The use of metformin in patients with renal impairment

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ABSTRACT

Metformin, a biguanide, is widely accepted to be the preferred first-line oral antihyperglycaemic agent to manage type 2 diabetes. There is considerable concern that patients receiving metformin therapy may be at an increased risk of developing lactic acidosis. The risk has traditionally been assumed to be increased in patients with chronic renal impairment, resulting in many patients being denied access to an effective first-line treatment agent. The overarching aims of this thesis were to explore the safe use of metformin and to create a renal dosing guideline that will mitigate the risk of lactic acidosis.

The safe use of metformin was explored by formally evaluating the association between metformin therapy and lactic acidosis in published case reports of metformin associated lactic acidosis (MALA) using two causality assessments. Metformin was found to play only a possible role in the development of lactic acidosis based on the results from the causality assessments. Almost all cases presented with other risk factors that could on their own have caused lactic acidosis.

A subgroup analysis was performed in MALA cases with a history of chronic renal impairment to explore the relationship between metformin dose, plasma concentration and lactic acidosis. Most cases presented with acute renal failure, confounding the relationship between metformin dose and plasma concentrations. The prescribed metformin dose exceeded the dosing recommendations in over 60% of cases with an estimated glomerular filtration rate of <60 mL/min by a median of 1000 mg/day. Despite this, based on simulations, the pre-admission plasma metformin concentrations measured pre-dose did not exceed the proposed upper limit of the therapeutic range of 5 mg/L.

A quantitative analysis was performed to explore the relationship between plasma metformin and lactate concentrations. Plasma metformin concentrations greater than 4.5 mg/L were found to be associated with severe hyperlactatemia. These findings suggest that metformin doses should be adjusted to maintain plasma concentrations below 4.5 mg/L to mitigate the risk of lactic acidosis.

A noncompartmental pharmacokinetic analysis was performed to explore the pharmacokinetics of metformin in renal impairment, from which an empirical renal dosing guideline for metformin was developed. Patients with poorer renal function were found to have lower apparent and renal clearance for metformin. These findings support the notion that metformin can be used safely for the treatment of type 2 diabetes mellitus in patients with chronic renal impairment provided plasma metformin concentrations are maintained within a safe therapeutic range.

A population pharmacokinetic model for metformin was developed and evaluated. A covariate analysis found that renal function and total body weight could describe patient variability in the apparent clearance and central compartment volume for metformin, respectively. The developed population pharmacokinetic model was used to assess the safety of the empirical renal dosing guidelines and the current published renal dosing guidelines in the New Zealand Formulary. Based on the simulations, plasma metformin concentrations are not expected to exceed the upper limit of safety of 4.5 mg/L under either of the dosing guidelines.

The influence of flip-flop pharmacokinetics in population pharmacokinetic models was explored using metformin as a motivating example. Approaches to address problems arising due to flip-flop in population pharmacokinetic models are presented in this thesis.

In conclusion, the findings in this thesis support the notion that metformin can be used safely for the treatment of type 2 diabetes mellitus provided plasma metformin concentrations are maintained within a safe range. In addition, an empirical renal dosing equation for metformin was developed and assessed for safety.

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PUBLICATIONS

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Oral Presentations

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GLOSSARY

ABBREVIATIONS

ADR	Adverse drug reaction
AIC	Akaike information criterion
AKI	Acute kidney injury
AMP	Adenosine monophosphate
ANZCTR	Australian and New Zealand Clinical Trials Registry
ARF	Acute renal failure
ATP	Adenosine triphosphate
BD	Twice a day
BMI	Body mass index
BQL	Below the quantification limit
BSV	Between subject variability
cAMP	Cyclic adenosine monophosphate
CI	Confidence interval
CKD	Chronic kidney disease
CLcr	Creatinine clearance
$C_{p,ss,ave}$	Steady-state plasma average concentration
$C_{p,ss,max}$	Steady-state plasma maximum concentration
$C_{p,ss,trough}$	Steady-state plasma trough concentration
CV	Coefficient of variation
CWRES	Conditional weighted residuals
DPP-4	Dipeptidyl peptidase 4
DV	Dependent variable
EBE	Empirical Bayesian estimates
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
FBPase	Fructose-1,6-biphosphatase
FFM	Fat free mass
GFR	Glomerular filtration rate
HbA1c	Glycated haemoglobin
IBW	Ideal body weight
IPRED	Individual predicted values
IV	Intravenous administration
KDIGO	Kidney Disease: Improving Global Outcomes
MALA	Metformin associated lactic acidosis
MATE1	Multidrug and toxin extrusion protein 1
MATE2-K	Multidrug and toxin extrusion protein 2-K
MeSH	Medical Subject Heading
MDRD	Modification of Diet in Renal Disease
mGFR	Measured glomerular filtration rate
mGPD	Mitochondrial glycerophosphate dehydrogenase
NCA	Noncompartmental analysis
NZF	New Zealand Formulary

OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
OCT3	Organic cation transporter 3
OD	Once a day
OFV	Objective function value
pCO2	Partial pressure of carbon dioxide
PD	Pharmacodynamics
pcVPC	Prediction corrected visual predictive check
PO	Oral administration
pO2	Partial pressure of oxygen
PK	Pharmacokinetics
pK_a	Acid dissociation constant
PKA	Protein kinase A
PMAT	Plasma membrane monoamine transporter
PRED	Model prediction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RSE	Relative standard error
RUV	Residual unexplained variability
se	Standard error
SGLT-2	Sodium glucose co-transporter 2
TID	Three times a day
VPC	Visual predictive check
WHO-UMC	World Health Organisation-Uppsala Monitoring Centre system for standardised case causality assessment

GLOSSARY

INDICIES AND SYMBOLS

A	Amount of drug in a compartment
AUC	Area under the plasma concentration curve
AUC_{0-24}	Area under the plasma concentration curve from time zero to 24 hours
$AUC_{0-\infty}$	Area under the plasma concentration curve from time zero to infinity
AUC_{0-last}	Area under the plasma concentration curve from time zero to time of last observed concentration above the limit of quantification
$AUC_{last-\infty}$	Area under the curve from time of last observed concentration above the limit of quantification to infinity
BMI	Body mass index
C	Concentration
C_{50}	Concentration at which a drug exhibits 50% of its maximum response
C_{last}	Last observed concentration above the limit of quantification
CL	Clearance
CL/F	Apparent clearance
$CLcr_{CG}$	Creatinine clearance estimated using the Cockcroft and Gault equation
CL_{renal}	Renal clearance
C_{max}	Maximum plasma concentration
CO_2	Carbon dioxide
D	Dose of drug administered
DI	Dose interval
E	Drug effect
E_0	Physiological baseline effect
$eGFR_{MDRD}$	Glomerular filtration rate estimated using the 4-variable Modification of Diet in Renal Disease equation
$eGFR_{CKDEPI}$	Glomerular filtration rate estimated using the Chronic Kidney Disease Epidemiology Collaboration equation

E_{max}	Maximum effect of a drug
F	Bioavailability
$f(\ \ )$	Mathematical function
$g(\ \ )$	Mathematical function
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
H_2CO_3	Carbonic acid
H_2O	Water
k	Elimination rate constant
k_a	Absorption rate constant
MDR	Maintenance dosing rate
$mGFR$	Measured glomerular filtration rate
p	Number of parameters
Q	Intercompartmental clearance
S_{cr}	Serum creatinine concentration
t	Time
$t_{1/2}$	Terminal half-life
TBW	Total body weight
T_{max}	Time at which maximum plasma concentration occurs
U	Concentration of renal function marker in urine
V	Volume of distribution
V_c/F	Apparent volume of the central compartment
V_p	Peripheral compartment
V_{urine}	Volume of urine excreted
wt	Weight
x	Independent variable
Y	Dependent variable
y	Dependent variable (observation)
Z	p-by-z vector of covariates
β	p-by-1 vector of individual parameters
ε	Residual unexplained variability or error
ε_{add}	Residual additive error
ε_{prop}	Residual proportional error

η	Random deviation of an individual's parameter estimate from the population typical value
λ	Hill coefficient
λ_z	Terminal elimination slope
Ω	Variance-covariance matrix of between subject variability
ω^2	Variance of between subject variability
σ^2	Variance of residual error
Θ	Fixed effect parameter
θ_{PD}	Pharmacodynamic parameter
θ_{PK}	Pharmacokinetic parameter

Chapter 1: Introduction

1.1. Introduction to the thesis

Each patient has the right to be treated with respect, privacy and services that take into account their individual needs, values and beliefs. In clinical practice, when pharmacological therapy is indicated, this involves upholding the biomedical ethical principles of beneficence (to do good) and non-maleficence (to avoid harm) through the selection of the right medication prescribed at the right dose and right time for the right patient. To achieve this goal of providing patients with the best care it requires that appropriate steps are taken to optimise medicines-related health outcomes for the patient as a fundamental principle.

Type 2 diabetes mellitus is a chronic metabolic condition characterised by hyperglycaemia due to an inadequate insulin response. The global prevalence of type 2 diabetes mellitus is increasing with a more rapid increase in low and middle income countries [1-4]. Chronic hyperglycaemia is recognised to result in a wide range of long term microvascular and macrovascular complications, including cardiopathy, nephropathy, retinopathy and neuropathy [5, 6]. The risk of hyperglycaemia related complications increases with the duration of diabetes and poor glycaemic control [7], highlighting the importance of effective glucose control in diabetic patients.

Metformin is an oral antihyperglycaemic agent widely regarded as the first line pharmacological treatment for type 2 diabetes mellitus. Metformin therapy may have several advantages over other antidiabetic treatments (e.g. insulin, sulfonylureas, thiazolidinediones), including a lower propensity to cause weight gain, a lower incidence of hypoglycaemia as well as a reduced risk of diabetes related cardiopathy, nephropathy, neuropathy and all-cause mortality [5, 8-11]. These benefits have been reported to persist in patients with a glomerular filtration rate as low as 30 mL/min/1.73m^2 [10], highlighting the usefulness of metformin therapy in patients with renal impairment.

There is concern that patients receiving metformin therapy may be at an increased risk of developing lactic acidosis – a rare but life-threatening metabolic condition. The risk has traditionally been assumed to be increased in patients

with renal impairment, resulting in many patients being denied access to an effective treatment. In the setting of acute overdose metformin is recognised to cause lactic acidosis [12, 13]. However, the relationship between the therapeutic use of metformin at usual doses and the risk of lactic acidosis is poorly understood. Furthermore, the relationship between metformin exposure and lactic acidosis risk is not well established.

In the setting of renal impairment, the use of standard doses is expected to lead to elevated plasma metformin concentrations [11]. It is suggested that the subsequent elevated metformin exposure will consequently result in an increased risk of lactic acidosis. While dose reduction should mitigate this risk, current renal dosing guidelines for metformin lack consensus and none appear to be evidence-based [14-16]. The result is considerable confusion for prescribers about the best practice for prescribing metformin to achieve plasma concentrations that are safe and effective, whilst mitigating the risk of adverse events. In this thesis, a systematic approach will be employed to understand how to safely dose metformin in patients with renal impairment. The evidence-base will be explored to better understand lactic acidosis risk, the influence of renal function on this risk, and to determine the safety limit for metformin plasma concentrations. Pharmacokinetic analyses will be conducted to understand metformin handling in renally impaired patients and to predict doses that will mitigate the risk of harm.

1.1.1. Aims of the thesis

The overarching goals of this thesis were to explore the safe use of metformin and to create a renal dosing guideline that will mitigate the risk of lactic acidosis. The specific objectives were to:

1. Investigate the causal role of metformin therapy in the development of lactic acidosis (Chapter 2)
2. Explore the role of pre-existing renal impairment in the development of metformin associated lactic acidosis in published case reports (Chapter 3)
3. Explore the association between metformin concentrations and the risk of lactic acidosis using lactate as a marker of lactic acidosis risk (Chapter 4)
4. Explore the pharmacokinetics of metformin in renal impairment (Chapter 5)
5. Explore the concentration time profile of metformin in renal impairment (Chapter 6)
6. Develop a population pharmacokinetic model for metformin and simulate different metformin dosing regimens in patients with varying degrees of renal function (Chapter 7)
7. Explore the absorption dependent pharmacokinetics of metformin (flip-flop) in population pharmacokinetic models (Chapter 8)

1.1.2. Overview of the introduction

The introduction chapter to the thesis is divided into four sections:

- Section 1.2 Metformin
- Section 1.3 The renal system
- Section 1.4 Acid-base homeostasis
- Section 1.5 Pharmacometrics

1.2. Metformin

Metformin is an oral antihyperglycaemic agent that is widely accepted as the first-line pharmacological treatment for type 2 diabetes mellitus. Metformin is part of the biguanide family and was developed from galegine, a guanidine derivative found in *Galega officinalis* [17, 18]. It works predominantly by inhibiting hepatic gluconeogenesis [19, 20]. In the clinical setting metformin is the preferred agent because unlike other anti-hyperglycaemic agents available it has a lower propensity to induce weight gain and is associated with a lower incidence of hypoglycaemia [9, 21, 22].

1.2.1. History of metformin

Figure 1.1 *Galega offinalis plant*

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The herbal lineage of metformin descents from the use of the plant *Galega officinalis* (shown in Figure 1.1), also known as French lilac, goat's rue, Italian fitch, professor weed and Spanish sainfoin [17, 18]. *Galega officinalis* was first used and indicated as a traditional medicine in medieval Europe for epilepsy, fever, pestilence and worms. It was only in 1772 that *Galega officinalis* was first recommended by John Hill to treat symptoms of diabetes – in particular thirst and urination [17].

Chemical analyses from the mid 1800's revealed *Galega officinalis* to be rich in guanidine and other related compounds, such as monoguanidines, diguanidines and biguanides. In 1918 guanidine was reported to reduce blood glucose concentrations in animals. Further investigations on galegine (a monoguanidine) and diguanidines during the 1920's were similarly found to exhibit an anti-hyperglycaemic effect in animals. Disappointingly, the therapeutic use of these compounds were restricted by their toxic effects [17].

The chemical genesis of metformin began in the 1800's. During the 1840's to 1860's Adolph Streker worked on the chemical synthesis of guanidine. Successive work conducted by Bernhard Rathke in 1879 focussed on fusing two guanidines to form a biguanide. However, it was only in 1922 that metformin was chemically synthesised by Werner and Bell. Despite the chemical structural similarities to monoguanidines and diguanidines, metformin and other biguanides were shown to exhibit antihyperglycaemic effects in rabbits and dogs, and, were reportedly less toxic than the monoguanidine and diguanidine moieties [17].

In 1957 Jean Sterne published an article describing the use of metformin to treat diabetes. Following this, metformin was first introduced in the United Kingdom and other European countries for the treatment of diabetes in 1958. However, it was only in 1994 that metformin was approved for use in the United States of America and introduced in 1995 [17].

1.2.2. Therapeutic use

Metformin is indicated as an antihyperglycaemic agent for the treatment of type 2 diabetes mellitus in adults and children over 10 years of age [23, 24]. It may also be used in combination therapy with sulfonylureas, DPP-4 inhibitors and SGLT-2 inhibitors [23]. Metformin is also accepted as an adjuvant therapy in type 1 diabetes mellitus (i.e. insulin-dependent diabetes), particularly in obese patients [25]. Metformin therapy is used in some patients with anovulatory infertility due to polycystic ovary syndrome (an unapproved indication) [23, 24].

1.2.3. Physicochemical properties

Metformin (1,1-dimethylbiguanide) is a guanidine derivative. The chemical formula of metformin is $C_4H_{11}N_5$ (structure shown in Figure 1.2) and its molecular weight is 129.17 g/mol. The guanidine moiety in the chemical structure of metformin is a strong base and has an acid dissociation constant value (pK_a) of 11.5 [18, 26]. Under physiological conditions metformin exists as a hydrophilic, cationic form (99% ionised) [18, 26]. Metformin exhibits poor lipophilicity with a logP value of -1.43 [18].

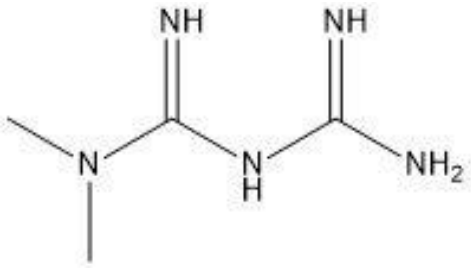

Figure 1.2 *Chemical structure of metformin*

1.2.4. Pharmacokinetics

1.2.4.1. Absorption

The majority of metformin absorption is reported to take place within 6 hours of drug administration [27]. The duodenum is reported to be the predominant site of metformin absorption, however, the entire small intestine is involved and vital for sufficient absorption [27, 28]. Only approximately 13 percent of the metformin dose is reported to be absorbed by the stomach over a 4 hour period [28]. The absorption of metformin from the gastrointestinal tract is incomplete with mass balance studies reporting around 30% of the drug recovered in the faeces [27, 29]. The absorption half-life of metformin is reported to be 2.63±0.18 hours [29].

The bioavailability of metformin is highly variable and has been reported to range between 0.32 and 0.61 [27, 29, 30]. An increase in the dose of metformin ingested has been associated with a decrease in bioavailability [27, 31]. In

addition, concomitant food intake was found to decrease the bioavailability of metformin, whereby the time to peak metformin concentration was prolonged [31].

1.2.4.2. Distribution

The apparent volume of distribution of metformin has been reported to range from 63 to 276 litres [27, 29, 30]. Metformin has not been found to bind to plasma proteins [27, 29, 30]. Metformin has been shown to distribute and accumulate in the liver, kidney, ureter, bladder, salivary glands, skeletal muscle, stomach, small intestine and red blood cells [18, 26, 32]. No uptake of metformin into the gallbladder, myocardium and brain has been observed [32]. An illustration of the main transporters involved in the distribution of metformin is shown in Figure 1.3.

Liver. The hepatic uptake of metformin occurs rapidly post drug administration resulting in significant accumulation of the drug in the liver [32]. Uptake of metformin into hepatocytes is mediated by organic cation transporter 1 (OCT1) and postulated to be mediated by organic cation transporter 3 (OCT3) [18, 20, 32-36]. Both transporters, OCT1 and OCT3, are located on the basolateral membrane [18, 33-35]. Multidrug and toxin extrusion protein 1 (MATE1) had previously been suggested to be involved in the transport of metformin from the hepatocyte to the biliary ducts [35], however, following a radiation and dosimetry biodistribution study of metformin, no uptake of metformin into the gallbladder has been observed [32, 33].

Kidney, ureter and bladder. Significant and rapid uptake, accumulation and excretion of metformin in the kidneys, ureter and bladder has been reported. The renal uptake of metformin from the blood to the renal epithelial cell has been shown to be mediated by organic cation transporter 2 (OCT2) and possibly OCT1 [18, 20, 32, 33, 35, 36]. Elimination of metformin by the kidneys into urine is mediated by MATE1 and multidrug and toxin extrusion protein 2-K (MATE2-K)

transporters [20, 33, 35, 36]. Reabsorption of metformin from the renal tubules into the renal epithelial cell is suggested to occur via the transporters OCT1 and plasma membrane monoamine transporter (PMAT) [32, 33].

Salivary glands. Metformin has been reported to accumulate in the salivary glands [32].

Skeletal muscle. The distribution of metformin into skeletal muscles occurs slowly [32]. Accumulation of metformin in skeletal muscle was minimal with concentrations being slightly greater than that of plasma [32].

Stomach and intestine. Following oral administration metformin was found in the stomach and intestine. A slow, steady accumulation of metformin was noted in the intestine. This has been suggested to be due to the low transport capacity of the basolateral membrane transporters [32]. The uptake of metformin into the enterocyte from the gastrointestinal lumen has been shown to be mediated by PMAT and is postulated to be mediated by OCT3 [18]. Whereby, the transport of metformin from the enterocyte into the systemic circulation has been postulated to be mediated by OCT1 [18].

1.2.4.3. Metabolism

Metformin has not been reported to undergo metabolism [29, 30].

1.2.4.4. Elimination

Metformin is predominantly cleared as unchanged drug by the kidneys [27, 29, 30]. The total plasma clearance of metformin was found to be similar to its renal clearance and is approximately four to five times creatinine clearance [27, 29]. It has been suggested that 80% of metformin is cleared via tubular secretion and the remaining 20% by glomerular filtration [32]. Pharmacokinetic studies in humans have shown that the terminal elimination phase of metformin was dissimilar following intravenous and oral administration. Following

intravenous administration the elimination half-life of metformin was reported to lie between 1.5 to 1.7 hours [29, 30], whilst following oral administration the half-life of metformin has been reported to range from 4 to 20 hours [18, 26]. These findings suggest that metformin exhibits flip-flop pharmacokinetics – the term 'flip-flop' is used to describe the scenario where the rate constant of absorption and elimination can swap around for extravascularly administered drugs. In addition, following an intravenous dose, metformin was completely recovered from urine 48 hours post administration with no traces of the drug found in the faeces [29].

1.2.4.5. The role of genetic variation in drug transporters as a contributing factor for variability in the pharmacokinetics of metformin

Genetic variation in drug transporters involved in the absorption, distribution and elimination of metformin have been reported to be a source of variability in the pharmacokinetics of metformin.

Polymorphisms in OCT1 have been associated with variations in the pharmacokinetic profile of metformin [37, 38]. In a study in healthy Caucasian-American volunteers an increase in the area under the concentration time curve and peak concentration and, decrease in the apparent volume of distribution was demonstrated in volunteers carrying reduced-function OCT1 alleles (G401S, G465R, M420del and R61C) relative to individuals carrying the reference allele [37]. Genetic variation in the OCT1 allele may have an influence on renal clearance, however more evidence is required to fully elucidate the impact of genetic variations in OCT1 on renal clearance [38, 39].

OCT2 has been shown to mediate the renal uptake of metformin from the systemic circulation to the renal epithelial cell. Hence, genetic variants in OCT2 are anticipated to influence the renal clearance of metformin. Several studies have associated specific genetic variants in OCT2 with changes in renal clearance and drug exposure. Three nonsynonymous single nucleotide polymorphisms (T199I, T201M and A270S) were associated with decreased metformin renal clearance and increased exposure, whereby, the renal clearance of metformin was found to decrease with each copy of the A270S variant of OCT2 [40-42].

MATE1 and MATE2-K are involved in the renal elimination of metformin. Polymorphisms in MATE1 and MATE2-K have been reported to contribute to variation in the disposition of metformin in healthy volunteers and patients with type 2 diabetes mellitus [43].

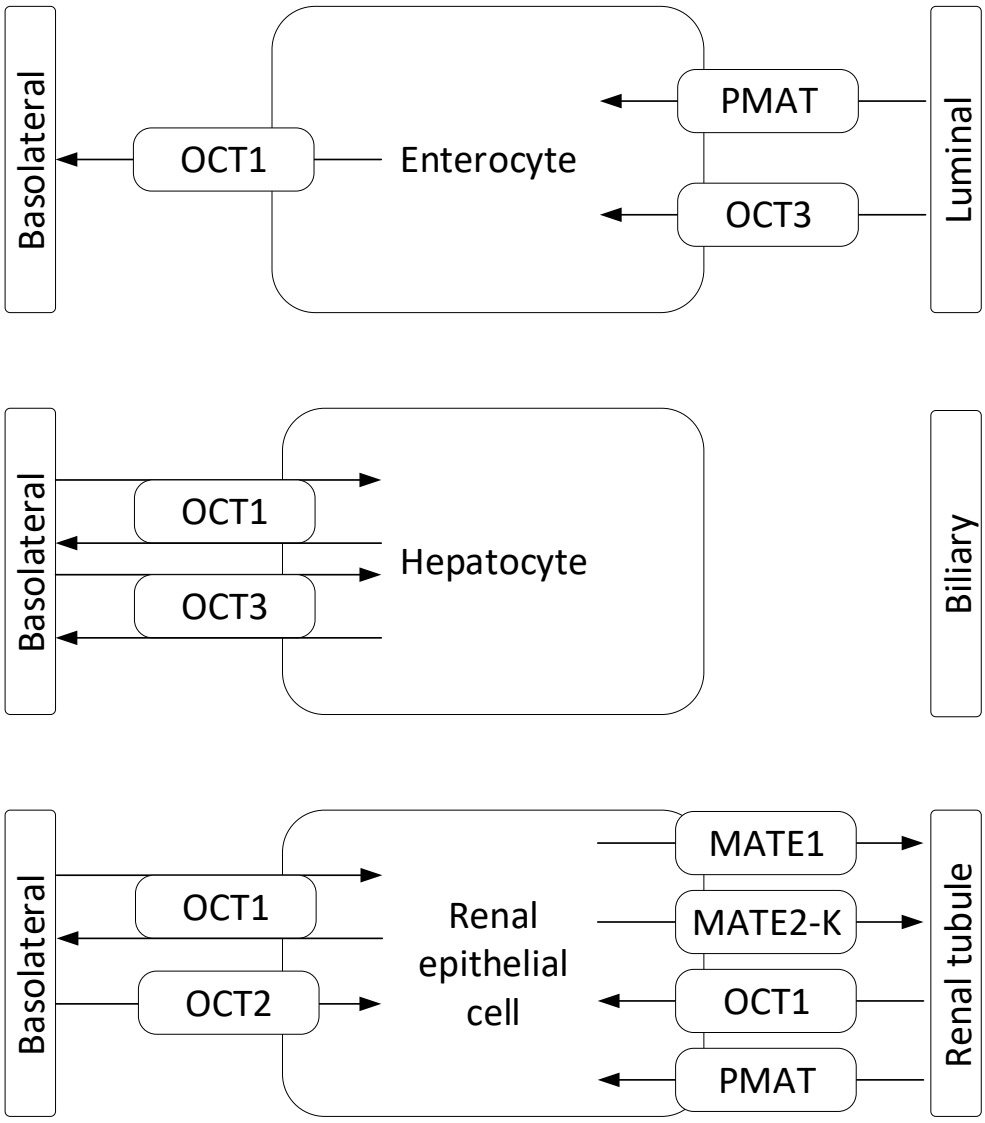

Figure 1.3 *Summary of the transporters involved in the distribution of metformin into and out of enterocytes, hepatocytes and renal epithelial cells. The arrows represent the movement of metformin via a transporter. In the diagram OCT1 is organic transporter 1, OCT2 is organic transporter 2, OCT3 is organic transporter 3, PMAT is plasma membrane monoamine transporter, MATE1 is multidrug and toxin extrusion 1, and, MATE2-K is multidrug and toxin extrusion 2-K.*

1.2.5. Pharmacodynamics

Metformin predominantly exerts its antihyperglycaemic effect by inhibiting hepatic gluconeogenesis and enhancing insulin suppression of endogenous glucose production [20]. To a lesser extent, metformin may also exert its effect by decreasing intestinal glucose absorption and possibly improving glucose uptake and use by peripheral tissues [20].

Metformin is actively transported into hepatocytes predominantly via OCT1 and to a lesser extent OCT3, where it goes on to partially inhibit mitochondrial respiratory-chain complex 1 [20]. This results in an accumulation of adenosine monophosphate (AMP) and a reduction in the concentration of adenosine triphosphate (ATP) [20]. This subsequent change and increase in the ratio of AMP to ATP limits hepatic gluconeogenesis by the following mechanisms: (i) decreased ATP concentrations result in an ATP deficit which otherwise would be required for glucose synthesis to occur, (ii) increased AMP concentrations resulting in reduced activity of fructose-1,6-bisphosphatase (FBPase) - a critical enzyme involved in gluconeogenesis, (iii) inhibition of mitochondrial glycerophosphate dehydrogenase (mGPD) resulting in an altered redox state as well as a decreased conversion of glycerol to glucose, and (iv) inhibition of adenylate cyclase cyclic AMP (cAMP) to protein kinase A (PKA) signalling [20]. In addition, the metformin caused change in the AMP to ATP ratio results in the activation of AMPK which ultimately goes on to suppress lipogenesis and exerts insulin sensitising effects [20].

1.2.6. Dosing recommendations

Metformin is available as an oral tablet in an immediate and controlled release formulation. It is also available in fixed-dose combinations containing dipeptidyl peptidase-4 inhibitors, sodium-glucose co-transporter 2 inhibitors and sulfonylureas. Note that only the 500 mg and 850 mg immediate release metformin tablets available in New Zealand are discussed in this thesis.

The dosing recommendations for metformin are indication specific. For the treatment of diabetes mellitus the usual starting dose for a patient initiating an

immediate release formulation of metformin is 500 mg once to thrice daily [23, 24]. This is followed by slow dose titration as tolerated by the patient to the maximum daily dose to achieve desired glucose control [23]. The usual starting dose for patients initiating a controlled release formulation of metformin is 500 mg once daily with slow dose titration [23]. For the treatment of polycystic ovary syndrome patients are initiated at 500 or 850 mg of metformin (immediate release) taken once daily with gradual weekly dose titration from once to thrice daily as tolerated [23]. The defined daily dose for metformin is 2,000 mg taken via oral administration.

1.2.6.1. Renal dosing recommendations

The safe use of metformin in patients with renal impairment is not well established. This is largely because until recently metformin was contraindicated in renally impaired patients with a creatinine clearance (CLcr) of <60 mL/min or a serum creatinine ≥1.5 mg/dL (≥132.6 μmol/L) in males and ≥1.4 mg/dL (≥123.8 μmol/L) in females [44, 45]. Recent work has proposed that metformin might be safe to use in patients with a CLcr of 30 mL/min or even as low as 15 mL/min [24, 46, 47]. Table 1.1 presents a summary of several renal dosing guidelines for metformin published worldwide showing a lack of consensus about the best dosing strategy in this patient group.

Table 1.1 *Summary of metformin renal dosing guidelines and contraindications published worldwide*

Country	Ref	Year	Renal estimation method	Renal dose adjustment		Renal contraindication
				Renal function	Recommended dose	
Australia	[44]	2016	CLcr			<60 mL/min
	[48]	2016	eGFR	30-60 mL/min/1.73m^2	Use with caution Reduce dose	<30 mL/min/1.73m^2
	[49]	2016	CLcr	60-90 mL/min 30-60 mL/min	Maximum: 2000 mg/day Maximum: 1000 mg/day	<30 mL/min
	[23]	2017	CLcr	<90 mL/min 15-30 mL/min	Reduce maximum dose Metformin may be considered for patients with stable renal function	15 mL/min
Canada	[50]	2015	eGFR	<60 mL/min	Reduce dose	-
	[51]	2015	eGFR	30-59 mL/min ≥60 mL/min	Reduce dose No dose adjustment	≤29 mL/min
	[47]	2016	CLcr eGFR	<60 mL/min	Caution	<30 mL/min
New Zealand	[52]	2014	CLcr	60-120 mL/min 30-60 mL/min 15-30 mL/min	Maximum: 2000 mg/day Maximum: 1000 mg/day Maximum: 500 mg/day	<15 mL/min

United States of America	[53]	2015	CLcr	60-120 mL/min	Maximum: 2000 mg/day	<15 mL/min
				30-60 mL/min	Maximum: 1000 mg/day	
				15-30 mL/min	Maximum: 500 mg/day	
	[54]	2015	-	60-120 mL/min	Maximum: 2000 mg/day	<15 mL/min
				30-60 mL/min	Maximum: 1000 mg/day	
				15-30 mL/min	Maximum: 500 mg/day	
	[55]	2015	eGFR	>90 mL/min/1.73m^2	Maximum: 3000 mg/day	Quantitative – *significant impairment or renal failure*
				60-90 mL/min/1.73^2	Maximum: 2000 mg/day	
				30-60 mL/min/1.73^2	Maximum: 1000 mg/day	
				<30 mL/min/1.73^2	Discuss with specialist	
	[56]	2016	CLcr	60-120 mL/min	Maximum: 2000 mg/day	<15 mL/min
				30-60 mL/min	Maximum: 1000 mg/day	
				15-30 mL/min	Maximum: 500 mg/day	
	[57]	2017	eGFR	60-120 mL/min/1.73m^2	Maximum: 2000 mg/day	<15 mL/min/1.73m^2
				30-60 mL/min/1.73^2	Maximum: 1000 mg/day	
				15-30 mL/min/1.73^2	Maximum: 500 mg/day	
	[58]	2016	eGFR	30-45 mL/min/1/73m^2	Not recommended Reassess benefit to risk	30 mL/min/1.73m^2
	[45]				Dose individualisation	Serum creatinine above normal upper limit for age Males: ≥1.5 mg/dL Females: ≥1.4 mg/dL

	[59]	2016	eGFR	30-45 mL/min/1.73m²	Dose reduction	Stop medication if GFR is low
				45-60 mL/min/1.73m²	Consider dose adjustments	
	[46]	2017	eGFR	30-44 mL/min/1.73m²	Consider dose adjustments	<30 mL/min/1.73m²
				<30 mL/min/1.73m²	Referral to a nephrologist	
United Kingdom	[60]	2015	eGFR	45 mL/min/1.73m²	Review dose	30 mL/min/1.73m²
	[61]	2015	CLcr	45-59 mL/min	Initial dose: 500 or 850 mg OD Maximum: 2000 mg/day	-
			eGFR	45-59 mL/min/1.73m²		
				60-89 mL/min	Maximum: 3000 mg/day	
	[62]	2016	GFR	45-59 mL/min	Maximum: 2000 mg/day	<30 mL/min
				30-44 mL/min	Maximum: 1000 mg/day	

1.2.7. Adverse reactions

Mild gastrointestinal adverse effects are very common in patients on metformin therapy (probable incidence: >1% of patients on metformin experience these symptoms) [45, 49, 53, 54]. The gastrointestinal adverse effects include abdominal pain, anorexia, nausea, vomiting and diarrhoea [24, 45, 53, 54]. These side effects are generally transient in nature and resolve on their own with continued therapy [53, 54]. However, these gastrointestinal side effects can be minimised by initiating metformin therapy in patients at a low dose with slow titration, and, taking metformin doses with food [49, 53, 54]. In some patients the gastrointestinal side effects of metformin are severe enough to stop therapy.

Infrequent adverse effects of metformin (probable incidence: 0.1-1%) include skin disorders, such as mild erythemia, puritus and urticarial rash [49, 53, 54]. Rare adverse effects (probable incidence <0.1%) include lactic acidosis (for a more detailed discussion on the relationship between metformin and lactic acidosis refer to section 1.2.7.1.) and acute hepatitis [49, 53, 54].

1.2.7.1. Metformin and lactic acidosis

There is concern that patients receiving metformin therapy may be at an increased risk of developing lactic acidosis, a rare but fatal metabolic condition. Metformin is recognised to cause lactic acidosis when ingested in supra-therapeutic doses, however when taken therapeutically its causal role is not well understood [12, 13, 63]. A more detailed look at the relationship between metformin therapy and lactic acidosis will be presented in Chapter 2.

1.2.8. Contraindications

Metformin is contraindicated in patients with severe hepatic impairment, significant renal impairment, cardiac and/or respiratory insufficiency, any hypoxic conditions, severe infection, alcohol abuse, use of radiographic contrast agents and pregnancy. Patients on metformin therapy should stop metformin prior to surgery and replace it with insulin; metformin should be restarted only when the patient is no longer fasting and renal function recovers to baseline [49].

1.3. The renal system

The renal system, also known as the urinary system, serves a principle role in regulating body homeostasis. In clinical practice, where pharmacological treatment is indicated, an understanding of the renal system is essential as approximately one-quarter of drugs available on the market are almost entirely renally cleared.

In this section a general overview of the anatomy, physiology and pathology of the renal system, as well as the methods used in clinical practice to estimate renal function is described.

1.3.1. Anatomy

The renal system comprises the kidneys, ureters, urinary bladder and urethra [64, 65]. The excretion of urine produced by the kidneys travels through the ureters, urinary bladder and urethra, respectively, prior to being eliminated from the body [64]. In the renal system, the kidneys are the principle functioning organ, whilst the ureters, urinary bladder and urethra are considered to be accessory organs [64]. A schematic of the gross anatomy of the renal system is shown in Figure 1.4.

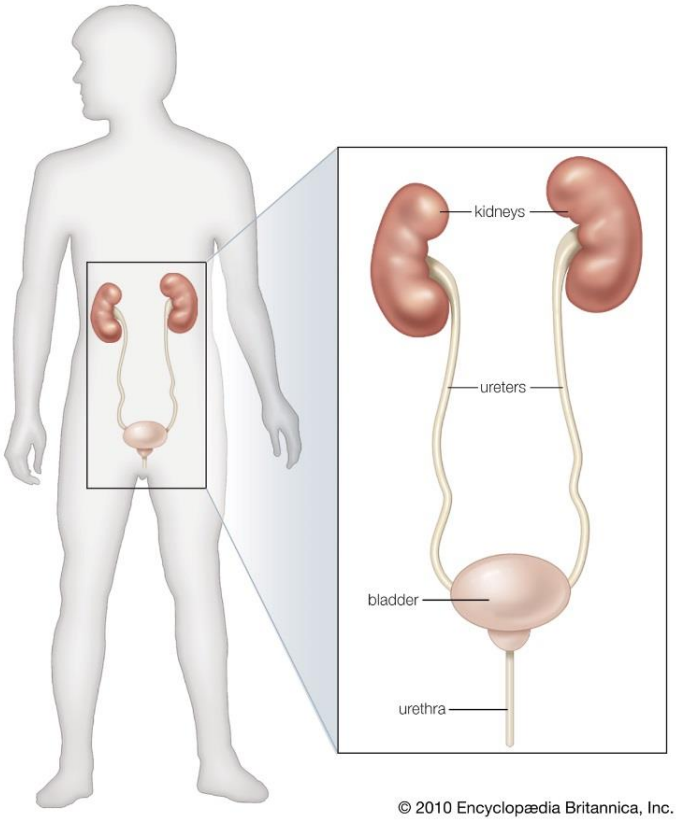

Figure 1.4 *General anatomy of the renal system [66]*

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1.3.1.1. The kidney

The kidneys are paired solid organs that are situated in the retroperitoneal cavity on either side of the vertebral column [64, 67, 68]. The kidneys are encased by a fibromuscular capsule that is surrounded by an additional protective adipose layer, known as peri-renal fat [68]. However, each kidney is held in position in the retroperitoneal space by additional layers comprising of a fibrous capsule of connective tissues known as the renal fasciae, and, a second layer of adipose cushion encasing each kidney known as the pararenal fat [64, 68].

The parenchyma of each kidney comprises two layers, which are: (i) an outer renal cortex and (ii) an inner renal medulla [68]. In the kidney, the renal cortex surrounds individual sections of the renal medulla, known as the renal pyramid [67].

Each kidney comprises approximately one million nephrons, known as the functional unit of the kidney (a schematic of a nephron is shown in Figure 1.5) [65, 68]. An individual nephron comprises two major structures: the renal corpuscle and the renal tubules [65, 68].

The renal corpuscle is the first segment of the nephron [64]. It is situated in the renal cortex and comprises the glomerulus and Bowman's capsule (also known as the glomerular capsule) [64, 65]. The glomerulus is a highly vascularised capillary network that is supplied blood via the afferent arteriole [64, 65, 68]. Blood from the glomerulus returns to the central bloodstream via the efferent arterioles [64, 65, 68]. The glomerular capillaries are made up of a single layer of fenestrated endothelial cells; the increased porosity of the glomerular capillaries are important as it permits filtration to occur at a rate critical for normal kidney function [64]. The Bowman's capsule is a hollow, cup-shaped structure that encloses the glomerulus [64]. The Bowman's capsule is composed of two layers: a parietal (outer) simple squamous epithelium layer and a visceral (inner) podocyte layer [64]. The gap between the glomerulus and the Bowman's capsule is known as the Bowman's space [64, 65].

The renal tubules are a hollow conduit extension of the renal corpuscle [64]. The renal tubules are divided into four main segments: the proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct [64, 68]. The proximal convoluted tubule is a continuous extension of the Bowman's capsule [64, 65]. It is composed of a single layer of epithelial cells with a brush border, formed by microvilli, on its luminal surface to optimise the surface area available for the absorption and secretion of substances between the tubules and bloodstream [64]. The loop of Henle follows the proximal convoluted tubule and comprises three segments [64, 65]. The initial segment comprises an initial thin descending limb that extends into the renal medulla, which then bends back towards the renal cortex in a U-shape manner firstly via the thin ascending limb followed by the thick ascending limb (forming the ascending loop of Henle) [64, 65]. The renal tubules return back to the renal cortex and form the distal convoluted tubules [64, 65].

The collecting duct system follows the distal convoluted tubules – this comprises an initial cortical collecting duct, followed by a medullary collecting duct [64, 65]. From here, several collecting ducts merge together to form a continuous conduit with the ureter [64]. The renal tubules are supplied blood via the peritubular capillaries [64].

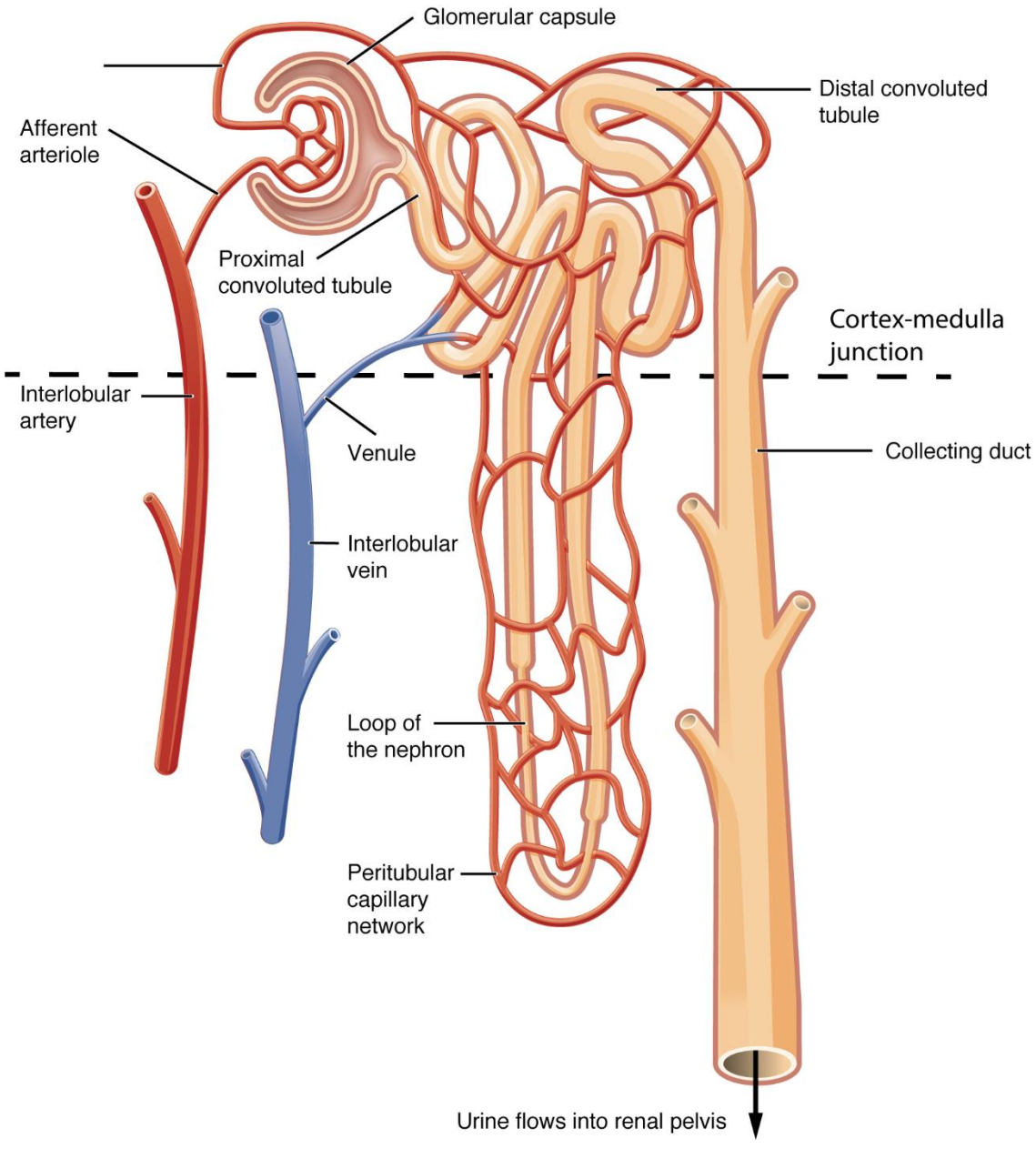

Figure 1.5 *General anatomy of a nephron [69]*

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1.3.1.2. The ureter

The ureters are paired hollow conduits that are responsible for the travel of urine from the kidneys to the urinary bladder [64, 67]. A single ureter is connected to the renal pelvis of each kidney and courses through to the bottom of the urinary bladder [64]. The ureter extends a further two centimetres into the bladder wall and opens at the floor of the bladder at a lateral angle to prevent the backflow of urine [64].

The ureter comprises the following tissue layers: (i) an inner mucosa transitional epithelium, (ii) a smooth muscle middle layer and (iii) a fibrous outer layer [64]. The inner transitional epithelium lining allows the ureter to stretch without injuring the epithelial lining [64] and the smooth muscle layer is important for transport of urine from the kidneys to the urinary bladder by peristalsis [64].

1.3.1.3. The urinary bladder

The urinary bladder serves two primary functions, which are: to serve as a storage reservoir for urine prior to being expelled, and, to expel urine alongside the urethra to outside of the body [64]. The urinary bladder is situated behind the pubic symphysis and below the parietal peritoneum [64].

The urinary bladder has a collapsible bag-like structure with three openings on the floor of the bladder; two openings from the ureters and a single opening into the urethra [64]. The wall of the urinary bladder is predominantly made up of smooth muscle tissue, commonly known as the detrusor muscle [64]. The urinary bladder is lined with mucous transitional epithelium - this inner layer is lined with rugae which allow for the extension and distension of the bladder [64].

1.3.1.4. The urethra

The urethra is a tube-like structure lined with transitional epithelium that extends from the bottom of the urinary bladder to the exterior of the body [64].

The position and structure of the urethra is different for females and males due to the different anatomical structure of the sex-specific organs.

In females the urethra is situated posterior to the pubic symphysis and anterior to the vagina [64]. The urethra extends from the bottom of the urinary bladder and courses through the muscular floor of the pelvis and ends at the external urinary meatus [64]. In males, the urethra extends from the urinary bladder and passes through the center of the prostate gland and penis before ending as the urinary meatus at the distal end of the penis [64]. In males, there are an additional two openings in the urethra - where it connects with the ejaculatory ducts as it passes through the prostate gland [64].

1.3.2. Physiology

The primary function of the renal system is to regulate body homeostasis. This involves the following fundamental physiological processes: (i) excretion, (ii) regulation, (iii) metabolism and (iv) endocrine functions. A description of each of the physiological processes is described below:

Excretion. The renal system is involved in the removal of metabolic (e.g. urea, uric acid and creatinine) and xenobiotic products from the bloodstream and elimination from the body [68]. It is also responsible for the formation of urine.

Regulation. The renal system is involved in the regulation and maintenance of fluid, electrolytes, osmolality, acid-base balance and blood pressure [68].

Metabolism. The renal system is involved in the metabolism of lactate, carbohydrates, proteins, lipids and other nutrients [70].

Endocrine. The renal system plays a fundamental role in the production and secretion of hormones and enzymes, such as erythropoietin and renin [68]. The renal system is also involved in glucose homeostasis via glucose utilisation, gluconeogenesis and glucose reabsorption from the glomerular filtrate [71].

The nephron, the functional unit of the kidney, is involved in the following processes; filtration, tubular reabsorption, tubular secretion and metabolism [65]. Note that glomerular filtration is widely used as an indicator for renal function.

1.3.3. Pathology

Renal impairment is a global health issue with increasing prevalence and health burden [72]. Renal impairment is divided into two broad categories based on the aetiology and duration of renal pathology, which are: (i) acute renal impairment and (ii) chronic renal impairment.

Acute renal impairment. Acute renal impairment is a potentially life-threatening clinical condition that is characterised by an abrupt decline in renal function, resulting in an accumulation of nitrogenous waste products (such as creatinine and urea nitrogen) with or without a decrease in urine output [73]. It is also known as acute kidney injury (AKI) and acute renal failure (ARF) [73]. The pathophysiology of acute renal impairment is described to occur by one or more of the three following mechanisms:

1. **Prerenal acute kidney injury**. This is characterised by a reduced, insufficient blood supply to the kidneys [73]. Common causes of reduced blood delivery to the kidneys include: a depletion in intravascular volume (i.e. haemorrhage, dehydration or gastrointestinal fluid loss), reduced cardiac output (i.e. congestive heart failure and myocardial infarction), hypotension and drugs [73].
2. **Intrinsic acute kidney injury**. Intrinsic acute kidney injury is caused by diseases or direct trauma that damage the integrity of the kidneys [73]. Common causes of intrinsic acute kidney injury include: toxins, drugs ingested in toxic amounts (e.g. aminoglycosides), as well as glomerular, interstitial and blood vessel diseases [73].

3. **Postrenal acute kidney injury**. Postrenal acute kidney injury is a result of an obstruction to urinary outflow [73]. Common causes include: pelvic tumours, benign prostatic hypertrophy and precipitation of renal calculi [73].

Chronic renal impairment. Chronic renal impairment - also known as chronic kidney disease (CKD), progressive kidney disease or nephropathy - is the term used to describe the presence of kidney damage or decreased glomerular filtration rate (GFR) that persists over a period of 3 months or more [73]. Chronic renal impairment is a progressive decline in renal function that is often irreversible and may persist for months to years [73]. The initiation of chronic renal impairment can arise from numerous risk factors, such as diabetes mellitus, hypertension, autoimmune disease, polycystic kidney disease, urinary tract abnormalities (including obstructions, stones and infections), and, drug toxicity [73]. However, it is worth noting that there is a natural decline in renal function following the second to forth decade of life; with an estimated decline in glomerular filtration rate by approximately 8 mL/min/1.73m^2 per each decade of life [74, 75]. Hence, it is possible for individuals to present with chronic renal impairment without presenting with any of the listed initiation risk factors.

The progressive nature of chronic renal impairment highlights the importance of its diagnosis, management and prevention from further deterioration. In clinical practice, chronic renal impairment is commonly classified using the Kidney Disease: Improving Global Outcomes (KDIGO) Chronic Kidney Disease guideline (shown in Table 1.2) into categories defined by a glomerular filtration rate range [76].

Table 1.2 *Chronic kidney disease classification categories published by the Kidney Disease: Improving Global Outcomes guideline [76]*

GFR category	Description	GFR range (mL/min/1.73m^2)
G1	Normal or high	≥90
G2	Mildly decreased	60-89
G3a	Mildly to moderately decreased	45-59
G3b	Moderately to severely decreased	30-44
G4	Severely decreased	15-29
G5	Kidney failure	<15

GFR: represents glomerular filtration rate

1.3.4. Estimating renal function

A reliable estimate of renal function is important in clinical practice and research for the diagnosis of pathology, monitoring of disease progression, and, for further evaluation and management of pathology. In addition, an estimate of renal function is important for the dose adjustment of renally cleared drugs.

1.3.4.1. Markers of renal function

In clinical practice, markers to estimate glomerular filtration rate as well as the use of urinary sediments are commonly used as a means to estimate renal function. A general overview of the markers and urinary sediments used to estimate renal function is described in this section.

1.3.4.1.1. Endogenous markers of renal function

The steady-state serum concentrations of endogenous markers are related to the reciprocal of glomerular filtration rate and hence, are used to estimate glomerular filtration rate. It is important to note that the synthesis and elimination of endogenous markers (e.g. tubular secretion, tubular reabsorption and extra-renal elimination) can change their serum concentrations within and between individuals significantly. Hence, the estimation of renal function using an endogenous serum marker concentration may vary significantly.

In the clinic, the use of endogenous markers is routinely used as it allows for a simple screening test that does not require the administration of an exogenous substance. A brief overview of the endogenous markers commonly used is provided as follows:

Creatinine. Creatinine is an amino acid derivative that is a metabolic breakdown product of creatine phosphate in skeletal muscle [77-79]. Creatinine synthesis is determined largely on muscle mass and dietary intake, which is likely to account for the variation in serum creatinine concentrations seen amongst various age groups, ethnicities and racial groups [77-79]. Creatinine is predominantly eliminated via glomerular filtration with up to fifteen percent being eliminated via active tubular secretion by the proximal tubular cells; hence, creatinine clearance exceeds glomerular filtration rate [77, 78]. The tubular secretion of creatinine varies within and between individuals, particularly in patients with mild to moderate renal impairment [77]. Extrarenal elimination of creatinine may be increased in advanced renal impairment due to increased degradation of creatinine by gastrointestinal bacteria [77, 78]. Note that some drugs, such as trimethoprim and cimetidine, are reported to inhibit creatinine clearance and hence, result in reduced creatinine clearance and elevated serum creatinine concentrations without influencing glomerular filtration rate [77].

Cystatin C. Cystatin C is a non-glycosylated basic protein that is produced at a fairly constant rate by all nucleated cells [77, 79]. It is freely filtered by the glomerulus, and, reabsorbed and catabolised by the tubular epithelial cells [77-79]. However, the serum concentration of Cystatin C is predominantly determined by glomerular filtration rate, making it a possible endogenous marker of glomerular filtration rate [79]. Only small amounts of Cystatin C are excreted in urine, hence its urinary clearance cannot be measured using urine excretion data [77].

Urea. Urea is a nitrogenous end product of amino acid and protein catabolism [79]. It is produced by the liver and is distributed in intracellular and extracellular fluid [79]. Urea is filtered by the glomerulus and is partially reabsorbed with water [79]. The clearance of urea is a poor marker for glomerular filtration rate as its synthesis is dependent on non-renal factors, such as urea cycle enzymes and diet [78, 79].

1.3.4.1.2. Exogenous markers of renal function

The use of exogenous markers is considered the gold standard as it provides a more accurate measure of glomerular filtration. This is because the exact amount of the exogenous marker administered is known and its concentration is not affected by other factors (e.g. physiological processes and/or dietary intake). However, the use of exogenous markers to measure renal function is not routinely used due to complexity associated with the administration and handling of the exogenous markers, cost and, onerous on health practitioners. The use of an exogenous marker of renal function is more commonly used in clinical situations where an accurate estimate of renal function is required (e.g. dosing of prescribed chemotherapy). A brief overview of the exogenous markers commonly used in the clinical practice to measure renal function is outlined as follows:

Inulin. Insulin is a polysaccharide fructose polymer that is found in dahlias, chicory and Jerusalem artichoke [78, 79]. Inulin is an ideal marker for measuring glomerular filtration rate as it is freely filtered at the glomerulus and does not undergo renal tubular reabsorption, secretion and metabolism [78]. However, the clinical use of inulin is limited as it is time consuming and requires a continuous intravenous infusion [78].

Radioisotopes. The use of an exogenous radioisotope is considered the gold standard for measuring glomerular filtration rate. The use of radioisotopes as markers of renal function have advantages over inulin as they do not require a

constant infusion nor do the radioisotopic methods necessitate the need for urine samples. However, the use of exogenous radioisotopes is disadvantageous as it requires additional precautions in handling and discarding of the radioactive substances [78]. Examples of exogenous radioisotopes commonly used in clinical practice include: ^{51}Cr-EDTA, ^{125}Iodine (i)-iothalamate and ^{99}mTc-DTPA.

Radiocontrast agents. The use of radiocontrast agents as markers of renal function have been used since the 1960s [78]. Historically, the use of these agents had been limited due to analytical difficulties and their relatively high amounts of free iodine, making radioisotopes the more favourable exogenous marker of renal function [78]. The majority of these problems have now been solved, with radiocontrast agents providing benefits over radioisotopes as they do not require extra precautions associated with radioactive substances [78]. Examples of exogenous radiocontrast agents used in practice include: iothalamate, siatrixoate meglumin and iohexol.

1.3.4.2. Methods to estimate renal function

For the purposes of this thesis, the methods for determining glomerular filtration rate have been divided into two categories: (i) estimated glomerular filtration rate (eGFR) and (ii) measured glomerular filtration rate (mGFR). An overview of these methods is provided in this section.

1.3.4.2.1. Estimated glomerular filtration rate

Regression equations have been developed to estimate glomerular filtration rate to circumvent the complexity and practical difficulties of formally measuring clearance (e.g. bolus dose followed by constant infusion required for inulin). The developed regression equations are simple straight-forward formulas that allow for routine clinical use. In this thesis, these equation based methods for determining glomerular filtration rate are referred to as estimated glomerular filtration rate (eGFR). An overview of the three more commonly used equations in the clinic (and equations used in this thesis) is given as follows:

Cockcroft and Gault equation. The Cockcroft and Gault equation was developed to predict creatinine clearance from serum creatinine, age, weight and sex [80]. The equation allows for a quick prediction of creatinine clearance using a single blood sample without the need for timed urine samples yielding it more convenient for routine clinical use. The equation is shown as follows;

$$CLcr_{CG}(mL/min) = \frac{(140 - age) \cdot (wt)}{72 \cdot S_{cr}} \cdot [0.85\ if\ female]$$

Equation 1.1 *Cockcroft and Gault equation [80]*

where, $CLcr_{CG}$ is creatinine clearance calculated using the Cockcroft and Gault equation in mL/min, age is age in years, wt is weight in kg and S_{cr} is serum creatinine concentration in mg/L. The Cockcroft and Gault equation was developed in a population of 249 males using their creatinine excretion data. The range of renal function in the study population ranged from 30 to 130 mL/min. Hence, a correction factor was empirically included to allow for its application to females. It is important to note that the Cockcroft and Gault equation systematically overestimates glomerular filtration rate due to the tubular secretion of creatinine. Furthermore, the equation tends to overestimate creatinine clearance in severe renal impairment, elderly and obese. The equation is likely to overestimate creatinine clearance in patients with severe renal impairment due to the equation being developed in patients with normal renal function. In addition, the Cockcroft and Gault equation is likely to overestimate creatinine clearance in obese patients if total body weight is used in the calculation.

Modification of Diet in Renal Disease formula. The Modification of Diet in Renal Disease (MDRD) equation was developed to determine glomerular filtration rate adjusted for body surface area. The MDRD equation was developed in 1,628 patients with chronic kidney disease. The original MDRD equation was referred to as the 6-variable MDRD as it included 6 variables in the

equation, which were: age, sex, race, and, serum concentrations of creatinine, urea and albumin [81]. This equation was later simplified to include 4 variables (i.e. age, sex, race and serum creatinine concentration) and was referred to as the 4-variable MDRD equation [82]. The 4-variable MDRD equation is as follows;

$$eGFR_{MDRD}(mL/min/1.73m^2) = 1.75 \cdot S_{cr}^{-1.154} \cdot age^{-0.203} \cdot [0.742\ if\ female] \cdot [1.212\ if\ African\ American]$$

Equation 1.2 *4-variable Modification of Diet in Renal Disease equation*

where, S_{cr} is serum creatinine concentration in mmol/L and age is age in years. Due to the MDRD equations being developed in patients with chronic renal failure their reliability in estimating glomerular filtration rate in patients with normal or mild kidney disease is questionable. Hence, the use of the MDRD equations is not recommended in patients with an estimated glomerular filtration rate greater than 60 mL/min/ 1.73m^2.

Chronic Kidney Disease Epidemiology Collaboration equation. The Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation was developed to better predict glomerular filtration rate in patients with normal to poor renal function [83]. The equation was developed using data from 8,254 study participants with and without renal impairment (i.e. mean measured glomerular filtration rate ranged from 2 to 190 mL/min/1.73m^2). The CKD-Epi equation predicts glomerular filtration rate from serum creatinine concentration, age and sex. The equation is as follows;

$$eGFR_{CKDEPI}(mL/min/1.73m^2) = 141 \cdot min(S_{cr}/\kappa, 1)^{\alpha} \cdot max(S_{cr}/\kappa, 1)^{-1.209} \cdot 0.993^{age} \cdot [1.018\ if\ female] \cdot [1.159\ if\ black]$$

Equation 1.3 *Chronic Kidney Disease Epidemiology Collaboration equation*

where, S_{cr} is serum creatinine in mg/dL, age is age in years, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and, max indicates the maximum of S_{cr}/κ or 1.

1.3.4.2.2. Measured glomerular filtration rate

In this thesis, the term measured glomerular filtration rate (mGFR) is used to define any method used to determine glomerular filtration rate that involves a pharmacokinetic experiment. The different mGFR methods can be divided into two categories: (i) noncompartmental analysis and (ii) compartmental analysis.

Noncompartmental analysis. Noncompartmental analysis, also known by its acronym NCA, provides a means for analysing pharmacokinetic data that do not make assumptions about hypothetical body compartments. The noncompartmental methods generally involve the use of algebraic equations to estimate pharmacokinetic metrics. Examples of commonly used non-compartmental analyses are described as follows:

- **Area under the plasma concentration-time curve**. The area under the plasma concentration-time curve method requires multiple plasma samples to characterise and describe the concentration time profile of the substance of interest. Using the plasma concentrations glomerular filtration can be determined using the following equation;

$$mGFR = \frac{dose}{AUC_{0-\infty}}$$

Equation 1.4 *Area under the plasma concentration-time curve equation for estimating glomerular filtration rate*

where, $mGFR$ is measured glomerular filtration rate, $dose$ is the dose of the marker of renal function administered to determine glomerular filtration rate, and, $AUC_{0-\infty}$ is the area under the plasma concentration curve from time zero to infinity.

- **Renal clearance of a marker determined using urine and plasma data**. This method involves the collection of urine samples over a period of at least 6 to 12 hours and a single blood sample. Using the collected urine and blood, the concentration of the renal function marker in plasma and urine, as well as the volume of urine excreted over the period of time is used to compute glomerular filtration rate using the following formula;

$$mGFR = \frac{U \cdot V_{urine}}{P}$$

Equation 1.5 *Equation for determine measured glomerular filtration rate using urine and plasma data*

where, $mGFR$ is measured glomerular filtration rate, U is the concentration of the marker of renal function in urine, V_{urine} is the volume of urine excreted and P is the plasma concentration of the marker of renal function.

Compartmental analysis. A compartmental analysis involves fitting a compartmental model to the drug concentration time data using hypothetical compartments. To estimate glomerular filtration rate, the concentration of a marker of renal function is fit to a compartmental model and the estimated clearance of the marker is measured glomerular filtration rate.

1.3.5. Renal drug dosing

Chronic renal impairment alters the clearance of renally cleared drugs and drug disposition, highlighting the importance for dose adjustment in patients with renal impairment to avoid potential drug accumulation, toxicity and/or treatment failure [84]. In patients with chronic renal impairment doses are adjusted according to renal function (calculated as creatinine clearance (CLcr) or glomerular filtration rate (GFR)) [84]. This makes the assumption that renal drug clearance is proportional to GFR (and/or CLcr), and, that the administered drug is entirely renally cleared via glomerular filtration. It is important to bear in mind

that this does not necessarily hold true for all drugs, as many drugs may also be cleared renally via tubular secretion such as metformin. Depending on the pharmacological treatment prescribed, recommended dose adjustment methods include a dose reduction and/or lengthening of the dosing interval [84].

1.4. Acid-base homeostasis

Acid-base homeostasis is important in the regulation and maintenance of pH for physiological processes to occur optimally [85]. In clinical practice acid-base balance is measured using the pH scale. Under normal physiological and steady state conditions a blood pH of 7.35 to 7.46 is required for biological systems to function [86]. To maintain acid-base homeostasis the human body has numerous physiological adaptations. In this section an overview of the physiology and pathophysiology of acid-base homeostasis is described.

1.4.1. Physiology

The homeostatic control of acid-base balance is controlled by intricate buffering systems to maintain physiological pH. In the context of acid-base homeostasis a buffer is defined as a substance that mediates and minimises substantial changes in pH. The two primary control systems involved in maintaining physiological pH are the chemical and physiological buffer systems.

1.4.1.1. Chemical buffer system

The chemical buffer system provides an immediate response to changes in body pH. In the body chemical buffers work by reacting with a relatively strong acid (or base) to transform it into a relatively weaker acid (or base) [64]. Hence, chemical buffers act by preventing drastic changes in physiological pH. In the body most buffers are present in pairs – commonly consisting of a weak acid and a salt of the corresponding weak acid [64]. The key chemical buffer systems (and buffer pairs) in the body include [64]:

- Bicarbonate buffer system
    - Example of buffer pair: $NaHCO_3$ and H_2CO_3
- Phosphate buffer system
    - Example of buffer pair: Na_2HPO_4 and NaH_2PO_4
- Protein buffer system
    - Example of buffer pair: $Na \cdot Proteinate$ and $Proteins$

1.4.1.2. Physiological buffer system

Physiological buffers serve as the secondary control mechanism when chemical buffers do not adequately prevent drastic changes to physiological pH. The respiratory and renal system are the main systems involved in regulating acid-base homeostasis in the physiological buffer system.

Respiratory system. Respiration plays a fundamental role in maintaining pH. Cellular metabolism processes in the body produce large amounts of carbon dioxide daily [86]. The produced carbon dioxide can go on to form hydrogen ions in red blood cells, resulting in the following reaction;

$$CO_2 + H_2O \xrightarrow{carbonic\ anhydrase} H_2CO_3 \rightarrow H^+ + HCO_3^-$$

Equation 1.6 *An equation describing the production of hydrogen ions from carbon dioxide*

where, CO_2 is carbon dioxide, H_2O is water, H_2CO_3 is carbonic acid, H^+ represents hydrogen ions and HCO_3^- is bicarbonate [64]. The process of expiration results in carbon dioxide and water leaving the body in expired air [64]. The carbon dioxide used in this process diffuses from venous blood in the lungs, resulting in lower concentrations of carbon dioxide in arterial blood leaving the lung capillaries [64]. Hence, in the setting of hyperventilation there is a net loss of carbon dioxide and hydrogen ions in the body which may result in an increase in arterial pH. Whilst in the setting of hypoventilation there is a net retention in carbon dioxide and hydrogen ions in the body which may lead to a decrease in arterial pH.

Renal system. The renal system regulates pH by reabsorbing and excreting substances that influence acidity or basicity. In the nephron, carbon dioxide diffuses from the tubule capillaries into renal tubule cells where it reacts with water to produce carbonic acid, which then dissociates to form bicarbonate and

hydrogen ions - note that this is the same reaction from which carbon dioxide produces hydrogen ions in red blood cells (shown in Equation 1.6)[64]. From here, the produced bicarbonate ions then travel down its concentration gradient from the tubular epithelial cells into the blood, whilst the hydrogen ions are secreted into the lumen [64]. In the lumen the hydrogen ions may combine with a bicarbonate buffer or a non-bicarbonate buffer (such as a phosphate buffer) [64]. Hydrogen ions that combine with a bicarbonate buffer produce both carbon dioxide and water which may be excreted or reabsorbed by the renal tubules, whilst, hydrogen ions that combine with a non-bicarbonate buffer form a hydrogen ion conjugate which is then excreted from the body [64].

1.4.2. Pathophysiology

Any disturbance and deviation of acid-base balance from physiological pH will result in either acidosis or alkalosis. Acidosis is the process in which there is an elevated hydrogen ion concentration in the arterial plasma [86]. Alkalosis describes the physiological state where there is a decrease in hydrogen ions in the arterial plasma [86]. For the purposes of this thesis only acidosis will be described.

1.4.2.1. Acidosis

Acidosis is the process in which there are excess hydrogen ions in the arterial plasma [86]. In clinical practice acidaemia is diagnosed in patients presenting with an arterial blood pH of less than 7.35. Acidosis can be categorised into two broad categories: respiratory acidosis and metabolic acidosis.

1.4.2.1.1. Respiratory acidosis

Respiratory acidosis occurs when there is a change in alveolar respiration that results in the retention of carbon dioxide in the blood [64]. It may occur as a result of hypoventilation or respiratory malfunction owing to a clinical condition such as pneumonia or emphysema [64]. In this altered state of ventilation the

rate carbon dioxide is eliminated by the respiratory system is slower than the production of carbon dioxide; hence, resulting in a net gain of carbon dioxide.

1.4.2.1.2. Metabolic acidosis

Metabolic acidosis is the term used to describe patients presenting with elevated arterial hydrogen ion concentration in the absence of a respiratory cause. Metabolic acidosis may occur during starvation or as a result of an underlying medical condition (e.g. diarrhoea, diabetes mellitus) [64]. Metabolic acidosis is characterised by an inverse relationship between arterial partial pressure of carbon dioxide and hydrogen ion concentrations [86].

Lactic acidosis is a subdivision of metabolic acidosis. It is characterised by the temporarily related events of an elevated lactate concentration (i.e >5 mmol/L) and a decreased arterial pH to less than 7.35 [87]. The Cohen and Woods classification categorises the causes of lactic acidosis into two broad groups, named Type A and Type B lactic acidosis [88]. Type A lactic acidosis describes causes of lactic acidosis where the patient presents with clinical evidence of tissue hypoxia (e.g. anaerobic muscle activity, post myocardial infarction and shock) [88]. Type B lactic acidosis is when a patient presents with lactic acidosis but no clinical evidence of hypoxia [88]. Type B lactic acidosis may occur as a result of an underlying medical condition (e.g. diabetes mellitus, malignancy, renal failure) or as a result of exposure to drugs (e.g. phenformin) or toxins [88].

1.5. Pharmacometrics

Pharmacometrics is a science concerned with the analysis and interpretation of data arising from pre-clinical and clinical drug studies. The discipline of pharmacometrics uses mathematical models to describe and quantify the complex interaction between xenobiotics and a system. Pharmacometric models may be used to describe and predict drug exposure, physiological and/or pathophysiological processes, drug action, clinical outcomes as well as disease progression. Note that pharmacometric models may also be used in pharmacoeconomics to better understand the economic impact a particular drug may have and hence, these pharmacometric techniques are not solely limited to use in biological systems.

1.5.1. Models

A pharmacometric model is a mathematical representation of a system given by a function (f) that describes the relationship between the input (i.e. independent variables) and the output (i.e. dependent variables), as follows;

$$Y = f(\theta, x) + \varepsilon$$

Equation 1.7 *General form of a fixed effect mathematical model*

where, Y is an n-by-1 vector of dependent variables, θ is an n_p-by-1 vector of fixed effects parameters that describes the relationship between Y and x, x is an n-by-n_p matrix of independent variables and ε is a vector the same size as Y that describes residual error.

1.5.1.1. Structural model

1.5.1.1.1. Pharmacokinetic model

Pharmacokinetics (PK) is the science that describes the movement of drugs into, within and out of the body. It is the study that relates the dose of an administered drug to its concentration in the body as a function of time. The

concentration time profile of a drug is governed by the pharmacokinetic processes absorption, distribution, metabolism and excretion.

A pharmacokinetic model describes drug concentration as a function of administered dose and time, and, is dependent on unknown pharmacokinetic parameters. In mathematical form, a general pharmacokinetic model is given as follows,

$$C(t) = f(D, t, \theta_{PK})$$

Equation 1.8 *General mathematical form of a pharmacokinetic model*

where, $C(t)$ is drug concentration, f represents function, D is the administered dose of drug administered, t is a vector of time points and θ_{PK} are pharmacokinetic parameters.

A fundamental part of pharmacokinetic modelling analyses involves estimating pharmacokinetic parameters that describe and characterise the concentration time profile of drugs following administration. The primary pharmacokinetic parameters of interest include clearance (CL), apparent volume of distribution (V), absorption rate constant (k_a), bioavailability (F) and the secondary pharmacokinetic parameter elimination rate constant (k), which is derived from clearance divided by apparent volume of distribution (i.e. CL/V). From a dosing perspective the most important primary and secondary pharmacokinetic parameters are clearance (determines the maintenance dose rate), apparent volume of distribution (determines the loading dose) and half-life (determines dosing interval and time to steady state). Note that clearance is a proportionality constant that describes the relationship between drug concentration and the rate of elimination. Apparent volume of distribution is the apparent volume into which a drug distributes. Bioavailability is the fraction of an extravascularly administered drug that reaches the systemic circulation.

Pharmacokinetic models can be built using compartment structure models. Compartment pharmacokinetic models describe the body by a finite number of compartments to characterise the time-course of drug movement into, within

and out of the body (i.e. the pharmacokinetic processes absorption, distribution, metabolism and elimination). Note that these hypothetical compartments do not necessarily represent true physiological tissues, spaces or organs. It is assumed that the drug homogenously distributes through all compartments.

The number of compartments in a pharmacokinetic model characterise the disposition kinetics of a drug. For instance, a one-compartment model is used to describe drugs that exhibit drug disposition that occurs very rapidly so that the process appears instantaneous. For drugs that exhibit one-compartment kinetics it is assumed that the drug distributes evenly throughout the body, as if it were a single, uniform compartment (refer to Figure 1.6 for a schematic of a one-compartment model). For drugs that exhibit one-compartment kinetics given via an intravenous bolus or a single extravenous dose it is anticipated that drug absorption (for extravenously administered drugs) and distribution is instantaneous. Drug elimination begins immediately after drug administration and would occur concurrently with drug absorption and distribution. For a one-compartment model, drug elimination would exhibit a mono-exponential decline if graphed on a semi log plot (shown in Figure 1.7). In a two-compartment model (schematic shown in Figure 1.8) an additional peripheral compartment is added to characterise the slower rate of distribution of some drugs, resulting in a bi-exponential decline in drug concentration (shown in Figure 1.9).

