with the reference subject aged 0.34 years, the median AUC was estimated to be 89% [90% CI: 54 to 134%] higher in an underweight subject aged 0.11 years and estimated to be 52% [90% CI: –63 to –40%] lower in an underweight subject aged 1.28 years. Considering the impact of weight and age on subjects with normal weight, a similar picture emerged. The variation of the Ross score did not affect the AUC. At a serum creatinine of 42 µmol/L, the median AUC was estimated to be 30% [90% CI: 11 to 58%] higher than in the reference subject, with a serum creatinine of 23.37 µmol/L.

The results for the $C_{max,ss}$ are almost the same as for the AUC. For $C_{max,1}$, there were two main differences from the previously reported results. Firstly, the variation in serum creatinine did not lead to a considerable change in $C_{max,1}$. Secondly, the Ross score showed an impact on the $C_{max,1}$. In comparison with the reference subject with a Ross score of 4, the median $C_{max,1}$ was estimated to be 40% [90% CI: –55 to –12%] lower in a subject with a Ross score of 0 and estimated to be 60% [90% CI: 13 to 106%] higher in a subject with a Ross score of 8.

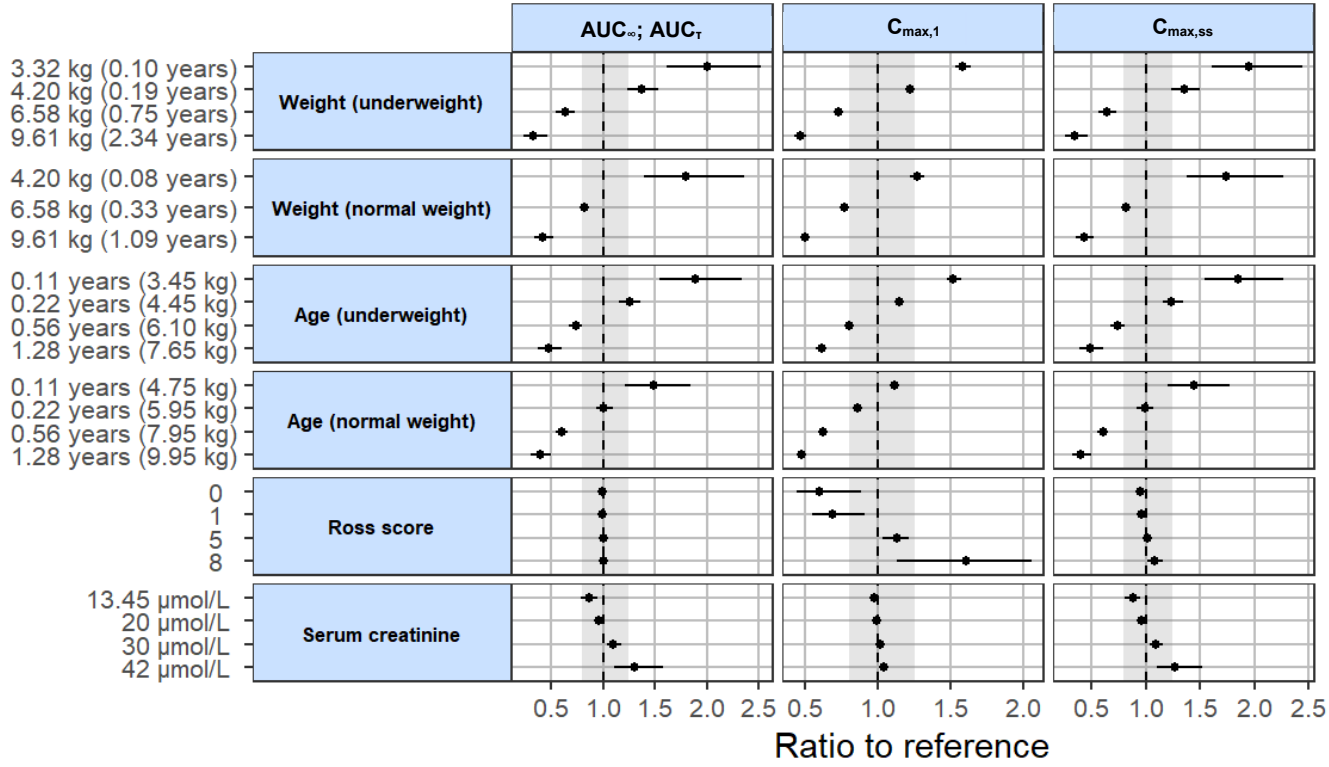

Figure 2.5 **Impact of weight and statistically significant covariates included in the final model on the predicted pharmacokinetic parameters of enalaprilat.** The plot shows the results for the pharmacokinetic parameters of enalaprilat after the first dose (AUC_{∞}, $C_{max,1}$) and at steady state (AUC_{τ}, $C_{max,ss}$). The predicted AUC_{∞}, AUC_{τ}, $C_{max,1}$ and $C_{max,ss}$ at the given weight and covariate values (5th, 25th, 75th and 95th percentiles of the analysed population) were compared with those predicted for a reference subject with a weight of 5 kg, an age of 0.34 years, a Ross score of 4 and a serum creatinine of 23.37 µmol/L. The black dots and error bars show the median of the ratio to reference with a 90% confidence interval (n = 988). The grey area shows the 80–125% range in relation to the reference, whereby the selected range is based on the standard bioequivalence limits. AUC_{∞}, area under the concentration–time curve from time zero to infinity; AUC_{τ}, area under the concentration–time curve during a dosage interval; $C_{max,1}$, maximum serum drug concentration after the first dose; $C_{max,ss}$, maximum steady state serum drug concentration during a dosage interval.

The higher the Ross score at the time of the first dose, the higher was the predicted median $C_{max,1}$ of enalaprilat after administration of 0.25 mg enalapril maleate (Figure 2.6). With a Ross score of 0, the predicted median $C_{max,1}$ was 1.85 µg/L (interquartile range 0.63), whereas with a Ross score of 11, the predicted median $C_{max,1}$ was 6.79 µg/L (interquartile range 2.81). Due to the exponential relationship, the stepwise increase in $C_{max,1}$ per Ross score point is greater with higher Ross scores. Normalised to dose and weight, the simulated $C_{max,1}$ was between 14.6 µg/L/mg·kg and 280.2 µg/L/mg·kg. In the previous non-compartmental analysis, $C_{max,1}$ ranged between 0 µg/L/mg·kg and 479.6 µg/L/mg·kg for the 32 included ACE inhibitor naïve subjects [52]. The smaller range of simulated values is not surprising, as only the covariate Ross score was varied for the simulation.

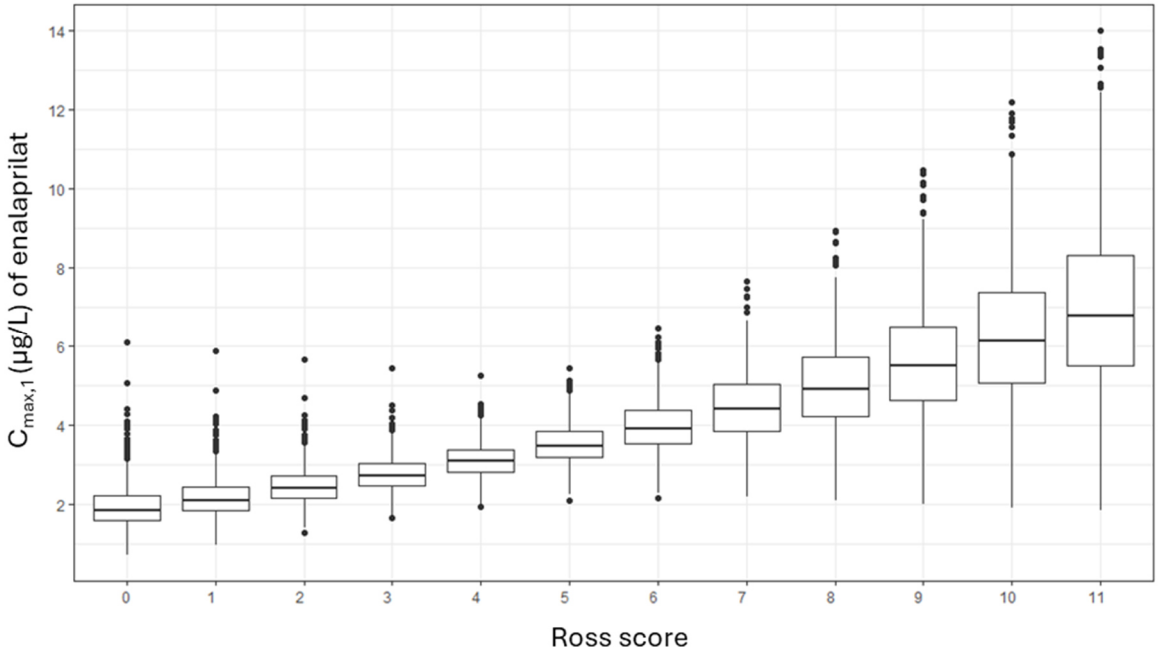

Figure 2.6 **Impact of the Ross score on the maximum enalaprilat serum concentration after the first dose.** A dose of 0.25 mg enalapril maleate was chosen for the simulations. The boxplot shows the predicted $C_{max,1}$ for subjects with a weight of 5 kg, an age of 0.34 years, a serum creatinine of 23.37 µmol/L and a Ross score between 0 and 11 (minimum to maximum Ross score of the analysed population). For each Ross score, 988 $C_{max,1}$ values were estimated. The upper whisker extends from the 75th percentile to the largest value no further than 1.5 times the interquartile range from the 75th percentile. The lower whisker extends from the 25th percentile to the smallest value no further than 1.5 times the interquartile range from the 25th percentile. Data beyond the end of the whiskers are outliers and are shown as black dots. $C_{max,1}$, maximum serum drug concentration after the first dose.

2.4 Discussion

The pharmacokinetics of enalapril and enalaprilat in ACE inhibitor naïve children with heart failure were adequately described by the final combined population pharmacokinetic model, consisting of a one-compartment model for enalapril coupled with a one-compartment model for enalaprilat with absorption lag. In addition to weight, which was included using allometric scaling, three covariate relationships were identified and included. Firstly, the weight-adjusted apparent clearance of enalaprilat increases with increasing age. Secondly, the weight-adjusted apparent clearance of enalaprilat decreases with increasing serum creatinine. Thirdly, the weight-adjusted apparent volume of distribution of enalaprilat decreases with increasing Ross score. The simulations suggested that age and weight are clinically relevant covariates for both the first dose and the steady state dose of enalapril. Similarly, the simulations suggested that serum creatinine above the normal reference range is a clinically relevant covariate with respect to the first dose and the steady state dose of enalapril. In addition, the simulations indicated that the Ross score is a clinically relevant covariate for the first dose of enalapril.

For the final combined population pharmacokinetic model, a one-compartment model was selected for both enalapril and enalaprilat. This is partly in line with a population pharmacokinetic model developed earlier in healthy adults [70]. The adult model also used a one-compartment model for enalapril. For healthy adults a two-compartment model was used for enalaprilat to describe the long lasting biphasic exponential decay of enalaprilat concentrations for about 48 hours after enalapril application. To comply with the recommended limits for blood loss in paediatrics, the total number of samples was less and the duration of sample collection for the full profiles in children with heart failure was only 12 hours, which limited the modelling in the elimination phase. Nevertheless, the individual predictions correlated well with the observed concentrations. By extrapolation of the estimated apparent clearances of the children with heart failure from this work to an adult weight of 70 kg, the apparent clearance of enalapril would be 64.5 L/h and the apparent clearance of enalaprilat 21.7 L/h. The healthy adults studied by Najib et al. [84] had an apparent clearance of enalapril of 77.0 L/h and an apparent clearance of enalaprilat of 30.2 L/h, calculated over the mean AUC_∞ and the dose given. The extrapolated apparent clearances of the children are in a similar range to those for adults but are lower for both enalapril and enalaprilat. The lower apparent clearances might be explained by the fact that the children investigated had a lower carboxylesterase 1 hydrolytic activity and a lower

glomerular filtration rate than adults owing to their age [32, 85]. The extrapolation of the estimated apparent volume of distribution of the children with heart failure from this work to an adult weight of 70 kg would result in an apparent volume of distribution of enalapril of 69.7 L. The apparent volume of distribution of enalapril according to the population pharmacokinetic model for adults, which also used a one-compartment model for enalapril, was 84.3 L [70]. The moderate difference between the extrapolated value and the value for adults might be explained by the fact that more sampling time points were available for the adults.

The first parameter that should be considered when dosing enalapril is the child's weight. In the population pharmacokinetic analysis of enalaprilat based on literature data from children with hypertension, weight was also incorporated into the final model, but the allometric exponents were estimated [72]. The presented population pharmacokinetic analysis implemented allometric scaling with fixed allometric exponents prior to covariate analysis so that other potential covariate effects could be distinguished from the effect of size [86]. The consideration of weight when dosing enalapril is also reflected in the product information of the authorised enalapril orodispersible minitablets (Aqumeldi®), where a weight-based recommended range for the initial and target dose is given [56].

The second parameter to consider when choosing the dosage of enalapril is the age of the child. A covariate effect of age on the apparent clearance of the active metabolite enalaprilat was found, but unexpectedly not on the apparent clearance of enalapril. Enalapril is eliminated by metabolism to a large extent, and enalaprilat is largely excreted renally. It is therefore suspected that the effect of age on renal excretion is greater than on metabolism in the population studied. In the age range from 35 to 198 days, which includes about 70% of the subjects studied, it was shown that carboxylesterase 1 expression and hydrolytic activity are similar [85]. One reason for the lack of effect of age on enalapril apparent clearance could therefore be that in the relatively narrow age range of the subjects, no major change in the metabolising enzyme carboxylesterase 1 takes place. Another reason could be that the number of subjects was too small to observe an effect of age on enalapril apparent clearance. The observed effect of age on enalaprilat apparent clearance might be explained by the large increase in glomerular filtration rate during the first year of life [32, 87]. Except for the recommendation to set the initial dose at the lower end of the recommended dose range for infants < 30 days of age, age is not currently considered in the dosage recommendations of

enalapril orodispersible minitablets [56]. In contrast, the dosing regimen developed for the LENA studies already took the covariate age into account.

The third parameter to be considered when dosing enalapril is the presence of elevated serum creatinine. At a serum creatinine value of 42 µmol/L, corresponding to a glomerular filtration rate of 55 mL/min/1.73 m² when using the Schwartz formula and an appropriate height for the simulated subject, the simulations showed a 30% higher enalaprilat AUC after the first dose and at steady state compared with the reference subject with a normal serum creatinine value [88]. Oguchi et al. [89] showed that adults with moderate renal impairment had a four-fold higher enalaprilat AUC than adults with normal renal function. The larger difference in AUC observed by Oguchi et al. [89] may be explained by the fact that their study group also comprised subjects with a glomerular filtration rate below 55 mL/min/1.73 m^2. In line with the simulation results, the product information recommends a reduction in the initial dose of enalapril orodispersible minitablets in the case of reduced glomerular filtration rate [56]. In the product information, a 50% reduced single dose is recommended as the initial dose if the glomerular filtration rate is between 30 and 50 mL/min/1.73 m^2. The simulations performed do not cover this range because only one subject had a glomerular filtration rate in this range at the start of the study, but they suggest that a dose reduction could be considered even at glomerular filtration rates above this threshold.

The fourth parameter that should be considered when dosing enalapril, or more precisely for the first dose, is the Ross score. The Ross score is used in children to classify the severity of heart failure, like the New York Heart Association classification for adults. The hypothesis is that greater severity of heart failure and a higher degree of heart failure symptoms are associated with a lower volume of distribution for enalaprilat. The covariate relationship between the Ross score and the apparent clearances was also tested. However, no impact of the Ross score on the apparent clearances could be determined. The majority of the subjects suffered from congenital heart disease. In contrast to adults with heart failure and reduced systolic function, children with congenital heart disease do not have reduced cardiac contractility but instead pulmonary overcirculation and systemic hypoperfusion due to intracardiac shunts or a patent ductus arteriosus [6]. Also, children with congenital heart disease show different symptoms of heart failure, for example, no peripheral oedema. The suggestion is that, owing to the different pathophysiology, an increase in heart failure symptoms, represented by the Ross Score, only affects the volume of distribution and not

the clearance in children. However, the detailed haemodynamic explanation needs further investigation. The dosage recommendations for enalapril orodispersible minitablets only include a note that the initial dose should be set at the lower end of the recommended dose range for less stable patients [56]. Thus, the severity of heart failure is not yet taken into account in the dosage recommendations for enalapril orodispersible minitablets.

2.5 Limitations

The population pharmacokinetic analysis is faced with limitations. Firstly, the glomerular filtration rate could not be used for the covariate analysis because the height of the subjects required for the calculation of the glomerular filtration rate was only available at the first and last visit of the study. Therefore, serum creatinine was used as a parameter for renal function in the covariate analysis. Secondly, the results primarily allow conclusions to be drawn for children with congenital heart disease, as these made up the majority of the subjects. Thirdly, the extent of the interindividual variability of the pharmacokinetic parameters is comparable to that of other population pharmacokinetic analyses, if the boundary conditions of this study are kept in mind. Nevertheless, other covariates for which no information was available could play a role. For example, polymorphisms of the metabolising enzyme carboxylesterase 1 or the hepatic uptake transporter organic anion-transporting polypeptide 1B1 could be partly responsible for the observed interindividual variability of the pharmacokinetic parameters. Several carboxylesterase 1 polymorphisms are known to affect carboxylesterase 1 activity and can lead to lower enalaprilat concentrations [90]. In subjects with an organic anion-transporting polypeptide 1B1 polymorphism, a significantly higher systemic exposure of enalapril at steady state and a significantly lower systemic exposure of enalaprilat after a single dose were observed [91]. Fourthly, a fixed value for the metabolised fraction was used because no urine data were available. The metabolised fraction influences the oral bioavailability of enalapril and the apparent volume of distribution of enalaprilat. Therefore, the metabolised fraction was selected based on data from the literature originating from children of a similar age. Despite these limitations, the pharmacokinetics of enalapril and enalaprilat in ACE inhibitor naïve children with heart failure were well predicted by the final model.

2.6 Conclusions

The results of the population pharmacokinetic analysis and simulations confirm the consideration of the currently used parameters weight and renal function in the dosage of enalapril in children with heart failure and provide further insights into these. The identified covariate age indicates that the age- and weight-dependent dosing regimen is preferable to sole weight-dependent dosing. In addition, the newly identified covariate Ross score indicates that the severity of heart failure should be considered for the choice of the initial dose of enalapril. To avoid high peak concentrations and a possible drop in blood pressure, the initial dose should be reduced depending on the Ross score. The subjects analysed were children with heart failure aged between 25 days and 2.1 years without previous ACE inhibitor treatment and without severe renal impairment, with the majority of children being under 1 year old and suffering from congenital heart disease. It should be noted that the results primarily allow conclusions to be drawn about patients within these criteria.

2.7 Disclosure

Parts of this chapter were previously published in a peer-reviewed journal:

Steichert, M., Cawello, W., & Laeer, S. (2025). Population Pharmacokinetic Analysis of Enalapril and Enalaprilat in Newly Treated Children with Heart Failure: Implications for Safe Dosing of Enalapril (LENA Studies). Clinical Pharmacokinetics, 64(7), 1103–1118. https://doi.org/10.1007/s40262-025-01520-5

The author of this thesis substantially contributed to the conception and design of the work. In addition, the author of this thesis was responsible for data preparation, population pharmacokinetic analysis, simulations, the draft of the manuscript, and critical revision of the manuscript.

3 Influence of Age, Heart Failure and ACE Inhibitor Treatment on Plasma Renin Activity

3.1 Background

In the population pharmacokinetic analysis, age and Ross score were identified as covariates for the pharmacokinetics of enalaprilat. Furthermore, it was observed that measurable enalaprilat levels were achieved with the given dosage of enalapril. This raises firstly the question whether age and heart failure also influence the pharmacodynamic parameter plasma renin activity. Secondly, the question arises whether the given dosage of enalapril also had a measurable influence on the pharmacodynamic parameter plasma renin activity.

Plasma renin activity, along with other markers such as NT-proBNP, is an important prognostic marker for adults with heart failure. This is based on the generally accepted view that neurohormonal activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system profoundly contributes to the pathophysiology of heart failure [92]. Moreover, Aimo et al. [93] and Vergaro et al. [94] found that plasma renin activity independently predicted cardiovascular death in adults with heart failure and concluded that plasma renin activity could be used besides other markers for prognostic stratification of heart failure patients.

As in adults with heart failure, plasma renin activity and the other markers are often measured in children with heart failure because the neurohumoral activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system also contribute to the pathophysiology of paediatric heart failure, although the aetiology of heart failure in children is somewhat different from adults [95]. So far, plasma renin activity has been measured in children with heart failure as a marker for renin–angiotensin–aldosterone system activity or to check whether clinical symptoms are related to plasma renin activity [96, 97]. Regarding clinical symptoms, plasma renin activity showed a correlation with respiratory rate and an inverse correlation with weight gain in children with heart failure [96].

To correctly assess plasma renin activity levels in daily practice, factors influencing plasma renin activity levels have to be identified and subsequently standardised or considered. It is already well known from healthy children that position of blood draw must be standardised because upright position during blood draw can increase plasma renin activity [98]. In

addition, sampling should be done at the same time of day because of the diurnal variation in plasma renin activity [99, 100]. For example, Dechaux et al. [99] recommend blood sampling at 7:00 AM in supine position. Since a low-sodium diet can increase plasma renin activity levels, if present, a low-sodium diet should be considered when evaluating plasma renin activity [101, 102].

Moreover, studies show that plasma renin activity decreases with increasing age [103–105]. Even though studies on the influence of age on plasma renin activity are available, they indicate a high variation in plasma renin activity levels. Because those studies have relatively small subject numbers, a systematic review for collecting as much information as possible, especially in young children, is mandatory to draw a precise picture on the magnitude of age dependency on plasma renin activity levels. Furthermore, the data collected during a systematic review would facilitate the differentiation between plasma renin activity in healthy children and children with heart failure.

Heart failure medication can also influence plasma renin activity levels. It is known from adults with heart failure that diuretic and ACE inhibitor therapy increase plasma renin activity, whereas beta-blocker and digoxin therapy decrease plasma renin activity [106–109]. For beta-blockers, the decrease in plasma renin activity was also shown in children with heart failure [110]. The studies on the effect of enalapril on plasma renin activity in children with heart failure are inconclusive. One study observed a significant increase in plasma renin activity after administration of enalapril, while another study found no significant difference in plasma renin activity before and during enalapril therapy [21, 23]. However, information on the influence and especially the magnitude of the influence of ACE inhibitors on plasma renin activity is important for the proper evaluation of plasma renin activity in children with heart failure on ACE inhibitor treatment.

The aim is therefore to evaluate the influence of age, heart failure and ACE inhibitor treatment on plasma renin activity levels in children. For that purpose, plasma renin activity was investigated in healthy children and children with heart failure on standard therapy (e.g., diuretics, digoxin, and beta blocker), with and without ACE inhibitor treatment. A systematic literature review was conducted and data from the European LENA project were analysed.

3.2 Methods

3.2.1 Data Base from Literature Search

A literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement using MEDLINE database [111]. Search terms were defined to identify literature on plasma renin activity in healthy children as well as in children with heart failure. In November 2021, the search term '(plasma renin activity) AND (Paediatric OR newborn OR infant OR toddler OR child) AND (Heart failure OR dilated cardiomyopathy OR congenital heart defect OR congenital heart disease)' was utilized to ascertain literature on plasma renin activity in children with heart failure. In January 2022, the search term '(plasma renin activity) AND (Paediatric OR newborn OR infant OR toddler OR child) AND (healthy OR 'control group')' was used to detect literature on plasma renin activity in healthy children. The following filters were set for both searches: Humans, English, German, Child: birth–18 years.

The inclusion criteria were set as follows. Studies were included if they provided data on plasma renin activity in healthy children or children with heart failure from birth to 18 years of age. In addition, for better comparability, plasma renin activity had to be reported in the study as arithmetic mean ± standard deviation (SD) or standard error (SE). Due to the smaller amount of data in children with heart failure, studies in which plasma renin activity was reported as arithmetic mean and range were also allowed. Age had to be reported in the study as arithmetic mean ± SD or SE or alternatively as range. SE was converted to SD for the graphs and tables. Healthy children were allowed to have no or only mild diseases without known influence on the renin–angiotensin–aldosterone system. Heart failure was allowed to be due to congenital or acquired heart defects.

The exclusion criteria were set as follows. Studies were excluded if age, type of statistical parameters used, or health status were not accurately reported. In addition, studies in which plasma renin activity was measured in preterm infants, in fetal blood or in umbilical cord blood were excluded. Further exclusion criteria were if only stimulated plasma renin activity or only renin concentration was measured. As the influence of ACE inhibitor treatment was to be investigated, studies on children with heart failure were excluded if no information on therapeutic medication was provided. Moreover, studies on children with heart failure were excluded if only postoperative or intraoperative plasma renin activity was measured.

In the first step of study selection, the titles and abstracts of all records identified with the above search terms were screened. Records that did not meet the inclusion criteria or fulfilled an exclusion criterion were excluded at this stage. Subsequently, the full text of the remaining records was sought. Reports for which neither a printed nor a digital version of the full text was available could not be considered further. In the next step, all available full texts were screened. Reports that did not meet the inclusion criteria or fulfilled an exclusion criterion were excluded. Finally, all studies that were not excluded and met the inclusion criteria were included in the review.

To obtain an overview of the renin–angiotensin–aldosterone system in children, a non-systematic literature search for all renin–angiotensin–aldosterone system parameters was conducted as a preliminary search before the systematic literature search. Additional publications found in the preliminary search that met all inclusion criteria were also included.

After inclusion of all suitable publications, it was evaluated how many publications contain information about plasma renin activity in young healthy children aged up to two years.

3.2.2 Data from Paediatric Clinical Studies

In addition to the literature review, data from the two pharmacokinetic bridging studies of the LENA project were analysed. To investigate the influence of ACE inhibitor treatment, only the data of the 35 subjects who were ACE inhibitor naïve at the onset of the LENA studies were examined. General information on the two pharmacokinetic bridging studies is described in Chapter 1.4.3, and information on the dosage of enalapril can be found in Chapter 2.2.2.

As part of the pharmacodynamic sampling, blood samples had been collected and analysed for plasma renin activity levels before, 4 hours after and within the first 8 days of enalapril treatment. Blood was collected in a cooled ethylenediaminetetraacetic acid (EDTA) tube, carefully mixed and immediately centrifugated under cooled conditions (0–4 °C). After centrifugation, the supernatant was transferred into a cryo tube and was stored at –80 °C until analysis. The sample was taken in supine position and, if possible, when the children were quiet. It was advised to collect the blood sample before 10:00 AM. If this time could not be kept, the sample collection should always take place around the same time to minimise the influence of the circadian rhythm as much as possible. Resting time and behaviour during

sampling (relaxed, moving or crying) were noted. Plasma renin activity was determined by using an validated in-house customised enzyme-linked immunosorbent assay [112].

As part of the clinical assessment during the studies, the modified Ross score was determined by the investigator [8]. To analyse the impact of heart failure severity on plasma renin activity, the children were divided in asymptomatic children (modified Ross score ≤ 2) and children with symptomatic heart failure (modified Ross score ≥ 3) according to the current guideline of the German Society for Paediatric Cardiology [2].

In addition to heart failure therapy, other drugs were also administered during the observation period that are not expected to influence plasma renin activity. Other concomitant medications were antiplatelet drugs, ampicillin/sulbactam, cephalosporins, chloral hydrate, folic acid, heparins, ibuprofen, iron supplement, levothyroxine, meropenem, methylprednisolone, morphine, palivizumab, paracetamol, polyethylene glycol, potassium, prednisone, ranitidine, red cell concentrate and vitamin D3.

3.2.3 Statistical Analysis

For the evaluation of the plasma renin activity level, the healthy children from the literature were divided in four groups after visual inspection of the data. In the visual inspection, age ranges in which the extent and variability of plasma renin activity was similar were defined as one age group. Based on mean age or centerpoint of age range of the study group, results reporting mean plasma renin activity were summarised into four groups of age ranges: Neonates up to 30 days of age, infants from 1–24 months, children from 2–10 years, and children and adolescents older than 10 years. For each age group, the weighted mean of the reported mean plasma renin activity was calculated as an overall approximation. The number of plasma renin activity measurements was used for weighing. The weighted mean of the four age groups was compared by calculating the percentage change between the groups.

As plasma renin activity and age were not normally distributed at all time points in the LENA studies, the median and, where appropriate, the range were reported for the analyses. For comparison with the literature data, the mean and SD of age and plasma renin activity were calculated to have better comparability with the literature data, which were all available as mean ± SD or mean and range. To compare the 35 ACE inhibitor naïve subjects with the literature, they were divided into four age groups (< 1 month, 1–3 months, 3–6 months and > 6 months).

Since the conditions regarding normal distribution for the application of the parametric tests for dependent and independent samples were not fulfilled, nonparametric tests were performed.

To analyse the effect of ACE inhibitor on plasma renin activity, plasma renin activity levels before, after 4 hours of enalapril treatment and within the first 8 days of enalapril treatment were compared. For this purpose, the Friedman test for more than two paired samples was conducted. After that, the Wilcoxon test for paired samples was conducted to compare the plasma renin activity before and after 4 hours of enalapril treatment as well as the plasma renin activity before and within the first 8 days of enalapril treatment. For both the Friedman test and the Wilcoxon test for paired samples, data from those children who had a complete dataset of three plasma renin activity measurements were used ($n = 29$).

The plasma renin activity of the asymptomatic children and children with symptomatic heart failure was compared before and within the first 8 days of enalapril treatment using the Wilcoxon test for unpaired samples. Similarly, the age of the asymptomatic children and the children with symptomatic heart failure was compared using the Wilcoxon test for unpaired samples.

P values < 0.05 were considered as indicator for statistical significance. If not otherwise indicated, all data mentioned were expressed as mean ± SD. Statistical analyses and graphics were produced with R software version 4.0.5 (The R Foundation for Statistical Computing, Vienna, Austria) and OriginPro 2021b version 9.8.5.201 (OriginLab Corporation, Northampton, Massachusetts, USA).

3.3 Results

In the literature search, a total of 168 records were identified, 62 for the children with heart failure and 106 for healthy children. Of the 168 records, nine records were identified as duplicates. Additionally, six records on plasma renin activity in healthy children were identified through a preliminary search. After screening the titles and abstracts, 21 records were excluded. A total of 12 reports were not available in print or digital form, and 100 reports were excluded after screening the full text. Finally, 32 studies fulfilled the criteria and were included in the review. One study contained information on plasma renin activity in both healthy children and children with heart failure. Thus, a total of 29 studies on plasma renin activity in healthy children and four studies on plasma renin activity in children with heart failure were identified. Further details on the literature search and exclusion criteria are provided in the PRISMA flow diagram (Figure 3.1).

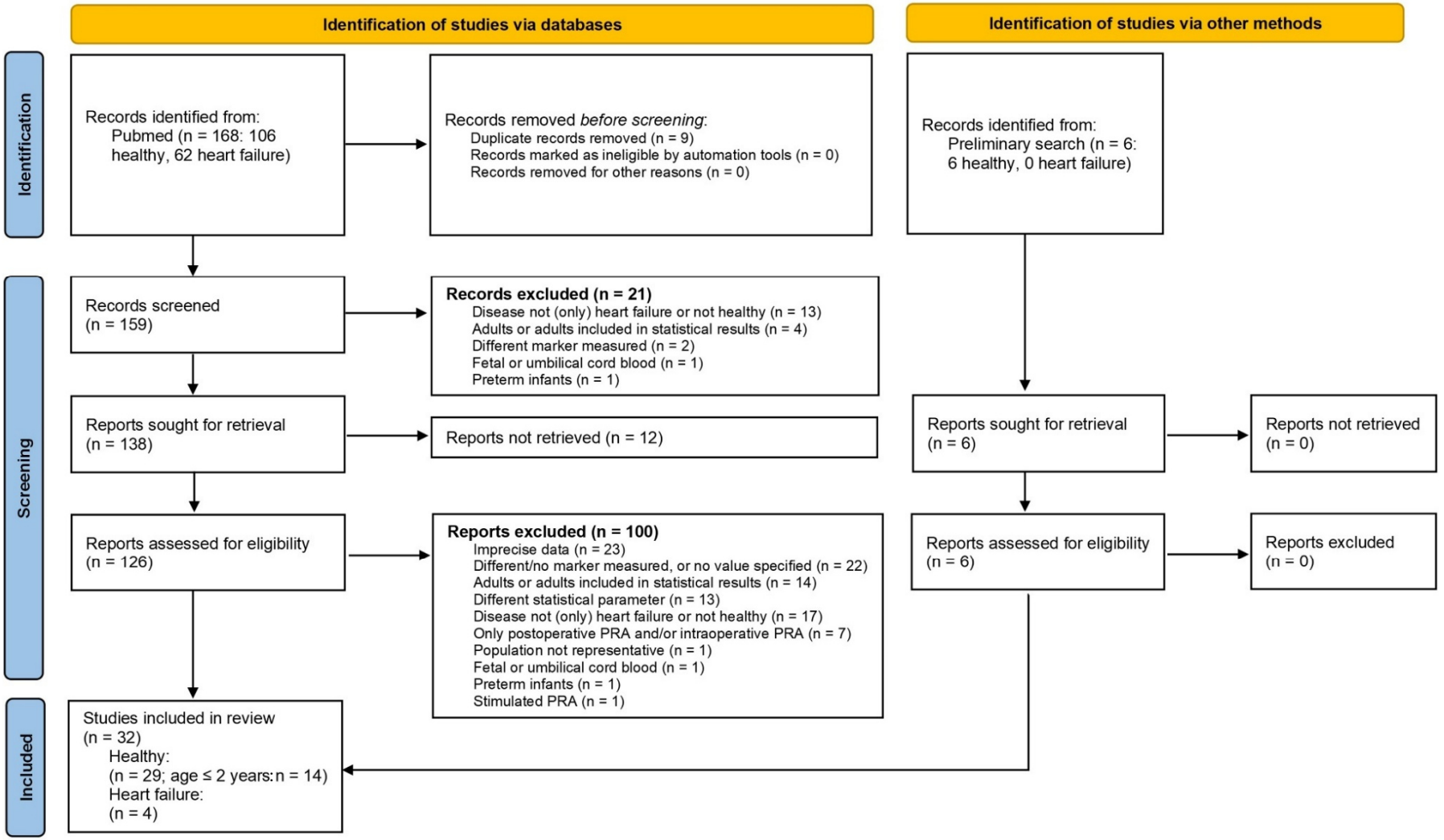

Figure 3.1 **Preferred reporting items for systematic reviews and meta-analyses flow diagram of the conducted literature search.** PRA, plasma renin activity.

3.3.1 Plasma Renin Activity in Healthy Children

The literature search yielded 29 publications on plasma renin activity in healthy children (Figure 3.1). The plasma renin activities reported in these 29 publications were from a total of 1482 healthy children. A total of 14 of the 29 publications provided values of plasma renin activity from healthy children younger than or equal to 2 years of age. Overall, 344 of the 1482 healthy children were younger than or equal to 2 years old.

Visual inspection of the literature data revealed roughly four groups: Neonates up to 30 days of age, infants from 1–24 months, children from 2–10 years, and children and adolescents older than 10 years. The results of plasma renin activity level evaluation in healthy subjects showed a decrease of plasma renin activity level over age (Figure 3.2, Table 3.1). The overall approximation of mean plasma renin activity levels for the four age ranges were 15.4, 11.8, 3.5, and 2.2 ng/mL/h. Compared with plasma renin activity levels of neonates up to 30 days of age, this means a drop of 23% in infants 1–24 months, 77% in children 2–10 years and 85% in children and adolescents older than 10 years. Comparing neonates with older children, plasma renin activity is up to 7 times higher in neonates than in older children. The percentage decrease in plasma renin activity between the different age groups is highest between infants aged 1 to 24 months and children aged 2 to 10 years. The highest mean plasma renin activity was determined by Vincent et al. [113] for 16 children between 6 and 30 days of age at 29.8 ± 28.6 ng/mL/h. Plasma renin activity levels were lower in children and adolescents older than 10 years. Nevertheless, the plasma renin activity in children and adolescents older than 10 years was still slightly above the values reported in adults. For example, Van Acker et al. [114] found a plasma renin activity of 0.96 ± 0.6 ng/mL/h in 20 adults aged 22 to 45 years. In the first two years of life, the reported plasma renin activity levels partially varied strongly between the different studies.

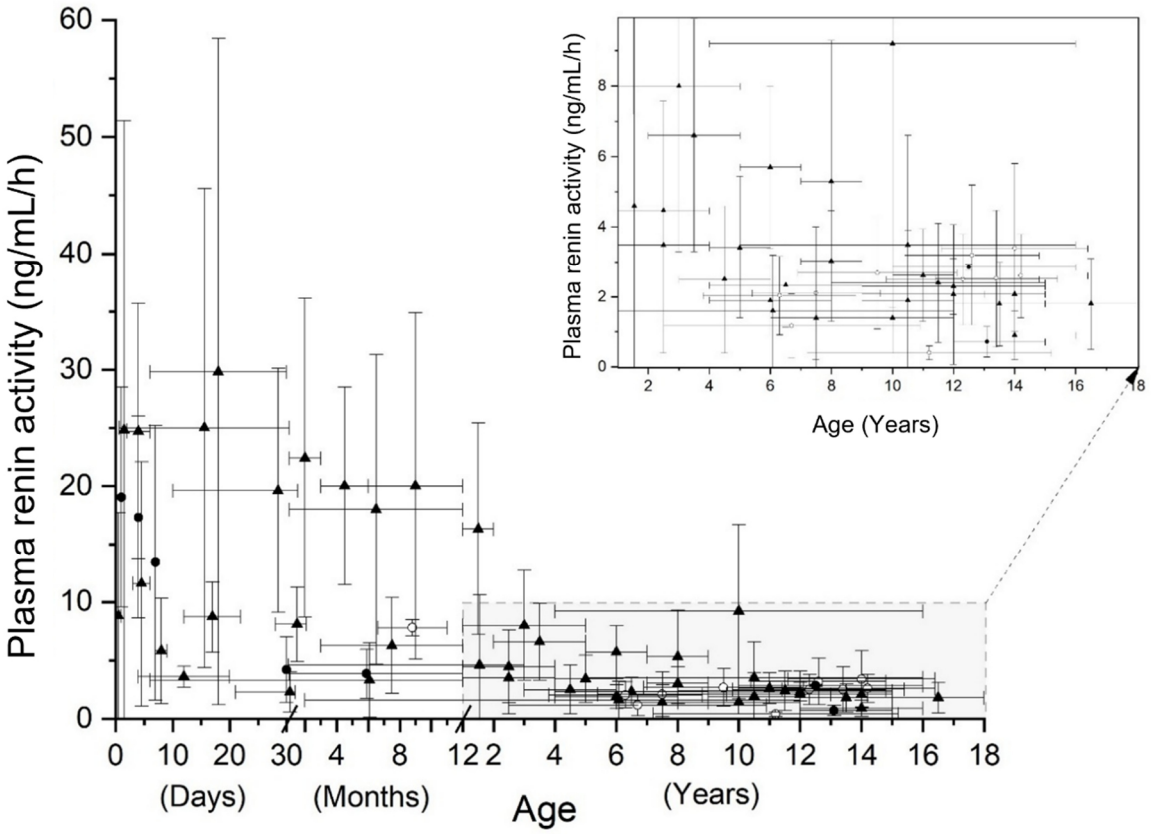

Figure 3.2 **Age-related change of plasma renin activity in healthy children from birth to 18 years of age.** Plasma renin activity is expressed as mean ± standard deviation. Age is expressed as ○: Mean ± standard deviation, ●: Mean and range or ▲: Centerpoint of range and range. A grey shaded box highlights plasma renin activity levels in healthy children from 1 to 18 years.

Table 3.1 **Plasma renin activity specification and demographic parameters of healthy children in the literature** (continued on the next three pages)

Age								Plasma renin activity (ng/mL/h)						
Mean	**SD**	**Centerpoint of range**	**Min**	**Max**	**Dimension**	**n**	**Sex**	**Mean**	**SD**	**Min**	**Max**	**n**	**Sampling procedure**	**Reference**
na	na	0.5	0	1	days	10	m/f	8.8	8.9[2]	0.6	30	10	Supine (2–3 h) in the morning (between 8:00 and 10:00 AM)	[115]
1	na	na	1	1	days	20	m/f	19.04	9.48	na	na	20	Sober (4 h) and supine (2 h) in the morning	[116]
na	na	1.5	1	2	days	10	m	24.8	26.6[2]	3.7	96	10	Supine (for at least 2 h) in the early morning	[117]
na	na	4.0	2	6	days	15	m/f	24.7	11[2]	na	na	15	Recumbent position in the morning (between 9:00 and 11:00 AM)	[118]
4	na	na	4	4	days	20	m/f	17.33	8.69	na	na	20	Sober (4 h) and supine (2 h) in the morning	[116]
na	na	4.5	3	6	days	15	m/f	11.6	10.5[2]	1.4	40	15	Supine (2–3 h) in the morning (between 8:00 and 10:00 AM)	[115]
7	na	na	7	7	days	12	m	13.41	11.8[2]	na	na	12	Sober (2 h) and supine (1–3 h) in the morning (9:00 AM)	[119]
na	na	8.0	7	9	days	9	m	5.8	4.5[2]	1.1	13.8	9	Supine (for at least 2 h) in the early morning	[117]
na	na	12.0	4	20	days	7	m/f	3.6	0.9	na	na	15	Sober (2 h)	[120]
na	na	15.6	0.667	30.5	days	17	m/f	25	20.6[2]	1.5	70	17	Supine (2 h)	[98]
na	na	17.0	12	22	days	10	m/f	8.73	3[2]	na	na	10	Recumbent position in the morning (between 9:00 and 11:00 AM)	[118]
na	na	18.0	6	30	days	16	m/f	29.8[3]	28.6[2,3]	na	na	16	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	28.5	10	47	days	5	m/f	19.6	10.5	5.2	31	5	Sober (2–3 h) and supine between 9:00 and 10:00 AM	[97]
30	na	na	30	30	days	25	m/f	4.2	2.8	na	na	25	na	[121]
na	na	31.5	21	42	days	8	m/f	2.3	1.7[2]	0.2	5.2	8	Supine (2–3 h) in the morning (between 8:00 and 10:00 AM)	[115]
na	na	45.5	28	63	days	9	m	8.1	3.2[2]	3.5	12.4	6	Supine (for at least 2 h) in the early morning	[117]
na	na	2.0	1	3	months	25	m/f	22.4[3]	13.7[2,3]	na	na	20	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	4.5	3	6	months	14	m/f	20[3]	8.5[2,3]	na	na	14	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
5.9	na	na	1	12	months	20	m/f	3.85	2.1[2]	na	na	20	Supine in the afternoon	[114]
na	na	6.5	1	12	months	11	m/f	18	13.3[2]	0.6	40	11	Supine (2 h)	[98]

Age								Plasma renin activity (ng/mL/h)						
Mean	**SD**	**Centerpoint of range**	**Min**	**Max**	**Dimension**	**n**	**Sex**	**Mean**	**SD**	**Min**	**Max**	**n**	**Sampling procedure**	**Reference**
na	na	7.5	3	12	months	18	m/f	6.27	4.1[2]	na	na	18	Supine (10 h) in the morning	[104]
8.8[6]	2.2[6]	na	na	na	months	8	m/f	7.8	0.7	na	na	8	Sober and supine	[122]
na	na	9.0	6	12	months	15	m/f	20[3]	14.9[2,3]	na	na	15	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	0.5	0.016	1	years	13	m/f	3.3[4,5]	3.2[4,5]	na	na	13	Recumbent (3 h) and sober in the morning (between 8:00 and 9:00 AM)	[123]
na	na	1.5	1	2	years	20	m/f	16.3[3]	9.1[2,3]	na	na	20	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	1.5	0.083	3	years	14	m/f	4.6	6[2]	na	na	14	Sitting except infants (supine)	[103]
na	na	2.5	1	4	years	16	m/f	4.47	3.1[2]	na	na	16	Supine (10 h) in the morning	[104]
na	na	2.5	1	4	years	8	m/f	3.5[4,5]	3.1[4,5]	na	na	8	Recumbent (3 h) and sober in the morning (between 8:00 and 9:00 AM)	[123]
na	na	3.0	1	5	years	10	m/f	8	4.7[2]	0.8	16.4	10	Supine (2 h)	[98]
na	na	3.5	2	5	years	15	m/f	6.6[3]	3.3[2,3]	na	na	15	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	4.5	3	6	years	17	m/f	2.5	2.1[2]	na	na	17	Sitting	[103]
na	na	5.0	4	6	years	36	m/f	3.42	2.02	na	na	36	Sober and supine in the morning (between 6:00 and 7:00 AM)	[105]
na	na	6.0	5	7	years	9	m/f	5.7[3]	2.3[2,3]	na	na	9	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	6.0	4	8	years	11	m/f	1.9[4,5]	1[4,5]	na	na	11	Recumbent (3 h) and sober in the morning (between 8:00 and 9:00 AM)	[123]
na	na	6.1	0.167	12	years	63	m/f	1.6	1.6[2]	0.33	6.4	63	Sober and supine in the morning (between 09:00 and 11:00 AM)	[124]
6.3	2.5	na	na	na	years	10	m/f	2.04	1.13	na	na	10	na	[125]
na	na	6.5	4	9	years	18	m/f	2.33	1.2[2]	na	na	18	Supine (10 h) in the morning	[104]
6.7	4.2	na	1	15	years	50	m/f	1.17	0.92	0.3	2.25	50	Supine (30 minutes)	[126]
na	na	7.5	6	9	years	24	m/f	1.4	1.5[2]	na	na	24	Sitting	[103]
7.5	2.1	na	6	9	years	2	m	2.1[5]	1.9[5]	na	na	2	Supine (1 h)	[127]

Age								Plasma renin activity (ng/mL/h)						
Mean	**SD**	**Centerpoint of range**	**Min**	**Max**	**Dimension**	**n**	**Sex**	**Mean**	**SD**	**Min**	**Max**	**n**	**Sampling procedure**	**Reference**
na	na	8.0	7	9	years	10	m/f	5.3[3]	4[2,3]	na	na	9	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
na	na	8.0	7	9	years	38	m/f	3.03	1.43	na	na	38	Sober and supine in the morning (between 6:00 and 7:00 AM)	[105]
9.5[1]	2.6[1]	na	7	15	years	8	m/f	2.68	1.6[2]	0.3	5.1	8	Sober and supine in the morning (7:00 AM)	[99]
na	na	10.0	8	12	years	21	m/f	1.4[4,5]	1[4,5]	na	na	21	Recumbent (3 h) and sober in the morning (between 8:00 and 9:00 AM)	[123]
na	na	10.0	4	16	years	50	m/f	9.2[5]	7.5[5]	na	na	50	Sitting in the morning (between 9:00 and 10:00 AM)	[128]
na	na	10.5	5	16	years	19	m/f	3.5	3.1[2]	0.6	11	19	Supine (2 h)	[98]
na	na	10.5	9	12	years	16	m/f	1.9	2[2]	na	na	16	Sitting	[103]
na	na	11.0	10	12	years	41	m/f	2.62	1.32	na	na	41	Sober and supine in the morning (between 6:00 and 7:00 AM)	[105]
11.2	4	na	3.1	16.7	years	32	m/f	0.4	0.2	na	na	32	Sober (6 h) and supine (15 minutes) in the morning	[129]
na	na	11.5	8	15	years	33	m/f	2.4[5]	1.7[5]	na	na	33	Supine (90 minutes) and sober in the morning	[130]
na	na	12.0	9	15	years	17	m/f	2.07	2[2]	na	na	17	Supine (10 h) in the morning	[104]
na	na	12.0	9	15	years	11	m/f	2.3[3]	0.8[2,3]	na	na	9	Recumbent (1 h) and sober (2 h) in the morning (before 10:00 AM)	[113]
12.3	2.5	na	na	na	years	24	m/f	2.5	1.3[2]	na	na	10	Supine (20 minutes) and sober in the morning (between 9:00 and 10:00 AM)	[131]
12.5	na	na	10	16	years	10	m/f	2.85	0.08	na	na	10	Supine in the morning	[132]
12.6	2.2	na	na	na	years	74	m/f	3.2	2	na	na	74	Sitting (30 minutes) in the morning (7:00 AM)	[133]
13.1	na	na	12	15	years	107	m/f	0.717	0.437	na	na	107	Sober and sitting in the morning	[134]
13.4	2	na	10	18	years	195	m/f	2.52	1.95	0.1	13.5	195	Sitting	[135]
na	na	13.5	12	15	years	16	m/f	1.8	1.2[2]	na	na	16	Sitting	[103]
na	na	14.0	13	15	years	41	m/f	2.07	1.14	na	na	41	Sober and supine in the morning (between 6:00 and 7:00 AM)	[105]
14	2.4	na	na	na	years	66	m/f	3.4	2.4	na	na	66	Sitting (30 minutes) in the morning (7:00 AM)	[133]

Age								Plasma renin activity (ng/mL/h)						
Mean	**SD**	**Centerpoint of range**	**Min**	**Max**	**Dimension**	**n**	**Sex**	**Mean**	**SD**	**Min**	**Max**	**n**	**Sampling procedure**	**Reference**
na	na	14.0	12	16	years	9	m/f	0.9[4,5]	0.7[4,5]	na	na	9	Recumbent (3 h) and sober in the morning (between 8:00 and 9:00 AM)	[123]
14.2	2.2	na	12	17	years	4	f	2.6[5]	1.2[5]	na	na	4	Supine (1 h)	[127]
na	na	16.5	15	18	years	10	m/f	1.8	1.3[2]	na	na	10	Sitting	[103]

In all studies, plasma renin activity was determined by radioimmunoassay. For age, the centerpoint of the range was only calculated if no mean value was available. *[1]Values calculated from raw data.* *[2]SD calculated from SE.* *[3]Values calculated from ng/L/min to ng/mL/h.* *[4]Values calculated from ng/mL/3h to ng/mL/h.* *[5]Values generated via GetData Graph Digitizer 2.26.0.20, mean of three times conducted.* *[6]Age-matched healthy control group: values calculated from patients age raw data.*

f, female; m, male; Max, maximum; Min, minimum; na, data not available; SD, standard deviation; SE, standard error.

3.3.2 Plasma Renin Activity in Children with Heart Failure without ACE Inhibitor Treatment

For children with heart failure, the literature search revealed four studies with plasma renin activity data from a total of 58 children (Figure 3.1). Plasma renin activity levels in patients with heart failure also show a tendency to decrease with age (Figure 3.3, Table 3.2). Plasma renin activity levels in children with heart failure younger than 6 months were greater than in healthy peers. In patients with heart failure at this age, plasma renin activity levels were 3 to 4 times higher than in healthy subjects comparing equal age ranges (Table 3.1, Table 3.2). Only the oldest children with heart failure, aged 6 ± 2 months, with a plasma renin activity of 10 ± 7 ng/mL/h had plasma renin activity levels that were within the range of mean plasma renin activity of healthy children of the same age [96]. None of the studies that met the predefined criteria included information on plasma renin activity in children with heart failure treated with ACE inhibitor. All children studied suffered from heart failure due to congenital heart disease with left-to-right shunts (Table 3.2).

The LENA studies also provided information on plasma renin activity in children with heart failure without ACE inhibitor treatment. A total of 35 subjects in the LENA studies were not pretreated with an ACE inhibitor. Therefore, the plasma renin activity measured before the first enalapril dose in these subjects can be compared with the literature data. Out of 35, 32 subjects had heart failure due to congenital heart disease and 3 due to dilated cardiomyopathy. The age of the 35 ACE inhibitor naïve subjects ranged from 25 days to 2.1 years. The mean plasma renin activity of all four age groups of the LENA subjects was comparable to the mean plasma renin activity in the literature. Three of the four groups also had a mean plasma renin activity that was above the plasma renin activity reported in healthy children. Analogous to the literature data, the oldest age group (11.1 ± 6.8 months) had with 11.2 ± 6.0 ng/mL/h a plasma renin activity that was within the range of the mean plasma renin activity reported in healthy children of this age.

In all four age groups, furosemide and spironolactone were included in the medication of the majority of the subjects. In addition, one subject in age group two and two subjects in age group four received digoxin. Moreover, one subject in age group three received milrinone and one subject in age group four received carvedilol. In age group three and four, one subject each received no concomitant heart failure medication. An overview of the concomitant heart failure medication and its dosage in the four age groups of the LENA subjects can be found in Appendix 9.5.