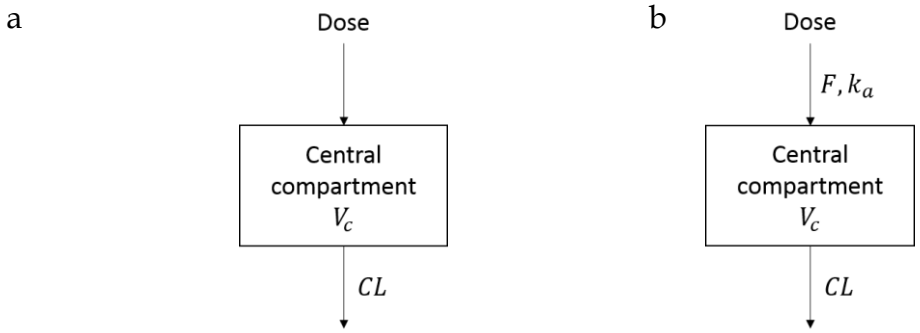


Figure 1.6 *A schematic representation of a one-compartment model for a drug administered intravenously (a) and extravenously (b). CL represents clearance, V_c represents the central compartment volume, F represents bioavailability and k_a represents the absorption rate constant.*

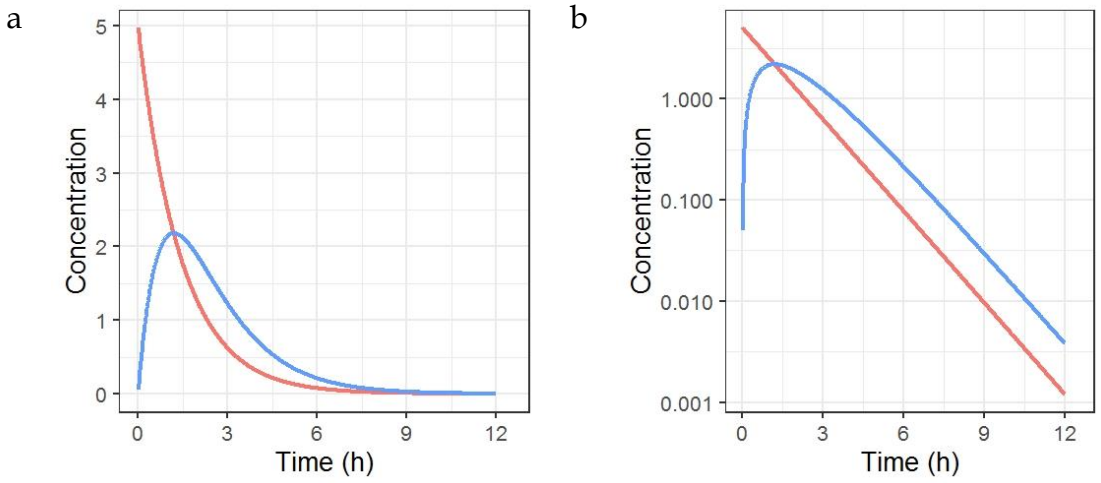


Figure 1.7 *Concentration time profile for a one-compartment model with first-order elimination for an intravenous bolus dose (red) and extravenously administered dose (blue) with first-order absorption on a Cartesian plot (a) and on a semi-log plot (b). Parameters: Dose = 5 units, F = 1, CL = log(2) h^{-1}, V = 1 L, k_a = 1 h^{-1} and k = CL/V. F represents bioavailability, CL represents clearance, V represents volume of distribution, k_a represents the absorption rate constant and k represents the elimination rate constant.*

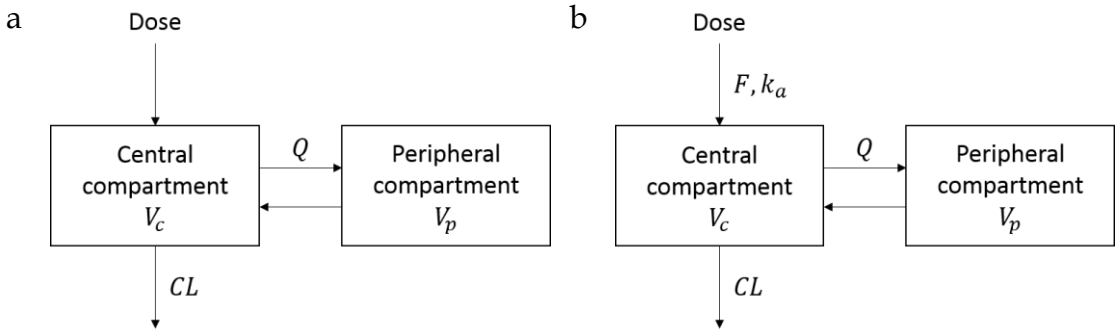


Figure 1.8 *A schematic representation of a two-compartment model for a drug administered intravenously (a) and extravenously (b). CL represents clearance, V_c represents the central compartment volume, V_p represents the peripheral compartment volume, F represents bioavailability, k_a represents the absorption rate constant and Q represents intercompartmental clearance.*

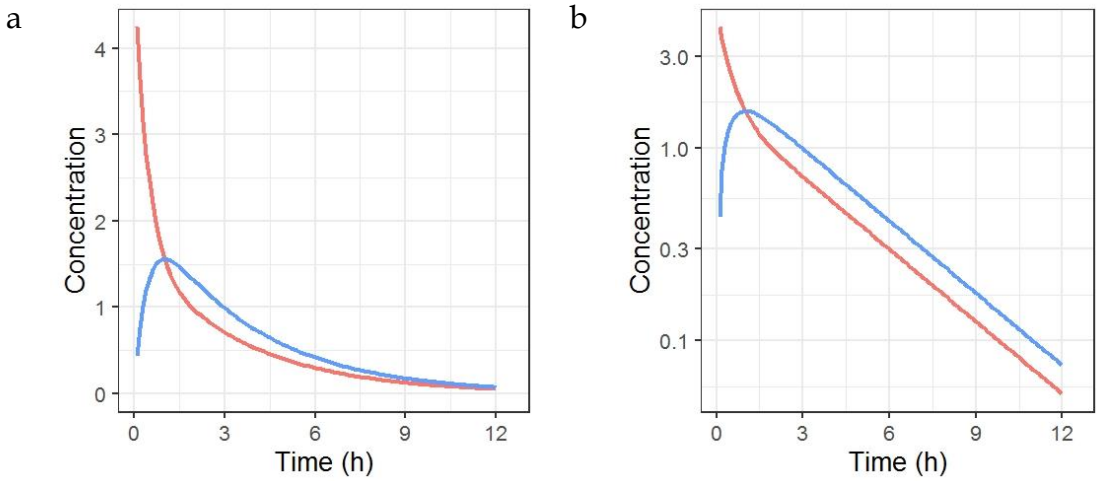


Figure 1.9 *Concentration time profile for a two compartment model with first-order elimination for an intravenous bolus dose (red) and extravenously administered dose (blue) with first-order absorption and on a Cartesian plot (a) and on a semi-log plot (b). Parameters: Dose = 5 units, F = 1, CL = log(2) h^{-1}, V_c = 1 L, Q = 1 h^{-1}, V_p = 1 L and k_a = 1 h^{-1}. F represents bioavailability, CL represents clearance, V_c represents central compartment volume, Q represents intercompartmental clearance, V_p represents peripheral compartment volume and k_a represents the absorption rate constant.*

The time course of drug concentrations for pharmacokinetic compartment models can be illustrated by a series of ordinary differential equations. For instance, for drug given extravenously a one-compartment model can be presented using the following ordinary differential equations;

$$\frac{dA_1}{dt} = -k_a \cdot A_1 \qquad ; \text{at } t = 0, A_1 = F \cdot D$$
$$\frac{dA_2}{dt} = k_a \cdot A_1 - k \cdot A_2 \qquad ; \text{at } t = 0, A_2 = 0$$

Equation 1.9 *Ordinary differential equations for a one-compartment pharmacokinetic model with first-order absorption and distribution*

where, $\frac{dA_1}{dt}$ is the rate of change in amount of drug from the administration site, $\frac{dA_2}{dt}$ is the rate of change in amount of drug from the central compartment, k_a is the absorption rate constant, A_1 is the amount of drug at the administration site, k is the elimination rate constant, A_2 is the amount of drug in the plasma, t represents time, D represents dose and F represents bioavailability. If the ordinary differential equations for the central compartment in Equation 1.9 are solved it provides the closed-form algebraic solution shown in Equation 1.10.

$$C = \frac{F \cdot D \cdot k_a}{V \cdot (k_a - k)} \cdot (e^{-k \cdot t} - e^{-k_a \cdot t})$$

Equation 1.10 *Closed-form algebraic solution for a one-compartment pharmacokinetic model with first-order absorption and elimination*

1.5.1.1.2. Pharmacodynamic model

Pharmacodynamics (PD) is the science that relates drug concentrations with effect. It is the study of the relationship between the drug concentration at the site of action and the observed biochemical and physiological effect. A pharmacodynamic model describes drug effect as a function of drug concentration and pharmacodynamic parameters. A mathematical form of a general pharmacodynamic model is given as follows;

$$E(C) = f(C, \theta_{PD})$$

Equation 1.11 *General mathematical form of a pharmacodynamic model*

where, E represents drug effect, C represents drug concentration, and θ_{PD} represents pharmacodynamic parameters. Note that drug effect is independent of time.

The relationship between drug concentration and pharmacological effect is commonly found to be non-linear. In other words doubling the drug concentration does not double the pharmacological effect. Rather, a hyperbolic function is commonly used to characterise the relationship between drug concentration and response. The extent of the response is dependent on drug concentration. However, as most drugs exhibit non-linear concentration to response relationships, the drug response will increase with increasing drug concentration until it reaches a maximum effect (E_{max}) after which the drug effect asymptotes with increasing drug concentrations.

Most of the principles used to describe the relationship between drug exposure and response are based on classical receptor-binding theory. In classical receptor theory a ligand is assumed to bind reversibly to a receptor, which subsequently results in a series of biochemical and physiological changes ultimately leading to an observed effect. From a pharmacometric perspective the ligand mentioned here can be interpreted as a drug, whereby the drug binds to a receptor resulting in a series of events that lead to pharmacological effect.

Bearing this interpretation of classical receptor theory in mind the relationship between drug concentration and effect can be given using the following model, known as the E_{max} model;

$$E = E_{max} \cdot \frac{C}{C_{50} + C}$$

Equation 1.12 *The E_{max} model*

where, E represents drug effect, E_{max} represents the maximum effect of the drug, C represents drug concentration and C_{50} is the drug concentration at which the drug exhibits 50% of its maximum response. The primary pharmacodynamic parameters of interest are E_{max} and C_{50}.

Several modifications have been made to the E_{max} model to increase its application to more settings. An example of a modification to the general E_{max} is the sigmoidal E_{max} model (shown in Equation 1.13). In the sigmoidal E_{max} model an empirical exponent, named the Hill coefficient (λ), was added to the E_{max} model to change the shape and steepness of the exposure-response curve. Another example of a modification to the general E_{max} model includes the addition of a physiological constant baseline (E_0) to predict what physiological conditions would be like in the absence of the drug (shown in Equation 1.14).

$$E = E_{max} \cdot \frac{C^{\lambda}}{C_{50}^{\lambda} + C^{\lambda}}$$

Equation 1.13 *The sigmoidal E_{max} model*

$$E = E_0 + E_{max} \cdot \frac{C}{C_{50} + C}$$

Equation 1.14 *The E_{max} model with physiological constant baseline*

1.5.1.2. Statistical models

The statistical model describes variability. In pharmacometrics a statistical model is commonly made up of two levels of hierarchy, which are: (i) between subject variability and (ii) residual unexplained variability.

1.5.1.2.1. Residual unexplained variability

Residual unexplained variability (RUV), also known as 'uncertainty', describes the variability of observations around model predictions. Residual unexplained variability may arise from: (i) measurement error, (ii) process error, (iii) within subject variability (also known as intraindividual variability) and, (iv) model misspecification.

A mathematical form of the residual error model is shown as follows;

$$y_i = f(t_i, \beta_i) + \varepsilon_i$$

Equation 1.15 *General form of a residual error model*

where, y_i is a n_i-by-1 vector of the observations from the i^{th} individual and $f(t_i, \beta_i)$ is the individual prediction given by the function (f) where t_i is a n_i-by-1 vector of sampling time and β_i is a p-by-1 vector of individual parameters. Note that n_i refers to the number of observations from the i^{th} individual and p is the number of parameters. The distribution of ε_i is assumed to be normally distributed with a mean of zero and a variance of σ^2 (shown in Equation 1.16).

$$\varepsilon_i \sim N(0, \sigma^2)$$

Equation 1.16 *The distribution of ε_i*

1.5.1.2.2. Between subject variability

The second level in the statistical model hierarchy is between subject variability (BSV), also known as interindividual variability (IIV). Between subject

variability describes unexplained variability that can be related to individual subject specific factors. A mathematical form of the between subject variability model is given;

$$\beta_i = g(\theta, Z_i; \eta_i)$$

Equation 1.17 *General form of a between subject variability model*

where, β_i is a p-by-1 vector of individual parameters for the i^{th} individual that is given by a function (g) where θ is a p-by-1 vector of population typical values of parameters, Z_i is a p-by-z vector of covariates and η_i is a p-by-1 vector that accounts for the random deviation of β_i from θ. The distribution of η_i for all subjects in the study population is often assumed to follow a normal distribution with a mean of zero (Equation 1.18) and a variance-covariance given by Ω (shown in Equation 1.19).

$$\eta_i \sim N(0, \Omega)$$

Equation 1.18 *The distribution of* η_i

$$\Omega = \begin{bmatrix} \omega_{11}^2 & \cdots & \omega_{1p}^2 \\ \vdots & \ddots & \vdots \\ \omega_{p1}^2 & \cdots & \omega_{pp}^2 \end{bmatrix}$$

Equation 1.19 *Variance-covariance matrix*

An exponential function is commonly used to describe between subject variability as many pharmacological parameters are positive real numbers. The exponential function is shown in Equation 1.20.

$$\beta_i = \theta \cdot exp(\eta_i)$$

Equation 1.20 *Exponential function used to describe between subject variability*

1.5.2. Modelling

The three main approaches for population analysis include: (i) naïve pooled data approach, (ii) two-stage approach and (iii) nonlinear mixed effects modelling.

1.5.2.1. Naïve pooled data approach

In the naïve pooled data approach all the data are combined (i.e. pooled) into a single dataset and the data analysed. In this method, data are analysed as if it had all arisen from a single individual, or, as if each observation had been collected from different individuals. The latter being the historical application of this method [89]. Due to all the data being pooled together in the naïve pooled approach there is only a single set of parameter values that is estimated for the corresponding model.

The simplicity of the naïve pooled data approach eases its use and allows for straightforward computation. The fundamental problem with the naïve pooled data approach is that it completely ignores any random variation that exists between individuals (i.e. between subject variability). Hence, lumping any variation arising between subjects together with residual error making the two indistinguishable. Consequently, the naïve pooled data approach cannot estimate the interindividual variability of parameters, neither can it estimate residual error that arises solely from intraindividual variability and measurement error – resulting in biased parameter estimates and inflated values of residual error [90]. In addition, parameter estimates can be biased if individuals provide different amounts of data and if the model is very nonlinear.

1.5.2.2. Two-stage approach

The two-stage approach, as can be inferred from its name, involves two stages. In the first stage, data from each individual is singly analysed to estimate pharmacokinetic parameters for each individual. These individual parameter estimates are then combined in the second stage to calculate the central tendency

(i.e. mean, logarithmic mean or median) and variability (i.e. variance and covariance) to yield population parameter estimates.

The two-stage approach provides a simple method to obtain estimates of population parameters and between subject variability. However, the problem with the two-stage approach is that it requires rich data from each individual. It has been shown that sparse data (i.e. up to 5 samples per individual) may result in poor individual parameter estimates, and, ultimately lead to biased and suboptimal results when combined with other individual parameter estimates [89]. In addition, the estimates of between subject variability tend to be inflated because the two-stage approach does not account for intraindividual variability.

1.5.2.3. Nonlinear mixed effects modelling

Nonlinear mixed effects modelling – also known as a full population approach – estimates population parameters, variability between individuals as well as residual variability. The hierarchical structure for this modelling approach includes the estimation of two types of effects – fixed effects and random effects – giving rise to the 'mixed effects' component of the name. The fixed effects represents structural parameters and the random effects represents the variance of distribution of a specific element of the model. This modelling approach for population analysis is robust as it is able to handle both sparse and rich data, as well as unbalanced/unstructured data. Nonlinear mixed effects modelling considers the population, as opposed to an individual, as the unit for the analysis. Hence, the individuality and variability between individuals is maintained.

Nonlinear mixed effects modelling is considered to be the best approach for population analysis, however, it is also the most complex method. The superiority of nonlinear mixed effects modelling illustrates its advantage over the naïve pooled data approach and two-stage approach when there are sparse data (e.g. commonly seen in paediatrics, geriatrics, intensive care patients and outpatients), interest in understanding sources of variability, as well as wanting

to develop a model to describe and/or to make future predictions using model-based simulations.

Chapter 2: The association between metformin therapy and lactic acidosis in published case reports

This chapter is based on the following peer-reviewed publication:

Kuan IHS, Savage RL, Duffull SB, Wright DFB (2019). *The Association between Metformin Therapy and Lactic Acidosis*. Drug Safety. 42(12): 1449-1469.

2.1. Preamble to the chapter

In this chapter the causal role of metformin therapy in the development of lactic acidosis was explored. A systematic literature review was performed to identify published case reports of metformin associated lactic acidosis. Demographic and clinical data were extracted from each identified case report and, the association between metformin therapy and lactic acidosis in the cases was assessed using two causality assessments. Case reports were also assessed for quality using a completeness scoring tool.

2.2. Introduction

Metformin is an oral antihyperglycaemic agent that is widely accepted as the first-line treatment for type 2 diabetes mellitus. It works predominantly by inhibiting hepatic gluconeogenesis [19, 20]. Metformin therapy may have several advantages over other antidiabetic treatments, including a neutral effect on body weight and a lower incidence of hypoglycaemia [9, 21, 22].

There is considerable concern that patients receiving metformin therapy may be at an increased risk of developing lactic acidosis, a rare but life threatening metabolic condition. The term "lactic acidosis" is used to characterise the temporarily related events of acidosis, characterised by a decreased arterial pH to less than 7.35, and hyperlactataemia, defined as a plasma lactate concentration of greater than 5 mmol/L [87]. Prompt diagnosis and management of lactic acidosis is critical to reduce mortality as it is associated with poor clinical outcomes [91, 92].

While the ingestion of supra-therapeutic metformin doses (e.g. > 20,000 mg) in the setting of intentional overdose has been known to cause lactic acidosis, the risk when metformin is taken chronically in standard therapeutic doses of 1,000-3,000 mg daily is not clear [12, 13, 63]. Phenformin, a predecessor of metformin, was removed from the market in the 1970s following reports of an association with fatal cases of lactic acidosis [93, 94]. The incidence of phenformin associated lactic acidosis was estimated to be 129 cases per 100,000

patient years, which is substantially higher than the incidence reported for metformin at 3.3-9 cases per 100,000 patient years [94-98]. Regardless, the possibility that lactic acidosis is causally related to metformin use at therapeutic doses has led to restricted use in some patient groups [99].

The incidence of lactic acidosis in diabetic patients not taking metformin is estimated to be about 9.7 cases per 100,000 patient years, a value roughly the same as the incidence reported for metformin [95-98]. This raises the possibility that metformin therapy may not be the primary cause of lactic acidosis in reported cases of metformin associated lactic acidosis. Indeed, other risk factors for lactic acidosis have been proposed as the primary drivers, including acute gastrointestinal illness, dehydration, and heart failure [100].

To date, a systematic review of the evidence-base around metformin associated lactic acidosis as presented in published case reports has not been undertaken. Well-presented case reports can provide sufficient detail to assess the impact of risk factors, other than metformin use, on the development of lactic acidosis in cases attributed to metformin therapy.

2.3. Objectives

The aims of this research were (i) to formally evaluate the association between metformin therapy and lactic acidosis in published case reports using two causality scoring systems, (ii) to determine the frequency of pre-existing independent risk factors in published metformin associated lactic acidosis cases, and, (iii) to investigate the association between risk factors and mortality in metformin associated lactic acidosis cases.

2.4. Methods

2.4.1. Data sources and search strategy

A systematic literature review was performed to identify case reports of metformin associated lactic acidosis. The literature search was conducted in Ovid MEDLINE (Ovid MEDLINE 1946 to July 2017), Ovid EMBASE (Ovid EMBASE 1946 to July 2017), Google Scholar (to May 2017) and SCOPUS (to May 2017). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria were used as a guide for conducting and reporting the literature review [101]. Articles were identified using three search strategies;

1. A static search in MEDLINE using Medical Subject Heading (MeSH) terms; 'Metformin', 'Biguanides', 'Acidosis, Lactic', 'Acidosis', 'Lactic Acid', 'Hyperlactatemia', 'Case reports' and key words 'Antidiabetic agent', 'Antihyperglycemic', 'Case series' and 'Case'. The static search was also conducted in EMBASE using the MeSH terms; 'Metformin', 'Biguanide derivative', 'Oral antidiabetic agent', 'Biguanide', 'Antidiabetic agent', 'Lactic acidosis', 'Acidosis', 'Lactic acid', 'Hyperlactatemia', 'Case report', 'Case study' and keywords 'Antihyperglycemic' and 'Case'. Google Scholar and SCOPUS were searched for completeness. The Boolean operators 'AND' and 'OR' were used to search for all relevant literature. The software Harzing's Publish or Perish (Windows 6.1.7601, version 6.16.7601.18837) was used to aid database searching for articles in Google Scholar.
2. A learning based approach was performed in Ovid MEDLINE. A detailed description of the learning based approach has been published elsewhere [102]. An article by Luft et al was used as the index article to search for relevant MeSH terms in Ovid MEDLINE [103]. Relevant MeSH terms obtained from the index article – 'Acidosis', 'Biguanides', 'Buformin', 'Diabetes complications', 'Diabetes mellitus', 'Hypoglycaemic agents', 'Lactate', 'Metformin'

and 'Phenformin' – were combined using the Boolean operator 'AND' to refine the search. A combination of five MeSH terms provided an acceptable number of article hits to review and all possible permutations were trialled. New relevant MeSH terms were added into subsequent searches in an iterative process until no new MeSH terms could be identified.

3. Studies were mined from the reference lists of identified review articles.

Note the learning based approach was used in addition to the standard static search and reference list mining. The learning based approach has the following advantages over the static search: (i) it is an iterative process with an adaptive feedback step so that each article located is reviewed for potential MeSH terms that were not previously identified to help increase search coverage, (ii) it involves the effective utilisation of MeSH terms by focussing on MeSH terms of relevance and (iii) it can link similar articles together without any previously known relationship between the articles. Hence, in theory the addition of the learning based approach should be able to identify articles that would not have been located if just a static search and/or reference list mining were performed. Details of the static and learning based approach search strategies are summarised in Appendix A1.1. Searches were limited to articles published in the English language and human studies. Duplicate studies sourced were removed using Endnote X8.

2.4.2. Article selection

The articles were initially screened by study title and abstract for relevance to metformin and lactic acidosis. For the purpose of this analysis, a therapeutic dose of metformin was defined as any dose reported in the case histories that had been prescribed and was taken chronically for a medical condition, usually type 2 diabetes mellitus. Relevant articles were then subjected to a full text review and assessed for inclusion and exclusion.

Publications were included if the following were reported (i) prior metformin use, (ii) a stated diagnosis of lactic acidosis (regardless of the reported pH and lactate concentration), and (iii) relevant laboratory investigations, (e.g. pH). Studies were excluded if they (i) were published in a language other than English, (ii) did not report human data, (iii) described an acute supra-therapeutic ingestion of metformin (i.e. an intentional overdose), and, (iv) reported only summary data from multiple cases (e.g. case series that only presented summary data). Duplicate cases reported in more than one publication were also excluded, with only the original publication retained. Note that case reports that involved an acute metformin overdose were excluded as the purpose of this study was to understand non-overdose cases.

Screening and study selection were initially conducted by I.H.S.K. Excluded studies and any discrepancies were resolved by a thesis supervisor.

2.4.3. Data extraction

The following data were extracted from each article where available; publication details (year of publication, author, title), case demographics (age, sex, weight, height and ethnicity), medical history (presenting complaints, diagnosis on admission, comorbidities, concomitant medications and dose, surgical/hospitalisation history), metformin maintenance dose, duration of metformin therapy to onset of lactic acidosis, metformin plasma concentrations, laboratory measurements (arterial blood gases, estimates of renal function (any estimate of glomerular filtration rate (GFR), including creatinine clearance (CLcr) calculated using the Cockcroft and Gault equation ([80]) or reported eGFR), and, plasma or serum concentrations of lactate, electrolytes (sodium, potassium, bicarbonate and chloride ions), creatinine, glucose and glycated haemoglobin (HbA1c), urea/blood urea nitrogen, concentrations of any concomitant intoxicants), medical interventions, and patient outcome. Clinical laboratory data presented in graphs were extracted using the MATLAB (R2016b, MathWorks, Natick, MA) script GRABIT. Data was extracted to three decimal points.

2.4.3.1. Independent risk factors for lactic acidosis

Pre-existing factors that are reported to increase the risk of developing lactic acidosis, other than metformin use, were extracted from each of the case reports. The risk factors were categorised as; (i) acute (presenting/diagnosis on admission), (ii) chronic (diagnosis of condition prior to admission) or (iii) acute on chronic (exacerbation of a chronic condition) at presentation. The risk factors used for this analysis are presented in Appendix A1.2 (Table A1.7). The list was developed by collating recognised risk factors summarised in standard medical texts [88, 104, 105] and was reviewed by two independent clinicians.

Two additional risk factors; acute gastrointestinal illness and dehydration, were not listed in most references as independent risk factors for lactic acidosis, but have been proposed to increase risk specifically in patients taking metformin [99]. The risk of lactic acidosis in this setting is suggested to be due to dehydration and hypovolemia leading to the possibility of acute renal failure and hence metformin accumulation [18, 100]. Information about these additional risk factors from each of the case reports were extracted.

2.4.4. Data analysis

2.4.4.1. Summary description of cases

Demographic and clinical features of cases with metformin associated lactic acidosis identified from the literature were summarised. The following graphs were plotted in R (version 3.3.3) for data visualisation: (i) arterial pH versus time, (ii) lactate concentration versus time, (iii) creatinine concentration versus time and (iv) metformin concentration versus time.

2.4.4.2. Causality assessment

The role of metformin in the development of lactic acidosis in each case report was assessed using the World Health Organisation-Uppsala Monitoring Centre (WHO-UMC) system for standardised case causality assessment [106] and the Naranjo adverse drug reaction (ADR) probability scale [107]. The two

causality assessments were applied to each identified case report of metformin associated lactic acidosis to allow for cross-validation and comparison between the results.

2.4.4.2.1. WHO-UMC system for standardised case causality assessment

The WHO-UMC system for standardised case causality assessment classifies the likelihood of a causal relationship between a pharmacological agent and an adverse event into six categories [106]. The tool assesses the causality likelihood based on clinical and pharmacological information provided in the case histories, as well as considering the quality of the information provided [106].

The WHO-UMC system for causality assessment was annotated with lactic acidosis specific diagnostic criteria under each causality category to allow for replicability of the results. The adapted WHO-UMC system for causality assessment with additions under assessment criteria is presented in Appendix A1.3 (Table A1.8).

2.4.4.2.2. Naranjo ADR probability scale

The Naranjo ADR probability scale is a questionnaire that describes the relationship between a pharmacological agent and an adverse reaction using a scoring system presented in Appendix A1.4 (Table A1.9) [107]. The relationship between the pharmacological agent and outcome is described by four categories, which are 'definite', 'probable', 'possible' and 'doubtful' shown in Appendix A1.4 (Table A1.10) [107].

2.4.4.3. Completeness (quality) score for published case reports

The information provided in the case reports was reviewed for quality using a bespoke completeness score. The completeness score was based on the quality scoring tool published by Stades et al [108].

Each case history was reviewed and a score assigned based on the availability of the following data; age, sex, height, weight, ethnicity, comorbidities, concomitant medications, doses of concomitant medications, co-

ingested substances (e.g. methanol), symptoms/illnesses prior to admission, ingested dose of metformin, duration of metformin therapy, time of last metformin ingestion, pH, partial pressure of carbon dioxide (pCO2), partial pressure of oxygen (pO2), anion gap, base excess, estimates of renal function (any estimate of GFR, including Cockcroft-Gault ([80])), medical interventions, patient outcome, and, plasma or serum concentrations of lactate, metformin, sodium, potassium, bicarbonate, chloride, creatinine, urea/blood urea nitrogen, and glucose. One point was allocated for each descriptor reported. An additional point was allocated to case histories with repeated measures data (e.g. serial plasma concentrations over time post-admission). The score could range from 0 to 31.

A sensitivity analysis was conducted to assess the influence of poor completeness scores on the causality assessment. Case reports with a completeness score in the lower quartile of possible scores (0 - 10) were excluded.

2.4.4.4. The frequency of pre-existing risk factors in metformin associated lactic acidosis cases

The overall frequency of each identified risk factor for lactic acidosis in the cohort of cases, stratified as acute, chronic or acute-on-chronic, was determined. For each individual case, the number of co-existing risk factors for lactic acidosis was tabulated and the frequency of identified risk factors per case was summarised.

Evidence of gastrointestinal illness (including both acute and chronic symptoms) and dehydration were recorded for each case and their prevalence summarised.

2.4.4.5. Risk factors and mortality in metformin associated lactic acidosis cases

The risk of mortality was assessed by determining the percentage of cases who did or did not survive in the overall cohort, as well as in subgroups divided on the basis of the number of risk factors for lactic acidosis present per case.

The association between each risk factor and mortality was assessed using a multivariable logistic regression analysis. This was conducted in R (version 3.3.3). The dependent variable was case outcome (i.e. mortality or survival) and the explanatory variables were the risk factors for lactic acidosis outlined in Appendix 1.2. Only explanatory variables reported in more than ten percent of cases were considered.

Each explanatory variable was added to the model one at a time in a univariate analysis. Risk factors were considered significant if they resulted in a decrease in the Akaike information criterion (AIC) relative to the null model. Risk factors which were imprecisely estimated, with a percent relative standard error (RSE%) of greater than 50%, were not considered further. Identified significant explanatory variables were then added to the null model one at a time using forward selection (i.e. firstly adding the risk factor that resulted in the greatest decrease in AIC), until no further reduction in AIC was observed following subsequent addition of explanatory variables to the model. The model was then subject to backwards deletion, which involved removing one explanatory variable at a time to confirm statistical significance. All possible second-level interactions between explanatory variables were individually added to the logistic regression model and tested for significance. Significant interaction terms were retained in the final model.

2.5. Results

2.5.1. Literature search

A total of 8,235 articles were identified. After the removal of duplicates, the remaining 4,166 articles were screened by study title and abstract. A total of 3,220 articles were excluded following the screening process. Of the 946 remaining articles, 699 were excluded for the following reasons: reported an intentional overdose of metformin (n=84), cases published in more than one paper (n=58), cases in a language other than English (n=64), no record of a lactic acidosis diagnosis (n=128), no record of either a lactic acidosis diagnosis and no record of metformin use (n=217), no record of metformin use (n=43), no laboratory data to support lactic acidosis diagnosis (n=31), case series with summary data (n=51), and articles that could not be accessed or located (n=23). In total, 247 articles were included in the final literature review representing 559 cases of metformin-associated lactic acidosis [8, 97, 100, 109-352]. A schematic of the PRISMA workflow for the systematic literature review is presented in Figure 2.1.

2.5.2. Identified literature

A total of 247 articles were identified in the literature. Identified articles included 181 case reports, 44 case series, 1 case control study, 2 cohort studies, 1 poison centre database and 18 retrospective cohort studies. Several publications included multiple case reports with individual patient data.

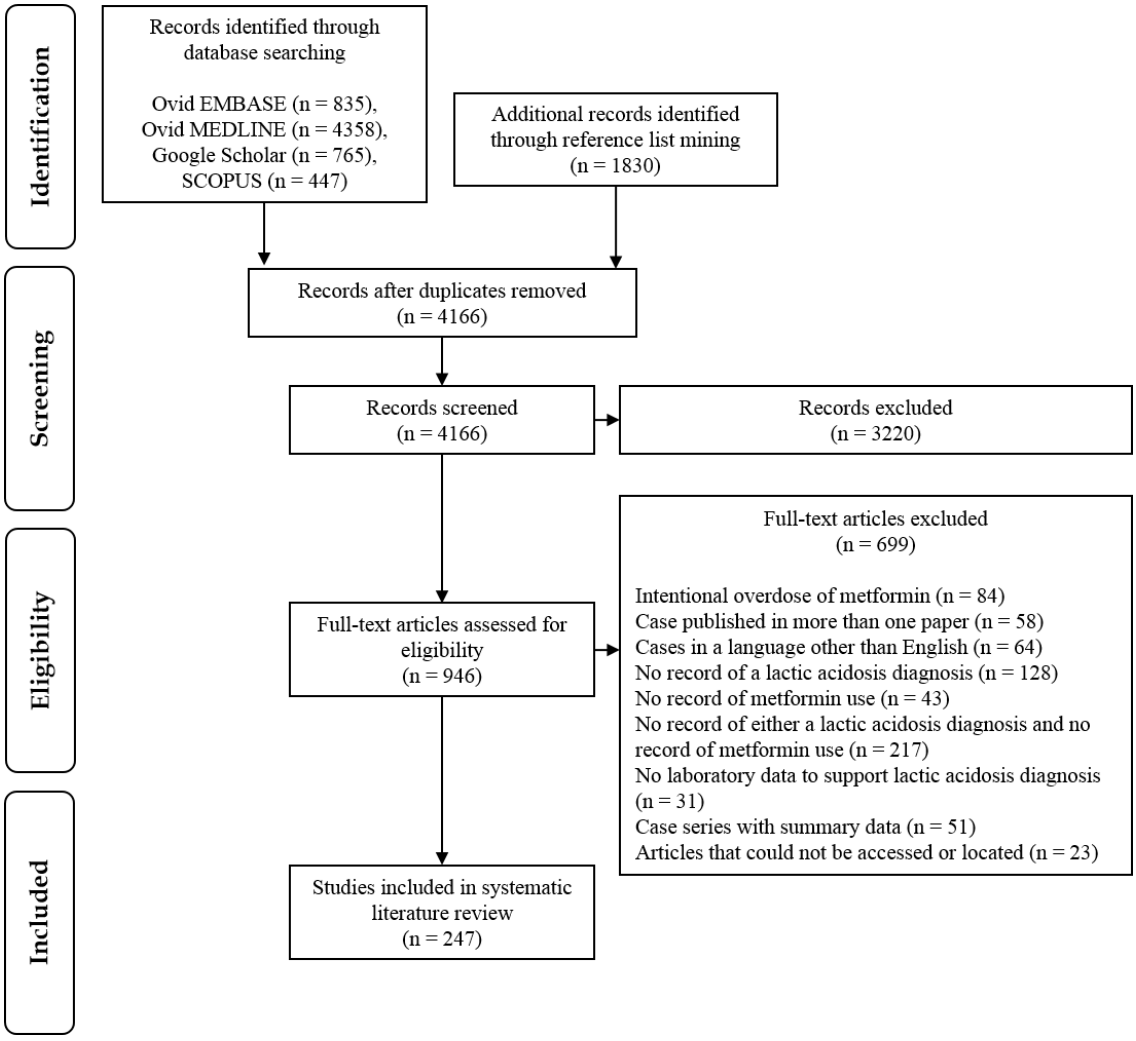


Figure 2.1 *PRISMA flow diagram for the systematic literature review*

2.5.3. Identified metformin associated lactic acidosis cases

A total of 559 metformin associated lactic acidosis cases were identified from the literature search. An online database of the metformin associated lactic acidosis cases was created and is available in the figshare repository, https://figshare.com/s/4a1129faa048322cfa0c. Please note that the online database also contains data extracted from 84 articles (of n=146 cases) that were excluded from this study due to having reported taking an intentional overdose of metformin.

2.5.4. Data analysis

2.5.4.1. Completeness scores

The completeness score was applied to each case report. A summary table of the completeness scores is shown in Appendix A1.5 (Table A1.11).

2.5.4.2. Demographics and clinical features of metformin associated lactic acidosis cases

A summary of the extracted demographic and clinical features of the cases is presented in Table 2.1. Missing data not recorded in the published case reports is also included. The median prescribed daily dose of metformin and duration of metformin therapy prior to admission was 1700 mg and 12 months, respectively. The median arterial pH on admission was 6.97 (range: 6.00 – 7.50) and the median lactate concentration was 14.5 mmol/L (range: 1.1 – 60).

A graph of arterial pH, creatinine, lactate and plasma metformin concentrations versus time post-admission is shown in Figure 2.2, 2.3, 2.4 and 2.5 respectively. As shown in Figure 2.2, it can be seen that many MALA cases had presented with severe acidosis (i.e. pH less than 7.35) on admission, which had later recovered back to normal physiological pH levels. In addition, in Figure 2.3, 2.4 and 2.5 it can be seen that many cases had presented with grossly elevated creatinine, lactate and metformin concentrations on admission which had subsequently returned back to normal physiological levels, respectively. The grossly elevated creatinine concentrations is likely to be a reflection of the fact that many of the cases had presented with acute renal failure. In the MALA cases, it is likely that the acidosis, as well as elevated concentrations of creatinine, lactate and metformin returned to normal values following medical interventions received during admission to a medical facility.

Table 2.1 *Demographics and clinical features of cases*

	Reference range		Cases with reported data (out of n = 559) [%]
Demographics			
Gender (F:M)		309:210	519 [93%]
Age (*years*)		69 [20–96] (60-76)	528 [94%]
Weight (*kg*)		72.8 [27-117] (55–78.3)	27 [5%]
Height (*m*)		1.64 [1.44–1.80] (1.56–1.66)	18 [3%]
BMI (kg/m^2)		29 [10.3-40] (22.2–30.9)	15 [3%]
Metformin			
Daily dose (*mg/day*)		1700 [500–6000] (1500-2550)	378 [68%]
Therapy duration (*months*)		12 [0.1–216] (1-60)	69 [12%]
Estimated GFR (baseline)			72 [13%]
Reported in *mL/min*		32 [1-105] (20.6–56.8)	14 [3%]
Reported in $mL/min/1.73m^2$		49 [8–98] (41–57.8)	58 [10%]
Key laboratory values on admission			
Arterial pH	7.35–7.45	6.97 [6.00–7.50] (6.80–7.17)	491 [88%]
Serum creatinine (*µmol/L*)	45–90[a] 50–110[b]	561 [9.7–2500] (221–795.6)	454 [81%]
Serum lactate (*mmol/L*)	0.5–1.6	14.5 [1.1–60] (9.5–20.0)	486 [87%]
Plasma metformin (*mg/L*)		31 [0–210] (8.9–54.2)	197 [35%]
History of diabetes (*Y:N*)		356:29	385 [69%]
Patient outcome (*Survived: Died*)		393:160	553 [99%]

Data presented as median [range] (interquartile range) unless otherwise specified. BMI = body mass index, GFR = glomerular filtration rate. [a]Reference range for laboratory values for females. [b]Reference range for laboratory values for males.

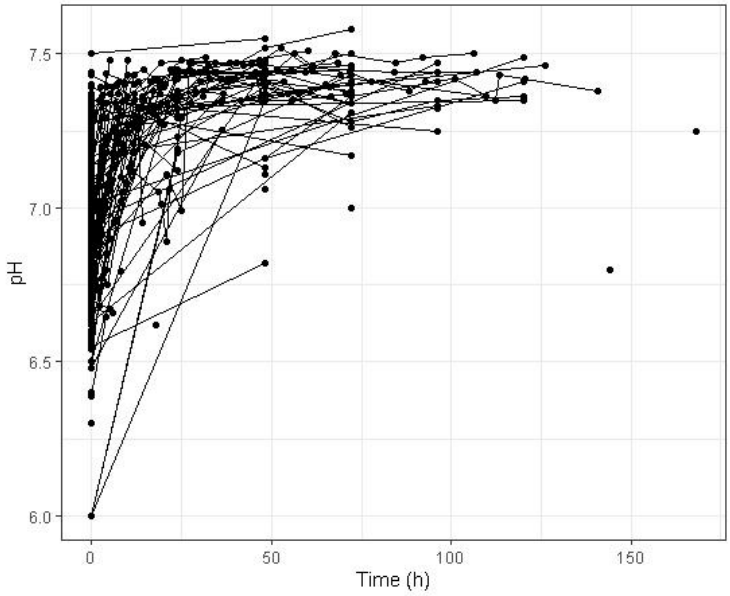


Figure 2.2 *Scatterplot of arterial pH versus time post-admission from metformin associated lactic acidosis cases (n=509). The dots represent single measures of pH and the lines link repeated measures data for a single case. Reported pH values below the limit of quantification were graphed as a pH of 6 to allow for visualisation of pH post-admission.*

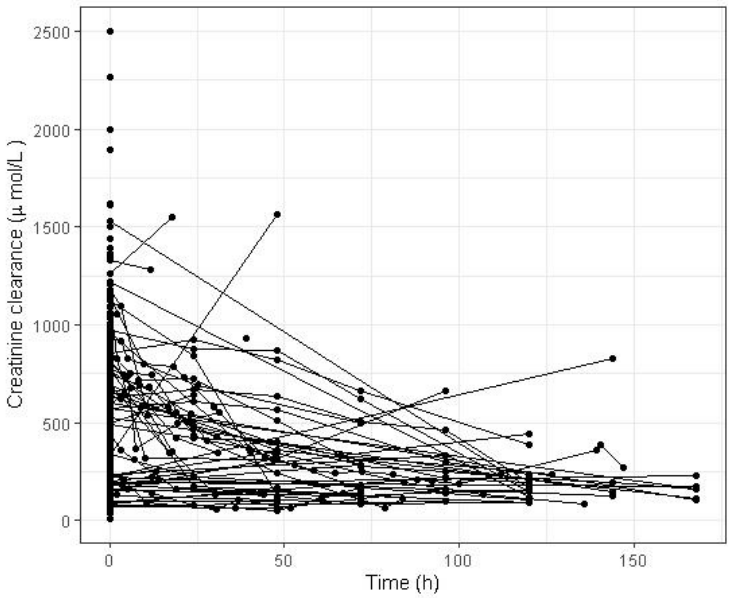


Figure 2.3 *Scatterplot of creatinine concentration versus time post-admission from metformin associated lactic acidosis cases (n=458). The dots represent single measures of creatinine concentration and the lines link repeated measures data for a single case.*

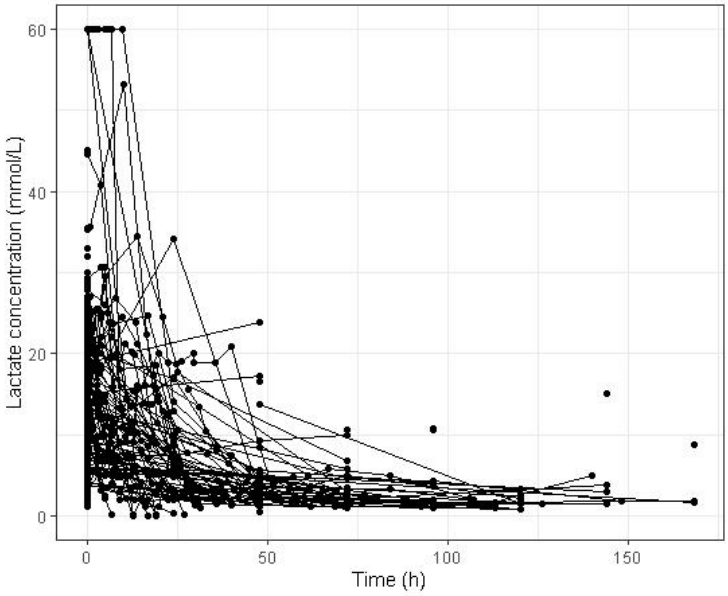


Figure 2.4 *Scatterplot of lactate concentration versus time post-admission from metformin associated lactic acidosis cases (n=505). The dots represent single measures of lactate concentration and the lines link repeated measures data for a single case. Reported pH values above the limit of quantification were graphed as a lactate concentration of 60 mmol/L to allow for visualisation of lactate concentrations post-admission.*

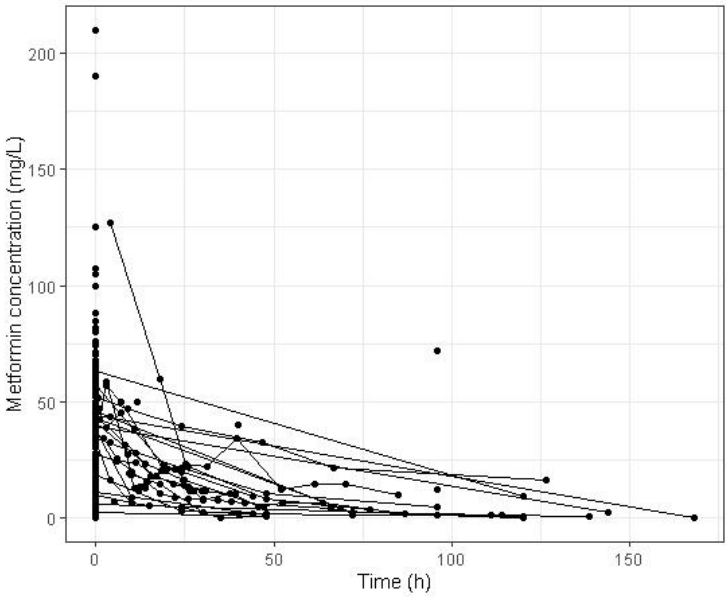


Figure 2.5 *Scatterplot of plasma metformin concentration versus time post-admission from metformin associated lactic acidosis cases (n=215). The dots represent single measures of metformin concentration and the lines link repeated measures data for a single case.*

2.5.4.3. Causality assessment

The results of the WHO-UMC and Naranjo causality assessments are presented in Table 2.2.

The WHO-UMC system for standardised case causality assessment categorised metformin as a 'probably/likely' cause of lactic acidosis in 17 cases (3.0%), a 'possible' cause in 473 cases (84.6%), an 'unlikely' cause in 2 cases (0.4%), 'conditional/unclassified' in 49 cases (8.8%) and 'not assessable/unclassifiable' in 18 cases (3.2%). Similarly, the Naranjo ADR probability scale found that metformin was a 'probable' cause of lactic acidosis in 22 cases (3.9%), a 'possible' cause in 536 cases (95.9%) and a doubtful cause in 1 case (0.2%). In the sensitivity analysis, where cases with a completeness score in the bottom quartile of possible completeness scores (≤10) were excluded, the causality scores did not change appreciably suggesting that cases of low quality did not influence the results (see Table 2.2).

Table 2.2 *Summary of the causality assessments using the World Health Organisation-Uppsala Monitoring Centre (WHO-UMC) system and Naranjo adverse drug reaction (ADR) probability scale, including a sensitivity analysis*

Causality Assessment	**Causality category**	**Cases (n = 559)**	**Sensitivity analysis excluding cases with a completeness score of ≤10 (n = 386)**
WHO-UMC system	Certain	-	-
	Probably/Likely	17 (3%)	11 (2.8%)
	Possible	473 (84.6%)	338 (87.6%)
	Unlikely	2 (0.4%)	1 (0.3%)
	Conditional/Unclassified	49 (8.8%)	24 (6.2%)
	Unassessable/Unclassifiable	18 (3.2%)	12 (3.1%)
Naranjo ADR probability scale	Definite	-	-
	Probable	22 (3.9%)	16 (4.3%)
	Possible	536 (95.9%)	369 (95.6%)
	Doubtful	1 (0.2%)	1 (0.3%)

2.5.4.4. Summary of independent risk factors for lactic acidosis

Of 559 cases, 540 (96.6%) presented with at least one independent risk factor for lactic acidosis other than metformin use. Many cases presented with multiple risk factors. The median number of co-existing risk factors per case was 2, ranging from 0 to 5 (Table 2.3).

A summary of the pre-existing 'acute', 'acute on chronic' or 'chronic' risk factors identified in the cases is presented in Table 2.4. Overall, the most prevalent pre-existing risk factors were renal impairment (n=454, 81%), shock (n=69, 12%), and hepatic impairment (n=55, 10%). When considering only acute conditions, acute renal impairment, shock and hypoglycaemia (n=292, n=69, and, n=49, respectively) were the most prevalent. It is worth bearing in mind that it may not be possible to tease apart some related acute conditions, such as shock and sepsis. Pre-existing chronic renal impairment, which did not progress to acute renal impairment, was reported to occur in only 36 cases.

A total of 186 of the 559 cases with metformin associated lactic acidosis reported symptoms of gastrointestinal illness on admission. On presentation, 184 of the 186 cases presented with acute symptoms of gastrointestinal upset, whilst the remaining 2 cases presented with chronic gastrointestinal symptoms that had lasted for 3 and 12 months. Dehydration was reported in 48 of the 559 cases on admission. In the cohort with acute gastrointestinal illness, 22/184 cases presented with dehydration. An additional analysis was conducted to explore the relationship between pre-existing gastrointestinal illness in patients on metformin therapy and lactic acidosis. However, due to the retrospective nature of the data a causal association between gastrointestinal illness, metformin therapy and lactic acidosis was difficult to establish. For more details refer to Appendix A1.6.

Fifty-four cases reported ingesting drugs and/or toxins known to be risk factors for lactic acidosis (shown in Table 2.5). Acetylsalicylic acid and alcohol were the two most common substances ingested.

Table 2.3 *Frequency of multiple independent risk factors for lactic acidosis*

Number of risk factors per case	Number of cases (n = 559)
0	19
1	198
2	195
3	100
4	38
5	9

Table 2.4 *Frequency of each risk factor for lactic acidosis*

Risk factor	Acute	Acute on chronic	Chronic	Total cases
Type A				
Anaemia	9	-	5	14 (2.5%)
Anaerobic muscle activity	3	-	-	3 (0.5%)
Asthma	-	1	6	7 (1.3%)
Heart failure	6	2	31	39 (7.0%)
Hypoxaemia	5	-	-	5 (0.9%)
Post myocardial infarction	41	2	11	54 (9.7%)
Regional tissue ischemia	23	-	5	28 (5.0%)
Shock	69	-	-	69 (12.3%)
Type B				
Hepatic impairment	37	4	14	55 (9.8%)
Human immunodeficiency virus	-	-	2	2 (0.4%)
Malignancy	2	-	21	23 (4.1%)
Renal impairment	292	126	36	454 (81.2%)
Sepsis	34	-	-	34 (6.1%)
SIRS	1	-	-	1 (0.2%)
Miscellaneous				
Diabetic ketoacidosis	5	-	-	5 (0.9%)
Hypoglycaemia	49	-	-	49 (8.8%)
Mitochondrial disease	2	-	1	3 (0.5%)
Gastrointestinal illness	184	-	2	186 (33.3%)

Table 2.5 *Summary of drugs and toxins ingested*

Substance ingested	Number of cases (n = 54)
Acetylsalicylic acid	26
Albuterol	5
Alcohol	18
Cocaine	1
Codeine	1
Didanosine	1
Emtricitabine	1
Isoniazid	1
Paracetamol	2
Propylene glycol	1
Salbutamol	1
Salicylates	1
Salmeterol	1
Stavudine	1
Tenofovir	1
Theophylline	2

2.5.4.5. Risk factors and mortality in metformin associated lactic acidosis cases

A total of 160 of the 553 cases with reported patient outcome did not survive. A summary of the mortality of metformin associated lactic acidosis based on the presence of risk factors is shown in Table 2.6.

Table 2.6 *Apparent risk of mortality versus the number of risk factors for lactic acidosis present in cases with reported patient outcome*

Number of risk factors per case	Death	Total number of cases	Cases that did not survive (%)
0	8	18	44.4
1	65	197	33.0
2	45	193	23.3
3	23	99	23.2
4	16	37	43.2

A small group of 19 cases were not reported to have any risk factors for lactic acidosis other than metformin use. Of these nineteen cases, 8 out of 18 (44%) cases with reported patient outcome did not survive. It was noted that the completeness scores for 76% of these cases were all in the lower range of scores (≤12), and n=8 cases were classed as either 'conditional/unclassified' or 'unassessable/unclassifiable' by the WHO-UMC causality assessment scheme. This suggests that the information presented in some of these cases was scant and may be unreliable.

A summary of the logistic regression analysis assessing the influence of risk factors for lactic acidosis and mortality is presented in Table 2.7. Hepatic impairment was associated with an increased risk of mortality (odds ratio of 1.926 (95% CI: 1.036-3.553)), whilst renal impairment was associated with a decreased risk of mortality (adjusted odds ratio of 0.305 (0.153-0.596)).

Table 2.7 *Logistic regression model for risk factors associated with mortality*

Covariate	β	se(β)	P value	Odds ratio (95% CI)
Intercept	0.3449	0.3078	0.262467	1.411 (0.779-2.625)
Renal impairment	-1.1860	0.3461	0.000611	0.305 (0.153-0.596)
Hepatic impairment	0.6553	0.3131	0.036332	1.926 (1.036-3.553)
Shock	0.5372	0.2883	0.062444	1.711 (0.967-3.004)
Renal0:DM1[a]	-1.6358	0.4479	0.000260	0.195 (0.079-0.458)
Renal1:DM1[b]	-0.5841	0.2331	0.012206	0.558 (0.353-0.883)

Note here that any form of renal impairment (i.e. chronic renal impairment or acute renal failure on admission) and hepatic impairment (i.e. chronic, acute or end stage hepatic failure) were included in the regression analysis. se: standard error, CI: confidence interval, [a]Interaction term used to describe when renal impairment is not present but diabetes mellitus is present. [b]Interaction term used to describe when both renal impairment and diabetes mellitus are present.

2.6. Discussion

The association between metformin and lactic acidosis remains controversial. Metformin is recognised to cause lactic acidosis when taken in supra-therapeutic doses, but when taken therapeutically its causal role is less clear. This study evaluated the association between metformin therapy and lactic acidosis and, investigated risk factors for lactic acidosis and their association with mortality in published metformin associated lactic acidosis cases. Metformin was found to only play a 'possible' role in the development of lactic acidosis when using two causality assessments. Almost all cases presented with risk factors for lactic acidosis other than metformin use.

Hepatic impairment (including chronic, acute or end stage hepatic failure) was found to be associated with increased mortality in cases of metformin associated lactic acidosis, whilst renal impairment (including chronic renal impairment or acute renal failure on admission), paradoxically, was associated with a decreased risk of death. The mechanism behind these associations are unknown, however, similar findings have been reported where an association between end stage hepatic failure and mortality in cases presenting with metformin associated lactic acidosis has been reported [108].

There is a growing body of evidence to support the idea that metformin, when used in therapeutic doses, may not be a primary cause of lactic acidosis. This is supported by the findings in this study, where nearly all cases reviewed presented with other risk factors for lactic acidosis. In a recent Cochrane review no cases of metformin associated lactic acidosis could be identified in 209 prospective comparative trials, 125 prospective cohort studies and 13 retrospective cohort studies, representing 69,642 patients on metformin therapy [353]. While the Cochrane review included studies with controlled clinical trial populations, which may not be representative of patients with type 2 diabetes in standard clinical care, results from studies using pharmacovigilance data and other cohorts also cast doubt on metformin as a primary cause of lactic acidosis. In a longitudinal observational study by Kamber et al, the incidence of lactic acidosis in patients on metformin was found to be about the same as those

treated with other anti-hyperglycaemic agents (i.e. 3 cases in 5,228 patient years and 2 cases in 7,238 patient years, respectively) [213]. In addition, the authors noted that most cases were in patients with risk factors for lactic acidosis other than metformin use [213]. Similarly, in a review of MALA case reports by Stades et al over 90% of the reviewed cases presented with acute risk factors for lactic acidosis leading the authors to conclude that the association between lactic acidosis and metformin is coincidental [108]. Findings from other published retrospective studies have collectively found that cases of MALA often presented with other risk factors that were more likely to account for the development of lactic acidosis [100, 233, 260]. Interestingly, current literature suggest that metformin may play a protective role in severe cases of lactic acidosis that are unrelated to metformin [14, 354].

The pathophysiology of metformin associated lactic acidosis is not well understood. It is generally assumed that excessive lactate production results in the release of free protons and thus leads to acidosis, although this hypothesis has been questioned by some researchers [355, 356]. At supra-therapeutic doses, metformin has been reported to interfere with the mitochondrial respiratory chain complex and increase lactate production which may be responsible for the development of lactic acidosis in patients who have taken large overdoses [298, 357, 358]. It is noteworthy that these reactions have not been observed in patients taking therapeutic doses of metformin, nor do therapeutic doses have an appreciable impact on plasma lactate concentrations [358-361]. Whether elevated systematic exposure to metformin in those taking therapeutic doses due to, for example, reduced renal function is of a magnitude sufficient to interfere with mitochondrial respiration is not known.

A total of nineteen published lactic acidosis cases were found with no other identifiable risk factors other than metformin use. These cases are of interest because they represent patients where metformin may be the primary cause of the lactic acidosis. However, it was found that many of these cases had lower completeness scores compared to the other reports (median: 10 (range: 5-25) versus median: 13 (range: 4-28), respectively). It is therefore possible that

additional information, such as other risk factors for lactic acidosis, were under-reported. In addition, eight of the nineteen cases were categorised as 'conditional/unclassified' or 'unassessable/unclassifiable' by the WHO-UMC causality assessment tool, indicating a lack of relevant information. It is therefore not possible to draw any conclusions about the role of metformin in these cases.

An interesting implication of this study is that the causality assessment methods used may lead to biased results for this type of work. In keeping with the study outcomes other authors have noted that the same two causality assessment methods most frequently assign causality to "possible" [362, 363].

The key criteria for causality assessment are (1) metformin use prior to lactic acidosis onset, (2) whether other drugs or clinical conditions may have caused or contributed to lactic acidosis and to what degree, (3) outcome on metformin dechallenge and (4) objective evidence of lactic acidosis. The majority of case reports fulfilled criteria (1) and (4) but (3) can be difficult to assess as metformin will inevitably be discontinued but other potential causes will also be corrected where possible and lactic acidosis treated. Criteria (2) is the key and the reason why metformin causality has been so contentious. Whatever causality assessment method is used clinical judgement is required. For the Naranjo method a decision has to be made whether there were alternative causes that could on their own have caused the adverse reaction. Even if the answer is "yes" a "possible" score can be obtained if metformin was started prior to lactic acidosis onset and there was objective evidence of lactic acidosis which, of course, was the case in almost all of the published reports.

The WHO-UMC criteria for "probable" include disease and other drugs being unlikely causes, "possible" criteria include disease and other drugs as potential alternative explanations and "unlikely" criteria include disease or other drugs providing plausible explanations. It is therefore evident that most reports with other risk factors for lactic acidosis will border between "possible" and "unlikely", and, "unlikely" may have been under-ascertained. What is important is that there are very few reports where metformin was clearly the sole or most important cause of the lactic acidosis. Another difference leading to more reports

being assigned to the "possible" category using the Naranjo algorithm was the absence in the latter of the WHO-UMC categories "unclassified" and "unclassifiable" which allow for reports to be categorised as unassessable if there is insufficient data. Including these in the "possible" category using the Naranjo algorithm is a bias towards a causal relationship.

2.7. Limitations

Lalau et al have provided a critical analysis of the pitfalls that will be encountered when examining the association between metformin and lactic acidosis [364]. The authors note that important diagnostic information such as the duration of metformin exposure, renal status, availability of a metformin assay, the ratio of metformin plasma to erythrocyte concentrations are required to fully assess metformin's role in lactic acidosis. It is important to acknowledge that the lack of these data represents an important limitation for the analysis of published case reports. In this study, data analysis was restricted by the availability of data reported in the literature. Published case histories identified are inconsistent, often lacking complete clinical and diagnostic information. Potentially influential risk factors for lactic acidosis (e.g. comorbidities, concomitant medicines and supplements) may not have been consistently documented. For instance, only 13% of cases reported estimated renal function prior to admission, limiting any inference about the relationship between renal impairment and metformin accumulation in the lactic acidosis cases. Furthermore, due to the retrospective nature of the data, it is difficult to infer whether the cause of acute renal impairment in these cases were metformin-related (i.e. toxicity) or metformin-independent. To better investigate the relationship between metformin therapy and renal impairment, it would be ideal to have a temporal series of pre-admission and post-admission estimates of renal function, as this would provide an insight on whether the cases' renal function were stable or deteriorating prior to admission; unfortunately, such information was rarely reported. Information regarding compliance to metformin therapy

was limited and hence, it was assumed that the cases were taking metformin as prescribed. Similarly, the time of last metformin ingestion relative to hospital admission was commonly not reported.

To account for the lack of consistency in the data presented in the case histories a sensitivity analysis was conducted, where the causality assessment results were re-analysed when case histories of poor quality (i.e. those with low completeness scores) were excluded.

It is important to note that the estimates of renal function reported in the published cases were measured several years to days prior to the development of lactic acidosis. Indeed, in the majority of cases it was unknown when renal function was measured relative to hospital admission.

2.8. Conclusion

Metformin was found to play only a possible role in the majority of published metformin associated lactic acidosis cases at therapeutic doses. Almost all cases presented with other risk factors that could on their own have led to lactic acidosis. This supports the suggestion that metformin may not play a role or only a contributory, rather than a primary role in the development of lactic acidosis when used in therapeutic doses. Traditionally, metformin had been contraindicated in patients with chronic kidney disease due to concerns of lactic acidosis. However, based on the findings from this study it suggests that many patients - particularly those with chronic kidney disease - may be denied metformin therapy, an otherwise effective therapy. This highlights the need for a formal evaluation of the relationship of between metformin therapy and lactic acidosis in patients with chronic renal impairment.

Chapter 3: The relationship between metformin therapy, chronic renal impairment and lactic acidosis

This chapter is based on the following peer-reviewed publication:

Kuan IHS, Savage RL, Duffull SB, Wright DFB (2019). *The Association between Metformin Therapy and Lactic Acidosis*. Drug Safety. 42(12): 1449-1469.

3.1. Preamble to the chapter

In the systematic literature review of published MALA cases presented in Chapter 2, it was found that most cases involved other risk factors for lactic acidosis. This raises the possibility that metformin therapy may not be the primary cause of lactic acidosis in many reported MALA cases. The use of metformin in patients with chronic kidney disease (CKD) has traditionally been restricted on the grounds that elevated metformin plasma concentrations when standard doses are used in this population will increase the risk of lactic acidosis. However, this relationship is poorly understood and it can be proposed that other risk factors are the primary cause of lactic acidosis in CKD patients. In this chapter, the database of MALA case reports generated in Chapter 2 will be interrogated for evidence of a relationship between chronic renal impairment, excessive metformin dosing and/or exposure and lactic acidosis.

3.2. Introduction

The safe use of metformin in patients with renal impairment is a matter of considerable debate. Traditionally, metformin was contraindicated in patients with an estimated creatinine clearance (CLcr) of <60 mL/min, but recent published guidelines suggest that this can be relaxed to CLcr <30 mL/min, or even as low as 15 mL/min [23, 24, 44, 59, 365, 366]. Regardless, there is a lack of consensus about the best dosing practice and little understanding of risk mitigation [14-16].

Metformin is primarily eliminated by the kidneys as unchanged drug so the use of standard doses in patients with impaired kidney function will result in increased plasma concentrations [19, 27, 29, 30]. Concentrations greater than 5 mg/L have been postulated to increase the risk of adverse effects, particularly lactic acidosis a life-threatening metabolic condition [45, 99]. The term "lactic acidosis" is used to characterise the temporally related events of acidosis, characterised by a decreased arterial pH to less than 7.35, and hyperlactatemia, defined as a plasma lactate concentration of greater than 5 mmol/L [87]. This has

led to the wide-spread practice of withdrawing metformin therapy in patients with impaired kidney function [367]. However, poor kidney function due to diabetic nephropathy is a common comorbidity with Type 2 diabetes so many patients may be denied an otherwise effective therapy. In addition, the risk of lactic acidosis due to elevated plasma concentrations could be mitigated, in theory, with dose reduction based on an understanding of how metformin exposure is altered by reduced renal function.

The relationship between chronic renal impairment, metformin dose and/or exposure and the development of lactic acidosis is poorly understood. A systematic review of published care reports to explore causality in these cases has not been conducted.

3.3. Objectives

The aims of this work were to; (i) formally evaluate the role of metformin therapy in the development of lactic acidosis in CKD patients reported in published case reports and (ii) explore the relationship between prescribed metformin doses, steady-state metformin plasma concentrations, and the development of lactic acidosis in CKD patients.

3.4. Methods

3.4.1. Database source

The case histories used in this analysis were sourced from the database of MALA cases generated in Chapter 2. In brief, the database was generated following a systematic literature review performed to identify cases of MALA. Relevant case histories were identified from Ovid MEDLINE (Ovid MEDLINE 1946 to July 2017), Ovid EMBASE (Ovid EMBASE 1946 to July 2017), Google Scholar (to May 2017) and SCOPUS (to May 2017). Studies were included if the publication reported (i) prior metformin ingestion, (ii) a diagnosis of lactic acidosis and (iii) relevant laboratory investigations (e.g. pH). Studies were excluded if they (i) were not in English, (ii) were not a human study, (iii) involved an acute metformin overdose, and, (iv) only reported summary data from multiple cases. Note that case reports that involved an acute metformin overdose were excluded as the purpose of this study was to understand non-overdose cases (i.e. the development of lactic acidosis in cases with chronic renal impairment prescribed therapeutic doses of metformin).

3.4.2. Case selection from the metformin associated lactic acidosis database

For the purposes of this analysis cases with a reported history of CKD prior to hospital admission for MALA were identified using the following criteria; (i) if pre-existing chronic renal impairment was reported as a comorbidity (regardless of the reported estimate of renal function), and/or, (ii) if pre-admission CLcr or estimated glomerular filtration rate (eGFR) was less than 60 mL/min or 60 mL/min/1.73m^2, respectively.

3.4.3. Data extraction

The database comprised demographic and clinical data extracted from each identified case of MALA. The following data were extracted from the database: article details (year of publication, author, title), demographic information (age, sex, weight, height and ethnicity), medical history (presenting complaints, diagnosis on admission, comorbidities, concomitant medications and dose,

surgical/hospitalisation history), metformin therapy related information (metformin maintenance dose, duration of metformin therapy to onset of lactic acidosis, metformin plasma concentrations), laboratory measurements (arterial blood gases, estimates of renal function (any estimate of glomerular filtration rate (GFR), including CLcr or reported eGFR), and, plasma or serum concentrations of lactate, electrolytes (sodium, potassium, bicarbonate and chloride ions), creatinine, glucose and glycated haemoglobin (HbA1c), urea/blood urea nitrogen, concentrations of any concomitant intoxicants), identified risk factors for lactic acidosis, medical interventions and patient outcome.

3.4.3.1. Independent risk factors for lactic acidosis

Pre-existing risk factors that are reported to increase the risk of developing lactic acidosis, other than metformin therapy, were extracted from each of the published cases. The risk factors were categorised as; (i) acute (presenting/diagnosis on admission), (ii) chronic (diagnosis of condition prior to admission) or (iii) acute on chronic (exacerbation of a chronic condition) at presentation. The list of risk factors is presented in Appendix A1.2.

3.4.3.2. Renal function metrics

In the absence of reported data in the case reports about body surface area, weight or height pre-admission CLcr and eGFR were considered to be interchangeable.

In the database the estimates of renal function were reported in: cc/min, L/week, mL/s, mL/min and mL/min/1.73m^2. The units for the estimates of renal function were converted to mL/min and mL/min/1.73m^2 as follows:

- mL/s to mL/min: estimate of renal function multiplied by 60
- cc/min was assumed to be equal to mL/min
- L/week to mL/min: estimate of renal function multiplied by 0.099

For cases that did not report pre-admission CLcr or eGFR, these metrics were calculated using the Cockcroft and Gault equation [80] and CKD-Epi

equation [83] respectively provided the publication reported the required demographic and laboratory data (e.g. pre-admission serum creatinine, weight, sex, age etc).

3.4.4. Data Analysis

3.4.4.1. Summary description of cases

The demographics and clinical presentation of MALA cases identified from the database were summarised and tabulated.

3.4.4.2. Completeness score

A completeness score was developed to assess the quality and availability of data in each case history first introduced in this thesis in Chapter 2 (section 2.4.4.3.). Each case history was assessed using the completeness score and assigned a score based on the availability of data for analysis. A maximum score of 31 was possible. The completeness score developed was an adaptation of the quality scoring tool published by Stades et al [108].

3.4.4.3. Causality assessment

The role of metformin therapy in the development of lactic acidosis in the MALA cases with a history of chronic renal impairment was assessed using the World Health Organisation-Uppsala Monitoring Centre (WHO-UMC) system for standardised case causality assessment [106] and the Naranjo adverse drug reaction (ADR) probability scale [107]. For the purposes of this study, the WHO-UMC causality assessment was adapted with lactic acidosis specific diagnostic criterion to allow for the replicability of the results. The adapted WHO-UMC causality assessment tool is presented in Appendix A1.3. The Naranjo ADR probability scale was used verbatim reproduced in Appendix A1.4 (Table A1.9).

A sensitivity analysis was performed to assess the influence of poor quality case histories on the causality assessment. Cases with a completeness score of 10 or lower were excluded and the results of the causality assessment were re-analysed and compared to the full cohort of cases.

3.4.4.4. Compliance to recommended renal dosing guidelines

The metformin doses reported in the MALA cases with renal impairment were compared to current renal dosing guidelines from the European Medicines Agency (EMA) assessment report and the New Zealand datasheet (derived from Duong et al 2013) [15, 368]. Here, the New Zealand datasheet was selected to represent a local renal dosing guideline, whilst the EMA assessment report was selected to represent an international renal dosing guideline. In addition, the New Zealand datasheet was selected as it was the least conservative guideline at the lower end of renal function (down to a CLcr or 15 mL/min), whilst the EMA guideline was selected as it was the least conservative guideline at the upper end of renal function.

3.4.4.5. Predicted pre-admission metformin plasma concentrations

Pre-admission steady state plasma concentrations were not available for any cases in the MALA database, although several publications reported measured metformin plasma concentrations once the patient had been admitted to hospital. MALA is often accompanied by a rapidly developing acute kidney injury. Therefore, the metformin plasma concentrations measured at the time of admission may be elevated due to an acute decline in kidney function. The goal of the analysis conducted here is to understand whether steady-state plasma concentrations, prior to admission and the development of acute kidney injury, were elevated and therefore contributed to the risk of lactic acidosis. In other words, to predict what metformin plasma concentrations would have been when the patient was well.

Pre-admission steady-state metformin plasma concentrations were predicted from a population pharmacokinetic model for metformin published by Duong et al [15]. Details of the model and the parameter estimates used for the simulations are summarised in Appendix A2.1. The model was implemented in MATLAB (R2016b, MathWorks, Natick, NA) and predictions were compared to the predicted plasma concentrations reported by Duong et al to ensure replicability.

Deterministic simulations to predict the steady-state trough ($C_{p,ss,trough}$), average ($C_{p,ss,ave}$) and peak ($C_{p,ss,max}$) concentration at day 20 were conducted for each MALA case with the following information available; the prescribed dose of metformin, the patients' body weight, and the estimated pre-admission CLcr or eGFR. Missing body weight was imputed at the median for the MALA database. The $C_{p,ss,ave}$ was determined via the following formula:

$$C_{p,ss,ave} = \frac{Dose}{CL \cdot DI}$$

Equation 3.1 *Formula to calculate* $C_{p,ss,ave}$

Here, $Dose$ represents the metformin dose used in the simulations, CL represents clearance of metformin in units of L/h, and, DI stands for dose interval and is the time difference between doses in hours. For cases that did not report information on metformin dosing frequency (i.e. only reported the total prescribed daily dose of metformin), each dose was assumed to be dosed twice or thrice daily based on reasonable division of the reported daily dose by available metformin tablet strengths. For example, a metformin dose of 2550 mg was divided into individual doses of 850 mg taken three times daily. The proportion of patients with steady state trough, average and peak concentrations prior to the development of MALA >5 mg/L (i.e. a concentration reported to be associated with increased risk of lactic acidosis) were determined [99, 369]. Cases were stratified into those with an estimated renal function of <60 mL/min and ≥60 mL/min and a boxplot was drawn using R (version 3.3.3).

3.5. Results

3.5.1. Identified cases

A total of 145 cases with a reported history of chronic renal impairment or CLcr/eGFR <60 mL/min (or 60 mL/min/1.73m2) were identified from the database.

3.5.2. Description of metformin associated lactic acidosis cases with chronic renal impairment

A description of the MALA cases with a history of chronic renal impairment is presented in Table 3.1. On admission 119 of the 145 (82%) cases presented with documented acute renal impairment.

The median prescribed dose of metformin was 1700 mg. Metformin had been taken for a median of 4 months prior to admission for lactic acidosis. The median arterial pH on admission was 6.97 (range: 6-7.36), and, the median lactate concentration on admission was 13.5 mmol/L (range: 1.1-60.0).

The median reported pre-admission estimates of renal function (CLcr or eGFR) were 32 in units of mL/min (range: 20-91 mL/min) and 43 mL/min/1.73m^2 (range: 8-92 mL/min/1.73m^2). On admission, the median estimate of renal function was 36 mL/min (only 2 patients) and 8 mL/min/1.73m^2.

A total of 64 cases reported metformin plasma concentrations. In all cases, the concentrations were measured on admission when many of the patients would be expected to be in acute renal failure. This is reflected in the data where a median concentration of 31 mg/L (range: 1.5-105 mg/L) was recorded. In some cases, repeated metformin plasma concentrations were measured during the course of the hospital admission to determine the impact of dialysis and other interventions. A plot of the reported time course of metformin plasma concentrations is presented in Figure 3.1.

Table 3.1 *Demographics and clinical data for MALA cases with pre-existing chronic renal impairment*

		Cases with reported data (n = 145)
Demographics		
Gender (F:M)	90: 50	140
Age (years)	71 [42 – 89] (65 - 76)	143
Weight (kg)	75.5 [46 – 94] (53.5 – 79.3)	14
Height (m)	1.62 [1.44 – 1.70] (1.51 – 1.67)	10
BMI (kg/m^2)	29 [17 – 30] (28.3 – 30.0)	5
Metformin		
Dose (mg/day)	1700 [500 – 6000] (1500 - 2500)	117
Therapy duration (months)	4 [0.1 - 204] (0.5 – 48.0)	19
Key laboratory values pre-admission[a]		
Renal function		
in mL/min	32 [20 – 91] (22.8 – 49.8)	10
in mL/min/1.73m^2	43 [8 – 92] (33.8 – 50.5)	40
Creatinine (µmol/L)	134 [56 – 1057] (108 – 173)	93
Key laboratory values after admission for lactic acidosis[b]		
Renal function		
in mL/min	36 [35 – 37] (35.5 – 36.5)	2
in mL/min/1.73m^2	8 [3 – 38] (6 – 29.7)	19
Creatinine (µmol/L)	585 [90 – 1502] (327 – 761)	133
Lactate concentration (mmol/L)	13.5 [1.1 – 60.0] (9.2 – 20.0)	131
pH	6.97 [6.00 – 7.36] (6.8 – 7.14)	130
Metformin (mg/L)	31 [1.5 – 105] (15.3 – 45.3)	64

Data presented as median [range] (interquartile range) unless otherwise specified. Renal function is a mixture of CLcr and eGFR. [a]In the case where there were multiple reported pre-admission values the most recent results were used. [b]The on-admission values are the first laboratory results analysed when cases were admitted to a clinic and do not include repeated measures. CLcr/ GFR was not calculated for cases that did not report renal function but had reported serum creatinine as the cases commonly lacked other demographic data required for calculation (e.g. age, weight).

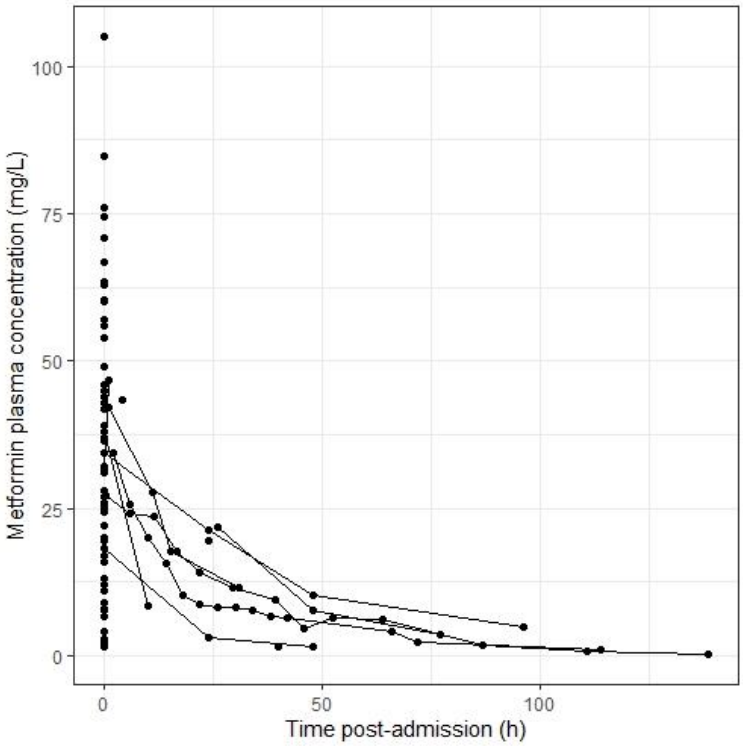


Figure 3.1 *Plasma metformin concentration from metformin associated lactic acidosis cases. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single case.*

3.5.3. Causality assessment

In the published cases of MALA with chronic renal impairment, the WHO-UMC system for standardised case causality assessment categorised metformin as a 'certain' cause of lactic acidosis in none of the cases, 'probably/likely' in 1 case (0.7%), 'possible' in 130 cases (89.7%), 'conditional/unclassified' in 10 cases (6.9%) and 'unassessable/unclassifiable' in 4 cases (2.8%). Using the Naranjo ADR probability scale metformin was categorised as 'probable' in 2 cases (1.4%), 'possible' in 143 cases (98.6%) and doubtful in none of the cases. Results from the sensitivity analysis showed no significant difference in the results when case histories with completeness scores of 10 or less were included in the causality assessment. A summary of the causality assessment results using the WHO-UMC system and Naranjo ADR probability scale is presented in Table 3.2.

Results from the completeness score are summarised in Appendix A2.2.

Table 3.2 *Summary of the WHO-UMC system and Naranjo ADR scale causality assessment results*

Causality assessment	Causality category	Cases (n = 145)	Sensitivity analysis (n = 125)
WHO-UMC system	Certain	-	-
	Probably/Likely	1 (0.7%)	1 (0.8%)
	Possible	130 (89.7%)	114 (91.2%)
	Unlikely	-	-
	Conditional/Unclassified	10 (6.9%)	8 (6.4%)
	Unassessable/Unclassifiable	4 (2.8%)	2 (1.6%)
Naranjo ADR scale	Definite	-	-
	Probable	2 (1.4%)	2 (1.6%)
	Possible	143 (98.6%)	123 (98.4%)
	Doubtful	-	-

3.5.4. Compliance to recommended renal dosing guidelines

Of 145 MALA cases with chronic renal impairment, only 50 included the prescribed metformin dose as well as an estimate of renal function (i.e. CLcr or eGFR) from prior to admission. Compliance of metformin prescribing to guidelines proposed by the EMA and Duong et al is presented in Table 3.3 [368, 370]. All prescribed doses aligned with the renal dosing guidelines in cases whose estimated CLcr or eGFR was equal or greater than 60 mL/min. However, with increasing severity of renal insufficiency approximately 50% of the cases received doses that exceeded the recommended dose. The prescribed doses exceeded the recommended dose by a median of 1500 mg for the EMA guideline and 1000 mg according to the guideline proposed by Duong et al (Table 3.4). Please note that some of the MALA cases were included in the analysis despite having an eGFR >60 mL/min, as these cases had been included on the basis of having reported a history of CKD despite having an eGFR >60 mL/min.