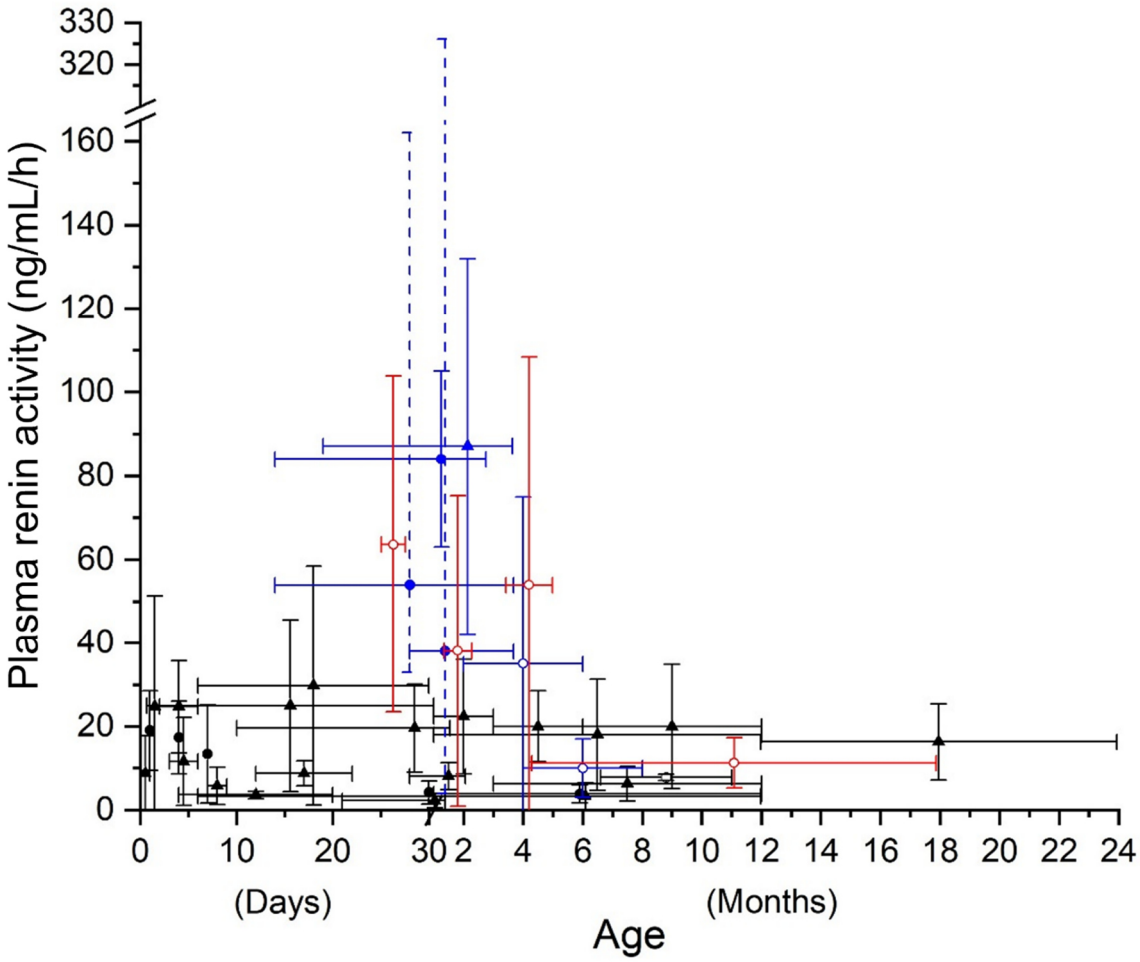

Figure 3.3 **Plasma renin activity of healthy children and children with heart failure.** The plasma renin activity data from the healthy children are from the literature (black, n = 344). The plasma renin activity data from the children with heart failure are from the literature (blue, n = 58) and from the LENA studies (red, n = 35). None of the children with heart failure had previously been treated with an ACE inhibitor. Plasma renin activity is expressed as mean ± standard deviation (solid lines) or as mean and range (dashed lines). Age is expressed as ○: Mean ± standard deviation, ●: Mean and range or ▲: Centerpoint of range and range. ACE, angiotensin-converting enzyme; LENA, Labeling of Enalapril from Neonates up to Adolescents.

Table 3.2 Plasma renin activity specification and demographic parameters of children with heart failure in the literature and in the LENA studies.

Age									Plasma renin activity (ng/mL/h)							
Mean	**SD**	**Centerpoint of range**	**Min**	**Max**	**Dimension**	**n**	**Sex**	**Indication**	**Mean**	**SD**	**Min**	**Max**	**n**	**Sampling procedure**	**Analytics**	**Reference**
28	na	na	14	112	days	11	m/f	severe congestive failure due to left-to-right shunts	54	na	33	162	11	na	na	[10]
38	na	na	14	84	days	11	m/f	CHD with left-to-right shunts (severe congestive heart failure)	84	21	57	126	11	Sober (2–3 h) and supine between 9:00 and 10:00 AM	RIA	[97]
42	na	na	28	112	days	11	m/f	severe congestive failure due to left-to-right shunts	38	na	4	326	11	na	na	[10]
na	na	65	19	111	days	8	m/f	CHD with left-to-right shunts (congestive heart failure)	87.1[1]	44.9[1]	22	183	8	Sober (2–3 h) and supine between 9:00 and 10:00 AM	RIA	[136]
4	2	na	na	na	months	18	m/f	CHD with left-to-right shunts	35	40	na	na	7	non-sedated infants	RIA	[96]
6	2	na	na	na	months	30	m/f	CHD with left-to-right shunts	10	7	na	na	10	non-sedated infants	RIA	[96]
26.3	1.3	na	25	27	days	3	m	CHD	63.6	40.2	30.7	108.4	3	Supine in the morning	ELISA	LENA studies
55.1	14.3	na	35	85	days	12	m/f	CHD	38.1	37.2	6.4	101.2	12	Supine in the morning	ELISA	LENA studies
4.2	0.8	na	3.2	5.9	months	13	m/f	CHD (n = 12) and DCM (n = 1)	54.0	54.3	4.2	183.9	13	Supine in the morning	ELISA	LENA studies
11.1	6.8	na	6.9	25.1	months	7	m/f	CHD (n = 5) and DCM (n = 2)	11.2	6.0	3.4	19.7	7	Supine in the morning	ELISA	LENA studies

For age, the centerpoint of the range was only calculated if no mean value was available.
[1]Values calculated from raw data.
CHD, congenital heart disease; DCM, dilated cardiomyopathy; ELISA, enzyme-linked immunosorbent assay; f, female; LENA, Labeling of Enalapril from Neonates up to Adolescents; m, male; Max, maximum; Min, minimum; na, data not available; RIA, radioimmunoassay; SD, standard deviation.

3.3.3 Plasma Renin Activity in Children with Heart Failure and Start of ACE Inhibitor Treatment

In contrast to the literature search, the LENA studies also provided information on plasma renin activity in children with heart failure treated with an ACE inhibitor. As mentioned earlier, 35 subjects (aged 25 days–2.1 years) had not received pretreatment with an ACE inhibitor. Their predose plasma renin activity was compared with the plasma renin activity 4 hours after the first enalapril dose and with the plasma renin activity within the first 8 days of enalapril treatment (Figure 3.4). The Friedman test showed a significant difference ($p < 0.01$) for the comparison of all three time points. The median predose plasma renin activity of 19.7 (n = 35) increased to 29.0 (n = 34, $p > 0.05$) 4 hours after the first enalapril dose, and to 89.1 ng/mL/h (n = 29, $p < 0.01$) after 4.7 ± 1.6 days of treatment. Compared with the median predose plasma renin activity, the plasma renin activity after 4.7 ± 1.6 days of treatment with enalapril is 4.5 times higher. The first dose administered was 0.06 ± 0.01 mg/kg enalapril maleate (n = 35). As daily dose, 0.10 ± 0.04 mg/kg enalapril maleate was administered on the first day of treatment (n = 35). At the time of plasma renin activity measurement after 4.7 ± 1.6 days, the daily dose was 0.12 ± 0.03 mg/kg enalapril maleate (n = 29). The enalapril dose was increased in 6 of 29 children within the first 8 days of enalapril treatment.

Of the 35 subjects, 33 received concomitant heart failure medication during the observation period (Table 3.3). Of 33 children who received furosemide, 29 had already taken it at least 3 days before starting enalapril therapy. One subject had been receiving furosemide for one day prior to enalapril administration but had received loop diuretics for a total of eight days prior to enalapril administration. All other medications had been taken at least 3 days before starting enalapril therapy. Changes in concomitant medication during the observation period were only carried out in two subjects. In one subject, milrinone was discontinued and the dosage of furosemide was reduced during the observation period. In another subject, the dosage of furosemide was increased only as part of the switch from intravenous to oral administration.

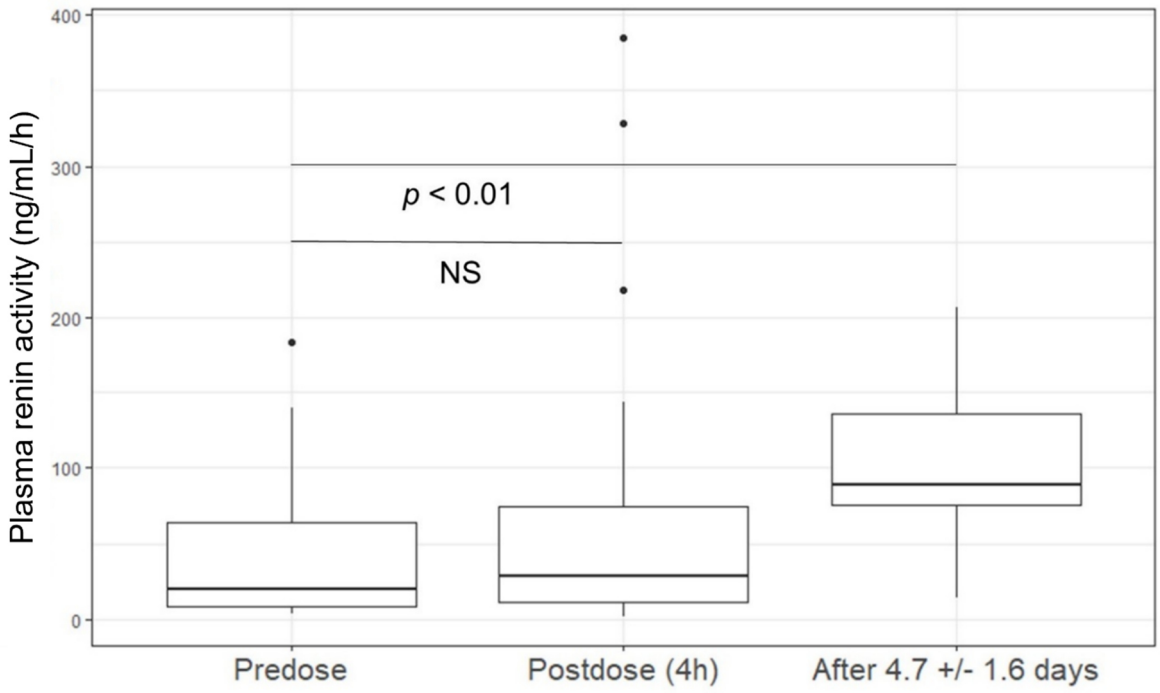

Figure 3.4 **Plasma renin activity in children with heart failure from the LENA studies at different time points of enalapril therapy.** Plasma renin activity data were available from 35 subjects (aged 25 days–2.1 years, median age = 3.6 months) predose, from 34 subjects 4 hours postdose, and from 29 subjects (aged 29 days–2.1 years, median age = 3.4 months) after 4.7 ± 1.6 days of enalapril therapy. The Wilcoxon test for paired samples was conducted with the data of the children with a complete dataset of three measurements (n = 29). LENA, Labeling of Enalapril from Neonates up to Adolescents; NS, not significant.

Table 3.3 Concomitant heart failure medication of the investigated LENA subjects.

Concomitant medication		Dosage at the start of the study		Duration of therapy before administration of enalapril		Change in dosage during observation period	Discontinuation of therapy during observation period
	n	Median (Range)	Unit	Range	Duration ≥ 3 days n (%)	n (%)	n (%)
Furosemide	33	1.42 (0.27–3.20)	mg/kg/day	1 day–4 months	29 (87.9)	2 (6.1)	0 (0)
Spironolactone	28	0.83 (0.27–1.88)	mg/kg/day	3 days–5 months	28 (100)	0 (0)	0 (0)
Digoxin	3	11.06 (10.91–14.93)	µg/kg/day	11–21 days	3 (100)	0 (0)	0 (0)
Carvedilol	1	0.55	mg/kg/day	19 days	1 (100)	0 (0)	0 (0)
Milrinone	1	0.30–0.45[1]	µg/kg/min	10 days	1 (100)	0 (0)	1 (100)
No concomitant medication	2	-	-	-	-	-	-

[1]*During the observation period, doses between 0.3 and 0.45 µg/kg/min were administered.*
LENA, Labeling of Enalapril from Neonates up to Adolescents.

3.3.4 Plasma Renin Activity and Heart Failure Severity in Children with and without ACE Inhibitor Treatment

Out of the 35 LENA participants without ACE inhibitor pretreatment, 8 had a Ross score less than or equal to 2 and were therefore classified as asymptomatic. Before the first enalapril dose, the median plasma renin activity of the children with asymptomatic heart failure was 9.3 ng/mL/h (Figure 3.5). In contrast, the LENA participants with symptomatic heart failure (Ross score ≥ 3) had a significantly higher median plasma renin activity of 31.8 ng/mL/h ($p < 0.05$). After 4.7 ± 1.6 days of treatment, 8 of 29 participants had a Ross score less than or equal to 2. The median plasma renin activity was 80.7 ng/mL/h in the children with asymptomatic heart failure and 99.8 ng/mL/h in the children with symptomatic heart failure. The difference in plasma renin activity between the asymptomatic and symptomatic children was no longer significant after 4.7 ± 1.6 days of enalapril therapy ($p > 0.05$).

In 17 out of 29 subjects, the Ross score improved during the observation period. For two subjects, the improvement in Ross score resulted in them being classified as now asymptomatic rather than symptomatic. In the remaining 12 subjects, the Ross score did not change during the observation period.

The median age of the symptomatic group is lower after 4.7 ± 1.6 days. The reason for this is that at this time only 21 subjects had a Ross score ≥ 3 due to missing data or an improvement of the Ross score. There was no significant difference in age between the children with asymptomatic heart failure and those with symptomatic heart failure, either before the first enalapril dose or after 4.7 ± 1.6 days of enalapril therapy ($p > 0.05$).

Prior to the first administration of enalapril, two of eight subjects with asymptomatic heart failure were not taking any concomitant heart failure medication, six subjects were receiving furosemide, and five subjects were receiving spironolactone. Of the 27 subjects with symptomatic heart failure prior to enalapril administration, all received furosemide, 23 subjects received spironolactone, 3 subjects received digoxin and 1 subject each received carvedilol and milrinone. The median dose of furosemide was 0.95 mg/kg/day in the children with asymptomatic heart failure and 1.57 mg/kg/day in the children with symptomatic heart failure. An overview of the concomitant heart failure medication and its dosage in the LENA subjects with asymptomatic and symptomatic heart failure before and after 4.7 ± 1.6 days of enalapril therapy can be found in Appendix 9.6.

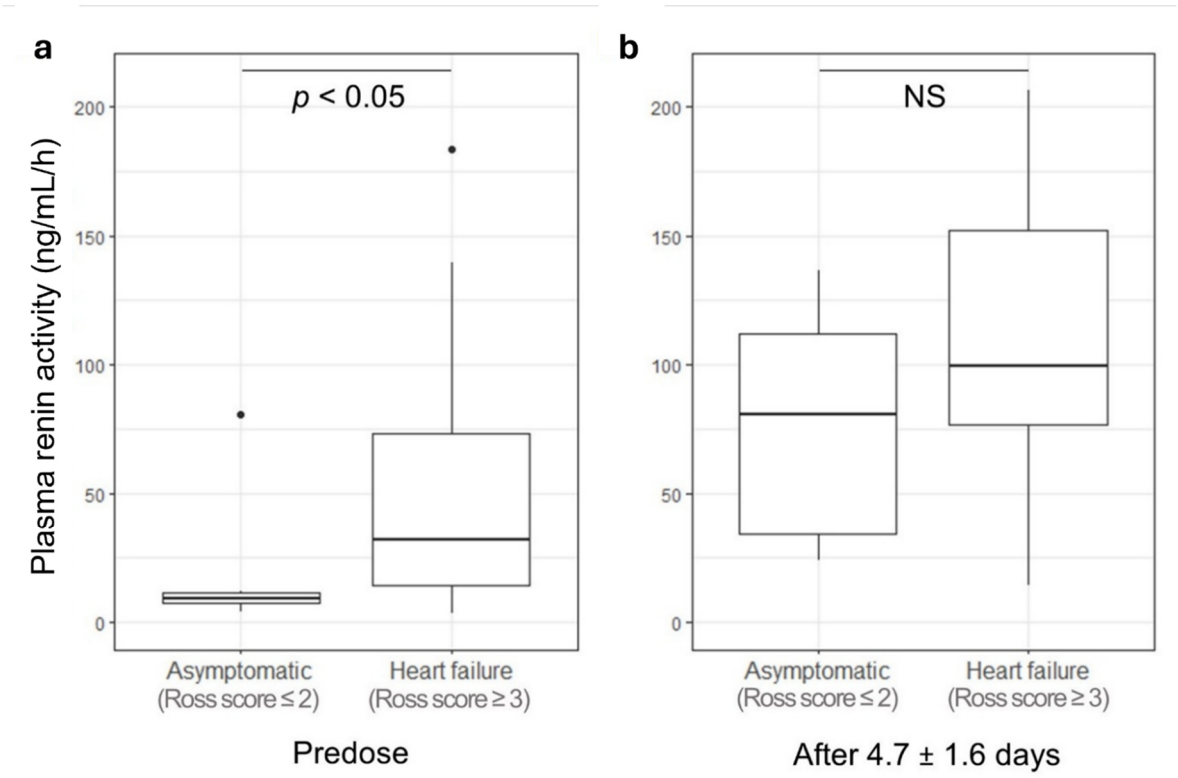

Figure 3.5 **Plasma renin activity in asymptomatic children and children with symptomatic heart failure from the LENA studies.** The Wilcoxon test for unpaired samples was conducted between the asymptomatic children with a Ross score ≤ 2 and children with symptomatic heart failure with a Ross score ≥ 3. In (a), plasma renin activity predose is shown (asymptomatic: n = 8, median age = 4.0 months, age range = 1.2–8.2 months; symptomatic heart failure: n = 27, median age = 3.6 months, age range = 25 days–2.1 years). In (b), plasma renin activity after 4.7 ± 1.6 days of enalapril therapy is shown (asymptomatic: n = 8, median age = 4.5 months, age range = 1.3 months–2.1 years; symptomatic heart failure: n = 21, median age = 2.3 months, age range = 29 days–8.2 months). LENA, Labeling of Enalapril from Neonates up to Adolescents; NS, not significant.

3.4 Discussion

The current literature review and the European LENA project on children with heart failure help to address the influence of age, heart failure and ACE inhibitor treatment on plasma renin activity levels in children more specifically. Firstly, age does have a profound effect on plasma renin activity levels in healthy children, as in neonates the plasma renin activity is up to about 7 times higher than in older children. Secondly, children with heart failure younger than 6 months show 3 to 4 times higher plasma renin activity levels than healthy children of comparable age. Thirdly, the ACE inhibitor enalapril further increased plasma renin activity levels by a factor of 4.5 in children with heart failure.

3.4.1 Age and Plasma Renin Activity

Of the three influencing factors analysed, age has the greatest influence on plasma renin activity in childhood according to the present analyses. The comparison of the results of the different studies in the systematic review confirms previous separate observations on the decrease of plasma renin activity with age and furthermore provides information on the extent of the age-related decrease of plasma renin activity in healthy children [103–105].

Considering the whole childhood, the analyses show a strong decrease of plasma renin activity by up to 85%. Such a strong decrease with age in childhood is not extraordinary and is also known for example for NT-proBNP [137].

The reasons for the increased plasma renin activity in children and especially in neonates and infants are not known with certainty. It is known that renin release is promoted by a decrease in renal perfusion pressure [138]. Consequently, the increase in blood pressure with age in children, could be a possible explanation for the age-related decrease of plasma renin activity in childhood [139].

Neonates and infants up to two years of age not only have the highest plasma renin activity levels, but also the greatest variability between the different studies. Possible reasons for the variability of plasma renin activity between the different studies could be differences in sampling or sodium intake of the subjects as well as crying during sampling. It is unlikely that the variability is due to the influence of a different position during sampling or a different time of sampling in the studies, as in most of the included studies with children under two years of age, sampling was performed in the morning in the supine position [98–100]. Different sodium intake could be one reason for the variability between the studies, as other

investigations show an inverse correlation between plasma renin activity and sodium intake [117, 140]. As sodium intake was rarely assessed in the included studies, differences in sodium intake between the studies could possibly explain parts of the variability between the different studies. In addition, crying during sampling increases plasma renin activity [141]. Neonates and infants are more likely to cry during blood collection than older children. A different proportion of crying subjects in the different studies could be another reason why the variability between the different studies is high for children under two years of age.

3.4.2 Heart Failure and Plasma Renin Activity

The second factor influencing plasma renin activity is heart failure, which appears associated with 3 to 4 times higher plasma renin activity levels in neonates and young infants compared with healthy peers. Children with heart failure younger than 6 months from both the literature and the LENA studies had higher plasma renin activity levels than healthy children of the same age.

The activation of the renin–angiotensin–aldosterone system in children with heart failure is part of the pathophysiology [95]. Consistent with the present findings in children with heart failure younger than 6 months, Nijst et al. [142] found about 5 times higher plasma renin activity in adults with chronic heart failure on optimal medical therapy compared with healthy controls. Anand et al. [143] reported a 9.5 times higher plasma renin activity in adults with severe clinical congestive cardiac failure. The greater difference between patients and healthy subjects in the latter study could be due to the fact that the subjects of Anand et al. [143] were completely untreated patients, while LENA subjects had been pretreated with various heart failure medications other than ACE inhibitor.

The assumption is that the plasma renin activity in children with heart failure is influenced by the severity of heart failure. The influence of the severity of heart failure on plasma renin activity is supported by the fact that the subjects with symptomatic heart failure (Ross score ≥ 3) had a significantly higher plasma renin activity than the subjects with asymptomatic heart failure (Ross score ≤ 2). However, no association could be found between the different severities of symptomatic heart failure, classified by the Ross score, and plasma renin activity. One reason for this could be that the Ross score includes various symptoms that are not all equally related to the renin–angiotensin–aldosterone system. Of course, children with symptomatic heart failure receive more intensive therapy than children with asymptomatic heart failure. Accordingly, the dosage of furosemide was higher in the

children with symptomatic heart failure. Since furosemide increases plasma renin activity, it is likely that current concomitant medication, in addition to the severity of heart failure, contributed to the fact that the children with symptomatic heart failure had a higher plasma renin activity [106]. Another indication of the influence of the severity of heart failure on plasma renin activity is the fact that Buchhorn et al. [96] found a correlation of plasma renin activity with respiratory rate and an inverse correlation of plasma renin activity with weight gain in children with heart failure.

Data from the literature and the LENA studies indicate that plasma renin activity levels, similar to healthy children, tend to decrease with age also in patients with heart failure. However, the amount of data available and the age range of children with heart failure were too small to interpret the influence of age in children with heart failure more precisely.

A surprising finding was that the oldest subjects with heart failure from the literature and the LENA studies even had plasma renin activity levels in the range of plasma renin activity of healthy children. Two reasons are hypothesised why plasma renin activity is not increased in older children with heart failure. The first reason could be that the older children have milder heart failure, as patients with more severe clinical symptoms due to congenital heart disease are usually operated earlier. The subjects in the oldest study group (aged 6 ± 2 months) from the literature had a respiratory rate < 50 min^{-1}, indicating milder heart failure considering their age [96]. In addition, the oldest group in the LENA studies had a Ross score that led to the classification as mild heart failure patients. However, the LENA patients with an age of 3 to 6 months and an increased plasma renin activity compared with healthy children were also classified as having mild heart failure according to the Ross score. Thus, there must be a second factor influencing plasma renin activity. The second reason could be that older children are more likely to have been stabilized on their medication. Furosemide, spironolactone and milrinone increase plasma renin activity whereas beta-blocker and digoxin decrease plasma renin activity [106, 108–110, 144, 145]. Consequently, a lower proportion of furosemide, spironolactone or milrinone and/or a higher proportion of beta-blocker or digoxin in the current medication of the respective study group could also lead to a lower plasma renin activity. The oldest study group in the literature also had the lowest percentage of diuretics in their current medication compared with the other groups in this study, which had higher plasma renin activity levels [96]. The oldest group of LENA subjects received the lowest percentage of concomitant medication that increased plasma renin activity and the highest percentage of concomitant medication that decreased plasma

renin activity. Therefore, plasma renin activity in children with heart failure is probably influenced by both the severity of heart failure and the current heart failure medication.

3.4.3 ACE Inhibitor Treatment and Plasma Renin Activity

The third factor influencing plasma renin activity is the ACE inhibitor treatment, which increases plasma renin activity by 4.5-fold in children with heart failure. Thus, the results suggest that treatment with an ACE inhibitor may have an even greater influence on plasma renin activity than heart failure itself.

Previous studies on plasma renin activity after captopril administration in children with heart failure either did not find a significant increase in plasma renin activity or did not state whether the increase in plasma renin activity was significant [10, 136, 146, 147]. The studies that did not find a significant increase investigated only a small number of 8 respectively 12 subjects [146, 147]. Similarly, the study by Lloyd et al. [23], in which no significant increase in plasma renin activity was observed during enalapril therapy, included only 10 subjects. In contrast, this analysis is based on a considerable higher number of 29 subjects. Seguchi et al. [21] examined 35 children with congestive heart failure between 1 month and 17 years of age. In the 18 subjects in whom plasma renin activity was measured, a significant increase in plasma renin activity was observed 3 hours after administration of enalapril. The reason why the increase was already significant three hours after administration of enalapril could be that a higher dose was administered.

In accordance with the present findings in children with heart failure, enalapril administration significantly increases plasma renin activity by approximately a factor of four in adults with heart failure [107]. Compared with adults with heart failure, the increase in plasma renin activity in children with heart failure was not yet significant 4 hours after administration, but only within the first 8 days of treatment. The delayed effect of enalapril in children with heart failure could have several reasons. One reason could be the higher starting dose used in the adult study compared with the starting dose in the LENA studies. In the study on enalapril in adults with heart failure a high dose between 10 and 40 mg was used as the first dose. The initial dose in the LENA studies was chosen with the aim of achieving exposure comparable to an initial dose of 2.5 mg in adults. Another reason may be that the maximum enalaprilat concentration is reached later in children under one year of age. The pharmacokinetic analysis of the LENA studies revealed that the subjects younger than one year had a median time to reach maximum enalaprilat concentration of 6 hours

[52]. Whereas in subjects older than one year, the median time to reach maximum enalaprilat concentration was 4 hours, comparable to that of adults [52, 148]. Since 28 of the 29 subjects analysed were under 1 year of age, the time of plasma renin activity measurement in these children may have been too early to observe a significant increase in plasma renin activity. As a third reason, the plasma renin activity increase may be due not only to a direct negative feedback of angiotensin II on renin release but also to an increase in renin synthesis, as treatment with enalapril for several days causes an increase in renin mRNA in rats [149].

As concomitant medication could have an impact on plasma renin activity during the observation period, the duration, changes and discontinuations of concomitant medication were investigated. Most of the concomitant medication was taken at least three days before the start of enalapril administration. It can therefore be assumed that the majority of subjects and their plasma renin activity had stabilized on their therapy before enalapril was administered. Since furosemide and milrinone increase plasma renin activity, reducing the dosage of furosemide and discontinuing milrinone therapy in one subject may at most have attenuated the observed increase in plasma renin activity due to enalapril [106, 145]. As the increase in furosemide dose in one subject only compensates for the reduced bioavailability due to the switch from intravenous to peroral administration, this change is not expected to have any effect. For the above reasons, an impact of concomitant medication on plasma renin activity during the observation period is considered as unlikely.

Of importance is the fact that the significant difference in plasma renin activity between asymptomatic children and children with symptomatic heart failure disappeared under enalapril therapy. The reason for this could be that, according to the present results, the influence of ACE inhibitor treatment appears to be greater than the influence of heart failure itself. However, the comparison between asymptomatic children and children with symptomatic heart failure is based on a small number of eight asymptomatic patients, and the improvement in heart failure with therapy may have attenuated the increase in plasma renin activity differently in the two groups.

The existing studies on plasma renin activity after captopril administration in children with heart failure had to be excluded from the systematic review due to missing information on plasma renin activity values, contradictory information on the unit or imprecise or missing information on age [10, 136, 146, 147]. The existing studies on plasma renin activity after enalapril administration in children with heart failure could not be found in the literature

search. In the case of the publication by Seguchi et al. [21], the reason was that it is not listed in the MEDLINE database. For the publication by Lloyd et al. [23], the abstract is not available in the MEDLINE database, and the title does not contain the search term plasma renin activity. However, these studies would have been excluded anyway due to the exclusion criteria. Seguchi et al. [21] only specified the age for the total group of 35 children and not for the subgroup of 18 children in whom plasma renin activity was measured. Lloyd et al. [23] did not specify the statistical parameters used for plasma renin activity. As far as is known, this is the first report on plasma renin activity following administration of an ACE inhibitor in children with heart failure that contains exact information on age and the statistical parameters used.

3.5 Limitations

The systematic review may not include all publications on plasma renin activity in healthy children and children with heart failure due to limitations of the method. Only publications listed in the MEDLINE database in English or German were considered. Publications in English and German for the literature search on plasma renin activity in healthy children and in children with heart failure, however, encompass the majority of all publications under the search terms used, with 92% and 90% respectively. Publications in which parts of the search terms are not mentioned in the title or abstract may have been overlooked. For healthy children, six additional publications from the preliminary search were included. In three of these cases were only the title and not the abstract available in the MEDLINE database. In the other three cases, other keywords were used for healthy children, for example, they were named normal children. Due to the nature of the literature data, it was not possible to analyse the literature data beyond descriptive statistics. The relatively strict inclusion criteria led to the exclusion of some studies. Excluding studies with inaccurate age information reduced the dataset, but as these analyses show, accurate age information is necessary to compare plasma renin activity values. Specifying the allowable statistical parameters in the inclusion criteria resulted in a smaller dataset but improved the comparability of the data. Despite these limitations, the systematic review contains a large amount of data on plasma renin activity from almost 1500 healthy children and almost 60 children with heart failure.

The age classification for the evaluation of the plasma renin activity level of the healthy children from the literature is based only on the visual inspection of the data. Both the age group of children from 2–10 years and the age group of children and adolescents over 10 years encompass a relatively wide age range. However, the age classification chosen is comparable to the age classification of the European Medicines Agency and the US Food and Drug Administration, where the age groups are defined as children aged 2–11 years and adolescents aged 12–18 years.

The analysis of plasma renin activity in children with heart failure from the LENA studies is faced with limitations. One limitation is that the number of subjects that could be analysed for the effect of enalapril on plasma renin activity was limited because only a part of the subjects was without ACE inhibitor pretreatment. However, almost all ACE inhibitor naïve children were under one year old. Considering this very young age, a large number of very young subjects with heart failure were studied. Furthermore, plasma renin activity was

analysed in children with heart failure of different aetiology. However, due to the small number of three ACE inhibitor naïve children with dilated cardiomyopathy, an analysis regarding a possible influence of the aetiology of heart failure was not possible. Data from the LENA studies cannot provide information on the prognostic value of plasma renin activity in children with heart failure. The number of 35 ACE inhibitor naïve subjects was unfortunately too small to perform a multiple regression analysis. Thus, an overlap of the influences of age, heart failure and ACE inhibitor treatment is possible. However, when comparing plasma renin activity between healthy children and children with heart failure, the LENA subjects were divided into four age groups to compare them with healthy peers and to keep the influence of age as low as possible. When comparing plasma renin activity between children with asymptomatic and symptomatic heart failure, there was no significant age difference between the two groups. The influence of enalapril on plasma renin activity was investigated in an observation period with a maximum of eight days. Therefore, the influence of age in the selected observation period is considered negligible. Since plasma renin activity decreases with age, age could at most have attenuated the observed effect of enalapril on plasma renin activity. As the Ross score improved in some of the subjects during the observation period, the improvement in heart failure may have attenuated the observed increase in plasma renin activity due to enalapril. However, as only two subjects had a change in Ross score large enough to change the heart failure classification, it is assumed that the effect of the improvement in heart failure on plasma renin activity will be rather small here. Moreover, the effect of enalapril on plasma renin activity in children with heart failure was analysed only in the first days of therapy. Further analysis of the studies data is required to determine whether plasma renin activity remains elevated with prolonged ACE inhibitor therapy.

3.6 Conclusions

In summary, it has been shown that age, heart failure and ACE inhibitor treatment have a notable influence on plasma renin activity. In children with heart failure, not only age but also ACE inhibitor treatment must be considered when assessing plasma renin activity as a clinically meaningful parameter, as ACE inhibitor treatment leads to a 4.5-fold increase of plasma renin activity that is not due to the disease state. In detail, it should be examined whether an ACE inhibitor is included in the medication and how long the ACE inhibitor treatment has already been given. In studies on plasma renin activity, subjects with and without ACE inhibitor should preferably be evaluated separately.

3.7 Disclosure

Parts of this chapter were previously published in a peer-reviewed journal:

Steichert, M., Cawello, W., Bajcetic, M., Breur, J. M. P. J., Dalinghaus, M., Male, C., Wildt, S. N. de, & Läer, S. (2023). Influence of Age, Heart Failure and ACE Inhibitor Treatment on Plasma Renin Activity in Children: Insights from a Systematic Review and the European LENA Project. Frontiers in Bioscience (Landmark Edition), 28(12), 335. https://doi.org/10.31083/j.fbl2812335

The author of this thesis substantially contributed to the conception and design of the work as well as to the analysis of the data. In addition, the author of this thesis was responsible for conducting the systematic review, drafting the manuscript, and critically revising the manuscript.

4 Angiotensin II/Angiotensin I Ratio as Pharmacodynamic Parameter for Population Modelling in Healthy Adults and Children with Heart Failure Treated with Enalapril

4.1 Background

Since the preceding work has shown that the pharmacokinetics of enalaprilat and the pharmacodynamic parameter plasma renin activity are influenced by age and heart failure, the question remains whether the pharmacodynamics of enalaprilat differ in children with heart failure compared with healthy adults. The previous model-independent analysis of plasma renin activity in the subjects of the LENA studies showed a significant increase in plasma renin activity after repeated administration of enalapril. Thereby, it was demonstrated that the administered dosage of enalapril in the LENA studies had a measurable effect on the renin–angiotensin–aldosterone system. However, the effect on plasma renin activity is caused by the reduced negative feedback of angiotensin II on renin secretion and is therefore the result of several steps following ACE inhibition by enalaprilat. To further investigate the effects of enalaprilat, a model-dependent population analysis shall be performed with another pharmacodynamic parameter that is closer to ACE inhibition.

Model-dependent pharmacodynamic analyses in adults used various pharmacodynamic parameters. The effect of the active metabolite enalaprilat on ACE activity or blood pressure in healthy adults and adults with hypertension was investigated using PK/PD modelling [71, 150–153]. In addition, two physiologically based PK/PD models and one population PK/PD model were developed, describing the effect of enalaprilat on the individual parameters of the renin–angiotensin–aldosterone system in adults [154–156].

Model-dependent pharmacodynamic analyses for enalaprilat based on paediatric data are limited. Kechagia et al. [72] developed a population PK/PD model using pharmacokinetic data from children and blood pressure data from adults with hypertension obtained from the literature. The population PK/PD model was then validated using blood pressure data from children with hypertension obtained from the literature.

Model-dependent population analyses in children with heart failure using suitable pharmacodynamic parameters could help to gain insights into the pharmacodynamic effects of enalaprilat in this population and possible differences compared with adults. Blood pressure is less suitable as a pharmacodynamic parameter in children with heart failure, as it

was observed that blood pressure did not change significantly during the study period in children with congenital heart disease who were treated with enalapril and participated in the LENA project [60]. Unlike blood pressure, ACE activity has the advantage that it is directly influenced by enalaprilat without any intermediate steps. As ACE activity is not always available, alternative pharmacodynamic parameters may be useful.

Since the angiotensin II/angiotensin I ratio is influenced by the administration of enalapril, it could serve as a pharmacodynamic parameter. In response to ACE inhibition by enalaprilat, angiotensin I levels increased significantly after administration of enalapril to healthy adults, while angiotensin II levels decreased significantly [20]. Therefore, the angiotensin II/angiotensin I ratio was used as a measure of the in vivo ACE activity in healthy adults [157, 158]. As mentioned above, a significant increase in plasma renin activity was observed in the subjects of the LENA studies after repeated administration of enalapril. Since renin converts angiotensinogen into angiotensin I, it is likely that the children with heart failure also showed an increase in angiotensin I after administration of enalapril. In another study in children with congestive heart failure, angiotensin II levels decreased significantly after administration of enalapril, and plasma renin activity also increased significantly [21]. Consequently, it can be assumed that the angiotensin II/angiotensin I ratio can also serve as a measure of in vivo ACE activity in children with heart failure. As far as is known, there are no model-dependent population analyses that use the angiotensin II/angiotensin I ratio as a pharmacodynamic parameter for healthy adults or children with heart failure.

The objectives were to gain insights into the effect of the active metabolite enalaprilat on the angiotensin II/angiotensin I ratio in children with heart failure and to identify possible differences compared with healthy adults. For this purpose, a population PK/PD model was developed that describes the effect of the active metabolite enalaprilat on the angiotensin II/angiotensin I ratio in healthy adults for whom extensive data were available. The predictions of the PK/PD model for adults were used to compare the angiotensin II/angiotensin I ratio after administration of enalapril in healthy adults and children with heart failure. In addition, a population pharmacodynamic model was developed based on simultaneous pharmacokinetic and pharmacodynamic measurements in ACE inhibitor naïve children with heart failure from the LENA project.

4.2 Methods

4.2.1 Adult Study: Study Design and Investigated Population

The adult data used were obtained from an open-label, single-sequence, single-dose study in nine healthy subjects [159]. The enalaprilat, angiotensin I, and angiotensin II measurements from this study were analysed. The study was conducted in 2013 at the Institute of Clinical Pharmacy and Pharmacotherapy at Heinrich Heine University Düsseldorf in Germany according to the Declaration of Helsinki and Good Clinical Practice recommendations. The Ethics Committee of the Medical Faculty of the Heinrich Heine University Düsseldorf granted the ethical approval (proposal no. 3809 [including all amendments]). The study was registered in the German Clinical Trials Register (DRKS00037310). Prior to participation, all subjects provided written informed consent.

Subjects were included in the study if they were at least 18 years old, had a body weight of more than 50 kg, and were free from any known organ diseases. Exclusion criteria were angioedema, urticaria, known allergies, or low blood pressure values in the past (< 90/60 mmHg), as well as pregnancy in female subjects.

4.2.2 Adult Study: Dosing and Sampling

After a fasting period of at least 10 hours, one tablet of 20 mg enalapril maleate (EnaHEXAL®, HEXAL, Holzkirchen, Germany) was administered to the subjects with 250 mL of water. The intake of xanthine-containing food or beverages and alcohol was prohibited for at least 48 hours after administration.

The time points for pharmacokinetic sampling on the first study day were predose, every 10 minutes for the first 3 hours after administration, every 20 minutes for the next 3 hours and thereafter every 30 minutes up to 8 hours after administration. Additional pharmacokinetic samples were collected 24, 48 and 72 hours after administration.

The time points for pharmacodynamic sampling were predose, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 48, and 72 hours after administration.

On the first day of the study, blood samples were taken through an indwelling cannula in a peripheral arm vein. The latter blood samples were taken through direct venipuncture. All blood samples were drawn after the participants had been lying in a supine position for at

least 30 minutes. The materials used and the sample preparation have been described elsewhere [159].

4.2.3 Adult Study: Analytical Methods

Pharmacokinetic serum samples (enalaprilat) were purified by solid-phase extraction and analysed using liquid chromatography–triple quadrupole tandem mass spectrometry [Shimadzu HPLC 10 (Shimadzu, Duisburg, Germany) coupled with AB Sciex API 2000 mass spectrometer (Sciex, Darmstadt, Germany)]. The LLOQ of enalaprilat was 0.70 µg/L.

Angiotensin I was determined using a commercial ^{125}I radioimmunoassay (Immunotech, Prague, Czech Republic) and had a calibration range of 0.2 to 30 µg/L. For the determination of angiotensin II, a solid-phase extraction was combined with a modified enzyme-linked immunosorbent assay (IBL, Hamburg, Germany). The calibration range of angiotensin II was between 1 and 125 pg/mL. Further information regarding the analytical methods can be found elsewhere [159].

4.2.4 Paediatric Studies: Study Design and Investigated Population

The sources for the paediatric data were the two pharmacokinetic bridging studies and the safety follow-up study of the LENA project. General information on the pharmacokinetic bridging studies and the safety follow-up study is described in Chapters 1.4.3 and 1.4.4. The three studies were carried out from 2016 to 2018 in hospitals in Austria, Hungary, the Netherlands (2 sites), Serbia (2 sites) and Germany (no subjects in the safety follow-up study). The ethics committees of the participating institutions approved all three studies. Prior to the inclusion of each subject in the respective study, informed parental consent was obtained. According to national requirements, the assent of the participating children was obtained. The studies were registered on the EU Clinical Trials Register (EudraCT 2015-002335-17, EudraCT 2015-002396-18 and EudraCT 2015-002397-21).

The inclusion and exclusion criteria for the pharmacokinetic bridging studies are listed in Chapter 2.2.1. In the safety follow-up study, subjects from the pharmacokinetic bridging studies who were still treated with enalapril orodispersible minitablets or who were treated with enalapril orodispersible minitablets for at least three days were included. There were no additional exclusion criteria in the safety follow-up study.

4.2.5 Paediatric Studies: Dosing and Sampling

The information on dosing in the two pharmacokinetic bridging studies can be found in Chapter 2.2.2. This information also applies to the safety follow-up study.

In the pharmacokinetic bridging studies, a PK/PD profile was usually obtained in ACE inhibitor naïve subjects at the initial dose visit, with pharmacokinetic samples collected before and 1, 2, 4, 6, and 12 hours after enalapril orodispersible minitablet administration, as well as pharmacodynamic samples collected before and 4 hours after administration. Alternatively, the PK/PD profile could also be obtained after reaching steady state at the optimal dose. Single pharmacokinetic and pharmacodynamic samples were collected in immediate succession during the remaining study visits. These samples were collected predose during titration and at the end of the studies. For all other visits, the investigator could determine the time of sampling. When the treatment with enalapril orodispersible minitablets was discontinued, no further pharmacokinetic samples were taken from the subjects concerned.

During the four study visits of the safety follow-up study, single pharmacokinetic samples were taken from subjects still treated with enalapril orodispersible minitablets, and single pharmacodynamic samples were taken from all subjects. For these samples, the investigator could determine the time of sampling. In subjects from whom pharmacokinetic and pharmacodynamic samples were taken, these samples were collected in immediate succession.

The predefined time points of the study visits are listed for the two pharmacokinetic bridging studies in Appendix 9.3 and for the safety follow-up study in Appendix 9.7. The exact sampling times were noted in the electronic case report forms for all pharmacokinetic and pharmacodynamic samples. The subjects were in a supine position for blood sampling. The materials used and the preparation of the pharmacokinetic samples can be found elsewhere [52].

For the collection of the analysed pharmacodynamic samples, EDTA Monovettes® (Sarstedt, Nuembrecht, Germany) were used, which were spiked with an inhibitor cocktail [dimethyl sulfoxide (AppliChem GmbH, Darmstadt, Germany), 1,10-phenanthroline (Sigma-Aldrich, Steinheim, Germany), pepstatin A (Sigma-Aldrich, Steinheim, Germany), 4-hydroxymercuri benzoic acid (Sigma-Aldrich, Steinheim, Germany), and EDTA (Carl

Roth GmbH + Co. KG, Karlsruhe, Germany)] to block degradation of humoral parameters. In situations where sampling with EDTA Monovettes® was not possible, micro collection tubes containing EDTA solution and the aforementioned inhibitor cocktail were used. The blood collection tubes were stored on ice before, during, and after blood collection. Immediately afterwards, the blood collection tubes were centrifuged for 10 minutes at 2000× *g* under cooled conditions (0–4 °C). The transferred supernatant was snap-frozen and stored at −80 °C until analysis.

4.2.6 Paediatric Studies: Analytical Methods

The analytical methods for the analysis of the pharmacokinetic serum samples with regard to enalaprilat are described in Chapter 2.2.4.

For the simultaneous determination of several angiotensin peptides, including angiotensin I and angiotensin II, a validated multiplex liquid chromatography high-resolution mass spectrometry method was applied [54]. Following precipitation and solid-phase extraction, the samples were analysed using a Nexera XR liquid chromatography system (Shimadzu, Duisburg, Germany) coupled to a TripleTOF® 6600 mass spectrometer from AB Sciex (Concord, ON, Canada). The calibration range was 25.4 to 1594.2 pg/mL for angiotensin I and 22.3 to 1395.8 pg/mL for angiotensin II.

4.2.7 Software

The population PK/PD and population pharmacodynamic analyses were conducted using the nonlinear mixed effects modelling program Monolix version 2024R1 (Lixoft, Antony, France). For the estimation of the population parameters, Monolix uses a stochastic approximation expectation-maximization algorithm. Excel® version 2406 (Microsoft, Redmond, WA, USA) and R version 4.2.2 (The R Foundation for Statistical Computing, Vienna, Austria) were used to generate the input files for Monolix, statistical analyses and additional graphics.

4.2.8 Model Development of the Pharmacokinetic/Pharmacodynamic Model for Healthy Adults

First, the angiotensin II/angiotensin I ratio was plotted against the enalaprilat concentration for each subject to check for hysteresis. Thereafter, a pharmacokinetic model for the active metabolite enalaprilat was developed. A one- and two-compartment model with first-order absorption and linear elimination was tested for enalaprilat. In addition, the implementation

of a lag time as well as transit compartments was tested. A combined residual error model with an additive and a proportional error was selected as the error model. The conditional sampling use for stepwise approach based on correlation tests (COSSAC) method was used for the covariate search [160]. The potential covariates sex, age, weight, and body mass index were tested as covariates for the model parameters with random effects. An exponential model was tested for the categorical covariate sex, and a power model scaled with the weighted mean of the respective covariates was tested for the continuous covariates age, weight, and body mass index. For the likelihood ratio threshold, the default setting $p = 0.01$ was used as the forward and backward threshold. Based on the scatter plots for each pair of random effects and the results of the correlation test using a t-test, the inclusion of potential correlations between random effects in the model was tested.

The pharmacokinetic model developed was then used for the pharmacokinetic part of the PK/PD model, with the parameter estimates of the pharmacokinetic model serving as initial estimates. For PK/PD modelling, the simultaneous approach was chosen, in which all model parameters are estimated together. A maximum inhibition (I_{max}) model with an effect compartment was selected for the pharmacodynamic part of the model. For the I_{max} model, partial inhibition and full inhibition were tested, as well as the addition of a sigmoidicity factor. For the pharmacodynamic part of the model, a proportional error model and a combined error model with an additive and a proportional error were tested as the residual error model. As described above, a covariate search was then performed, and the inclusion of correlations between random effects was tested.

Interindividual variability was incorporated exponentially for the parameters, as it was assumed that the interindividual variability of the parameters is lognormally distributed. For model selection, the change in the objective function, the change in the residual and interindividual variability, the change in the relative standard errors of the parameter estimates, and the visual inspection of the goodness-of-fit plots were considered. Bioavailability could not be determined based on the available data. Therefore, the apparent clearance, the apparent intercompartmental clearance, the apparent central volume of distribution and the apparent peripheral volume of distribution are reported. Predose pharmacokinetic samples were excluded because no concentration was expected at this time in healthy adults without prior treatment with enalapril. Pharmacokinetic samples with concentrations below the limit of quantification were treated as censored, and a lower limit of zero was set (equivalent to the M4 method in NONMEM®) [79]. For pharmacodynamic

samples with concentrations below the limit of quantification, the reported value was used to calculate the angiotensin II/angiotensin I ratio.

4.2.9 Model Evaluation of the Pharmacokinetic/Pharmacodynamic Model for Healthy Adults

To evaluate the final PK/PD model, the goodness-of-fit plots and visual predictive checks were visually inspected. For the visual predictive checks, 500 Monte Carlo simulations were performed with the final PK/PD model and the design structure of the original dataset.

4.2.10 Comparison of the Time Course of the Effect in Healthy Adults and Children with Heart Failure

To compare the time course of the angiotensin II/angiotensin I ratio after enalapril administration in healthy adults and children with heart failure, the corresponding visual predictive check of the final PK/PD model for healthy adults was used. The observed data from children with heart failure were added to the visual predictive check. In contrast to the adult data, data were also available for children with heart failure after repeated administration of enalapril. For this data, the time since the last dose was calculated. If the angiotensin II value was below the LLOQ, the angiotensin II value was replaced by LLOQ/2 before calculating the angiotensin II/angiotensin I ratio. Observations in which both angiotensin I and angiotensin II were below the LLOQ were excluded.

4.2.11 Change in the Angiotensin II/Angiotensin I Ratio After Initial Enalapril Dose in Children with Heart Failure

The percentage change in the angiotensin II/angiotensin I ratio was calculated in children with heart failure in whom an angiotensin II/angiotensin I ratio was available before the first dose of enalapril and four hours afterwards.

4.2.12 Model Development of the Pharmacodynamic Model for Children with Heart Failure

The angiotensin II/angiotensin I ratio and the simultaneously measured enalaprilat concentrations were used as input data for the pharmacodynamic model. An I_{max} model was selected as the pharmacodynamic model. Analogous to the adult model, partial inhibition and full inhibition were tested, as well as the addition of a sigmoidicity factor. A proportional error model and a combined error model with an additive and a proportional error were investigated to describe residual variability.

Exponential models were used for the interindividual variability of the parameters, as a lognormal distribution of the interindividual variability of the parameters was assumed. For the model selection, the same points were considered as for the adult model. The angiotensin II value was substituted by LLOQ/2 for the calculation of the angiotensin II/angiotensin I ratio if the angiotensin II value was below the LLOQ. Observations were excluded if angiotensin I and angiotensin II were below the LLOQ.

4.2.13 Model Evaluation of the Pharmacodynamic Model for Children with Heart Failure

The final pharmacodynamic model was evaluated by visual inspection of the goodness-of-fit plots and the visual predictive check. A total of 500 Monte Carlo simulations using the final pharmacodynamic model and the design structure of the original dataset were performed for the visual predictive check.

4.3 Results

4.3.1 Adult Data

A total of 288 serum enalaprilat concentrations from 9 healthy subjects were included in the analysis. Of these, 47 (16.3%) concentrations were below the LLOQ and were treated as censored, with the lower limit set at zero (M4 method). In 2 subjects, 9 of the 34 scheduled pharmacokinetic samples were not available. Additionally, pharmacokinetic samples taken prior to administration of enalapril (n = 9) were excluded because no concentration was expected at that time in healthy adults who had not previously been treated with enalapril.

In total, 99 calculated angiotensin II/angiotensin I ratios from the 9 healthy subjects were included in the analysis. The angiotensin II/angiotensin I ratios 48 and 72 hours after administration of enalapril were excluded because the angiotensin II/angiotensin I ratio rose above the baseline effect in some subjects. Furthermore, the maximum dosage interval for enalapril therapy in patients is 24 hours. Five (5.1%) angiotensin I values and one (1.0%) angiotensin II value were below the LLOQ. Due to the low number of values below the LLOQ, the reported values were used to calculate the angiotensin II/angiotensin I ratio. The patient characteristics of the nine healthy subjects are summarised in Table 4.1.

Table 4.1 Characteristics of the healthy adults and children with heart failure.

Characteristic	Number of Observations (%)	Mean (SD)	Median (Range)
Healthy adults (n = 9)			
Age (years)	9 (100)	22.7 (4.0)	21 (19–30)
Weight (kg)	9 (100)	69.8 (13.6)	74 (47–88)
Body mass index (kg/m^2)	9 (100)	21.3 (2.4)	22 (18–25)
Sex			
Male	6 (66.7)	-	-
Female	3 (33.3)	-	-
Children with heart failure (n = 27)			
Age (years)	54 (100)	0.49 (0.47)	0.36 (0.07–2.24)
Weight (kg)	54 (100)	5.5 (2.2)	4.8 (3.2–13.0)
Ross score	54 (100)	4.0 (2.6)	4 (0–9)
Sex			
Male	12 (44.4)	-	-
Female	15 (55.6)	-	-
Aetiology of heart failure			
Dilated cardiomyopathy	3 (11.1)	-	-
Congenital heart disease	24 (88.9)	-	-

SD, standard deviation.

4.3.2 Paediatric Data

Overall, 54 simultaneous serum concentrations of enalaprilat and angiotensin II/angiotensin I ratios from 27 children with heart failure were included. The angiotensin II value was replaced by LLOQ/2 in 55.6% of the cases because the angiotensin II value was below the LLOQ. Of the enalaprilat serum concentrations, only the measurements prior to the first dose of enalapril were below the LLOQ and were set to zero. Measurements were excluded from analysis because angiotensin I was above the calibration range (n = 10), enalapril therapy was discontinued or interrupted (n = 10), the timepoint of discontinuation of enalapril therapy was unclear (n = 8), angiotensin I and angiotensin II were below the LLOQ (n = 2), the time since the last dose was more than 24 hours (n = 1), or other uncertainties existed (n = 2).

The included observations could be divided into observations prior to the first dose of enalapril (n = 16), observations after the first dose of enalapril (n = 12), and observations after repeated doses of enalapril (n = 26). Observations after repeated doses of enalapril ranged from 3.9 days to 11.8 months after the first dose of enalapril. Two subjects did not receive their enalapril orodispersible minitablets on the evening before the measurement. The patient characteristics of the 27 children with heart failure are given in Table 4.1.

4.3.3 Population Pharmacokinetic/Pharmacodynamic Model for Healthy Adults

The diagrams showing the angiotensin II/angiotensin I ratio versus the enalaprilat concentration revealed varying degrees of clockwise hysteresis in the individual subjects. Exemplary, Figure 4.1 shows the effect concentration curve of one healthy adult subject, with the data points connected in chronological order of measurement. A clockwise hysteresis can be seen, as a given concentration at a later measurement time point leads to a lower angiotensin II/angiotensin I ratio. Since a greater reduction in the angiotensin II/angiotensin I ratio represents a stronger effect, the clockwise hysteresis shows that the effect increased with time at a given concentration. An effect compartment was integrated into the PK/PD model because pronounced clockwise hysteresis was observed in some subjects.