Table 3.3 *Compliance of prescribed metformin dose to renal dosing guidelines by EMA and Duong et al*

Guideline	eGFR/CLcr	Recommendation	Case	
	(mL/min)	Maximum dose (mg/day)	Maintenance dose within guidelines	Maintenance dose exceeded the guidelines
EMA	>59	3000	4 (8%)	-
	45-59	2000	13 (26%)	6 (12%)
	30-45	1000	3 (6%)	12 (24%)
	<30	Contraindicated	-	12 (24%)
Duong et al	90-120	3000	2 (4%)	-
	60-90	2000	1 (2%)	-
	30-60	1000	6 (12%)	29 (58%)
	15-30	500	-	10 (20%)
	<15	Contraindicated	-	2 (4%)

Table 3.4 *Quantity of dose prescribed exceeding the renal dosing guidelines by EMA and Duong et al*

Amount of dose exceeding the renal dosing guidelines (mg)	Guideline	
	EMA (n = 30)	Duong et al (n = 41)
0 – 500	3	7
>500 – 1000	11	16
>1000 – 2000	12	14
>2000 – 3000	3	3
>3000	1	1

3.5.5. Predicted pre-admission steady-state metformin plasma concentrations

Predictions from the metformin pharmacokinetic model aligned with the plots produced by Duong et al suggesting that the model was implemented correctly and replicated published predictions (details can be found in Appendix A2.3).

Pre-admission metformin steady state concentrations were predicted for 50 cases, all with recorded metformin doses and estimates of pre-admission renal function available. The median age of the 50 cases was 72 years (range: 54-89) with two-thirds of the cases being female. Weight was not reported for 46 cases and hence was imputed as the median weight for the MALA cases. The results from the simulations are summarised in Table 3.5.

Table 3.5 *Summary of simulated metformin plasma concentration*

Metformin plasma concentration (mg/L)	Median [range]
$C_{p,ss,max}$[a]	3.33 [1.41-8.77]
$C_{p,ss,ave}$ [b]	2.75 [0.64-8.20]
$C_{p,ss,trough}$[c]	1.38 [0.17-6.49]

[a]$C_{p,ss,max}$: maximum plasma concentration at steady state, [b] $C_{p,ss,ave}$: steady state average plasma concentration, [c] $C_{p,ss,trough}$: trough plasma concentration at steady state

The results from the simulations indicate that 94% of the MALA cases are predicted to have trough plasma metformin concentrations below 5 mg/L. Furthermore, it is predicted that 86% and 80% of cases would have steady state average and peak concentrations below 5 mg/L, respectively.

The predicted pre-dose metformin plasma concentrations are shown in Figure 3.2. When stratifying cases based on their renal function, it can be seen that cases with an estimated renal function less than 60 mL/min had higher predicted steady-state pre-dose concentrations in comparison to cases with estimates of renal function greater than 60 mL/min whose plasma concentrations were all below 1 mg/L.

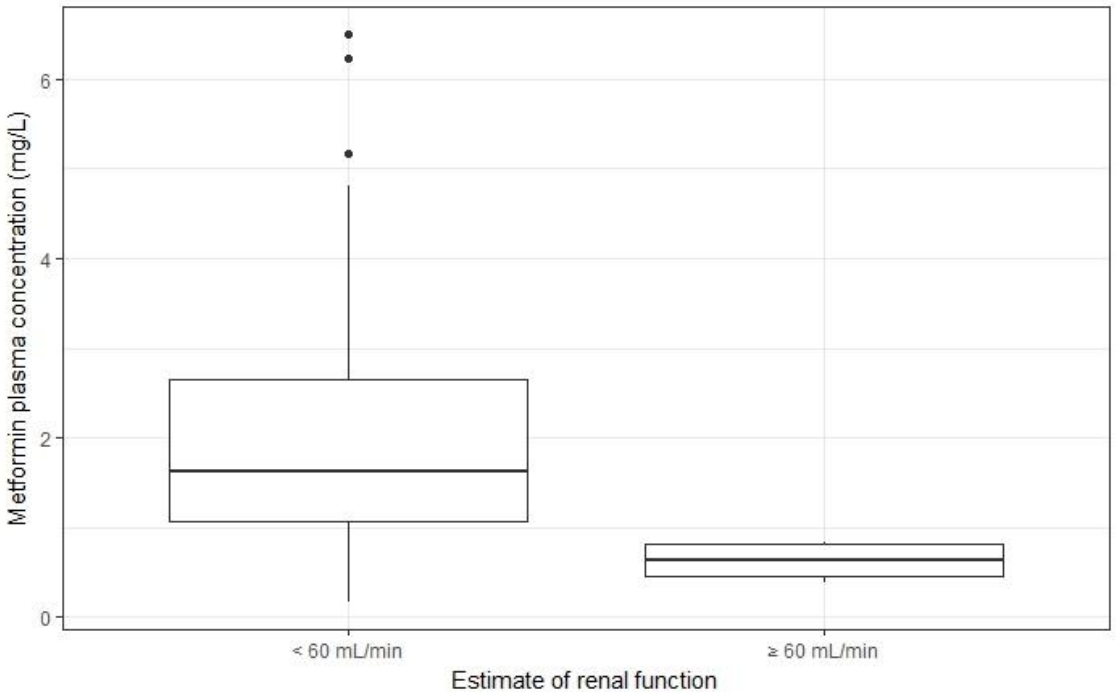


Figure 3.2 *Boxplot of simulated pre-admission plasma metformin concentrations for 50 cases whom had their prescribed metformin dose and estimate of renal function from prior to admission available for analysis. Cases were stratified into two groups based on renal function: cases with an estimate of renal function of less than 60 mL/min and cases with an estimate greater than and equal to 60 mL/min.*

3.6. Discussion

In this study, metformin was found to be a 'possible' cause of lactic acidosis in 90 percent of metformin associated lactic acidosis cases with chronic renal impairment. Interpretation of the causality term 'possible' is analogous for both causality algorithms, depicting a scenario where metformin played a possible role in the development of lactic acidosis however, there were other equally likely risk factors present in the cases that could have also led to the adverse event. In this study, almost all cases presented with acute renal impairment on admission, complicating any ability to assess the cause and effect relationship between metformin therapy, chronic renal impairment and lactic acidosis.

However, in this study, it is worth noting that it is impossible to score 'doubtful' when using the Naranjo ADR scale. This is because all MALA cases identified from the database would score positively on the questionnaire for firstly having taken the drug (i.e. metformin) and secondly, having objective evidence for lactic acidosis (e.g. pH and lactic acid concentrations). If the scores assigned for these two questions had been removed and the probability categories re-assigned it is likely that the finding that metformin played a 'possible' role can be interpreted instead as 'doubtful'. If consequently metformin was found to be a 'doubtful' cause, this would then be interpreted that the event of lactic acidosis was likely to be related to factors other than metformin.

Comparison of the prescribed metformin dose relative to published renal dosing guidelines found that cases with moderate to normal renal function (i.e. >60 mL/min/1.73m2) received doses within the dosing guidelines whilst, in cases with impaired renal function (i.e. <60 mL/min/1.73m2), an increasing proportion received doses above the dosing guidelines. Based on the results from the simulations, the steady state pre-dose plasma metformin concentrations prior to admission would not have exceeded 5 mg/L in the majority of patients who developed lactic acidosis, however, it is worth noting that the predicted pre-dose concentrations were about two times higher than reported in the literature [15, 45, 371]. When stratifying the predicted pre-dose, steady-state

concentrations by renal function, it can be seen that cases with good renal function (i.e. >60 mL/min) all had pre-dose metformin concentrations below 1 mg/L, however, for cases with poor renal function (i.e. <60 mL/min) the pre-dose steady-state concentrations were much higher. It is unknown what the reason is for this, but it could possibly be a result of the inappropriate renal dosing observed in this cohort. These findings suggest that if the recommended renal dose adjustments had been applied the predicted concentrations would lie within the proposed upper limit of 5 mg/L in the cases with poor renal function.

In addition, it is worth noting that the results from our simulations are 5-10 fold less than the metformin plasma concentrations observed in the cases reported to have stable chronic renal impairment on admission for lactic acidosis. This is suggestive that there may be other underlying processes simultaneously occurring in these cases that are yet to be explored. A possible explanation is that these cases had acute renal impairment on admission for lactic acidosis, however this was not reported in the case report. It is also possible that the simulations performed excluded cases with extremely low clearance and hence did not represent the MALA cases with chronic renal impairment.

In patients with renal impairment on metformin therapy, it has been proposed that the initial trigger leading to lactic acidosis may be acute renal failure. This being the case, acute renal failure will result in the increase in metformin concentrations [372]. The upper end of the therapeutic range for metformin is usually considered to be 5 mg/L, although a recently revised upper limit of 2.5 mg/L has been proposed [45, 369]. The majority of published cases reviewed in this study reported plasma metformin concentrations that were much greater than 5 mg/L on admission with concomitant acute renal impairment. It is unclear if the excessive metformin plasma concentrations observed on admission led to the acute renal impairment, or the acute renal impairment led to metformin accumulation (or indeed a combination of both). In the former, unadjusted doses of metformin in patients with poor renal function might be implicated as the primary risk factor for lactic acidosis. The mechanism for this has been proposed to be due to metformin intoxication

leading to symptoms of gastroenteritis, dehydration and/or circulatory collapse, and, acute renal failure, respectively [18, 373]. In the latter, metformin is more likely to be an innocent bystander. Similar findings have been reported in the literature where many cases presenting with MALA had often presented with acute renal impairment on admission [272, 372, 374].

Results from this study suggest that pre-admission plasma concentrations would be below the proposed upper limit of the therapeutic range of 5 mg/L and the revised upper limit of 2.5 mg/L in the majority of cases. This is despite the use of doses in excess of those recommended by current guidelines proposed by the EMA and Duong et al. The overarching impression is that metformin use in patients with renal impairment, even at doses 1000-1500 mg above the recommended doses, is not expected to produce excessive accumulation of pre-dose concentrations and may therefore not be the primary cause of lactic acidosis in the published cases. This conjecture is supported by the causality assessment where most cases were found to have other risk factors for lactic acidosis (results shown in Chapter 2). Therefore, future work on quantifying an upper therapeutic limit for metformin is necessary to help guide renal drug dosing.

Metformin has been shown to be tolerated in patients with severe renal impairment (i.e. CLcr of 20 mL/min) and in patients on dialysis [371, 375]. Current metformin renal dosing guidelines worldwide lack agreement and informative advice on how to safely dose metformin in renal impairment. Some guidelines state that metformin therapy should be contraindicated in renal impairment, whilst others advise to 'review the dose', 'reassess the benefit to risk', 'caution', 'referral to a nephrologist', a dose adjustment or no dose adjustment [23, 45, 60, 376, 377]. It has been reported that up to 19% of patients prescribed metformin have a renal contraindication to the drug [136, 213, 233, 378-382]. Therefore, research to determine the safe dosing of metformin in renal impairment is warranted.

3.7. Limitations

Due to the retrospective nature of the data it is difficult to infer whether the cause of acute renal impairment in these cases was metformin-related (i.e. toxicity) or metformin-independent. To better investigate the relationship between metformin therapy and renal impairment, it would be ideal to have a temporal series of pre-admission and post-admission estimates of renal function, as this would provide an insight on whether the cases' renal function were stable or deteriorating prior to admission; unfortunately, such information was rarely reported. In addition to this, it is important to note that the reported estimates of renal function in the case reports ranged from being measured several years to days prior to the development of lactic acidosis, and, in the majority of cases it was unknown when the estimate of renal function was estimated. Similarly, it is worth noting that the cases with reported estimates of renal function from prior to and on admission for lactic acidosis are not necessarily the same.

Data reported in case reports is inconsistent and lacking. For instance, of the 145 MALA cases with CKD only 14 cases had reported weight. This was a limitation in the simulations performed to predict what pre-admission steady state plasma concentrations would have been prior to admission for lactic acidosis as weight had to be imputed for 46 cases.

3.8. Conclusion

Metformin played a possible role in 90% of the metformin associated lactic acidosis cases with renal impairment. Most cases presented with acute renal failure, confounding the relationship between metformin dose and plasma concentration. The prescribed metformin dose exceeded the dosing recommendations in more than 60% of cases with an eGFR of <60 mL/min by a median amount of 1000 mg/day. Despite this finding, the predicted pre-admission metformin plasma concentrations measured pre-dose did not exceed the proposed upper limit of the therapeutic range of 5 mg/L in most cases, suggesting that chronic renal impairment is not the cause of metformin accumulating to high concentrations.

Chapter 4: The relationship between metformin concentrations and lactic acidosis

This chapter is based on the following peer-reviewed publication:

Kuan IHS, Wright DFB, Duffull SB, Zhu X (2020). *Understanding the association between metformin plasma concentrations and lactate*. Br J Clin Pharmacol. doi: 10.1111/bcp.14394.

4.1. Introduction

There is concern that patients with renal impairment who are prescribed metformin therapy may be at an increased risk of developing lactic acidosis. This is due to the fear that, if standard doses are used, the reduced metformin elimination in this patient group would lead to elevated plasma concentrations and an increased risk of lactic acidosis. In theory, dose reduction along with plasma metformin concentration monitoring to ensure that concentrations do not exceed a safe upper limit should help mitigate the risk of lactic acidosis in patients with renal impairment. However, little is known about the relationship between metformin plasma concentrations and the risk of lactic acidosis. Metformin is recognised to cause lactic acidosis when taken in acute overdose [12, 63]. However, when used in therapeutic doses for type 2 diabetes there is a growing body of evidence to suggest that metformin may not be the primary cause of lactic acidosis [108, 353] - as was found in Chapter 2 and 3 of this thesis. Nonetheless, a better understanding of the relationship between plasma metformin concentrations and the risk of lactic acidosis will help guide dosing to mitigate the risk of lactic acidosis to patients prescribed metformin therapy.

4.2. Objectives

The aims of this work were to (i) determine the current consensus on the upper limit of safety for plasma metformin concentrations in the published literature and (ii) to quantitatively explore the relationship between plasma metformin concentrations and plasma lactate concentrations using data from published case reports. In this work, plasma lactate concentrations are used as a marker of lactic acidosis risk. This will be discussed further in the discussion. The metformin concentration associated with severe hyperlactatemia is assumed to define the upper limit of safety for metformin.

4.3. Methods

4.3.1. Literature review to clarify the upper limit of safety for plasma metformin concentrations

4.3.1.1. Data source

A literature search was conducted to clarify the upper limit of safety for plasma metformin concentrations. The literature review was conducted using a static search performed in Ovid MEDLINE (1946 to March 2020) and Ovid EMBASE (1946 to March 2020). The search was performed in Ovid MEDLINE using the Medical Subject Heading (MeSH) terms; 'Metformin', 'Acidosis, Lactic', 'Dose-Response Relationship, Drug' and keywords 'therapeutic concentration', 'therapeutic limit' and 'toxic concentration'. In Ovid EMBASE the search was performed using the MeSH terms; 'Metformin', 'Lactic acidosis', 'Dose response' and keywords 'upper limit', 'therapeutic concentration', 'therapeutic limit' and 'toxic concentration'. The Boolean operators 'AND' and 'OR' were used to search for relevant literature. Details of the static search strategy is shown in Appendix A3.1. The search was limited to studies conducted in humans and those published in the English language. Duplicate articles sourced from the databases were removed.

The sourced articles were initially screened by study title and abstract for relevance to metformin and reference to an upper limit of safety for plasma metformin concentrations. Relevant screened articles were then eligible for a full text review and assessed for inclusion and exclusion. Studies were included if there was a reported upper limit of safety for plasma metformin concentrations. Studies were excluded if they: (i) were not human studies and (iii) were not written in the English language.

4.3.1.2. Data extraction

The following data were extracted from each article; publication details (author(s), year of publication), reported upper limit of safety for plasma metformin concentration, and, whether the study had defined the upper safety limit by means of a formal analysis (e.g. exposure-response study).

4.3.2. Quantitative analysis of metformin and lactate plasma concentrations from published reports

4.3.2.1. Data source

A retrospective analysis of plasma metformin and lactate concentrations extracted from published case reports and research studies was conducted. The data were derived from two primary sources:

1. An observational study conducted by Duong et al [371] investigating the plasma concentrations of metformin and lactate in type 2 diabetic patients with chronic renal impairment who were taking therapeutic doses (i.e. between 250-2000 mg daily) of metformin. No patients in this cohort developed lactic acidosis.
2. A database of metformin associated lactic acidosis (MALA) cases derived from case reports in Chapter 2 of this thesis. All patients in these case reports presented with or developed lactic acidosis. Only cases that had ingested an intentional overdose of metformin were extracted and included in the analysis. Non-overdose cases were excluded from the analysis to avoid the potential of other risk factors reported in the case reports (e.g. renal impairment and heart failure as identified in Chapter 2) being the cause of the elevated metformin and/or lactate concentrations.

Note that the analysis was conducted using data from metformin overdose and non-overdose cases.

4.3.2.2. Data selection

All paired metformin and lactate concentrations from the observational study by Duong et al were included in the analysis. Concentration data from this cohort of patients represented the typical patient population taking metformin for therapeutic uses.

Data extracted from cases in the published database were included if there were (i) paired metformin and lactate concentrations, (ii) no interventions (e.g.

renal replacement therapy) that could alter metformin and lactate concentrations prior to measurement, (iii) patient-level data (summary data was not analysed), (iv) the case had ingested an overdose of metformin, and, (v) metformin and lactate concentrations were measured on admission. Data from the database were excluded if the metformin and lactate concentrations did not have reported measurement units.

4.3.2.3. Data extraction

Metformin and lactate concentrations were extracted from cases in the published database that met the inclusion criteria. Data provided in graphical form were extracted using the MATLAB (R2016b, MathWorks, Natick, MA) script GRABIT.

4.3.2.4. Data analysis

Data sourced from the paper by Duong et al [371] and the published MALA database were combined, and, the data analysed by fitting a concentration-response (E_{max}) model with baseline. A sigmoidal E_{max}model with baseline was also tried. Here plasma metformin concentration was the independent variable and plasma lactate the response variable. The E_{max} and sigmoidal E_{max} models with baseline are shown in Equation 4.1 and Equation 4.2 respectively, as follows;

$$E = E_0 + \frac{(E_{max} - E_0) \cdot C}{EC_{50} + C}$$

Equation 4.1 *The E_{max} model with baseline*

$$E = E_0 + \frac{(E_{max} - E_0) \cdot C^{\lambda}}{EC_{50}{}^{\lambda} + C^{\lambda}}$$

Equation 4.2 *The sigmoidal E_{max} model with baseline*

where, E represents lactate concentration (mmol/L), E_0 is the basal lactate concentration in the absence of metformin (mmol/L), E_{max} is the maximum lactate concentration (mmol/L), EC_{50} is the concentration of metformin (mg/L) at which the drug produces half its maximum effect, C is the observed concentration of metformin (mg/L) and λ is the Hill coefficient. Note that the basal lactate concentration was estimated in both the E_{max} and sigmoidal E_{max} models with baseline. The E_{max} and sigmoidal E_{max} models with baseline were compared for fit based on: (i) visual inspection of the model fit to the data and (ii) the biological plausibility of parameter estimates.

A naïve pooled analysis was conducted using NONMEM v7.4 (ICON Development Solutions, Hanover, MD, USA). In brief, a naïve pooled approach involves pooling all data available together as if the data were collected from a single individual or each observation were obtained from different individuals, refer to Chapter 1 (section 1.1.4.1.) for more information. Note a naïve pooled analysis was used to analyse the data because there were repeated paired metformin and lactate concentration data extracted from the publication by Duong et al that was not patient identifiable. The first-order (FO) estimation method was used. Pre- and post- processing was conducted using R (version 3.5.3) to manipulate the data into a NONMEM readable form and for plotting graphs.

For the purposes of this analysis it was assumed that blood lactate and plasma lactate concentrations were interchangeable. The difference between blood and plasma lactate concentrations have been shown to be negligibly different at rest (ratio 1:1) [383, 384].

The median and 95% confidence interval (CI) of plasma metformin concentrations associated with hyperlactatemia and severe hyperlactatemia were predicted from the models. Hyperlactatemia was defined as a lactate concentration of >2 mmol/L and severe hyperlactatemia was defined as a lactate concentration of >5 mmol/L (considered the lower limit for the diagnosis of associated acidosis).

4.3.3. Quantitative analysis of metformin and pH from published reports

The concentration-response relationship between plasma metformin concentrations and pH was conducted using data extracted from the published database of MALA cases [385]. All paired metformin and pH concentrations were extracted from the database and included in the analysis if there was: (i) no intervention made that could affect the plasma metformin concentrations and pH, (ii) patient-level data, (iii) the case had ingested an acute overdose of metformin and (iv) the plasma metformin concentration and pH had been measured on admission. The extracted plasma metformin concentrations and pH data were analysed by fitting an E_{max} and sigmoidal E_{max} model with baseline (as described in section 4.3.2.4.). The median and 95% CI of plasma metformin concentrations associated with acidosis (i.e. pH <7.35) were predicted from the models.

4.4. Results

4.4.1. Literature review

4.4.1.1. Identified literature

The search identified a total of 173 articles. Duplicate articles were removed and a total of 161 articles were screened. Only 30 of the 161 screened articles were subject to a full-text analysis. Following the full-text analysis, 25 articles were excluded for the following reasons: no reference to an upper limit of safety for metformin (n=21) and article could not be accessed or located (n=4). Five articles met the inclusion criteria and were included in the literature review. A schematic of the workflow for the literature review is presented in Figure 4.1.

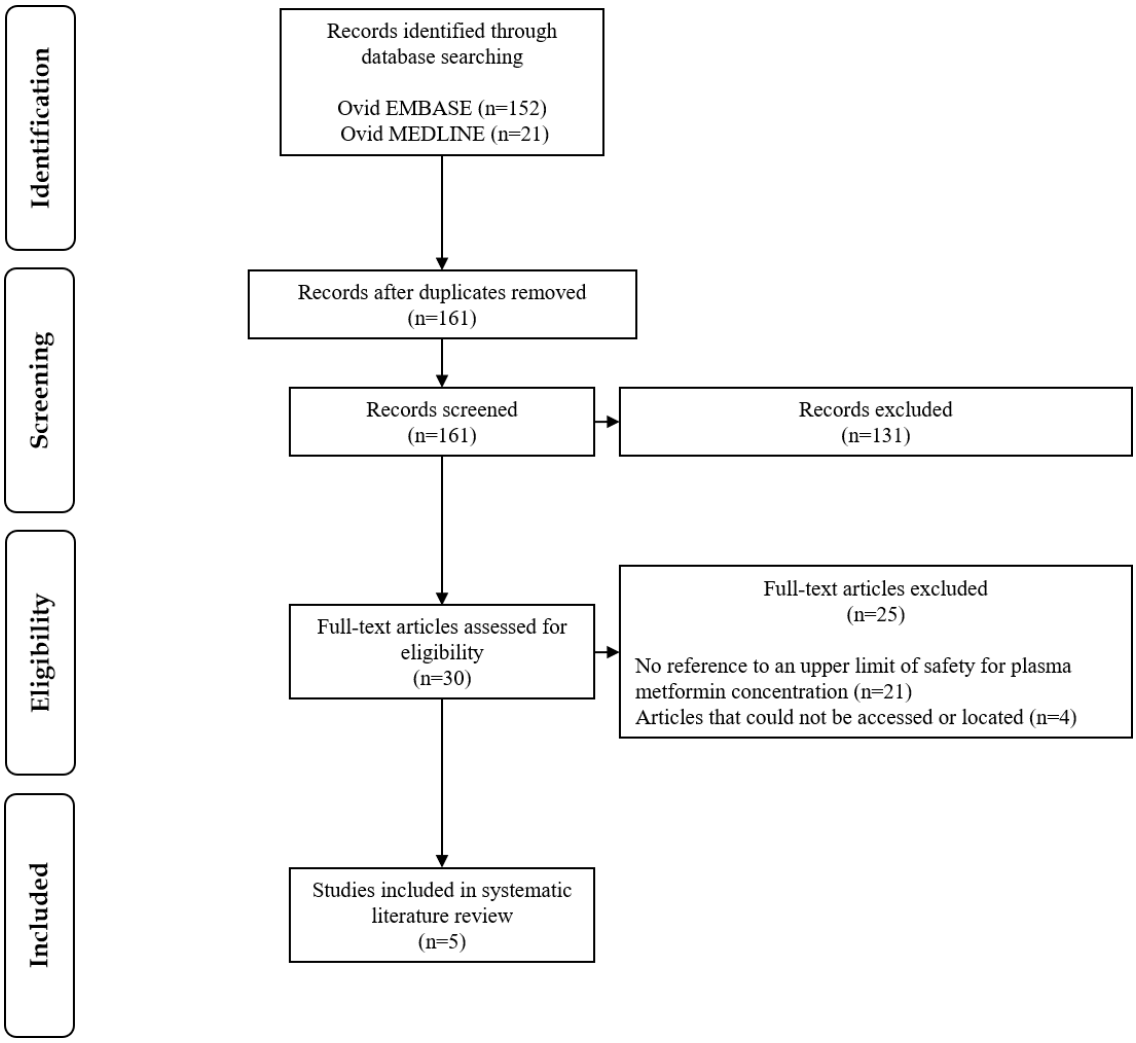


Figure 4.1 *Flow diagram for the literature review*

4.4.1.2. Upper limit of safety for plasma metformin concentration

A total of 5 articles reported an upper limit of safety for plasma metformin concentration (Table 4.1). The reported upper limit of safety for plasma metformin concentrations was reported to range from 2 to 5 mg/L [122, 386-389]. However, none of the identified articles conducted a formal analysis to support these values. It remains unclear how this safety limit had been defined.

Table 4.1 *Summary of reported upper safety limit for plasma metformin concentration in the published literature*

Author	Year	Ref	Study conducted an analysis to define upper limit (Y/N)	Upper safety limit for plasma metformin concentration (mg/L)
Carland et al	2018	[386]	N	5
Gil et al	2018	[387]	N	5
Goo et al	1996	[388]	N	5
Zoppellari et al	2013	[122]	N	2
Zoppellari et al	2019	[389]	N	4

4.4.2. Quantitative analysis of plasma metformin and lactate concentrations

4.4.2.1. Data source

Data were extracted from 24 patients in the publication by Duong et al and 31 case histories were identified from the published MALA case report database. A flowchart of the inclusion and exclusion process for the cases in the database is shown in Figure 4.2. A total of 103 paired metformin and lactate concentrations were collected from 55 individuals (demographics shown in Table 4.2).

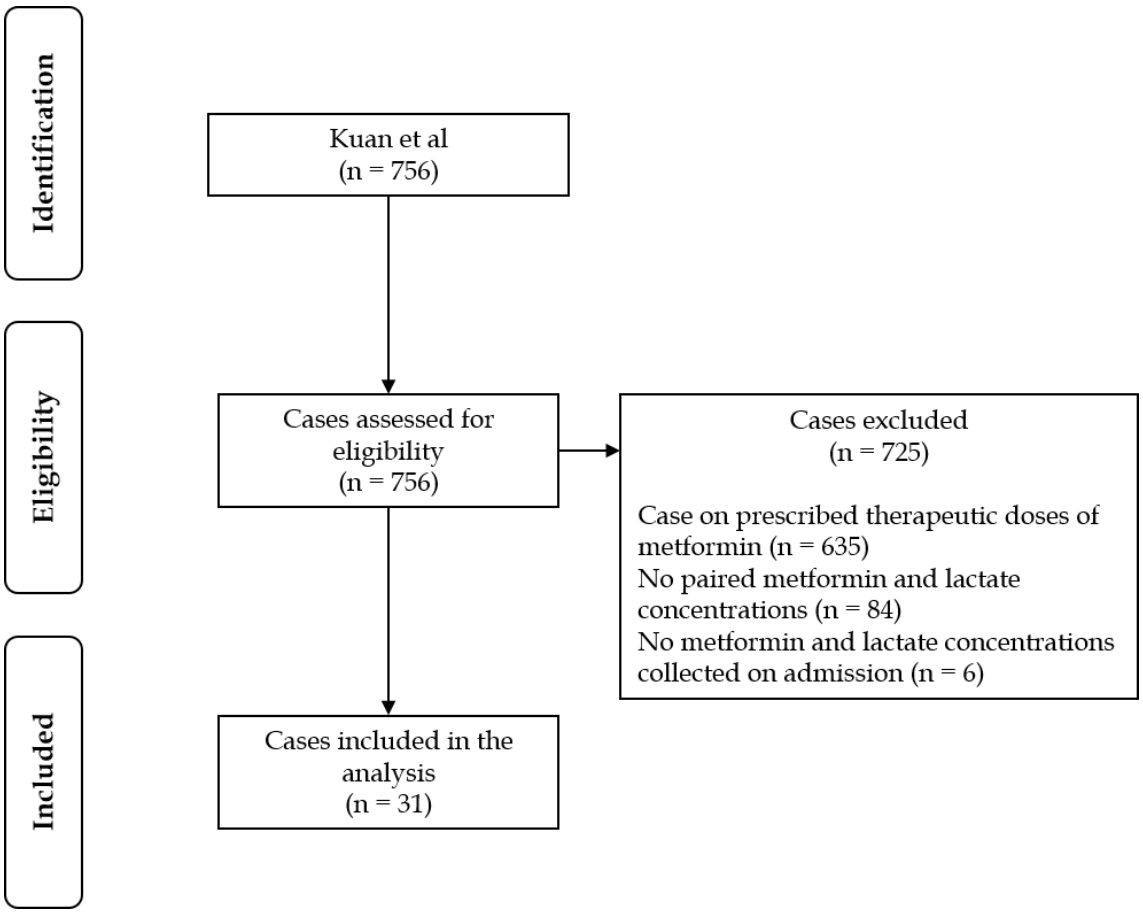


Figure 4.2 *Flow diagram for the inclusion and exclusion criteria of cases identified from the published database*

Table 4.2 *Summary demographic and clinical characteristics of patients*

Demographics	**Duong et al [371] (n=24)**	**Kuan et al [390] (n=31)**
Number of samples	72	31
Age (years)	73 [51-86]	51 [15-74]
Weight (kg)	87 [60-126]	-[b]
Metformin dose (mg/day)	1000 [250-2000]	60000 [12750-76500][c]
Lactate concentration (mmol/L)[a]	1.7 [0.8-5.5][d]	14.5 [2.4-39.0]
Metformin concentration (mg/L)[a]	1.5 [0.11-4.5]	66.0 [0.3-749.0]

[a]Lactate and metformin concentrations for cases extracted from the database were those measured on admission. [b]No cases from the database reported weight. [c]The ingested metformin dose was only available in 11 cases from the database. [d]Note that 18 lactate concentrations from the study by Duong et al met the criteria for hyperlactatemia (lactate >2 mmol/L).

4.4.2.2. Metformin and lactate concentration-response curve

The E_{max}model provided a reasonable description of the metformin and lactate concentration-response curve (Figure 4.3). The model predicted that a median plasma metformin concentration of 1.57 mg/L (95% CI: 1.05-2.36 mg/L) and 7 mg/L (95% CI: 4.5-10.9 mg/L) was associated with hyperlactatemia and severe hyperlactatemia, respectively. Model parameter estimates and relative standard errors are presented in Table 4.3.

The sigmoidal E_{max} model was also trialled for fit to the metformin and lactate data (shown in Figure 4.4). In theory, the sigmoidal E_{max} should provide a better fit to the data due to increased degrees of freedom, however, on visual inspection the sigmoidal E_{max} does not appear to capture the data as well as the E_{max} model. The sigmoidal E_{max} model predicted that a median plasma metformin concentration of 3.95 mg/L (95% CI: 2.57-6.08) and 5.6 mg/L (95% CI: 4.6-6.8 mg/L) was associated with hyperlactatemia and severe hyperlactatemia, respectively. Model parameter estimates and relative standard errors for the sigmoidal E_{max} model are shown in Table 4.4.

The E_{max} model estimated a reasonable basal plasma lactate concentration of 0.96 mmol/L (normal reference plasma lactate range: 0.5-1 mmol/L). However, the sigmoidal E_{max} model estimated a basal plasma lactate concentration of 1.69 mmol/L, a value outside the normal plasma lactate concentration reference range. These results indicate that the E_{max} model with baseline provided a more reasonable description. However, regardless of whether the E_{max} or sigmoidal E_{max} model with baseline was used to describe the data the lower bound of the 95% CI associated with severe hyperlactatemia was approximately 4.5 mg/L.

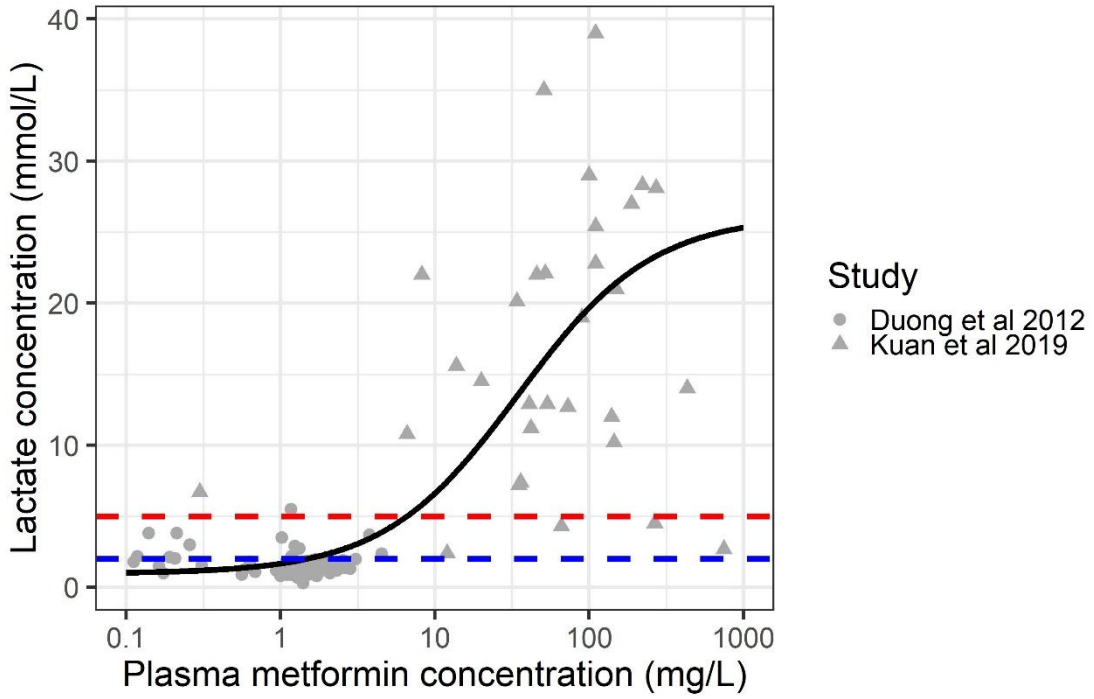


Figure 4.3 *The relationship between plasma metformin concentrations and lactate. The line of best fit from the E_{max} model is superimposed on the data. The circles and triangles in the plot represent data extracted from the study by Duong et al [371] and Kuan et al [390], respectively. The blue and red dashed line represents a lactate concentration of 2 and 5 mmol/L, respectively.*

Table 4.3 *Model parameter estimates for the E_{max} model*

Parameters	Definition	Estimates [RSE%]
E_{max} $(mmol/L)$	Maximum lactate concentration	25.2 [16%]
$E_0(mmol/L)$	Basal lactate concentration in the absence of metformin	0.96 [31%]
$EC_{50}(mg/L)$	Concentration of metformin at which it produces half its maximum effect	34.7 [17%]

RSE is relative standard error calculated as the standard error divided by the parameter estimate.

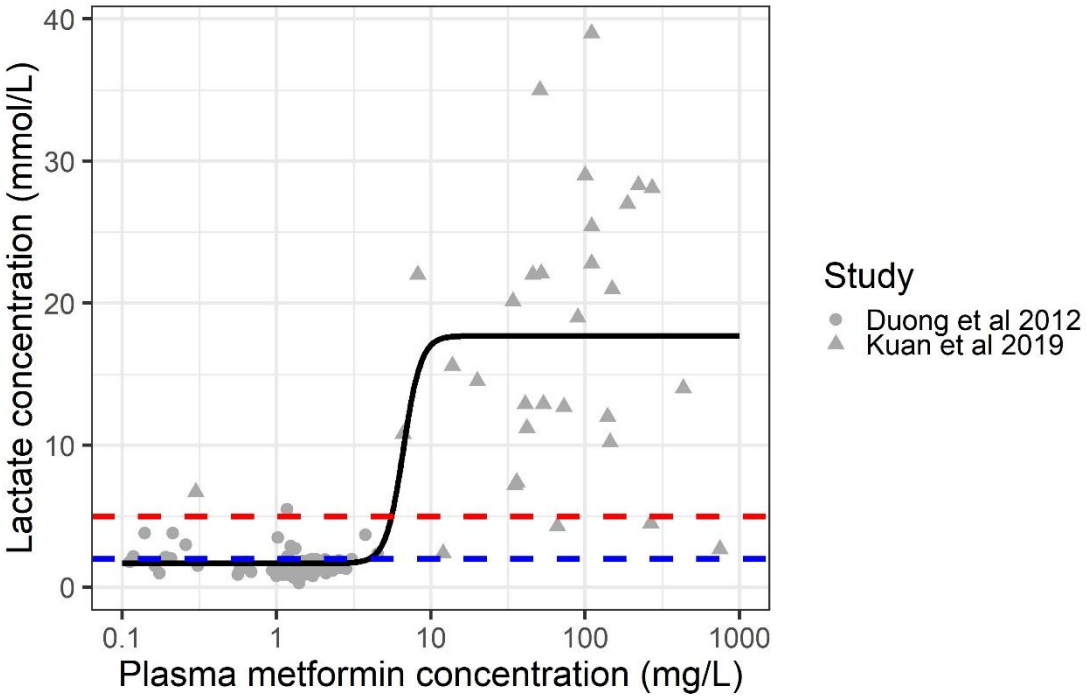


Figure 4.4 *The relationship between plasma metformin concentrations and lactate. The line of best fit from the sigmoidal E_{max} model is superimposed on the data. The circles and triangles in the plot represent data extracted from the study by Duong et al [371] and Kuan et al [390], respectively. The blue and red dashed line represents a lactate concentration of 2 and 5 mmol/L, respectively.*

Table 4.4 *Model parameter estimates for the sigmoidal E_{max} model*

Parameters	Definition	Estimates [RSE%]
$E_{max}\ (mmol/L)$	Maximum lactate concentration	17.7 [10%]
$E_0(mmol/L)$	Basal lactate concentration in the absence of metformin	1.69 [8%]
$EC_{50}(mg/L)$	Concentration of metformin at which it produces half its maximum effect	6.64 [3%]
$Hill\ coefficient$		7.66 [41%]

RSE is relative standard error calculated as the standard error divided by the parameter estimate.

4.4.3. Quantitative analysis of plasma metformin concentrations and pH

There was insufficient data in the published sources to conduct a meaningful analysis of the relationship between plasma metformin concentrations and pH. There were no paired metformin concentration and pH measurements in patients taking therapeutic doses of metformin. In addition, the range of reported pH data collected from metformin overdose cases was very narrow (i.e. pH of 6.05 to 7.39) making a meaningful analysis difficult. A raw plot of paired metformin concentration and pH measurements is presented in Figure 4.5.

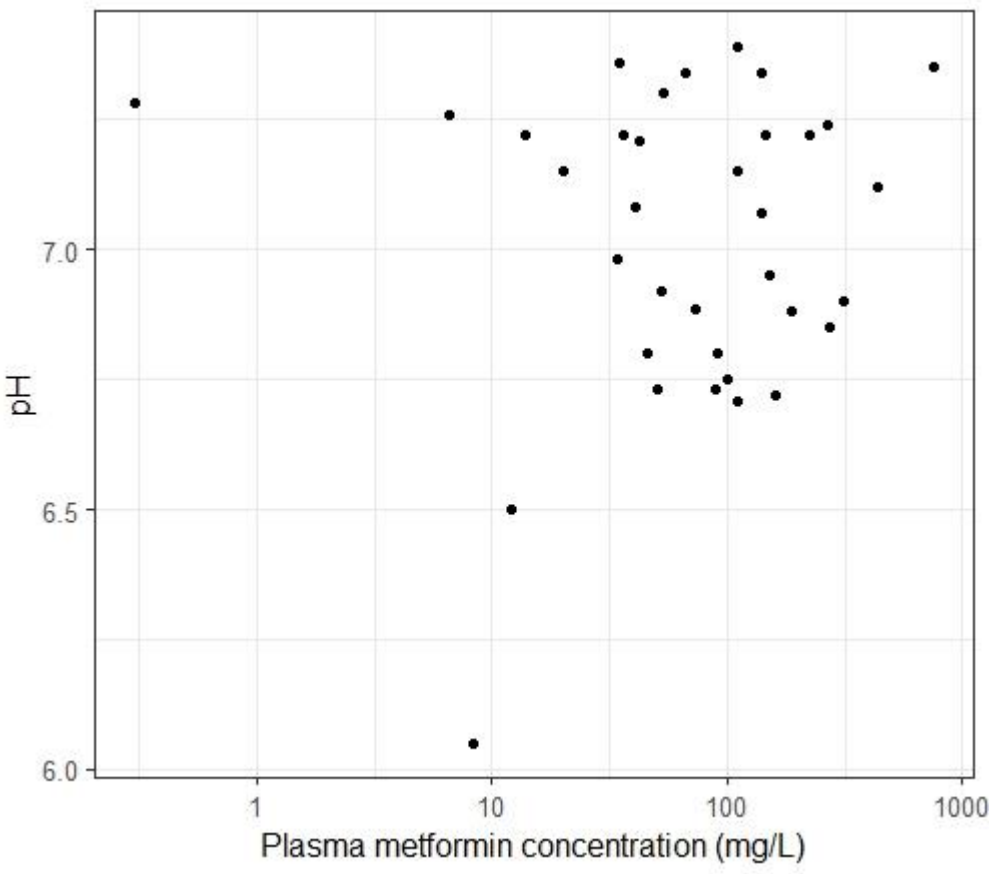


Figure 4.5 *Semi-log plot of paired plasma metformin concentration and pH measured on admission to a medical facility from cases whom had ingested an overdose of metformin from the published metformin associated lactic acidosis database.*

4.5. Discussion

In this study, the relationship between plasma metformin concentrations and lactate was explored by quantitatively analysing the concentration-response relationship across a wide range of metformin doses, including intentional overdoses. This study found that metformin plasma concentrations greater than 4.5 mg/L (the lower bound of the 95% confidence interval) are associated with severe hyperlactatemia and, based on the data analysed here, is proposed as the upper limit of safety. Dose adjustment to maintain metformin plasma concentrations below 4.5mg /L should mitigate the risk of lactic acidosis. In addition, no studies were found in the literature review to have explored the upper therapeutic limit for metformin using a safety endpoint.

There has been little research focussed on establishing a therapeutic range for plasma metformin concentrations, although our findings are in agreement with the small number of studies in the published literature. In a systematic literature review of metformin studies, Kajbaf et al found that the majority of studies supported a therapeutic plasma metformin concentration range between 0.1 and 4 mg/L [369]. This is supported by findings from a retrospective study that found that plasma metformin concentrations within 0.5±0.4 (standard deviation) mg/L were well tolerated in diabetic patients with normal renal function [391]. Similarly, in a study by Lalau et al, 798 plasma metformin concentrations were analysed from 467 patients [392]. Of these measured plasma concentrations only approximately 7% were found to be greater than 5 mg/L [392].

4.6. Limitations

The results of this study should be interpreted in light of the following limitations.

It is assumed in this work that the association between metformin concentrations and the risk of lactic acidosis can be explored using lactate concentration as a marker of lactic acidosis risk in patients taking therapeutic and supra-therapeutic doses of metformin. While it is not necessarily implied

that metformin will cause lactate concentrations to increase directly, and that this in turn will cause lactic acidosis, it is assumed that lactic acidosis will cause a severe hyperlactatemia in many cases. Hence, while the association may exist, it does not imply cause and effect. In addition, lactate may be a fairly poor safety metric, since lactate concentrations may fluctuate significantly for many reasons, such as exercise and underlying pathology (e.g. chronic airway obstruction) [393].

All cases extracted from the database had taken an acute overdose of metformin and were diagnosed with lactic acidosis in the case reports. These cases therefore all had increased lactate concentrations by definition. In the overdose cases included in this analysis it was assumed that the supra-therapeutic dose of metformin was the dominant cause of lactic acidosis. None of the patients extracted from Duong et al had taken an intentional overdose and none experienced hyperlactataemia or acidosis [371]. Overall, the results from this chapter are only able to show an association between metformin and lactate concentrations.

An assumption of this analysis was that there is no significant delay between elevated metformin concentrations resulting in an increased production of lactate as a consequence of lactic acidosis. However, four cases analysed in the study were noted to have on admission metformin concentrations greater than 10 mg/L and lactate concentrations less than 5 mmol/L (as can be seen in Figure 4.3 seen below the red dashed cut-off line). Upon closer investigation of these cases it was found that subsequent lactate concentrations that were measured post-admission to a medical facility were all greater than 5 mmol/L. This is a possible explanation why the E_{max} model does not appear to provide a reasonable description for the paired concentration data for these cases.

4.7. Conclusion

In summary, the relationship between plasma metformin and lactate concentrations was explored quantitatively by analysing the concentration-response relationship across a wide range of metformin doses. This study found

that plasma metformin concentrations >4.5 mg/L (the lower bound of the 95% CI) are associated with a risk of severe hyperlactatemia. Findings from this study suggest that dose adjustment to maintain plasma metformin concentrations to <4.5 mg/L should mitigate the risk of lactic acidosis.

Chapter 5: The pharmacokinetics of metformin in renal impairment

5.1. Preamble to the chapter

In Chapter 4 of this thesis plasma metformin concentrations greater than 4.5 mg/L were found to be associated with an increased risk of lactic acidosis. Here, a better understanding of the pharmacokinetics of metformin in patients with reduced renal function will help guide dosing to maintain concentrations below this upper limit of safety. In this chapter a noncompartmental analysis was performed to explore the pharmacokinetics of metformin in renally impaired patients.

5.2. Introduction

Metformin is predominantly cleared from the body as unchanged drug by the kidneys [27, 29, 30]. It has been suggested that 80% of metformin is cleared via active tubular secretion by the kidneys and the remaining 20% by glomerular filtration [32]. Patients with renal impairment who are prescribed metformin therapy have been found to have reduced metformin clearance [394]. Metformin has traditionally been contraindicated in renally impaired patients due to the concern that accumulation of the drug will lead to an increased risk of lactic acidosis. Recent changes to dosing guidelines propose that metformin can be used in renally impaired patients provided that doses are reduced to account for reduced renal function [14, 24]. However, current dosing guidelines show little agreement and few appear to be evidence based, leaving prescribers with unclear messages about how to safely dose metformin in patients with renal impairment.

The impact of reduced renal function on the handling of metformin may be more complex than previously assumed. According to first principles, dose reduction will normalise metformin exposure between patients with different levels of renal impairment and mitigate the risk of adverse effects. In theory, this could be achieved by reducing the dose in proportion to glomerular filtration rate (GFR), where it is assumed that metformin dosing requirements and GFR follow a simple linear relationship. However, it has been proposed that

compounds eliminated mainly by tubular secretion, like metformin, may display a non-linear relationship with GFR [395, 396]. It is therefore unclear if creatinine-based equations used to estimate GFR (eGFR) or creatinine clearance (CLcr) in the clinical setting provide accurate predictions of metformin clearance. A quantitative analysis of metformin pharmacokinetics in patients with renal impairment is required to guide dosing.

5.3. Objectives

The aim of this chapter was to explore the pharmacokinetics of metformin in renal impairment to inform renal dosing. The specific objectives of this work were to:

i. To conduct a pharmacokinetic analysis of metformin in subjects with different degrees of renal function
ii. Compare the ability of different GFR equations used in the clinical setting to predict metformin clearance, and to determine which equation will provide the best guide for metformin dosing
iii. Develop an empirical renal dosing equation for metformin using the Cockcroft and Gault equation, 4-variable Modification of Diet in Renal Disease equation and Chronic Kidney Disease Epidemiology Collaboration equation and determine the maintenance dose range for patients in different CKD groups to achieve a target steady-state plasma concentration of 1 mg/L.

5.4. Methods

5.4.1. Data

Data from an observation study conducted by the Nephrology Department at Dunedin Public Hospital (New Zealand) was available for analysis. The study was approved by the New Zealand Health and Disability Ethics Committee, application number: 14/STH/156/AM01. The study was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR), number: ACTRN12614001180616. All study subjects gave written and informed consent.

A brief overview of the study procedures is provided however note that the collection of data was not part of this thesis. Thirty-four study participants with varying degrees of renal function were enrolled in the study. Participants were stratified into three groups based on their renal function: (i) those with stable eGFR ≥60 mL/min, (ii) those with stable eGFR 30-60 mL/min, and, (iii) those with stable eGFR <30 mL/min. Study participants fasted overnight prior to the first study day. On the first study day, a blood sample was taken for the measurement of baseline plasma metformin and creatinine concentrations. Subjects then received a single dose of metformin 500 mg orally. Metformin was administered with a minimum of 250 mL of filtered water and an intake of 100 mL/h was maintained for the following four hours to ensure adequate urine output. Blood samples were collected at the following times post drug administration: 15-30 minutes, 30-60 minutes, 90-120 minutes, 3 hours, 5-6 hours, 8-12 hours and 24 hours for the measurement of metformin and creatinine. An additional blood sample was collected at 30-36 hours for the measurement of metformin. Timed urine samples were collected at times 0-3 hours, 3-8 hours and 8-24 hours post drug administration. The collected blood and urine samples were stored at -80^{o}C. For a detailed overview of the study procedures refer to Appendix A4.1.

A summary of the study participants' demographics stratified by their enrolment groups is presented in Table 5.1.

Table 5.1 *Demographics of participants in the Dunedin Public Hospital*

	Group 1 ***(n=17)***	**Group 2** ***(n=6)***	**Group 3** ***(n=11)***	**Pooled data** ***(n=34)***
Age (years)	32.0 [20.0-59.0] (23.0-37.0)	65.0 [42.0-70.0] (56.0-68.0)	67.0 [47.0-79.0] (63.5-71.0)	51.5 [20.0–79.0] (32.3–66.0)
Sex (F:M)	2:15	1:5	2:9	5:29
Height (cm)	179 [164-195] (171-184)	171 [160-181] (164-175)	172 [157-182] (167-174)	174 [157–195] (168–181)
Weight (kg)	82.2 [48.0-98.4] (71.0-87.5)	82.2 [76.0-106.7] (81.0-89.9)	79.0 [65.1-149.5] (72.8-88.0)	82.1 [48.0–149.5] (75.1–87.7)
BMI (kg/m^2)[a]	24.8 [17.8-33.5] (23.0-26.1)	29.3 [26.6-35.0] (26.9-32.3)	28.6 [22.9-48.8] (25.2-29.9)	26.3 [17.8–48.8] (24.2–28.9)
FFM (kg)[b]	62.3 [33.9-69.9] (54.5-64.7)	58.2 [49.5-72.1] (55.7-61.1)	60.6 [41.5-80.5] (52.2-62.5)	61.5 [33.9–80.5] (54.4–63.8)
Serum creatinine (µmol/L)	89 [52.0-126.0] (82.0-94.0)	172.5 [111.0-196.0] (146.5-188.0)	395.0 [242.0-546.0] (322.5-416.0)	118.5 [52.0–546.0] (89.5–300.0)
$CLcr_{CG}$[c] (mL/min)	114.1 [85.7-167.0] (93.3-119.2)	33.7 [27.8-61.4] (29.8-49.6)	15.4 [9.5-19.6] (13.9-17.6)	73.5 [9.5-167.0] (18.1-113.2)
$eGFR_{MDRD}$[d] ($mL/min/1.73m^2$)	87.7 [59.5-118.5] (80.3-101.0)	34.2 [29.9-48.6] (31.3-42.2)	13.4 [8.9-18.7] (12.3-15.9)	54.1 [8.9–118.5] (17.4–86.7)
$eGFR_{MDRD}$ (adjusted)[f] (mL/min)	97.3 [75.7-142.7] (93.9-104.0)	38.7 [34.1-55.9] (36.2-48.2)	15.6 [11.0-19.3] (13.8-18.5)	65.8 [11.0-142.7] (18.5-97.2)

Table 5.1 cont *Demographics of participants in the Dunedin Public Hospital*

	Group 1 ***(n=17)***	**Group 2** ***(n=6)***	**Group 3** ***(n=11)***	**Pooled data** ***(n=34)***
$eGFR_{CKDEPI}$[e] (mL/min/1.73m²)	100.1 [66.9-122.4] (87.2-115.7)	34.0 [30.0-53.5] (31.3-45.3)	13.2 [8.2-17.3] (11.9-15.5)	60.2 [8.2–122.4] (17.2–98.7)
$eGFR_{CKDEPI}$ (adjusted)[f] (mL/min)	85.1 [85.1-151.1] (104.4-119.1)	38.7 [34.2-61.5] (35.8-51.8)	15.6 [10.0-18.7] (13.3-17.4)	73.2 [10.0-151.1] (17.9-109.2)

Data are presented as median [range] (interquartile range) unless otherwise specified. Group 1: participants with stable eGFR ≥60 mL/min. Group 2: participants with stable eGFR 30-60 mL/min. Group 3 participants with stable eGFR <30 mL/min.[a]BMI is body mass index. [b]FFM is fat free mass calculated using the equation by Janmahasatian et al [397]. [c]$CLcr_{CG}$ is creatinine clearance estimated using the Cockcroft and Gault equation [80]. Note that ideal body weight [398] was used in the Cockcroft and Gault equation as a body size metric. [d]$eGFR_{MDRD}$ is glomerular filtration rate estimated using the 4-variable Modification of Diet in Renal Disease equation [82]. [e]$eGFR_{CKDEPI}$ is glomerular filtration rate estimated using the Chronic Kidney Disease Epidemiology Collaboration equation [83]. [f]Note that $eGFR_{MDRD}$ (adjusted) and $eGFR_{CKDEPI}$ (adjusted) were adjusted for the individual body surface area measurements for each subject calculated using the Du Bois Method [399].

5.4.2. Pharmacokinetic analysis

The pharmacokinetic analysis was performed in Microsoft® Excel® 2016 and R (version 3.5.3) using the pk.nca function in the PKNCA package (v0.9.2). The R code is presented in Appendix A4.2.1. Unless stated, the metrics reported in section 5.5.1 were determined using Microsoft® Excel® 2016 (the outputs from R were used to quality assure the calculations). The following pharmacokinetic metrics for metformin were determined; (i) maximum plasma concentration achieved (C_{max}) following a single oral dose, (ii) time when maximum plasma concentration was achieved (T_{max}), (iii) terminal elimination slope (λ_z), (iv) terminal elimination half-life ($t_{1/2}$), (v) area under the curve (AUC) from time zero to time of last observed concentration above the limit of quantification (AUC_{0-last}) and time zero to infinity ($AUC_{0-\infty}$), (vi) apparent clearance (CL/F),

and, (vii) renal clearance (CL_{renal}). A description of how each of the pharmacokinetic metrics was determined is described as follows.

C_{max} and T_{max}. The C_{max} achieved following a single dose of metformin was determined by identifying the maximum observed plasma metformin concentration. The T_{max} for metformin was determined by identifying the time at which the maximum plasma metformin concentration was observed. The C_{max} and T_{max} were determined in Microsoft® Excel® 2016 and the mean and standard deviation was calculated.

Terminal elimination slope. To derive λ_z a linear regression was drawn through the last 2 observations on a semi-log plot of drug concentration versus time using the following equation;

$$\lambda_z = \frac{\ln(C_i/C_{i+1})}{\Delta t}$$

Equation 5.1 *Formula for the terminal elimination slope*

where, C_i is the concentration (mg/L) taken at time i, C_{i+1} is the concentration (mg/L) taken at time $i + 1$ (in hours), and, Δt is the time interval between the observations. Note that visual inspection of the data was performed prior to calculation of the terminal elimination slope. Based on the data available only the last 2 observations were used to determine the terminal elimination slope to avoid the use of the maximum observed plasma metformin concentration and to account for terminal phase samples being below the limit of quantification.

Terminal elimination half-life. The terminal elimination half-life was calculated using the following equation;

$$t_{1/2} = \frac{\ln(2)}{\lambda_z}$$

***Equation* 5.2** *Formula for terminal elimination half-life*

where, $t_{1/2}$ is the terminal elimination half-life (h^{-1}) and λ_z is the terminal elimination slope (described above).

Area under the curve. The area under the curve was calculated using the trapezoidal rule. The AUC_{0-last} was interpolated using Equation 5.3 as follows;

$$AUC_{i-i+1} = \frac{C_i + C_{i+1}}{2} (t_{i+1} - t_i)$$

***Equation* 5.3** *Formula to calculate the area under the curve for each trapezoid*

where, AUC_{i-i+1} is the area under the curve (mg·h/L) from time i to time $i + 1$, C_i is the concentration of the drug at time i, C_{i+1} is the concentration of the drug at time $i + 1$, t_i is the time at i and t_{i+1} is the time at $i + 1$. To calculate the AUC from time 0 to the first observed concentration it was assumed that the plasma concentration at time zero was equal to 0, under the assumption that drug absorption at time zero is negligible. The area under the curve from the time of last observed concentration above the limit of quantification to infinity ($AUC_{last-\infty}$) was calculated using the following equation;

$$AUC_{last-\infty} = \int_{t=t_{last}}^{t=\infty} C\ dt = \frac{C_{last}}{\lambda_z}$$

***Equation* 5.4** *Formula to calculate the extrapolated area under the curve*

where, t_{last} is the time of last observed concentration above the limit of quantification, C is the drug concentration, C_{last} is the last observed concentration above the limit of quantification and λ_z is the terminal elimination slope. To calculate $AUC_{0-\infty}$ the interpolated trapezoid areas from t_0 to t_{last} and the extrapolated area from t_{last} to t_∞ were summated.

Apparent clearance. Apparent clearance was determined using the following equation,

$$CL/F = \frac{dose}{AUC_{0-\infty}}$$

Equation 5.5 *Formula for area under the curve from time zero to infinity*

where, CL/F is apparent clearance, $dose$ is the amount of drug administered (in mg) and $AUC_{0-\infty}$ is the area under the curve from time zero to infinity.

Renal clearance. Renal clearance was calculated using plasma and urine concentration data. Renal clearance was determined using the following equation;

$$CL_{renal} = \frac{amount\ excreted}{AUC_{0-24}}$$

Equation 5.6 *Formula to calculate renal clearance*

where $amount\ excreted$ is the product of the concentration of the drug in urine and volume of urine eliminated over a 24 hour period (in units of mg), and, AUC_{0-24} is the area under the curve from time 0 to 24 hours (i.e. time of last observed urine collection).

Study participants were stratified into their Chronic Kidney Disease (CKD) classification category, and, the mean and standard deviation of the estimated

pharmacokinetic metrics C_{max}, T_{max}, λ_z, $t_{1/2}$, AUC_{0-last}, $AUC_{0-\infty}$, CL/F and CL_{renal} for metformin were determined and tabulated.

5.4.3. The relationship between eGFR/ CLcr and metformin clearance

Creatinine clearance was estimated using the Cockcroft and Gault equation ($CLcr_{CG}$) (as shown in Equation 5.7) [80]. Note that ideal body weight was used in place of total body weight in the Cockcroft and Gault equation as per usual practice in New Zealand. In the Cockcroft and Gault equation, age is in years, IBW is ideal body weight [398] in kilograms, S_{cr} is serum creatinine in mg/100mL and $CLcr_{CG}$ is creatinine clearance in units of mL/min. Serum creatinine was converted from units of μmol/L to mg/100mL by dividing by 88.42. Creatinine clearance was estimated using the Cockcroft and Gault equation in R (version 3.5.3). The equation for ideal body weight (IBW) is shown in Equation 5.8, where $height$ is in inches.

$$CLcr_{CG} = \frac{(140 - age)(IBW)}{72 \cdot S_{cr}} \cdot 0.85\ (if\ female)$$

Equation 5.7 *Cockcroft and Gault equation*

$$Ideal\ body\ weight\ (male) = 50kg + 2.3kg \cdot (height - 60)$$
$$Ideal\ body\ weight\ (female) = 45.5kg + 2.3kg \cdot (height - 60)$$

Equation 5.8 *Ideal body weight equation* [398]

Estimated glomerular filtration rate was determined using the 4-variable Modification of Diet in Renal Disease (MDRD) [82] and Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) [83] creatinine-based estimating equations. In this chapter estimated glomerular filtration calculated using the 4-variable MDRD and CKD-Epi equations are referred to as $eGFR_{MDRD}$ and $eGFR_{CKDEPI}$, respectively. The 4-variable MDRD equation used is shown in Equation 5.9. In the 4-variable MDRD equation, S_{cr} represents serum creatinine in mmol/L and age is in years. The CKD-Epi equation is shown in Equation 5.10,

where, S_{cr} is serum creatinine in mg/dL, age is age in years, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and, max indicates the maximum of S_{cr}/κ or 1. The calculation of eGFR using the creatinine-based estimating equations was performed in R (version 3.5.3).

$$eGFR_{MDRD} = 1.75 \cdot {S_{cr}}^{-1.154} \cdot age^{-0.203} \cdot 0.742\ (if\ female)$$

Equation 5.9 *4-variable Modification of Diet in Renal Disease equation*

$$eGFR_{CKDEPI} = 141 \cdot min(S_{cr}/\kappa, 1)^{\alpha} \cdot max(S_{cr}/\kappa, 1)^{-1.209} \cdot 0.993^{age} \cdot [1.018\ if\ female] \cdot [1.159\ if\ black]$$

Equation 5.10 *Chronic Kidney Disease Epidemiology Collaboration equation*

Metformin apparent clearance (CL/F) and renal clearance (CL_{renal}) were regressed against; (i) $CLcr_{CG}$, (ii) $eGFR_{MDRD}$, and, (iii) $eGFR_{CKDEPI}$. For the purposes of this analysis $eGFR_{MDRD}$ and $eGFR_{CKDEPI}$ were adjusted for by body surface area (i.e. $eGFR$ was reported in units of mL/min instead of its standard units of mL/min/1.73m^2). Total body weight was used to determine body surface area.

A regression analysis was conducted in R (version 3.5.3). The R-squared values were determined and informally compared.

5.4.4. Developing empirical renal dosing equations for metformin

The regression equations were used to determine an empirical estimate of CL/F for an individual using individual estimates of eGFR/CLcr to guide the renal dosing of metformin using Equation 5.11. In Equation 5.11, MDR is maintenance dosing rate (mg/day), $C_{p,ss,ave}(target)$ is the target average steady-state plasma drug concentration (mg/L) and CL/F is apparent clearance (L/h). Note that the therapeutic range for metformin efficacy is poorly defined. Hence, an a priori $C_{p,ss,ave}(target)$of 1 mg/L was selected as a consensus value from

several studies where values of 0.1-2 mg/L have commonly been proposed [26, 323, 369, 400]. The maintenance dose-rate range was predicted for the lower and upper bound of each CKD category. The predicted doses were rounded to the nearest pragmatic metformin dose based on available tablet strengths.

$$MDR = C_{p,ss,ave}(target) \cdot CL/F \cdot 24$$

Equation 5.11 *Formula to calculate the maintenance dosing rate*

5.5. Results

A total of 257 plasma metformin concentrations and 102 timed urine samples from 34 study participants were available for analysis. Thirty-seven plasma concentrations were below the quantification limit and were excluded from the analysis. A graph of the plasma metformin concentrations versus time for study participants stratified by their CKD classification group is shown in Figure 5.1.

5.5.1. Pharmacokinetics of metformin at different degrees of renal function

The pharmacokinetic metrics of metformin in 34 study participants stratified by their CKD classification group is presented in Table 5.2. The maximum plasma metformin concentration achieved (i.e. C_{max}) was lowest in study participants with CKD 1 and 2 and was highest in study participants with CKD 5. The mean C_{max} achieved in study participants with CKD 1, 2, 3, 4 and 5 was 0.84, 0.84, 1.66, 2.34 and 2.36 mg/L, respectively. The time to achieve the maximum plasma concentration following a single dose of metformin was longer in study participants with CKD 3, 4 and 5. This was shown by the T_{max} for metformin in study participants with CKD 1 and 2 being approximately 2.4 hours versus a T_{max} of 2.92, 2.98 and 3.20 hours in study participants with CKD 3, 4 and 5, respectively. The $t_{1/2}$ of metformin was higher in subjects with CKD 3, 4 and 5. This is shown by the mean $t_{1/2}$ of metformin in CKD 1 and 2 being 3.38 and 3.75 hours versus the $t_{1/2}$ of 6.19, 8.09 and 6.76 hours in CKD 3, 4 and 5, respectively. Metformin $AUC_{0-\infty}$ was higher in patients with increasingly severe renal impairment. This can be seen in Table 5.2 where both AUC_{0-last} and $AUC_{0-\infty}$ increased from 3.81 and 4.44 mg·h/L in CKD 1 to 38.31 and 42.33 mg·h/L in CKD 5, respectively. The apparent clearance (CL/F) and renal clearance (CL_{renal}) of metformin was shown to decrease from CKD 1 to CKD 4.

Outputs from the pharmacokinetic analysis conducted in R are presented in Appendix A4.2.2. The pharmacokinetic metrics values determined in R were similar to the results obtained manually. Note a formal comparison was not conducted.

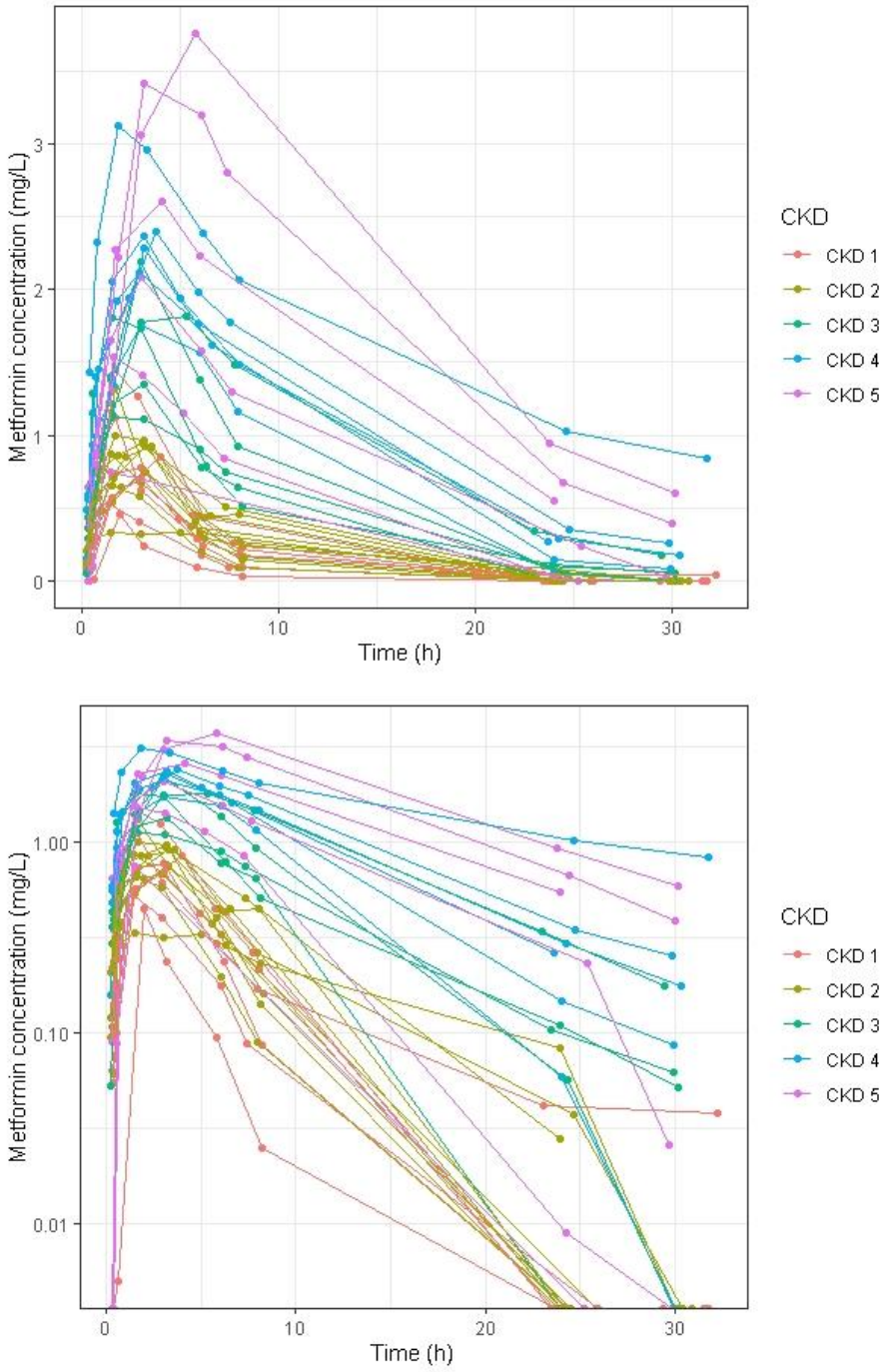


Figure 5.1 *Plasma metformin concentrations for study participants stratified by CKD renal group. The top is a Cartesian plot and the bottom is a semi-log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant. In the plot, CKD 1 represents a glomerular filtration rate ≥90 mL/min, CKD 2 represents a glomerular filtration rate of 60-89 mL/min, CKD 3 represents a glomerular filtration rate of 30-59 mL/min, CKD 4 represents a glomerular filtration rate of 15-29 mL/min and CKD 5 represents a glomerular filtration rate <15 mL/min.*

Table 5.2 *Pharmacokinetic metrics for study participants stratified by their CKD classification group*

Parameter	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5
Sample size (n)	7	10	5	6	6
C_{max} (mg/L)	0.84±0.35	0.84±0.26	1.66±0.42	2.34±0.45	2.36±1.14
T_{max} (h)	2.41±0.95	2.38±0.88	2.92±1.56	2.98±0.64	3.20±1.62
λ_z (h^{-1})	0.31±0.14	0.24±0.11	0.12±0.03	0.11±0.05	0.13±0.08
$t_{1/2}$ (h)	3.38±3.26	3.75±2.56	6.19±1.26	8.09±5.00	6.76±3.01
AUC_{0-last} (mg·h/L)	3.81±1.75	4.67±2.36	16.55±7.19	32.17±10.84	38.31±18.42
$AUC_{0-\infty}$ (mg·h/L)	4.44±2.11	5.51±2.13	18.47±6.20	37.13±18.80	42.33±21.73
CL/F (L/h)	150.89±105.85	108.87±63.56	29.11±7.70	15.62±5.52	15.74±10.33
CL_{renal} (L/h)	49.58±33.27	31.27±11.49	8.31±2.32	4.32±2.58	4.42±2.84

Data are presented as mean ± standard deviation. CKD 1 represents a glomerular filtration rate ≥90 mL/min. CKD 2 represents a glomerular filtration rate of 60-89 mL/min. CKD 3 represents a glomerular filtration rate of 30-59 mL/min. CKD 4 represents a glomerular filtration rate of 15-29 mL/min. CKD 5 represents a glomerular filtration rate of <15 mL/min.

5.5.2. The relationship between eGFR/CLcr and metformin clearance

Plots of the apparent clearance for metformin versus different measures of renal function are presented in Figure 5.2. Note that in each of the plots there are three subjects with a particularly high apparent clearance but normal measure of renal function (presented as a red dot in each of the graphs). On inspection these subjects only had plasma metformin concentrations above the quantification limit within 8 hours post-drug administration (i.e. within 4 hours after C_{max}). It is speculated that only the distribution phase of metformin was observed in these subjects due to concentrations after 8 hours post-drug administration being below the quantification limit - resulting in a high apparent clearance. These data were excluded from the correlation analysis. A linear relationship was found between the apparent clearance of metformin and $CLcr_{CG}$ estimated using the Cockcroft and Gault equation (R^2=0.85), $eGFR_{MDRD}$ estimated using the 4-variable MDRD equation (R^2=0.86) and $eGFR_{CKDEPI}$ estimated using the CKD-Epi equation (R^2=0.87).

Plots of the renal clearance for metformin versus the different measures of renal function are presented in Figure 5.3. Similarly, a linear relationship was identified between the renal clearance of metformin and $CLcr_{CG}$ (R^2=0.56), $eGFR_{MDRD}$ (R^2=0.53) and $eGFR_{CKDEPI}$ (R^2=0. 54).

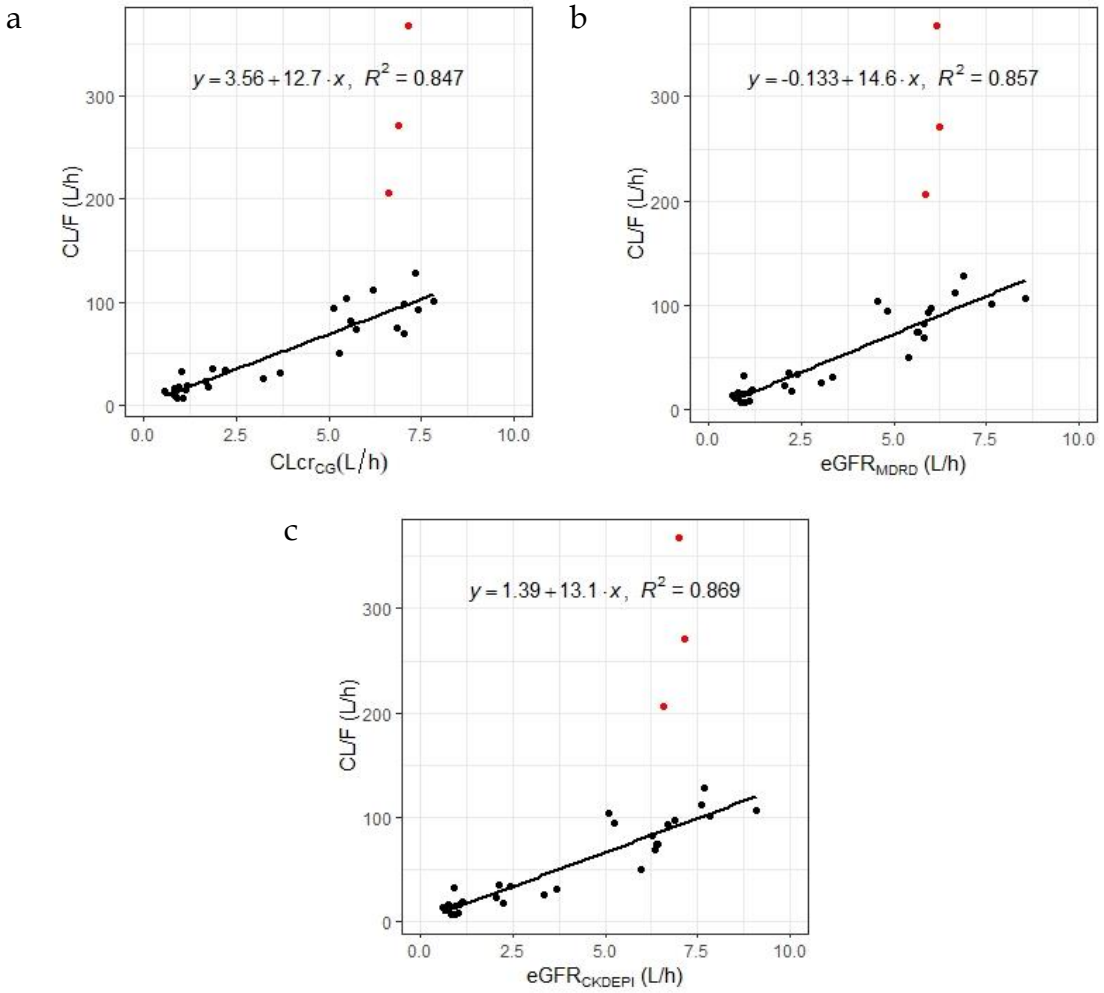


Figure 5.2 *Apparent clearance for metformin compared to different measures of renal function, including* ***a*** *$CLcr_{CG}$ (creatinine clearance estimated using the Cockcroft and Gault equation),* ***b*** *$eGFR_{MDRD}$ (glomerular filtration rate estimated using the 4-variable MDRD equation) and* ***c*** *$eGFR_{CKDEPI}$ (glomerular filtration rate estimating using the CKD-Epi equation). Each point represents a study participant. The line is a linear regression drawn through the data. The regression and R-squared value for each plot are reported. The red dots represent three outliers that were excluded from the correlation analysis.*

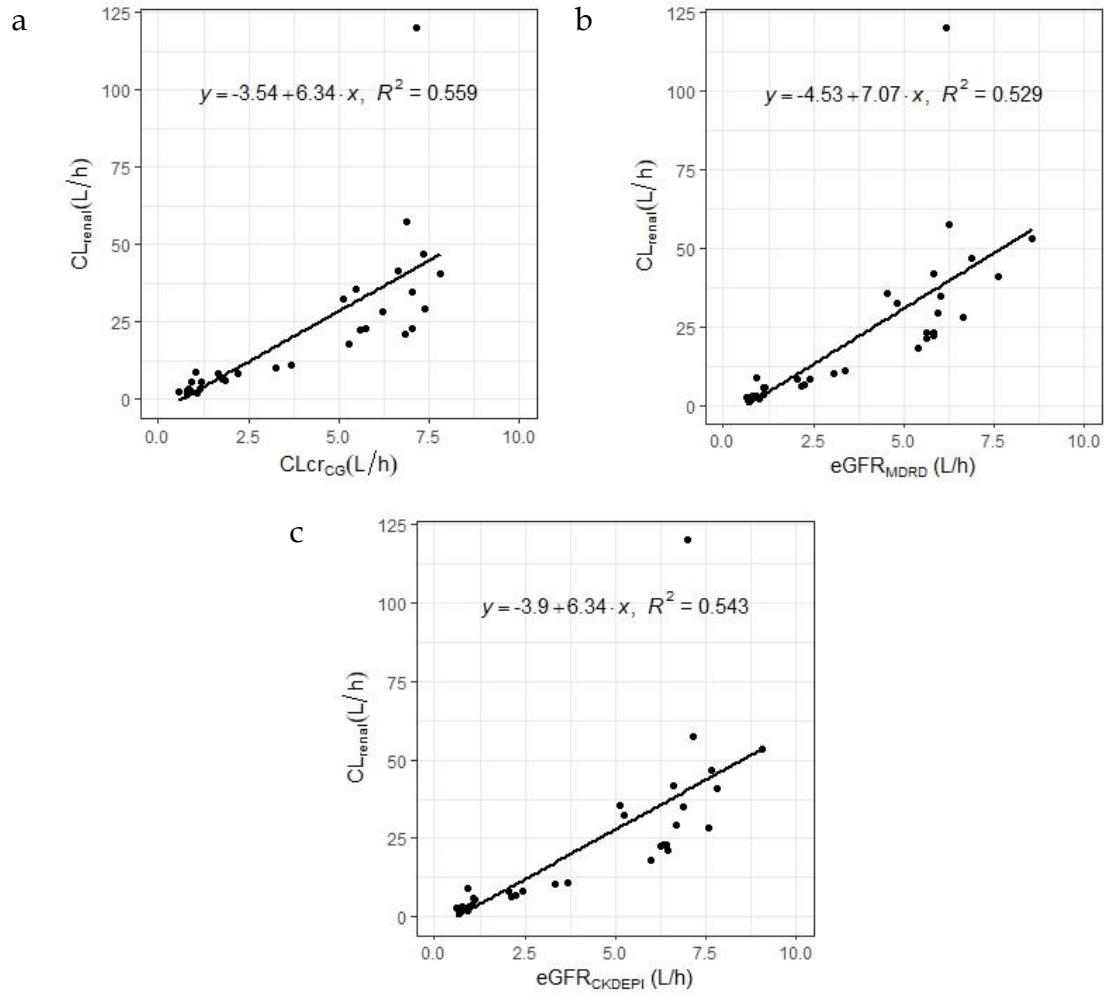


Figure 5.3 *Renal clearance for metformin compared to different measures of renal function, including* ***a*** *$CLcr_{CG}$ (creatinine clearance estimated using the Cockcroft and Gault equation),* ***b*** *$eGFR_{MDRD}$ (glomerular filtration rate estimated using the MDRD equation) and* ***c*** *$eGFR_{CKDEPI}$ (glomerular filtration rate estimating using the CKD-Epi equation). Each point represents a study participant. The line is a linear regression drawn through the data. The regression and R-squared value for each plot are reported.*

5.5.3. Empirical equations to guide the renal dosing of metformin

The empirical equations developed to guide the renal dosing of metformin using $CLcr_{CG}$, $eGFR_{MDRD}$ and $eGFR_{CKDEPI}$ are given as follows;

$$Daily\ dose = C_{p(target)} \cdot (3.56 + 12.7 \cdot CLcr_{CG}) \cdot 24$$

Equation 5.12 *An empirical equation for the renal dosing of metformin using the Cockcroft and Gault equation*

$$Daily\ dose = C_{p(target)} \cdot (-0.133 + 14.6 \cdot eGFR_{MDRD}) \cdot 24$$

Equation 5.13 *An empirical equation for the renal dosing of metformin using the 4-variable MDRD equation*

$$Daily\ dose = C_{p(target)} \cdot (1.39 + 13.1 \cdot eGFR_{CKDEPI}) \cdot 24$$

Equation 5.14 *An empirical equation for the renal dosing of metformin using the CKD-Epi equation*

where, $Daily\ dose$ is the daily dose of metformin required to achieve the target plasma metformin concentration in units of mg/day, $C_{p(target)}$ is the target plasma metformin concentration in mg/L, $CLcr_{CG}$ is creatinine clearance estimated using the Cockcroft and Gault equation in units of L/h, $eGFR_{MDRD}$ is glomerular filtration rate estimated using the 4-variable MDRD equation in units of L/h adjusted for body surface area and $eGFR_{CKDEPI}$ is glomerular filtration rate estimated using the CKD-Epi equation in units of L/h adjusted for body surface area. The predicted maximum daily metformin dose for each CKD eGFR/CLcr range using the developed empirical equations is presented in Table 5.3. As shown in Table 5.3 the predicted doses for metformin are the same amongst the different empirical equations for CKD 3 to 5, but not for CKD 1 to 2.

Table 5.3 *Predicted maximum daily metformin doses using the developed empirical equations stratified by CKD group*

CKD	GFR range (mL/min)	Predicted maximum daily metformin dose (mg)		
		$CLcr_{CG}$ method	$eGFR_{MDRD}$ method	$eGFR_{CKDEPI}$ method
1	≥90[a]	1700-2250	2000-2550	1700-2250
2	60-89	1000-1700	1000-2000	1000-1700
3	30-59	500-1000	500-1000	500-1000
4	15-29	250-500	250-500	250-500
5	<15	250	250	250

$CLcr_{CG}$ method represents using Equation 5.12 to predict metformin dose. $eGFR_{MDRD}$ method represents using Equation 5.13 to predict metformin dose. $eGFR_{CKDEPI}$ method represents using Equation 5.14 to predict metformin dose. [a]The upper end of the GFR range CKD 1 was set as 120 mL/min.

5.6. Discussion

In this study the pharmacokinetics of metformin in renal impairment was explored to understand the exposure metrics and how these related to covariates of interest. Whereby, the elimination curve of metformin was of particular interest given that metformin is recognised to exhibit flip-flop pharmacokinetics. The C_{max}, T_{max}, $t_{1/2}$, AUC_{0-last} and $AUC_{0-\infty}$ of metformin were found to increase with poorer levels of renal function, whilst the CL/F and CL_{renal} of metformin were found to decrease with poorer levels of renal function. Findings from other published pharmacokinetic studies have collectively found the same trend in patients with renal impairment [30, 394].

A linear relationship was found between the apparent clearance of metformin and estimates of renal function calculated using the creatinine-based estimating equations (i.e. CLcr estimated using the Cockcroft and Gault equation and eGFR estimated using the 4-variable MDRD and CKD-Epi equations). Similarly, a linear relationship was found between the renal clearance of metformin and estimates of renal function calculated using the creatinine-based estimating equations. Based on these results it can be inferred that the commonly used creatinine-based estimating equations will provide a reasonable reflection of the apparent clearance of metformin. Thus, the commonly used creatinine-based estimating equations can be used with ease to guide the dosing of metformin in the range of renal function explored in this analysis.

As part of the analysis three empirical renal dosing equations were developed for metformin using the Cockcroft and Gault, 4-variable MDRD and CKD-Epi equations. Using the empirical renal dosing equations, a maximum metformin daily dose of 2250, 1700, 1000, 500 and 250 mg was recommended in patients with CKD 1, 2, 3, 4 and 5, respectively. The maximum daily dose determined for metformin from the empirical equations aligned with published renal dosing guidelines (as shown in Table 5.4). The maximum daily dose of metformin determined from the empirical equations lay within the recommendations published by the European Medicines Agency for each CKD category [368]. Relative to the dosing guidelines in the New Zealand Formulary,

the maximum daily dose of metformin calculated using the empirical equations were found to lie within the dosing recommendations for subjects with a creatinine clearance less than 30 mL/min, but were found to overestimate the maximum daily dose in those with a creatinine clearance above 30 mL/min [24]. Furthermore, the maximum daily dose of metformin calculated using the empirical equations was found to be higher than the recommendations provided in the Australian Medicines Handbook.

Table 5.4 *Summary of metformin renal dosing guidelines*

Guideline	**Ref**	**Renal estimation method**	**Renal dose adjustment**	
			Renal function (mL/min)	Maximum dose (mg/day)
Australian Medicines Handbook	[49]	CLcr	60-90	2000
			30-60	1000
European Medicines Agency	[368]	GFR	>59	3000
			45-59	2000
			30-45	1000
New Zealand Formulary		eGFR	60-120	2000
			30-60	1000
			15-30	500
Developed empirical equation				
$CLcr_{CG}$ method		CLcr	≥90	2250
			60-89	1700
			30-59	1000
			15-29	500
			<15	250
$eGFR_{MDRD}$ method		eGFR	≥90	2550
			60-89	2000
			30-59	1000
			15-29	500
			<15	250
$eGFR_{CKDEPI}$ method		eGFR	≥90	2250
			60-89	1700
			30-59	1000
			15-29	500
			<15	250

5.7. Limitations

The results of this analysis should be viewed in light of the following limitations associated with noncompartmental pharmacokinetic analyses. A total of 37 plasma metformin concentrations were excluded in the analysis due to being below the limit of quantification; whereby, 33 of the 37 plasma concentrations below the limit of quantification were the final 24-hour and/or 32-hour plasma concentrations collected from 20 study participants. Here, it is likely that only the distribution phase for metformin was observed in the study participants with both their 24 and 36 hour concentrations reported to being below the quantification limit because the elimination phase for metformin has been reported to only occur around 20 hours post metformin ingestion [18]. In these study participants it is likely that the estimates for λ_z (and pharmacokinetic statistics derived from λ_z) will be overestimated. In addition, due to the 24- and 32-hour plasma metformin concentrations being below the limit of quantification only 2 concentrations were used to calculate the λ_z in affected study participants. Here, the use of only the last two concentrations to determine the terminal elimination slope can result in biased results, particularly in the setting when at least one of the two samples was taken when drug distribution is not in equilibrium. In future pharmacokinetic metformin studies it is recommended that a higher dose of metformin is administered to patients with normal renal function to avoid terminal phase metformin data being below the limit of concentration.

The dose predictions are limited by the assumption that a linear relationship exists between the apparent clearance for metformin and GFR. In addition, the dose predictions were based on a single covariate (i.e. an estimate of renal function). However, there are other factors that may determine metformin dose variability between patients, such as body weight or genetic variation in drug transporters.

5.8. Conclusion

In this study the pharmacokinetics of metformin was explored in patients with varying levels of renal function. A linear relationship was found between the renal clearance of metformin and the widely used creatinine-based equations, suggesting that the creatinine-based equations can be used to guide the dosing of metformin in renal impairment. Furthermore, in this current piece of analysis an empirical equation to guide the renal dosing of metformin was developed. However, a clinical trial would be warranted to validate the empirical renal dosing equation prior to use in patients.

Chapter 6: The concentration time profile of metformin in renal impairment

6.1. Introduction

The pharmacokinetic profile of metformin is altered in patients with renal impairment. In Chapter 5 of this thesis, the maximum plasma metformin concentration achieved following a single dose (C_{max}) and the time at which it occurred were found to be higher in individuals with renal impairment as opposed to those with normal renal function. Similarly, the elimination half-life ($t_{1/2}$), area under the curve from time zero to last observation (AUC_{0-last}), as well as the area under the curve from time zero to infinity ($AUC_{0-\infty}$) for metformin were found to increase with poorer levels of renal function. Several hypotheses have been proposed to describe the possible physiological changes that occur in renal impairment that influence the pharmacokinetics of metformin. These proposed hypotheses include: (i) a decrease in renal clearance (CL_{renal}) [27, 30], (ii) a decrease in volume of distribution (V) [30, 34], (iii) a decrease in bioavailability (F) and (iv) flip-flop pharmacokinetics [18, 27, 29, 30]. It is not clear whether one or more of the proposed hypotheses are possible mechanisms to describe the pharmacokinetic profile of metformin in renal impairment. Simulations provide a means of assessing each of the proposed mechanisms by testing to see which of the proposed hypotheses can recreate the metformin concentration time profiles seen in patients with varying degrees of renal impairment.

6.2. Objectives

The aim of this research was to test the hypotheses proposed to describe the pharmacokinetics of metformin in patients with renal impairment.

6.3. Methods

The four hypotheses proposed to influence the pharmacokinetic profile of metformin in renal impairment (i.e. a reduction in CL, a decrease in V, a decrease in F and flip-flop pharmacokinetics) were implemented under a standard one-compartment model (termed generic signature profile) and a published metformin pharmacokinetic model by Duong et al (termed metformin signature profile) [15]. Details of the simulations performed using a standard one-compartment model and the published model by Duong et al are described, respectively, as follows;

Generic signature profile. Deterministic simulations were performed under a standard one compartment model with first order absorption using MATLAB (R2016b, MathWorks, Natick, NA). A reference generic signature profile was simulated using the parameter values presented in Table 6.1 for comparison to the profiles simulated under the different proposed hypotheses.

Table 6.1 *Parameter and parameter values for the reference generic profile*

Parameters		**Values**
Dosing	Dose number	1
	Dose (mg)	100
	Dose interval (h)	24
Pharmacokinetic	F	1
	CL $(L \cdot h^{-1})$	10
	$k_a(h^{-1})$	1
	V (L)	20

Dose number: the number of doses administered; Dose interval: the time interval between administering individual doses. F represents bioavailability, CL represents clearance, k_a represents absorption rate constant and V represents the volume of distribution

Deterministic simulations were performed with decreasing imputed values of CL (i.e. 10, 8, 6, 4 and 2 L/h) to explore the impact a reduction in CL would have on the pharmacokinetic profile. For the purposes of this analysis, the

remainder of parameter values in the model were kept the same as the reference generic signature profile (shown in Table 6.1). Similarly, decreasing values of V (i.e. 20, 16, 12, 8 and 4 L) and F (i.e. 1.0, 0.8. 0.6, 0.4 and 0.2) were imputed into the one-compartment model, respectively. The influence of flip-flop on the pharmacokinetic profile of metformin was explored by simulating several scenarios describing flip-flop situations, which were: (i) constant elimination rate constant (k) and changing absorption rate constant (k_a), and, (ii) changing k and constant k_a.

Metformin signature profile. Deterministic simulations were performed under a published metformin population pharmacokinetic model by Duong et al in MATLAB (R2016b, MathWorks, Natick, NA) [15]. In brief, the metformin population pharmacokinetic model published by Duong et al was a two-compartment model with first-order absorption and elimination processes (refer to Appendix 2.1 for a more detailed description of the model by Duong et al).

A reference metformin signature profile using the model by Duong et al was simulated using the parameter values shown in Table 6.2. Deterministic simulations were performed to investigate the influence a reduction in CL would have on the pharmacokinetic profile by imputing the following creatinine clearance values into the model one at a time: 90, 60, 30 and 15 mL/min. The remainder of the parameter values were kept the same as the reference values presented in Table 6.2. Likewise, the influence a reduction in V and F would have on the concentration time profile for metformin was explored separately by individually imputing the following values of volume of distribution for the central compartment volume (20, 16, 12, 8 and 4 L) and F (1, 0.8, 0.6, 0.4 and 0.2) into the model. The influence of flip-flop on the pharmacokinetic profile of metformin was performed by simulating the following two scenarios of flip-flop in the published model: (i) constant macro-constant describing the terminal decline in drug concentration (β) and changing k_a, and, (ii) changing β and constant k_a.

Table 6.2 *Parameter values for the reference metformin pharmacokinetic profile*

Parameters		**Values**
Dosing	Dose number	1
	Dose (mg)	500
	Dose interval (h)	24
Pharmacokinetic	Bioavailability	1
	CL (L/h)	72
	V_c (L)	149
	Q (L/h)	203
	V_p (L)	182
	k_a (h^{-1})	0.35
	$TLag$ (h)	0.38

Dose number: number of doses administered. Dose interval: time interval between administering individual doses. CL represents clearance, V_c represents central compartment volume, Q represents intercompartmental clearance, V_p represents peripheral compartment volume, k_a represents absorption rate constant and $TLag$ corresponds to the time taken for a drug to appear in the systemic circulation following extravascular drug administration.

6.4. Results

The reference generic signature profile is shown in Figure 6.1. Simulated concentration time profiles exploring the influence of a reduction in clearance, volume of distribution and bioavailability are presented in Figure 6.2, 6.3 and 6.4, respectively. Simulated concentration profiles exploring the influence of flip-flop when (i) k is constant and k_a is changing and (ii) k is changing and k_a is constant are shown in Figure 6.5 and 6.6, respectively.

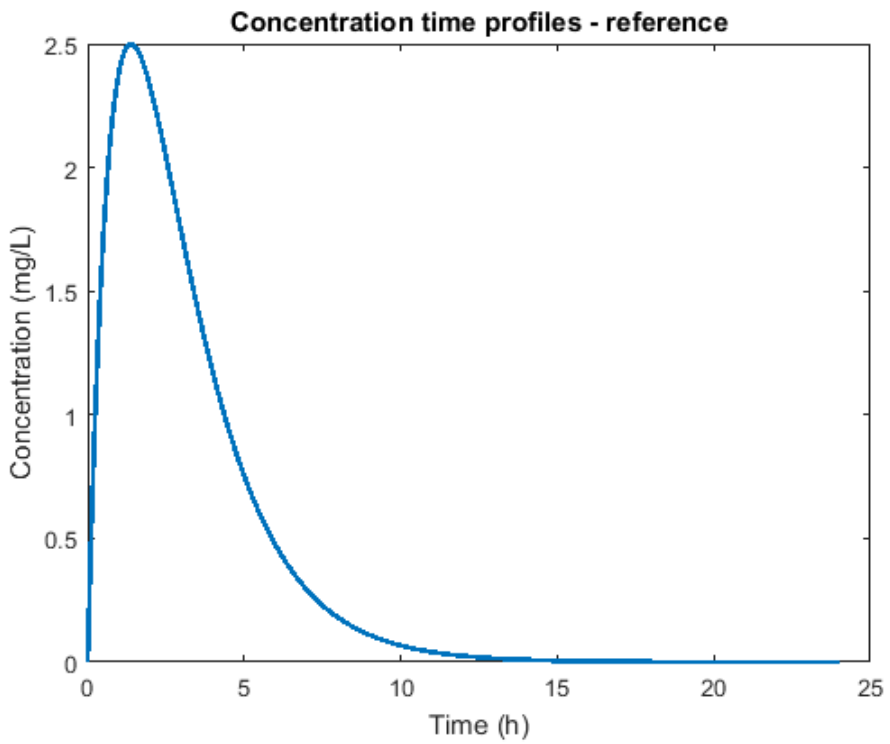
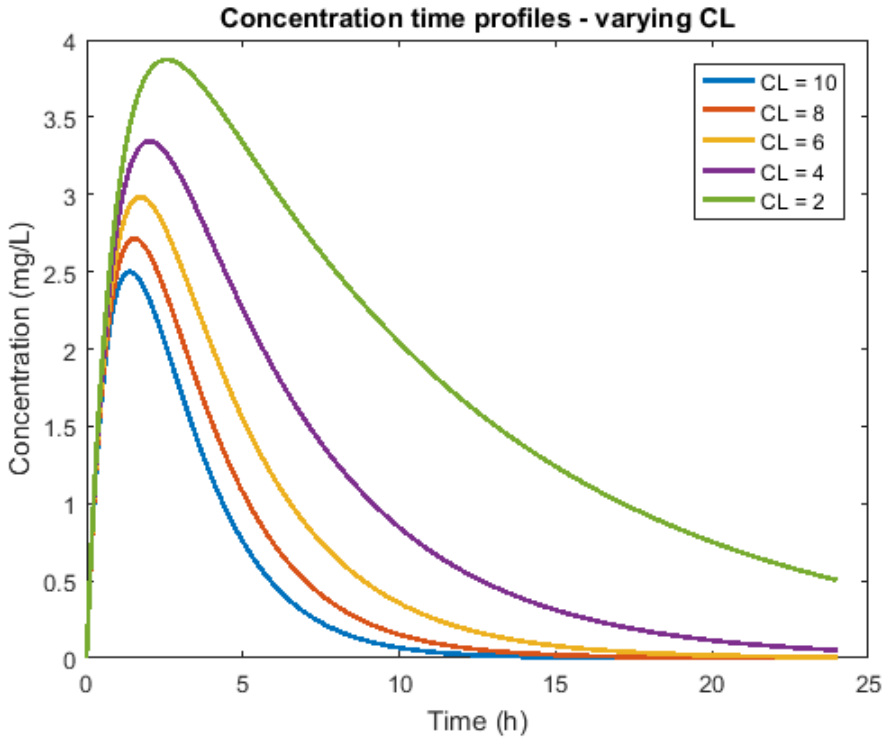


Figure 6.1 *Simulated reference generic signature profile of concentration versus time*

a



b

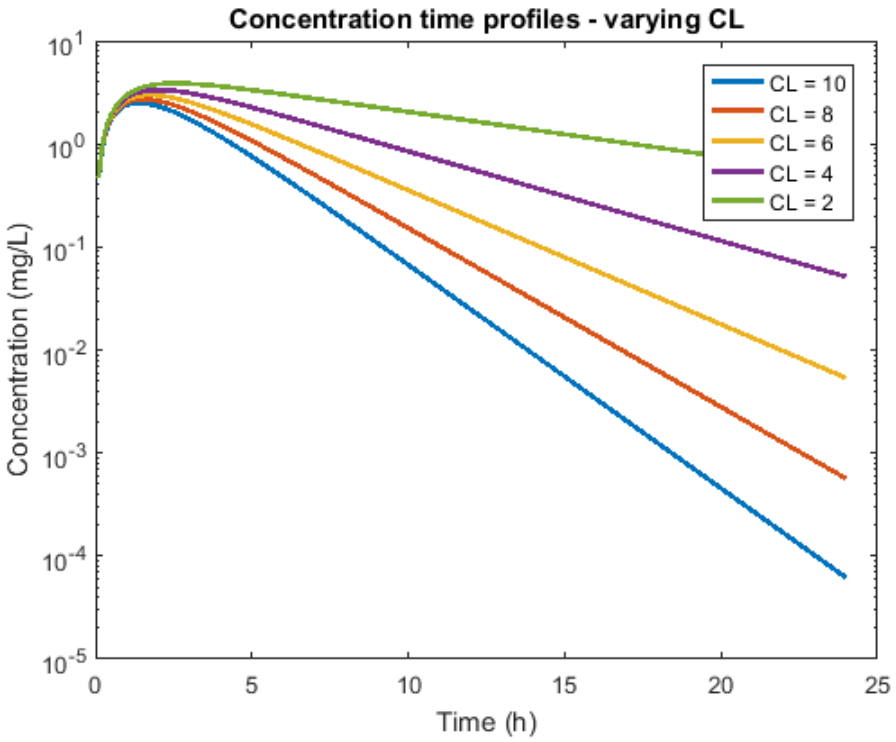
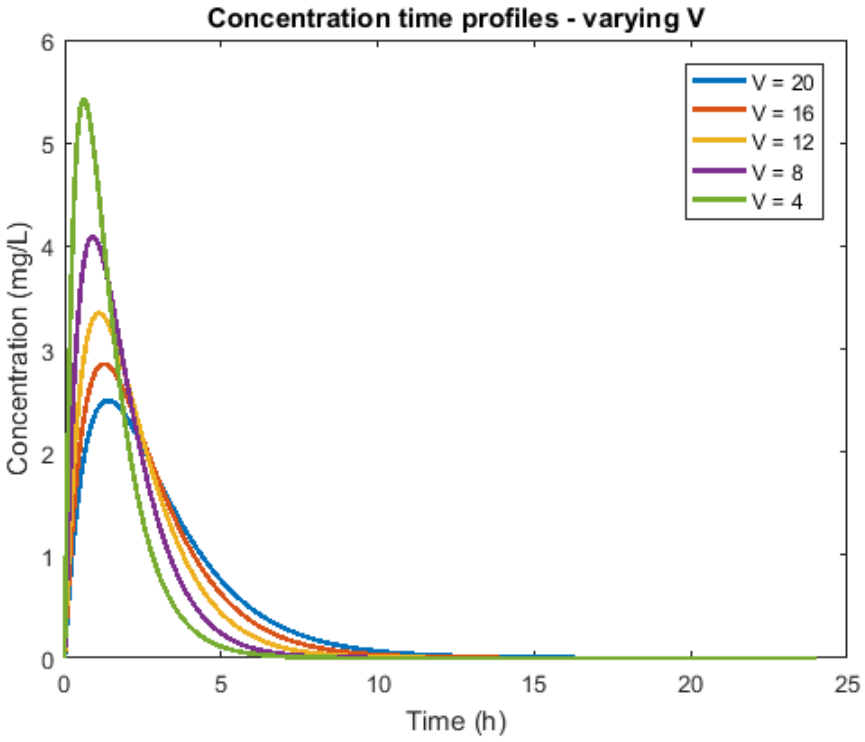


Figure 6.2 *Simulated generic signature profile of concentration versus time exploring the influence of a reduction in clearance on a Cartesian plot (a) and semi-log plot (b)*

a



b

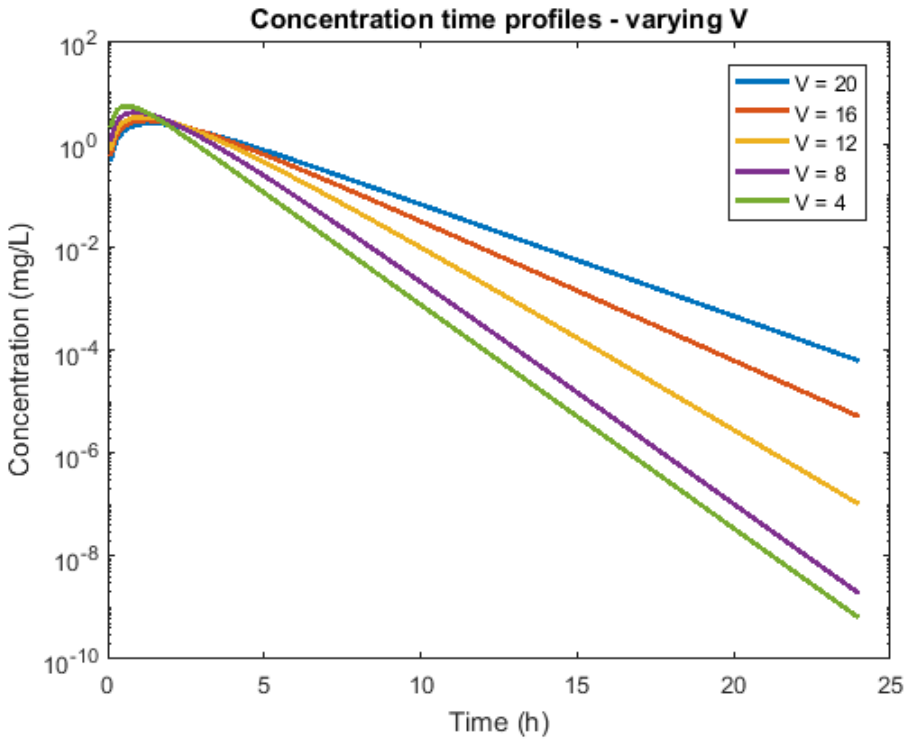
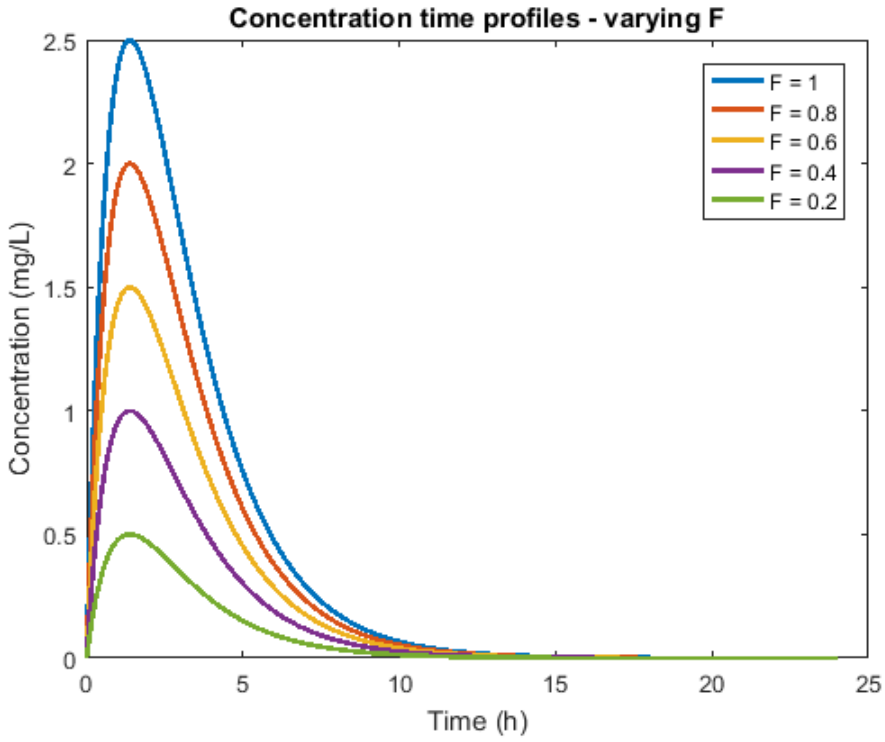


Figure 6.3 *Simulated generic signature profile of concentration versus time exploring the influence of a reduction in volume of distribution on a Cartesian plot (a) and semi-log plot (b)*

a



b

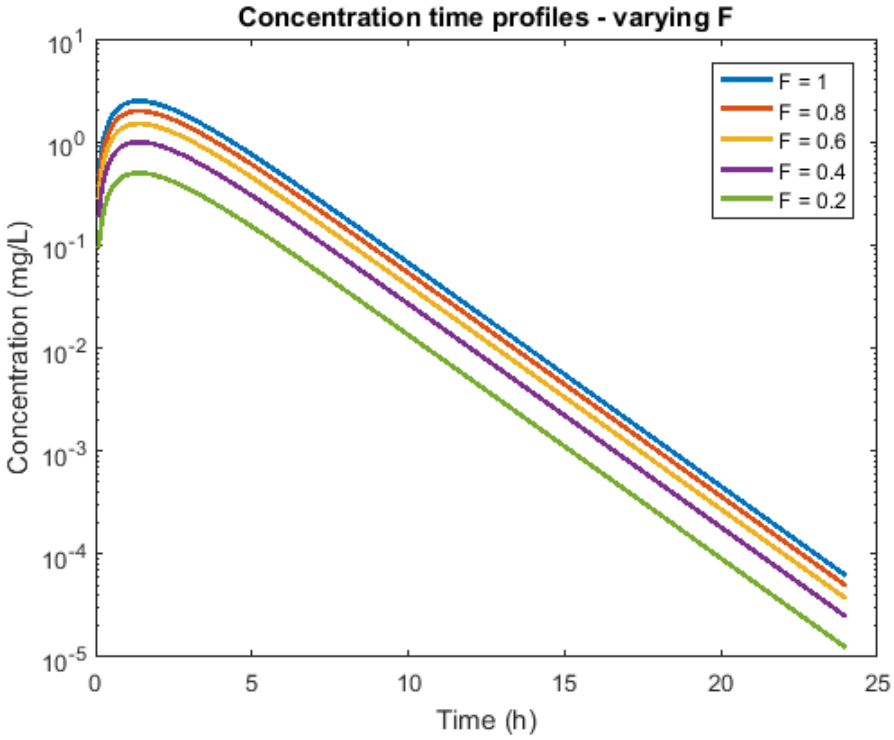
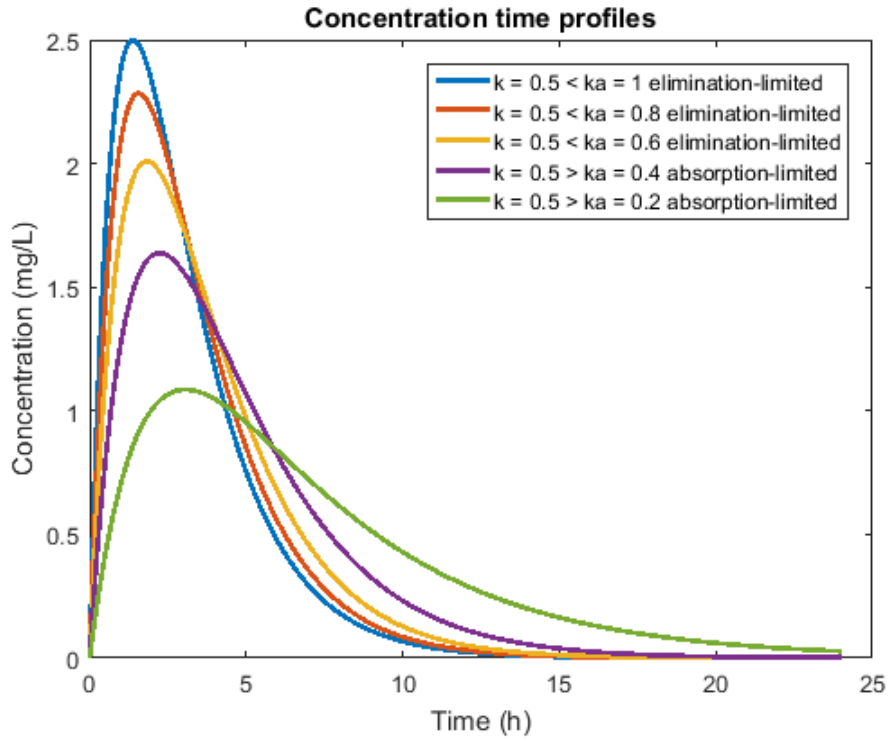


Figure 6.4 *Simulated generic signature profile of concentration versus time exploring the influence of a reduction in bioavailability on a Cartesian plot (a) and semi-log plot (b)*

a



b

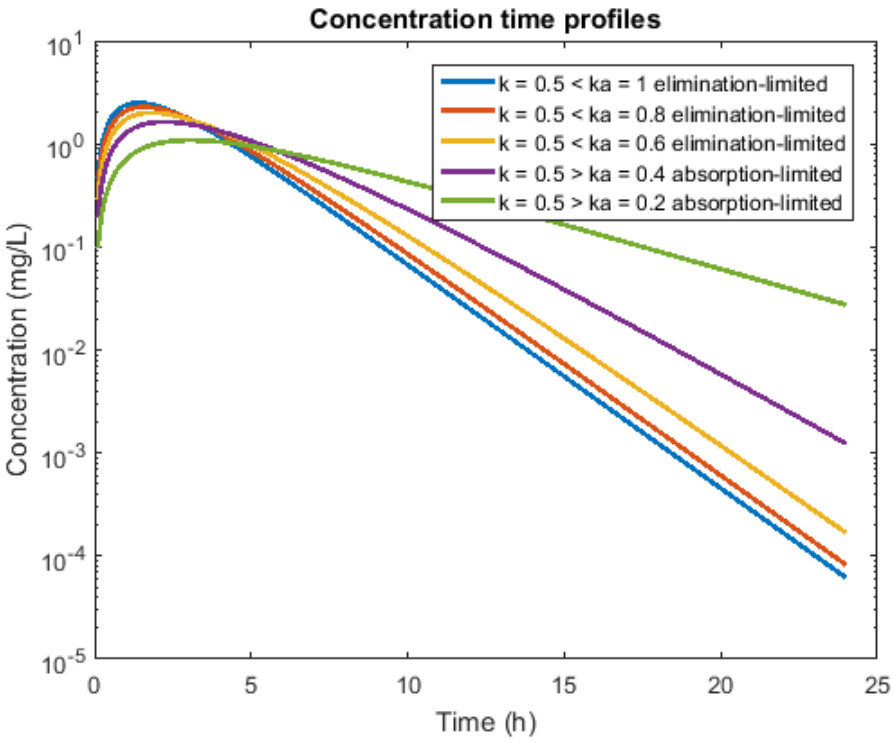
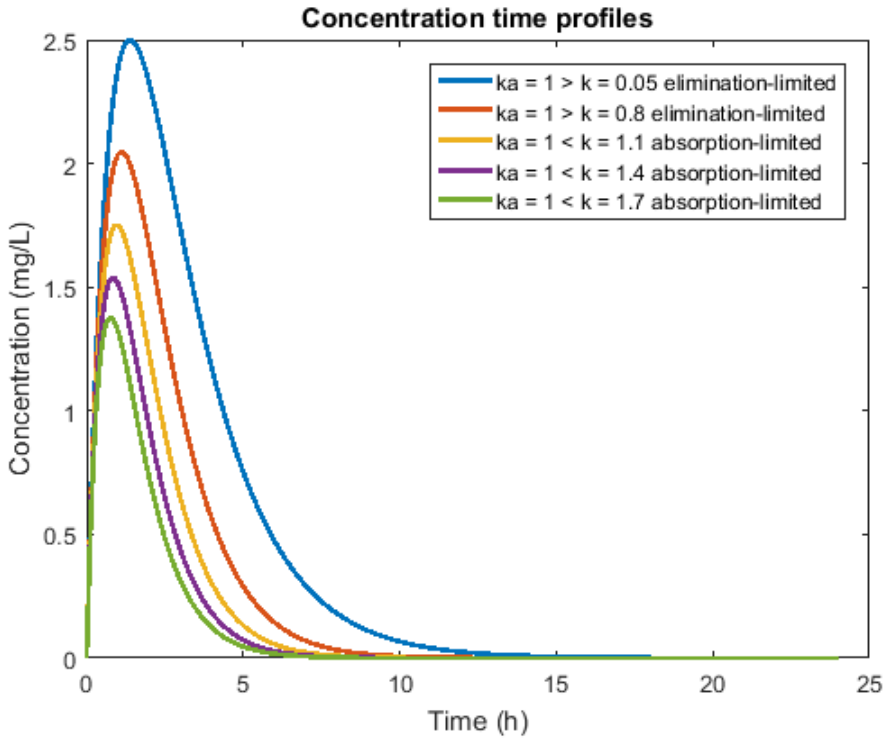


Figure 6.5 *Simulated generic signature profile of concentration versus time exploring the influence of flip-flop where the rate of elimination (k) is kept constant and the rate of absorption (k_a) is changing on a Cartesian plot (a) and semi-log plot (b)*

a



b

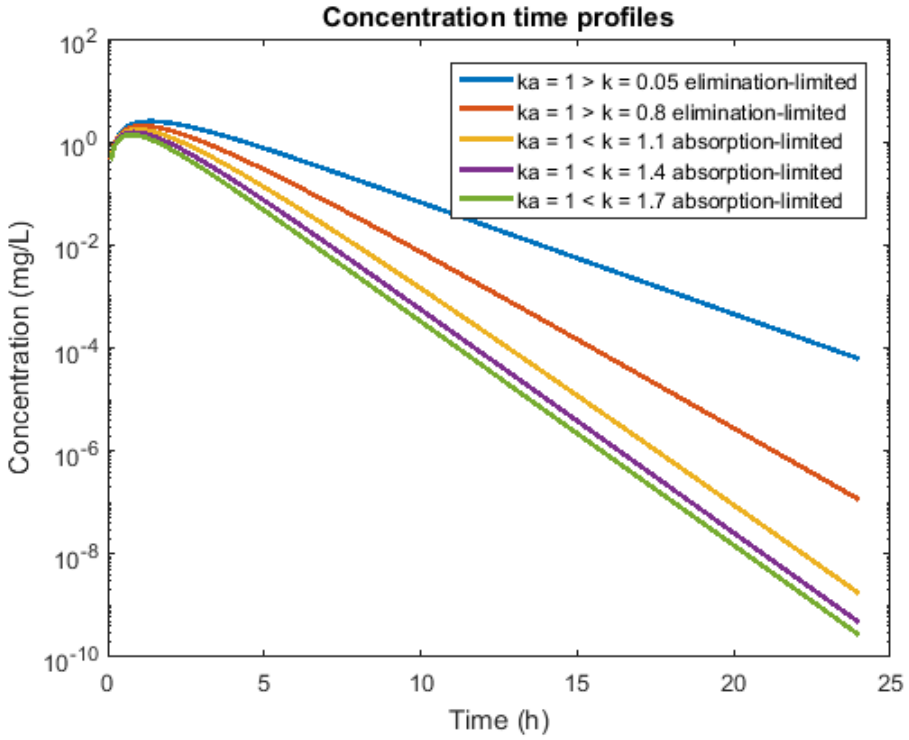


Figure 6.6 *Simulated generic signature profile of concentration versus time exploring the influence of flip-flop where the rate of elimination (k) is changing and the rate of absorption (k_a) is kept constant on a Cartesian plot (a) and semi-log plot (b)*

The reference metformin signature profile simulated using the published model by Duong et al is shown in Figure 6.7. Simulated metformin concentration time profiles exploring the impact of a reduction in creatinine clearance, volume of distribution and bioavailability are shown in Figure 6.8, 6.9 and 6.10, respectively. The simulated flip-flop scenarios where (i) β is constant and k_a is changing and (ii) β is changing and k_a is constant is shown in Figure 6.11 and Figure 6.12, respectively.

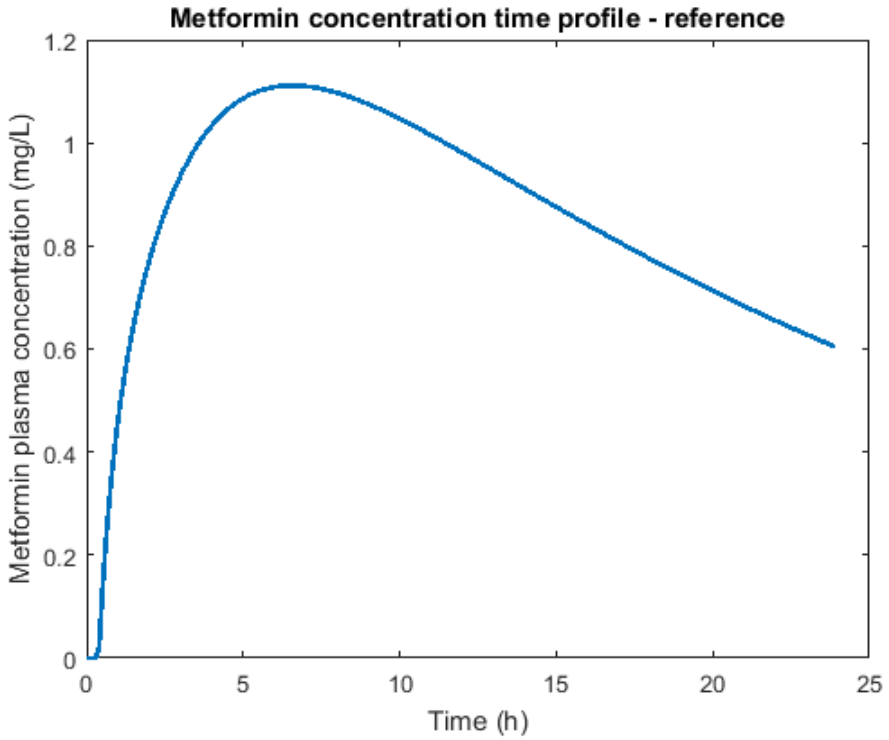
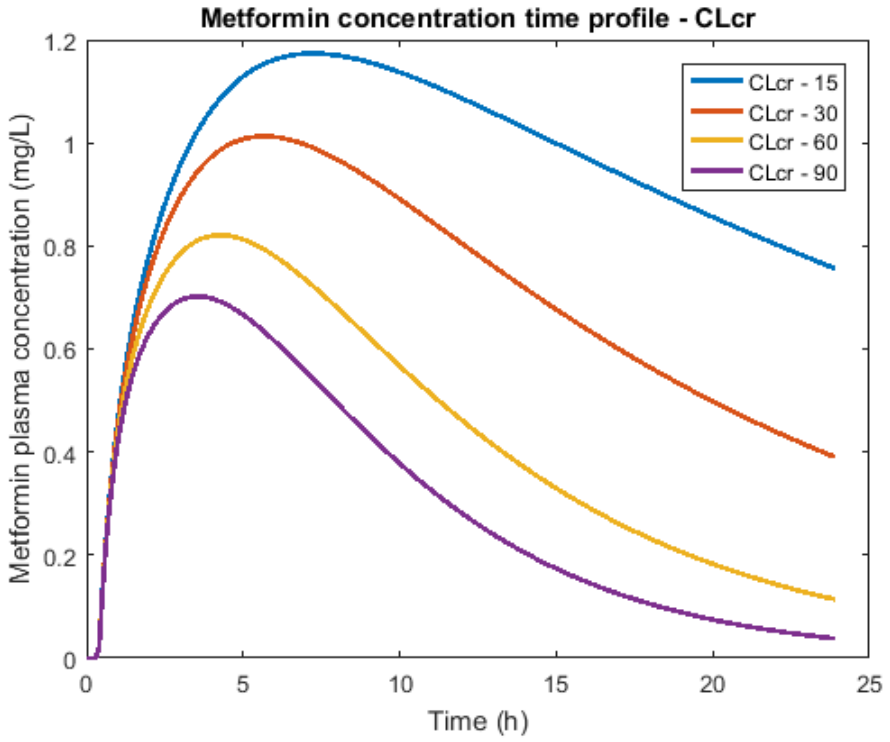


Figure 6.7 *Simulated reference metformin signature profile for metformin concentration versus time*

a



b

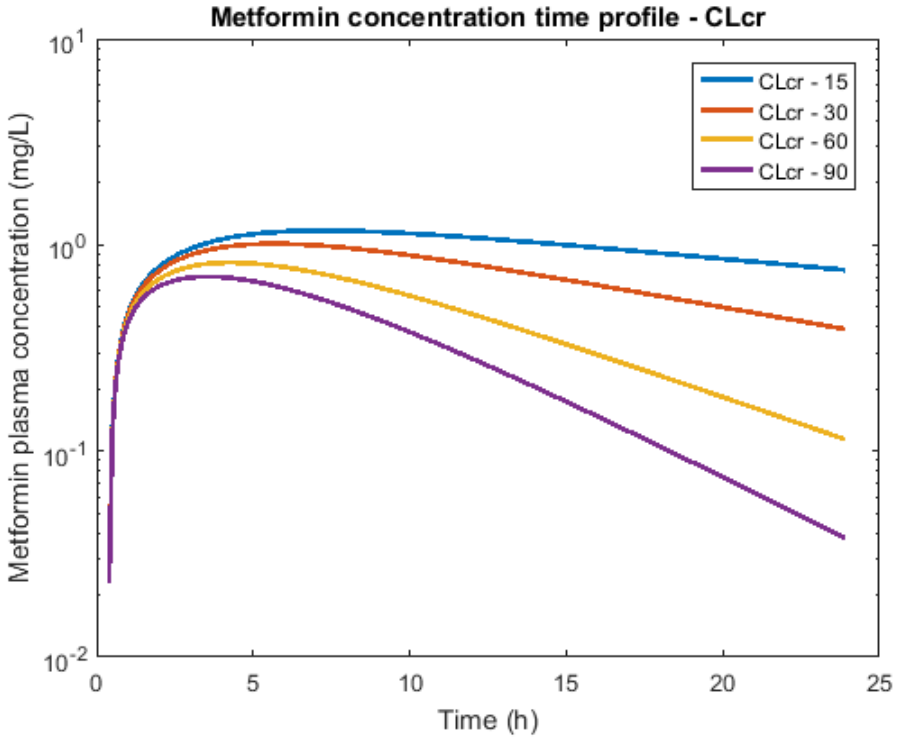
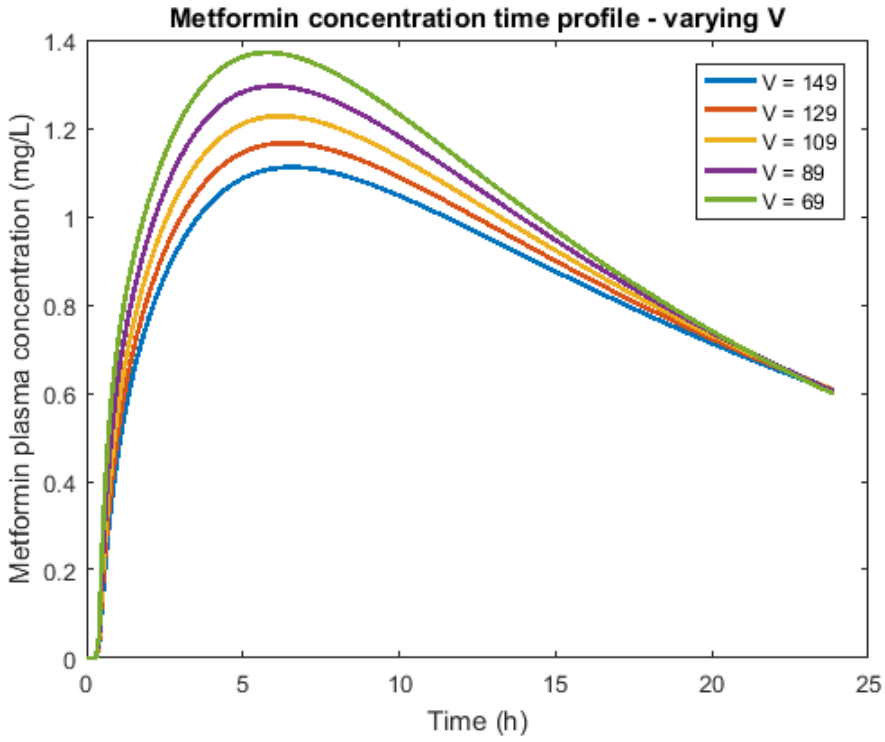


Figure 6.8 *Simulated metformin signature profile of concentration versus time exploring the influence of a reduction in creatinine clearance on a Cartesian plot (a) and semi-log plot (b)*

a



b

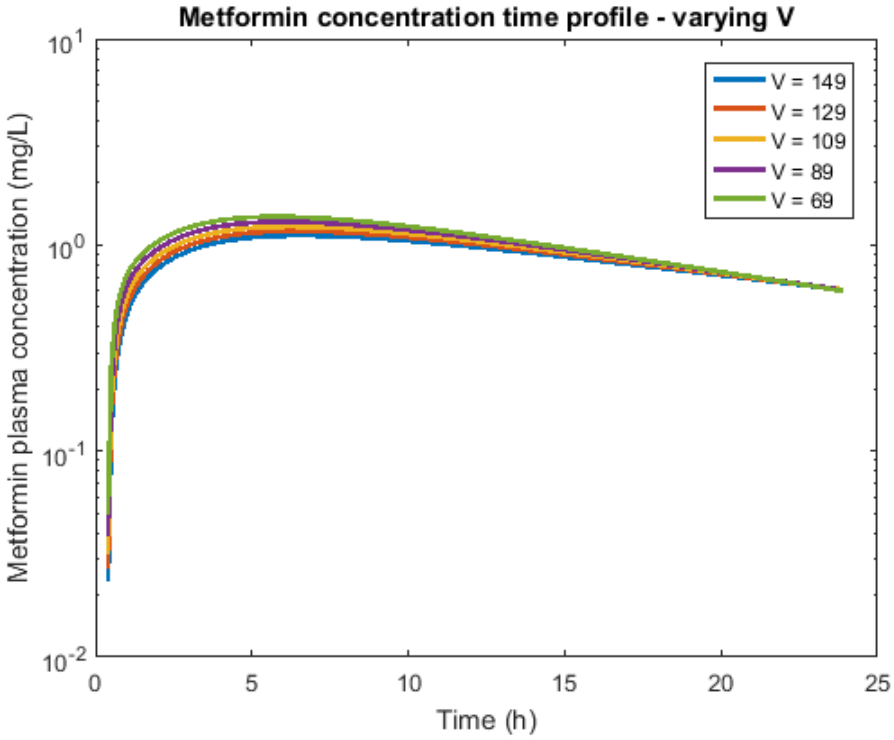
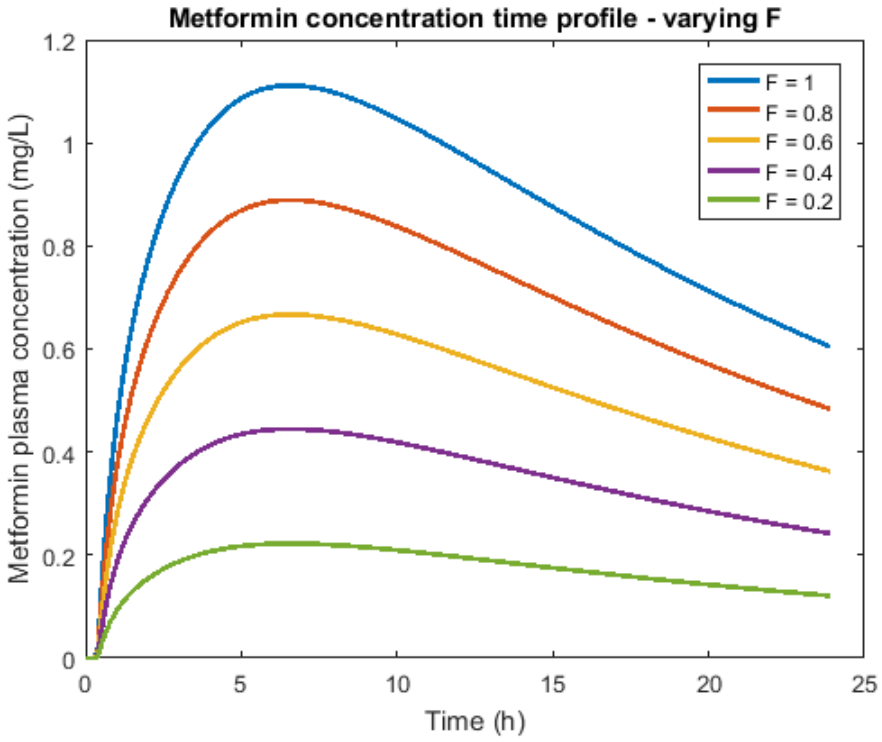


Figure 6.9 *Simulated metformin signature profile of concentration versus time exploring the influence of a reduction in volume of distribution on a Cartesian plot (a) and semi-log plot (b)*

a



b

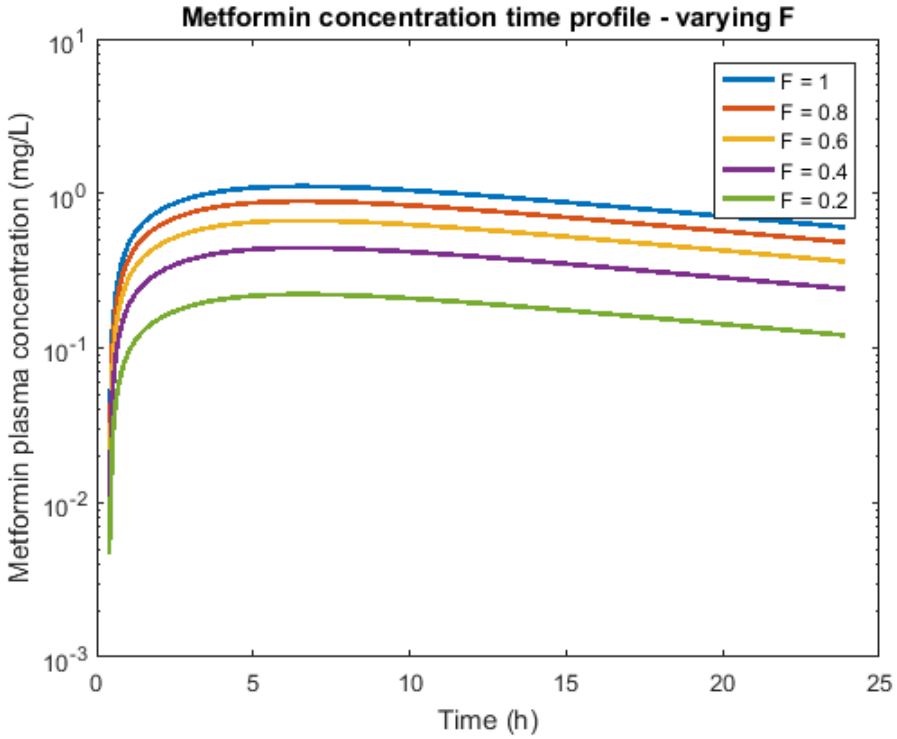
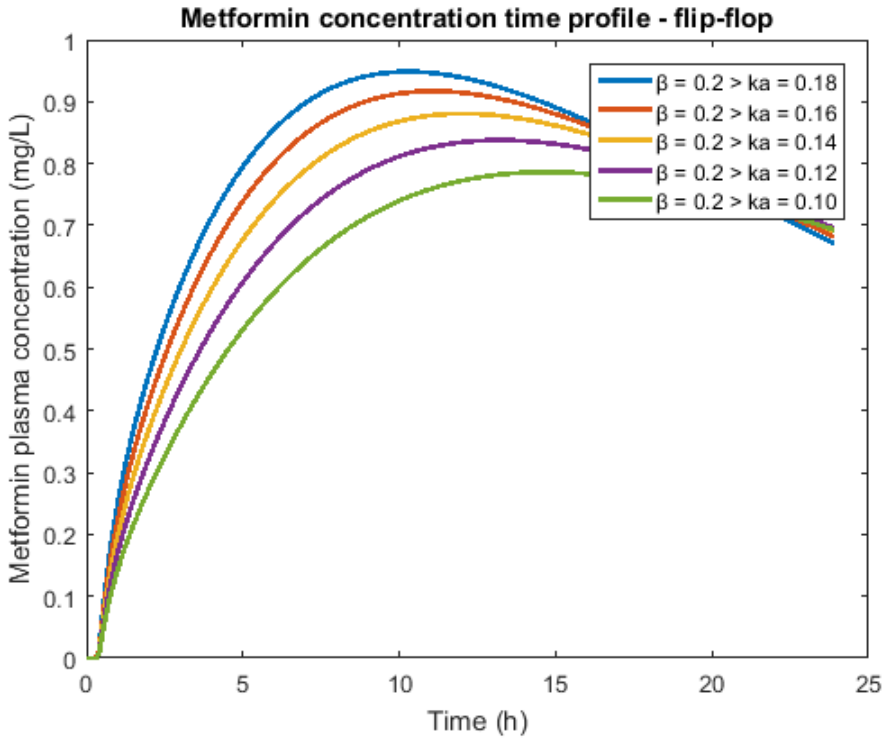


Figure 6.10 *Simulated metformin signature profile of concentration versus time exploring the influence of a reduction in bioavailability on a Cartesian plot (a) and semi-log plot (b)*

a



b

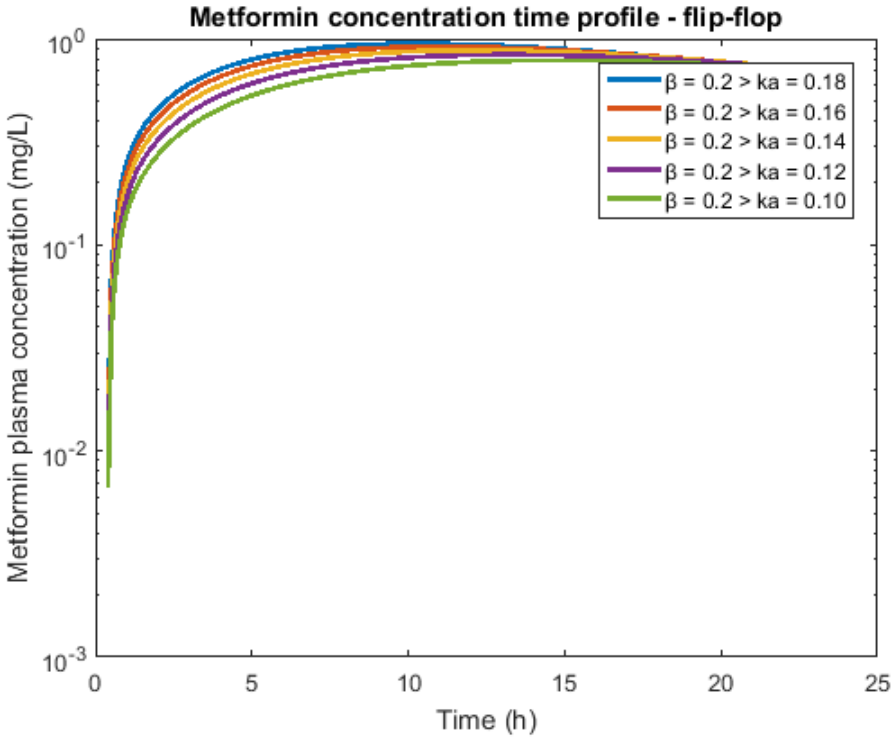
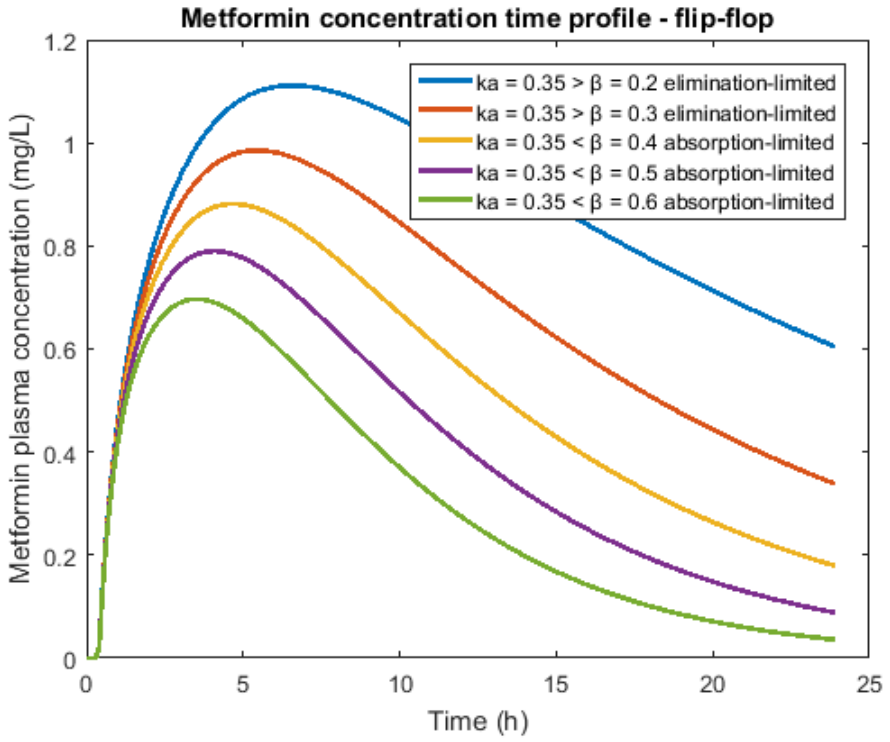


Figure 6.11 *Simulated metformin signature profile of concentration versus time exploring the influence of flip-flop where the macro-constant describing the terminal decline (β) is kept constant and the rate of absorption (k_a) is changing on a Cartesian plot (a) and semi-log plot (b)*

a



b

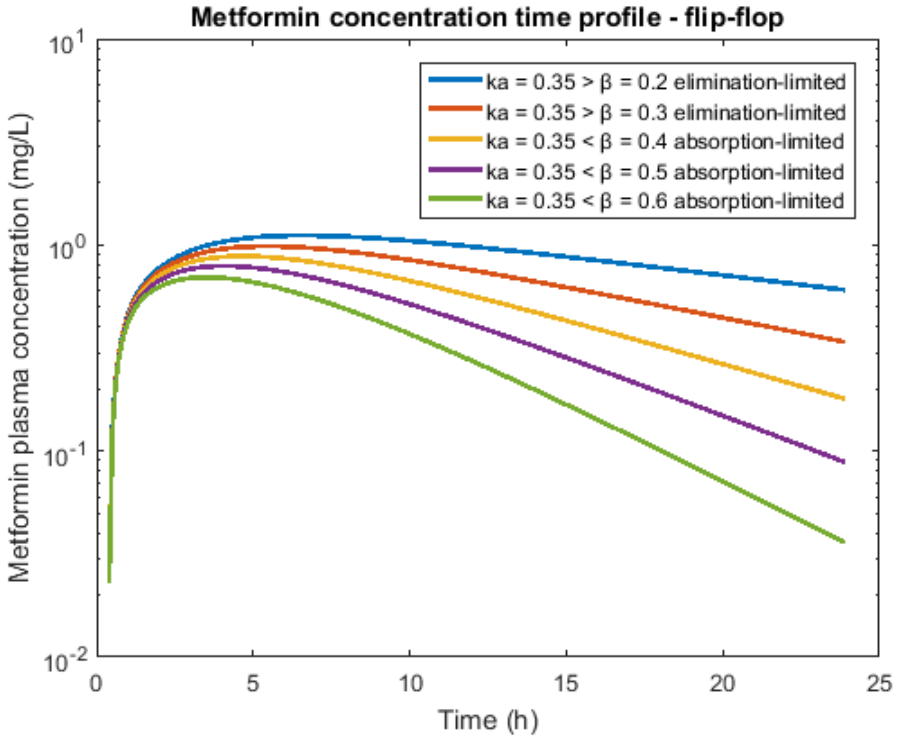


Figure 6.12 *Simulated metformin signature profile of concentration versus time exploring the influence of flip-flop where the macro-constant describing the terminal decline (β) is changing and the rate of absorption (k_a) is kept constant on a Cartesian plot (a) and semi-log plot (b)*

A description of the influence a reduction in clearance, reduction in volume of distribution, reduction in bioavailability, and, flip-flop had on the C_{max}, T_{max}, $t_{1/2}$ and AUC is given as follows;

Reduction in clearance. A reduction in clearance led to an increase in C_{max}, T_{max} and AUC (as shown in Figure 6.2). In addition, as shown in Figure 6.2(b), the gradient of the terminal elimination slope became less steep with a decrease in clearance. On this basis it can be inferred that $t_{1/2}$ increased with a reduction in clearance.

Reduction in volume of distribution. A reduction in volume of distribution resulted in an increase in C_{max}, whilst, resulting in a decrease in T_{max} and $t_{1/2}$. A reduction in volume of distribution had negligible effects on AUC.

Reduction in bioavailability. A reduction in bioavailability resulted in a drop in C_{max} and AUC, whilst $T_{max}$and $t_{1/2}$ were unchanged (Figure 6.4).

Flip-flop. In the simulated flip-flop scenario where k was kept the same and k_a was changing C_{max} decreased with decreasing values of $k_a$whilst T_{max}increased with reduced $k_a$values. In the second flip-flop scenario where the value of k were changing and the k_a was kept the same, both $C_{max}$and T_{max}decreased with increasing values of k. In both flip-flop scenarios the gradient of the terminal slope of decline was the same when the values of the k_a were smaller than the values of k. However, when the values of k_a were greater than k, the gradient of the terminal slope was different.

6.5. Discussion

Understanding the influence of renal impairment on the pharmacokinetics of metformin is important to guide dosing. Based on the results of this simulation study a reduction in clearance or flip-flop pharmacokinetics (where k is changing and k_a is constant) could possibly recreate the concentration time profile of metformin seen in renally impaired patients (Figure 5.1). This was on the basis of achieving the same trends seen in the pharmacokinetic metrics (i.e. C_{max}, T_{max}, $t_{1/2}$, AUC_{0-last} and $AUC_{0-\infty}$) found in Chapter 5 (presented in bold).

C_{max} was higher with increasing severity of renal impairment. This same trend was apparent in the simulated signature profiles exploring a reduction in clearance and flip-flop. A reduction in the volume of distribution had similarly resulted in an increase in C_{max}. However, from the simulated profiles exploring the influence of a reduction in volume of distribution, the concentration time curves cross over when the volume of distribution is reduced to a certain extent – a characteristic not evident in the observed metformin concentration time profiles. A reduction in bioavailability resulted in a decrease in C_{max}, which is inverse of the trend seen in the pharmacokinetics of metformin in renal impairment.

T_{max} was longer with poorer levels of renal function. From the deterministic simulations, this pharmacokinetic characteristic could only be recreated by a reduction in clearance or flip-flop kinetics. Whereby, neither a reduction in volume of distribution nor bioavailability could recreate the pharmacokinetic profile seen for metformin in study participants with varying degrees of renal impairment.

$t_{1/2}$was longer with poorer levels of renal function. Based on the simulated signature profiles, both a reduction in clearance and the flip-flop kinetics hypotheses could recreate the increase in half-life with poorer levels of renal function. A reduction in the volume of distribution resulted in a decrease in half-

life which is the inverse of the trend noted and a reduction in bioavailability did not influence the half-life.

***AUC* increased with increasing severity of renal impairment.** This characteristic of the concentration time curve could be recreated under the scenario where there was a reduction in clearance or flip-flop. A reduction in the volume of distribution had negligible effects on the area under the curve, whilst a reduction in bioavailability resulted in a reduction in the area under the curve.

6.6. Conclusion

In conclusion, the proposed hypothesis of a reduction in clearance and flip-flop kinetics could recreate the concentration time profile of metformin in patients with renal impairment.

Chapter 7: A population pharmacokinetic model for metformin

7.1. Preamble to the chapter

In Chapter 5 an empirical dosing equation for metformin was developed and renal doses predicted. However, it is not known if the calculated doses will produce plasma metformin concentrations that remain within the upper limit of safety (i.e. 4.5 mg/L) defined in Chapter 4. In this chapter the development and evaluation of a population pharmacokinetic model for metformin in renal impairment is described. Simulations were performed to predict the plasma metformin concentration profile for metformin under the doses predicted in Chapter 5 and those published in the New Zealand Formulary to assess for safety. Note, that in this chapter, new data from a metformin study conducted at Middlemore Hospital (New Zealand) became available and was used in the model development along with the data from Chapter 5.

7.2. Introduction

Current renal dosing guidelines for metformin lack agreement and few appear to be evidence based. Some dosing guidelines state that metformin should not be used in patients with renal impairment, while others recommend caution, no dose adjustment, or a reduced dosage [5, 24, 47, 48, 51, 99]. This leaves prescribers with unclear messages on how to safely use and dose metformin in patients with chronic renal impairment. In theory, metformin therapy in renally impaired patients could be monitored for safety by measuring plasma metformin concentrations to ensure that they do not exceed the upper limit of safety defined in Chapter 4 (4.5 mg/L). However, this is not currently recommended in clinical practice.

A nonlinear mixed effects population pharmacokinetic model presents a platform for which plasma metformin concentrations can be predicted across patient populations, including those with poor renal function, as a means of assessing safe dosing. Previous studies have described the pharmacokinetics of metformin in patients with moderate renal impairment (creatinine clearance (CLcr) 30-60 mL/min) [14, 15, 30, 370]. However, few studies have looked at the

pharmacokinetics of metformin in patients with severe renal impairment (CLcr <30 mL/min) (see [15] for an exception) – and only a handful of population analyses exist [14-16, 370, 401]. Only a few population pharmacokinetic studies have proposed dosing recommendations in patients with severe renal impairment (creatinine clearance <30 mL/min). Hence, the purpose of this study was to prove whether metformin could be used in patients with CKD 4 or CKD 5 (creatinine clearance 15-29 mL/min).

7.3. Objectives

The specific objectives of this chapter were to (i) develop a population pharmacokinetic model for metformin in patients with varying degrees of renal function and (ii) simulate metformin concentration time profiles for patients with varying renal function under the empirical renal dosing recommendations developed in Chapter 5 and the published New Zealand Formulary renal dosing guidelines. The purpose of the simulations was to understand what percentage of patients would be expected to have plasma metformin concentrations above the upper limit of 4.5 mg/L (defined in Chapter 4).

7.4. Methods

7.4.1. Data

Data available for this analysis was collected from two sources: (i) a study conducted at the Dunedin Public Hospital (New Zealand) and (ii) a study conducted at Middlemore Hospital (New Zealand). The same data used in Chapter 5 from the Dunedin Public Hospital was used in this Chapter – refer to Chapter 5 (section 5.4.1.) for information about the data available from the Dunedin Public Hospital. The data from Middlemore Hospital was only made available after the completion of Chapter 5 and hence is firstly used and introduced in this chapter. A brief description of the new data from Middlemore Hospital is provided in the following section (section 7.4.1.1.). An overview of the data from the Dunedin Public Hospital and Middlemore Hospital is provided in this thesis, however, note that the collection of data was not part of this thesis.

A summary of demographic data of study participants from the Dunedin Public Hospital and Middlemore Hospital is presented in Table 7.1.

7.4.1.1. Middlemore Hospital

Data from an open-label, prospective, phase I, safety study conducted at Middlemore Hospital (Auckland, New Zealand) was available for analysis [16]. The study was approved by the New Zealand Health and Disability Ethics Committees, reference number: NTX/11/12/112. All patients provided written and informed consent.

Eighteen patients with type 2 diabetes mellitus and stable stage 4 chronic kidney disease (CKD) were enrolled in the study. Study volunteers were included in the study if they presented: between 30 and 75 years of age, had a diagnosis of type two diabetes for at least two years, an HbA1c level between 6% and 11% and stable stage 4 CKD. Stage 4 CKD was defined by a stable eGFR value between 15-30 mL/min/1.73m^2 over the preceding three months. Study volunteers were excluded if they presented with: a history of metformin intolerance, pregnancy, breastfeeding, pre-existing metabolic acidosis or having

significant risk factors for metabolic acidosis. Significant risk factors for metabolic acidosis included: morbid obesity (>160 kg), unstable ischemic heart disease, a planned radiocontrast examination within the following six months and/or relevant medical comorbidities (e.g. severe chronic obstructive pulmonary disease, unstable congestive heart failure and significant liver disease).

Study participants were randomised into one of three study arms to receive either 250, 500 or 1000 mg of metformin orally once daily for four weeks. Participants randomised to receive the lowest dose of metformin (i.e. 250 mg PO OD) were the first to complete the study. Participants in the second and third study arm then completed the study if metformin safety and tolerability were reported to be satisfactory, respectively.

Study participants fasted overnight prior to the first study day. On the first study day, blood samples were collected to assess baseline fasting metabolic control profiles (glucose, insulin, lipids and HbA1c) and a safety profile (serum lactate, bicarbonate, venous pH, renal function, electrolytes, liver enzymes and a full blood count). A continuous capillary glucose monitoring system was used to assess glycaemic control over a 72 hour period. On study day four, study participants received a single daily dose of metformin followed by a standard breakfast. Blood samples were taken at 0 (baseline), 2, 4, 6, 8 and 24 hours after the first dose of metformin was administered. Study participants continued their daily metformin therapy for four weeks, returning to the clinic on a weekly basis to measure trough metformin concentrations, monitor their safety profiles and to assess for adverse events. The 72 hour continuous capillary glucose monitoring system was repeated on the last three days of the study and HbA1c was measured on the last day of the study.

The same assay was used to determine plasma metformin concentrations in the Dunedin Public Hospital and Middlemore Hospital metformin studies. For more additional details regarding the assay used refer to Appendix A4.1.2.