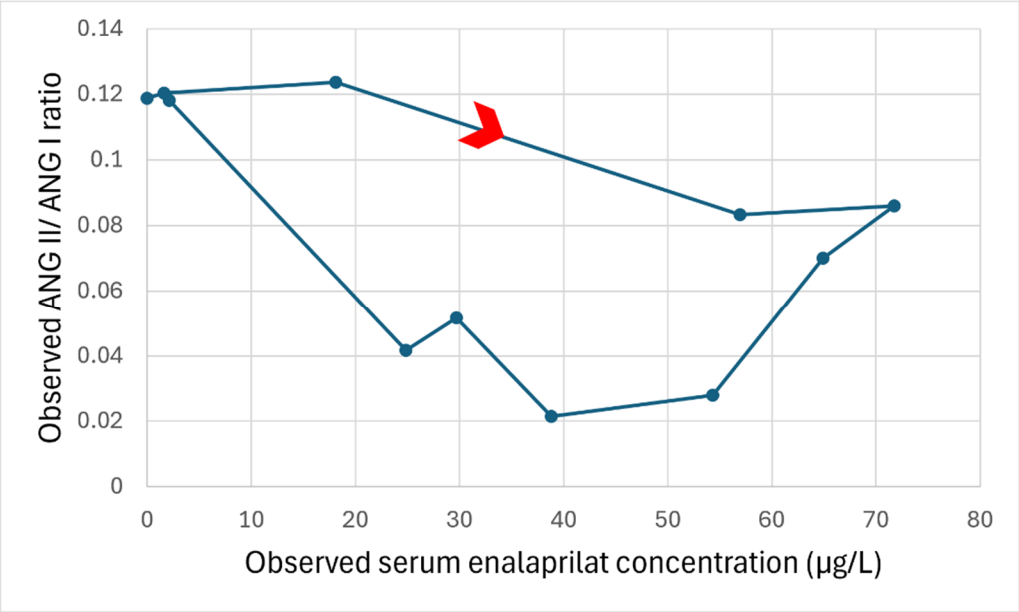

Figure 4.1 **Effect concentration curve of a healthy adult subject.** The points in the plot are connected in chronological order of measurement. The first measurement was the one with an observed serum enalaprilat concentration of zero. The red arrow indicates the direction of the chronological order. ANG I, angiotensin I; ANG II, angiotensin II.

For the pharmacokinetic part of the PK/PD model, a two-compartment model with first-order absorption and linear elimination with transit compartments was selected based on the predefined model selection criteria. In contrast to the one-compartment model, the two-compartment model captured the observed biphasic exponential decline in enalaprilat concentrations. The delayed increase in enalaprilat concentration was better captured with transit compartments than with a lag time. The delay is due to the time required for enalapril to be absorbed and converted to enalaprilat. In this model, the absorption rate constant therefore considers the absorption of enalapril and its conversion to enalaprilat.

For the pharmacodynamic part of the PK/PD model, an I_{max} model with full inhibition and a sigmoidicity factor was selected in accordance with the predefined model selection criteria. Full inhibition was chosen, as the model with partial inhibition estimated an I_{max} of 99% for all subjects. Adding the sigmoidicity factor contributed to achieving a relative standard error of less than 40% for all parameters.

A combined residual error model with an additive and a proportional error was used to describe the residual variability of the pharmacokinetic, while a proportional error model was used to describe the residual variability of the pharmacodynamic. Since the predefined thresholds were not reached, no covariate relationship was included in the model. A positive correlation between the random effects of the apparent central volume of distribution of enalaprilat and the apparent clearance of enalaprilat was identified and included in the model. Interindividual variability for the absorption rate constant, the apparent intercompartmental clearance of enalaprilat, the apparent peripheral volume of distribution of enalaprilat, the effect compartment transfer rate constant, and the sigmoidicity factor was removed because the data were not sufficient to leave interindividual variability for all parameters in the model. The parameter estimates of the final PK/PD model for healthy adults are shown in Table 4.2.

Table 4.2 Parameter estimates of the final pharmacokinetic/pharmacodynamic model for healthy adults.

Parameter	Unit	Estimate	Relative Standard Error (%)
k_{tr}	h^{-1}	5.31	25.7
Mtt	h	1.46	11.7
k_a	h^{-1}	1.19	8.6
CL/F	L/h	36.39	10.2
V_1/F	L	223.71	15.3
Q/F	L/h	6.38	13.7
V_2/F	L	108.26	27.2
k_{e0}	h^{-1}	0.48	21.9
γ	-	2.02	14.9
E_0	-	0.043	39.1
IC_{50}	µg/L	30.01	27.8
Interindividual variability			
IIV k_{tr}	CV%	72.89	25.6
IIV Mtt	CV%	33.7	24.1
IIV CL/F	CV%	30.17	25.2
IIV V_1/F	CV%	46.61	23.6
IIV E_0	CV%	141.9	24.0
IIV IC_{50}	CV%	79.35	30.4
Correlations			
Correlation V_1/F, CL/F	-	0.84	12.5
Residual variability pharmacokinetic model			
Proportional error	-	0.072	12.2
Additive error	µg/L	0.42	8.7
Residual variability pharmacodynamic model			
Proportional error	-	0.41	8.9

The pharmacokinetic parameters refer to enalaprilat. Further details can be found in the text. CL/F, apparent clearance; CV, coefficient of variation; E_0, baseline effect; γ, sigmoidicity factor; IC_{50}, half-maximal inhibitory concentration; IIV, interindividual variability; k_a, absorption rate constant; k_{e0}, effect compartment transfer rate constant; k_{tr}, transit rate constant; Mtt, Mean transit time; Q/F, apparent intercompartmental clearance; V_1/F, apparent central volume of distribution; V_2/F, apparent peripheral volume of distribution.

4.3.4 Model Evaluation of the Pharmacokinetic/Pharmacodynamic Model for Healthy Adults

Visual inspection of the goodness-of-fit plots suggests a good model performance of the final PK/PD model for healthy adults (Figure 4.2, Figure 4.3). A uniform distribution around the unity line is evident in the diagrams in which the observations are plotted against the individual predictions. The scatter around the unity line is lower for pharmacokinetic than for pharmacodynamic. The individual weighted residuals versus the individual predictions and versus time are evenly distributed around zero. Furthermore, most of the individual weighted residuals lie between −2 and 2.

The visual predictive checks indicate that the PK/PD model adequately describes the data (Figure 4.2, Figure 4.3). In the relevant time range up to 24 hours after administration of enalapril, the 10th, 50th and 90th percentiles of the observed data are within the corresponding 90% prediction interval. A slight underprediction of late enalaprilat concentrations was observed, as the 10th percentile of observed enalaprilat serum concentrations after 36 hours is slightly above the corresponding 90% prediction interval (Figure 4.4).

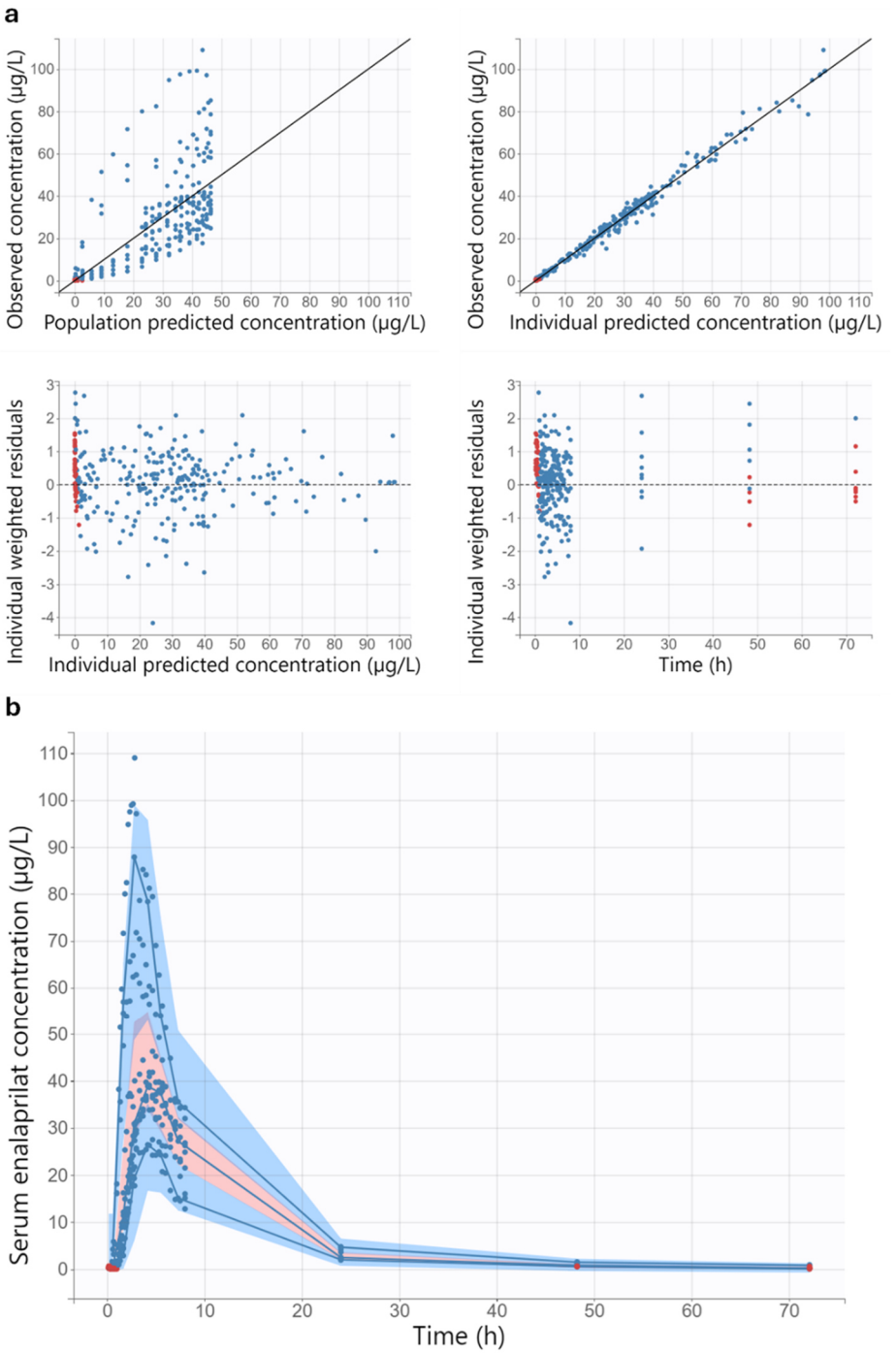

Figure 4.2 **Goodness-of-fit plots (a) and visual predictive check (b) for the pharmacokinetic part of the final pharmacokinetic/pharmacodynamic model for healthy adults.** All concentrations mentioned in the figure refer to the enalaprilat concentration. In all diagrams, the red dots indicate the censored data, and the blue dots indicate the observed data. In the two upper diagrams in (a), the solid black line is the line of unity. In the two lower diagrams in (a), the dashed black line represents the theoretical mean. In the visual predictive check (b), the solid blue lines with blue dots are the 10th, 50th, and 90th percentiles of the observed data. The shaded areas represent the 90% prediction intervals of the 10th (blue), 50th (pink), and 90th (blue) percentiles of the simulated data. The purple areas are the areas where the 90% prediction interval of the 50th percentile of the simulated data overlaps with the 90% prediction interval of the 10th or 90th percentile of the simulated data.

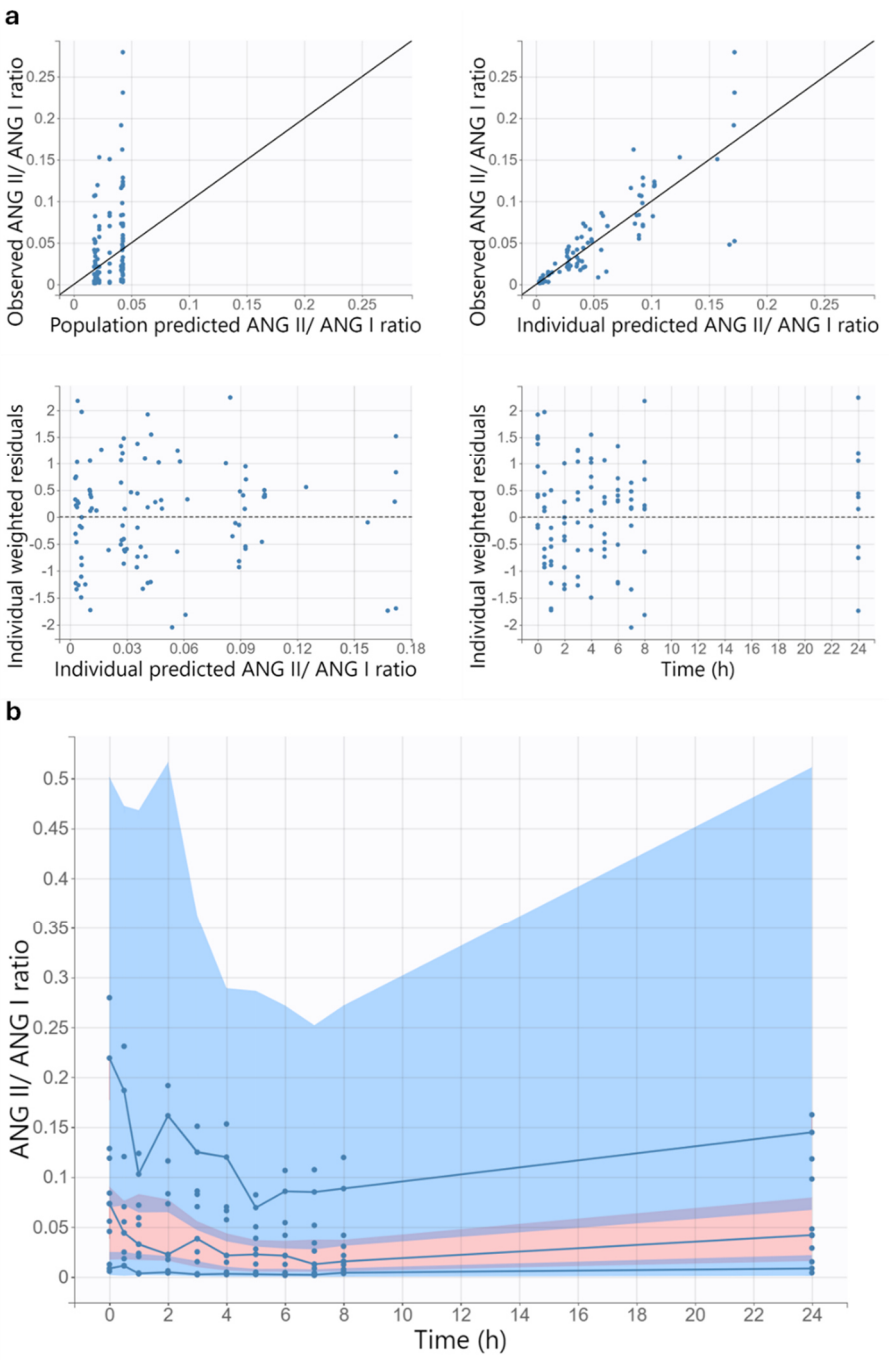

Figure 4.3 **Goodness-of-fit plots for the pharmacodynamic part of the final pharmacokinetic/pharmacodynamic model for healthy adults (a) and visual predictive check of the final pharmacokinetic/pharmacodynamic model for healthy adults (b).** In all diagrams, the blue dots indicate the observed data. In the two upper diagrams in (a), the solid black line is the line of unity. In the two lower diagrams in (a), the dashed black line represents the theoretical mean. In the visual predictive check (b), the solid blue lines with blue dots are the 10th, 50th, and 90th percentiles of the observed data. The shaded areas represent the 90% prediction intervals of the 10th (blue), 50th (pink), and 90th (blue) percentiles of the simulated data. The purple areas are the areas where the 90% prediction interval of the 50th percentile of the simulated data overlaps with the 90% prediction interval of the 10th or 90th percentile of the simulated data. ANG I, angiotensin I; ANG II, angiotensin II.

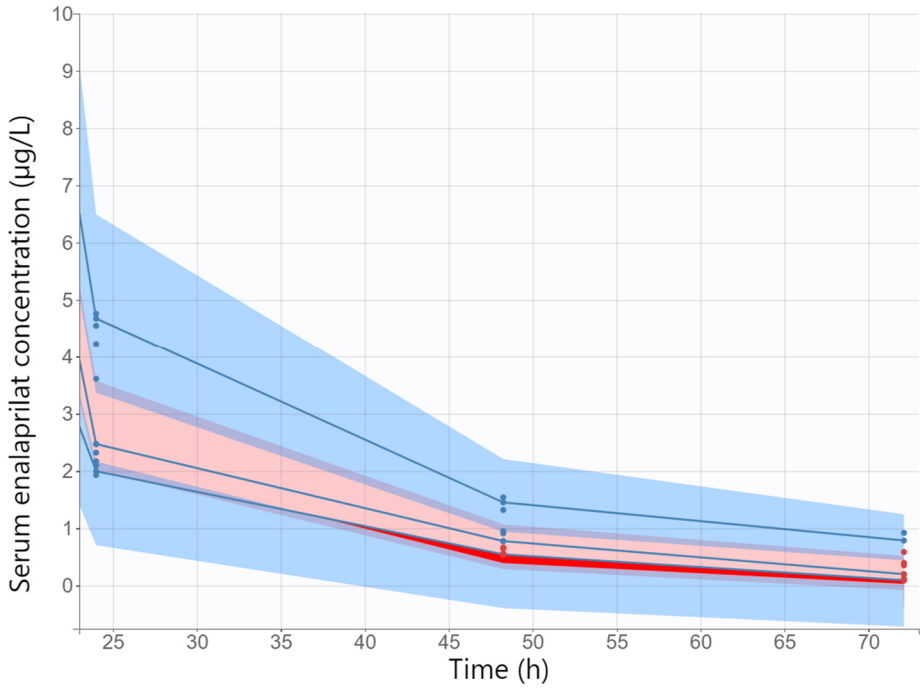

Figure 4.4 **Visual predictive check for the pharmacokinetic part of the final pharmacokinetic/pharmacodynamic model for healthy adults in the period from 24 to 72 hours after administration of enalapril.** The red dots indicate the censored data, and the blue dots indicate the observed data. The solid blue lines with blue dots are the 10th, 50th, and 90th percentiles of the observed data. The shaded areas represent the 90% prediction intervals of the 10th (blue), 50th (pink), and 90th (blue) percentiles of the simulated data. The purple areas are the areas where the 90% prediction interval of the 50th percentile of the simulated data overlaps with the 90% prediction interval of the 10th or 90th percentile of the simulated data. Red areas indicate the areas where the empirical percentile lies outside the prediction interval.

4.3.5 Comparison of the Time Course of the Effect in Healthy Adults and Children with Heart Failure

The healthy adults received a median dose of enalapril maleate of 0.27 mg/kg (range 0.23–0.43 mg/kg), and the highest measured enalaprilat concentration was 109 µg/L. In contrast, the children with heart failure received a median first dose of enalapril maleate of 0.06 mg/kg (range 0.03–0.08 mg/kg). With repeated administration of enalapril orodispersible minitablets, the dosing interval varied between subjects. One subject received a dose of 0.06 mg/kg enalapril maleate once daily. The other subjects received enalapril orodispersible minitablets twice daily at a median daily dose of enalapril maleate of 0.11 mg/kg (range 0.06–0.27 mg/kg). The highest measured enalaprilat concentration in children with heart failure was 18.3 µg/L.

Although the dosage was lower, children with heart failure achieved a similar angiotensin II/angiotensin I ratio after administration of enalapril as healthy adults (Figure 4.5). The time course of the angiotensin II/angiotensin I ratio after administration of enalapril is broadly similar in children with heart failure and in healthy adults. Most angiotensin II/angiotensin I ratios prior to the first dose of enalapril in children with heart failure are within the 90% prediction interval of the 90th percentile of the simulated data. This suggests that the angiotensin II/angiotensin I ratio is slightly higher in children with heart failure prior to the first dose of enalapril than in healthy adults.

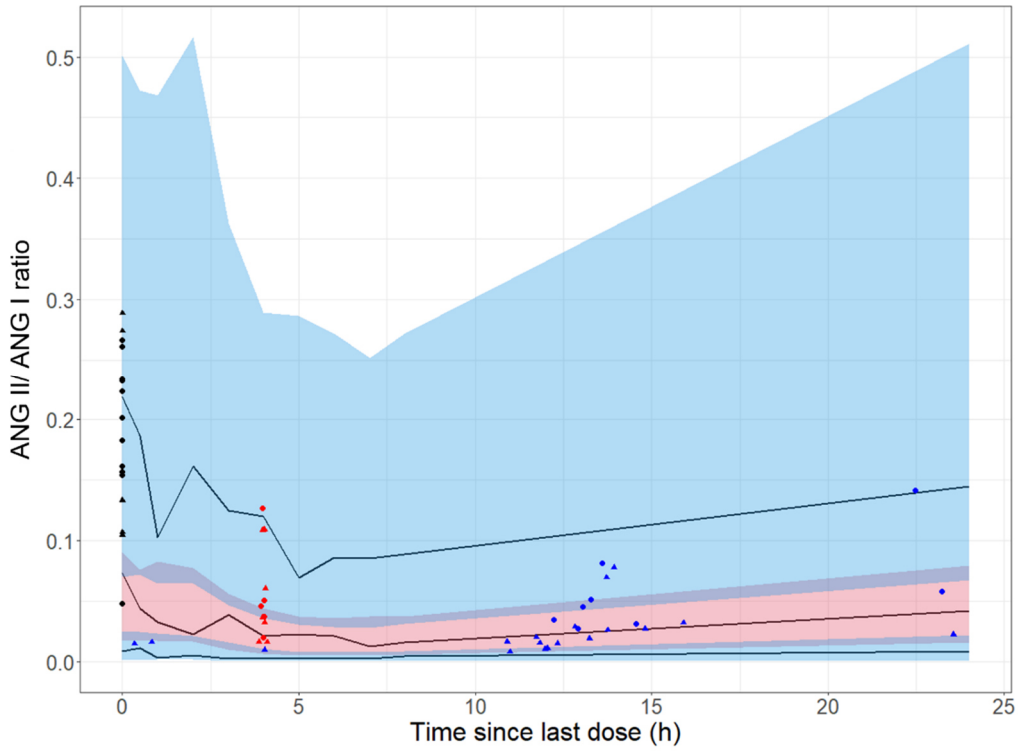

Figure 4.5 **Comparison of the time course of the angiotensin II/angiotensin I ratio after enalapril administration in healthy adults and children with heart failure.** The solid black lines are the 10th, 50th, and 90th percentiles of the observed data in healthy adults. The shaded areas represent the 90% prediction interval of the 10th (blue), 50th (pink), and 90th (blue) percentiles of the simulated data. The purple areas are the areas where the 90% prediction interval of the 50th percentile of the simulated data overlaps with the 90% prediction interval of the 10th or 90th percentile of the simulated data. The simulated data originate from 500 Monte Carlo simulations performed with the final pharmacokinetic/pharmacodynamic model of healthy adults. The symbols indicate the observed data in children with heart failure. The black symbols represent observations prior to the first dose of enalapril, the red symbols represent observations after the first dose of enalapril, and the blue symbols represent observations after later doses of enalapril. Observations in which the angiotensin II value was within the calibration range are shown as circles. Observations in which the angiotensin II value was below the LLOQ and was replaced by LLOQ/2 are shown as triangles. ANG I, angiotensin I; ANG II, angiotensin II; LLOQ, lower limit of quantification.

4.3.6 Change in the Angiotensin II/Angiotensin I Ratio After Initial Enalapril Dose in Children with Heart Failure

For 8 children (median age 0.22 years, age range 0.07–0.58 years) with heart failure, an angiotensin II/angiotensin I ratio was available before the first dose of enalapril and 4 hours afterwards. Four hours after the first dose of enalapril (median 0.06 mg/kg enalapril maleate, range 0.04–0.08 mg/kg enalapril maleate), the angiotensin II/angiotensin I ratio in these subjects decreased by a median of 79.3% (range 21.4–92.7%).

4.3.7 Population Pharmacodynamic Model for Children with Heart Failure

An I_{max} model with full inhibition was selected as a pharmacodynamic model for children with heart failure. The addition of a sigmoidicity factor was omitted because the models with and without the sigmoidicity factor barely differed in terms of the predefined model selection criteria. A proportional error model was used to describe the residual variability. The interindividual variability for the baseline effect was removed because the data were not sufficient to estimate it with an acceptable relative standard error. For the population of children with heart failure, the baseline effect was estimated to be 0.19 and the half-maximal inhibitory concentration to be 1.19 µg/L. Further parameter estimates of the final pharmacodynamic model for children with heart failure can be found in Table 4.3.

Table 4.3 Parameter estimates of the final pharmacodynamic model for children with heart failure.

Parameter	Unit	Estimate	Relative Standard Error (%)
E_0	-	0.19	8.2
IC_{50}	µg/L	1.19	17.9
Interindividual variability			
IIV IC_{50}	CV%	59.92	24.6
Residual variability			
Proportional error	-	0.37	14.2

CV, coefficient of variation; E_0, baseline effect; IC_{50}, half-maximal inhibitory concentration; IIV, interindividual variability.

4.3.8 Model Evaluation of the Pharmacodynamic Model for Children with Heart Failure

The goodness-of-fit plots of the final pharmacodynamic model suggest acceptable model performance (Figure 4.6). Since the final model does not include interindividual variability for the baseline effect, the model predicted the same angiotensin II/angiotensin I ratio at baseline for all subjects. Almost all individual weighted residuals lie between −2 and 2.

At enalaprilat concentrations above 14 µg/L, the visual predictive check suggests a slight underprediction of the angiotensin II/angiotensin I ratio, as the 10th percentile of the observed angiotensin II/angiotensin I ratios is slightly above the corresponding 90% prediction interval (Figure 4.6). Apart from this, the empirical percentiles lie within the corresponding 90% prediction interval, indicating that the pharmacodynamic model adequately describes the data.

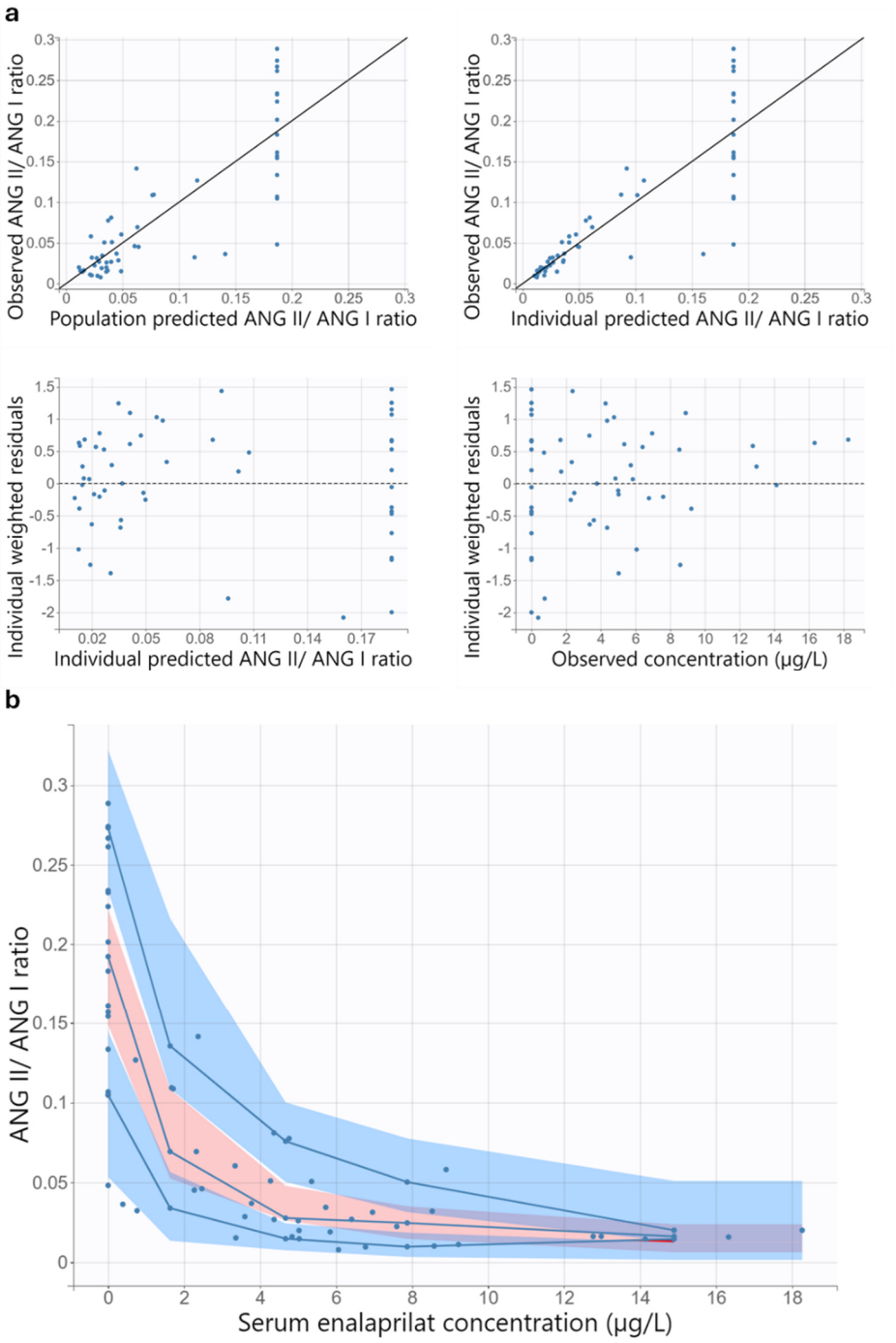

Figure 4.6 **Goodness-of-fit plots (a) and visual predictive check (b) for the final pharmacodynamic model for the children with heart failure.** All concentrations mentioned in the figure refer to the enalaprilat concentration. In all diagrams, the blue dots indicate the observed data. In the two upper diagrams in (a), the solid black line is the line of unity. In the two lower diagrams in (a), the dashed black line represents the theoretical mean. In the visual predictive check (b), the solid blue lines with blue dots are the 10th, 50th, and 90th percentiles of the observed data. The shaded areas represent the 90% prediction intervals of the 10th (blue), 50th (pink), and 90th (blue) percentiles of the simulated data. The purple areas are the areas where the 90% prediction interval of the 50th percentile of the simulated data overlaps with the 90% prediction interval of the 10th or 90th percentile of the simulated data. Red areas indicate the areas where the empirical percentile lies outside the prediction interval. ANG I, angiotensin I; ANG II, angiotensin II.

4.4 Discussion

The angiotensin II/angiotensin I ratio was a suitable pharmacodynamic parameter for model-dependent population analyses in healthy adults and children with heart failure who have been administered enalapril. The effect of the active metabolite enalaprilat on the angiotensin II/angiotensin I ratio was adequately described by the PK/PD model developed for healthy adults and the pharmacodynamic model developed for children with heart failure. The first finding was that the population estimate for the angiotensin II/angiotensin I ratio prior to the first administration of enalapril was higher in children with heart failure than in healthy adults. Secondly, based on the change in the angiotensin II/angiotensin I ratio, it can be assumed that effective ACE inhibition was achieved with the administered dose in children with heart failure. Thirdly, the population estimate for the half-maximal inhibitory concentration was lower in children with heart failure than in healthy adults.

For the pharmacokinetic part of the PK/PD model for healthy adults, a two-compartment model with transit compartments for enalaprilat was most appropriate. The two-compartment model with a central compartment and a peripheral compartment captured the observed biphasic exponential decline in enalaprilat concentration. In the two-compartment model, the initial rapid decline in concentration is attributed to distribution into the peripheral compartment, while the subsequent slower decline is attributed to back diffusion from the peripheral compartment. A two-compartment model was also used for enalaprilat in a previously developed combined model for enalapril and enalaprilat in healthy adults [70]. Since the focus of this study was on the relationship between enalaprilat and the angiotensin II/angiotensin I ratio, the prodrug enalapril was not included in the model. Kechagia et al. [72] also considered only the active metabolite enalaprilat for the pharmacokinetic part of their PK/PD model and likewise selected a two-compartment model. By extrapolating the estimated parameters for enalaprilat in children from this model to an adult weight of 70 kg, the apparent clearance of enalaprilat would be 18.2 L/h, and the apparent central volume of distribution of enalaprilat would be 168.8 L. The parameter estimates are thus lower than the apparent clearance of enalaprilat of 36.4 L/h and the apparent central volume of distribution of enalaprilat of 223.7 L from this study. One reason for the difference could be that the PK/PD model of Kechagia et al. [72] is based on literature data of age groups and not of individuals.

An I_{max} model with full inhibition and sigmoidicity factor for healthy adults and without sigmoidicity factor for children with heart failure was appropriate to describe the relationship between the angiotensin II/angiotensin I ratio and enalaprilat. Due to the observed time delay between measured enalaprilat concentration and effect in healthy adults, an effect compartment was included. As far as is known, no model-dependent analyses of the angiotensin II/angiotensin I ratio as a measure of in vivo ACE activity are available for comparison. However, there are several model-dependent analyses based on in vitro ACE activity. For the relationship between enalaprilat and the inhibition of ACE activity in adults, Ribeiro et al. [161], Donnelly et al. [151], and Zapater et al. [153] used an E_{max} model without a sigmoidicity factor, while Ajayi et al. [71] and Hockings et al. [150] used an E_{max} model with a sigmoidicity factor. The sigmoidicity factor, also known as the Hill coefficient, influences the steepness of the concentration–effect curve. The reason that E_{max} models were used instead of I_{max} models is that the inhibition of ACE activity and not the ACE activity itself was investigated. Only Donnelly et al. [151], who investigated both the inhibition of ACE activity and blood pressure reduction as pharmacodynamic parameters, also used an effect compartment. One reason for an increasing effect over time, as observed in this study for the angiotensin II/angiotensin I ratio and by Donnelly et al. [151] for blood pressure reduction, could be that an indirect physiological response is triggered [162]. Although the reduction in angiotensin II is a direct consequence of ACE inhibition, the increase in angiotensin I results indirectly from the absence of negative feedback from angiotensin II on renin secretion and could therefore be a reason for the hysteresis observed [163].

Comparison of the adult model predictions with paediatric data and population analyses showed that the angiotensin II/angiotensin I ratio is higher in children with heart failure than in healthy adults prior to the first administration of enalapril. The review of the raw data revealed that the higher angiotensin II/angiotensin I ratio in the children with heart failure studied was mainly due to the higher angiotensin II levels compared with healthy adults. The higher angiotensin II levels in children with heart failure could be partly age-related, as a study has shown that normal children have significantly higher angiotensin II levels than adults [124]. Another study found no significant difference in angiotensin II levels between healthy children and healthy adults, but this could also be due to the fact that the samples were taken from children in a supine position and from adults in an upright position [114]. In addition to age, the higher angiotensin II levels in children with heart failure could also be due to the disease. A compensatory mechanism in paediatric heart failure is the activation

of the renin–angiotensin–aldosterone system, which leads to an increase in angiotensin II [3].

Four hours after the first administration of enalapril, a median percentage reduction in the angiotensin II/angiotensin I ratio of 79.3% was achieved, suggesting effective ACE inhibition at the median initial dose of 0.06 mg/kg enalapril maleate. In eight infants with congestive heart failure, a similar mean inhibition of ACE activity of 75.5% was observed after four hours, but after a single dose of 0.25 mg/kg enalapril maleate [164]. One reason for the higher dose required could be that the congestive heart failure was described as poorly controlled with digoxin and diuretics. Secondly, the pharmacodynamic parameter examined was not exactly the same. Thirdly, extemporaneous formulations of the 5 mg tablet for adults were used, which, in contrast to the orodispersible minitablets used in the present study, carry a higher risk that parts of the dose will not be swallowed by the infant.

For the pharmacodynamic parameter angiotensin II/angiotensin I ratio, the population estimate for the half-maximal inhibitory concentration in healthy adults was 30.01 µg/L, whereas in children with heart failure it was only 1.19 µg/L. This suggests that children with heart failure may be more sensitive to enalaprilat than healthy adults. An explanation could be that more unbound enalaprilat is available for ACE inhibition in children with heart failure than in healthy adults. It is known that the amount of plasma proteins is lower in newborns and young infants [32]. Therefore, it is to be expected that the unbound fraction of enalaprilat is higher at this age. However, plasma protein binding of enalaprilat in adults is less than 50%, so this would probably only explain part of the difference [33]. Since the change in the angiotensin II/angiotensin I ratio results from ACE inhibition, a different binding affinity of enalaprilat to ACE in children with heart failure could be another possible reason. Molecular experiments are required to investigate this assumption.

4.5 Limitations

Although the population analyses have yielded new insights, there are also limitations. Firstly, despite the relatively high interindividual variability of pharmacodynamic parameters in healthy adults, no covariates could be identified. Reasons for this could be that the number of subjects was not large enough or that the covariates examined were too similar among the subjects. Since the population analysis in healthy adults served to provide a comparison for children with heart failure, explaining pharmacodynamic variability in healthy adults was not the main objective. Secondly, the proportion of angiotensin II values below the LLOQ was relatively high in children with heart failure. However, this is not surprising, as the production of angiotensin II is inhibited by enalaprilat. The relatively high proportion of angiotensin II values below the LLOQ therefore also indicates effective ACE inhibition. To avoid excluding measurements with angiotensin II below the LLOQ, angiotensin II values below the LLOQ were replaced by LLOQ/2 for the calculation of the angiotensin II/angiotensin I ratio. Thirdly, a different analytical method was used to determine angiotensin I and angiotensin II in healthy adults and children with heart failure. However, all analysis methods used were validated at least for intra- and inter-run accuracy and precision. Differences in the measured angiotensin I and angiotensin II values due to different analysis methods should therefore be minimal. Fourthly, the pharmacodynamic model for the children with heart failure slightly underpredicted the angiotensin II/angiotensin I ratio at enalaprilat concentrations above 14 µg/L. This is probably due to the limited data available in this area. The number of samples that could be evaluated was limited by the fact that the determination of angiotensin peptides using liquid chromatography and high-resolution mass spectrometry was an additional analytical determination that was only performed for some of the pharmacodynamic samples from the LENA studies. However, in the LENA studies, more than 90% of all measured enalaprilat concentrations in ACE inhibitor naïve subjects were below 14 µg/L. For the concentration range in which most measured enalaprilat concentrations lie, the pharmacodynamic model therefore adequately describes the data.

4.6 Conclusions

The analyses of the angiotensin II/angiotensin I ratio indicate that effective ACE inhibition and thus the prerequisite for clinical effects were achieved in children with heart failure at the given dosage. However, further studies on the effects of enalapril on clinical endpoints are necessary. The differences between children with heart failure and healthy adults identified in population analyses suggest that children with heart failure may be more sensitive to enalaprilat than healthy adults. The pharmacodynamic model developed for children with heart failure could be used in conjunction with the existing population pharmacokinetic model for enalapril and enalaprilat to simulate the effect of a given dose of enalapril on the angiotensin II/angiotensin I ratio.

4.7 Disclosure

Parts of this chapter were previously published in a peer-reviewed journal:

Steichert, M., Cawello, W., Burckhardt, B. B., Suessenbach, F. K., Laeer, S., & on behalf of the LENA Consortium. (2025). Angiotensin II/Angiotensin I Ratio as a New Pharmacodynamic Parameter for Population Modelling in Healthy Adults and Children with Heart Failure Treated with Enalapril. Pharmaceutics, 17(10). https://doi.org/10.3390/pharmaceutics17101345

The author of this thesis substantially contributed to the conceptualisation, methodology, and data curation. In addition, the author of this thesis was responsible for the formal analysis, visualisation, the draft of the manuscript, and critical revision of the manuscript.

5 Overall Conclusion and Perspective

This thesis provides new and comprehensive insights into the pharmacokinetics and pharmacodynamics in ACE inhibitor naïve children with heart failure who were treated with enalapril orodispersible minitablets. The model-independent and model-dependent analyses enabled the investigation of the influence of age and heart failure on pharmacokinetics and pharmacodynamics.

In the first part, a suitable combined population pharmacokinetic model was developed for enalapril and its active metabolite. In addition to the simulations already performed, the model offers the opportunity to carry out further simulations. For example, the enalapril and enalaprilat concentrations could be simulated after a different dose or dosing interval. Furthermore, it would be possible to simulate a missed dose. Beyond the size-related changes captured by allometric scaling, an influence of age on the pharmacokinetics of enalaprilat was identified. Concretely, the weight-adjusted apparent clearance of enalaprilat increases with increasing age. Moreover, the weight-adjusted apparent volume of distribution of enalaprilat decreases with increasing Ross score. Since the Ross score is a measure of the severity of heart failure, it has been demonstrated that the pharmacokinetics of enalaprilat are also influenced by heart failure. In addition, renal function has an influence on the pharmacokinetics of enalaprilat, as the weight-adjusted apparent clearance of enalaprilat decreases with increasing serum creatinine. To summarise, three factors influencing the pharmacokinetics of enalaprilat were identified in addition to weight. These findings also provide initial indications which parameters should be recorded and tested as covariates in future studies on drugs with similar physicochemical properties in children with heart failure. The clinical relevance of the identified covariates for the initial dose and steady state dose of enalapril was assessed using simulations. Age, weight and renal function should be considered for the selection of the initial dose and steady state dose of enalapril. The severity of heart failure should be considered for the first dose of enalapril. Knowledge of these clinically relevant covariates provides the fundament for improved selection of the initial dose and steady state dose based on patient characteristics. Improved dosage selection reduces the likelihood of the occurrence of side effects and thereby increases safety. A lower incidence of side effects in turn reduces the likelihood of therapy discontinuation due to side effects.

In the second part, the nature and extent of the influence of age, heart failure and ACE inhibitor treatment on the pharmacodynamic parameter plasma renin activity were successfully evaluated. A detailed overview of the development of plasma renin activity in healthy children from birth to 18 years of age was provided based on comprehensive data from the systematic literature review. Thereby, a remarkable influence of age on plasma renin activity in healthy children was substantiated, as plasma renin activity in neonates was up to seven times higher than in older children. The three to four times higher plasma renin activity in children with heart failure younger than six months than in healthy children of a comparable age is probably attributable to the disease itself as well as to the drug therapy for heart failure. With the model-independent analysis, it was demonstrated that repeated administration of enalapril in the LENA studies had a measurable effect on the renin–angiotensin–aldosterone system. Plasma renin activity in children with heart failure increased by a factor of 4.5 due to the repeated administration of enalapril. Altogether, information was generated that facilitates the assessment of plasma renin activity in children with heart failure with and without ACE inhibitor treatment.

In the third part, a population PK/PD model for healthy adults and a pharmacodynamic model for children with heart failure were developed, which adequately described the effect of the active metabolite enalaprilat on the angiotensin II/angiotensin I ratio. Thereby, it was demonstrated that the angiotensin II/angiotensin I ratio is suitable as a pharmacodynamic parameter for population modelling and can also be utilised in future population analyses. Comparison with healthy adults revealed that the angiotensin II/angiotensin I ratio was higher in children with heart failure prior to the first dose, which is likely due to the influence of both age and heart failure on the renin–angiotensin–aldosterone system. The analyses regarding the angiotensin II/angiotensin I ratio indicate that the administered dose of enalapril leads to effective ACE inhibition. Effective ACE inhibition is the precondition for the positive effects of ACE inhibitors in heart failure known from adults, such as preload and afterload reduction as well as inhibition of cardiac remodelling. The analyses thus indicate that the precondition for positive clinical effects in the subjects of the LENA studies was fulfilled with the given dosage of enalapril. However, further research on the clinical effects of enalapril in children with heart failure is required. The population estimate for the half-maximal inhibitory concentration was lower in children with heart failure than in healthy adults, suggesting that children with heart failure may be more sensitive to enalaprilat than healthy adults. Overall, the knowledge about the effects of enalaprilat in ACE inhibitor naïve children with heart failure has been expanded and impetus has been

given for further research on this topic. The use of the population pharmacokinetic model for enalapril and enalaprilat in conjunction with the pharmacodynamic model would open the possibility of simulating the effect of a specific dose of enalapril on the angiotensin II/angiotensin I ratio.

6 References

1. Irving C, Azeka E, Adorisio R, Blume ED, Bogle C, Chubb H, et al. The International Society for Heart and Lung Transplantation Guidelines for the Management of Pediatric Heart Failure (Update From 2014). J Heart Lung Transplant. 2025;44:e21-e71. doi:10.1016/j.healun.2025.06.003.
2. Guidelines for the Management of Congenital Heart Diseases in Childhood and Adolescence. Cardiol Young. 2017;27:S1-S105. doi:10.1017/S1047951116001955.
3. Masarone D, Valente F, Rubino M, Vastarella R, Gravino R, Rea A, et al. Pediatric Heart Failure: A Practical Guide to Diagnosis and Management. Pediatr Neonatol. 2017;58:303–12. doi:10.1016/j.pedneo.2017.01.001.
4. Hoffman JIE, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39:1890–900. doi:10.1016/S0735-1097(02)01886-7.
5. Lipshultz SE, Sleeper LA, Towbin JA, Lowe AM, Orav EJ, Cox GF, et al. The incidence of pediatric cardiomyopathy in two regions of the United States. N Engl J Med. 2003;348:1647–55. doi:10.1056/NEJMoa021715.
6. Kantor PF, Lougheed J, Dancea A, McGillion M, Barbosa N, Chan C, et al. Presentation, diagnosis, and medical management of heart failure in children: Canadian Cardiovascular Society guidelines. Can J Cardiol. 2013;29:1535–52. doi:10.1016/j.cjca.2013.08.008.
7. Ross RD, Bollinger RO, Pinsky WW. Grading the severity of congestive heart failure in infants. Pediatr Cardiol. 1992;13:72–5. doi:10.1007/BF00798207.
8. Läer S, Mir TS, Behn F, Eiselt M, Scholz H, Venzke A, et al. Carvedilol therapy in pediatric patients with congestive heart failure: a study investigating clinical and pharmacokinetic parameters. Am Heart J. 2002;143:916–22. doi:10.1067/mhj.2002.121265.
9. Hsu DT, Pearson GD. Heart failure in children: part II: diagnosis, treatment, and future directions. Circ Heart Fail. 2009;2:490–8. doi:10.1161/CIRCHEARTFAILURE.109.856229.
10. Buchhorn R, Ross RD, Hulpke-Wette M, Bartmus D, Wessel A, Schulz R, Bürsch J. Effectiveness of low dose captopril versus propranolol therapy in infants with severe congestive failure due to left-to-right shunts. Int J Cardiol. 2000;76:227–33. doi:10.1016/s0167-5273(00)00384-3.

11. Amdani S, Conway J, George K, Martinez HR, Asante-Korang A, Goldberg CS, et al. Evaluation and Management of Chronic Heart Failure in Children and Adolescents With Congenital Heart Disease: A Scientific Statement From the American Heart Association. Circulation. 2024;150:e33-e50. doi:10.1161/CIR.0000000000001245.
12. Hartupee J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. Nat Rev Cardiol. 2016;14:30–8. doi:10.1038/nrcardio.2016.163.
13. The CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med. 1987;316:1429–35. doi:10.1056/NEJM198706043162301.
14. Smeets NJL, Schreuder MF, Dalinghaus M, Male C, Lagler FB, Walsh J, et al. Pharmacology of enalapril in children: a review. Drug Discov Today. 2020;25:1957–70. doi:10.1016/j.drudis.2020.08.005.
15. MacFadyen RJ, Meredith PA, Elliott HL. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. An overview. Clin Pharmacokinet. 1993;25:274–82. doi:10.2165/00003088-199325040-00003.
16. Ulm EH. Enalapril maleate (MK-421), a potent, nonsulfhydryl angiotensin-converting enzyme inhibitor: absorption, disposition, and metabolism in man. Drug Metab Rev. 1983;14:99–110. doi:10.3109/03602538308991383.
17. Thomsen R, Rasmussen HB, Linnet K. In vitro drug metabolism by human carboxylesterase 1: focus on angiotensin-converting enzyme inhibitors. Drug Metab Dispos. 2014;42:126–33. doi:10.1124/dmd.113.053512.
18. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical Renin-Angiotensin system in kidney physiology. Compr Physiol. 2014;4:1201–28. doi:10.1002/cphy.c130040.
19. Kaschina E, Unger T. Angiotensin AT1/AT2 Receptors: Regulation, Signalling and Function. Blood Pressure. 2003;12:70–88. doi:10.1080/08037050310001057.
20. Nussberger J, Wuerzner G, Jensen C, Brunner HR. Angiotensin II suppression in humans by the orally active renin inhibitor Aliskiren (SPP100): comparison with enalapril. Hypertension. 2002;39:E1-8. doi:10.1161/hy0102.102293.
21. Seguchi M, Nakazawa M, Momma K. Effect of enalapril on infants and children with congestive heart failure. Cardiol Young. 1992;2:14–9. doi:10.1017/S1047951100000524.

22. Sluysmans T, Styns-Cailteux M, Tremouroux-Wattiez M, Lame PA de, Lintermans J, Rubay J, Vliers A. Intravenous enalaprilat and oral enalapril in congestive heart failure secondary to ventricular septal defect in infancy. Am J Cardiol. 1992;70:959–62. doi:10.1016/0002-9149(92)90749-O.
23. Lloyd TR, Mahoney LT, Knoedel D, Marvin WJ, Robillard JE, Lauer RM. Orally administered enalapril for infants with congestive heart failure: a dose-finding study. J Pediatr. 1989;114:650–4. doi:10.1016/s0022-3476(89)80715-2.
24. Frenneaux M, Stewart RA, Newman CM, Hallidie-Smith KA. Enalapril for severe heart failure in infancy. Arch Dis Child. 1989;64:219–23. doi:10.1136/adc.64.2.219.
25. Hsu DT, Zak V, Mahony L, Sleeper LA, Atz AM, Levine JC, et al. Enalapril in infants with single ventricle: results of a multicenter randomized trial. Circulation. 2010;122:333–40. doi:10.1161/CIRCULATIONAHA.109.927988.
26. Leversha AM, Wilson NJ, Clarkson PM, Calder AL, Ramage MC, Neutze JM. Efficacy and dosage of enalapril in congenital and acquired heart disease. Arch Dis Child. 1994;70:35–9. doi:10.1136/adc.70.1.35.
27. Lipshultz SE, Lipsitz SR, Sallan SE, Simbre VC, Shaikh SL, Mone SM, et al. Long-term enalapril therapy for left ventricular dysfunction in doxorubicin-treated survivors of childhood cancer. J Clin Oncol. 2002;20:4517–22. doi:10.1200/JCO.2002.12.102.
28. Nakamura H, Ishii M, Sugimura T, Chiba K, Kato H, Ishizaki T. The kinetic profiles of enalapril and enalaprilat and their possible developmental changes in pediatric patients with congestive heart failure. Clinical Pharmacology & Therapeutics. 1994;56:160–8. doi:10.1038/clpt.1994.119.
29. Ohuchi H, Hasegawa S, Yasuda K, Yamada O, Ono Y, Echigo S. Severely impaired cardiac autonomic nervous activity after the Fontan operation. Circulation. 2001;104:1513–8. doi:10.1161/hc3801.096326.
30. van den Anker J, Reed MD, Allegaert K, Kearns GL. Developmental Changes in Pharmacokinetics and Pharmacodynamics. J Clin Pharmacol. 2018;58 Suppl 10:S10-S25. doi:10.1002/jcph.1284.
31. Remko M. Acidity, lipophilicity, solubility, absorption, and polar surface area of some ACE inhibitors. Chemical Papers. 2007;61:133–41. doi:10.2478/s11696-007-0010-y.
32. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. N Engl J Med. 2003;349:1157–67. doi:10.1056/NEJMra035092.

33. Davies RO, Gomez HJ, Irvin JD, Walker JF. An overview of the clinical pharmacology of enalapril. Br J Clin Pharmacol. 1984;18 Suppl 2:215S-229S. doi:10.1111/j.1365-2125.1984.tb02601.x.
34. Boberg M, Vrana M, Mehrotra A, Pearce RE, Gaedigk A, Bhatt DK, et al. Age-Dependent Absolute Abundance of Hepatic Carboxylesterases (CES1 and CES2) by LC-MS/MS Proteomics: Application to PBPK Modeling of Oseltamivir In Vivo Pharmacokinetics in Infants. Drug Metab Dispos. 2017;45:216–23. doi:10.1124/dmd.116.072652.
35. Mulla H. Understanding Developmental Pharmacodynamics. Paediatr Drugs. 2010;12:223–33. doi:10.2165/11319220-000000000-00000.
36. Suessenbach FK, Burckhardt BB. Levels of angiotensin peptides in healthy and cardiovascular/renal-diseased paediatric population-an investigative review. Heart Fail Rev. 2019;24:709–23. doi:10.1007/s10741-019-09797-y.
37. Makowski N. Development and validation of a low-volume LC-HRMS assay for the analysis of aldosterone, its precursor and main metabolite tailored for paediatric research in a GCLP-compliant environment [PhD thesis]. Düsseldorf: Heinrich Heine University Düsseldorf; 16.07.2019.
38. Burdman I, Burckhardt BB. Prorenin and active renin levels in paediatrics: a bioanalytical review. Clin Chem Lab Med. 2020;59:275–85. doi:10.1515/cclm-2020-0857.
39. Koch HJ. Veränderung der klinischen Pharmakokinetik durch Herzinsuffizienz. NeuroGeriatrie. 2013;10:143–8.
40. Mangoni AA, Jarmuzewska EA. The influence of heart failure on the pharmacokinetics of cardiovascular and non-cardiovascular drugs: a critical appraisal of the evidence. Br J Clin Pharmacol. 2019;85:20–36. doi:10.1111/bcp.13760.
41. Abdel Jalil MH, Abdullah N, Alsous MM, Saleh M, Abu-Hammour K. A systematic review of population pharmacokinetic analyses of digoxin in the paediatric population. Br J Clin Pharmacol. 2020;86:1267–80. doi:10.1111/bcp.14272.
42. Schwartz JB, Taylor A, Abernethy D, O'Meara M, Farmer J, Young J, et al. Pharmacokinetics and pharmacodynamics of enalapril in patients with congestive heart failure and patients with hypertension. J Cardiovasc Pharmacol. 1985;7:767–76. doi:10.1097/00005344-198507000-00023.