Table 7.1 *Summary statistics of study participants by study*

	Dunedin Public Hospital (n=34)	**Middlemore Hospital (n=18)**	**Pooled dataset (n=52)**
Age (years)	51.5 [20.0–79.0] (32.3–66.0)	66.0 [40.0–75.0] (62.0–68.0)	61.5 [20.0-79.0] (39.3-68.0)
Sex (F:M)	5:29	3:15	8:44
Height (cm)	174 [157–195] (168–181)	172 [145–183] (168–176)	173 [145-195] (168-179)
Weight (kg)	82.1 [48.0–149.5] (75.1–87.7)	111.7 [77.2–149.8] (91.0–125.8)	85.7 [48.0-149.8] (77.6-104.4)
BMI (kg/m^2)[a]	26.3 [17.8–48.8] (24.2–28.9)	38.0 [25.8–51.4] (32.6–42.3)	28.9 [17.8-51.4] (25.3-35.4)
FFM (kg)[b]	61.5 [33.9–80.5] (54.4–63.8)	68.2 [44.4–83.3] (58.8–72.8)	61.8 [33.9-83.3] (56.3-68.8)
Serum creatinine (µmol/L)	118.5 [52.0–546.0] (89.5–300.0)	259.5 [197.0–370.0] (219.3–300.0)	215.5 [52.0-546.0] (95.5-301.3)
$CLcr_{CG}$[c] (mL/min)	73.5 [9.5-167.0] (18.1-113.2)	23.4 [11.4-37.3] (19.0-28.3)	29.0 [9.5-167.0] (18.4-93.1)
$eGFR_{MDRD}$[d] ($mL/min/1.73m^2$)	54.1 [8.9–118.5] (17.4–86.7)	20.4 [14.5–29.3] (17.7–25.7)	28.0 [8.9-118.5] (17.5-78.9)
$eGFR_{MDRD}$ (adjusted)[f] (mL/min)	65.8 [11.0-142.7] (18.5-97.2)	24.8 [17.2-39.1] (20.8-35.8)	35.9 [11.0-142.7] (19.1-91.1)

Table 7.1 cont *Summary statistics of study participants by study*

	Dunedin Public Hospital (n=34)	Middlemore Hospital (n=18)	Pooled dataset (n=52)
$eGFR_{CKDEPI}$ [e] (mL/min/1.73m²)	60.2 [8.2–122.4] (17.2–98.7)	20.1 [14.2–29.6] (17.3–25.8)	28.3 [8.2-122.4] (17.2-86.8)
$eGFR_{CKDEPI}$ (adjusted)[f] (mL/min)	73.2 [10.0-151.1] (17.9-109.2)	24.7 [17.0-40.4] (20.2-35.6)	35.6 [10.0-151.1] (18.6-100.8)

Data presented as median [range] (interquartile range) unless otherwise specified. [a]BMI is body mass index. [b]FFM is fat free mass calculated using the equation by Janmahasatian et al [397]. [c]$CLcr_{CG}$ is creatinine clearance estimated using the Cockcroft and Gault equation [80]. Note that ideal body weight [398] was used in the Cockcroft and Gault equation as a body size metric. [d]$eGFR_{MDRD}$ is glomerular filtration rate estimated using the 4-variable Modification of Diet in Disease in Renal Disease equation [82]. [e]$eGFR_{CKDEPI}$ is glomerular filtration rate estimated using the Chronic Kidney Disease Epidemiology Collaboration equation [83]. [f]Note that $eGFR_{MDRD}$ (adjusted) and $eGFR_{CKDEPI}$ (adjusted) were adjusted the individual body surface area measurements for each subject calculated using the Du Bois Method [399].

7.4.2. General analytical approach and software

A population analysis was conducted in NONMEM v7.3 using the first-order conditional estimation method with interaction. The model runs were executed in Perl-speaks-NONMEM (v4.8.1). Pre- and post-processing was conducted in R (version 3.5.3) using the R packages xpose (version 0.4.4) and xpose4 (version 4.6.1).

7.4.3. Data management

Data were saved in an electronic spreadsheet and were converted into a NONMEM compatible format in R (version 3.5.3) using the tidyverse (version 1.2.1) package.

7.4.3.1. Handling below the limit of quantification

Data below the quantification limit (BQL) were handled differently based on the percentage of data below the quantification limit. If less than 5% of the observations in the study were BQL, the BQL data were ignored. If more than 5 percent of the observations were BQL the M6 method described by Beal [402] was used where the first BQL observation was imputed as half of the lower limit of quantification and subsequent data discarded. If more than 10 percent of the observations were BQL then a likelihood-based method to determine the probability of the data being BQL was used (M3 method described by Beal [402]).

7.4.3.2. Handling outliers

A preliminary analysis of the data using an exploratory model in NONMEM was used to identify outliers. Outliers were identified by review of the conditional weighted residuals (CWRES). Absolute values >5 units were considered to be outliers. An influential outlier was defined as an outlier that resulted in a change in a parameter value of more than 10%. Influential outliers were excluded from the analysis. The developed final model was then used to reanalyse the full data set (i.e. including outliers), a sensitivity analysis was conducted and the difference in the results discussed.

7.4.4. Model development

7.4.4.1. Pharmacokinetic model

A one-, two- and three-compartment structural model with first-order absorption and elimination were considered to describe the pharmacokinetics of metformin. Covariance between clearance and volume parameters was considered. The typical value for bioavailability was fixed to 0.55 in the models. Bioavailability was fixed to a consensus value of 0.55 on the basis of several studies proposing metformin bioavailability to range between 0.32 to 0.61 and, to allow for comparison of the results to other previously published population pharmacokinetic models for metformin that had also fixed the value for bioavailability to 0.55.

Statistical models were implemented to describe variability. The between subject variability (BSV) was modelled on an exponential scale to ensure population pharmacokinetic parameters were constrained to values greater than 0. The parameter variability between individuals took the generic form;

$$\theta_{ip} = \hat{\mu}_p \cdot exp^{\eta_{ip}}$$

Equation 7.1 *Model for between subject variability*

where, θ_{ip} is the estimate of the p^{th} parameter θ for the i^{th} individual, $\hat{\mu}_p$ is the population estimate of the p^{th} parameter, and, η_{ip} is the deviation of the p^{th} parameter for the i^{th} individual from the population estimate. η was assumed to be normally distributed with a mean of zero and a variance of ω^2.

An additive, proportional and combined (i.e. additive and proportional) error model was tested to describe residual unexplained error. A description of an additive, proportional and combined residual error model is given as follows:

- **Additive error model**. The additive error model describes variability that is constant at all concentrations. It can be given by the following equation;

$$Y = F + \varepsilon_{add}$$

Equation 7.2 *Additive error model*

where, Y represents observed dependent concentration, F is the individual specific model prediction and ε_{add} is additive error.

- **Proportional error model**. The proportional error model describes variability that is proportional to the magnitude of concentration. It is given by the following equation;

$$Y = F \cdot (1 + \varepsilon_{prop})$$

Equation 7.3 *Proportional error model*

where, ε_{prop} represents proportional error.

- **Combined error model**. The combined error model is a combination of the additive and proportional error model (shown in Equation 7.4).

$$Y = F \cdot (1 + \varepsilon_{prop}) + \varepsilon_{add}$$

Equation 7.4 *Combined error model*

7.4.4.2. Covariate model and structure

Covariates were considered in the model if there was a significant correlation between continuous covariates and the empirical Bayes estimates from the base model, or a significant *p* value from a *t* test for binary covariates. Covariates considered included age, sex, weight, fat free mass and creatinine clearance. Creatinine clearance was estimated using the Cockcroft and Gault equation [80]. Note that ideal body weight [398] was used in the Cockcroft and Gault equation. Fat free mass (FFM) was determined using the formula developed by Janmahasatian et al presented in Equation 7.5 [397];

$$FFM(male) = \frac{9.27 \cdot 10^3 \cdot TBW}{6.68 \cdot 10^3 + 216 \cdot BMI}$$

$$FFM(male) = \frac{9.27 \cdot 10^3 \cdot TBW}{8.78 \cdot 10^3 + 244 \cdot BMI}$$

Equation 7.5 *Fat free mass equation developed by Janhamasatian et al [397]*

where, TBW is total body weight in kilograms and BMI is body mass index in units of kilograms per square metre.

7.4.4.3. Model selection

Model selection was based on: (i) a reduction in objective function value (OFV) of 3.84 units (Chi-square [χ^2], $p < 0.05$) for nested models with one degree of freedom, (ii) graphical goodness of fit plots (including visual predictive checks), (iii) a reduction in between subject variability, (iv) parameter precision (i.e. relative standard errors less than 50%) and (v) biological plausibility of the parameter estimates.

7.4.4.4. Model building

The model building process first involved the development of a base model – this included the structural components and statistical models for between subject variability and residual unexplained variability. Covariates were subsequently tested in the base model one at a time to evaluate fit to data. A likelihood ratio test was performed and statistically significant covariates that resulted in a reduction in objective function value (OFV) of 3.84 units (Chi-square [χ^2], $p < 0.05$) were retained for further model building. Statistically significant covariates were sequentially added to the model in descending order of objective function value change from the base model (i.e. forward selection). The full covariate model was then subject to backwards deletion, which involved removing one covariate at a time to confirm statistical significance in the final model. Covariates that increased the objective function value by >6.6 units (Chi-square [χ^2], $p < 0.01$) following backwards deletion were retained in the final model.

7.4.4.5. Model evaluation

The final model was evaluated using standard diagnostic plots (i.e. dependent variable versus model predictions (DV v PRED), dependent variable versus individual predicted values (DV v IPRED), conditional weighted residuals versus model predictions (CWRES v PRED) and conditional weighted residuals versus time (CWRES v TIME)), a prediction corrected visual predictive check (pcVPC) and a non-parametric bootstrap. The median parameter values

and the 95% confidence intervals were determined from 1000 non-parametric bootstrap runs. The pcVPCs were produced in R (version 3.5.3) using the package xpose4 (version 4.6.1). A thousand datasets were simulated under the final model and the 5th, 50th and 95th percentiles were plotted against the same percentiles from the original dataset.

7.4.5. Simulations

Stochastic simulations were performed to predict the plasma metformin concentrations from the final population pharmacokinetic model for metformin. Plasma metformin concentrations were predicted for a one-week period under two guidelines;

1. the doses predicted by the empirical dosing guideline developed in Chapter 5 (see Table 5.3)
2. the doses recommended by the New Zealand Formulary (NZF) renal dosing guidelines (presented in Table 7.2).

A summary of the simulated doses under the empirical renal dosing guideline and the NZF is presented in Table 7.2.

The simulations were implemented in R (version 3.5.3) using the R package RxODE (version 0.9.0-7). RxODE is a platform that facilitates simulation from models implemented with ordinary differential equation models in R that was designed for pharmacometric use. A total of 1000 virtual patients were simulated for each renal dosing band under each guideline. Creatinine clearance was generated from a uniform distribution based on the renal function range defined in the CKD categories defined in Chapter 5 and the NZF renal dosing guidelines. Weight was fixed to 70 kilograms. The probability of achieving a plasma metformin concentration exceeding the upper safety limit of 4.5 mg/L (as defined in Chapter 4) was determined. The simulation code is presented in Appendix A5.1.

Table 7.2 *Simulated renal doses for the empirical renal dosing guideline (Chapter 5) and the NZF guideline*

Dosing guideline	Creatinine clearance (mL/min)	Maximum daily dose (mg)	Simulated dose (mg)
Developed empirical equation			
$CLcr_{CG}$	≥90	2250	750 TID
method	60-89	1700	850 BD
	30-59	1000	500 BD
	15-29	500	500 OD
	<15	250	250 OD
$eGFR_{MDRD}$	≥90	2550	850 TID
method	60-89	2000	1000 BD
	30-59	1000	500 BD
	15-29	500	500 OD
	<15	250	250 OD
$eGFR_{CKDEPI}$	≥90	2250	750 TID
method	60-89	1700	850 BD
	30-59	1000	500 BD
	15-29	500	500 OD
	<15	250	250 OD
Published dosing guideline			
New Zealand	60-120	2000	1000 BD
Formulary	30-60	1000	500 BD
	15-30	500	500 OD

OD: once a day. BD: twice a day, TID: three times a day.

7.5. Results

7.5.1. Data

A total of 321 plasma metformin concentrations from 52 study participants were available for analysis. A total of 77 plasma metformin concentrations collected from Middlemore Hospital were omitted from the analysis due to protocol violation. In the study protocol blood samples were to be collected 0, 2, 4, 6, 8 and 24 hours post administration of the first metformin dose, with the 24 hour blood sample to be measured prior to study participants receiving their second dose. However, in 5 study participants their 24 hour plasma metformin concentrations were greater than their 8 hour concentrations. Hence, these five aberrant concentrations were excluded from the analysis. It is likely that these participants ingested their second dose of metformin prior to the measurement of their 24 hour metformin trough concentration. In addition, in the protocol, weekly blood samples were to be collected from study participants prior to them ingesting their daily dose. However, in a large majority of the study participants their weekly trough concentrations measured were below the limit of quantification; this is likely to be due to issues surrounding patient compliance. For these reasons, data from unobserved doses were excluded from the analyses.

Thirty-six plasma metformin concentrations were below the quantification limit. Data that were below the limit of quantification were accounted for using the M6 method described by Beal [402]. No data were identified as outliers.

A Cartesian and semi-log concentration time profile of the combined raw data is presented in Figure 7.1.

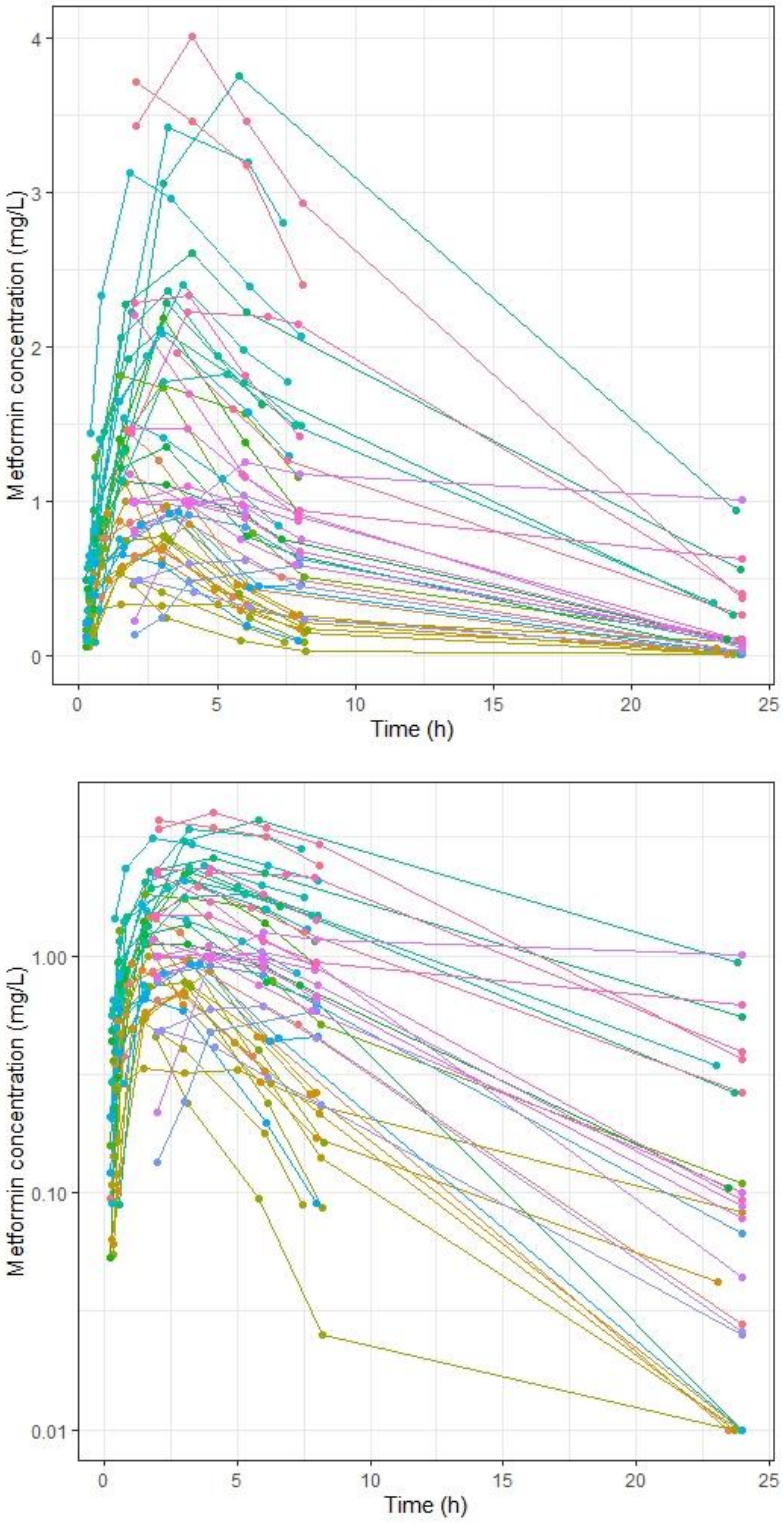


Figure 7.1 *Plasma metformin concentrations following a single oral dose of metformin on a Cartesian (top) and semi-log (bottom) plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single study participant.*

7.5.2. Population pharmacokinetic model for metformin

A two-compartment model with first-order absorption and elimination provided the best fit to the data. The final model to describe the pharmacokinetics of metformin is shown as follows;

$$CL = \theta_{CL} \cdot (CLcr_{CG}/100)^{CG\_EFF}$$
$$V_c = \theta_{V_c} \cdot (WTKG/70)^{WTKG\_EFF}$$
$$Q = \theta_Q$$
$$V_p = \theta_{V_p}$$
$$k_a = \theta_{k_a}$$
$$F = 0.55$$

Equation 7.6 *Final pharmacokinetic model for metformin*

where CL is the clearance of metformin (L/h), θ_{CL} is the mean population value for metformin clearance (L/h), $CLcr_{CG}$ is creatinine clearance calculated using the Cockcroft and Gault equation using ideal body weight as the body size metric, CG_EFF is the estimated exponent on the covariate effect for $CLcr_{CG}$, V_c is the central compartment volume (L), θ_{V_c} is the mean population value for the central compartment volume (L), $WTKG$ is total body weight in kg, $WTKG\_EFF$ is the exponent on the effect of $WTKG$ on the central compartment volume that was fixed to 1, Q is intercompartmental clearance (L/h), θ_Q is the mean population value for intercompartmental clearance (L/h), V_p is the peripheral compartment volume (L), θ_{V_p} is the mean population value for the peripheral compartment volume (L), k_a is the absorption rate constant (h^{-1}), θ_{k_a} is the mean population value for the absorption rate constant (h^{-1}) and F is bioavailability that was fixed to 0.55. Between subject variability on the peripheral compartment volume (V_p) and intercompartmental clearance (Q) were not supported under the current data and were not included in the final model. The parameter estimates and bootstrap results for the final population pharmacokinetic model for metformin are shown in Table 7.3.

Creatinine clearance and total body weight were found to be significant covariates on clearance and the central compartment volume, respectively. The addition of creatinine clearance as a covariate on clearance resulted in a reduction in BSV from 105% to 46% and the addition of total body weight on the central compartment volume resulted in a reduction in BSV from 37% to 30%. Subsequent removal of either of the covariates resulted in a poorer global fit.

Diagnostic plots for the final model are presented in Figure 7.2. The conditional-weighted residual plots show no apparent bias for the model predictions. A pcVPC for the final metformin pharmacokinetic model is presented in Figure 7.3. The median, 5th and 95th percentiles of the population pharmacokinetic model predicted plasma metformin concentrations that follow the percentiles of the observed data well, suggesting a good model fit to the data. The non-parametric bootstrap results (shown in Table 7.3) are similar to the final model suggesting that the developed model is stable.

The NONMEM control file for the final model is presented in Appendix A5.2.

Table 7.3 *Parameter estimates and bootstrap results for the final PK model*

Parameter	**Parameter estimates (RSE%)**	**Bootstrap results [95% confidence interval]**
θ_{CL} (L/h)	90.5 (8)	89.6 [75.4-108.1]
θ_{V_c} (L/70kg)	147 (9)	143.8 [106.2-181.0]
θ_Q (L/h)	3.7 (10)	3.6 [0.9-8.3]
θ_{V_p} (L)	57.2 (18)	53.6 [12.8-515.0]
θ_{k_a} (h^{-1})	0.4	0.4 [0.3-0.5]
θ_F	0.55 FIX	0.55 FIX
θ_{CG_EFF}	0.8 (11)	0.8 [0.6-1.0]
θ_{WT_EFF}	1 FIX	1 FIX
Between subject variability		
ω_{CL} (CV%)	46.9 (12)	46.6 [35.0-61.0]
ω_{V_c} (CV%)	29.3 (18)	28.5 [12.5-39.1]
ω_{k_a} (CV%)	39.5 (24)	41.3 [18.2-60.0]
Covariance $\omega_{CL}, \omega_{V_c}$	0.0697	0.0605 [-0.0305-0.1513]
Residual error		
σ_{add} (mg/L)	0.0337 (42)	0.0316 [0.0069-0.1012]
σ_{prop} (CV%)	24.1 (15)	24.0 [7.1-30.9]
Shrinkage (%)		
η-shrinkage (CL)	6	
η-shrinkage (V_C)	25	
η-shrinkage (k_a)	24	
ε-shrinkage	15	

θ_{CL} mean population value for metformin clearance, θ_{V_c} mean population value for the central compartment volume, θ_Q mean population value for intercompartmental clearance, θ_{V_p} mean population value for the peripheral compartment volume, θ_{k_a} mean population value for the absorption rate constant, θ_F mean population value for bioavailability, ω_{CL} between subject variability for clearance, ω_{V_c} between subject variability for the central compartment volume, ω_{k_a} between subject variability for the absorption rate constant, σ_{add} additive residual error, σ_{prop} proportional residual error.

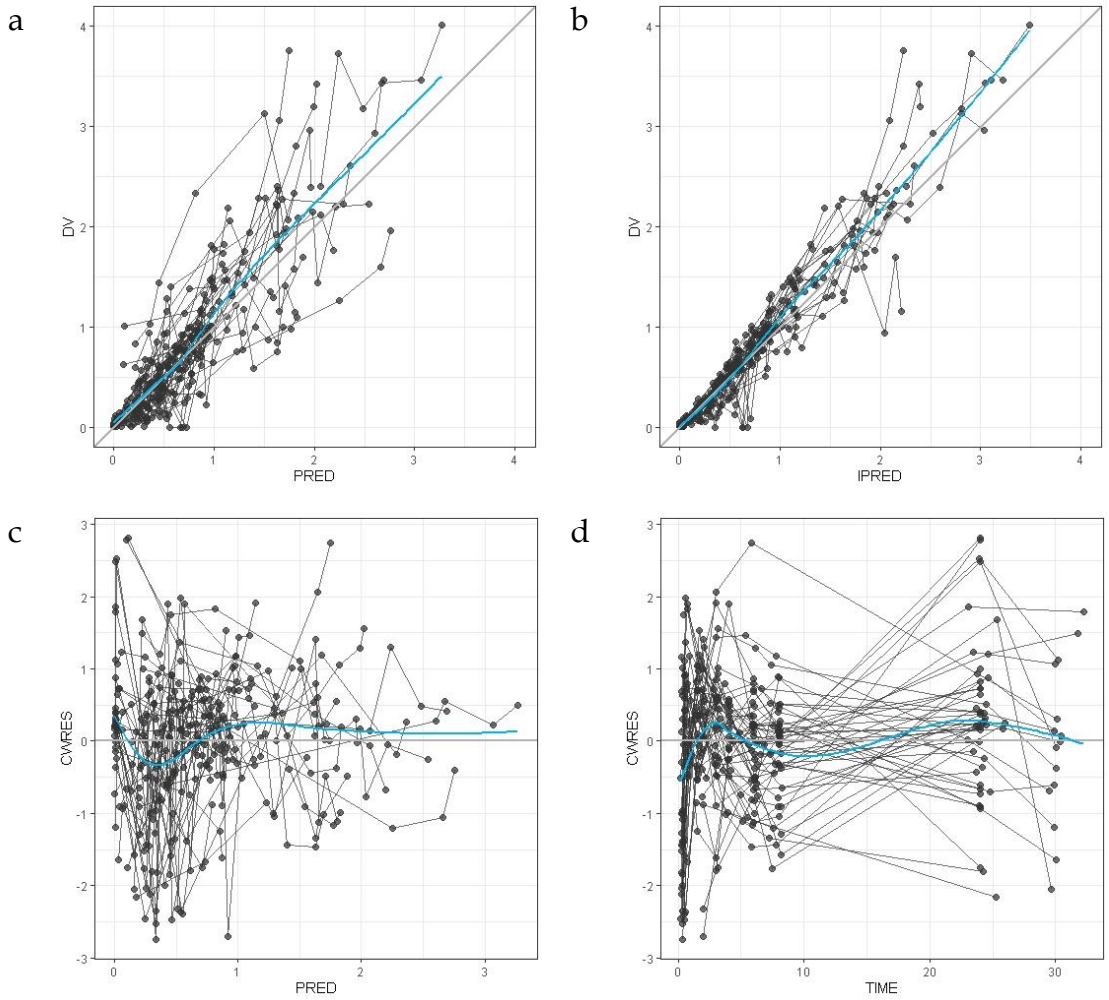


Figure 7.2 *Goodness of fit plots for the final population pharmacokinetic model.* ***a*** *observed plasma metformin concentration data (DV) plotted against the population prediction for plasma metformin concentrations (PRED).* ***b*** *observed plasma metformin concentration data (DV) plotted against the individual prediction for plasma metformin concentrations.* ***c*** *conditional weighted residuals (CWRES) plotted against population predictions for plasma metformin concentrations.* ***d*** *conditional weighted residuals (CWRES) plotted against time.*

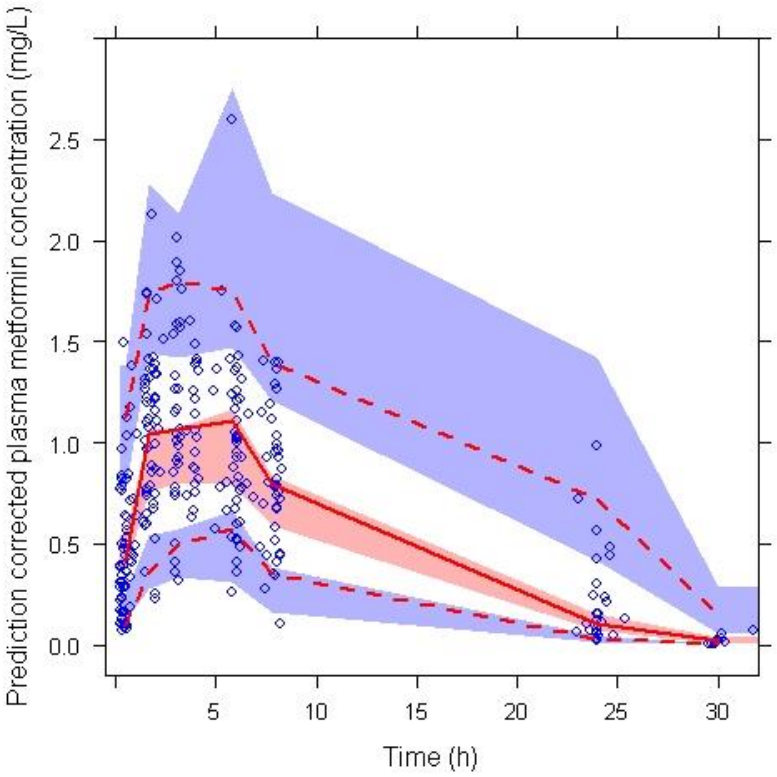


Figure 7.3 *Prediction corrected visual predictive check for the final pharmacokinetic model. The plot shows the observed plasma metformin concentrations (open blue circles) and the 5th, 50th and 95th percentiles for the model-predicted plasma metformin concentration (red lines, dashed for the 5th and 95th percentiles and a solid red line for the 50th percentiles) with the 95% confidence interval around the percentiles.*

7.5.3. Simulations

Simulations were performed using the parameter estimates shown in Table 7.3. The simulated plasma metformin concentration profiles under the empirical renal dosing recommendations using the Cockcroft and Gault, 4-variable MDRD and CKD-Epi equations and, the NZF guidelines are presented in Figures 7.4, Figure 7.5, Figure 7.6 and Figure 7.7, respectively. The plasma metformin concentration was not found to exceed 4.5 mg/L under any of the simulated renal dosing guidelines. This finding is reflected in the simulated plasma metformin concentration profiles where the 95th percentiles all are all well within 3 mg/L.

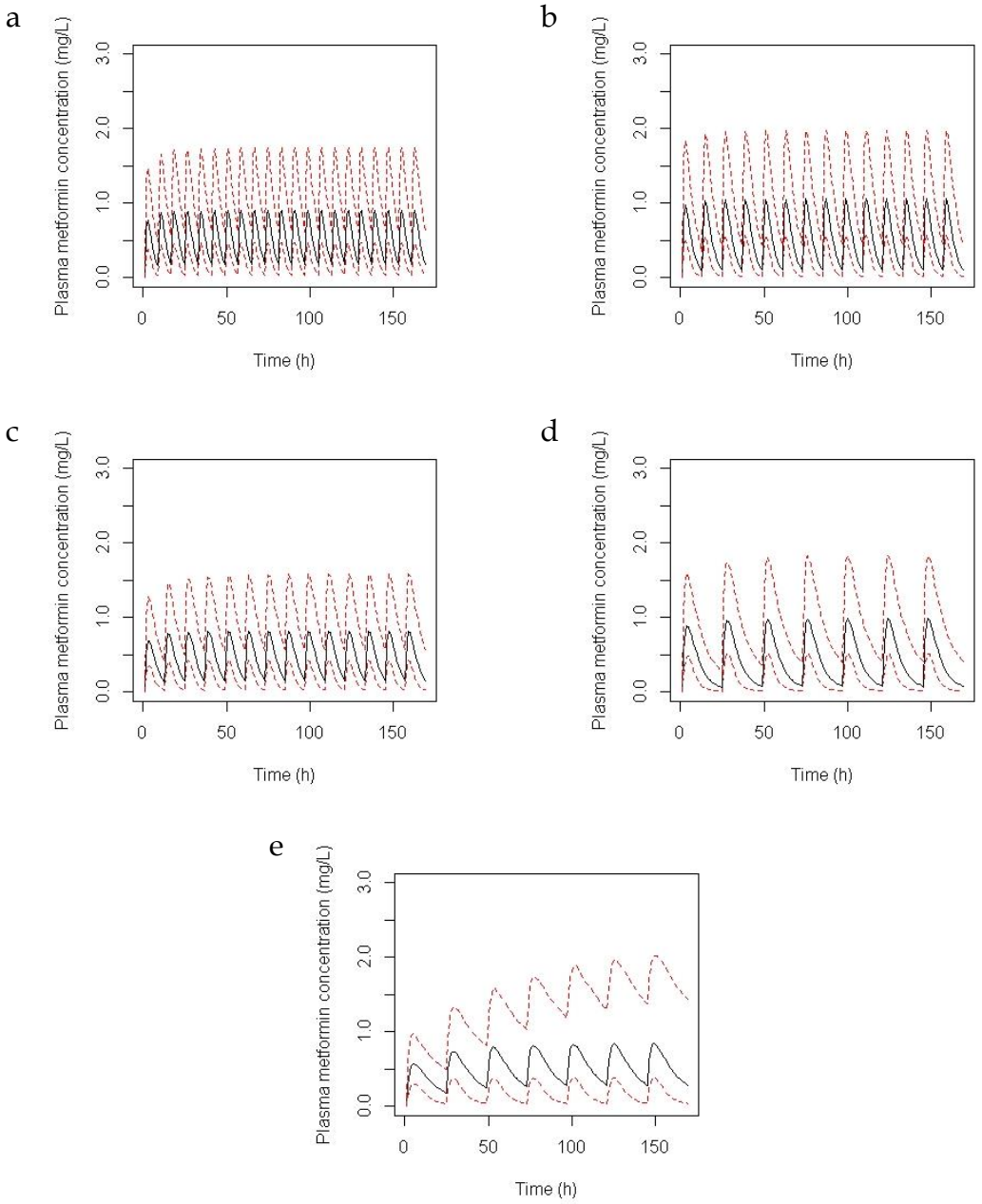


Figure 7.4 *Simulated plasma metformin concentration versus time profiles under the empirical $CLcr_{CG}$ method.* ***a*** *750 mg metformin given thrice daily, creatinine clearance 90-120 mL/min.* ***b*** *850 mg metformin given twice daily, creatinine clearance 60-89 mL/min.* ***c*** *500 mg metformin given twice daily, creatinine clearance 30-59 mL/min.* ***d*** *500 mg metformin given once daily, creatinine clearance 15-29 mL/min.* ***e*** *250 mg metformin given once daily, creatinine clearance <15 mL/min. The red dashed lines represent the 5th and 9th percentiles. The black line is the median percentile.*

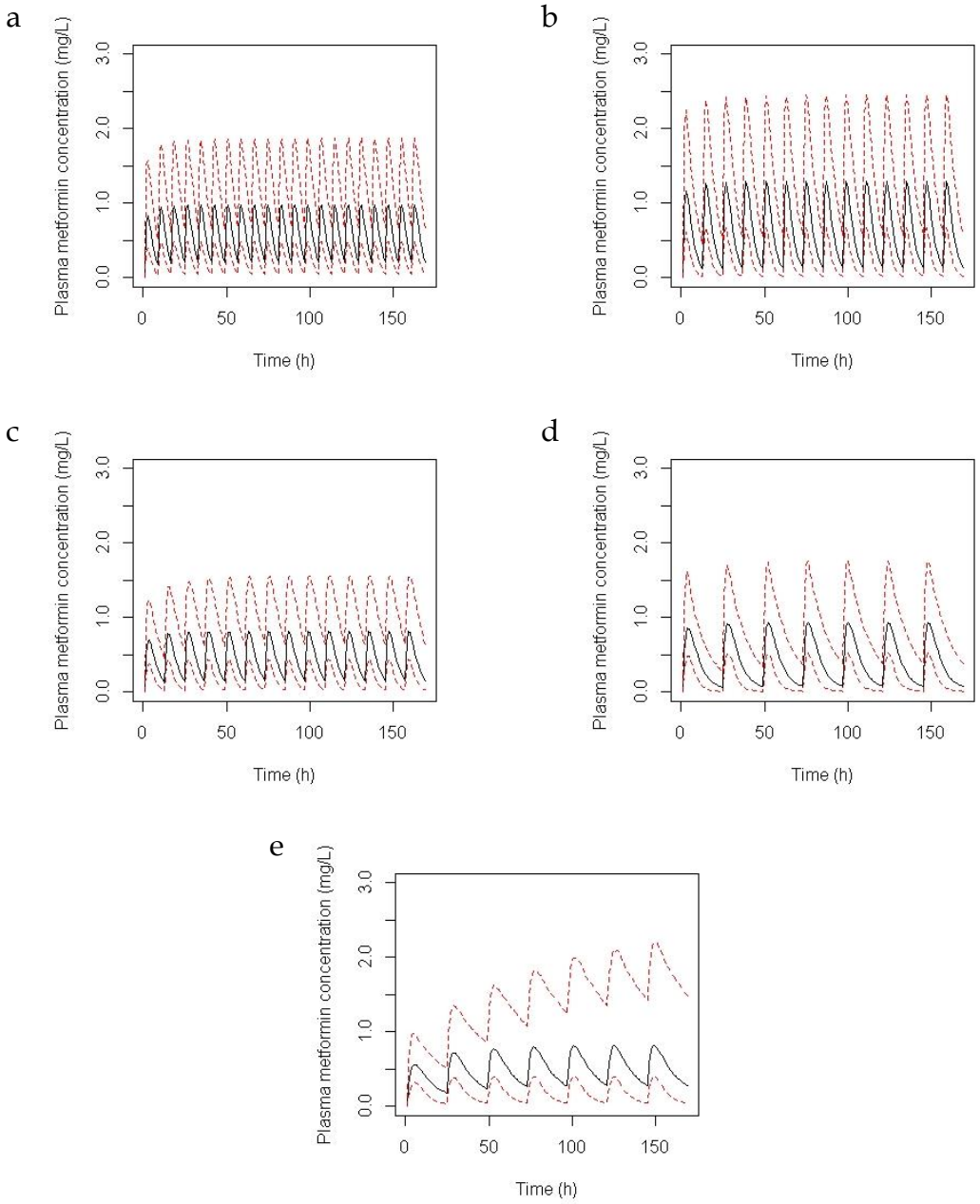


Figure 7.5 *Simulated plasma metformin concentration versus time profiles under the empirical $eGFR_{MDRD}$ method.* ***a*** *750 mg metformin given thrice daily, creatinine clearance 90-120 mL/min.* ***b*** *850 mg metformin given twice daily, creatinine clearance 60-89 mL/min.* ***c*** *500 mg metformin given twice daily, creatinine clearance 30-59 mL/min.* ***d*** *500 mg metformin given once daily, creatinine clearance 15-29 mL/min.* ***e*** *250 mg metformin given once daily, creatinine clearance <15 mL/min. The red dashed lines represent the 5^{th} and 9^{th} percentiles. The black line is the median percentile.*

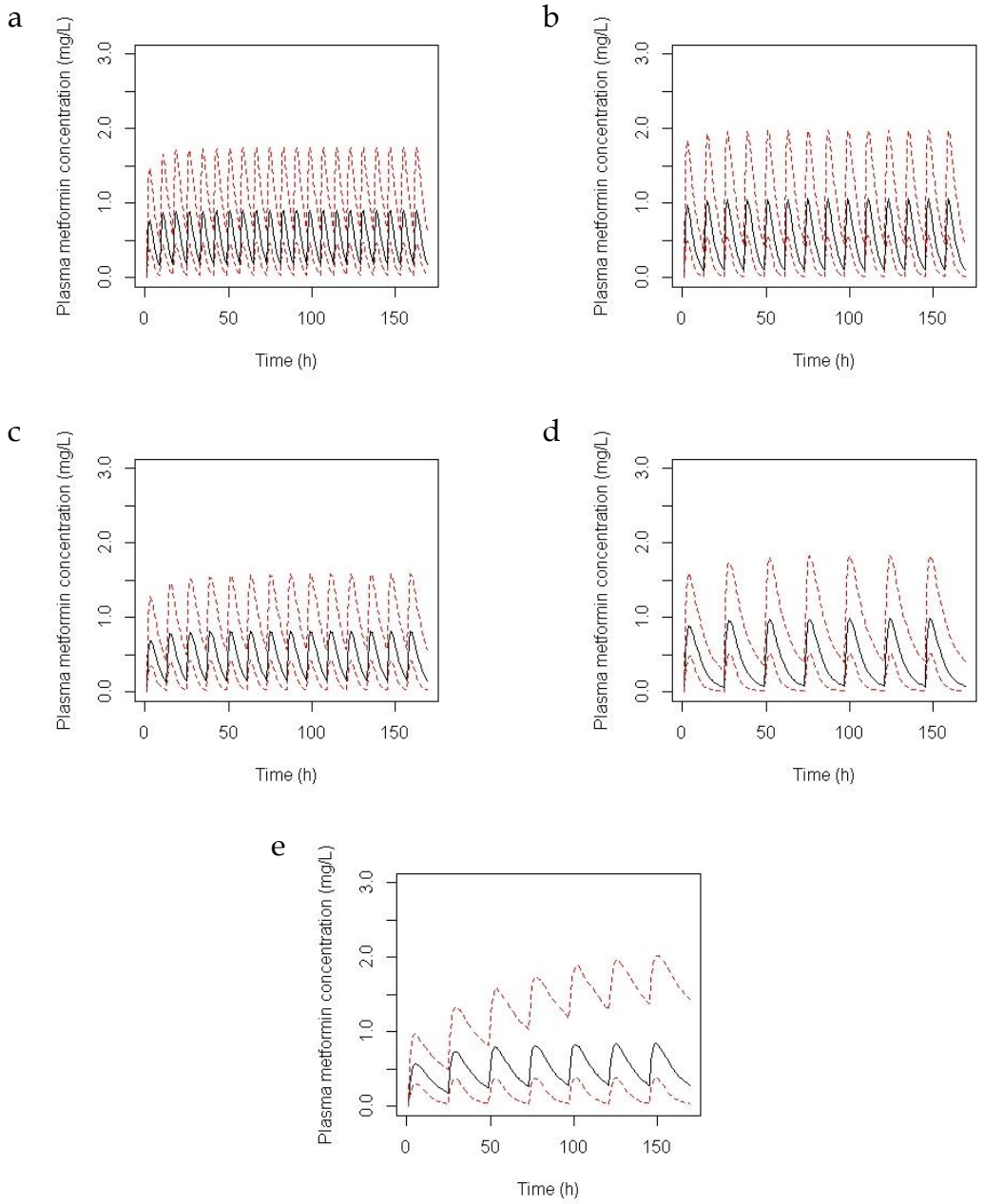


Figure 7.6 *Simulated plasma metformin concentration versus time profile under the empirical $eGFR_{CKDEPI}$ method.* ***a*** *750 mg metformin given thrice daily, creatinine clearance 90-120 mL/min.* ***b*** *850 mg metformin given twice daily, creatinine clearance 60-89 mL/min.* ***c*** *500 mg metformin given twice daily, creatinine clearance 30-59 mL/min.* ***d*** *500 mg metformin given once daily, creatinine clearance 15-29 mL/min.* ***e*** *250 mg metformin given once daily, creatinine clearance <15 mL/min. The red dashed lines represent the 5^{th} and 9^{th} percentiles. The black line is the median percentile.*

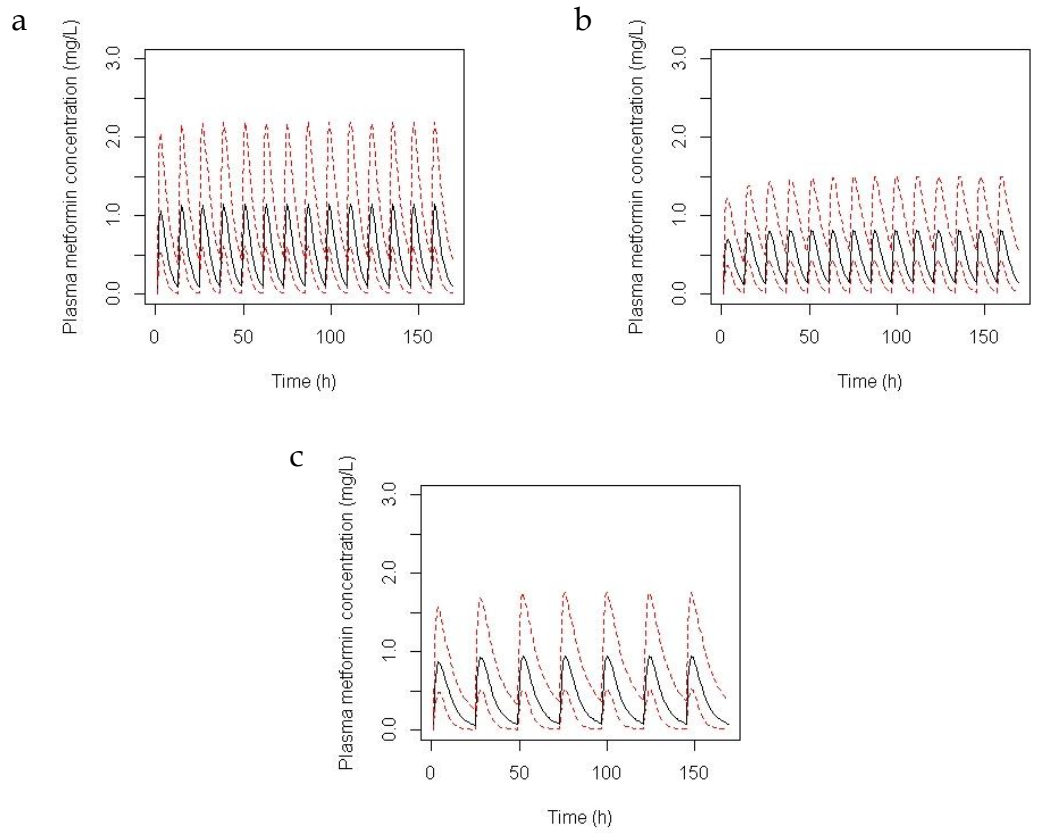


Figure 7.7 *Simulated plasma metformin concentration versus time profiles under each renal dosing band in the New Zealand Formulary.* ***a*** *1000 mg metformin given twice daily, creatinine clearance 60-120 mL/min.* ***b*** *500 mg metformin given twice daily, creatinine clearance 30-60 mL/min.* ***c*** *500 mg metformin given once daily, creatinine clearance 15-30 mL/min. The red dashed lines represent the 5th and 9th percentiles. The black line is the median percentile.*

7.6. Discussion

In this study a population pharmacokinetic model for metformin was developed and evaluated. A two-compartment model with first-order absorption and elimination was found to best describe the pharmacokinetics of metformin in study participants with varying levels of renal function. Both creatinine clearance and total body weight were identified as significant covariates on the apparent clearance and the central compartment volume for metformin. Based on the simulations performed in this study it was predicted that under the doses predicted using the empirical equations developed in Chapter 5 and the current NZF renal dosing guidelines the plasma metformin concentration would not exceed the 4.5 mg/L upper limit of safety for metformin (defined in Chapter 4).

Several published studies have described the pharmacokinetics of metformin in patients with normal and poor renal function [14-16, 370]. These published analyses have generally been designed to develop a renal dosing guideline for metformin in the clinic. However, only a few of these studies involved the development of a population pharmacokinetic model for metformin. Published population pharmacokinetic models have commonly found that a two-compartment structural model best described the absorption and disposition of metformin following an oral dose [15, 370, 403, 404]. The covariates identified in this study to predict the pharmacokinetics of metformin are similar to those in published studies [15, 404]. In a population pharmacokinetic model by Duong et al both creatinine clearance and total body weight were found to be significant covariates on the apparent clearance and central compartment volume for metformin, respectively, as identified in this study [15].

The intact nephron hypothesis provides a general model for renal impairment [405]. It assumes that each nephron is either fully functional (intact) or non-functional (lost), whereby changes in glomerular filtration are proportional to changes in tubular reabsorption and tubular secretion [405]. The implications of the intact nephron hypothesis for drug therapy are that only

glomerular filtration rate is required to predict the impact of renal pathology on renal drug handling. Recent studies have suggested that the intact nephron hypothesis may not be a suitable general model for summarising renal drug handling, particularly in drugs that also undergo tubular secretion and/or reabsorption [406, 407]. However, for metformin, glomerular filtration and metformin renal clearance were shown to share a linear relationship (results from Chapter 5). This suggests that glomerular filtration rate (and creatinine clearance) can be used to sufficiently describe renal impairment and guide dosing and, is sufficient as a covariate on clearance for metformin.

7.7. Limitation

The results of this chapter should be viewed and interpreted in light of the influence flip-flop pharmacokinetics may have on nonlinear mixed effects modelling. As mentioned in Chapter 1 (section 1.2.4.4.) metformin has been reported to exhibit flip-flop pharmacokinetics. Noncompartmental analyses - such as those performed in Chapter 5 – are unaffected by flip-flop. However, it is unclear if flip-flop will present problems in population pharmacokinetic models due to issues of local identifiability. This warrants the need for further analyses to be performed to explore the influence of flip-flop in population pharmacokinetic models.

7.8. Conclusion

In this study a population pharmacokinetic model for metformin was developed and evaluated. A two-compartment structural model with first-order absorption and elimination provided the best fit to the metformin data from study participants with varying levels of renal impairment. Predictions from the model under the empirical renal dosing guideline (developed in Chapter 5) and the NZF renal dosing guidelines suggest that plasma metformin concentrations will not exceed the proposed upper limit of safety of 4.5 mg/L proposed in Chapter 4. This suggests that the empirical and NZF renal dosing guidelines can be used safely in patients with renal impairment.

Chapter 8: Exploring the influence of flip-flop

8.1. Preamble to the chapter

In Chapter 7 of this thesis a population pharmacokinetic model was developed for metformin using nonlinear mixed effects modelling techniques. Metformin is known to exhibit so called 'flip-flop' pharmacokinetics [27, 29]. Flip-flop is a pharmacokinetic phenomenon where the rate constant of absorption and elimination for extravascularly administered drugs can interchange. In compartmental nonlinear mixed effects models flip-flop presents a problem due to issues of local identifiability in the parameter estimates. This chapter explores the influence of flip-flop in population pharmacokinetic modelling using metformin as an example.

8.2. Introduction

The term 'flip-flop' is used to describe the scenario where the rate constant of absorption and rate constant of elimination for extravascularly administered drugs can swap over. Drugs that undergo absorption limited elimination (i.e. elimination rate constant (k) > absorption rate constant (k_a)) are often said to be 'flip-flop' but in reality they are usually just 'flip' ($k > k_a$) or 'flop' ($k < k_a$). Of note, flip-flop can occur between patients for a drug with slow absorption from the gastrointestinal tract and with rapid and extensive renal clearance. In this setting, for patients with normal renal function the usual finding will be that $k > k_a$, however in patients with impaired renal function the finding may be $k < k_a$.

Flip-flop pharmacokinetics is in reality a permutation of the rank order of the parameter values and is therefore an issue of local identifiability in that there exists a finite set of parameter values (rather than a single set) that solves the problem. Any mammillary pharmacokinetic model that can be constructed from multiple exponential functions will also only be locally identifiable. The simplest example is a one-compartment model with first-order input and output which has two sets of permutations of parameter values that provide the same input-output relationship. The possible permutations using a CL, V, k_a and k, V, k_a parameterisation are shown in Table 8.1 – where CL is clearance and V is volume

of distribution. Here it can be seen that the k, V, k_a parameterisation is a complete permutation of the parameters, whereby the values for k, V and k_a are completely different in permutation 1 and permutation 2. However, in Table 8.1 it can be seen that the CL, V, k_a parameterisation is only a partial permutation, whereby V becomes a function of CL and k_a, k_a becomes a function of CL and V and, CL is invariant to flip-flop (i.e. CL' remains equal to CL in both possible permutations) where $AUC = Dose/CL$ is irrespective of whether the system is in a state of 'flip' or 'flop' (i.e. $k > k_a$ or $k < k_a$). Note here that noncompartmental analyses are unaffected by a model being in either a 'flip' or a 'flop' state. Under this model, flip-flop can be considered a mathematical abstraction and a special case of local identifiability problem in that it is not just a finite set of parameter values but a partial permutation of the set. Refer to Appendix A6.1 for an extended discussion on the different sets of permutations of parameter values for pharmacokinetic compartmental models.

Table 8.1 *Possible permutations for a one-compartment model using CL, V, k_a and k, V, k_a parameterisation*

	Parameterisation	
	$\boldsymbol{CL, V, k_a}$	$\boldsymbol{k, V, k_a}$
Permutation 1	$CL' = CL$	$k' = k$
	$V' = V$	$V' = V$
	$ka' = ka$	$ka' = ka$
Permutation 2	$CL' = CL$	$k' = ka$
	$V' = CL/ka$	$V' = (V \cdot k)/ka$
	$ka' = CL/V$	$ka' = k$

In theory, the issue of local identifiability (flip-flop behaviour) can be addressed by incorporating a mechanistic model of the absorption and elimination that accounts for the underlying processes. This is, however, generally not possible in a standard top-down estimation setting. A simpler alternative is to consider that there is a level of functioning of the elimination organ at which the absorption and elimination rate constants flip around and

that this can be estimated as a transition cut-off value. The model could then be stabilised into either its flip or flop state for any given individual and hence avoid flip-flop to yield a globally identifiable model.

The concept of a transition cut-off was applied to pharmacokinetic data arising from metformin. Metformin is an antihyperglycaemic agent that is reported to exhibit absorption mediated elimination [27, 29]. In published human pharmacokinetic studies the terminal elimination phase of metformin was dissimilar following intravenous (IV) and oral (PO) administration, whereby there was an evident slower decline in metformin concentrations following PO administration [27, 29]. Whereby, the terminal plasma elimination half-life of metformin has been reported to be 1.74±0.11 and 8.41±0.58 hours (mean±standard error) following intravenous and oral administration, respectively [29]. The slower apparent elimination of metformin following PO administration has been suggested to be a result of the slow gastrointestinal absorption of the drug [29, 30]. This has led to speculation that the terminal slope of metformin following PO administration in patients with normal renal function is absorption rate limited, whilst in patients with poor renal function it is elimination rate limited. Metformin is predominantly renally eliminated as unchanged drug via tubular secretion [29]. However, it is not known if and at what level of renal impairment the terminal slope of metformin's concentration profile changes from being absorption rate limited to elimination rate limited.

8.3. Objectives

The aim of this research was to explore the influence of flip-flop in population pharmacokinetic models using metformin as a motivating example. The specific objectives were:

i. To determine whether it is possible to estimate the flip-flop transition point in order to parameterise a model that disallows flip-flop

ii. To investigate the application of constraining parameters to address flip-flop
iii. To investigate whether the inclusion of intravenous data addresses issues associated with flip-flop
iv. To investigate the influence of flip-flop pharmacokinetics on covariate modelling

8.4. Methods

8.4.1. Data

8.4.1.1. Data source

Data available for analysis arose from three sources: (i) Dunedin Public Hospital, (ii) Middlemore Hospital and (iii) a study by Pentikainen et al. The data sourced from Dunedin Public Hospital and Middlemore Hospital have previously been described in Chapter 5 (section 5.4.1.) and Chapter 7 (section 7.4.1.1.) of this thesis, respectively. A description of the newly introduced data from the study by Pentikainen et al is provided in the following section. A summary of the demographic data of study participants from the three sources combined is shown in Table 8.2.

8.4.1.1.1. Metformin pharmacokinetic study by Pentikainen et al

Data was extracted from a study by Pentikainen et al [29]. In the study the pharmacokinetics of metformin following IV and PO administration in humans was investigated. The study included 5 healthy volunteers. Study volunteers fasted the night prior to the study. A single dose of metformin 500 mg was administered, either PO or IV, in the morning following the overnight fast. All study volunteers received an oral dose of metformin, with three of the volunteers receiving an additional IV bolus dose. The PO and IV doses were administered in random order and were given a minimum of three weeks apart. The oral dose of metformin was administered with 200 mL of water. Blood samples were collected at 0 (baseline), 5, 10, 20, 30, and 45 minutes, and, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours post metformin PO and IV administration. Additional blood samples were collected following IV administration at the end of the injection and, 2 and 15 minutes post drug administration.

Demographic, clinical and metformin pharmacokinetic data were extracted for the 3 study volunteers that had received both PO and IV doses of metformin. Demographic and clinical data extracted included: age, sex, height, weight and

serum creatinine. Data presented in the form of graphs was extracted using the MATLAB (R2016b, MathWorks, Natick, MA) code GRABIT.

Table 8.2 *Summary statistics of study participants by study*

	Dunedin Public Hospital (n = 34)	**Middlemore Hospital (n = 18)**	**Pentikainen et al (n = 3)**	**Pooled dataset (n = 55)**
Age (years)	51.5 [20.0–79.0] (32.3–66.0)	66.0 [40.0–75.0] (62.0–68.0)	38.0 [36.0–39.0] (37.0–38.5)	61.0 [20.0–79.0] (37.5–67.5)
Sex (F:M)	5:29	3:15	2:1	10:45
Height (cm)	174 [157–195] (168–181)	172 [145–183] (168–176)	171 [161–171] (166–171)	172 [145–195] (168–178)
Weight (kg)	82.1 [48.0–149.5] (75.1–87.7)	111.7 [77.2–149.8] (91.0–125.8)	60.0 [58.0–63.0] (59.0–61.5)	84.6 [48.0–149.8] (76.0–102.8)
BMI (kg/m²)[a]	26.3 [17.8–48.8] (24.2–28.9)	38.0 [25.8–51.4] (32.6–42.3)	21.5 [19.8–23.1] (20.7–22.3)	28.6 [17.8–51.4] (24.8–34.5)
FFM (kg)[b]	61.5 [33.9–80.5] (54.4–63.8)	68.2 [44.4–83.3] (58.8–72.8)	39.5 [38.6–51.5] (39.0–45.5)	61.5 [33.9–83.3] (55.0–67.8)
Serum creatinine (μmol/L)	118.5 [52.0–546.0] (89.5–300.0)	259.5 [197.0–370.0] (219.3–300.0)	71.0 [71.0–75.0] (71.0–73.0)	206 [52.0–546.0] (91.5–300.0)
$CLcr_{CG}$[c] (mL/min)	73.5 [9.5-167.0] (18.1-113.2)	23.4 [11.4-37.3] (19.0-28.3)	95.3 [79.1-111.6] (89.2-103.5)	29.0 [9.5-167.0] (18.4-93.1)
$eGFR_{MDRD}$[d] (mL/min/1.73 m²)	54.1 [8.9–118.5] (17.4–86.7)	20.4 [14.5–29.3] (17.7–25.7)	80.8 [79.5-101.1] (80.1-90.9)	28.0 [8.9-118.5] (17.5-78.9)
$eGFR_{MDRD}$ (adjusted)[f] (mL/min)	65.8 [11.0-142.7] (18.5-97.2)	24.8 [17.2-39.1] (20.8-35.8)	78.4 [74.9-101.6] (76.6-90.0)	35.9 [11.0-142.7] (19.1-99.1)

Table 8.2 cont *Summary statistics of study participants by study*

	Dunedin Public Hospital (n = 34)	Middlemore Hospital (n = 18)	Pentikainen et al (n = 3)	Pooled dataset (n = 55)
$eGFR_{CKDEPI}$ [e] (mL/min/1.73 m²)	60.2 [8.2–122.4] (17.2–98.7)	20.1 [14.2–29.6] (17.3–25.8)	94.4 [92.4-110.6] (93.4-102.5)	28.3 [8.2-122.4] (17.2-89.8)
$eGFR_{CKDEPI}$ (adjusted)[f] (mL/min)	73.2 [10.0-151.1] (17.9-109.2)	24.7 [17.0-40.4] (20.2-35.6)	91.5 [87.1-111.2] (89.3-101.3)	35.6 [10.0-151.1] (18.6-100.8)

Data presented as median [range] (interquartile range) unless otherwise specified. [a]BMI is body mass index. [b]FFM is fat free mass calculated using the equation by Janmahasatian et al [397]. [c]$CLcr_{CG}$ is creatinine clearance estimated using the Cockcroft and Gault equation [80]. Note that ideal body weight [398] was used in the Cockcroft and Gault equation as a body size metric. [d]$eGFR_{MDRD}$ is glomerular filtration rate estimated using the 4-variable Modification of Diet in Disease in Renal Disease equation [82]. [e]$eGFR_{CKDEPI}$ is glomerular filtration rate estimated using the Chronic Kidney Disease Epidemiology Collaboration equation [83]. [f]Note that $eGFR_{MDRD}$ (adjusted) and $eGFR_{CKDEPI}$ (adjusted) were adjusted the individual body surface area measurements for each subject calculated using the Du Bois Method [399].

8.4.1.2. Data management

Data were handled as previously described in Chapter 7 (section 7.4.3.).

8.4.2. Models for examining flip-flop with metformin

For the purposes of this analysis a simplified one-compartment pharmacokinetic model was used to examine flip-flop with metformin. A simplified model was used as this work is illustrative for any drug rather than definitive for metformin and the issue of local identifiability becomes more complicated when the number of mammillary-compartments (n) increases; whereby, the possible number of permutations of parameter values that provide the same input-output relationship for a given compartmental model is $n + 1$ (counting only disposition compartments).

In order to accommodate the effects of flip-flop, three levels of parameter constraints were considered (i) no constraints (i.e. flip-flop was allowed) termed

an unconstrained model, (ii) constraints at the population level to avoid flip-flop at the population parameter level (termed a partially constrained model) and (iii) constraints at the individual level to avoid flip-flop at the individual parameter level (termed a fully constrained model). The models were parameterised using either CL, V, k_a or k, V, k_a. The parameter constraints, when applied, were applied to force the model into either its 'flip' or 'flop' state. The transition point (when $CL/V = k_a$) was attempted to be estimated. The constraints were applied such that: (i) $k > k_a$ in subjects with a creatinine clearance ($CLcr_{CG}$) calculated using the Cockcroft and Gault equation greater than the flip-flop transition point, and, (ii) $k < k_a$ in subjects with a $CLcr_{CG}$ less than the transition point. In this setting k was either the primary parameter or a secondary parameter (calculated from CL and V). The model constrained designs parameterised using CL, V, k_a and k, V, k_a is shown as follows;

CL, V, k_a parameterisation

$$k_a = \begin{cases} \dfrac{(CL/V)}{1 + k_a^*}, & CLcr_{CG} \geq transition\ point \\ (CL/V) \cdot (1 + k_a^*), & CLcr_{CG} < transition\ point \end{cases}$$

k, V, k_a parameterisation

$$k = \begin{cases} k_a \cdot (1 + k^*), & CLcr_{CG} \geq transition\ point \\ \dfrac{k_a}{1 + k^*}, & CLcr_{CG} < transition\ point \end{cases}$$

where, $CLcr_{CG}$ is creatinine clearance determined using the Cockcroft and Gault equation [80], $transition\ point$ is an estimated value of creatinine clearance at which CL/V is believed to equal k_a. In the CL, V, k_a parameterisation, CL, V and k_a^* are estimated parameters, and, k_a is a calculated parameter. In the k, V, k_a parameterisation k_a, V and k^* are estimated parameters and k is a calculated parameter. Note that the values of k_a and k^* were constrained to values greater than 0.

Between subject variability was modelled using an exponential model to ensure individual pharmacokinetic parameters were constrained to values greater than 0. Covariance was considered for each parameterisation separately. A combined error model was used to describe residual unexplained variability (as described in Chapter 7 section 7.4.4.1.).

The pharmacokinetic models were run with PO and IV concentration data combined and, also PO data only. Two sets of initial parameter estimates were used for the unconstrained models where $k < k_a$ or $k > k_a$ (shown in Table 8.3).

Table 8.3 *Initial estimates used for the unconstrained models*

Parameter values	$\boldsymbol{k < k_a}$	$\boldsymbol{k > k_a}$
CL (L/h)	25	25
V (L)	100	50
k_a (h^{-1})	0.5	0.25
k (h^{-1})	0.25	0.5

8.4.3. Performance of model constrained designs

The performance of the model constrained designs were assessed by calculating the percentage of cases whose empirical Bayesian estimates (EBE) were as anticipated (i.e. $k > k_a$ in subjects with $CLcr_{CG} > transition\ point$ and $k < k_a$ in subjects with a $CLcr_{CG} < transition\ point$).

8.4.4. Assessing flip-flop in covariate modelling

A covariate analysis was conducted for each of the unconstrained models to assess the influence of flip-flop. In the unconstrained models parameterised using CL, V, k_a creatinine clearance calculated using the Cockcroft and Gault equation ($CLcr_{CG}$) was added as a covariate to (i) only k_a, (ii) only CL and (iii) both k_a and CL. Similarly, $CLcr_{CG}$ was added as a covariate to (i) k_a, (ii) k and (iii) both k_a and k in the unconstrained models parameterised using k, V, k_a. A significant reduction in the objective function value (i.e. 3.84 units Chi-square [χ^2], p≤0.05) was used to test for significance.

8.4.5. Model evaluation

A prediction corrected visual predictive check (pcVPC) was produced to assess whether a one-compartment model provided a reasonable fit to the data. Models were evaluated using standard diagnostic plots (described in Chapter 7 section 7.4.4.5.) and pcVPCs. For the pcVPCs 1000 datasets were simulated under the models and the 5th, 50th and 95th percentiles were plotted against the same percentiles from the original dataset. The standard diagnostic plots and pcVPCs were produced using R (version 3.5.3).

8.4.6. Modelling software

The models were implemented using NONMEM v7.3 (ICON Development Solutions, Ellicott City, MD, USA) using the first-order conditional estimation method with interaction for parameter estimation. Pre- and post- processing was conducted using Perl-speaks-NONMEM (version 4.9.0) and R (version 3.5.3). The convergence criterion for estimation was set to 3 significant digits.

8.5. Results

8.5.1. Data

The data analysed included a total of 426 plasma metformin concentrations available from 55 study participants. Cartesian and semi log plots of the plasma concentration time curves for the combined data are shown in Figure 8.1. Plots of each of the studies graphed separately is shown in Appendix A6.2.

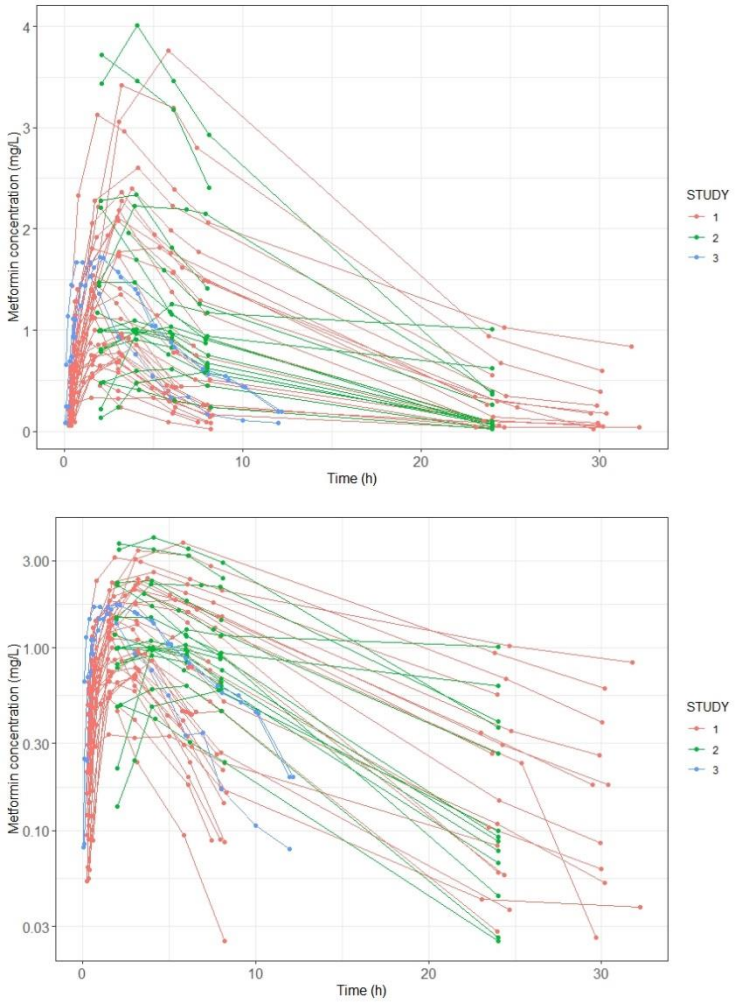


Figure 8.1 *Plasma metformin concentrations following a single oral dose of metformin on a Cartesian (top) and semi-log (bottom) plot. The dots represent single measures of plasma metformin concentrations and the lines link repeated measures data for a single study participant. The red, green and blue lines reresent the metformin studies conducted by Dunedin Public Hospital, Middlemore Hospital and Pentikainen et al, respectively.*

Seventy-seven plasma metformin concentrations collected from Middlemore Hospital were omitted from the analysis due to protocol violation (refer to Chapter 7 section 7.5.1. for additional details). Overall, eight percent of the plasma concentrations were below the quantification limit and were accounted for using the M6 method described by Beal [402].

Seventeen study participants had normal renal function (i.e. chronic kidney disease (CKD) category 1), and, 7, 16, 14 and 1 participant(s) had CKD 2, 3, 4 and 5, respectively. Refer to Chapter 1 section 1.3.3. for more information about CKD categories. A histogram of the range of renal functions in the study population is shown in Figure 8.2.

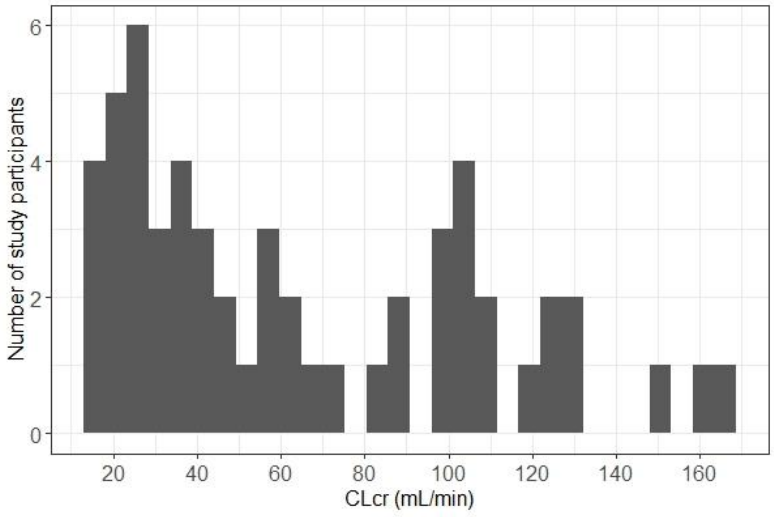


Figure 8.2 *Histogram of the range of renal function represented by creatinine clearance in the combined dataset*

8.5.2. Models exploring flip-flop

Sixteen models with different constrained designs, parameterisations and initial estimates were run with and without IV metformin concentration data. In all cases a one-compartment model with first-order input and output provided a reasonable description of the data. A pcVPC of the base model and goodness of fit plots for the sixteen models are presented in Appendix A6.3. Parameter estimates for the models run with concentration data following PO administration only and those following PO and IV administration are shown in Table 8.4 and Table 8.5, respectively. Examples of the NONMEM control files for

the unconstrained, partially constrained and fully constrained models parameterised using CL, V, k_a are presented in Appendix A6.4.

The parameter estimate for clearance was similar within each model parameterisation (i.e. CL, V, k_a and k, V, k_a) across the unconstrained models. This is shown in Table 8.4 and 8.5 where the model estimates for clearance were approximately 24 L/h under the CL, V, k_aparameterisation, whilst the estimates of clearance determined by taking the product of k and V in the models parameterised using k, V, k_a also appear to be approximately the same with a calculated value of approximately 44 L/h. The results from both tables suggest that the parameter estimates for clearance in the unconstrained models were not affected by the initial estimates used for k and k_a. It is worth noting that the estimates of clearance are similar across the partially and fully constrained designs. However, it appears that the remaining estimated parameters - k, V and k_a- were dissimilar across the different model designs.

A comparison of the variance of the EBEs (var_η) and their relative estimated variance (Ω) is shown in Table 8.6 (models run with PO data only) and Table 8.7 (models run with PO and IV data). From both tables there is evidence of Ω shrinkage (i.e. $var_\eta > \Omega$) and Ω inflation ($var_\eta < \Omega$). It is only possible to describe the relative characteristics of Ω and var_η and, it is possible that Ω shrinkage may be due to η inflation. It is assumed that η follows a normal distribution. However, in the setting of flip-flop, it is possible that some subjects are estimated to be in 'flip' whilst others in are in 'flop' (i.e. some subjects are estimated to exhibit absorption mediated elimination whilst others exhibit elimination mediated elimination). This may lead to a bimodal distribution for η, hence violating the assumption of normality on which Ω is predicated and causing Ω to be significantly inflated.

The variance for k_a appears to be larger under the constrained designs, and even more so under the fully constrained design (as shown in Table 8.6 and Table 8.7). This is shown in the Table 8.6 and Table 8.7 where the variance for k_a in the unconstrained models ranged between 0.08 to 0.43, whilst the variance for k_a in the partially and fully constrained models range from 0.44 to 0.81 and 0.21 to

2.34, respectively. In the fully constrained design the variance for k_a is likely to be larger as the constrained design forces the value for k_a to either be in a 'flip' or 'flop' state based on the relative relationship between a study participants' creatinine clearance and the transition point. Consequently, due to flip-flop, the distribution of Ω may have high skewness (i.e. heavy tail) and thus violate the normality assumption that predicates the use of the variance (Ω) to be a descriptor of dispersion. This can be seen in the histogram of the EBEs for k_a from the fully constrained models presented in Figure 8.3.

Table 8.4 *Parameter estimates for models developed using metformin concentrations obtained following orally administered metformin*

Parameters	Model description							
	Unconstrained				Partially constrained		Fully constrained	
	CL, V, k_a		k, V, k_a		CL, V, k_a	k, V, k_a	CL, V, k_a	k, V, k_a
	Rank order of model initial estimates used for k and k_a							
	$k < k_a$	$k > k_a$	$k < k_a$	$k > k_a$				
θ_{CL}	24.03	27.27			44.64		42.26	
θ_k			0.17	0.57				
$\theta_{k(SMALL)}$						0.79		1.48
$\theta_{k(BIG)}$						0.43		7.14E-5
θ_V	134.44	67.00	259.78	74.44	157.40	133.42	172.92	172.06
θ_{k_a}	0.54	0.23	0.61	0.17		0.29		0.38
$\theta_{k_a(SMALL)}$					1.32E-4		9.97E-6	
$\theta_{k_a(BIG)}$					0.360		0.694	
Between subject variability								
ω_{CL} (CV%)	102.1	85.6			104.4		113.2	
ω_k (CV%)			78.0	78.3		36.5		127.4
ω_V (CV%)	46.5	102.4	53.5	115.1	50.5	68.6	43.9	40.2
ω_{k_a} (CV%)	68.6	50.6	50.7	61.2	100	101.9	237.1	51.7
$corr_{CL,V}$	0.797	0.519			0.565		0.795	
$corr_{CL,k_a}$	0.078	0.260			0.185		-0.707	
$corr_{k,V}$			0.009	-0.498		-0.014		-0.100
$corr_{k,k_a}$			-0.428	-0.251		-0.489		0.891
$corr_{V,k_a}$	0.447	0.900	0.637	0.865	0.900	0.879	0.052	0.362
Residual error								
σ_{add}	0.033	0.031	0.018	0.011	0.018	0.046	0.049	0.116
σ_{prop}	0.264	0.251	0.263	0.265	0.258	0.222	0.219	0.098
OFV	-610.78	-606.58	-618.84	-619.00	-620.79	-623.84	-607.18	-608.96

θ_{CL} is the mean population value for clearance (L/h). θ_K is the mean population value for the elimination rate constant (h^{-1}). $\theta_{k(SMALL)}$ is an adjusting factor used to constrain $k<k_a$ in the partially and fully constrained models. $\theta_{k(BIG)}$ is an adjusting factor used to constrain $k>k_a$ in the partially and fully constrained models. θ_V is the mean population value for the volume of distribution (L). θ_{k_a} is the mean population value for the absorption rate constant (h^{-1}). $\theta_{k_a(SMALL)}$ is an adjusting factor used to constrain $k>k_a$ in the partially and fully constrained models.

$\theta_{k_a(BIG)}$ is an adjusting factor used to constrain $k < k_a$ in the partially and fully constrained models. ω_{CL} is between subject variability for clearance, ω_k is between subject variability for the elimination rate constant. ω_V is between subject variability for the volume of distribution. ω_{k_a} is between subject variability for the absorption rate constant. σ_{add} is additive residual error (mg/L). σ_{prop} is proportional residual error (CV%). $corr$ stands for correlation. OFV stands for objective function value.

Table 8.5 *Parameter estimates for models developed using metformin concentrations obtained following oral and intravenous metformin*

Parameters	Model description							
	Unconstrained				Partially constrained		Fully constrained	
	CL, V, k_a		k, V, k_a		CL, V, k_a	k, V, k_a	CL, V, k_a	k, V, k_a
	Rank order of model initial estimates used for k and k_a							
	$k < k_a$	$k > k_a$	$k < k_a$	$k > k_a$				
θ_{CL}	24.37	24.17			42.84		42.19	
θ_k			0.19	0.65				
$\theta_{k(SMALL)}$						0.47		9.61E-5
$\theta_{k(BIG)}$						1.50		2.46
θ_V	128.01	37.27	227.04	66.79	148.62	104.11	172.96	83.64
θ_{k_a}	0.54	0.16	0.51	0.16		0.24		0.19
$\theta_{k_a(SMALL)}$					9.95E-5		5.13E-6	
$\theta_{k_a(BIG)}$					0.22		0.51	
Between subject variability								
ω_{CL} (CV%)	100.4	98.2			101.6		100	
ω_k (CV%)			98.6	10.5		39.1		97.9
ω_V (CV%)	68.3	102.4	86.0	109.7	113.7	108.8	63.5	79.2
ω_{k_a} (CV%)	50.9	72.8	75.2	73.4	111.4	99.9	569.6	59.1
$corr_{CL,V}$	0.372	0.989			0.537		0.712	
$corr_{CL,k_a}$	-0.038	0.835			0.340		-0.147	
$corr_{k,V}$			-0.426	-0.558		-0.571		-0.750
$corr_{k,k_a}$			-0.567	-0.442		-0.697		-0.998
$corr_{V,k_a}$	0.637	0.828	0.737	0.836	0.820	0.897	0.590	0.790
Residual error								
σ_{add}	0.331	0.025	0.020	0.022	0.024	0.023	0.014	0.022
σ_{prop}	0.027	0.342	0.379	0.384	0.371	0.377	0.453	0.395
OFV	-507.92	-524.40	-416.76	-449.68	-410.73	-446.57	-303.87	-434.13

θ_{CL} is the mean population value for clearance (L/h). θ_K is the mean population value for the elimination rate constant (h^{-1}). $\theta_{k(SMALL)}$ is an adjusting factor used to constrain $k<k_a$ in the partially and fully constrained models. $\theta_{k(BIG)}$ is an adjusting factor used to constrain $k>k_a$ in the partially and fully constrained models. θ_V is the mean population value for the volume of distribution (L). θ_{k_a} is the mean population value for the absorption rate constant (h^{-1}). $\theta_{k_a(SMALL)}$ is an adjusting factor used to constrain $k>k_a$ in the partially and fully constrained models.

$\theta_{k_a(BIG)}$ is an adjusting factor used to constrain $k < k_a$ in the partially and fully constrained models. ω_{CL} is between subject variability for clearance, ω_k is between subject variability for the elimination rate constant. ω_V is between subject variability for the volume of distribution. ω_{k_a} is between subject variability for the absorption rate constant. σ_{add} is additive residual error (mg/L). σ_{prop} is proportional residual error (CV%). $corr$ stands for correlation. OFV stands for objective function value.