43. Brunner-La Rocca HP, Weilenmann D, Kiowski W, Maly FE, Follath F. Plasma levels of enalaprilat in chronic therapy of heart failure: relationship to adverse events. J Pharmacol Exp Ther. 1999;289:565–71.
44. Dunne J. The European Regulation on medicines for paediatric use. Paediatr Respir Rev. 2007;8:177–83. doi:10.1016/j.prrv.2007.04.004.
45. EMA. Revised priority list for studies into off-patent paediatric medicinal products. 2009. https://siope.eu/media/wp-content/uploads/2013/09/EMEA_Off-patent_list_2009.pdf. Accessed 9 Sep 2025.
46. EMA. Report on the Expert Group Meeting of Paediatric Heart Failure, London 29 November 2010: Clinical trials in Paediatric Heart Failure. 2010. https://www.ema.europa.eu/en/documents/other/report-expert-group-meeting-paediatric-heart-failure-london-29-november-2010_en.pdf. Accessed 9 Sep 2025.
47. Castro Díez C, Khalil F, Schwender H, Dalinghaus M, Jovanovic I, Makowski N, et al. Pharmacotherapeutic management of paediatric heart failure and ACE-I use patterns: a European survey. BMJ Paediatr Open. 2019;3:e000365. doi:10.1136/bmjpo-2018-000365.
48. Standing JF, Tuleu C. Paediatric formulations--getting to the heart of the problem. Int J Pharm. 2005;300:56–66. doi:10.1016/j.ijpharm.2005.05.006.
49. Walsh J. Reflection on the Pharmaceutical Formulation Challenges Associated with a Paediatric Investigation Plan for an Off-Patent Drug. AAPS PharmSciTech. 2017;18:250–6. doi:10.1208/s12249-016-0527-x.
50. Thabet Y, Walsh J, Breitkreutz J. Flexible and precise dosing of enalapril maleate for all paediatric age groups utilizing orodispersible minitablets. Int J Pharm. 2018;541:136–42. doi:10.1016/j.ijpharm.2018.02.037.
51. van Hecken A, Burckhardt BB, Khalil F, Hoon J de, Klingmann I, Herbots M, et al. Relative Bioavailability of Enalapril Administered as Orodispersible Minitablets in Healthy Adults. Clin Pharmacol Drug Dev. 2020;9:203–13. doi:10.1002/cpdd.728.
52. Laeer S, Cawello W, Burckhardt BB, Ablonczy L, Bajcetic M, Breur JMPJ, et al. Enalapril and Enalaprilat Pharmacokinetics in Children with Heart Failure Due to Dilated Cardiomyopathy and Congestive Heart Failure after Administration of an Orodispersible Enalapril Minitablet (LENA-Studies). Pharmaceutics. 2022;14:1163. doi:10.3390/pharmaceutics14061163.
53. Bajcetic M, Wildt SN de, Dalinghaus M, Breitkreutz J, Klingmann I, Lagler FB, et al. Orodispersible minitablets of enalapril for use in children with heart failure (LENA):

Rationale and protocol for a multicentre pharmacokinetic bridging study and follow-up safety study. Contemp Clin Trials Commun. 2019;15:100393. doi:10.1016/j.conctc.2019.100393.

54. Suessenbach FK. The Renin-Angiotensin-Aldosterone System in the Paediatric Population: Reliable Highly Sensitive Bioanalytical Investigations via Immunoassay and Innovative Multiplex High-resolution Mass Spectrometry [PhD thesis]. Düsseldorf: Heinrich Heine University Düsseldorf; 14.07.2020.
55. EMA. Aqumeldi (enalapril): European public assessment report. 2023. https://www.ema.europa.eu/en/documents/overview/aqumeldi-epar-medicine-overview_en.pdf. Accessed 19 Aug 2025.
56. Proveca Pharma Limited. AQUMELDI: Summary of product characteristics. 2023. https://www.ema.europa.eu/en/documents/product-information/aqumeldi-epar-product-information_en.pdf. Accessed 18 Sep 2025.
57. Ethypharm GmbH. Fachinformation Noyada 5 mg/5 ml Lösung zum Einnehmen. 2022. https://www.ethypharm.de/wp-content/uploads/2023/06/Fachinformation-Noyada-5-mg-2022-12.pdf. Accessed 18 Sep 2025.
58. EMA. Entresto (sacubitril/valsartan): European public assessment report. 2023. https://www.ema.europa.eu/en/documents/overview/entresto-epar-summary-public_en.pdf. Accessed 19 Aug 2025.
59. Shaddy R, Burch M, Kantor PF, Solar-Yohay S, Garito T, Zhang S, et al. Sacubitril/Valsartan in Pediatric Heart Failure (PANORAMA-HF): A Randomized, Multicenter, Double-Blind Trial. Circulation. 2024;150:1756–66. doi:10.1161/CIRCULATIONAHA.123.066605.
60. Bijelic M, Djukic M, Vukomanovic V, Parezanovic V, Lazic M, Pavlovic A, et al. Clinical and Hemodynamic Outcomes with Enalapril Orodispersible Minitablets in Young Children with Heart Failure Due to Congenital Heart Disease. J Clin Med. 2024;13:4976. doi:10.3390/jcm13174976.
61. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. Journal of Pharmacokinetics and Biopharmaceutics. 1977;5:445–79. doi:10.1007/BF01061728.
62. Mould, Upton RN. Basic Concepts in Population Modeling, Simulation, and Model-Based Drug Development. CPT Pharmacometrics Syst Pharmacol. 2012;1:1–14. doi:10.1038/psp.2012.4.

63. Bauer RJ. NONMEM Tutorial Part I: Description of Commands and Options, With Simple Examples of Population Analysis. CPT Pharmacometrics Syst Pharmacol. 2019;8:525–37. doi:10.1002/psp4.12404.
64. Traynard P, Ayral G, Twarogowska M, Chauvin J. Efficient Pharmacokinetic Modeling Workflow With the MonolixSuite: A Case Study of Remifentanil. CPT Pharmacometrics Syst Pharmacol. 2020;9:198–210. doi:10.1002/psp4.12500.
65. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometrics Syst Pharmacol. 2013;2:e38. doi:10.1038/psp.2013.14.
66. Owen JS, Fiedler-Kelly J. Introduction to population pharmacokineticpharmacodynamic analysis with nonlinear mixed effects models. Hoboken New Jersey: Wiley; 2014.
67. Cock RFW de, Piana C, Krekels EHJ, Danhof M, Allegaert K, Knibbe CAJ. The role of population PK-PD modelling in paediatric clinical research. Eur J Clin Pharmacol. 2011;67 Suppl 1:5–16. doi:10.1007/s00228-009-0782-9.
68. Anderson BJ, Allegaert K, Holford NHG. Population clinical pharmacology of children: modelling covariate effects. Eur J Pediatr. 2006;165:819–29. doi:10.1007/s00431-006-0189-x.
69. Wells T, Rippley R, Hogg R, Sakarcan A, Blowey D, Walson P, et al. The pharmacokinetics of enalapril in children and infants with hypertension. J Clin Pharmacol. 2001;41:1064–74. doi:10.1177/00912700122012661.
70. Faisal M, Cawello W, Burckhardt BB, Hoon J de, Laer S. Simultaneous Semi-Mechanistic Population Pharmacokinetic Modeling Analysis of Enalapril and Enalaprilat Serum and Urine Concentrations From Child Appropriate Orodispersible Minitablets. Front Pediatr. 2019;7:281. doi:10.3389/fped.2019.00281.
71. Ajayi AA, Campbell BC, Kelman AW, Howie C, Meredith PA, Reid JL. Pharmacodynamics and population pharmacokinetics of enalapril and lisinopril. Int J Clin Pharmacol Res. 1985;5:419–27.
72. Kechagia I-A, Kalantzi L, Dokoumetzidis A. Extrapolation of enalapril efficacy from adults to children using pharmacokinetic/pharmacodynamic modelling. J Pharm Pharmacol. 2015;67:1537–45. doi:10.1111/jphp.12471.
73. EMA. Ethical considerations for clinical trials on medicinal products conducted with the paediatric population. 2008. https://health.ec.europa.eu/system/files/2016-11/ethical_considerations_en_0.pdf. Accessed 5 Jul 2024.

74. Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005;79:241–57. doi:10.1016/j.cmpb.2005.04.005.
75. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. Comput Methods Programs Biomed. 2004;75:85–94. doi:10.1016/j.cmpb.2003.11.003.
76. Elmokadem A, Riggs MM, Baron KT. Quantitative Systems Pharmacology and Physiologically-Based Pharmacokinetic Modeling With mrgsolve: A Hands-On Tutorial. CPT Pharmacometrics Syst Pharmacol. 2019;8:883–93. doi:10.1002/psp4.12467.
77. Marier J-F, Teuscher N, Mouksassi M-S. Evaluation of covariate effects using forest plots and introduction to the coveffectsplot R package. CPT Pharmacometrics Syst Pharmacol. 2022;11:1283–93. doi:10.1002/psp4.12829.
78. Faisal M, Steichert M, Cawello W, Laeer S, and the LENA consortium. Enalapril orodispersible minitablets for pediatric heart failure - a population pharmacokinetic analysis from multicenter Phase II/III LENA clinical trials. 2023. www.page-meeting.org/?abstract=10341. Accessed 5 Jul 2024.
79. Ahn JE, Karlsson MO, Dunne A, Ludden TM. Likelihood based approaches to handling data below the quantification limit using NONMEM VI. J Pharmacokinet Pharmacodyn. 2008;35:401–21. doi:10.1007/s10928-008-9094-4.
80. Byon W, Smith MK, Chan P, Tortorici MA, Riley S, Dai H, et al. Establishing best practices and guidance in population modeling: an experience with an internal population pharmacokinetic analysis guidance. CPT Pharmacometrics Syst Pharmacol. 2013;2:e51. doi:10.1038/psp.2013.26.
81. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 2011;13:143–51. doi:10.1208/s12248-011-9255-z.
82. World Health Organization. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age; methods and development. Geneva: WHO Press; 2006.
83. Gastonguay M, Eltahtawy A. Effect of NONMEM minimization status and number of replicates on bootstrap parameter distributions for population pharmacokinetic models: A case study. Clinical Pharmacology & Therapeutics. 2005;77:P2-P2. doi:10.1016/j.clpt.2004.11.010.

84. Najib NM, Idkaidek N, Adel A, Admour I, Astigarraga REB, Nucci G de, et al. Bioequivalence evaluation of two brands of enalapril 20 mg tablets (Narapril and Renitec) in healthy human volunteers. Biopharm Drug Dispos. 2003;24:315–20. doi:10.1002/bdd.368.
85. Shi D, Yang D, Prinssen EP, Davies BE, Yan B. Surge in expression of carboxylesterase 1 during the post-neonatal stage enables a rapid gain of the capacity to activate the anti-influenza prodrug oseltamivir. J Infect Dis. 2011;203:937–42. doi:10.1093/infdis/jiq145.
86. Meibohm B, Läer S, Panetta JC, Barrett JS. Population pharmacokinetic studies in pediatrics: issues in design and analysis. AAPS J. 2005;7:E475-87. doi:10.1208/aapsj070248.
87. Chen N, Aleksa K, Woodland C, Rieder M, Koren G. Ontogeny of drug elimination by the human kidney. Pediatr Nephrol. 2006;21:160–8. doi:10.1007/s00467-005-2105-4.
88. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr. 1984;104:849–54. doi:10.1016/S0022-3476(84)80479-5.
89. Oguchi H, Miyasaka M, Koiwai T, Tokunaga S, Hora K, Sato K, et al. Pharmacokinetics of temocapril and enalapril in patients with various degrees of renal insufficiency. Clin Pharmacokinet. 1993;24:421–7. doi:10.2165/00003088-199324050-00006.
90. Hussain M, Basheer S, Khalil A, Haider QUA, Saeed H, Faizan M. Pharmacogenetic study of CES1 gene and enalapril efficacy. J Appl Genet. 2024;65:463–71. doi:10.1007/s13353-024-00831-w.
91. Tian L, Liu H, Xie S, Jiang J, Han L, Huang Y, Li Y. Effect of organic anion-transporting polypeptide 1B1 (OATP1B1) polymorphism on the single- and multiple-dose pharmacokinetics of enalapril in healthy Chinese adult men. Clin Ther. 2011;33:655–63. doi:10.1016/j.clinthera.2011.04.018.
92. Packer M. Evolution of the neurohormonal hypothesis to explain the progression of chronic heart failure. Eur Heart J. 1995;16 Suppl F:4–6. doi:10.1093/eurheartj/16.suppl_f.4.
93. Aimo A, Prontera C, Passino C, Emdin M, Vergaro G. Norepinephrine, plasma renin activity and cardiovascular mortality in systolic heart failure. Heart. 2021;107:989–95. doi:10.1136/heartjnl-2020-318791.

94. Vergaro G, Emdin M, Iervasi A, Zyw L, Gabutti A, Poletti R, et al. Prognostic value of plasma renin activity in heart failure. Am J Cardiol. 2011;108:246–51. doi:10.1016/j.amjcard.2011.03.034.
95. Auslender M. Pathophysiology of pediatric heart failure. Prog Pediatr Cardiol. 2000;11:175–84. doi:10.1016/s1058-9813(00)00048-5.
96. Buchhorn R, Hammersen A, Bartmus D, Bürsch J. The pathogenesis of heart failure in infants with congenital heart disease. Cardiol Young. 2001;11:498–504. doi:10.1017/s1047951101000725.
97. Scammell AM, Diver MJ. Plasma renin activity in infants with congenital heart disease. Arch Dis Child. 1987;62:1136–8. doi:10.1136/adc.62.11.1136.
98. Blazy I, Guillot F, Laborde K, Dechaux M. Comparison of plasma renin and prorenin in healthy infants and children as determined with an enzymatic method and a new direct immunoradiometric assay. Scand J Clin Lab Invest. 1989;49:413–8. doi:10.1080/00365518909089115.
99. Dechaux M, Broyer M, Lenoir G, Limal JM, Sachs C. Nyctohemeral rhythm of plasma renin activity and plasma aldosterone in children. Pediatr Res. 1982;16:354–6. doi:10.1203/00006450-198205000-00007.
100. Raaijmakers A, Zhang Z-Y, Claessens J, Cauwenberghs N, van Tienoven TP, Wei F-F, et al. Does Extremely Low Birth Weight Predispose to Low-Renin Hypertension? Hypertension. 2017;69:443–9. doi:10.1161/HYPERTENSIONAHA.116.08643.
101. Legris GJ, Dearborn D, Stern RC, Geiss CL, Hopfer U, Douglas JG, Doershuk CF. Sodium space and intravascular volume: dietary sodium effects in cystic fibrosis and healthy adolescent subjects. Pediatrics. 1998;101:48–56. doi:10.1542/peds.101.1.48.
102. Fiselier T, Monnens L, van Munster P, Jansen M, Peer P, Lijnen P. The renin-angiotensin-aldosterone system in infancy and childhood in basal conditions and after stimulation. Eur J Pediatr. 1984;143:18–24. doi:10.1007/BF00442742.
103. Stalker HP, Holland NH, Kotchen JM, Kotchen TA. Plasma renin activity in healthy children. J Pediatr. 1976;89:256–8. doi:10.1016/s0022-3476(76)80460-x.
104. van Acker KJ, Scharpe SL, Deprettere AJ, Neels HM. Renin-angiotensin-aldosterone system in the healthy infant and child. Kidney Int. 1979;16:196–203. doi:10.1038/ki.1979.121.
105. Fukushige J, Shimomura K, Ueda K. Influence of upright activity on plasma renin activity and aldosterone concentration in children. Eur J Pediatr. 1994;153:284–6. doi:10.1007/BF01954521.

106. Westheim AS, Bostrøm P, Christensen CC, Parikka H, Rykke EO, Toivonen L. Hemodynamic and neuroendocrine effects for candoxatril and frusemide in mild stable chronic heart failure. J Am Coll Cardiol. 1999;34:1794–801. doi:10.1016/S0735-1097(99)00435-0.
107. Cody RJ, Covit AB, Schaer GL, Laragh JH. Evaluation of a long-acting converting enzyme inhibitor (enalapril) for the treatment of chronic congestive heart failure. J Am Coll Cardiol. 1983;1:1154–9. doi:10.1016/s0735-1097(83)80119-3.
108. Jansson K, Dahlström U, Karlberg BE, Karlsson E, Nylander E, Nyquist O, Karlberg KE. The circulating renin-angiotensin system during treatment with metoprolol or captopril in patients with heart failure due to non-ischaemic dilated cardiomyopathy. J Intern Med. 1999;245:435–43. doi:10.1046/j.1365-2796.1999.00458.x.
109. van Veldhuisen DJ, Man in 't Veld AJ, Dunselman PH, Lok DJ, Dohmen HJ, Poortermans JC, et al. Double-blind placebo-controlled study of ibopamine and digoxin in patients with mild to moderate heart failure: results of the Dutch Ibopamine Multicenter Trial (DIMT). J Am Coll Cardiol. 1993;22:1564–73. doi:10.1016/0735-1097(93)90579-p.
110. Giardini A, Formigari R, Bronzetti G, Prandstraller D, Donti A, Bonvicini M, Picchio FM. Modulation of neurohormonal activity after treatment of children in heart failure with carvedilol. Cardiol Young. 2003;13:333–6.
111. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
112. Suessenbach FK, Tins J, Burckhardt BB. Customisation and validation of a low-volume plasma renin activity immunoassay: Enabling of regulatory compliant determination in paediatric trials. Pract Lab Med. 2019;17:e00144. doi:10.1016/j.plabm.2019.e00144.
113. Vincent M, Dessart Y, Annat G, Sassard J, Francois R, Cier JF. Plasma renin activity, aldosterone and dopamine beta-hydroxylase activity as a function of age in normal children. Pediatr Res. 1980;14:894–5. doi:10.1038/pr.1980.226.
114. van Acker KJ, Scharpé SL, Lynen PJ, Amery AK. Comparative study of active and inactive plasma renin in healthy infants and adults. J Clin Chem Clin Biochem. 1983;21:775–8. doi:10.1515/cclm.1983.21.12.775.

115. Kotchen TA, Strickland AL, Rice TW, Walters DR. A study of the renin-angiotensin system in newborn infants. J Pediatr. 1972;80:938–46. doi:10.1016/S0022-3476(72)80005-2.

116. Gemelli M, Mamì C, Luca F de, Stelitano L, Bonaccorsi P, Martino F. Atrial natriuretic peptide and renin-aldosterone relationship in healthy newborn infants. Acta Paediatr Scand. 1991;80:1128–33. doi:10.1111/j.1651-2227.1991.tb11799.x.

117. Godard C, Geering JM, Geering K, Vallotton MB. Plasma renin activity related to sodium balance, renal function and urinary vasopressin in the newborn infant. Pediatr Res. 1979;13:742–5. doi:10.1203/00006450-197906000-00004.

118. Del García Río C, Acuña D, Bustamante M, Soler A, García-Torres L, Osorio C. Increased activity of the renin-angiotensin-aldosterone system during the perinatal period. Rev Esp Fisiol. 1982;38:171–6.

119. Sulyok E, Varga F, Csaba IF, Németh M, Tényi I, Györy E, Ertl T. Function of the renin-angiotensin-aldosterone system in relation to electrolyte balance in the small-for-date neonate. Acta Paediatr Acad Sci Hung. 1980;21:153–7.

120. Richer C, Hornych H, Amiel-Tison C, Relier JP, Giudicelli JF. Plasma renin activity and its postnatal development in preterm infants. Preliminary report. Biol Neonate. 1977;31:301–4. doi:10.1159/000240979.

121. Matsuoka OT, Shibao S, Leone CR. Blood pressure and kidney size in term newborns with intrauterine growth restriction. Sao Paulo Med J. 2007;125:85–90. doi:10.1590/s1516-31802007000200004.

122. Assadi FK, Ziai M. Impaired renal acidification in infants with fetal alcohol syndrome. Pediatr Res. 1985;19:850–3. doi:10.1203/00006450-198508000-00015.

123. Stark P, Beckerhoff R, Leumann EP, Vetter W, Siegenthaler W. Control of plasma aldosterone in infancy and childhood. A study of plasma renin activity, plasma cortisol and plasma aldosterone. Helv Paediatr Acta. 1976;30:349–56.

124. Broughton Pipkin F, Smales OR, O'Callaghan M. Renin and angiotensin levels in children. Arch Dis Child. 1981;56:298–302. doi:10.1136/adc.56.4.298.

125. El-Raziky MS, Gohar N, El-Raziky M. Study of substance P, renin and aldosterone in chronic liver disease in Egyptian children. J Trop Pediatr. 2005;51:320–3. doi:10.1093/tropej/fmi060.

126. Youmbissi TJ, Tedong F, Fairbank ST, Blackett-Ngu K, Mbede J. Plasma renin activity studies in a group of African neonates and children. J Trop Pediatr. 1990;36:128–30. doi:10.1093/tropej/36.3.128.

127. Tiosano D, Schwartz Y, Braver Y, Hadash A, Gepstein V, Weisman Y, Lorber A. The renin-angiotensin system, blood pressure, and heart structure in patients with hereditary vitamin D-resistance rickets (HVDRR). J Bone Miner Res. 2011;26:2252–60. doi:10.1002/jbmr.431.
128. Goldfarb D, Sack J, Iaina A, Eliahou H. Sodium, potassium and age: possible determinants of plasma renin activity and aldosterone during childhood (age 4-16). Clin Endocrinol (Oxf). 1981;15:29–36. doi:10.1111/j.1365-2265.1981.tb02744.x.
129. Simões e Silva AC, Diniz JSS, Pereira RM, Pinheiro SVB, Santos RAS. Circulating renin Angiotensin system in childhood chronic renal failure: marked increase of Angiotensin-(1-7) in end-stage renal disease. Pediatr Res. 2006;60:734–9. doi:10.1203/01.pdr.0000246100.14061.bc.
130. Uchiyama M, Otsuka T, Shibuya Y, Sakai K. Hormonal factors influencing fractional excretion of filtered sodium in normal children. Exp Clin Endocrinol. 1986;87:104–7. doi:10.1055/s-0029-1210528.
131. Simsolo R, Grunfeld B, Gimenez M, Lopez M, Berri G, Becú L, Barontini M. Long-term systemic hypertension in children after successful repair of coarctation of the aorta. Am Heart J. 1988;115:1268–73. doi:10.1016/0002-8703(88)90020-8.
132. Otto-Buczkowska E, Kokot F. Verhalten der Reninaktivität im Blutplasma bei diabetischen Kindern. Monatsschr Kinderheilkd. 1979;127:91–4.
133. Harshfield GA, Alpert BS, Pulliam DA, Willey ES, Somes GW, Stapelton FB. Sodium excretion and racial differences in ambulatory blood pressure patterns. Hypertension. 1991;18:813–8. doi:10.1161/01.hyp.18.6.813.
134. Goldberg S, Krishan I, Hames CB, Knight M, Spierto FW. Elevated renin levels in normotensive adolescents. Pediatrics. 1974;54:596–8.
135. Harshfield GA, Alpert BS, Pulliam DA. Renin-angiotensin-aldosterone system in healthy subjects aged ten to eighteen years. J Pediatr. 1993;122:563–7. doi:10.1016/s0022-3476(05)83536-x.
136. Scammell AM, Diver MJ. Plasma aldosterone and renin activity. Arch Dis Child. 1989;64:139–41. doi:10.1136/adc.64.1.139.
137. Nir A, Lindinger A, Rauh M, Bar-Oz B, Laer S, Schwachtgen L, et al. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. Pediatr Cardiol. 2009;30:3–8. doi:10.1007/s00246-008-9258-4.
138. Kurtz A. Renin release: sites, mechanisms, and control. Annu Rev Physiol. 2011;73:377–99. doi:10.1146/annurev-physiol-012110-142238.

139. Task Force on Blood Pressure Control in Children. Report of the Second Task Force on Blood Pressure Control in Children-1987. Pediatrics. 1987;79:1–25.
140. Varga F, Sulyok E, Németh M, Tényi I, Csaba IF, Györi E. Activity of the renin-angiotensin-aldosterone system in full-term newborn infants during the first week of life. Acta Paediatr Acad Sci Hung. 1981;22:123–30.
141. Shimomura K, Fukushige J, Ueda K. Influence of crying on plasma renin activity and aldosterone concentration. Eur J Pediatr. 1989;149:18–9. doi:10.1007/BF02024326.
142. Nijst P, Verbrugge FH, Martens P, Bertrand PB, Dupont M, Francis GS, et al. Plasma renin activity in patients with heart failure and reduced ejection fraction on optimal medical therapy. J Renin Angiotensin Aldosterone Syst. 2017;18:1-9. doi:10.1177/1470320317729919.
143. Anand IS, Ferrari R, Kalra GS, Wahi PL, Poole-Wilson PA, Harris PC. Edema of cardiac origin. Studies of body water and sodium, renal function, hemodynamic indexes, and plasma hormones in untreated congestive cardiac failure. Circulation. 1989;80:299–305. doi:10.1161/01.cir.80.2.299.
144. Barr CS, Lang CC, Hanson J, Arnott M, Kennedy N, Struthers AD. Effects of adding spironolactone to an angiotensin-converting enzyme inhibitor in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol. 1995;76:1259–65. doi:10.1016/s0002-9149(99)80353-1.
145. Chiu YJ, Hu SH, Reid IA. Inhibition of phosphodiesterase III with milrinone increases renin secretion in human subjects. J Pharmacol Exp Ther. 1999;290:16–9.
146. Stern H, Weil J, Genz T, Vogt W, Bühlmeyer K. Captopril in children with dilated cardiomyopathy: acute and long-term effects in a prospective study of hemodynamic and hormonal effects. Pediatr Cardiol. 1990;11:22–8. doi:10.1007/BF02239543.
147. Pereira CM, Tam YK, Collins-Nakai RL. The pharmacokinetics of captopril in infants with congestive heart failure. Ther Drug Monit. 1991;13:209–14. doi:10.1097/00007691-199105000-00004.
148. Weisser K, Schloos J, Lehmann K, Düsing R, Vetter H, Mutschler E. Pharmacokinetics and converting enzyme inhibition after morning and evening administration of oral enalapril to healthy subjects. Eur J Clin Pharmacol. 1991;40:95–9. doi:10.1007/BF00315146.
149. Johns DW, Peach MJ, Gomez RA, Inagami T, Carey RM. Angiotensin II regulates renin gene expression. Am J Physiol. 1990;259:F882-887. doi:10.1152/ajprenal.1990.259.6.F882.