Table 8.6 *Variance of empirical Bayesian estimates and their relative estimated variance values for models developed using metformin concentrations following oral metformin administration only*

Parameters	**Model constraint design**															
	Unconstrained								Partially constrained				Fully constrained			
	CL, V, k_a				k, V, k_a				CL, V, k_a		k, V, k_a		CL, V, k_a		k, V, k_a	
	Rank order of model initial estimates used for k and k_a															
	$k < k_a$		$k > k_a$		$k < k_a$		$k > k_a$									
	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω
CL	0.70	0.71	0.70	0.55					0.71	0.74			0.71	0.73		
k					0.36	0.48	0.30	0.48			0.24	0.13			0.83	0.96
V	0.15	0.20	0.94	0.72	0.19	0.25	0.64	0.84	0.19	0.23	0.25	0.39	0.18	0.21	0.14	0.15
k_a	0.26	0.39	0.30	0.23	0.10	0.23	0.24	0.32	0.57	0.69	0.60	0.71	1.36	1.63	0.21	0.24

var_η: represents the variance of the empirical Bayes estimates, Ω: represents the model estimated variance

Table 8.7 *Variance of empirical Bayesian estimates and their relative estimated variance values for models developed using metformin concentrations following oral and intravenous administered metformin*

Parameters	**Model constraint design**															
	Unconstrained								Partially constrained				Fully constrained			
	CL, V, k_a				k, V, k_a				CL, V, k_a		k, V, k_a		CL, V, k_a		k, V, k_a	
	Rank order of model initial estimates used for k and k_a															
	$k < k_a$		$k > k_a$		$k < k_a$		$k > k_a$									
	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω	var_η	Ω
CL	0.65	0.70	0.64	0.68					0.67	0.71			0.66	0.71		
k					0.45	0.68	0.002	0.01			0.05	0.14			0.56	0.67
V	0.28	0.38	0.66	0.72	0.43	0.55	0.73	0.79	0.40	0.83	0.63	0.78	0.37	0.78	0.44	0.49
k_a	0.08	0.23	0.37	0.43	0.20	0.45	0.37	0.43	0.44	0.81	0.53	0.69	1.24	2.34	0.25	0.3

var_η: represents the variance of the empirical Bayes estimates, Ω: represents the model estimated variance

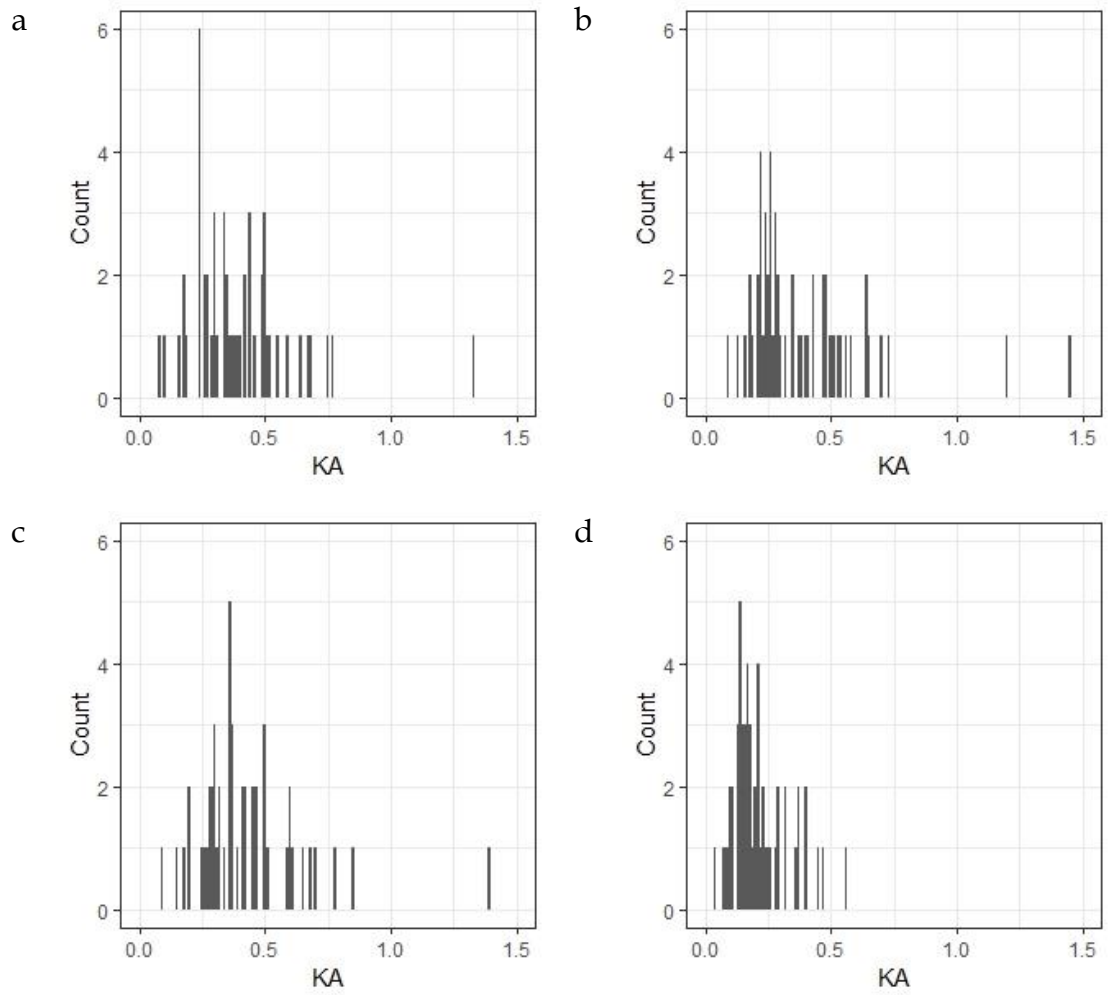


Figure 8.3 *Histogram of empirical Bayesian estimates for k_a from the fully constrained models.* ***a*** *is the fully constrained model parameterised using CL, V, k_a run using oral data.* ***b*** *is the fully constrained model parameterised using CL, V, k_a run using oral and intravenous data.* ***c*** *is the fully constrained model parameterised using k, V, k_a run using oral data.* ***d*** *is the fully constrained model parameterised using k, V, k_a run using oral and intravenous data*

8.5.2.1. Determining a flip-flop transition point

A flip-flop transition point could not be estimated from the data available for analysis. A sensitivity analysis was performed by fixing the flip-flop transition point to $CLcr_{CG}$ values of 10 to 100 mL/min in 10 mL/min increments, but the modelling results were not sensitive to the changes. Instead, a flip-flop transition point of $CLcr_{CG}$ 30 mL/min was selected based on theoretical considerations (discussed in Appendix A6.5).

8.5.2.2. Performance of constrained designs

Initial estimates were found to influence the model parameter estimates in the unconstrained models. This was demonstrated by the model parameter estimates sharing the same rank order as the model initial estimates used. The percentage of subjects with k values less than or greater than k_a when using different rank orders for k and k_a as the initial estimates is shown in Table 8.8.

Table 8.8 *Percentage of subjects with empirical Bayes estimates of k less than or greater than k_a when using different initial estimates of k and k_a in the unconstrained model*

Model description		$\boldsymbol{k > k_a}$ **(% of subjects)**	$\boldsymbol{k < k_a}$ **(% of subjects)**
Parameterisation	Rank order of initial estimates		
Models run using oral metformin concentration data only			
CL, V, k_a	$k < k_a$	5.8%	94.2%
	$k > k_a$	80.8%	19.2%
V, k_a, k	$k < k_a$	0.0%	100.0%
	$k > k_a$	96.2%	3.8%
Models run using oral and intravenous concentration data			
CL, V, k_a	$k < k_a$	10.9%	89.0%
	$k > k_a$	98.2%	1.8%
V, k_a, k	$k < k_a$	7.3%	92.7%
	$k > k_a$	98.2%	1.8%

A summary of the performance of the parameter constrained designs is shown in Table 8.9. The fully constrained model performed the best at constraining the rank order of model parameters. This was demonstrated by the rank order of EBEs (as shown in Table 8.9) always being as theoretically anticipated when using the fully constrained model.

Table 8.9 *Percentage of cases with output EBEs theoretically anticipated based on the transition point (creatinine clearance of 30 mL/min)*

Models description		CLcr >30 mL/min $k > k_a$ (%true)		CLcr ≤30 mL/min $k < k_a$ (% true)	
Constrain design	Rank order of initial estimates	*PO*	*IV & PO*	*PO*	*IV & PO*
Unconstrained					
CL, V, k_a	$k < k_a$	8.1	15	100	100
	$k > k_a$	75.7	97.5	6.7	0
V, k_a, k	$k < k_a$	0	10	100	100
	$k > k_a$	94.6	97.5	0	0
Partially constrained					
CL, V, k_a		48.6	62.5	86.7	93.3
V, k_a, k		59.5	95	93.3	86.7
Fully constrained					
CL, V, k_a		100	100	100	100
V, k_a, k		100	100	100	100

8.5.2.3. Accounting for important covariates

In the unconstrained models parameterised using CL, V, k_a the univariate addition of $CLcr_{CG}$ to either CL or k_a was found to be significant on each parameter – with the exception of the addition of $CLcr_{CG}$ to k_a in the unconstrained model run with PO and IV data with initial estimates of $k > k_a$. In the unconstrained models parameterised using CL, V, k_a the greatest reduction in objective function value followed the univariate addition of $CLcr_{CG}$ as a covariate on CL.

The univariate addition of $CLcr_{CG}$ to k was found significant in all unconstrained models parameterised using k, V, k_a. However, the univariate addition of $CLcr_{CG}$ to k_a was only found significant in the unconstrained model parameterised using k, V, k_a with initial estimates of $k < k_a$ run with PO and IV data. As was found in the unconstrained models parameterised using CL, V, k_a, the greatest reduction in objective function value in the unconstrained models parameterised using k, V, k_a followed the univariate addition of $CLcr_{CG}$ to k.

Furthermore, in the unconstrained models, the order $CLcr_{CG}$was added as a covariate on CL (or k) or k_a and then both CL (or k) and k_a were found to influence model findings. When $CLcr_{CG}$was first added as a covariate on CL (or k), the further addition of $CLcr_{CG}$ on k_a did not result in any further improvement in global fit (shown in Table 8.10). However, when $CLcr_{CG}$was first added as a covariate on k_a, the further addition of $CLcr_{CG}$on CL improved the global fit.

Table 8.10 *Objective function value for unconstrained base and covariate models*

Model description			**OFV**	
Data	Rank order of initial estimates	Model	CL, V, k_a	V, k_a, k
PO	$k < k_a$	Base model	-610.777	-618.839
		$CLcr_{CG}$ on k_a	-619.756	-613.612
		$CLcr_{CG}$ on CL (or k)	-664.627	-664.544
		$CLcr_{CG}$ on k_a and CL (or k)	-664.576	-668.277
	$k > k_a$	Base model	-606.575	-619.003
		$CLcr_{CG}$ on k_a	-618.988	-612.479
		$CLcr_{CG}$ on CL (or k)	-664.076	-658.409
		$CLcr_{CG}$ on k_a and CL (or k)	-665.584	-657.125
PO & IV	$k < k_a$	Base model	-507.917	-416.763
		$CLcr_{CG}$ on k_a	-524.710	-450.193
		$CLcr_{CG}$ on CL (or k)	-566.119	-472.453
		$CLcr_{CG}$ on k_a and CL (or k)	-565.131	-470.012
	$k > k_a$	Base model	-524.404	-449.678
		$CLcr_{CG}$ on k_a	-524.710	-450.193
		$CLcr_{CG}$ on CL (or k)	-566.119	-473.763
		$CLcr_{CG}$ on k_a and CL (or k)	-560.991	-471.527

OFV represents objective function value

8.6. Discussion

In this study the influence of issues associated with flip-flop in population pharmacokinetic modelling was explored and an approach to solve flip-flop was proposed. The following five inferences can be made from this work;

Inference 1. Clearance is invariant to flip-flop

Clearance is not affected by flip-flop. For instance, in a one-compartment model with first-order input and output parameterised using CL, V, k_a there are two sets of permutations of parameter values that will provide the same input-output relationship. In both permutations clearance remains the same and is independent of other parameters (shown in Table 8.1) and also it is seen theoretically when permuting the parameter values for the CL , V , k_a parameterisation.

The product of k and V (under the k, V, k_a) parameterisation also remains unaffected by flip-flop although their respective values are affected.

Inference 2. Unconstrained models require a covariate on clearance

If an unconstrained model is used to model a drug with flip-flop pharmacokinetics, a covariate on clearance describing the elimination process is required if there is no paired oral and intravenous data. A strong covariate relationship should be able address issues of local identifiability due to flip-flop and hence, stabilise the model. Note that inference 2 would not apply to a covariate that also influences other processes, such as absorption. This is because the covariate would be confounded by the absorption and elimination processes and hence, would no longer be able to address issues of local identifiability.

Inference 3. A fully constrained design is needed if there is no IV data and no covariate on clearance

A fully constrained model should be used to model drugs with flip-flop pharmacokinetics if there is no paired IV and PO data and, no covariate available

to describe clearance. The fully constrained design should be used if the volume of distribution and absorption rate constant are parameters of interest.

Inference 4. The k, V, ka parameterisation should not be used when investigating flip-flop

The k, V, k_a parameterisation should not be used to model drugs with flip-flop pharmacokinetics as all parameters in this parameterisation set are influenced by flip-flop (shown in Table 8.1). However, it is worth noting that the product of k and V (i.e. CL) is not affected. Instead, the CL, V, k_a parameterisation should be used when dealing with drugs exhibiting flip-flop pharmacokinetics.

Inference 5. Flip-flop may result in spurious relationships being found if important covariates are not accounted for

A relationship was identified between $CLcr_{CG}$ and k_a for the unconstrained model when the relationship $CLcr_{CG}$ and CL (or k) had not been accounted for. This relationship between $CLcr_{CG}$and k_a is likely to be spurious.

In Chapter 7 of this thesis, a population pharmacokinetic model for metformin was developed. The findings from this chapter indicate that the developed model would not be affected by flip-flop. This is for two reasons. The first is because the model was parameterised using CL, V, k_a; hence, the estimate of CL would not be affected and can reasonably be used in simulations exploring the renal dosing of metformin. Secondly, the developed model was an unconstrained model but had a covariate (i.e. $CLcr_{CG}$) on clearance, and hence, would stabilise the model into 'flip' or 'flop' by preventing it from 'flip-flop'.

There are very few published compartmental population pharmacokinetic models where flip-flop pharmacokinetics were observed that also explain how the data were analysed. In these studies flip-flop pharmacokinetics was commonly exhibited in drugs that were either in a modified drug release formulation or had been administered via a zero order process (e.g. continuous infusion) [408-410]. The methodology used to solve the issues of local

identifiability due to flip-flop pharmacokinetics in population pharmacokinetic modelling ranged from methods that simply ignored flip-flop to studies that had applied constraints in the structural model [408-410]. In addition, only one study was identified that explicitly stated how constraints were applied to maintain a certain rank order amongst model parameters [408].

8.7. Limitations

An important limitation for extrapolation of these study results is the dependence of the inferences on metformin data. While it is reasonable that some of the concepts could be generalised to other drugs that are predicted to exhibit 'flip-flop' we cannot state the degree of certainty or power of such analyses. Future work would need to be performed to explore the truth behind these findings, such as a stochastic simulation estimation method.

8.8. Conclusions

In this study, the influence of the 'flip-flop' problem in modelling was explored and approaches to solve the 'flip-flop' problem in population pharmacokinetic modelling were proposed. In the absence of IV data a fully constrained design is needed. The CL, V, k_a parameterisation should be used when dealing with drugs with flip-flop pharmacokinetics.

Chapter 9: Discussion

9.1. Synopsis of this thesis

In this thesis different approaches were used to explore the safe use of metformin and to create a renal dosing guideline to mitigate the risk of lactic acidosis.

In Chapter 2 a systematic literature review was performed to identify published case reports of metformin associated lactic acidosis (MALA) and the association between metformin therapy and lactic acidosis was formally evaluated in the published case reports using the World Health Organisation-Uppsala Monitoring Centre system for case causality assessment (WHO-UMC) and the Naranjo Adverse Drug Reaction (ADR) Probability Scale [106, 107]. As part of this work a database of MALA case reports identified from the systematic literature review was developed and is available to other researchers in the figshare repository; https://figshare.com/s/4a1129faa048322cfa0c.

In Chapter 3 a subgroup analysis was performed to explore the relationship between metformin dose, plasma metformin concentrations and lactic acidosis in published MALA cases with chronic renal impairment to assess whether metformin therapy could safely be used in patients with renal impairment.

In Chapter 4 a pragmatic literature search was performed to clarify the upper limit of safety for metformin. This was complimented by a quantitative analysis that identified an upper limit of safety for metformin by fitting a concentration response curve to plasma metformin and lactate concentrations.

In Chapter 5 a pharmacokinetic analysis was performed to explore the pharmacokinetics of metformin in renal impairment to inform dosing and, from which an empirical renal dosing guideline for metformin was developed.

In Chapter 6 simulations were performed to explore the concentration time profile of metformin in patients with renal impairment.

In Chapter 7 a population pharmacokinetic model for metformin was developed and evaluated. Simulations were performed from the model to assess the safety of the dose recommendations from the empirical renal dosing guideline and the New Zealand Formulary (NZF)[24].

Lastly, in Chapter 8, an analysis was performed to explore the influence of flip-flop pharmacokinetics in population pharmacokinetic models.

9.2. Synopsis of the thesis findings

9.2.1. Metformin therapy and lactic acidosis

The causal association between therapeutic doses of metformin and lactic acidosis is well-debated. In this thesis, metformin was found to play only a possible role in the development of lactic acidosis at therapeutic doses in the published cases of MALA according to analyses using the WHO-UMC and Naranjo causality assessments [106, 107]. Almost all identified cases presented with risk factors, other than metformin, that could on their own have led to lactic acidosis. These findings support the growing argument in literature that therapeutic doses of metformin may not be a primary cause of lactic acidosis. Rather, that metformin may play a contributory role in the development of lactic acidosis alongside other risk factors. Published work by other authors have collectively reported that the majority of MALA cases had presented with other risk factors for lactic acidosis that could better explain the development of lactic acidosis (e.g. septic shock) [100, 213, 260], and thus, has led to the notion that the presence of metformin in these cases is coincidental and that it may merely be an innocent bystander. Overall, these results support the notion that metformin can safely be used in patients at therapeutic doses.

9.2.1.1. Metformin therapy and lactic acidosis in renal impairment

In a subgroup analysis looking at MALA cases with a history of chronic renal impairment, metformin was similarly found to play only a possible role in the development of lactic acidosis when using the WHO-UMC and Naranjo causality assessments. Most of the cases with a history of chronic renal impairment presented with acute renal failure on admission, which confounded the relationship between metformin dose and plasma concentrations. However, it is worth noting that the prescribed metformin dose was found to exceed published renal dosing guidelines in over 60% of cases with an eGFR less than

60 mL/min by a median of 1000 mg/day. However, despite this, simulations performed using a published pharmacokinetic model for metformin predicted that pre-admission plasma metformin concentrations measured pre-dose were not expected to exceed the proposed upper limit of the therapeutic range of 5 mg/L in most cases. These findings suggest that chronic renal impairment is unlikely to be the primary cause of the highly elevated plasma metformin concentrations seen in MALA cases presenting with concomitant acute renal failure. Here, these findings reinforce the idea that metformin can be used safely for the treatment of type 2 diabetes mellitus in patients with or without chronic renal impairment provided that plasma metformin concentrations are maintained within a safe therapeutic range.

9.2.2. Metformin dosing in renal impairment

9.2.2.1. An upper limit of safety for metformin

To date, the upper limit of safety for metformin is not well established. In a pragmatic literature search no studies were identified to have reported an upper limit of safety for metformin that was defined by means of a formal exposure response analysis. Historically, a plasma metformin concentration of 5 mg/L has been nominated as the upper limit of safety for metformin. However, the origin of this proposed upper safety limit for metformin is unclear and has been questioned [45, 386]. Findings from an exploration of the concentration response relationship between plasma metformin and lactate concentrations suggested that plasma metformin concentrations greater than 4.5 mg/L were associated with severe hyperlactatemia – a value in the same ballpark as the nominated upper limit of safety for metformin of 5 mg/L.

Overall, the proposed upper limit of 4.5 mg/L provides a metric that can be used to guide the safe dosing of metformin, particularly in renal impairment. The findings suggest that metformin doses should be adjusted to maintain plasma metformin concentrations below 4.5 mg/L to mitigate the risk of lactic acidosis. The proposed upper limit of safety for metformin was used in Chapter 7 to assess the safety of renal dosing guidelines for metformin.

9.2.2.2. Pharmacokinetics of metformin in renal impairment

The pharmacokinetic profile of metformin was found to be influenced by renal impairment. Patients with poorer renal function were found to have lower apparent and renal clearances for metformin. In a correlation analysis, the decrease in the apparent and renal clearance for metformin in patients with varying levels of renal impairment could reasonably be predicted by estimates of renal function determined using the Cockcroft and Gault, 4-variable Modification Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equations [80, 82, 83]. These findings highlight the idea that metformin can continued to be used in renal impairment provided doses are reduced in proportion to renal function to maintain plasma concentration within a safe therapeutic range. In addition, the results suggest that the Cockcroft and Gault, 4-variable MDRD and CKD-Epi equations can be used to interchangeably to inform the renal dosing of metformin [80, 82, 83]. Furthermore, a simulation study found that a reduction in clearance and/or flip-flop kinetics could recreate the concentration time profile of metformin seen in patients with renal impairment.

A population pharmacokinetic model for metformin was developed in healthy subjects and patients with severe renal impairment. The covariate analysis found that renal function and total body weight could describe patient differences in metformin apparent clearance and volume of distribution, respectively. The addition of creatinine clearance as a covariate on the apparent clearance of metformin resulted in a reduction in between subject variability from 105% to 46%. Similarly, the addition of total body weight as a covariate on the central compartment volume was found to reduce between subject variability from 37% to 30%. Here, it is worth noting, that the model structure, parameter estimates and covariates of the final developed population pharmacokinetic model for metformin were similar to published models of metformin developed in subjects with and without renal impairment [15, 370].

9.2.2.3. Dosing metformin in patients with stable chronic renal impairment

An empirical renal dosing guideline for metformin using the Cockcroft and Gault, 4-variable MDRD and CKD-Epi equations was developed, and, the maintenance dose range was determined for patients in different CKD category groups. In simulations performed using the developed population pharmacokinetic model under the dose predictions from the empirical equations and the NZF guidelines it was predicted that plasma concentrations would not exceed the proposed upper limit of safety of 4.5 mg/L.

To date, there are a number of published renal dosing guidelines for metformin, though few appear to be evidence based. Here, it is worth noting that the published metformin renal dosing guidelines recommended the use of doses around the same magnitude as the doses predicted from the empirical equations. This suggests, that on this basis, it can be inferred that the plasma metformin concentrations in the published renal dosing guidelines are also unlikely to exceed the upper limit of safety of 4.5 mg/L, and thus, furthermore suggests that the published renal dosing guidelines do provide reasonable dose recommendations that will maintain plasma concentrations within a safe range.

9.2.3. Flip-flop pharmacokinetics in population pharmacokinetic models

The influence of flip-flop pharmacokinetics in population pharmacokinetic models was explored and an approach to solve problems due to flip-flop was proposed. In the absence of paired intravenous (IV) and oral (PO) concentration data, it was found that a covariate on clearance is required in an unconstrained model to avoid issues of local identifiability due to flip-flop. However, in the absence of paired IV and PO data as well as a covariate on clearance, a fully constrained model is required. Published population pharmacokinetic models dealing with flip-flop commonly addressed flip-flop in the context of compounds delivered using controlled release dosage forms (i.e. the elimination rate constant (k) is larger than the absorption rate constant (k_a)) [408]. However, in this thesis, novel approaches to address issues of local identifiability when $k < k_a$ and $k < k_a$ can occur in a single dataset are proposed.

9.3. *Thesis limitations*

The findings presented in this thesis should be interpreted in light of the following limitations.

An important limitation of the causality assessment work was that the Naranjo Adverse Drug Reaction Probability Scale was not designed to assess causality retrospectively. Rather, the Naranjo causality assessment was designed for use in the clinic where clinicians could request for laboratory tests to be performed. Therefore, the Naranjo causality assessment was used beyond its original intention. Despite this, the results from the Naranjo causality assessment supported the results from the WHO-UMC causality assessment which was designed to assess causality using retrospective data presented in case reports.

In addition, the association between metformin therapy and lactic acidosis was assessed using retrospective data from case reports. From an epidemiological stance, case reports are commonly recognised to be poor in quality and incomplete in terms of data reported. Furthermore, due to the retrospective nature of the data presented in the case reports the causation of metformin in the development of lactic acidosis could not be proven [411]. Rather the published case reports could only be used to provide evidence for and to show the strength of an association between metformin therapy and lactic acidosis [411]. Only by means of a randomised controlled trial could the causation of metformin therapy in the development of lactic acidosis be established.

The use of the empirical renal dosing equations should viewed in light of the following limitations. Previous literature has suggested that the bioavailability of metformin decreases with increasing dose [27, 31]. However, the data from which metformin concentrations were sampled from came from study participants that were all given a single oral 500 mg dose of metformin. However, here, the dose predictions from the empirical equation assumes that bioavailability is the same between the different doses. In addition, the developed empirical equation only had a single covariate (i.e. renal function) to

describe patient variability. Though, it is possible that there are other patient characteristics that may also help inform dosing.

Although it was not addressed in this thesis it is important to consider the impact of obesity on the estimation of renal function. In obese patients, the impact of obesity may result in overestimated values for clearance and renal function, which may lead to higher doses of a drug being prescribed than truly required. This is particularly evident in the Cockcroft and Gault equation (shown in Equation 1.1) as the formula uses weight to predict creatinine clearance. Hence, in obese patients, it is commonly recommended that an alternate body size descriptor is used (e.g. lean body weight) instead of total body weight when using a renal function estimating equation that relies on body weight.

9.4. Future prospects

In Chapter 2 and 3 the use of the WHO-UMC and Naranjo causality assessments may have produced biased results in this study. It was noted that both the causality assessments frequently assigned causality to the "possible" category – a finding raised by other authors in the literature [362, 363]. Future work investigating the reason for the noted bias in the causality criteria could guide areas where the current published causality assessments could be modified and improved.

In this thesis metformin doses were assessed for safety by predicting whether plasma metformin concentrations would exceed the identified upper limit of safety. However, as this thesis did not explore the pharmacodynamics of metformin, it is not known whether the doses recommended are efficacious. A study is needed to define the therapeutic range (i.e. both upper and lower limits for safety and efficacy) for metformin. The identification of a therapeutic range for metformin would help inform the dosing of metformin to provide a dose that is both safe and efficacious.

In Chapter 5 a dose banding approach was used to guide the renal dosing of metformin when using the empirical renal dosing guidelines. However, it is unclear whether patients at the upper end of a dose band cut-off are more

susceptible to treatment failure and whether patients at the lower end of the dose band cut-off are more susceptible to drug toxicity due to increased drug exposure. Future work to explore the impact of being near the renal function cut-offs on treatment failure and drug toxicity would provide resolution on whether dose banding is an appropriate method to guide dosing or whether other methods are warranted.

Future work could involve the development of a model-based dose banding guideline from the developed population pharmacokinetic model in Chapter 7. Here, the empirical renal dosing guideline and model-based dosing guidelines could be compared using a clinical trial simulation design involving stochastic simulations to identify the best dosing approach. This work could then be followed by a randomised controlled trial to assess the proposed renal dosing guideline in patients with type 2 diabetes mellitus patients.

In Chapter 8 the influence of flip-flop pharmacokinetics in population pharmacokinetic models was explored using metformin data. Thus, the findings in this setting are true for metformin but not all concepts are necessarily generalisable to other drugs. Here, a future stochastic simulation estimation study to explore the influence of flip-flop pharmacokinetics in population pharmacokinetic models could be conducted to evaluate these findings and their relevance to other drugs.

9.5. Conclusion

Metformin was found to play only a possible role in the majority of published MALA cases with and without renal impairment at therapeutic doses. Almost all cases presented with risk factors other than metformin that could on their own have caused to lactic acidosis.

The concentration-response relationship between plasma metformin and lactate concentrations was explored across a wide range of metformin doses. Plasma metformin concentrations >4.5 mg/L were found to be associated with an increased risk of severe hyperlactatemia. This suggests that dose adjustment

to maintain plasma metformin concentrations to <4.5 mg/L should mitigate the risk of lactic acidosis.

A pharmacokinetic analysis was performed to explore the exposure metrics of metformin in patients with renal impairment. The results suggest that metformin exposure in renal impairment is influenced by a reduction in clearance and/or flip-flop. The creatinine-based equations for estimating renal function were found to provide reasonable predictions of the renal clearance of metformin and can be used interchangeably to inform the dosing.

An empirical renal dosing guideline was developed for metformin. A population pharmacokinetic model for metformin was developed and was used to assess the safety of the developed empirical renal dosing guideline for metformin and the current NZF renal dosing guidelines. Based on the simulation results plasma concentrations of metformin were predicted to not exceed the upper safety limit of 4.5 mg/L. This suggests that both the empirical and NZF renal dosing guidelines can be used safely in patients with renal impairment.

The influence of the flip-flop in modelling was explored and approaches to solve the 'flip-flop' problem in population pharmacokinetic modelling were proposed.

Appendix 1: Appendices to Chapter 2

A1.1. Systematic literature review search strategy

In this section the search strategies used to perform the systematic literature review are presented. The search strategies are divided into the following two sections:

- Appendix A1.1.1 Static search strategy
- Appendix A1.1.2 Learning based approach search strategy

A1.1.1. Static search strategy

The static search strategies conducted in Ovid EMBASE, Ovid MEDLINE, Google Scholar and SCOPUS are presented in Table A1.1, A1.2, A1.3 and A1.4, respectively.

Table A1.1 *Ovid EMBASE static search strategy*

Source	Search strategy
Ovid EMBASE *Covering period 1946 to July 2017*	1. exp Metformin/ or Biguanide derivative/ or Oral antidiabetic agent/ 2. exp biguanide 3. Antidiabetic agent/ 4. Antihyperglycemic.mp 5. 1 or 2 or 3 or 4 6. exp Lactic acidosis 7. exp Acidosis 8. exp Lactic acid 9. exp Hyperlactatemia 10. 6 or 7 or 8 or 9 11. Case report/ 12. exp Case study/ 13. Case.mp 14. 11 or 12 or 14 15. 5 and 10 and 14 Results: 1025 16. Limit 15 to (English language and human) Results: 835

Table A1.2 *Ovid MEDLINE static search strategy*

Source	Search strategy
Ovid MEDLINE *Covering period 1946 to July 2017*	1. exp Metformin 2. Biguanides 3. Antidiabetic agent.mp 4. Antihyperglycemic.mp 5. 1 or 2 or 3 or 4 6. exp Acidosis, Lactic/ 7. exp Acidosis/ 8. exp Lactic Acid/ 9. exp Hyperlactatemia/ 10. 6 or 7 or 8 or 9 11. exp Case reports/ 12. Case series.mp 13. Case.mp 14. 11 or 12 or 13 15. 5 and 10 and 14 Results: 306 16. Limit 15 to (English language and humans) Results: 219

Table A1.3 *Google Scholar static search strategy*

Source	Search strategy
Google Scholar *Search was conducted using the software Harzing's Publish and Perish*	***All of the words***: metformin, lactic acidosis, case, patient, lactate, pH ***Any of the words***: metformin, lactic acidosis ***The phrase***: metformin associated lactic acidosis Results: 765

Table A1.4 *SCOPUS static search strategy*

Source	Search strategy
SCOPUS	1. exp Metformin 2. Lactic acidosis 3. Case Results: 609 4. Limit 4 to (Human, Humans and English) Results: 447

A1.1.2. Learning based approach search strategy

Two searches using the learning based approach were conducted in Ovid MEDLINE. The first was a general search of metformin associated lactic acidosis case reports (Table A1.5) and the second was a search to identify case reports of metformin associated lactic acidosis following metformin overdose (Table A1.6).

Table A1.5 *General learning based approach search strategy*

Source	Iterations	Medical subject headings
Ovid MEDLINE *Covering period 1946 to July 2017*	1	1. exp Acidosis 2. exp Biguanides 3. exp Buformin 4. exp Diabetes Complications 5. exp Diabetes Mellitus 6. exp Hypoglycaemic Agents 7. exp Lactates 8. exp Metformin 9. Phenformin Results: 1473
	2	10. exp Diabetes Mellitus 11. exp Hypoglycaemic Agents 12. exp Metformin 13. exp Acidosis, Lactic 14. exp Cohort Studies 15. exp Hyperlactatemia 16. exp Case-control Studies 17. exp Pharmacovigilance 18. exp Drug-related side effects 19. exp Biguanides 20. exp Patients Results: 1670

Table A1.5 cont *General learning based approach search strategy*

Source	Iterations	Medical subject headings
		1 OR 2
		Results: 2557
		Limit to English language
		Results: 2128
		Limit to Humans
		Results: 2036

Table A1.6 *Overdose learning based approach search strategy*

Source	Iterations	Medical subject headings
Ovid MEDLINE *Covering period 1946 to July 2017*	1	1. exp Acidosis 2. exp Biguanides 3. exp Buformin 4. exp Diabetes Complications 5. exp Diabetes Mellitus 6. exp Hypoglycaemic Agents 7. exp Lactates 8. exp Metformin 9. exp Phenformin Results: 1473
	2	10. exp Diabetes Mellitus 11. exp Hypoglycaemic Agents 12. exp Metformin 13. exp Acidosis, Lactic 14. exp Lactates 15. exp Cohort Studies 16. exp Hyperlactatemia 17. exp Case-Control Studies 18. exp Inappropriate Prescribing 19. exp Adverse Drug Reaction Reporting Systems 20. exp Pharmacovigilance 21. exp Biguanides 22. exp Patients

Table A1.6 cont *Overdose learning based approach search strategy*

Source	Iterations	Medical subject headings
	3	1. exp Suicide 2. exp Drug Overdose 3. exp Drug Dosage Calculations Results: 1714 4. exp Hypoglycaemic Agents 5. exp Metformin 6. exp Case-Control Studies 7. exp Diabetes Mellitus 8. exp Acidosis, Lactic 9. exp Cohort Studies 10. exp Hyperlactatemia 11. exp Lactates 12. exp Drug Dosage Calculations 13. exp Biguanides 14. exp Drug Overdose 15. exp Inappropriate Prescribing 16. exp Patients 17. exp Adverse Drug Reaction Reporting Systems 18. exp Pharmacovigilance 19. exp Suicide 20. exp Medication Errors Results: 1766 1 or 2 or 3 Results: 2648 Limit to English language Results: 2217 Limit to Humans Results: 2122

A1.2. List of pre-existing risk factors for lactic acidosis

Presented in this section is a table of known risk factors for lactic acidosis.

Table A1.7 *List of risk factors for lactic acidosis*

Risk factors for lactic acidosis
Type A[a]
Anaerobic muscle activity
Seizures
Post-cardiac arrest
Regional tissue ischemia
Mesenteric ischemia
Limb ischemia
Burns
Trauma
Compartment syndrome
Necrotising soft tissue infections
Severe anaemia
Severe asthma
Severe hypoxemia
Shock
Cardiogenic
Distributive
Hypovolemic
Obstructive
Septic
Type B[a]
Type B_1 (lactic acidosis occurring in association with an underlying disease)
Diabetes mellitus
Liver dysfunction/failure
Malignancy
Sepsis
Pheochromocytoma
Thiamine deficiency
Renal failure
Systemic inflammatory response syndrome
Human immunodeficiency virus

Table A1.7 cont *List of risk factors for lactic acidosis*

Risk factors for lactic acidosis
Type B_2 (lactic acidosis due to drugs/toxins)
Alcohol
Ethanol[b, c]
Methanol[c]
Biguanides
Metformin
Phenformin
Carbon monoxide
Cocaine
Ethylene glycol
Salicylates[c]
Paracetamol
Epinephrine
Ritodrine
Cyanide
Nitroprusside
Isoniazid
Propylene glycol[c]
Linezolid
Nucleoside reverse transcriptase inhibitors
Propofol
$Beta_2$ agonist
Theophylline
Type B_3 (lactic acidosis due to inborn errors of metabolism)
Glucose-6-phosphatase deficiency (von Gierke's disease)
Fructose-1,6-diphosphatase deficiency
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase deficiency
Oxidative Phosphorylation defects

Table A1.7 cont *List of risk factors for lactic acidosis*

Risk factors for lactic acidosis
Miscellaneous
D-Lactic acidosis
Diabetic ketoacidosis
Hypoglycaemia
Mitochondrial disease

[a]The Cohen and Woods classification was used to categorise the risk factors into Type A (clinical evidence of tissue hypoxia) and Type B (no evidence of tissue hypoxia) lactic acidosis. [b]Ethanol is known to increase the risk of lactic acidosis when taken alongside other risk factors for lactic acidosis. [c]These drugs and/or toxins are recognised to cause lactic acidosis when taken in toxic concentrations.

A1.3. WHO-UMC system for standardised case causality assessment

The adapted WHO-UMC causality assessment annotated with lactic acidosis specific diagnostic criterion under each causality category is presented in Table A1.8.

Table A1.8 *Adapted WHO-UMC causality assessment*

Causality term	Assessment criteria

Certain	Event or laboratory test abnormality, with plausible time relationship to drug intake
	• *Involves metformin ingestion prior to lactic acidosis*
	Cannot be explained by disease or other drugs
	• *Patient presents with no independent comorbidities, concomitant medications or risk factors for lactic acidosis*[a]
	Response to withdrawal plausible (pharmacologically, pathologically)
	• *Metformin withdrawal results in resolution of lactic acidosis symptoms*
	Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon)
	• *Diagnosis of lactic acidosis:*
	  ○ *Lactate plasma concentration >5 mmol/L*
	  ○ *pH <7.35*
	Rechallenge satisfactory, if necessary
	• *Metformin rechallenge results in lactic acidosis*

Probably/ Likely	Event or laboratory test abnormality, with reasonable time relationship to drug intake
	• *Involves metformin ingestion prior to lactic acidosis*
	• *Diagnosis of lactic acidosis:*
	  ○ *Lactate plasma concentration >5 mmol/L*
	  ○ *pH <7.35*
	Unlikely to be attributed to disease or other drugs
	• *Patient presents with independent comorbidities, concomitant medications and other risk factors for lactic acidosis*[a] *that are unlikely to be a causative agent or none of these*
	Response to withdrawal clinically reasonable
	• *Metformin withdrawal results in resolution of lactic acidosis*
	Rechallenge not required

Table A1.8 cont *Adapted WHO-UMC causality assessment*

Causality term	Assessment criteria
Possible	Event or laboratory test abnormality, with reasonable time relationship to drug intake • *Involves metformin ingestion prior to lactic acidosis* • *Diagnosis of lactic acidosis:* o *Lactate plasma concentration >5 mmol/L* o *pH <7.35* Could also be explained by disease or other drugs • *Patient presents with comorbidities and/or concomitant medications that are risk factors for lactic acidosis[a] and could explain the adverse event* Information on drug withdrawal may be lacking or unclear • *If available: outcome of metformin withdrawal is unclear or lacking*
Unlikely	Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • *Involves metformin ingestion prior to lactic acidosis* • *Diagnosis of lactic acidosis:* o *Lactate plasma concentration >5 mmol/L* o *pH <7.35* Disease or other drugs provide plausible explanations • *Patient presents with comorbidities and/or concomitant medications that are risk factors for lactic acidosis[a] more likely to be the cause than metformin*
Conditional/ Unclassified	Event or laboratory test abnormality • *Event involves history of metformin ingestion with no time restraint* More data for proper assessment needed, or additional data under examination • *Uncertain diagnosis of lactic acidosis* • *Lacking/unavailable clinical information required to support diagnosis*
Unasssessable/ Unclassifiable	Reported suggesting an adverse reaction • *Medical report suggests lactic acidosis diagnosis in patient* Cannot be judged because information is insufficient or contradictory • *Insufficient and/or contraindicatory information to diagnose lactic acidosis* Data cannot be supplemented or verified

Annotations of lactic acidosis specific diagnostic criteria are presented in italics. [a]Risk factors were defined using the list of risk factors for lactic acidosis summarised in Table A1.7.

A1.4. Naranjo adverse drug reaction probability scale

Presented in this section is the Naranjo adverse drug reaction probability scale that was used to assess the association between metformin therapy and lactic acidosis in Chapter 2. The Naranjo adverse drug reaction probability scale questionnaire is shown in Table A1.9 and interpretation of the Naranjo adverse drug reaction probability categories is shown in Table A1.10.

Table A1.9 *Naranjo adverse reaction probability scale*

To assess the drug reaction, please answer the following questionnaire and give the pertinent score					
		Yes	No	Do not know	Score
1	Are there previous conclusive reports on this reaction?	+1	0	0	
2	Did the adverse event appear after the suspected drug was administered?	+2	-1	0	
3	Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0	
4	Did the adverse reaction reappear when the drug was readministered?	+2	-1	0	
5	Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	+2	0	
6	Did the reaction reappear when a placebo was given?	-1	+1	0	
7	Was the drug detected in the blood (or other fluids) in concentrations known to be toxic?	+1	0	0	
8	Was the reaction more severe when the dose was increased, or less when the dose was decreased?	+1	0	0	
9	Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	
10	Was the adverse event confirmed by any objective evidence?	+1	0	0	

Table A1.10 *Interpretation of the Naranjo adverse drug reaction probability categories*

Probability category	Score	Interpretation
Definite	≥9	• Followed a reasonable temporal sequence after a drug or in which a toxic drug level had been established in body fluids or tissues • Followed a recognised response to the suspected drug • Confirmed by improvement on withdrawing the drug and reappeared on re-exposure
Probable	5-8	• Followed a reasonable temporal sequence after a drug • Followed a recognised response to the suspected drug • Confirmed by withdrawal but not by exposure to the drug • Could not be reasonably explained by the known characteristics of the patient's clinical state
Possible	1-4	• Followed a temporal sequence after a drug • Possibly followed a recognised pattern to the suspected drug • Could be explained by characteristics of patient's disease
Doubtful	≤0	• Likely related to factors other than a drug

A1.5. Completeness scores for identified metformin associated lactic acidosis case reports

A summary of the completeness score results for the identified metformin associated lactic acidosis case reports is shown below in Table A1.11.

Table A1.11 *Completeness scores for each metformin associated lactic acidosis case report*

Quality score	Cases (n = 559)
4	11
5	12
6	6
7	15
8	22
9	29
10	79
11	63
12	32
13	38
14	34
15	39
16	28
17	28
18	24
19	15
20	21
21	20
22	16
23	14
24	9
25	2
26	2
27	1

A1.6. Gastrointestinal illness and metformin associated lactic acidosis

In patients on metformin therapy it has been proposed that gastrointestinal illness may be an independent risk factor for lactic acidosis. Several hypotheses have been proposed that describe the series of events linking acute gastrointestinal illness to lactic acidosis in patients on metformin therapy [18, 373]. However, it is unknown whether the hypothesised scenarios, if any, are a true representation of the series of events that cases of metformin associated lactic acidosis present with. Knowledge of whether gastrointestinal illness does play a role in the development of lactic acidosis would allow for preventative measures to be implemented to safeguard the use of metformin.

Three of the proposed scenarios postulated to describe the series of events from acute gastrointestinal illness to lactic acidosis in patients on metformin therapy are described as follows:

- Scenario one.

  Acute gastroenteritis leads to dehydration which then leads to acute renal failure and consequently lactic acidosis

- Scenario two

  Acute gastrointestinal illness leads to dehydration followed by acute renal failure, resulting in metformin accumulation and lactic acidosis, respectively.

- Scenario three
  Metformin toxicity leads to symptoms of gastroenteritis which lead to acute renal failure and ultimately, lactic acidosis.

A1.6.1. Objectives

The aims of this study were to investigate the role of gastrointestinal illness in the development of metformin associated lactic acidosis from published case reports in the literature. The specific objectives of this study were:

i. To determine the prevalence of gastrointestinal illness in published case reports of metformin associated lactic acidosis
ii. To describe the clinical and demographic features of the cases presenting with gastrointestinal illness
iii. To test each hypothesised scenario linking gastrointestinal illness to metformin associated lactic acidosis

A1.6.2. Methods

A1.6.2.1. Data source

The database of published metformin associated lactic acidosis case reports identified in the systematic literature in Chapter 2 were used to address the research question. For the purposes of this analysis only case histories that had ingested a therapeutic dose of metformin (identified from the database) were included in the analysis.

A1.6.2.2. Data analysis

Description and clinical presentation. A summary of the demographic and clinical presentation of metformin associated lactic acidosis cases with reported gastrointestinal illness was described. Data analysis and graphing was conducted using R (version 3.3.3).

Postulated scenario hypothesis testing. The three scenarios hypothesised to link gastrointestinal illness to lactic acidosis in patients on metformin therapy were tested for feasibility using available data extracted from case reports. Each of the hypotheses were tested and were deemed feasible if all of the components of the series of events were present in the cases.

Metformin concentrations were extracted from the database, if available, for each case history. Metformin accumulation was defined as plasma metformin concentrations that exceeded 5 mg/L at any time throughout the case's admission to a medical facility.

A1.6.3.Results

Description of cases identified

A total of 186 metformin associated lactic acidosis cases were identified to have reported symptoms of gastrointestinal illness. A summary of the demographics for these cases is presented in Table A1.12.

The duration of gastrointestinal illness in these cases varied greatly. On presentation, 184 of the 186 (98.9%) cases presented with acute gastrointestinal symptoms that were present for less than a month. The remainder two cases presented with gastrointestinal symptoms that lasted for 3 and 12 months. The most common identifiable causes for the gastrointestinal symptoms were gastrointestinal infection, recent use of antibiotics, post-op nausea and recent bowel preparation for colonoscopy.

Acute renal impairment was noted in 154 cases. Forty-four cases of those with acute renal impairment had pre-existing chronic renal impairment. Twenty-two cases presented in a clinical state of dehydration.

Table A1.12 *Demographics of cases with reported gastrointestinal illness*

Demographics		**Cases with reported data (n = 186)**
Gender (F: M)	118:68	186
Age (years)	69 [20 – 90]	186
Weight (kg)	68.5 [27 – 117]	12
Height (m)	1.631.53 – 1.65]	9

Data presented as median[range] unless otherwise specified

Postulated scenario hypothesis testing

Only a total of 21, 5 and 42 cases fulfilled the series of events described in scenario one, two and three, respectively.

A1.6.4.Discussion

The presence of acute gastrointestinal illness, leading to vomiting, diarrhoea and dehydration, has been proposed to increase the risk of MALA. While vomiting and diarrhoea are recognised side effects of metformin it is anticipated that these side effects are minimal in most patients already stabilised on metformin therapy [99]. The causal relationship between gastrointestinal illness and lactic acidosis involving a combination of gastrointestinal illness, dehydration, acute kidney injury and metformin accumulation, is hard to determine in the published cases reviewed here due to limitations arising from the retrospective nature of the data.

Appendix 2: Appendices to Chapter 3

A2.1. Description of population pharmacokinetic model for metformin by Duong et al

In the metformin population pharmacokinetic study by Duong et al, metformin was described by a two compartment model with first order absorption for immediate release formulations and zero-order absorption for extended release formulations (a schematic of the published model by Duong et al is presented in Figure A2.1). In the model Duong et al, creatinine clearance and total body weight were found to be significant covariates on metformin CL/F and V_1/F, respectively. The published equations for CL/F and $V_1/F$are as follows:

$$CL/F = \left(\theta_{CL/F} \times (CL_{CR}/6)\right) \times e^{PPVCL}$$

$$V_1/F = \left(\theta_{V_1/F} \times (TBW/70)\right) \times e^{PPVV1}$$

where $\theta_{CL/F}$ is the mean population value for CL/F, θ_{V_1} is the mean population value for V_1/F, $PPVCL$ is the sum of inter-individual variability and inter-occasion variability for CL/F, $PPVV1$ is the sum of the inter-individual variability and inter-occasion variability for V_1/F, CL_{CR} is creatinine clearance estimated using the Cockcroft Gault equation using lean body weight, and, TBW is total body weight.

The simulations performed using the published model by Duong et al were deterministic. Case specific characteristics (i.e. metformin dose, weight and pre-admission estimate of renal function) were entered into the model for each case where the data was available. The median weight for the cohort of metformin associated lactic acidosis cases was imputed if weight was not reported. The parameter values used in the simulation were the same as the published model by Duong et al and are presented in Table A2.1. Parameter values for between subject or between occasion variability and residual unexplained variance were not used in the simulations so are not reported in Table A2.1 below

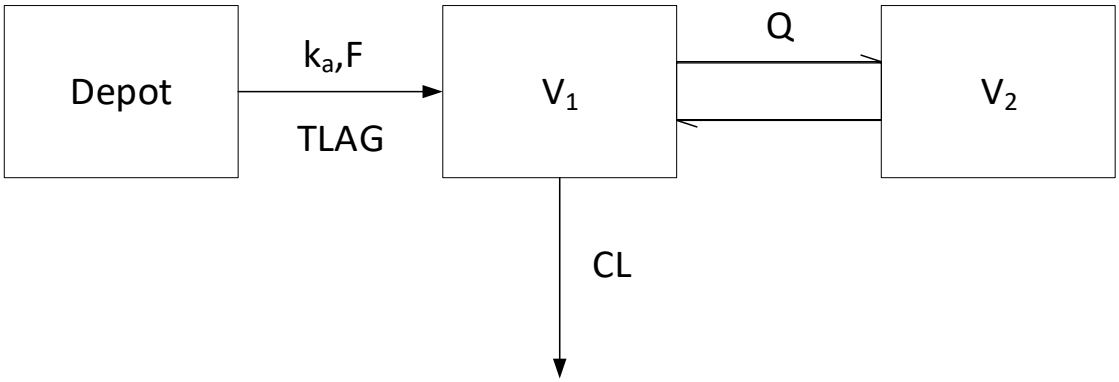


Figure A2.1 *Schematic of published population pharmacokinetic model for metformin by Duong et al. CL is clearance, F is bioavailability, k_a is the absorption rate constant, Q is intercompartmental clearance, TLAG is lag time for absorption, V_1 is the central compartment volume and V_2 is the peripheral compartment volume.*

Table A2.1 *Parameter values in the published model by Duong et al to predict pre-admission plasma concentration for metformin associated lactic acidosis patients*

Parameter	Values
$\theta_{CL/F}$ (mL/min)	72
$\theta_{V_1/F}$ (L)b	149
$\theta_{Q/F}$ (mL/min)	203
$\theta_{V_2/F}$ (L)	182
θ_{k_a} (h^{-1})	0.35
θ_{TLAG} (h^{-1})	0.38

The final covariate model for metformin clearance was $CL/F = \left(\theta_{CL/F} \cdot (CLcr_{CG}/6)\right) \cdot e^{PPVCL}$, where $\theta_{CL/F}$ is the population estimate for metformin clearance, $CLcr_{CG}$ is creatinine clearance calculated using the Cockcroft and Gault equation [80] and $PPVCL$ is the sum of the interindividual and interoccassion variability for CL. The final covariate model for metformin central compartment volume was $V_1/F = \left(\theta_{V_1/F} \cdot (TBW/70)\right) \cdot e^{PPVV1}$, where $\theta_{V_1/F}$ is the population estimate for the central compartment volume for metformin, TBW is total body weight and $PPVV1$ is the sum of the inter-individual and inter-occassion variability for V_1. $\theta_{Q/F}$ is population estimate for intercompartmental clearance. $\theta_{V_2/F}$ is the population estimate for the peripheral compartment volume. θ_{k_a} is the population estimate for the absorption rate constant. θ_{TLAG} is the population estimate for the lag time for absorption. θ_F is the population estimate for bioavailability that was fixed to a value of 1 for the purposes of the simulations.

A2.2. Completeness score results

A completeness score was developed to assess the availability and quality of data provided in each case history. Below is a table summarising the results from the completeness score. A maximum score of 31 could be obtained.

Table A2.2 *Summary results for completeness scores allocated to MALA cases with a history of chronic renal impairment from the database*

Completeness Score	Number of case histories (n = 145)
7	2
8	3
9	6
10	9
11	24
12	12
13	16
14	9
15	17
16	8
17	6
18	3
19	3
20	3
21	11
22	3
23	7
24	2
28	1

A2.3. Implementation of the metformin population pharmacokinetic model by Duong et al

The simulated metformin plasma concentration time profiles in the publication by Duong et al were replicated to ensure the model was correctly implemented for the purposes of analysis. Stochastic simulations were performed as described in the publication by Duong et al by using the maximum doses for patients with varying levels of renal impairment (shown in Table A2.2). The median weight (i.e. 65 kg) for the study population in the publication by Duong et al was imputed as the weight for the simulations. Each simulation was replicated 1000 times to day 25 (i.e. steady state). The simulations were performed in MATLAB (R2016b, MathWorks, Natick, NA) and the 5th, 50th and 95th percentiles of the simulated concentrations were plotted.

Table A2.3 *Dosing regimen used for the stochastic simulations*

Renal function in creatinine clearance (mL/min)	Metformin dose (mg)
15	500
30	1000
60	2000
120	3000

The replicated stochastic simulations performed under the different renal dosing regimens are presented side by side with the published original plots by Duong et al in Figure A2.2. The replicated predictions from the metformin pharmacokinetic model are similar to the published plots produced by Duong et al for the scenarios with a creatinine clearance of 15, 30 and 120 mL/min. However, in the simulations where creatinine clearance is equal to 60 mL/min the 5th, 50th and 95th percentiles appear to overestimate the metformin concentrations relative to the published plots. In addition, in all the plots the 50th percentiles of the simulated data appear higher than the measured values. In the publication by Duong et al the dosing frequency for each scenario was not reported. Hence, it was assumed that metformin was given as a single dose. This could be a potential reason for deviation seen in each of the plots.

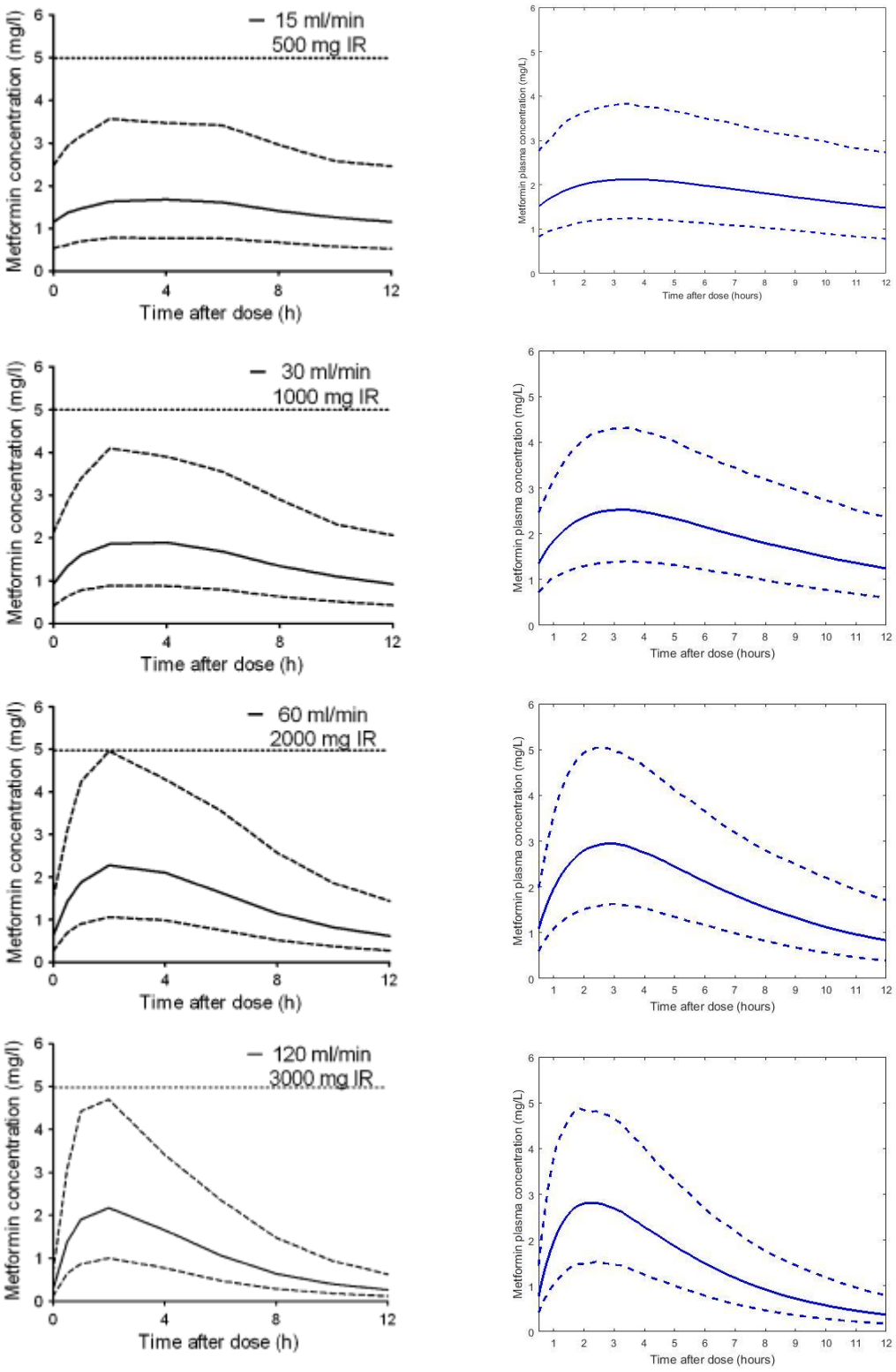


Figure A2.2 *Original plots by Duong et al (left) and replicated (right) stochastic simulations of plasma metformin concentrations at the maximum recommended dose for patients with varying levels of renal impairment. The 5th, 50th and 95th percentiles of the predicted concentrations are shown in the plot.*

Appendix 3: Appendices to Chapter 4

A3.1. Literature review search strategy

The static search strategy performed in Ovid MEDLINE and Ovid EMBASE are presented in Table A3.1 and A3.2, respectively.

Table A3.1 *Ovid MEDLINE static search strategy*

Source	Search strategy
Ovid MEDLINE *Covering period 1946 to March 2020*	17. Metformin 18. Acidosis, Lactic 19. upper limit.mp 20. therapeutic concentration.mp 21. therapeutic limit.mp 22. toxic concentration.mp 23. Dose-Response Relationship, Drug 24. 3 or 4 or 5 or 6 or 7 25. 1 and 2 and 8 26. Limit 9 to humans 27. Limit 10 to English language Results: 21

Table A3.2 *Ovid EMBASE static search strategy*

Source	Search strategy
Ovid EMBASE *Covering period 1946 to March 2020*	1. Metformin 2. Lactic acidosis 3. upper limit.mp 4. therapeutic concentration.mp 5. therapeutic limit.mp 6. toxic concentration 7. dose response 8. 3 or 4 or 5 or 6 or 7 9. 1 and 2 and 8 10. Limit 9 to humans 11. Limit 10 to English language Results: 152

Appendix 4: Appendices to Chapter 5

A4.1. Details of the study procedure for data available from the Dunedin Public Hospital

Additional details of the study procedures for the metformin study conducted at the Dunedin Public Hospital is described in this section.

A4.1.1. Study participant inclusion/exclusion criteria

Study participants were excluded if they presented with: inability to give written informed consent, type 2 diabetes or were currently taking metformin, had evidence of >25% change in eGFR in the past month, pregnancy, known allergy to medications used in the study (i.e. biguanides or aminoglycosides), or, were taking drugs known or suspected to interact with the renal tubular transport of metformin or creatinine (i.e. antibiotics, atenolol, calcium channel blockers, antiarrhythmic drugs, histamine (H_2) antagonists, thiazide diuretics, antituberculosis drugs and probenecid). During the eligibility screening process baseline information, including study participant demographics and concomitant medications were recorded.

A4.1.2. Metformin assay

A high-performance liquid chromatography (HPLC) assay was used to determine plasma metformin concentrations. The assay used has been described and validated by Zhang et al [412]. The standard curve of metformin were found to be linear over the concentration range of 0.02-4 mg/L. The assay lower limit of quantification was reported to be around 0.02 mg/L. The intra- and inter-day coefficients of variation were reported to be less than 9.0%.

A4.2. Pharmacokinetic analysis conducted in R

In this section the R code and outputs from the pharmacokinetic analysis are presented in section A4.2.1. and A4.2.2., respectively.

A4.2.1.R code

```
setwd("Z:/Projects/2_PopPK/4_Analysis/1_Exploratory
 analysis/3_Calculations/NCA/Metformin")

library(tidyverse)
library(readxl)
library(dplyr)
library(NonCompart)
library(PKPDmisc)
library(PKNCA)

#---------------------------------------------------------------
# DATA
#---------------------------------------------------------------
metforminstudy <- read.csv("metformin.csv")

initialconc <- metforminstudy %>%
 filter(study == 1) %>%
 select(id) %>%
 distinct %>%
 mutate(time = 0,
        dv = 0)

metforminconc <- metforminstudy %>%
 filter(study == 1) %>%
 filter(cmt == 2) %>%
 filter(mdv == 0) %>%
```

```
  mutate(dv = as.numeric(as.character(dv))) %>%
  select(id, time, dv) %>%
  full_join(initialconc, by = c("id", "time", "dv")) %>%
  arrange(id, time) %>%
  rename(conc = dv)

metformindose <- metforminstudy %>%
  filter(study == 1) %>%
  filter(evid == 1) %>%
  select(id, time, amt) %>%
  rename(dose = amt)

#---------------------------------------------------------------
# DATA VISUALIATION
#---------------------------------------------------------------

met.obs1 <- metforminconc %>%
  mutate(id = as.factor(id)) %>%
  ggplot(mapping = aes(x = time,
                       y = as.numeric(conc),
                       group = id,
                       colour = id)) +
  geom_line() +
  geom_point() +
  ylab("Metformin concentration (mg/L)") +
  xlab("Time (h)") +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        axis.line = element_line(colour = "black")) +
  ggtitle("Metformin plasma concentrations versus time")
```

```
met.obs1

met.obs2 <- metforminconc %>%
 mutate(id = as.factor(id)) %>%
 ggplot(mapping = aes(x = time,
                      y = as.numeric(conc))) +
 geom_line() + geom_point() +
 facet_wrap(~id, nrow = 5) +
 geom_line() +
 geom_point() +
 ylab("Metformin concentration (mg/L)") +
 xlab("Time (h)") +
 theme(panel.grid.major = element_blank(),
       panel.grid.minor = element_blank(),
       panel.background = element_blank(),
       axis.line = element_line(colour = "black")) +
 ggtitle("Metformin plasma concentrations versus time")
met.obs2

#---------------------------------------------------------------
# NCA ANALYSIS
#---------------------------------------------------------------
PKNCA.options()
conc <- PKNCAconc(data = metforminconc, formula = conc~time|id)
dose <- PKNCAdose(metformindose, dose~time|id)
data <- PKNCAdata(conc, dose)

my.results <- pk.nca(data)
summary(my.results)
raw <- my.results$result
```

```
head(raw)
results <- raw %>%
 select(id, PPTESTCD, PPORRES) %>%
 spread(PPTESTCD, PPORRES)

metformin.urine <- metforminstudy %>%
 mutate(dv = as.numeric(as.character(dv)),
       amt = as.numeric(as.character(amt)),
       mdv = as.numeric(as.character(mdv)),
       uvol = as.numeric(as.character(uvol))) %>%
 filter(mdv == 0 & cmt == 4) %>%
 mutate(amt = 500) %>%
 rename(dose = amt,
             conc = dv,
             volume = uvol) %>%
 mutate(AE = conc*volume) %>%
 group_by(id) %>%
 summarise(ae = sum(AE)) %>%
 ungroup

table <- results %>%
 left_join(metformin.urine, by = ("id")) %>%
 mutate(fe=ae/500,
            cl.last = 500/auclast,
            cl.obs = 500/ aucinf.obs,
            vz.obs=cl.obs/lambda.z,
            vd.obs=500/(aucinf.obs*lambda.z),
            clr.last=ae/auclast,
            clr.obs=ae/aucinf.obs)

units <- (setNames((c("UNITS", "", "mg/L.h", "mg/L.h",
```

```
                                "mg/L", "mg/L", "mg/L", "h", "1/h", "",
                                "h", "", "", "h", "h", "mg", "", "L/h", "L/h",
                                "L", "L", "L/h", "L/h")),
                              c(colnames(table))))
n <- table %>%
      summarise_all(funs(round(sum(!is.na(.)),0))) %>%
      mutate(id="n")
mean <- table %>%
            summarise_all(funs(round(mean(., na.rm=T))),2) %>%
            mutate(id="MEAN")
sd <- table %>%
       summarise_all(funs(round(sd(., na.rm=T))),2) %>%
       mutate(id="SD")
colnames(table)

table <- rbind(units,table,mean,sd,n)

write.csv(table,"NCA.csv")
```

A4.2.2. Output from noncompartmental analysis conducted in R

Presented in this section of the appendix is the pharmacokinetic metric output from the PKNCA R package.

Table A4.1 *Pharmacokinetic metric estimates for study participants stratified by their CKD classification group*

Parameter	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5
Sample size (n)	7	10	5	6	6
C_{max} (mg/L)	0.84±0.35	0.84±0.26	1.66±0.42	2.34±0.45	2.36±1.14
T_{max} (h)	2.41±0.95	2.38±0.88	2.92±1.56	2.98±0.64	3.20±1.62
λ_z (h^{-1})	0.28±0.19	0.25±0.14	0.13±0.03	0.11±0.05	0.13±0.08
$t_{1/2}$ (h)	4.21±4.10	4.25±3.48	5.69±1.40	8.01±4.62	6.71±2.83
AUC_{0-last} (mg·h/L)	2.91±2.19	4.15±0.73	13.51±5.88	15.81±5.31	20.66±18.31
$AUC_{0-\infty}$ (mg·h/L)	3.30±2.60	5.06±1.13	16.52±5.97	33.71±19.52	38.18±20.88
CL/F (L/h)	225.27±152.69	103.95±29.49	32.85±9.21	17.99±7.29	19.05±15.04
CL_{renal} (L/h)	68.63±55.39	35.56±4.59	11.87±4.08	9.69±5.49	8.97±6.08

Data are presented as mean ± standard deviation

Appendix 5: Appendices to Chapter 7

A5.1. Simulation code

Presented in this section is the code used to simulate plasma metformin concentration profiles as described in Chapter 7 (section 7.4.5.).

```
# Load R packages
library(RxODE)
library(MASS)
library(tidyverse)

set.seed <- 123456

#---------------------------------------------------------------
# Define model
#---------------------------------------------------------------

ode <- "
C2 = centr/V2;
C3 = peri/V3;
d/dt(depot) = -KA*depot;
d/dt(centr) =  F1*KA*depot - CL*C2 - Q*C2 + Q*C3
d/dt(peri)  =                Q*C2 - Q*C3;
"
# Compile model
mod001 <- RxODE(model = ode, modName = "mod001")

#---------------------------------------------------------------
# Create virtual patients
#---------------------------------------------------------------
# number of subjects
nsub <- 1000
```

```
# Creatinine Clearance (Cockcroft-Gault Equation)
CG <- runif(nsub, min = [minimum value of creatinine clearance] ,
                  max = [maximum value of creatinine clearance])
WT <- 70

# eta1: CL, eta2: V2, eta3: KA
omega <- matrix(c(0.199,   0.0697,   0,
                  0.0697,  0.118,    0,
                  0,       0,        0.145),3,3)

eta    <- mvrnorm(n=nsub, rep(0,3), omega)

# Population typical parameter values
CG_EFF      <- 0.797
WT_EFF      <- 1
F1          <- 0.55

TVCL        <- 90.5*(CG/100)^CG_EFF
TVV2        <- 147*(WT/70)^WT_EFF
TVQ         <- 3.74
TVV3        <- 57.2
TVKA        <- 0.4
prop_err_ed <- 0.241
add_err_ed  <- 0.0337

# Individual parameter values
virtual_patients <- data.frame(ID = 1:nsub,
                               CG = CG,
                               F1 = F1) %>%
 mutate(ETA1 = eta[,1],
```

```
        ETA2 = eta[,2],
        ETA3 = eta[,3]) %>%
  mutate(CL = TVCL*exp(ETA1),
        V2 = TVV2*exp(ETA2),
        Q  = TVQ,
        V3 = TVV3,
        KA = TVKA*exp(ETA3),
        F1 = F1)

params.all <- cbind(CL = virtual_patients$CL,
                    V2 = virtual_patients$V2,
                    Q  = virtual_patients$Q,
                    V3 = virtual_patients$V3,
                    KA = virtual_patients$KA,
                    F1 = virtual_patients$F1)

#-------------------------------------------------------------
# Simulate a single dose
#-------------------------------------------------------------

# Initialize event table
ev <- eventTable()

# Specify dose
ev$add.dosing(dosing.to="depot",dose = [insert dose], nbr.doses = [insert
number doses], dosing.interval= [insert dosing interval])

# Specify sampling
ev$add.sampling(0:168)

# Initial conditions
```

```
inits <- c(0,0,0)

res <- NULL #Create an empty matrix for storing results

# Loop through each row of parameter values and simulate
for (i in 1:nsub)  {
  params <- params.all[i,]
  temp <- mod001$run(params, ev, inits = inits)
  res <- cbind(res,temp[,"C2"])
}

#The same can be achieved more efficiently by replacing the above for-loop with:
res <- apply(params.all, 1, function(params) mod001$run(params, ev, inits)[, "C2"])

# Plot results
par(mfrow = c(2), mar = c(4,4,1,1))
matplot(res, type = "l",  ylab = "Concentration", xlab = "Time (h)")

# Calculate and plot quantiles
res.q.t <- apply(res, 1, quantile, prob = c(.05, .5, .95))
matplot(t(res.q.t), type = "l", lty = c(2,1,2), col = c(2,1,2),
      ylab = "Plasma metformin concentration (mg/L)", xlab = "Time (h)", ylim=c(0,2))
legend("topright", lty = c(1,2), col = c("black","red"), cex = .8)
```

A5.2. NONMEM control file for the final population pharmacokinetic model for metformin

```
$PROBLEM METFORMIN PK

$INPUT C STUDY ID STUDYID TIME DV AMT CMT1=CMT CMT2 CMT3
 EVID DVID MDV BLQ RATE FORM OCC AGE SEX HTCM WTKG BMI
FFM IBW GENTCL CR CG CGIBW MDRD CKDEPI DWTKG BLACT
BBICAR DSTART DSTOP DRETURN BFR DFR SA DIAL

$DATA metformin.csv
    IGNORE = C
    IGNORE = (BLQ.EQ.1)
    IGNORE = (STUDY.EQ.3)
    IGNORE = (STUDY.EQ.4)
    IGNORE = (STUDY.EQ.5)
    IGNORE = (STUDY.EQ.6)
    IGNORE = (STUDY.EQ.7)
    IGNORE = (STUDY.EQ.8)
    IGNORE = (STUDY.EQ.9)
    IGNORE = (STUDY.EQ.10)
    IGNORE = (STUDY.EQ.11)
    IGNORE = (CMT1.EQ.3)
    IGNORE = (CMT1.EQ.-3)
    IGNORE = (FORM.EQ.2)

$SUBROUTINES ADVAN13 TOL=9

$MODEL
COMP(DEPOT)
COMP(CENTRAL, DEFOBS)
```

```
COMP(PERIPH)

$PK
; COVARIATE MODEL
TVCL=THETA(1)*((CGIBW/100)**THETA(7))
TVV2=THETA(2)*((WTKG/70)**THETA(8))
TVQ=THETA(3)
TVV3=THETA(4)
TVKA=THETA(5)
TVF1=THETA(6)

TVK23=TVQ/TVV2
TVK32=TVQ/TVV3
TVK20=TVCL/TVV2

; BETWEEN SUBJECT VARIABILITY
CL=TVCL*EXP(ETA(1))
V2=TVV2*EXP(ETA(2))
Q=TVQ*EXP(ETA(3))
V3=TVV3*EXP(ETA(4))
KA=TVKA*EXP(ETA(5))

K23=Q/V2
K32=Q/V3
K20=CL/V2

; SCALE CONCENTRATIONS
S2 = V2

$DES
DADT(1) = -KA*A(1)
```

```
DADT(2) = KA*A(1)-(K23+K20)*A(2)+K32*A(3)
DADT(3) = K23*A(2)-K32*A(3)

$ERROR
IPRED = F
   W = SQRT(THETA(9)**2*IPRED**2 + THETA(10)**2)
   Y = IPRED + W*EPS(1)
 IRES = DV-IPRED
IWRES = IRES/W

$THETA
(0, 98)         ; CL
(0, 181)        ; V2
(0, 4.2)        ; Q
(0, 47)         ; V3
(0, 0.38)       ; KA
0.55 FIX        ; F1
(0, 1)          ; CG_EFF
1 FIX           ; WT_EFF
(0, 0.258)      ; Prop
(0, 0.02)       ; Add

$OMEGA BLOCK(2)
0.1             ; IIV CL
0.01    0.1     ; IIV V2

$OMEGA
0 FIX           ; IIV Q
0 FIX           ; IIV V3
(0.20)          ; IIV KA
```

```
$SIGMA
1 FIX ; Proportional PK S1

$EST METHOD=COND INTER MAXEVAL=9999 NOABORT SIGL=9
NSIG=3 PRINT=10

$COV

; Xpose
$TABLE STUDY ID TIME DV MDV EVID CMT1 CMT2 CMT3 BLQ IPRED
IWRES CWRES ONEHEADER NOPRINT FILE=sdtab56
$TABLE STUDY ID CL V2 Q V3 KA ETA1 ETA2 ETA3 ETA4 ETA5
 ONEHEADER NOPRINT FILE=patab56
```

Appendix 6: Appendices to Chapter 8

A6.1. Extended theoretical consideration

In this section, all possible permutations of parameter values for a one-, two- and three-compartment mamillary pharmacokinetic model are explicitly shown. The pharmacokinetic mathematical models for a one-, two- and three-compartment model are shown as follows:

One-compartment model

$$C = \frac{F \cdot D \cdot k_a}{V_c(k_a - k)} \left(e^{-k \cdot t} - e^{-k_a \cdot t}\right)$$

Two-compartment model

$$\mathrm{C} = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21} - \alpha)exp^{-\alpha \cdot t}}{(k_a - \alpha)(\beta - \alpha)}\right) + \left(\frac{(k_{21} - \beta)exp^{-\beta \cdot t}}{(k_a - \beta)(\alpha - \beta)}\right) + \left(\frac{(k_{21} - k_a)exp^{-k_a \cdot t}}{(\alpha - k_a)(\beta - k_a)}\right)\right]$$

Three-compartment model

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21} - \alpha)(k_{31} - \alpha)exp^{-\alpha \cdot t}}{(k_a - \alpha)(\alpha - \beta)(\alpha - \gamma)}\right) + \left(\frac{(k_{21} - \beta)(k_{31} - \beta)exp^{-\beta \cdot t}}{(k_a - \beta)(\beta - \alpha)(\beta - \gamma)}\right) + \left(\frac{(k_{21} - \gamma)(k_{31} - \gamma)exp^{-\gamma \cdot t}}{(k_a - \gamma)(\gamma - \alpha)(\gamma - \beta)}\right) + \left(\frac{(k_{21} - k_a)(k_{31} - k_a)exp^{-k_a \cdot t}}{(\alpha - k_a)(\beta - k_a)(\gamma - k_a)}\right)\right]$$

Here, C represents concentration, F is bioavailability, D is the dose, k_a is the absorption rate constant, k is the elimination rate constant, V_c is the central compartment volume, t is time, and, both k_{21} and k_{31} are the first-order transfer rate constants of a drug from the peripheral compartment volume to the central compartment volume. In the two- and three-compartment models α is a macro-constant that describes the initial decline in drug concentrations. In a two-compartment model β is a macro-constant that describes the terminal decline in drug concentrations. In the three-compartment model β and γ are macro-constants that describe the intermediate and terminal decline in drug concentrations, respectively.