150. Hockings N, Ajayi AA, Reid JL. Age and the pharmacokinetics of angiotensin converting enzyme inhibitors enalapril and enalaprilat. Br J Clin Pharmacol. 1986;21:341–8. doi:10.1111/j.1365-2125.1986.tb05205.x.
151. Donnelly R, Meredith PA, Elliott HL, Reid JL. Kinetic-dynamic relations and individual responses to enalapril. Hypertension. 1990;15:301–9. doi:10.1161/01.hyp.15.3.301.
152. Cortés-Ríos J, Hermida RC, Rodriguez-Fernandez M. Dosing time optimization of antihypertensive medications by including the circadian rhythm in pharmacokinetic-pharmacodynamic models. PLoS Comput Biol. 2022;18:e1010711. doi:10.1371/journal.pcbi.1010711.
153. Zapater P, Novalbos J, Gallego-Sandín S, Hernández FT, Abad-Santos F. Gender differences in angiotensin-converting enzyme (ACE) activity and inhibition by enalaprilat in healthy volunteers. J Cardiovasc Pharmacol. 2004;43:737–44. doi:10.1097/00005344-200405000-00018.
154. Claassen K, Willmann S, Eissing T, Preusser T, Block M. A detailed physiologically based model to simulate the pharmacokinetics and hormonal pharmacodynamics of enalapril on the circulating endocrine Renin-Angiotensin-aldosterone system. Front Physiol. 2013;4:4. doi:10.3389/fphys.2013.00004.
155. Ramusovic S, Laeer S. An integrated physiology-based model for the interaction of RAA system biomarkers with drugs. J Cardiovasc Pharmacol. 2012;60:417–28. doi:10.1097/FJC.0b013e3182676f06.
156. van Nguyen A, Zhang L, Kagan L, Rowland M, Mager DE. Target Reserve and Turnover Parameters Determine Rightward Shift of Enalaprilat Potency From its Binding Affinity to the Angiotensin Converting Enzyme. J Pharm Sci. 2024;113:167–75. doi:10.1016/j.xphs.2023.10.025.
157. Juillerat L, Nussberger J, Ménard J, Mooser V, Christen Y, Waeber B, et al. Determinants of angiotensin II generation during converting enzyme inhibition. Hypertension. 1990;16:564–72. doi:10.1161/01.hyp.16.5.564.
158. Biollaz J, Schelling JL, Des Jacot Combes B, Brunner DB, Desponds G, Brunner HR, et al. Enalapril maleate and a lysine analogue (MK-521) in normal volunteers; relationship between plasma drug levels and the renin angiotensin system. Br J Clin Pharmacol. 1982;14:363–8. doi:10.1111/j.1365-2125.1982.tb01992.x.
159. Burckhardt BB, Tins J, Ramusovic S, Läer S. Tailored Assays for Pharmacokinetic and Pharmacodynamic Investigations of Aliskiren and Enalapril in Children: An

Application in Serum, Urine, and Saliva. J Pediatr Pharmacol Ther. 2015;20:431–52. doi:10.5863/1551-6776-20.6.431.

160. Ayral G, Si Abdallah J-F, Magnard C, Chauvin J. A novel method based on unbiased correlations tests for covariate selection in nonlinear mixed effects models: The COSSAC approach. CPT Pharmacometrics Syst Pharmacol. 2021;10:318–29. doi:10.1002/psp4.12612.
161. Ribeiro W, Muscará MN, Martins AR, Moreno H, Mendes GB, Nucci G de. Bioequivalence study of two enalapril maleate tablet formulations in healthy male volunteers. Pharmacokinetic versus pharmacodynamic approach. Eur J Clin Pharmacol. 1996;50:399–405. doi:10.1007/s002280050130.
162. Louizos C, Yáñez JA, Forrest ML, Davies NM. Understanding the hysteresis loop conundrum in pharmacokinetic/pharmacodynamic relationships. J Pharm Pharm Sci. 2014;17:34–91. doi:10.18433/J3GP53.
163. Triebel H, Castrop H. The renin angiotensin aldosterone system. Pflugers Arch. 2024;476:705–13. doi:10.1007/s00424-024-02908-1.
164. Dutertre JP, Billaud EM, Autret E, Chantepie A, Oliver I, Laugier J. Inhibition of angiotensin converting enzyme with enalapril maleate in infants with congestive heart failure. Br J Clin Pharmacol. 1993;35:528–30. doi:10.1111/j.1365-2125.1993.tb04180.x.

7 Funding

The research work is based on the data of the LENA project, which was funded by a European Union Seventh Framework Program (FP7/2007-2013) under the grant agreement no. 602295.

Participation in the 19th ESDPPP congress was supported by the Heine Research Academies.

Open access funding for the publication entitled ‘Population Pharmacokinetic Analysis of Enalapril and Enalaprilat in Newly Treated Children with Heart Failure: Implications for Safe Dosing of Enalapril (LENA Studies)’ was enabled and organised by Projekt DEAL.

Open access funding for the publication entitled ‘Angiotensin II/Angiotensin I Ratio as a New Pharmacodynamic Parameter for Population Modelling in Healthy Adults and Children with Heart Failure Treated with Enalapril’ was enabled by Proveca Pharma Limited (Dublin, Ireland).

8 Statement on the Use of Artificial Intelligence

DeepL Pro, an artificial intelligence-powered and data protection-compliant translator, was used for translations. All translations were reviewed and adjusted where necessary.

Since March 2025, Elicit and Web of Science Research Assistant have been used to search for literature. These are artificial intelligence-powered research assistants. The suggested literature was always reviewed and checked for suitability.

No artificial intelligence was used for the systematic review conducted in 2021 and 2022.

The text of this thesis was not generated by artificial intelligence.

9 Appendix

Appendix 9.1 Members of the LENA Consortium.

LENA beneficiaries and principal investigators: Stephanie Läer, Germany (Coordinator). Jörg Breitkreutz, Germany. Ingrid Klingmann, Belgium. Florian Lagler, Austria. Jan de Hoon, Belgium. Michiel Dalinghaus, the Netherlands. Milica Bajcetic, Serbia. Saskia de Wildt, the Netherlands. Anne Keatley Clarke, United Kingdom. Johannes Breur, the Netherlands. Christoph Male, Austria. Laszlo Ablonczy, Hungary. Thomas Mir, Germany. Vladislav Vukomanovic, Serbia. Milan Dukic, Serbia. Ida Jovanovic, Serbia.

Advanced scientists: Björn Burckhardt, Willi Cawello, Karl Kleine, Angelika Moder, Emina Obarcanin, Peter Wagner, Jennifer Walsh, Anne van Hecken and Lucie Spatenkova.

Scientists and young scientists: Mohsin Ali, Bojana Božić, Maja Bijelić, Ilja Burdman, Agnes Ciplea, Muhammad Faisal, Samieh Farahani, Martin Feickert, Tanja Gangnus, Milica Lazic, Nina Makowski, Fabian Süssenbach, Marijke van der Meulen, Saša Popović, Miro Parezanović, Nori Smeets and Vanessa Swoboda.

Clinical investigators: Dragana Bojanin, Stefan Đorđević, Jasminka Dragić, Ann-Kathrin Holle, Bosiljka Jovičić, Jovan Košutić, Gordana Kozomara, Haidara Majid, Jadranka Mitrović, Sanja Ninić, Miro Parezanovic, Vojislav Parezanovic, Andrija Pavlović, Sergej Prijić, Branislava Rebić, Igor Stefanović, Daniel Tordas and Irena Vulićević.

Study nurses, technicians, social workers: Anke Bartel, Andjelka Čeko, Marissa Herborts, Annelies Hennink, Bosiljka Kosanović, Sanja Kostic, Ljiljana Isailović, Jasmina Maksimovic, Badies Manai, Nada Martinović, Gyöngyi Máté, Miloš Perišić, Jelena Reljić, Regina Pirker, Marta Salamomovic, Claudia Schlesner, Jutta Tins and Eva Wissmann.

Appendix 9.2 Dosing regimen for enalapril orodispersible minitablets.

Age	Dose	Type of dose	Enalapril daily dose (ODMT)	Enalapril daily dose (mg)	Enalapril morning dose (ODMT)	Enalapril evening dose (ODMT)
1 day to below 6 months	1st	Titration dose	10% of 1 ODMT 0.25 mg[1]	0.025	10% of 1 ODMT 0.25 mg	-
(ca. 2.5 to 7 kg)	2nd	Titration dose	50% of 1 ODMT 0.25 mg[1]	0.125	50% of 1 ODMT 0.25 mg	-
	3rd	Titration dose	1 ODMT 0.25 mg	0.25	1 ODMT 0.25 mg	-
	4th	Titration dose	2 ODMT 0.25 mg	0.5	1 ODMT 0.25 mg	1 ODMT 0.25 mg
	5th	Target dose	4 ODMT 0.25 mg	1	2 ODMT 0.25 mg	2 ODMT 0.25 mg
		Maximum dose	2 ODMT 1 mg	2	1 ODMT 1 mg	1 ODMT 1 mg
6 months to below 3 years	1st	Titration dose	1 ODMT 0.25 mg	0.25	1 ODMT 0.25 mg	-
(ca. 8 to 15 kg)	2nd	Titration dose	2 ODMT 0.25 mg	0.5	1 ODMT 0.25 mg	1 ODMT 0.25 mg
	3rd	Titration dose	4 ODMT 0.25 mg	1	2 ODMT 0.25 mg	2 ODMT 0.25 mg
	4th	Target dose	2 ODMT 1 mg	2	1 ODMT 1 mg	1 ODMT 1 mg
		Maximum dose	4 ODMT 1 mg	4	2 ODMT 1 mg	2 ODMT 1 mg
3 to below 8 years	1st	Titration dose	2 ODMT 0.25 mg	0.5	1 ODMT 0.25 mg	1 ODMT 0.25 mg
(ca. 16 to 25 kg)	2nd	Titration dose	4 ODMT 0.25 mg	1	2 ODMT 0.25 mg	2 ODMT 0.25 mg
	3rd	Titration dose	2 ODMT 1 mg	2	1 ODMT 1 mg	1 ODMT 1 mg
	4th	Target dose	4 ODMT 1 mg	4	2 ODMT 1 mg	2 ODMT 1 mg
		Maximum dose	8 ODMT 1 mg	8	4 ODMT 1 mg	4 ODMT 1 mg
8 to below 12 years	1st	Titration dose	4 ODMT 0.25 mg	1	2 ODMT 0.25 mg	2 ODMT 0.25 mg
(ca. 26 to 40 kg)	2nd	Titration dose	2 ODMT 1 mg	2	1 ODMT 1 mg	1 ODMT 1 mg
	3rd	Titration dose	4 ODMT 1 mg	4	2 ODMT 1 mg	2 ODMT 1 mg
	4th	Target dose	8 ODMT 1 mg	8	4 ODMT 1 mg	4 ODMT 1 mg
		Maximum dose	16 ODMT 1 mg	16	8 ODMT 1 mg	8 ODMT 1 mg

[1]*Option for very young and low weight patients in whom the investigator considers an initial dose of 1 x 0.25 mg enalapril ODMT to be too high.*
ODMT, orodispersible minitablets.

Appendix 9.3 Predefined time points of the study visits for the pharmacokinetic bridging studies.

Visit	Time point
Screening Visit[1]	Day –21 to Day –1
Initial Dose Visit	Day 0
Titration Visits[2]	Day 2–Day x (Visit Window 2–7 days from the previous visit)
Dose Confirmation Visit[3]	Day 3 to Day 8 from last Titration Visit
First Study Control Visit	Day 14 ± 2 days
Second Study Control Visit	Day 28 ± 2 days
Third Study Control Visit	Day 42 ± 2 days
End-of-Study Visit of the pharmacokinetic bridging studies	Day 56 ± 2 days

[1]*Screening Visit and Initial Dose Visit could be combined in one visit, if the children had a weight of more than 4.2 kg.*
[2]*Number of Titration Visits was dependent on the age of the patient and the judgement of the investigator. For infants from 1 to 6 months a maximum of 4 titration visits were planned and for all other children older than 6 months a maximum of 3 titration visits were planned.*
[3]*Dose Confirmation Visit and First Study Control Visit could be combined in one visit.*

Appendix 9.4 NONMEM® code for the final combined population pharmacokinetic model for enalapril and enalaprilat.

```
;; 1. Based on: run5

;; 2. Description: Combi model ALAG WP8 WP9 WT Norm SCM VII final

;; x1. Author: Steichert

$PROBLEM    Base model

$INPUT      ID DV TIME CMT AMT II ADDL EVID WT ROSS BUN CREA AGE

            SEX WP MARKER TAD LDOS DT CMTDT

$DATA       PopPK_MS_Inputdatei_WTcarryforward.csv IGNORE=#
            IGNORE=(MARKER=999) IGNORE=(WP=10) IGNORE=(DV=1.567)

$SUBROUTINE ADVAN7 TRANS=1

$MODEL      NCOMP=3 COMP(GUT) COMP(CENTRAL) COMP(METACC)

$PK

;;; CLENAATCREA-DEFINITION START

IF(CREA.EQ.-99) THEN

  CLENAATCREA = 1

ELSE

  CLENAATCREA = EXP(THETA(9)*(CREA - 23.37))

ENDIF

;;; CLENAATCREA-DEFINITION END


;;; V3ROSS-DEFINITION START

IF(ROSS.EQ.-99) THEN

  V3ROSS = 1

ELSE

  V3ROSS = EXP(THETA(8)*(ROSS - 4))

ENDIF

;;; V3ROSS-DEFINITION END
```

```
;;; V3-RELATION START
V3COV=V3ROSS
;;; V3-RELATION END

;;; CLENAATAGE-DEFINITION START
  CLENAATAGE = ((AGE/0.34)**THETA(7))
;;; CLENAATAGE-DEFINITION END

;;; CLENAAT-RELATION START
CLENAATCOV=CLENAATAGE*CLENAATCREA
;;; CLENAAT-RELATION END

K12 = THETA(1); Rate constant of absorption of enalapril.

TVCLENA=THETA(2)*(WT/5)**0.75; Total clearance of enalapril
CLENA=TVCLENA*EXP(ETA(1))
FM=0.7 ; fraction enalapril metabolised
CLM = CLENA*FM; Enalapril cleared to metabolite
CLR=CLENA*(1-FM); Enalapril cleared through urine
TVCLENAAT=THETA(3)*(WT/5)**0.75; Total clearance of enalaprilat
TVCLENAAT = CLENAATCOV*TVCLENAAT
CLENAAT1=TVCLENAAT
CLENAAT=CLENAAT1*EXP(ETA(2))

TVV2 = THETA(4)*(WT/5); Volume of distribution of enalapril
V2=TVV2*EXP(ETA(3))
TVV3 = THETA(5)*(WT/5); Volume of distribution of enalaprilat
```

```
TVV3 = V3COV*TVV3
V3 = TVV3*EXP(ETA(4))

K23=CLM/V2
K20=CLR/V2
K30=CLENAAT/V3
S2=V2
S3=V3

TVALAG1=THETA(6)
ALAG1= TVALAG1

$ERROR
C2=A(2)/V2
C3=A(3)/V3

IPRED=F
IF(IPRED.LE.0) IPRED=0.0001
IF(CMT.EQ.1) IPRED=0
W=1/IPRED/IPRED

IF(CMT.EQ.2)Y=IPRED*(1+ERR(1))+ERR(2)
IRES  = IPRED-DV
IWRES = IRES/W
IF(CMT.EQ.3)Y=IPRED*(1+ERR(3))
IRES  = IPRED-DV
IWRES = IRES/W
```

```
$THETA

(0.6) FIX ; KA

(0, 4.41) ; CLENA

(0, 1.43) ; CLENAAT

(0, 3.76) ; V2

(0, 31.4) ; V3

(0, 0.66) ; ALAG1

(-100, 0.303,100000) ; CLENAATAGE1

(-100, -0.145,100000) ; V3ROSS1

(-100, -0.0125,100000) ; CLENAATCREA1

$OMEGA 0.483

 0.143

 0.594

 0.828

$SIGMA 0.259

 1.79

 0.155


$ESTIMATION MAX=9999 METHOD=1 INTER SIG=5

$COVARIANCE MATRIX=R SIGL=12 TOL=12 PRINT=E

$TABLE ID TIME CMT DV AMT II ADDL EVID IPRED PRED IRES CWRES AGE
CLENA CLENAAT V2 V3 ALAG1 TAD LDOS DT NOPRINT ONEHEADER
NOAPPEND FILE=mytab33.TAB

$TABLE ID TIME CMT DV AMT II ADDL EVID IPRED IWRES CWRES CPRED TAD
LDOS DT NOPRINT ONEHEADER FILE=sdtab33

$TABLE ID TIME CMT DV AMT IPRED PRED IRES K12 CLENA CLENAAT V2 V3
ALAG1 AGE WT ROSS BUN CREA SEX ETA1 ETA2 ETA3 ETA4 TAD LDOS DT
NOPRINT ONEHEADER NOAPPEND FILE=patab33

$TABLE ID SEX NOPRINT ONEHEADER FILE=catab33

$TABLE ID AGE WT ROSS BUN CREA NOPRINT ONEHEADER FILE=cotab33
```

Appendix 9.5 Frequency and dosage of concomitant heart failure medication in the different age groups of the LENA subjects.

	Age group 1 (n = 3)		**Age group 2 (n = 12)**		**Age group 3 (n = 13)**		**Age group 4 (n = 7)**	
Concomitant medication	Frequency	Dosage	Frequency	Dosage	Frequency	Dosage	Frequency	Dosage
	n (%)	Median (Range)	n (%)	Median (Range)	n (%)	Median (Range)	n (%)	Median (Range)
Furosemide (mg/kg/day)	3 (100)	2.30 (1.71–3.05)	12 (100)	1.22 (0.80–1.94)	12 (92.3)	1.40 (0.63–3.2)	6 (85.7)	1.27 (0.27–2.25)
Spironolactone (mg/kg/day)	3 (100)	1.15 (0.86–1.83)	11 (91.7)	0.78 (0.40–1.88)	9 (69.2)	0.85 (0.39–0.98)	5 (71.4)	0.65 (0.27–0.88)
Digoxin (µg/kg/day)	0 (0)	-	1 (8.3)	14.93	0 (0)	-	2 (28.6)	10.98 (10.91–11.06)
Carvedilol (mg/kg/day)	0 (0)	-	0 (0)	-	0 (0)	-	1 (14.3)	0.55
Milrinone (µg/kg/min)	0 (0)	-	0 (0)	-	1 (7.7)	0.30–0.45[1]	0 (0)	-
No concomitant medication	0 (0)	-	0 (0)	-	1 (7.7)	-	1 (14.3)	-

[1]*During the observation period, doses between 0.3 and 0.45 µg/kg/min were administered.*
LENA, Labeling of Enalapril from Neonates up to Adolescents.

Appendix 9.6 Frequency and dosage of concomitant heart failure medication in the LENA subjects with asymptomatic and symptomatic heart failure.

	Predose				**After 4.7 ± 1.6 days of enalapril therapy**			
	Asymptomatic heart failure (n = 8)		**Symptomatic heart failure (n = 27)**		**Asymptomatic heart failure (n = 8)**		**Symptomatic heart failure (n = 21)**	
Concomitant medication	Frequency	Dosage	Frequency	Dosage	Frequency	Dosage	Frequency	Dosage
	n (%)	Median (Range)	n (%)	Median (Range)	n (%)	Median (Range)	n (%)	Median (Range)
Furosemide (mg/kg/day)	6 (75)	0.95 (0.63–1.39)	27 (100)	1.57 (0.27–3.20)	7 (87.5)	1.18 (0.95–1.77)	21 (100)	1.57 (0.27–3.05)
Spironolactone (mg/kg/day)	5 (62.5)	0.95 (0.57–0.98)	23 (85.2)	0.8 (0.27–1.88)	7 (87.5)	0.88 (0.57–0.98)	19 (90.5)	0.85 (0.27–1.88)
Digoxin (µg/kg/day)	0 (0)	-	3 (11.1)	11.06 (10.91–14.93)	1 (12.5)	11.06	1 (4.8)	14.93
Carvedilol (mg/kg/day)	0 (0)	-	1 (3.7)	0.55	1 (12.5)	0.55	0 (0)	-
Milrinone (µg/kg/min)	0 (0)	-	1 (3.7)	0.30–0.45[1]	0 (0)	-	0 (0)	-
No concomitant medication	2 (25)	-	0 (0)	-	1 (12.5)	-	0 (0)	-

[1]*During the observation period, doses between 0.3 and 0.45 µg/kg/min were administered.*
LENA, Labeling of Enalapril from Neonates up to Adolescents.

Appendix 9.7 Predefined time points of the study visits for the safety follow-up study.

Visit	Time point
First Follow-up Study Visit[1]	Day 56 ± 2 days
Second Follow-up Study Visit	Month 3 ± 7 days
Third Follow-up Study Visit	Month 6 ± 7 days
Fourth Follow-up Study Visit	Month 9 ± 7 days
Follow-up Study Close-out Visit	Month 12 ± 7 days

[1]*The First Follow-up Study Visit was, de facto, the End-of-Study Visit of the respective pharmacokinetic bridging study.*

Appendix 9.8 Monolix code of the structural model of the final pharmacokinetic/pharmacodynamic model for healthy adults.

```
DESCRIPTION:

PKPD model. The PK data must be tagged with the lowest OBSERVATION ID value.

The administration is extravascular with a first order absorption (rate constant ka) with
transit compartments (mean transit time Mtt, transit rate Ktr).

The PK model has a central compartment (volume V1), a peripheral compartment (volume
V2, intercompartmental clearance Q), and a linear elimination (clearance Cl).

The PD model is a Imax model with effect compartment and a full inhibition (Imax=1) at
high concentrations (rate constant to effect compartment ke0, baseline effect E0, and half-
maximal inhibitory concentration IC50).

The parameter gamma accounts for the sigmoidicity of the drug effect.

[LONGITUDINAL]

input = {Ktr, Mtt, ka, Cl, V1, Q, V2, ke0, gamma, E0, IC50}


EQUATION:

odeType = stiff


PK:

;====== PK part of the model

; Parameter transformations

V = V1

k12 = Q/V1

k21 = Q/V2
```

; PK model definition and effect compartment

{Cc, Ce} = pkmodel (Ktr, Mtt, ka, ke0, V, Cl, k12, k21)

EQUATION:

;====== PD part of the model

E = E0 * (1 – max (Ce,0)^gamma/ (max (Ce,0)^gamma+IC50^gamma))

OUTPUT:

output = {Cc, E}

table = {Ce}

Appendix 9.9 Monolix code of the structural model of the final pharmacodynamic model for the children with heart failure.

```
DESCRIPTION:

The PD model is a Imax model.

[LONGITUDINAL]

input = {IC50, S0}


EQUATION:

Cc_sat = max (t, 0)


A = 1 - Cc_sat/ (Cc_sat + IC50)

S = S0

E = A * S


OUTPUT:

output = {E}
```

Note: The column in the dataset containing the concentrations was tagged as time to enable continuous predictions of the response.

10 List of Publications

Some parts of this thesis have already been published in international peer-reviewed journals or presented at conferences:

Publications in international peer-reviewed journals:

1. Steichert, M., Cawello, W., Burckhardt, B. B., Suessenbach, F. K., Laeer, S., & on behalf of the LENA Consortium. (2025). Angiotensin II/Angiotensin I Ratio as a New Pharmacodynamic Parameter for Population Modelling in Healthy Adults and Children with Heart Failure Treated with Enalapril. Pharmaceutics, 17(10). https://doi.org/10.3390/pharmaceutics17101345

2. Steichert, M., Cawello, W., & Laeer, S. (2025). Population Pharmacokinetic Analysis of Enalapril and Enalaprilat in Newly Treated Children with Heart Failure: Implications for Safe Dosing of Enalapril (LENA Studies). Clinical Pharmacokinetics, 64(7), 1103–1118. https://doi.org/10.1007/s40262-025-01520-5

3. Dabidian, A., Kinny, F., Steichert, M., Schlottau, S., Bartel, A., Schwender, H., & Laeer, S. (2024). Impact of a Clinical Decision Support System on the Efficiency and Effectiveness of Performing Medication Reviews in Community Pharmacies: A Randomized Controlled Trial. Healthcare (Basel, Switzerland), 12(23). https://doi.org/10.3390/healthcare12232491

4. Sharkas, A. R., Ali Sherazi, B., Sayyed, S. A., Kinny, F., Steichert, M., Schwender, H., & Laeer, S. (2024). Development and Evaluation of Interprofessional High-Fidelity Simulation Course on Medication Therapy Consultation for German Pharmacy and Medical Students-A Randomized Controlled Study. Pharmacy (Basel, Switzerland), 12(4). https://doi.org/10.3390/pharmacy12040128

5. Steichert, M., Cawello, W., Bajcetic, M., Breur, J. M. P. J., Dalinghaus, M., Male, C., Wildt, S. N. de, & Läer, S. (2023). Influence of Age, Heart Failure and ACE Inhibitor Treatment on Plasma Renin Activity in Children: Insights from a Systematic Review and the European LENA Project. Frontiers in Bioscience (Landmark Edition), 28(12), 335. https://doi.org/10.31083/j.fbl2812335

Publications in German journals without peer review:

1. Sharkas, A.R., Ali Sherazi, B., Sayyed, S.A., Kinny, F., Steichert, M., Schwender, H., Läer, S. (2025). Kollaboratives Lernen in Aktion: Innovative Technologien zur Patientensimulation in der Arzneimittelberatung. Medizinische Monatsschrift für Pharmazeuten, 48(1), 19–25.

Oral presentation:

1. Steichert, M., Cawello, W., Breur, J. M., Male, C., Wildt, S. N. de, & Läer, S. (2023). 23 Plasma renin activity in young children with heart failure: influence of age, disease and ACE inhibitor treatment. Archives of Disease in Childhood, 108(6), A8.1-A8. https://doi.org/10.1136/archdischild-2023-esdppp.23
   19th European Society for Developmental, Perinatal and Paediatric Pharmacology Congress, 2022, Liverpool, United Kingdom

Poster presentation:

1. Steichert, M., Cawello, W., Suessenbach, F.K., Laeer, S. (2025). Angiotensin II/Angiotensin I Ratio as Pharmacodynamic Parameter in Healthy Adults and Children with Heart Failure Treated with Enalapril: Insights from an Adult Population Pharmacokinetic-Pharmacodynamic Model and the LENA Studies. Paediatric Drugs, 27(4), 503–532. https://doi.org/10.1007/s40272-025-00705-62025
   21st European Society for Developmental, Perinatal and Paediatric Pharmacology Congress, 2025, Nijmegen, the Netherlands

2. Steichert, M., Laeer, S., & Cawello, W. (2024). Abstract 4141312: Safe Initial Dosing of Innovative Enalapril Orodispersible Minitablets in Newly Treated Newborns, Infants and Young Children with Heart Failure – Results of a Population Pharmacokinetic Analysis of the LENA Trials. Circulation, 150(Suppl_1). https://doi.org/10.1161/circ.150.suppl_1.4141312
   American Heart Association Scientific Sessions, 2024, Chicago, United States of America

3. Faisal, M., Steichert, M., Cawello, W., Laeer, S., & and the LENA consortium. (2023). Enalapril orodispersible minitablets for pediatric heart failure - a population pharmacokinetic analysis from multicenter Phase II/III LENA clinical trials. www.page-meeting.org/?abstract=10341
   31st Population Approach Group Europe Meeting, 2023, A Coruña, Spain