There are two possible permutations of parameter values that provide the same input and output relationship in a one-compartment mamillary model. The two possible permutations are:

Permutation 1:

$$k_a = k_a$$
$$k = k$$
$$V_c = V_c$$

Permutation 2:

$$k_a' = k$$
$$k' = k_a$$
$$V_c' = \frac{V_c \cdot k}{ka}$$

As shown in Permutation 2, the volume of distribution (V_c') becomes a function of the absorption rate constant (k_a) and the elimination rate constant (k). If the parameter values for Permutation 2 are substituted in the one-compartment model and simplified the same input-output relationship can be obtained as Permutation 1. The substitution and simplification of Permutation 2 into the one-compartment model is shown as follows:

Permutation 2

$$k_a' = k$$
$$k' = k_a$$
$$V_c' = \frac{V_c \cdot k}{ka}$$

Substituted into a one-compartment model

$$C = \frac{F \cdot D \cdot k_a}{V_c(k_a - k)} (e^{-k \cdot t} - e^{-k_a \cdot t})$$

$$C = \frac{F \cdot D \cdot k}{\frac{V_c \cdot k}{k_a}(k - k_a)} (e^{-k_a \cdot t} - e^{-k \cdot t})$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V_c\,(k_a - k)}(e^{-k \cdot t} - e^{-k_a \cdot t})$$

For a two-compartment pharmacokinetic model there are three possible permutations of parameter values that can give the same input-output relationship. The three possible permutations of sets of parameter values are:

Permutation 1:

$$\alpha = \alpha$$
$$\beta = \beta$$
$$k_a = k_a$$
$$V_c = V_c$$

Permutation 2:

$$\alpha' = k_a$$
$$\beta' = \beta$$
$$k_a' = \alpha$$
$$V_c' = \frac{V_c \cdot \alpha}{k_a}$$

Permutation 3:

$$\alpha' = \alpha$$
$$\beta' = k_a$$
$$k_a' = \beta$$
$$V_c' = \frac{V_c \cdot \beta}{k_a}$$

Here, Permutation 2 and 3 can be substituted into the two-compartment model and provide the same answer as Permutation 1. The mathematical substitution of Permutation 2 and 3 into a two-compartment model followed by mathematical simplification is as follows:

Permutation 2

$$\alpha' = k_a$$
$$\beta' = \beta$$
$$k_a' = \alpha$$
$$V_c' = \frac{V_c \cdot \alpha}{k_a}$$

Substituted into the two-compartment model:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\beta-\alpha)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\alpha-\beta)}\right)+\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)}\right)\right]$$

$$C = \frac{F \cdot D \cdot \alpha}{\frac{V_c \cdot \alpha}{k_a}}\left[\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta \cdot t}}{(\alpha-\beta)(k_a-\beta)}\right)+\left(\frac{(k_{21}-\alpha)exp^{-\alpha t}}{(k_a-\alpha)(\beta-\alpha)}\right)\right]$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)exp^{-\alpha t}}{(k_a-\alpha)(\beta-\alpha)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\alpha-\beta)}\right)+\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)}\right)\right]$$

Permutation 3

$$\alpha' = \alpha$$
$$\beta' = k_a$$
$$k_a' = \beta$$
$$V_c' = \frac{V_c \cdot \beta}{k_a}$$

Substituted into the two-compartment model:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\beta-\alpha)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\alpha-\beta)}\right)+\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)}\right)\right]$$

$$C = \frac{F \cdot D \cdot \beta}{\frac{V_c \cdot \beta}{k_a}}\left[\left(\frac{(k_{21}-\alpha)exp^{-\alpha \cdot t}}{(\beta-\alpha)(k_a-\alpha)}\right)+\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\beta-k_a)(\alpha-k_a)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta t}}{(\alpha-\beta)(k_a-\beta)}\right)\right]$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V}\left[\left(\frac{(k_{21}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\beta-\alpha)}\right)+\left(\frac{(k_{21}-\beta)exp^{-\beta t}}{(k_a-\beta)(\alpha-\beta)}\right)+\left(\frac{(k_{21}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)}\right)\right]$$

For a three-compartment model the four possible permutations are:

Permutation 1:

$$\alpha = \alpha$$
$$\beta = \beta$$
$$\gamma = \gamma$$
$$k_a = k_a$$
$$V_c = V_c$$

Permutation 2:

$$\alpha' = k_a$$
$$\beta' = \beta$$
$$\gamma' = \gamma$$
$$k_a' = \alpha$$
$$V_c' = \frac{V_c \cdot \alpha}{k_a}$$

Permutation 3:

$$\alpha' = \alpha$$
$$\beta' = k_a$$
$$\gamma' = \gamma$$
$$k_a' = \beta$$
$$V_c' = \frac{V_c \cdot \beta}{k_a}$$

Permutation 4:

$$\alpha' = \alpha$$
$$\beta' = \beta$$
$$\gamma' = k_a$$
$$k_a' = \gamma$$
$$V_c' = \frac{V_c \cdot \gamma}{k_a}$$

As previously shown for the two-compartment model, Permutations 2, 3 and 4 can be substituted into the three-compartment model to provide the same input-output relationship as Permutation 1. The mathematical substitution and simplification is given as follows:

Permutation 2

$$\alpha' = k_a$$
$$\beta' = \beta$$
$$\gamma' = \gamma$$
$$k_a' = \alpha$$
$$V_c' = \frac{V_c \cdot \alpha}{k_a}$$

Substituted into the three-compartment model:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\alpha-\beta)(\alpha-\gamma)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\beta-\alpha)(\beta-\gamma)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(k_a-\gamma)(\gamma-\alpha)(\gamma-\beta)}\right)+\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)(\gamma-k_a)}\right)\right]$$

$$C = \frac{F \cdot D \cdot \alpha}{\frac{V_c \cdot \alpha}{k_a}}\left[\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(k_a-\beta)(k_a-\gamma)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\alpha-\beta)(\beta-k_a)(\beta-\gamma)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\alpha-\gamma)(\gamma-k_a)(\gamma-\beta)}\right)+\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\beta-\alpha)(\gamma-\alpha)}\right)\right]$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\beta-\alpha)(\gamma-\alpha)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\alpha-\beta)(\beta-k_a)(\beta-\gamma)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\alpha-\gamma)(\gamma-k_a)(\gamma-\beta)}\right)+\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(k_a-\beta)(k_a-\gamma)}\right)\right]$$

Permutation 3

$$\alpha' = \alpha$$
$$\beta' = k_a$$
$$\gamma' = \gamma$$
$$k_a' = \beta$$
$$V_c' = \frac{V_c \cdot \beta}{k_a}$$

Substituted into the three-compartment model:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\alpha-\beta)(\alpha-\gamma)}\right) + \left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\beta-\alpha)(\beta-\gamma)}\right) + \left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(k_a-\gamma)(\gamma-\alpha)(\gamma-\beta)}\right) + \left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)(\gamma-k_a)}\right)\right]$$

$$C = \frac{F \cdot D \cdot \beta}{\frac{V_c \cdot \beta}{k_a}}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(\beta-\alpha)(\alpha-k_a)(\alpha-\gamma)}\right) + \left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\beta-k_a)(k_a-\alpha)(k_a-\gamma)}\right) + \left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\beta-\gamma)(\gamma-\alpha)(\gamma-k_a)}\right) + \left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\alpha-\beta)(k_a-\beta)(\gamma-\beta)}\right)\right]$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(\beta-\alpha)(\alpha-k_a)(\alpha-\gamma)}\right) + \left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\alpha-\beta)(k_a-\beta)(\gamma-\beta)}\right) + \left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\beta-\gamma)(\gamma-\alpha)(\gamma-k_a)}\right) + \left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\beta-k_a)(k_a-\alpha)(k_a-\gamma)}\right)\right]$$

Permutation 4

$$\alpha' = \alpha$$
$$\beta' = \beta$$
$$\gamma' = k_a$$
$$k_a' = \gamma$$
$$V_c' = \frac{V_c \cdot \gamma}{k_a}$$

Substituted into the three-compartment model:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(k_a-\alpha)(\alpha-\beta)(\alpha-\gamma)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(k_a-\beta)(\beta-\alpha)(\beta-\gamma)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(k_a-\gamma)(\gamma-\alpha)(\gamma-\beta)}\right)+\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\alpha-k_a)(\beta-k_a)(\gamma-k_a)}\right)\right]$$

$$C = \frac{F \cdot D \cdot \gamma}{\frac{V_c \cdot \gamma}{k_a}}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(\gamma-\alpha)(\alpha-\beta)(\alpha-k_a)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\gamma-\beta)(\beta-\alpha)(\beta-k_a)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\gamma-k_a)(k_a-\alpha)(k_a-\beta)}\right)+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\alpha-\gamma)(\beta-\gamma)(k_a-\gamma)}\right)\right]$$

Rearranged:

$$C = \frac{F \cdot D \cdot k_a}{V_c}\left[\left(\frac{(k_{21}-\alpha)(k_{31}-\alpha)exp^{-\alpha \cdot t}}{(\gamma-\alpha)(\alpha-\beta)(\alpha-k_a)}\right)+\left(\frac{(k_{21}-\beta)(k_{31}-\beta)exp^{-\beta \cdot t}}{(\gamma-\beta)(\beta-\alpha)(\beta-k_a)}\right)\right.$$
$$\left.+\left(\frac{(k_{21}-\gamma)(k_{31}-\gamma)exp^{-\gamma \cdot t}}{(\alpha-\gamma)(\beta-\gamma)(k_a-\gamma)}\right)+\left(\frac{(k_{21}-k_a)(k_{31}-k_a)exp^{-k_a \cdot t}}{(\gamma-k_a)(k_a-\alpha)(k_a-\beta)}\right)\right]$$

Here, it is can be seen that the issue of local identifiability becomes a greater problem when the number of compartments (n) increases [413]. The number of possible permutations of sets of parameter values for a given compartment model is $n + 1$.

Simulations under all the possible permutations for a one-, two- and three-compartment model were performed to illustrate that the same input-output profile could be produced under different sets of parameter values. The simulations were performed in R (version 3.5.3) using the parameter values shown in Table A6.1.

The simulated pharmacokinetic profiles for a one-, two- and three-compartment model are shown in Figure A6.1. Here, it can be seen that the different permutations are superimposed on one another and provide the same input-output profile.

Table A6.1 *Parameter values used in the simulation*

Parameter	Parameter values		
	One- compartment	Two-compartment	Three-compartment
F	1	1	1
D	1	1	1
k_a	0.5	0.5	0.5
k	0.1		
V_c	10	2	2
α		0.2	0.2
β		0.05	0.05
γ			0.01
k_{21}		0.1	0.1
k_{31}			0.15
t	0:100	0:100	0:100

F is bioavailability; D is the dose; k_a is the absorption rate constant; k is the elimination rate constant; V_c is the central compartment volume; t is time; k_{21} and k_{31} are the first-order transfer rate constants of a drug from the peripheral compartment volume to a central compartment volume; α is a macro-constant that describes the initial decline in drug concentrations. In a two-compartment model β is a macro-constant that describes the terminal decline in drug concentrations and in a three-compartment model it describes the intermediate decline in drug concentrations; γ is a macro-constant that describes the terminal decline in a three-compartment model.

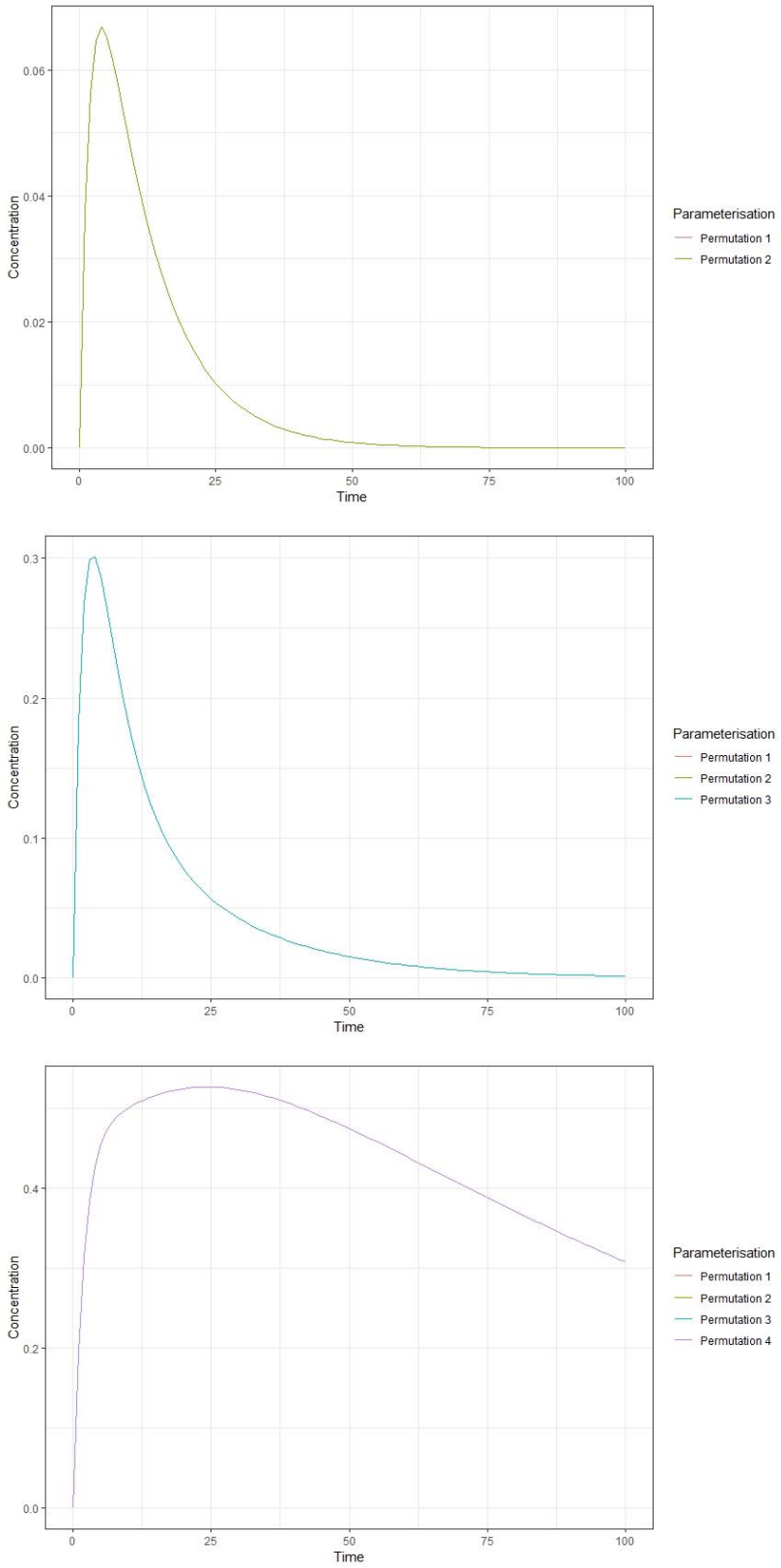


Figure A6.1 *Simulations of permutations that provide the same input-output relationship for a one- (top), two- (middle) and three- (bottom) compartment model.*

A6.2. Plasma metformin concentration time profiles of data analysed stratified by study

In this section the plasma metformin concentration time profiles stratified by study are presented. This comprised data from the following sources:

1. Dunedin Public Hospital
2. Middlemore Hospital
3. Metformin pharmacokinetic study by Pentikainen et al

Dunedin Public Hospital. Plasma metformin concentration data sourced from the Dunedin Public Hospital are shown in Figures A6.2 and A6.3. Figure A6.2 is a plot of the metformin plasma concentration time profiles stratified by study participants, whilst Figure A6.3 is a plot of the concentration profiles of study participants stratified by their study enrolment group.

Middlemore Hospital. Metformin concentration data sourced from Middlemore Hospital are shown in Figures A6.4 and A6.5. Figure A6.4 is a plot of the plasma metformin concentration time profiles stratified by study participants and Figure A6.5 is a plot of the plasma metformin concentration time profiles collected from the study participants stratified by the dose of metformin received.

Metformin pharmacokinetic study by Pentikainen et al. The plasma metformin concentrations following oral administration of metformin in the study by Pentikainen et al is shown in Figure A6.6. The metformin plasma concentrations following intravenous administration of metformin is shown in Figure A6.7.

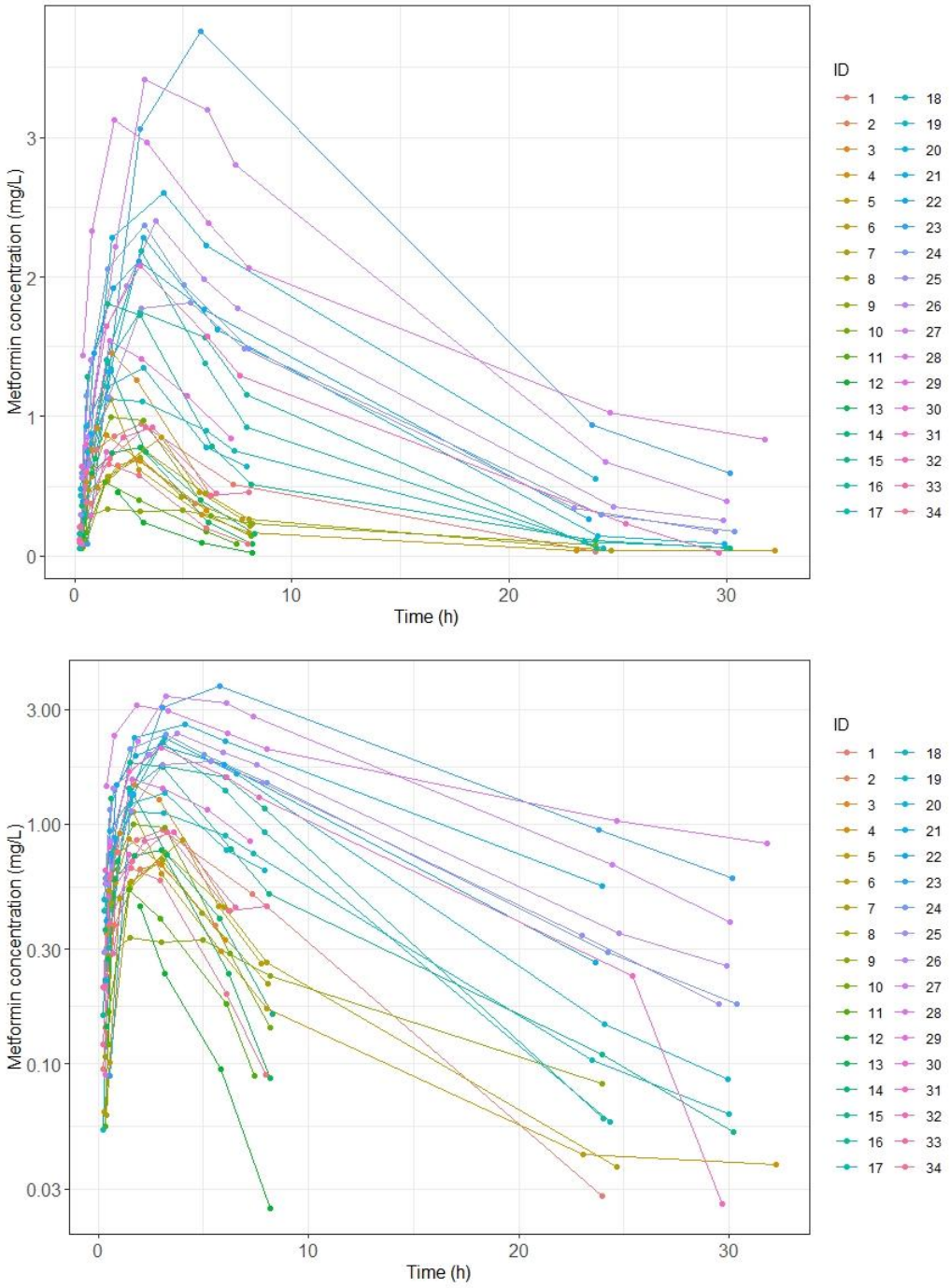


Figure A6.2 *Plasma metformin concentrations of study participants in the Dunedin Study. The top plot is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant.*

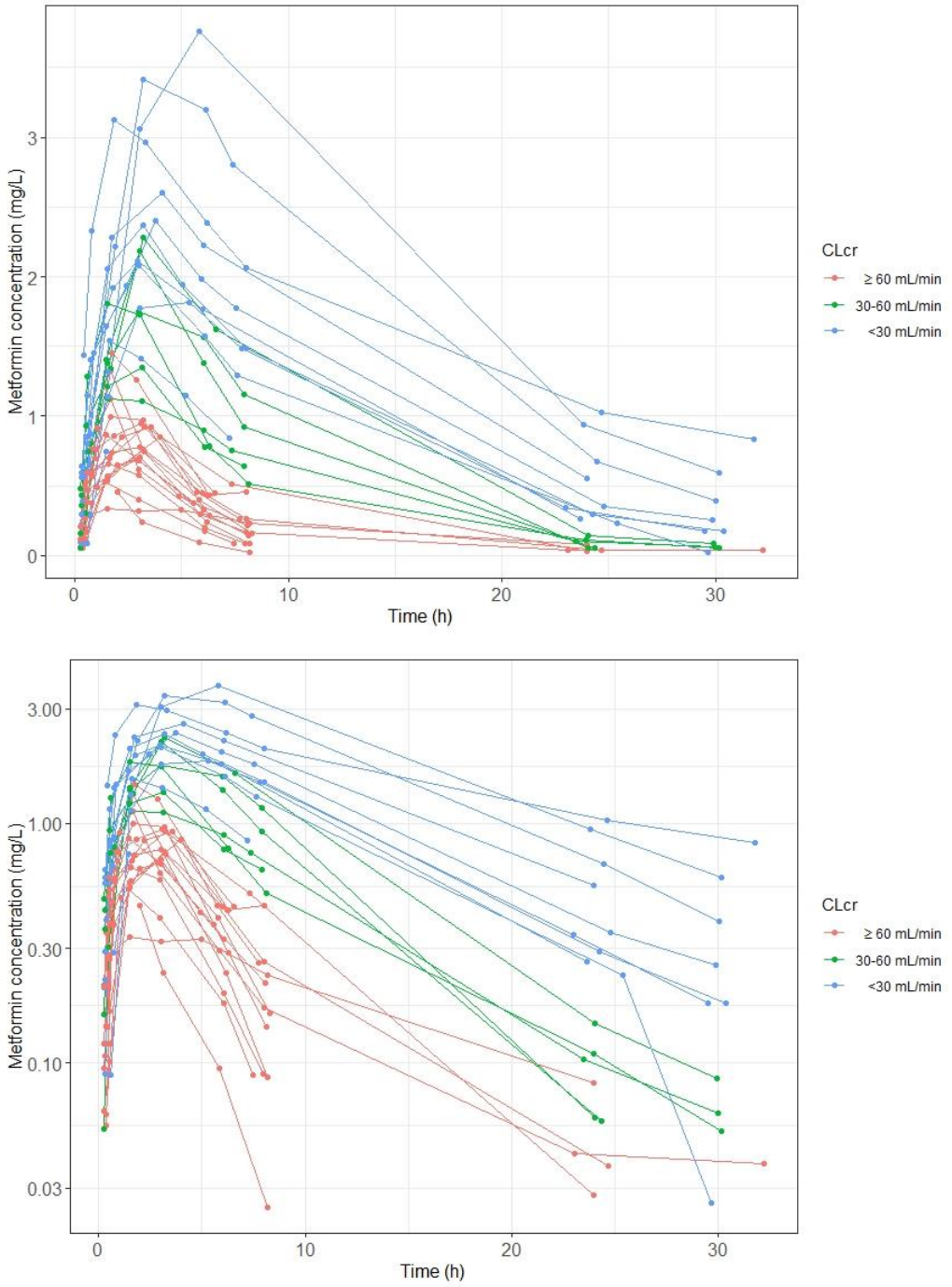


Figure A6.3 *Plasma metformin concentrations of study participants in the Dunedin Study stratified by renal enrolment groups. The top plot is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant.*

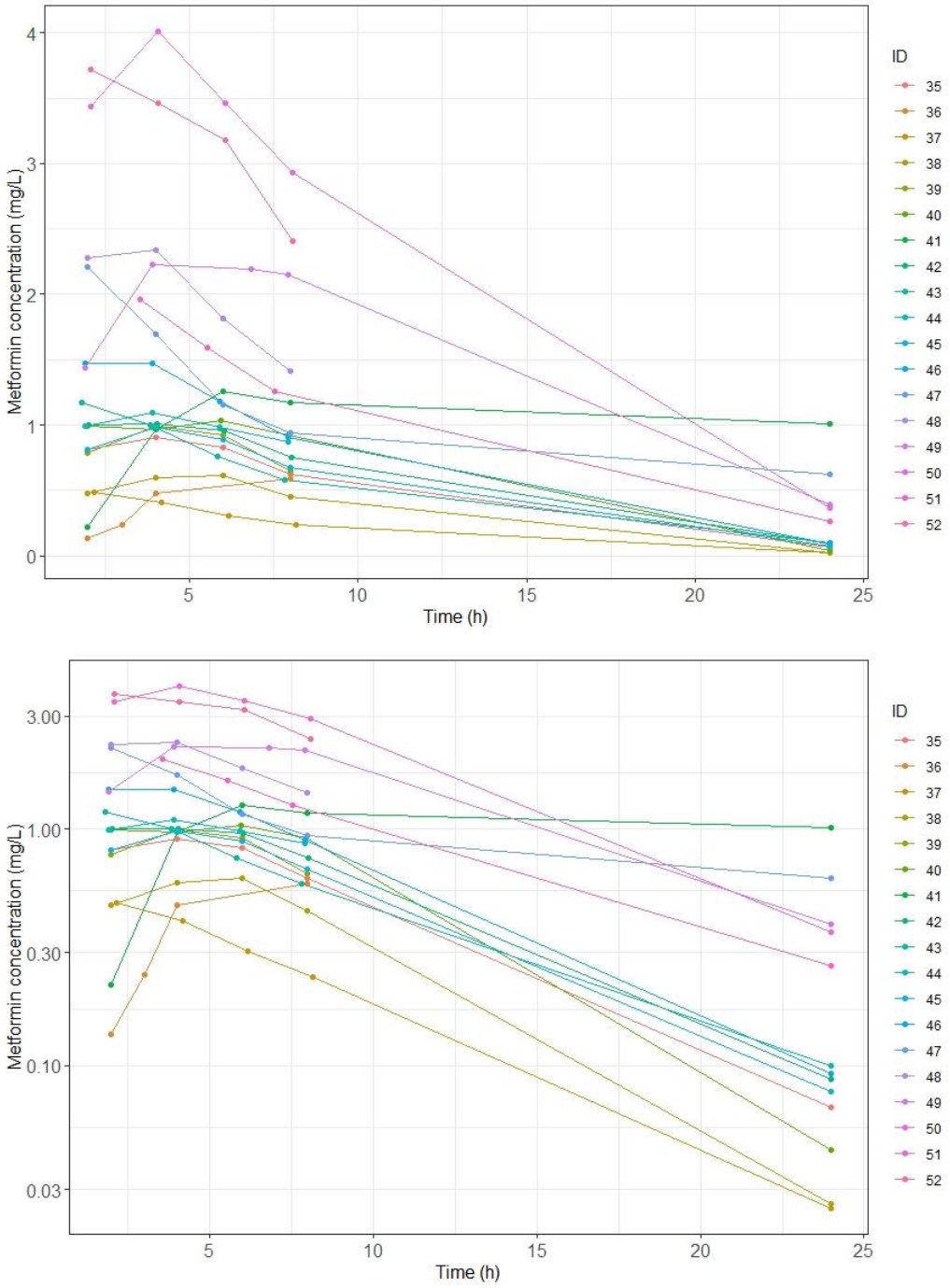


Figure A6.4 *Plasma metformin concentrations of study participants in the Middlemore Study. The top graph is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant*

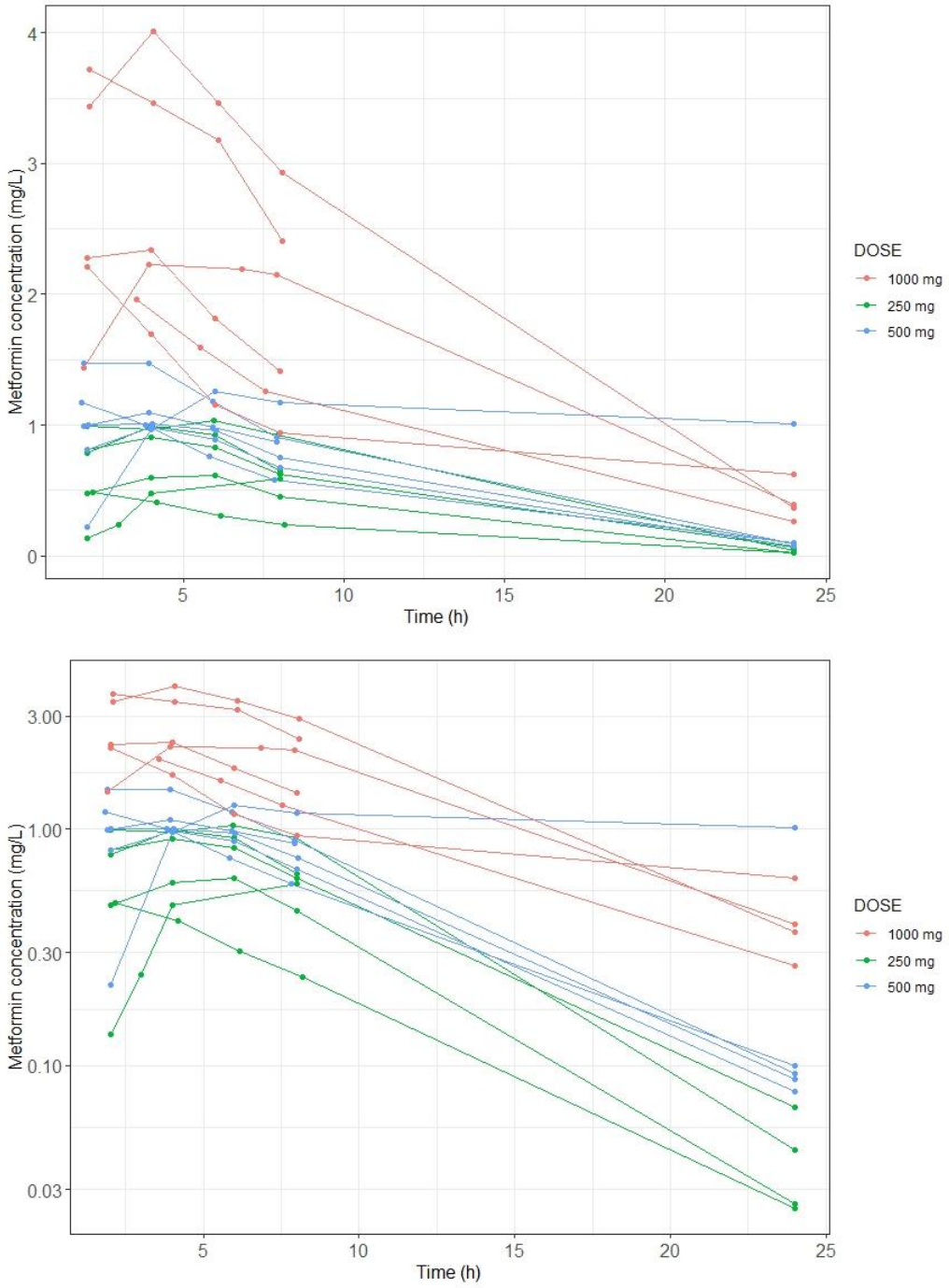


Figure A6.5 *Plasma metformin concentrations of study participants in the Middlemore Study stratified by metformin dose received. The top graph is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant.*

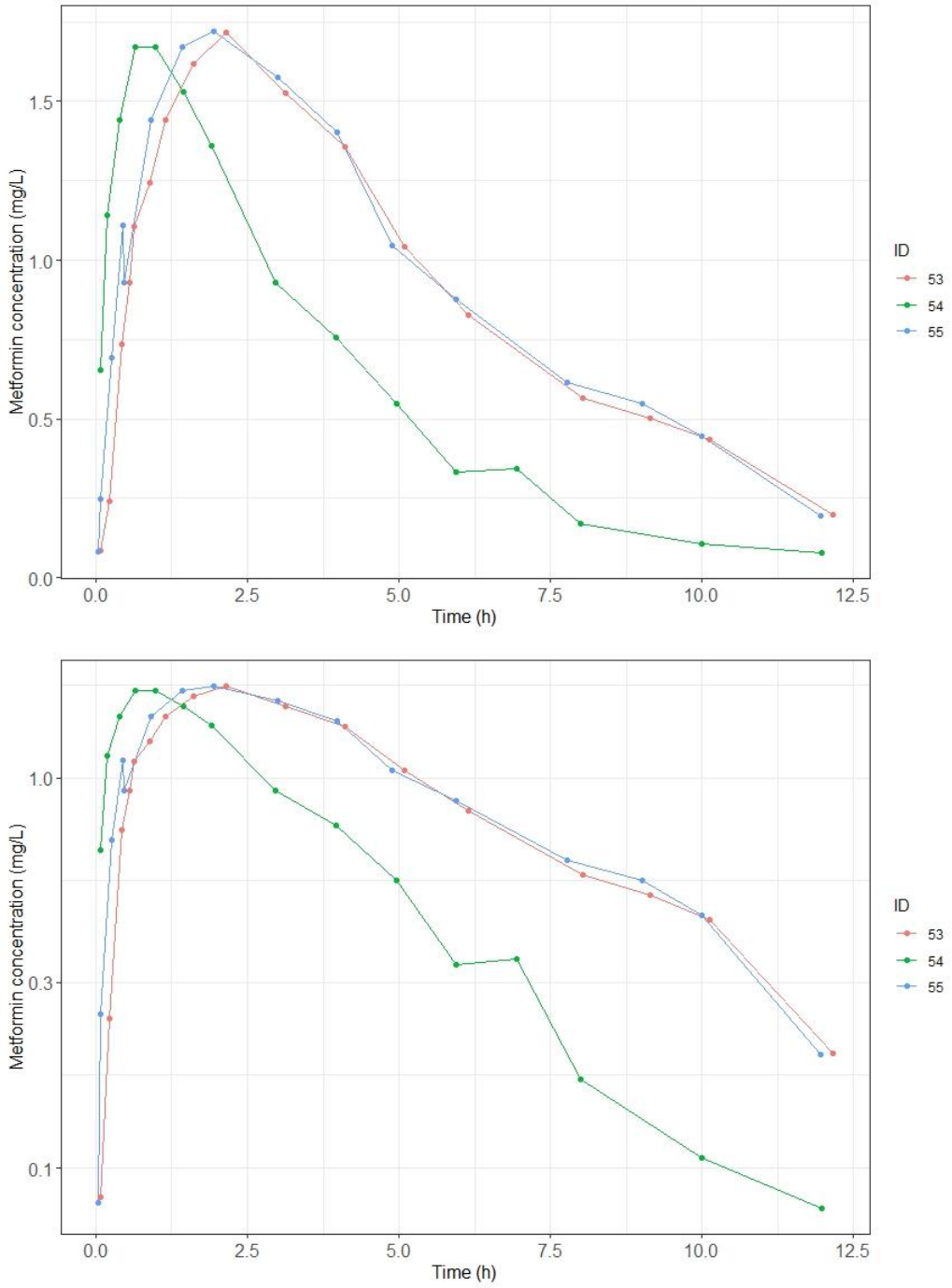


Figure A6.6 *Plasma metformin concentrations of study participants in the Pentikainen et al study following a single oral metformin dose. The top graph is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant.*

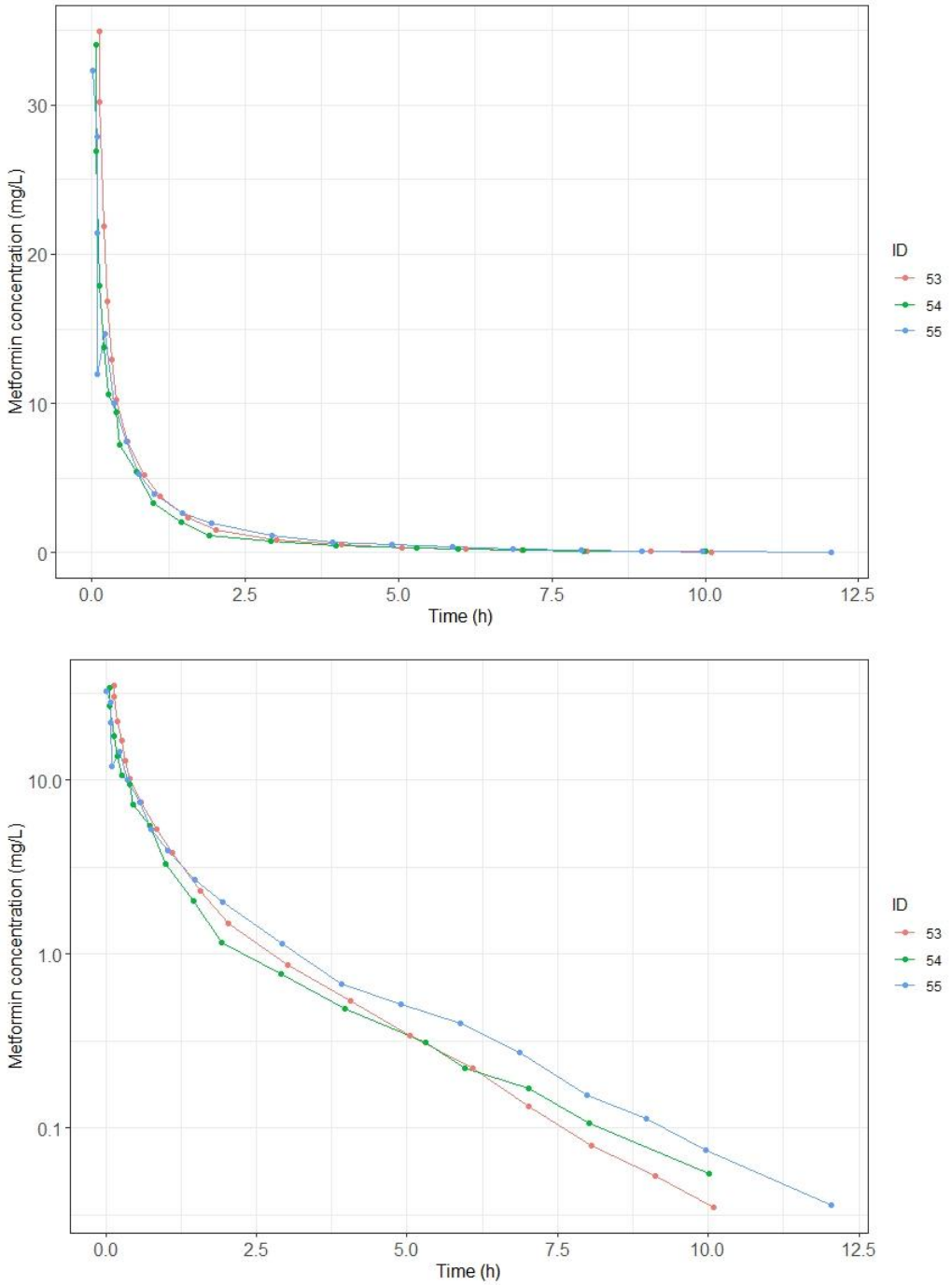


Figure A6.7 *Plasma metformin concentrations of study participants in the Pentikainen et al study following an intravenous dose of metformin. The top graph is a Cartesian plot and the bottom is a semi log plot. The dots represent single measures of plasma metformin concentration and the lines link repeated measures data for a single participant.*

A6.3. Model evaluation

In this section, the following diagnostic and evaluation plots are presented for the models explored in Chapter 8:

i. Prediction corrected visual predictive check (section A6.3.1.)
ii. Goodness of fit plots (section A6.3.2.)
iii. ETA distributions (section A6.3.3.)

A6.3.1. Prediction corrected visual predictive check

A prediction corrected visual predictive check (pcVPC) for the base model parameterised using CL, V and k_a is shown in Figure A6.8. The median, 5th and 95th percentiles of the population pharmacokinetic model predicted plasma metformin concentrations follow the percentiles of the data well, suggesting that a one-compartment model provided a good fit to the data.

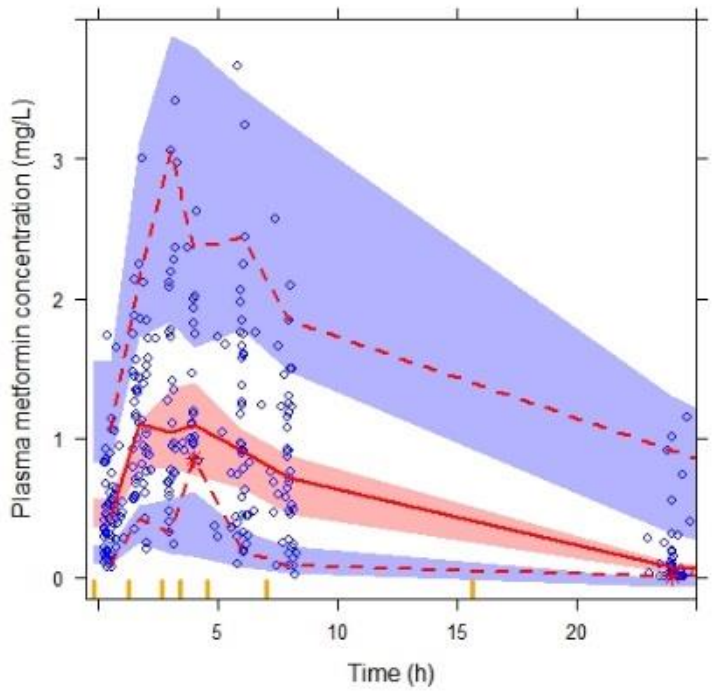


Figure A6.8 *Prediction corrected visual predictive check of the unconstrained base model parameterised using CL, V, k_a developed using plasma metformin concentration data following oral administration only*

A6.3.2. Goodness of fit plots

For the models developed in Chapter 8 the following goodness of fit plots were graphed:

1. DV versus PRED
2. DV versus IPRED
3. CWRES versus PRED
4. CWRES versus TIME

In these plots, DV stands for dependent variable and is the observed metformin concentration (mg/L), PRED stands for prediction and is the model predicted metformin concentration (mg/L), IPRED is the individual prediction of metformin concentration (mg/L), CWRES stands for conditional weighted residuals and TIME is time post metformin ingestion in hours.

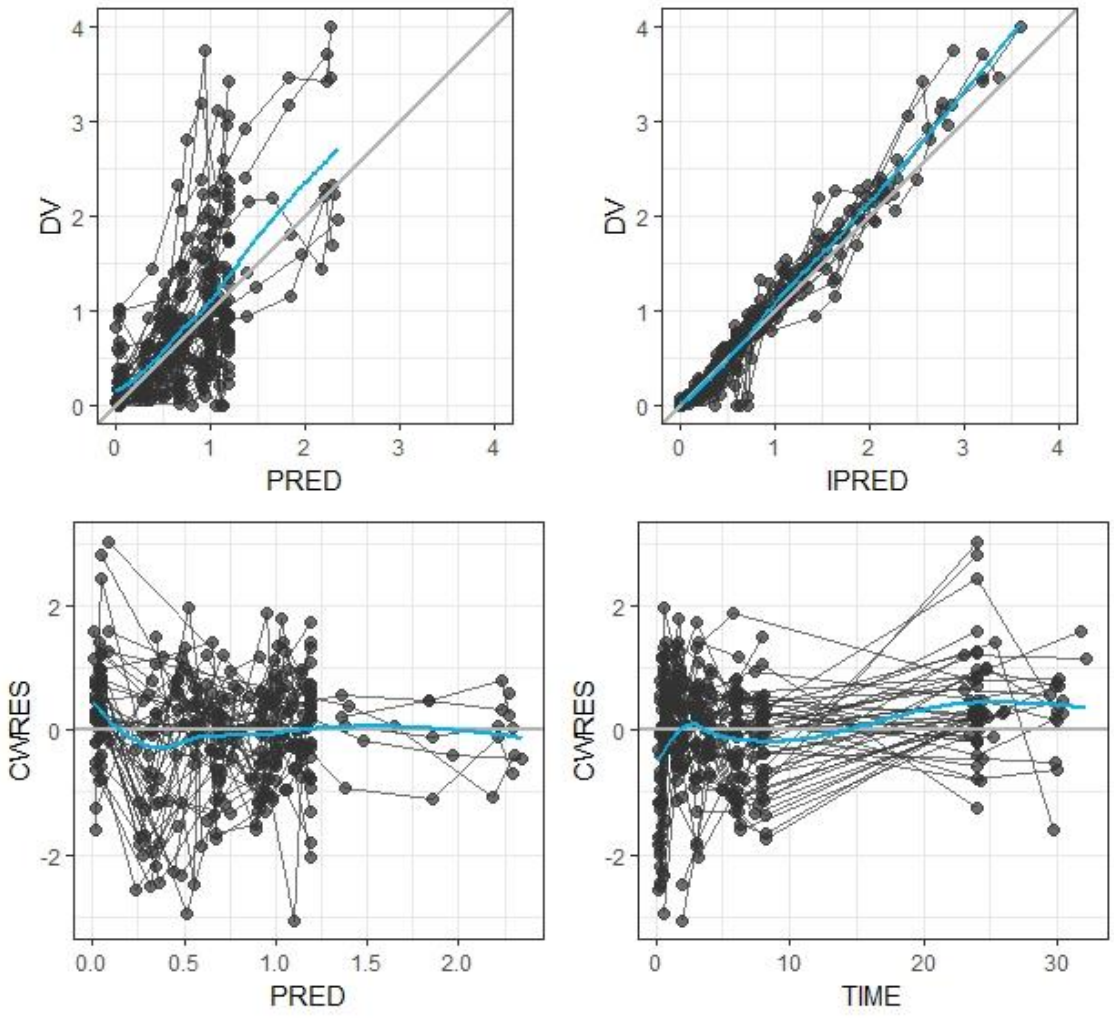


Figure A6.9 *Goodness of fit plots for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral metformin administration only.*

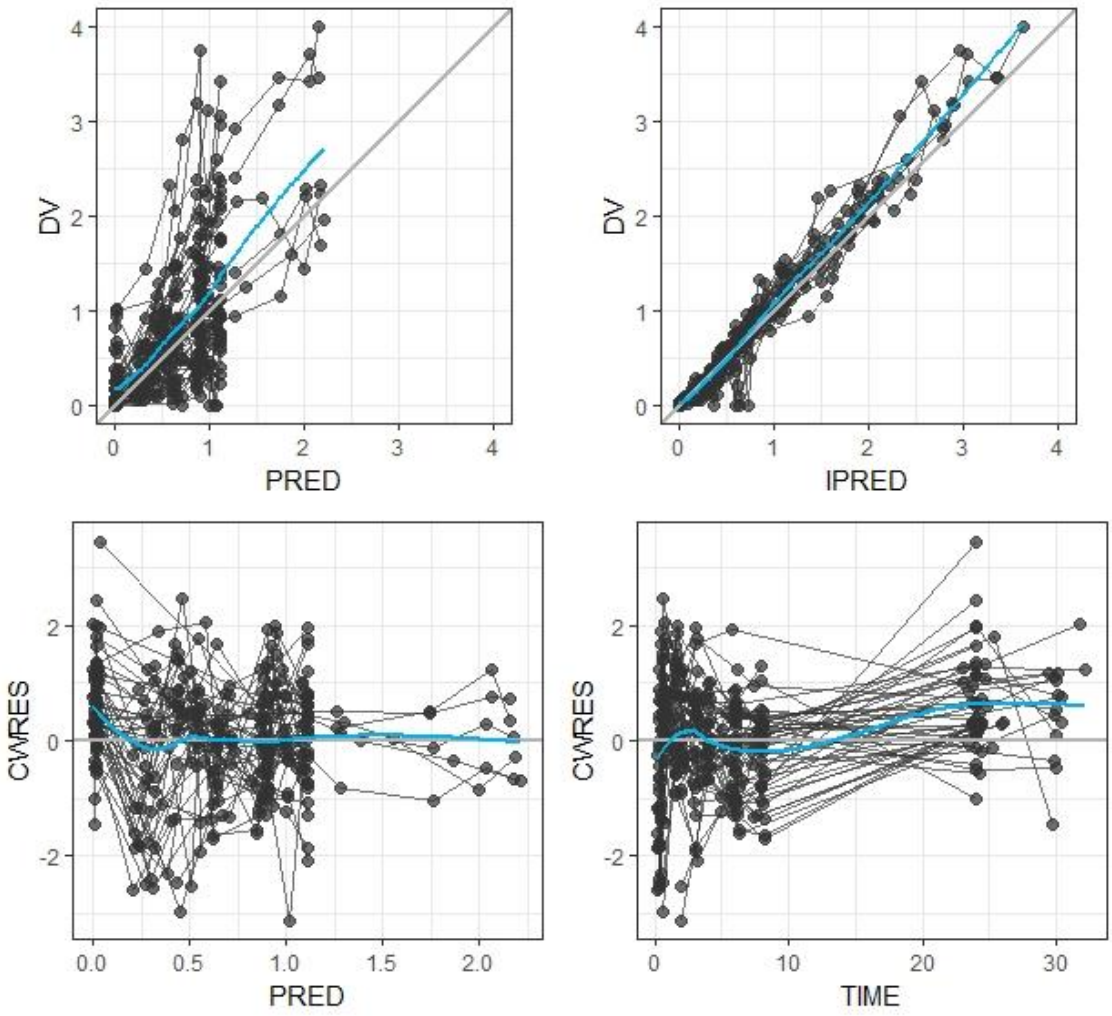


Figure A6.10 *Goodness of fit plots for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral metformin administration only.*

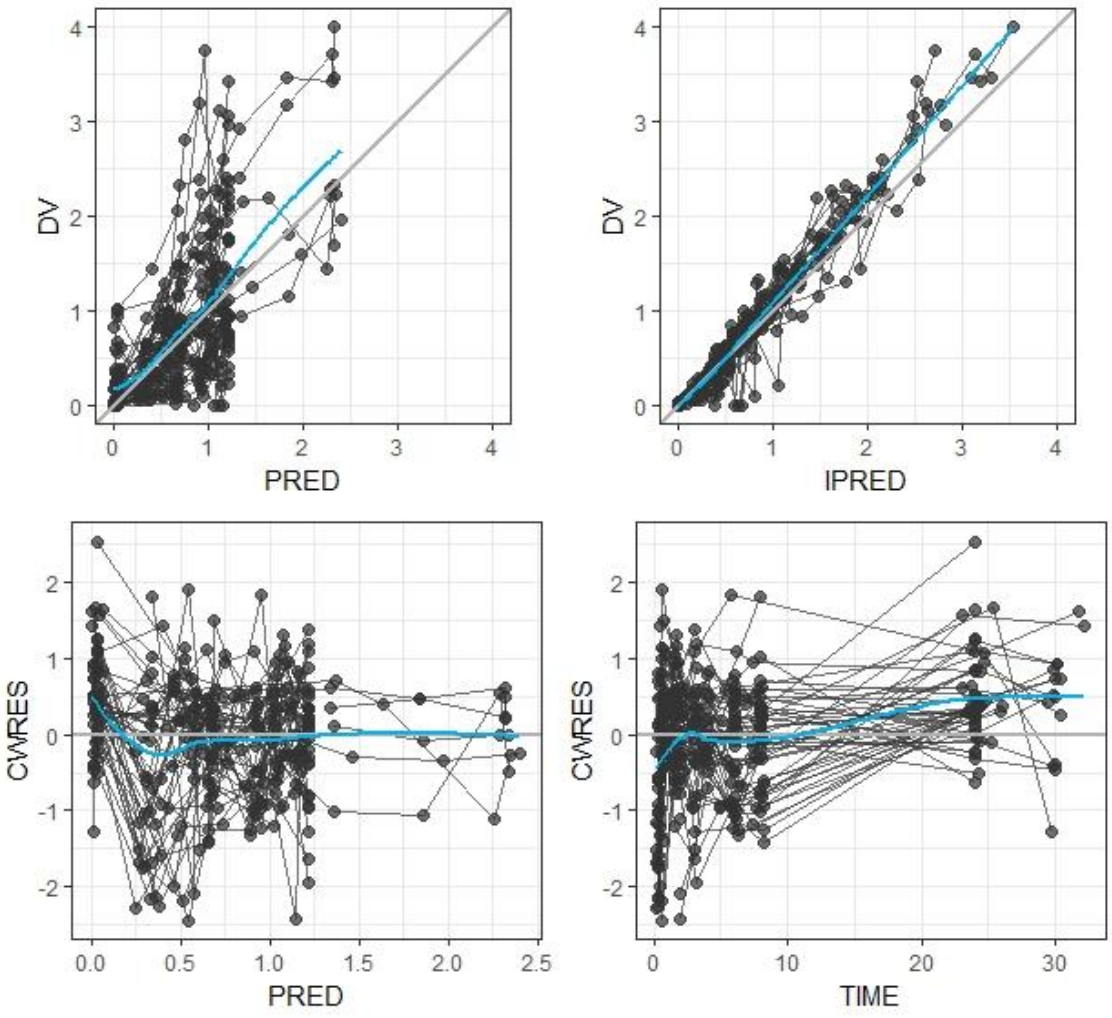


Figure A6.11 *Goodness of fit plots for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral and intravenous metformin administration.*

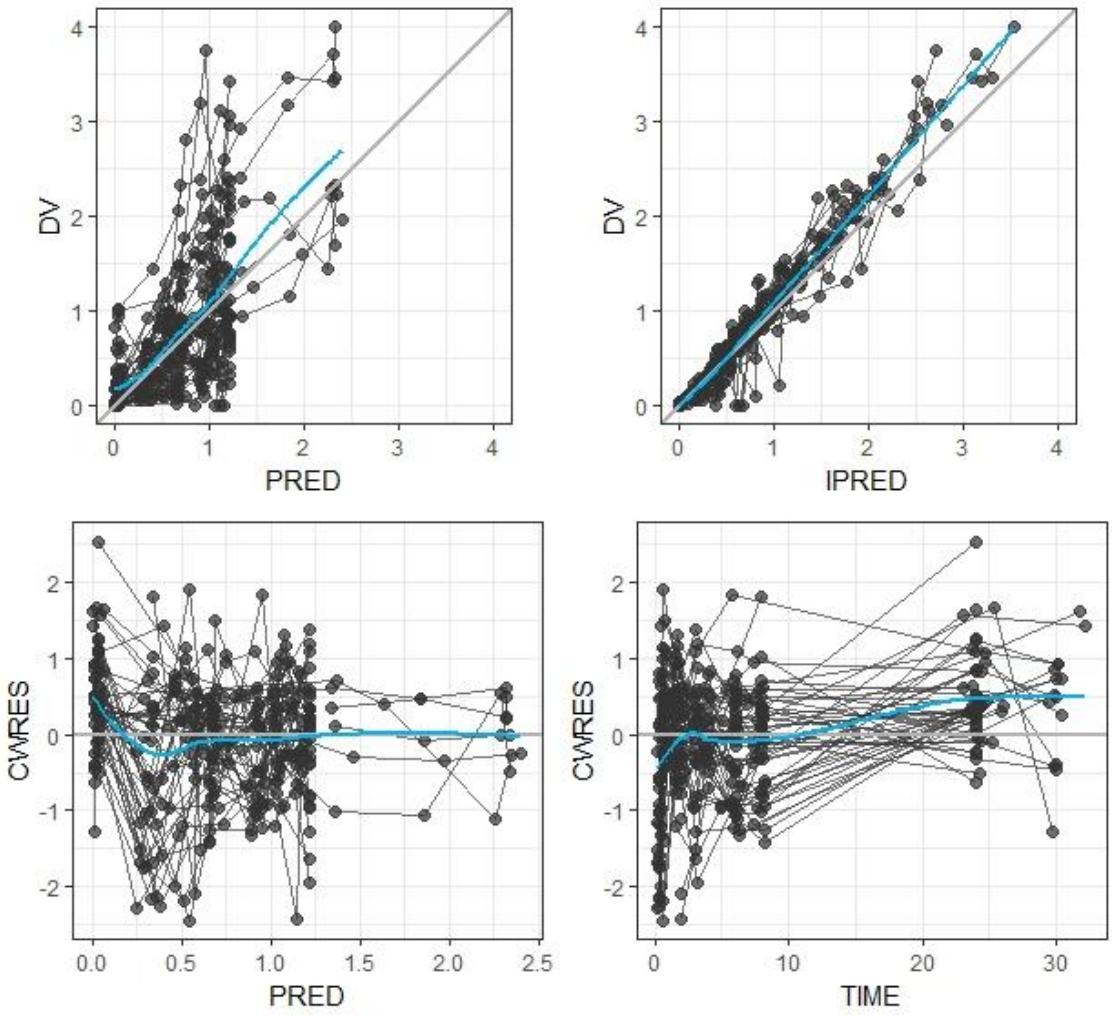


Figure A6.12 *Goodness of fit plots for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral and intravenous metformin administration.*

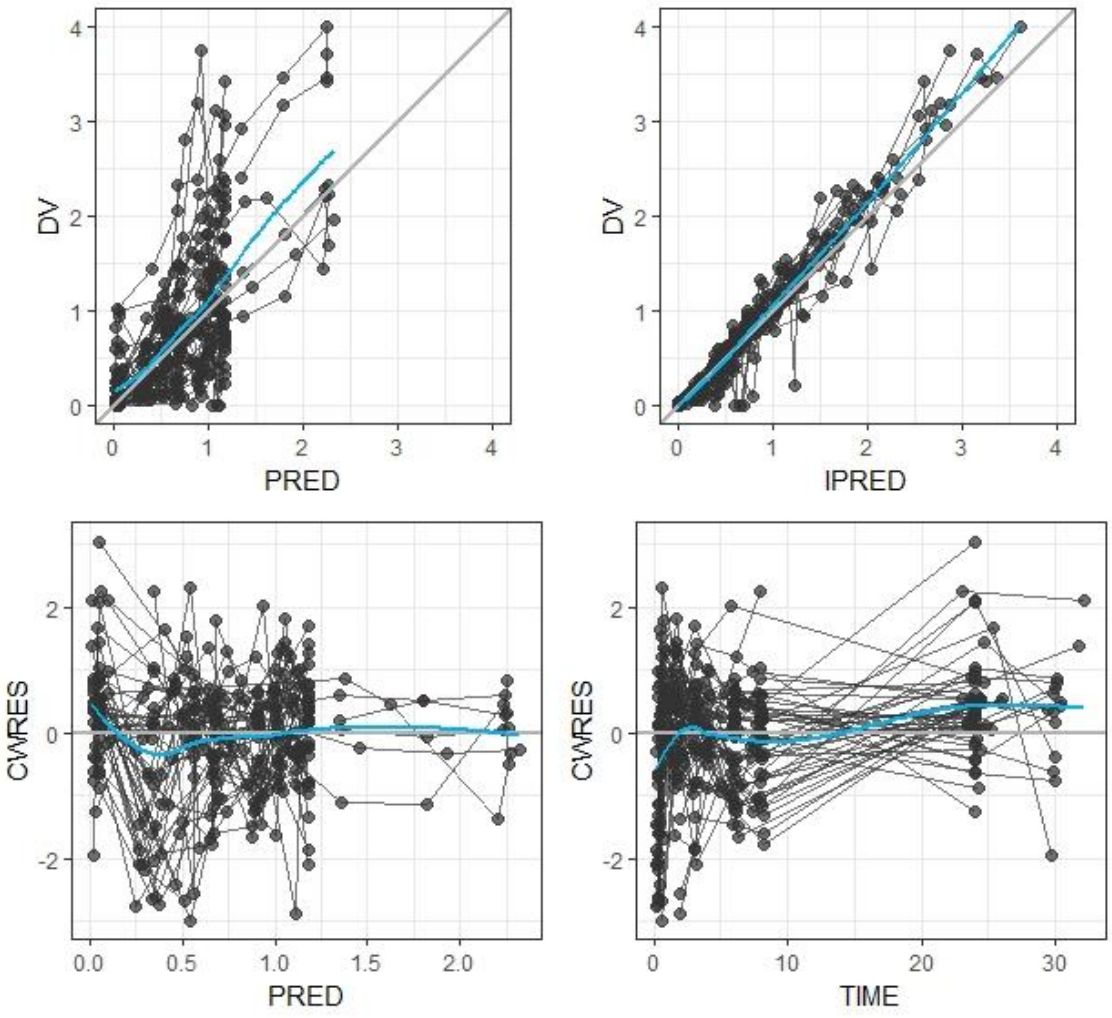


Figure A6.13 *Goodness of fit plots for the unconstrained k, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral metformin administration only.*

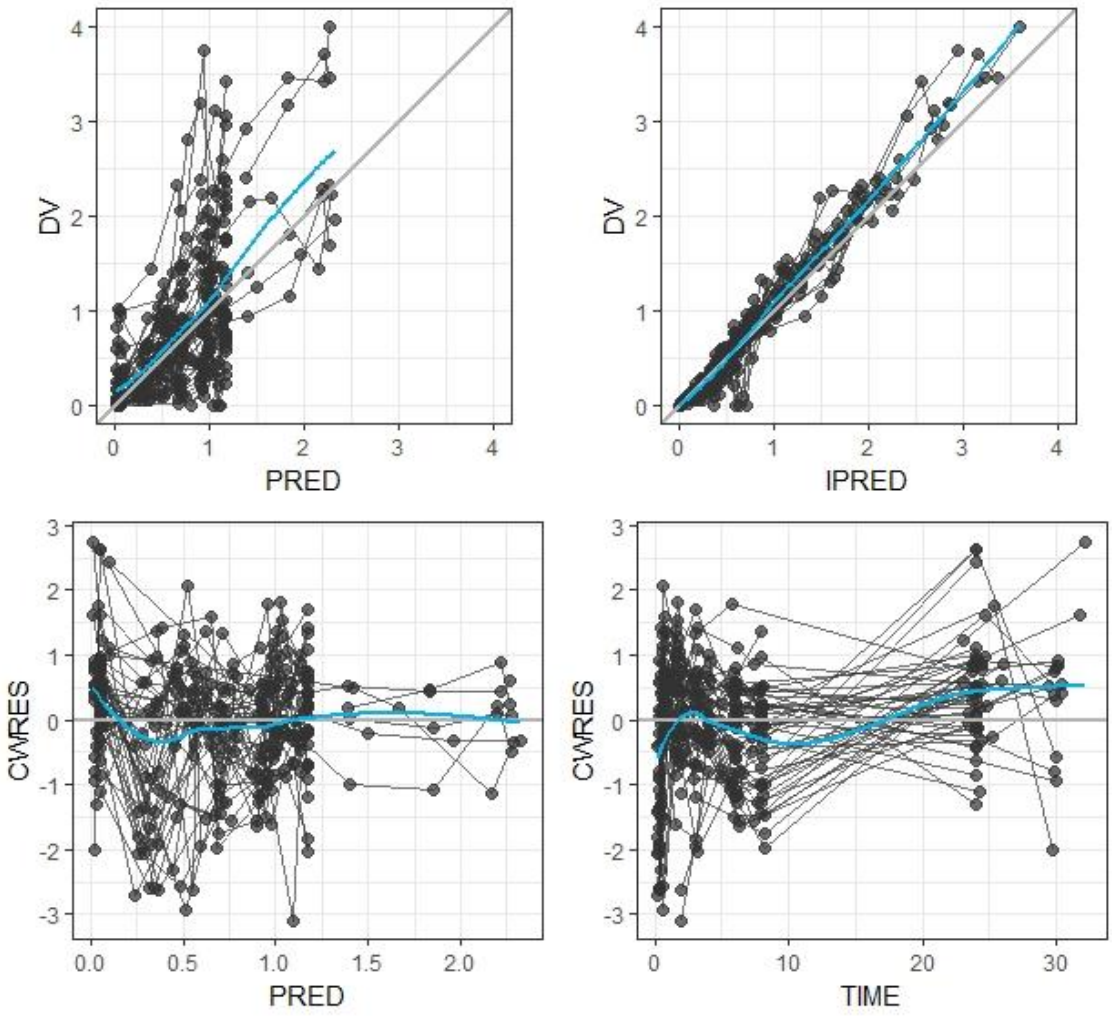


Figure A6.14 *Goodness of fit plots for the unconstrained k, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral metformin administration only.*

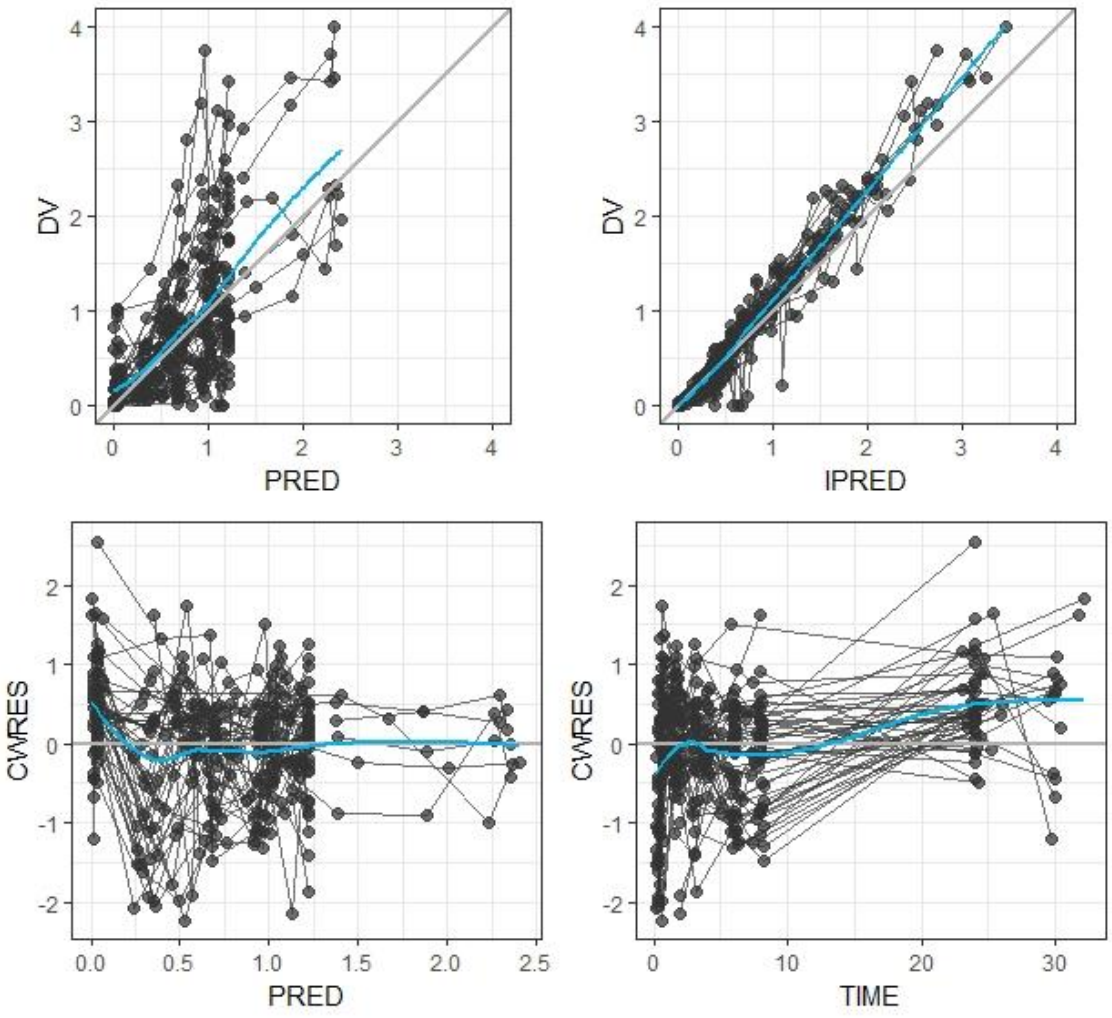


Figure A6.15 *Goodness of fit plots for the unconstrained k, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral and intravenous metformin administration.*

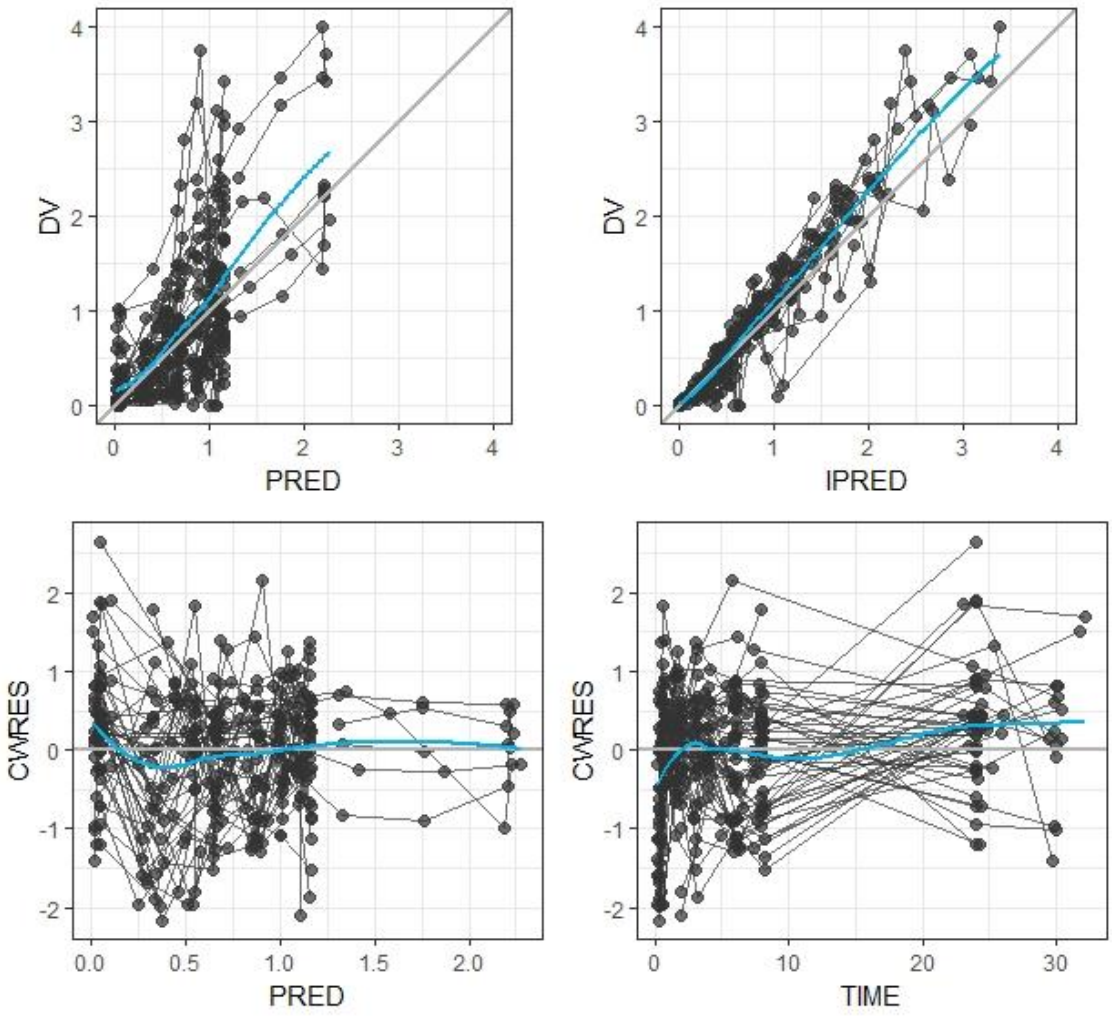


Figure A6.16 *Goodness of fit plots for the unconstrained k, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral and intravenous metformin administration.*

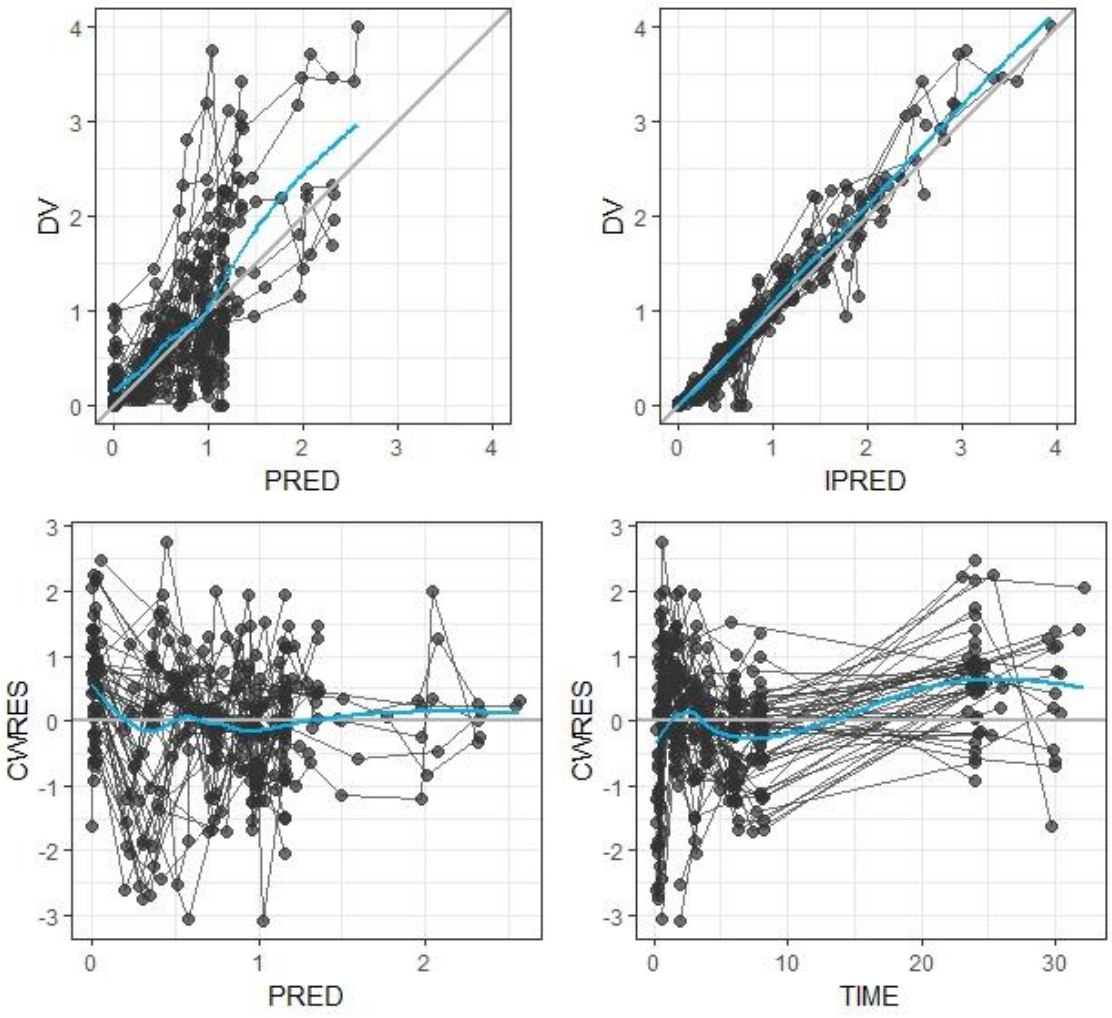


Figure A6.17 *Goodness of fit plots for the partially constrained CL, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only.*

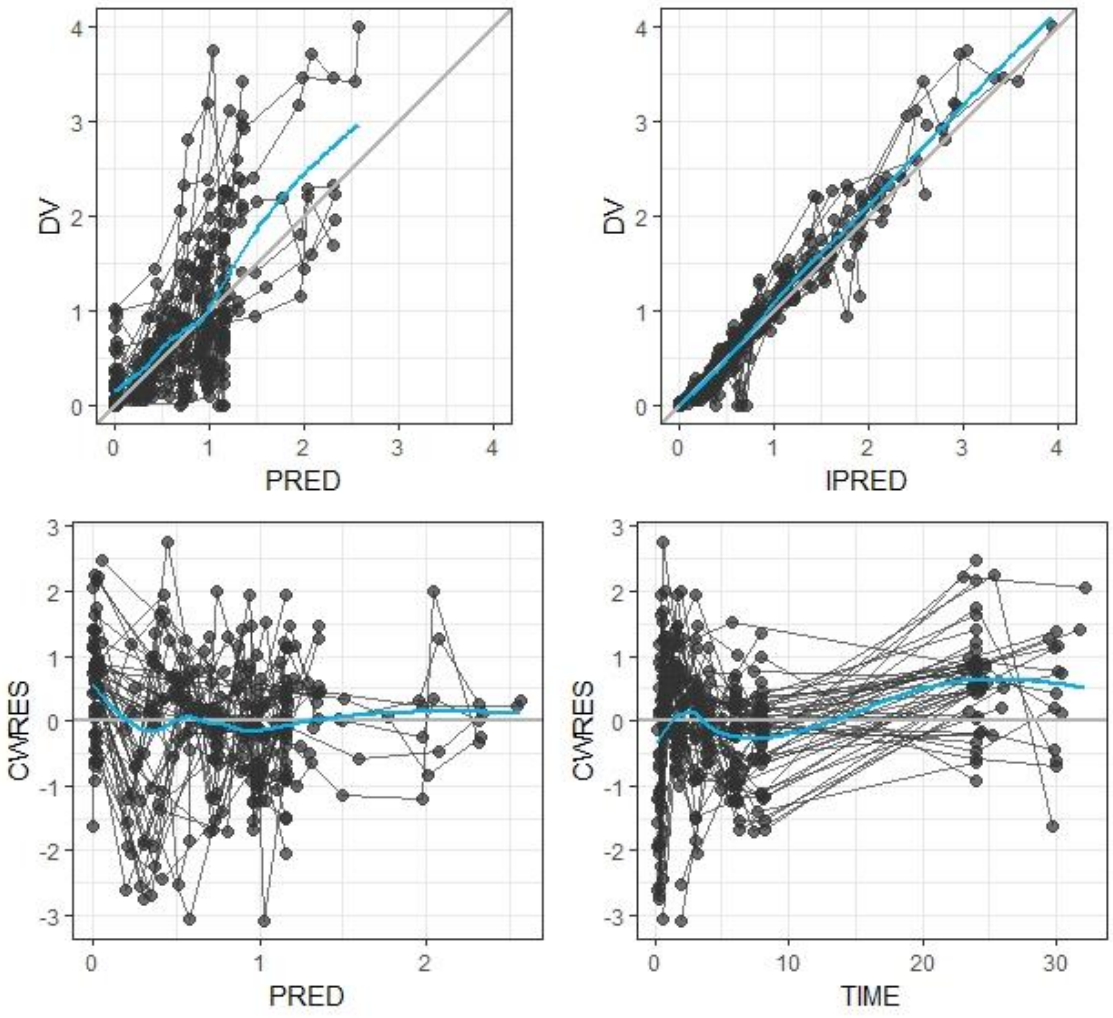


Figure A6.18 *Goodness of fit plots for the partially constrained CL, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration.*

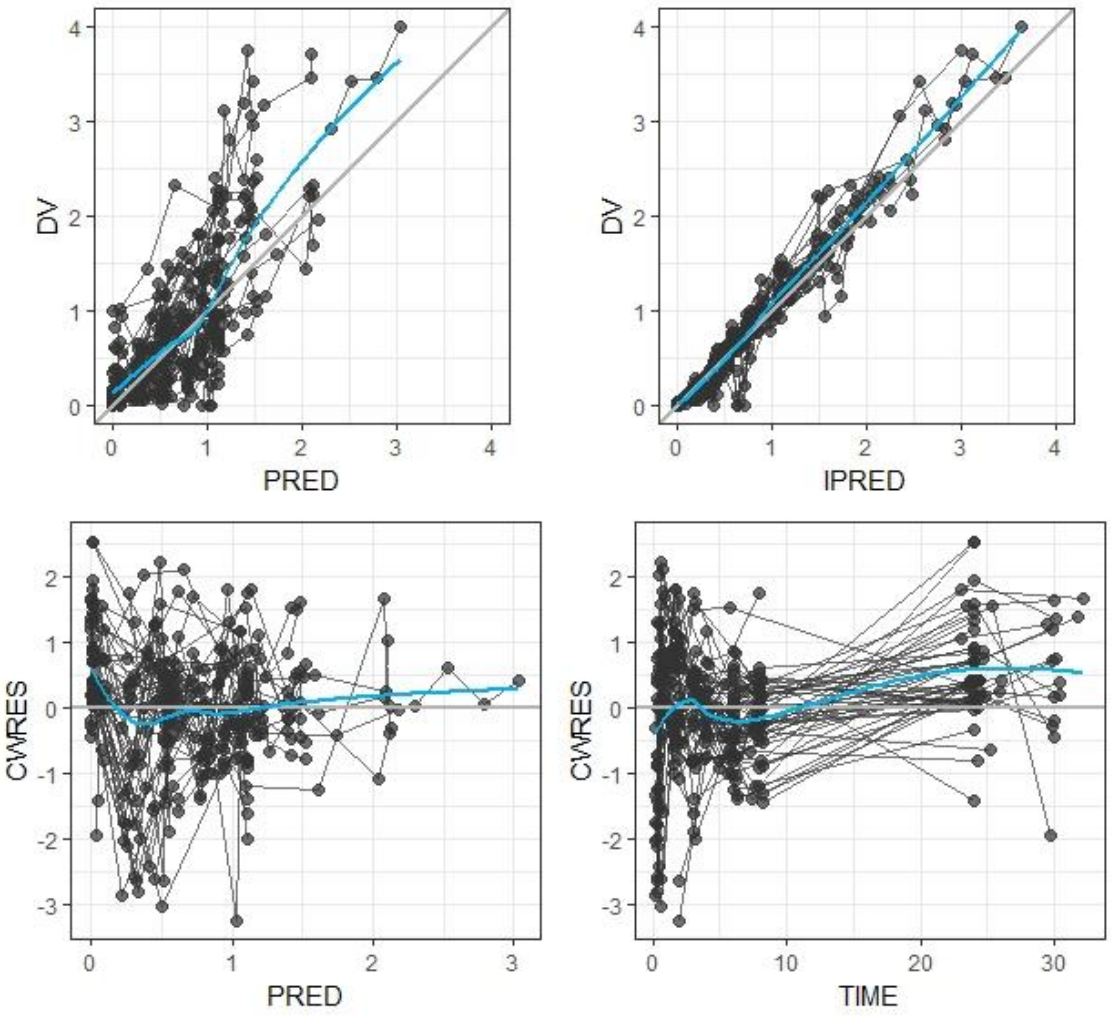


Figure A6.19 *Goodness of fit plots for the partially constrained k, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only.*

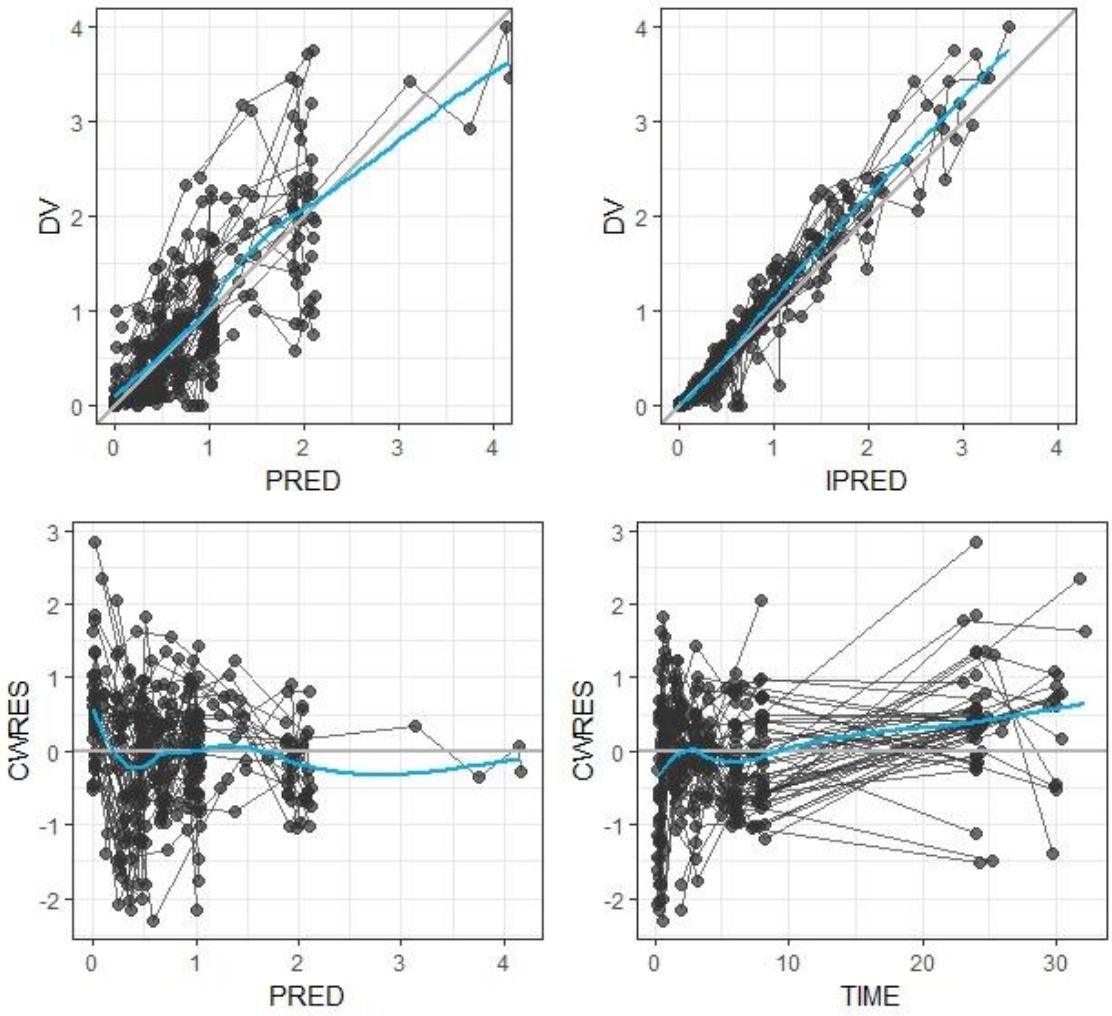


Figure A6.20 *Goodness of fit plots for the partially constrained k, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration.*

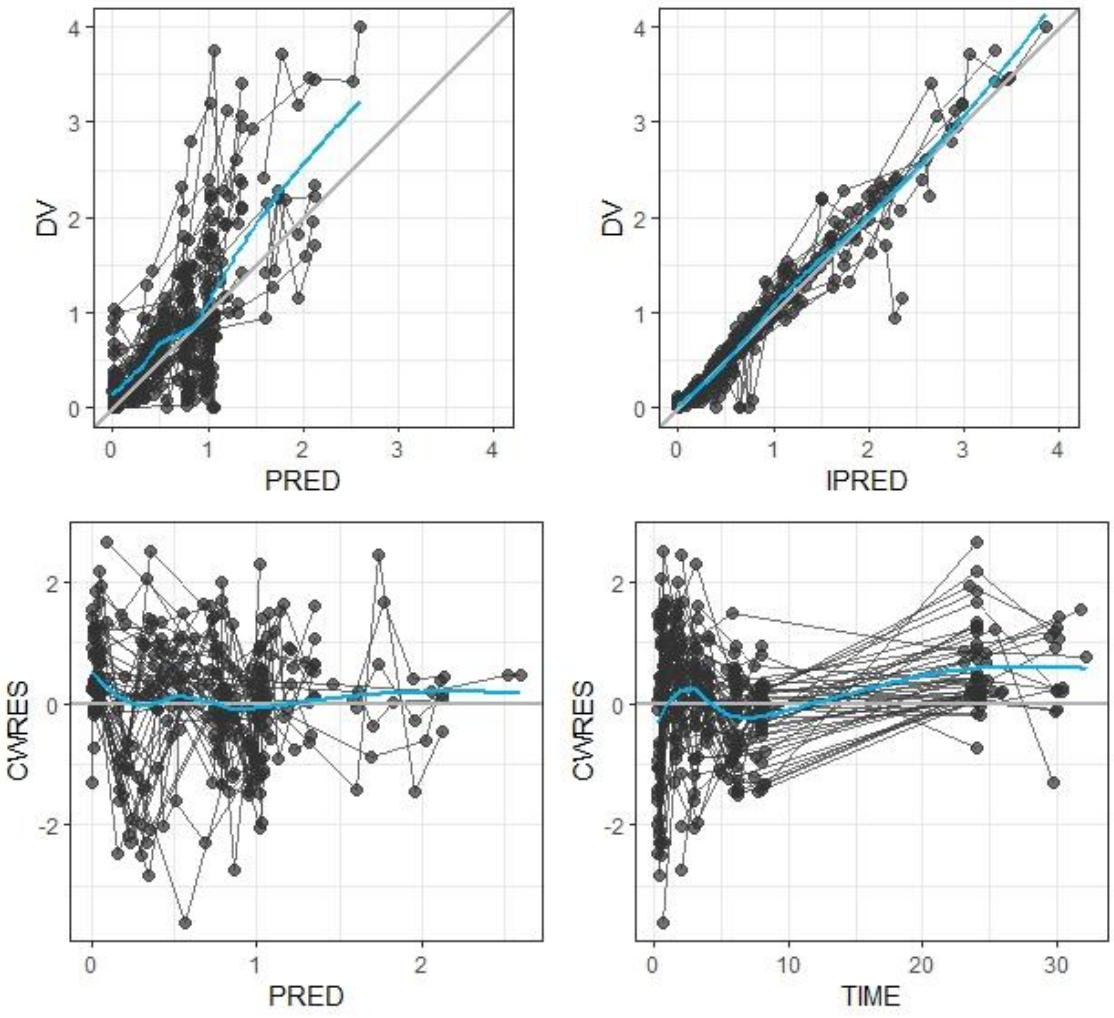


Figure A6.21 *Goodness of fit plots for the fully constrained CL, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only.*

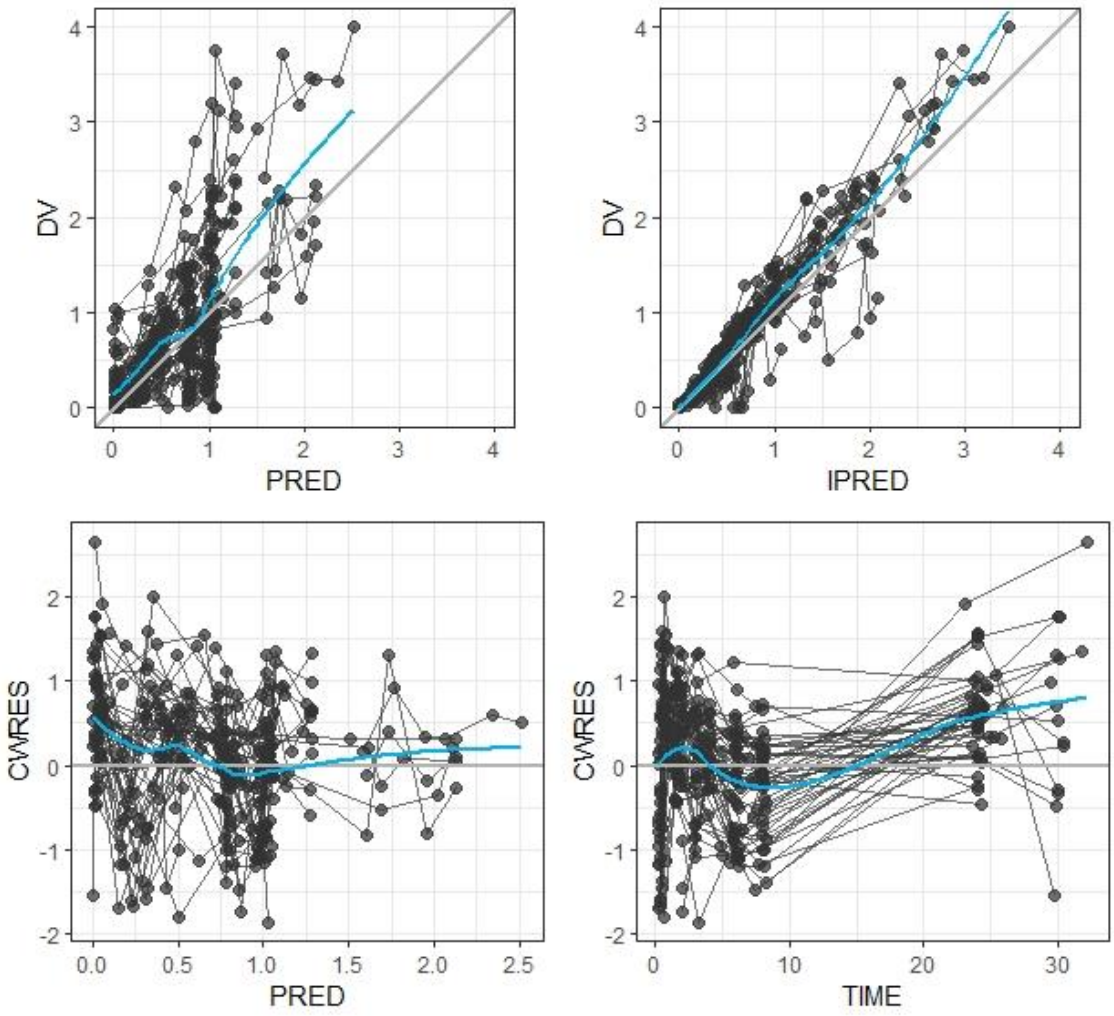


Figure A6.22 *Goodness of fit plots for the fully constrained CL, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration.*

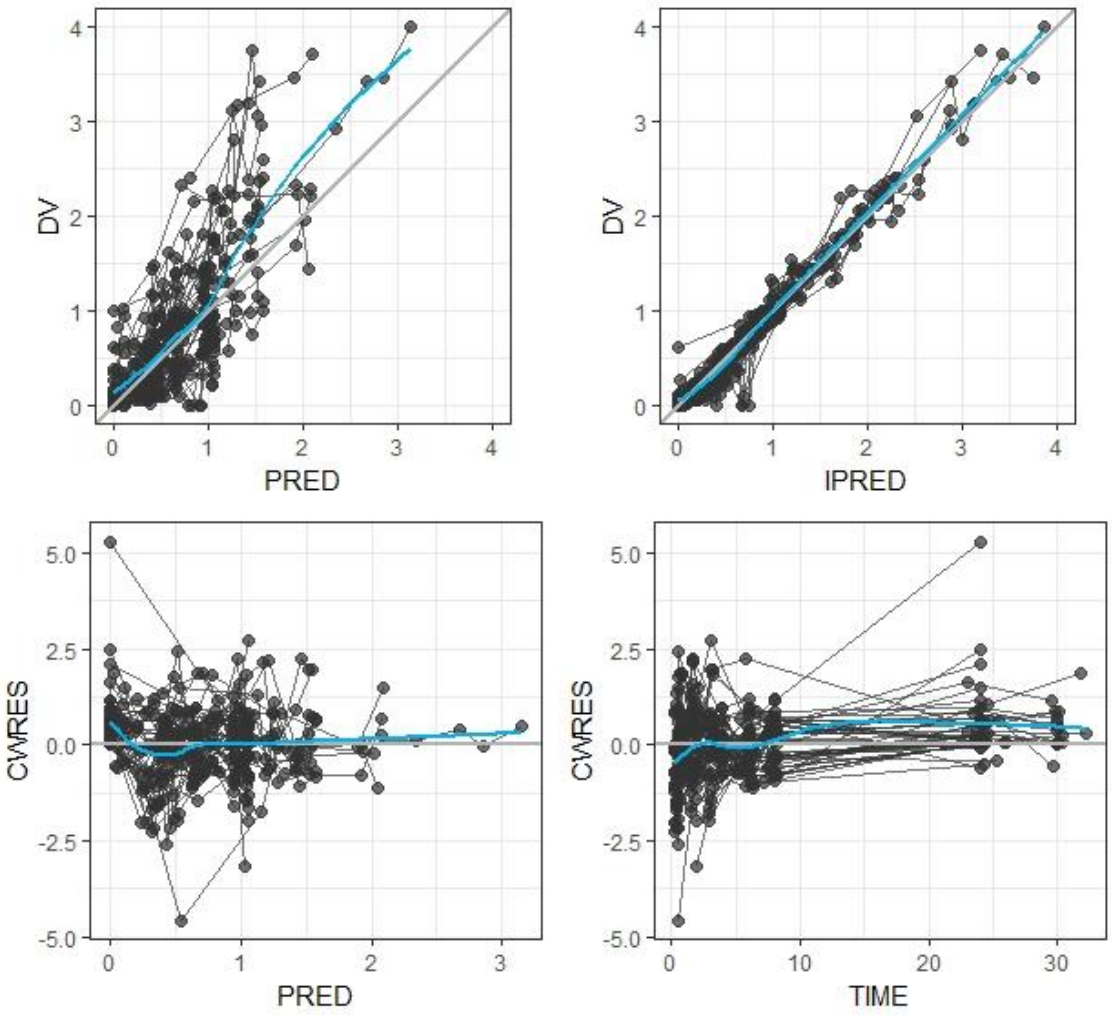
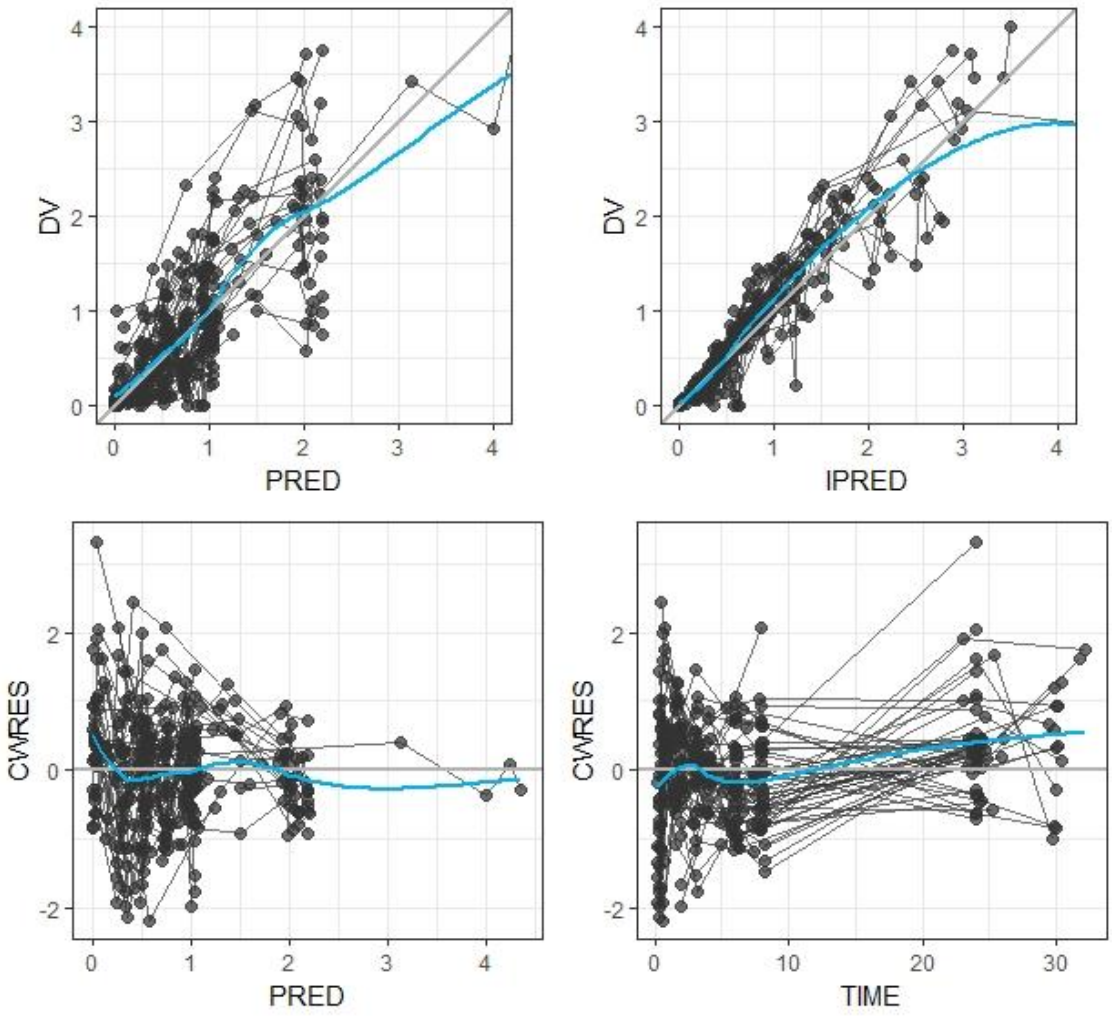


Figure A6.23 *Goodness of fit plots for the fully constrained k, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only.*



FigureA6.24 *Goodness of fit plots for the fully constrained k, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration.*

A6.3.3.η distribution

The following are plots of histograms of individual η values.

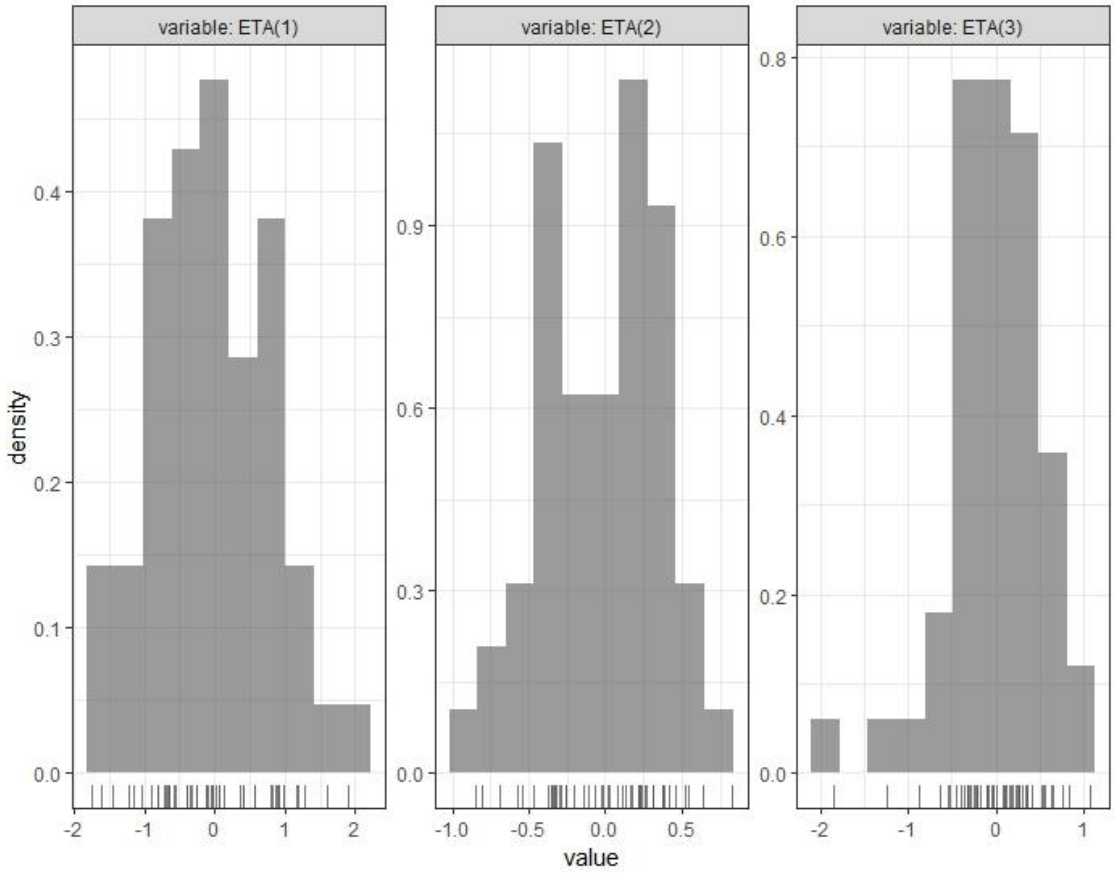


Figure A6.25 *η distribution for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral metformin administration only.*

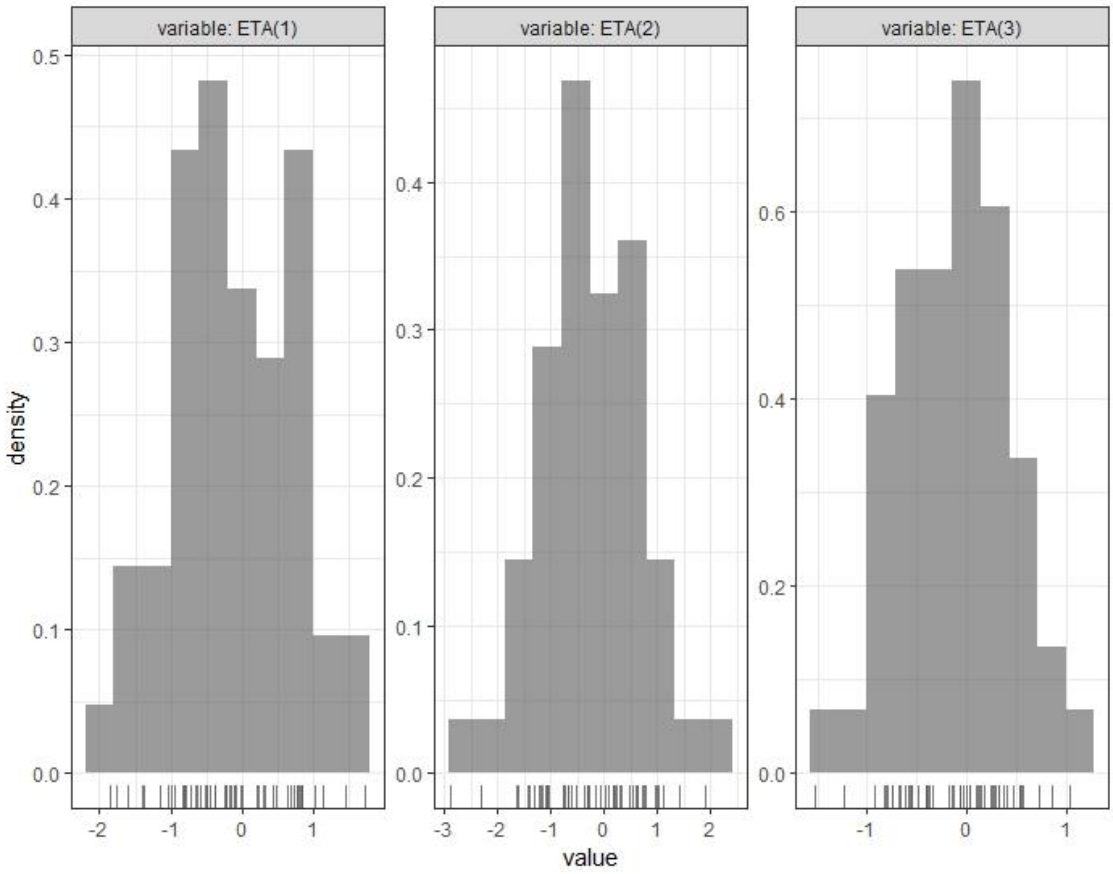


Figure A6.26 *η distribution for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral metformin administration only.*

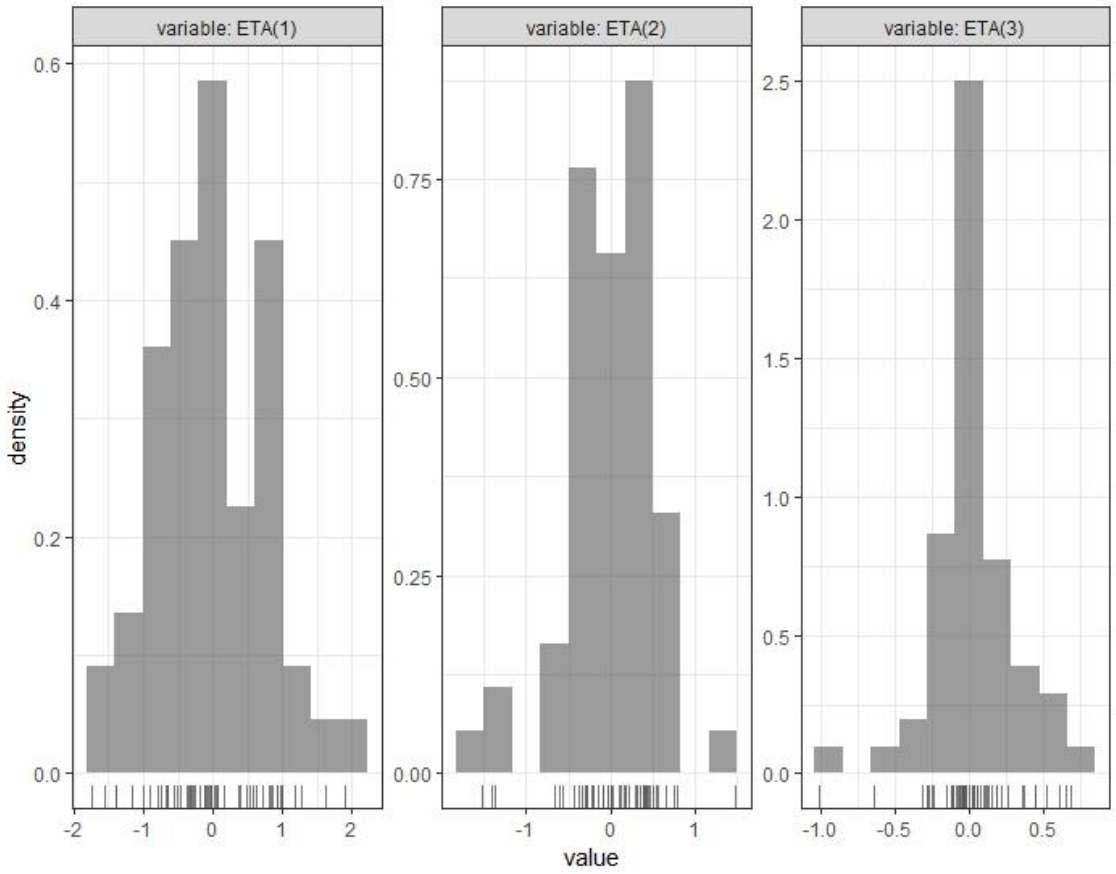


Figure A6.27 *η distribution for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral and intravenous metformin administration.*

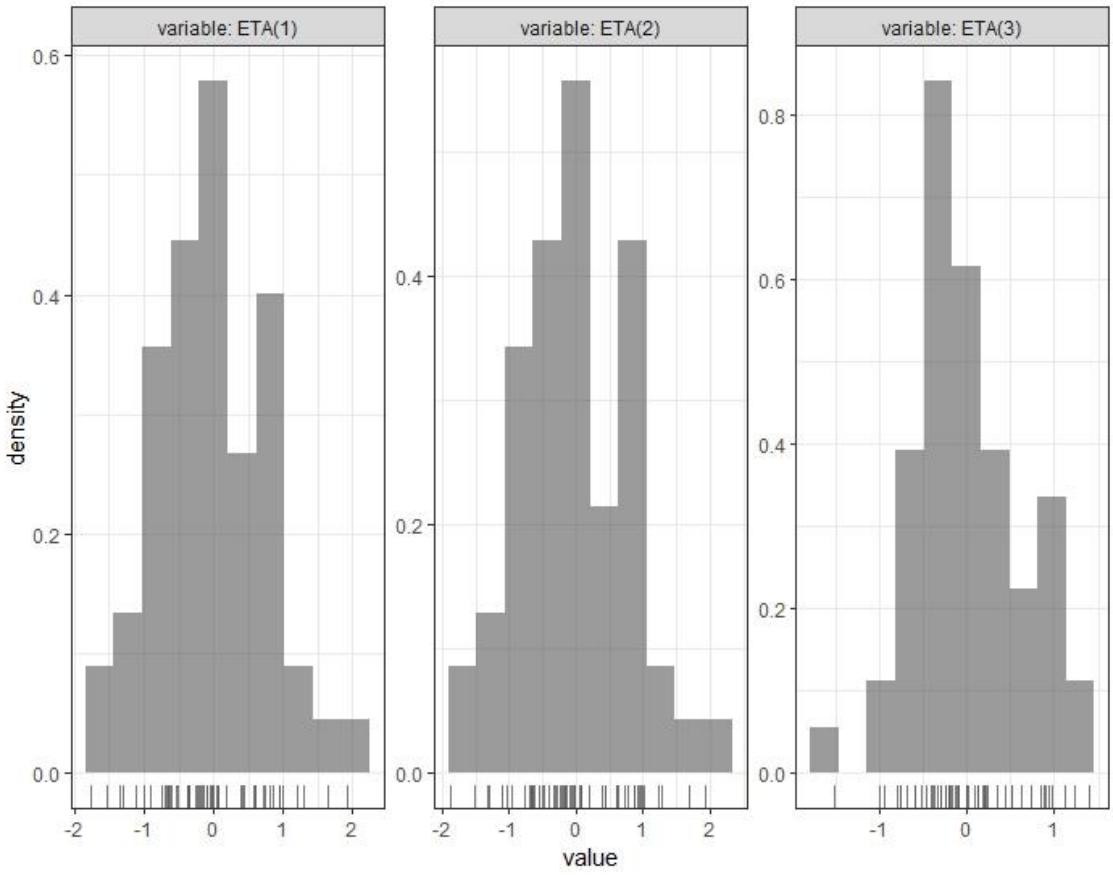


Figure A6.28 *η distribution for the unconstrained CL, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral and intravenous metformin administration.*

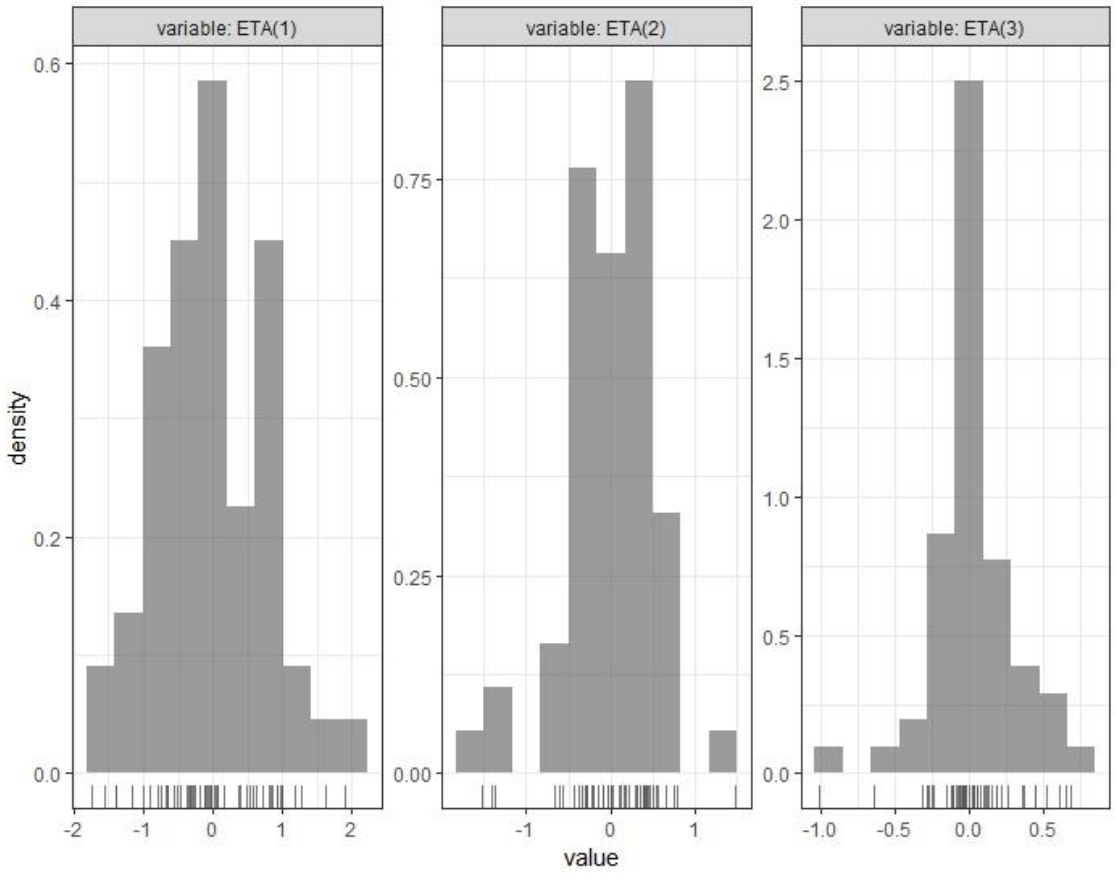


Figure A6.29 *η distribution for the unconstrained k, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral metformin administration only.*

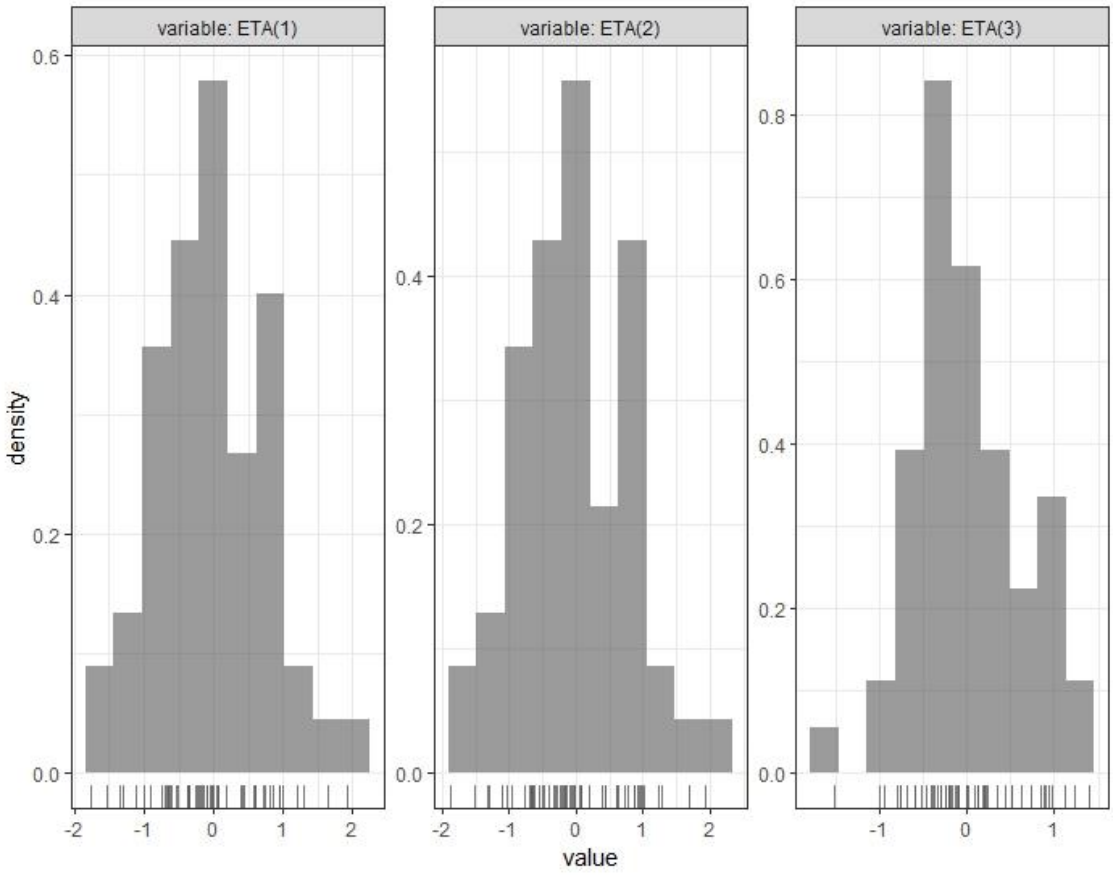


Figure A6.30 *η distribution for the unconstrained k, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral metformin administration only.*

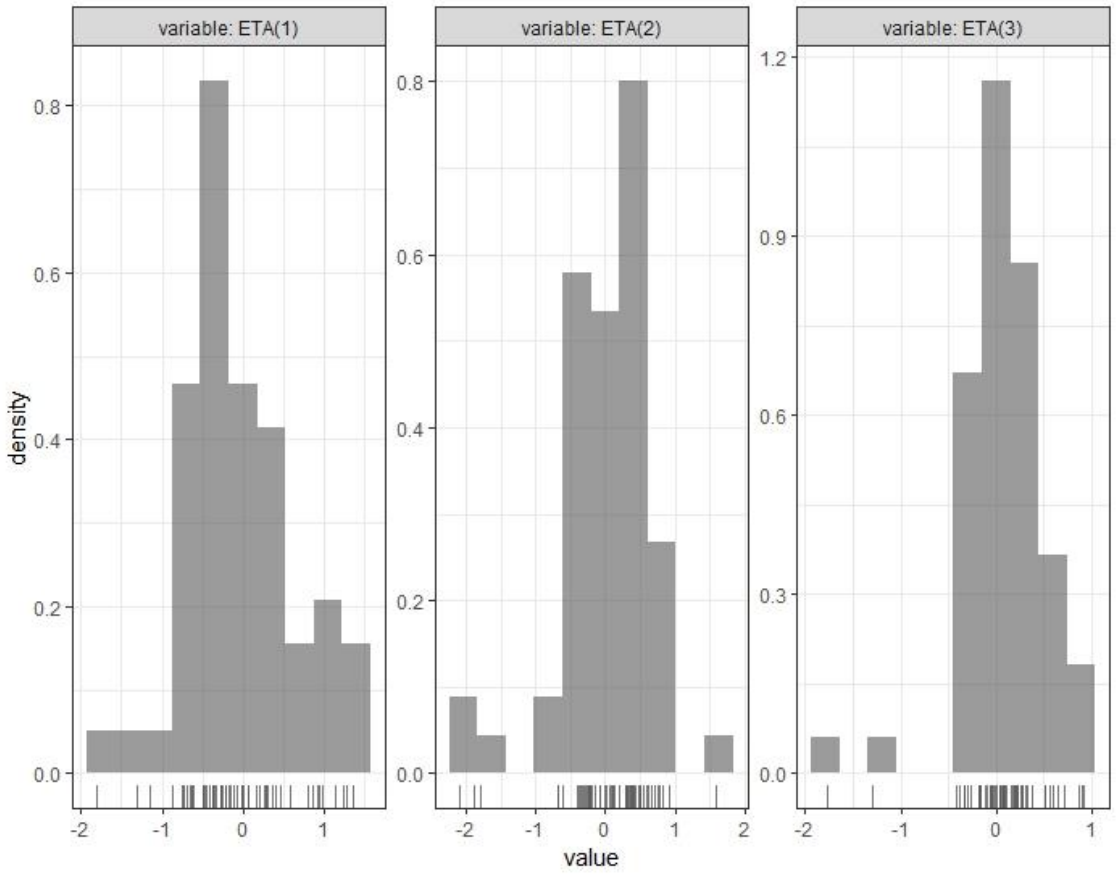


Figure A6.31 *η distribution for the unconstrained k, V, k_a parameterised model developed using initial estimates of k smaller than k_a and metformin concentrations following oral and intravenous metformin administration.*

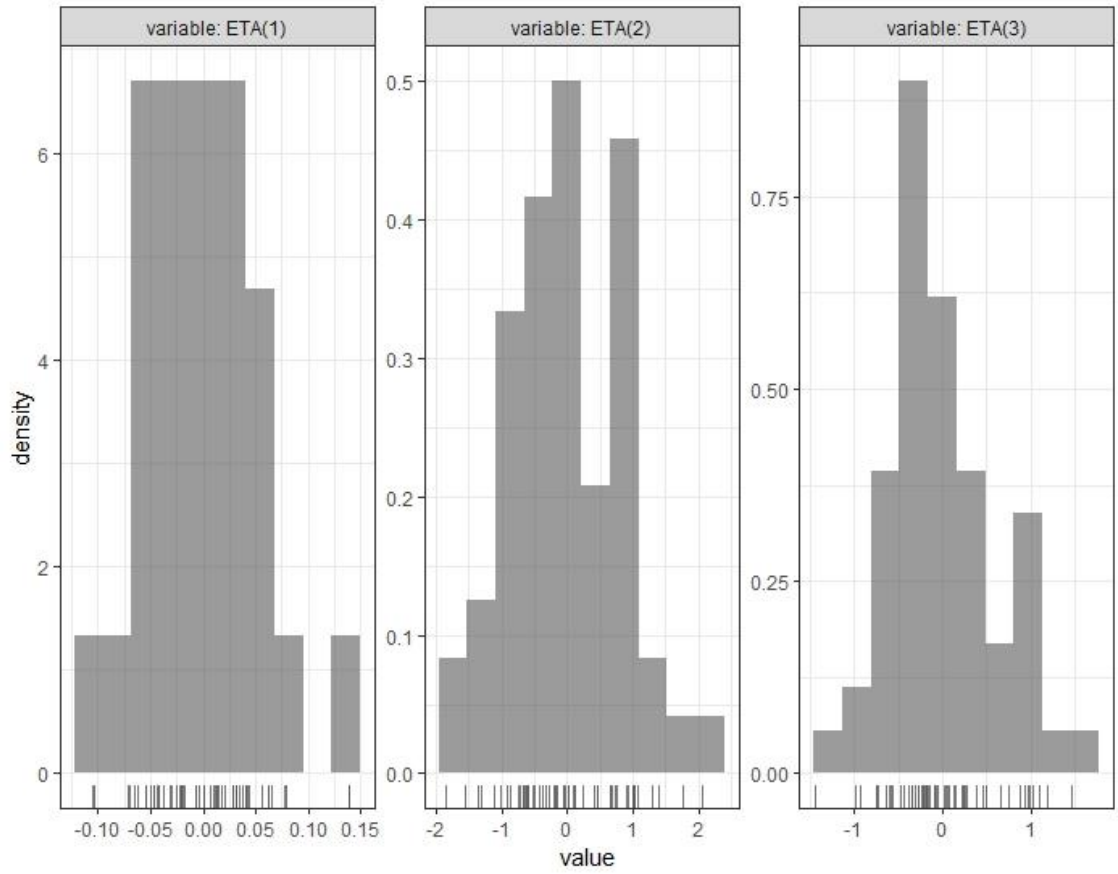


Figure A6.32 *η distribution for the unconstrained k, V, k_a parameterised model developed using initial estimates of k larger than k_a and metformin concentrations following oral and intravenous metformin administration.*

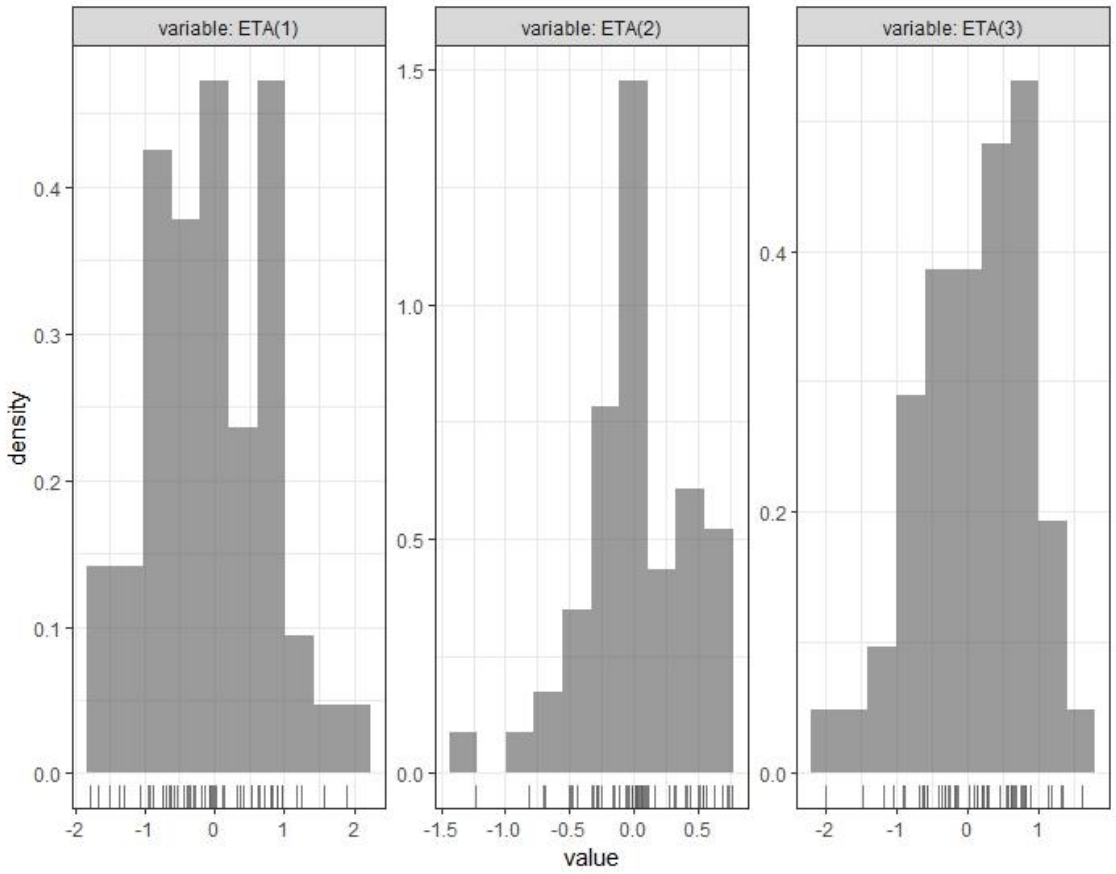


Figure A6.33 *η distribution for the partially constrained CL, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only*

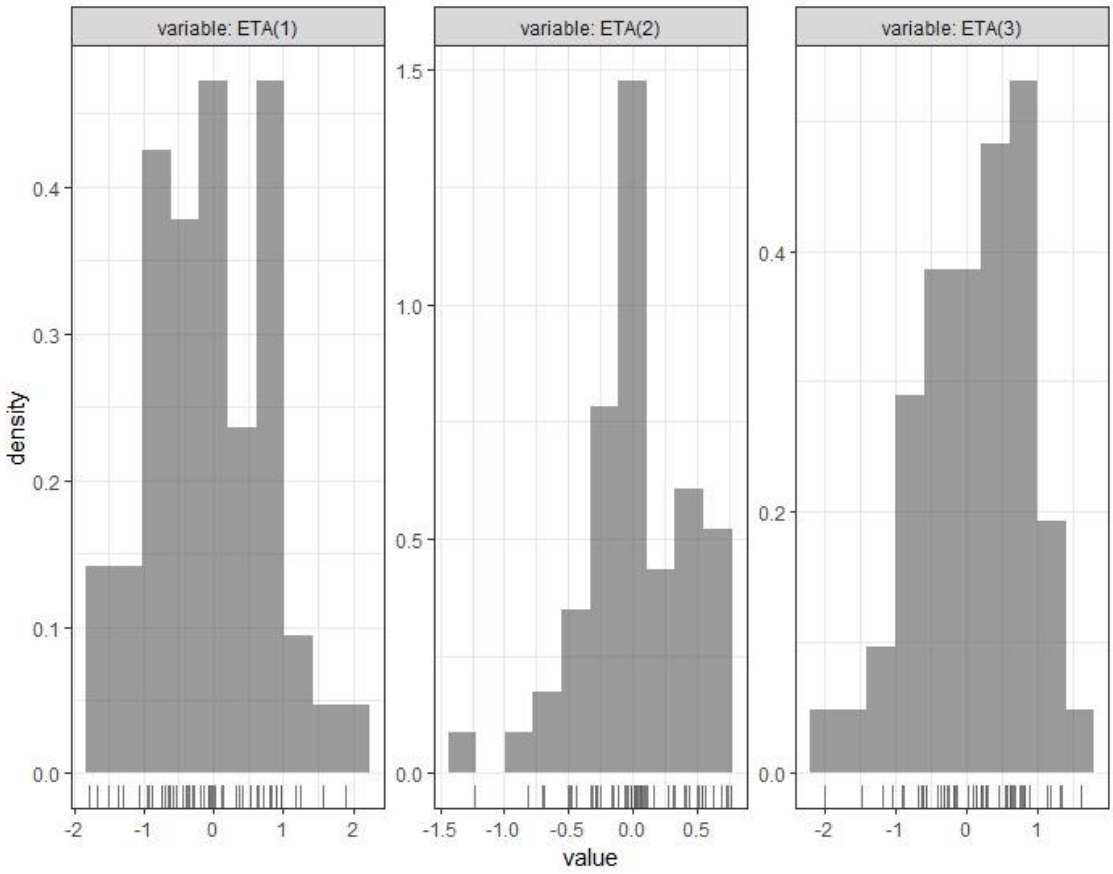


Figure A6.34 *η distribution for the partially constrained CL, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration.*

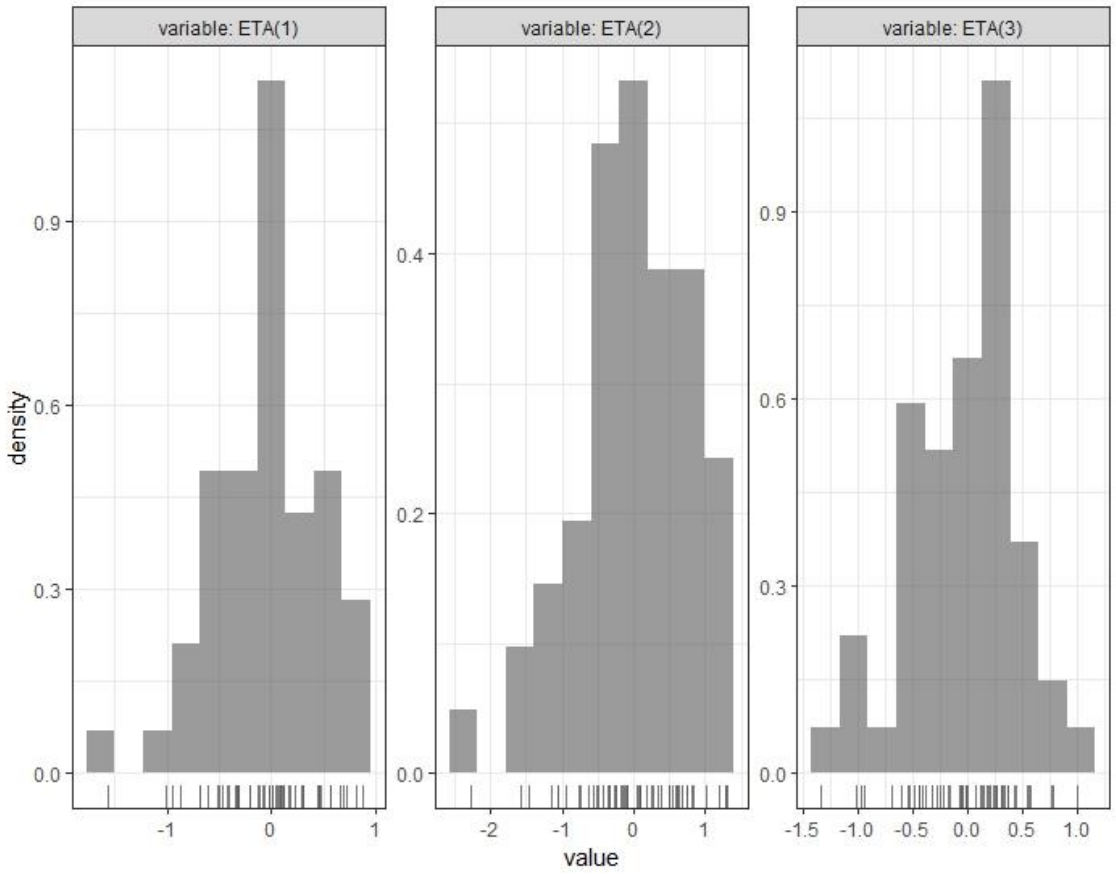


Figure A6.35 *η distribution for the partially constrained k, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only*

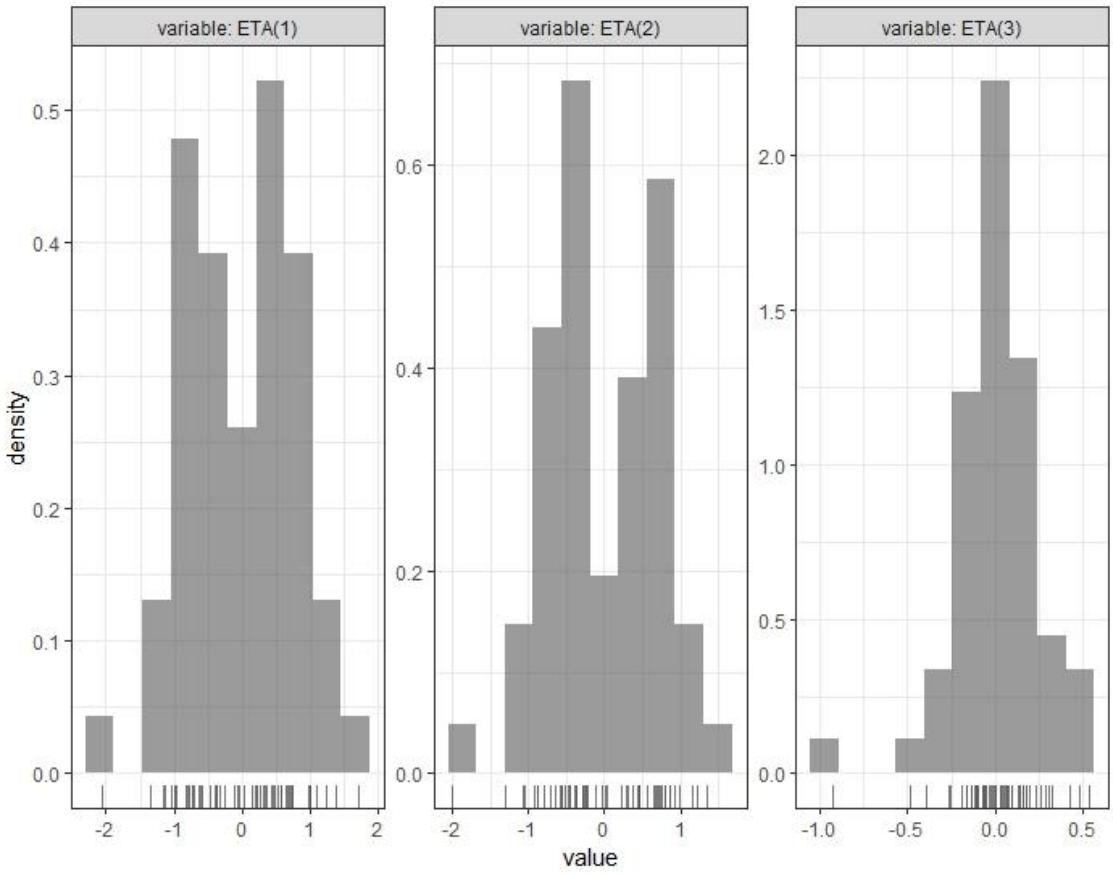


Figure A6.36 *η distribution for the partially constrained k, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration*

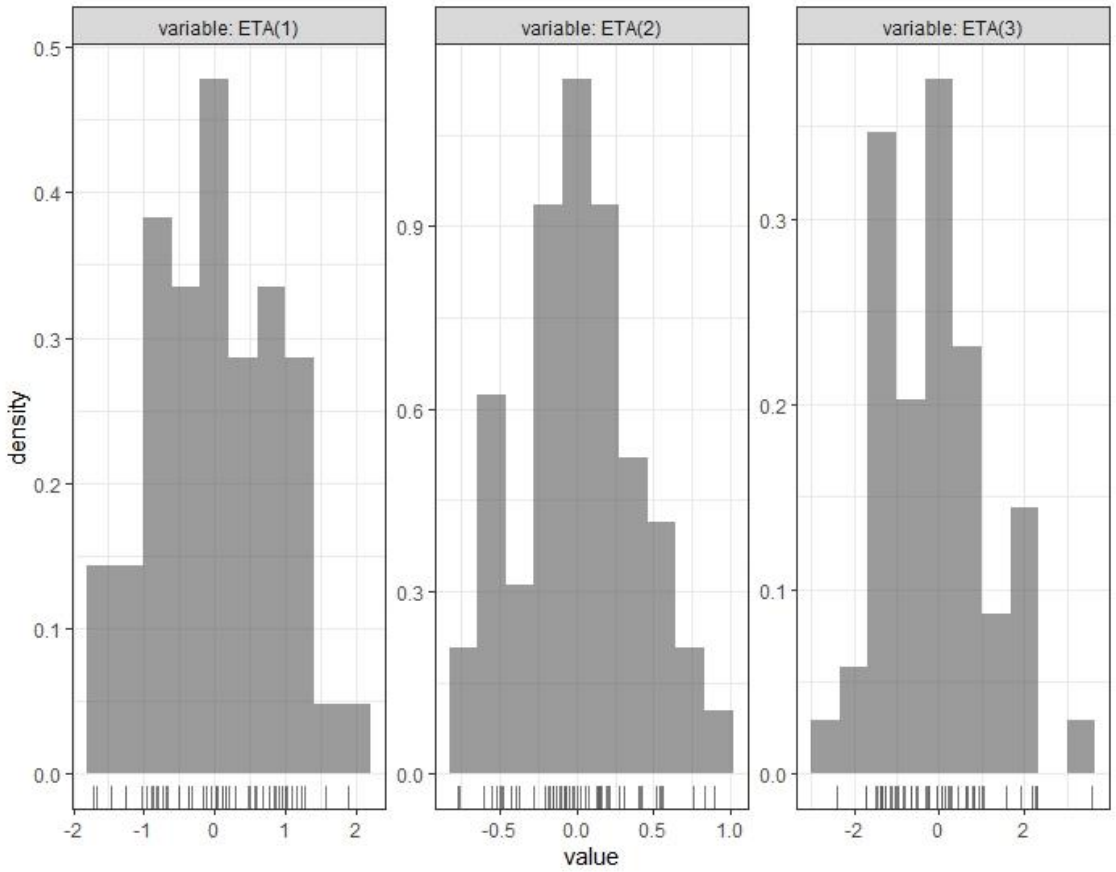


Figure A6.37 *η distribution for the fully constrained CL, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only*

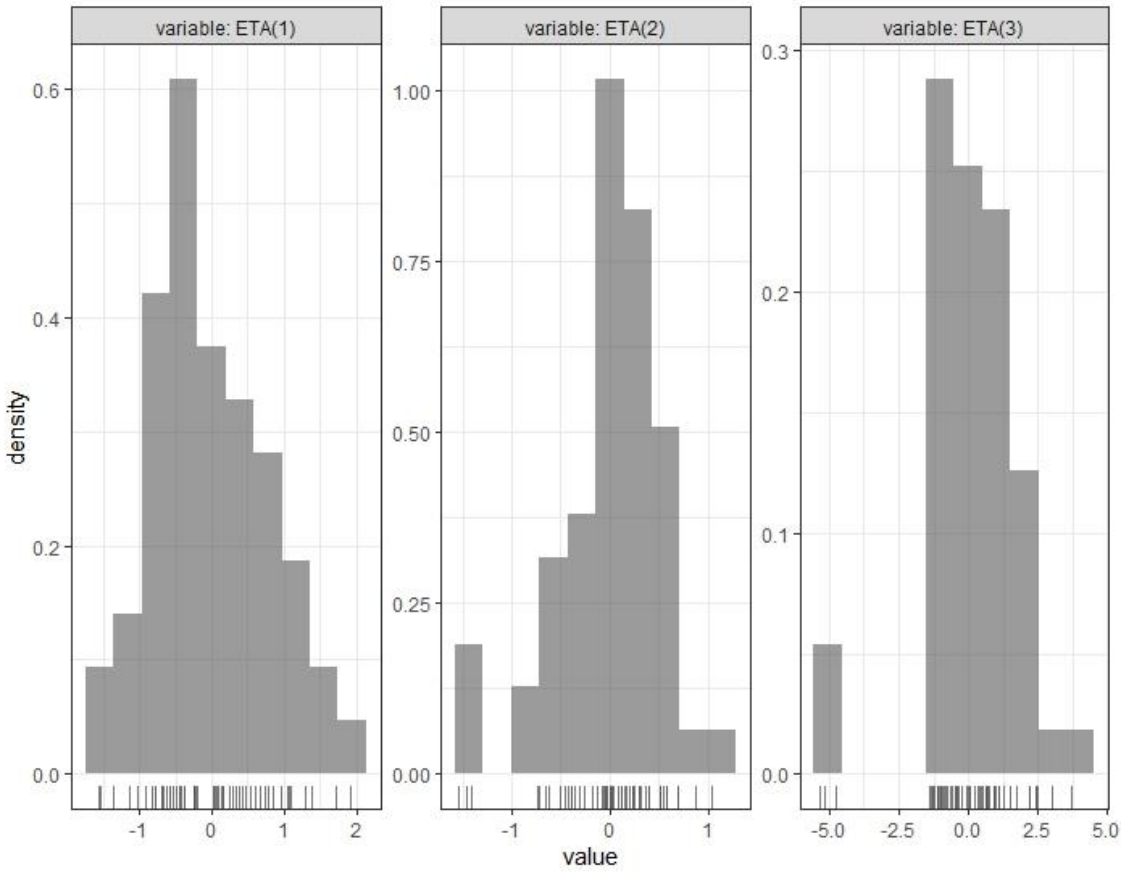


Figure A6.38 *η distribution for the fully constrained CL, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration*

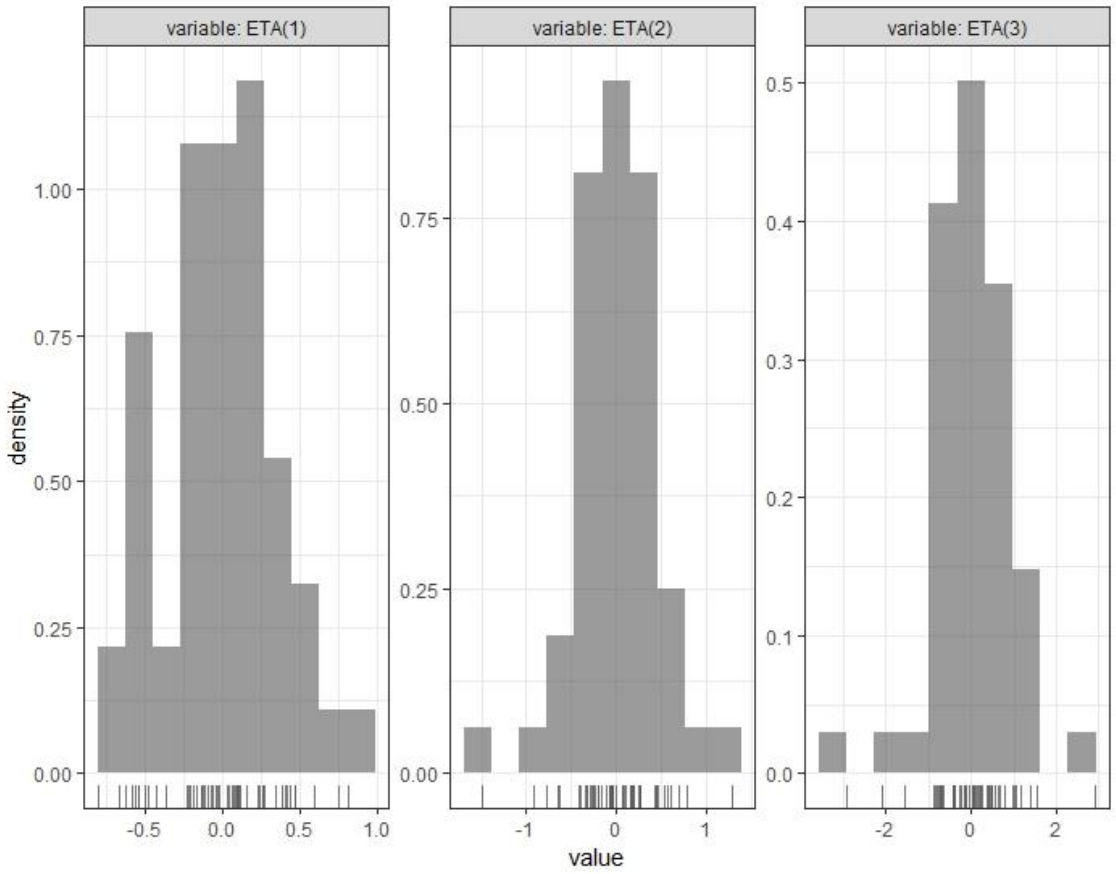


Figure A6.39 *η distribution for the fully constrained k, V, k_a parameterised model developed using metformin concentrations following oral metformin administration only*

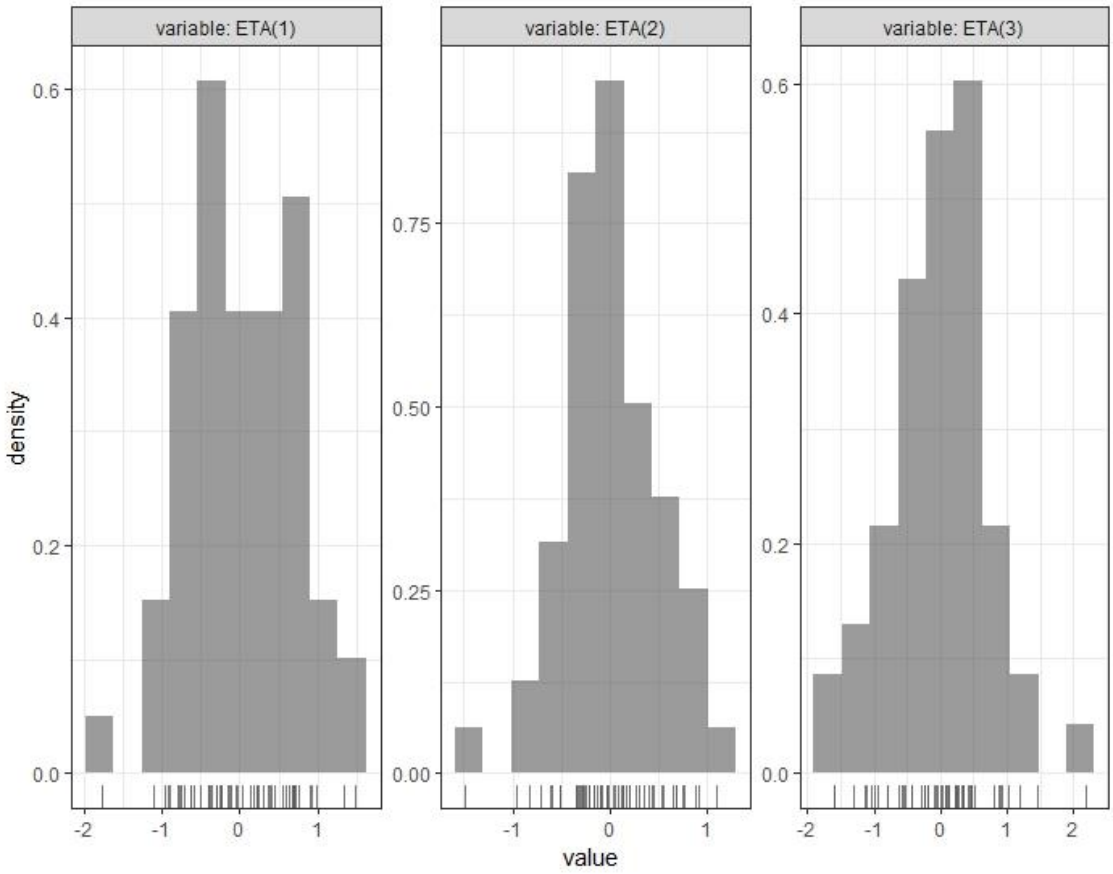


Figure A6.40 *η distribution for the fully constrained k, V, k_a parameterised model developed using metformin concentrations following oral and intravenous metformin administration*

A6.4. Example NONMEM control files for the unconstrained, partially constrained and fully constrained models

Presented in this section are examples of the NONMEM control files for the unconstrained, partially constrained and fully constrained models parameterised using CL, V, k_a. The example NONMEM control files shown are in the following sections as follows:

i. Unconstrained model with initial estimates for the elimination rate constant being smaller than the absorption rate constant (section A6.4.1.)
ii. Partially constrained model (section A6.4.2.)
iii. Fully constrained model (section A6.4.3.)

A6.4.1. Example NONMEM control file for an unconstrained model

```
$PROBLEM METFORMIN PK

$INPUT C STUDY ID STUDYID TIME TAD UVOL DV AMT CMT=CMT1
CMT2 CMT3 EVID DVID MDV BLQ AGE SEX HTCM WTKG BMI FFM
GENTCL CR CG MDRD CKDEPI DWTKG BLACT BBICAR DSTART
DSTOP DRETURN BFR DFR SA DIAL

$DATA metformin_singledose3.csv
    IGNORE = C
    IGNORE = (BLQ.EQ.1)
    IGNORE = (STUDY.EQ.3)
    IGNORE = (STUDY.EQ.4)
    IGNORE = (CMT1.EQ.3)
    IGNORE = (CMT1.EQ.-3)

$SUBROUTINES ADVAN13 TOL=9

$MODEL
COMP(DEPOT)
COMP(CENTRAL, DEFOBS)

$PK
; COVARIATE MODEL
TVCL=THETA(1)
TVV2=THETA(2)
TVKA=THETA(3)
TVF1=THETA(4)

; BETWEEN SUBJECT VARIABILITY
CL=TVCL*EXP(ETA(1))
```

```
V2=TVV2*EXP(ETA(2))
KA=TVKA*EXP(ETA(3))

TVLOGIT=LOG(TVF1/(1-TVF1))
LOGIT=TVLOGIT+ETA(4)
F1=1/(1+EXP(-LOGIT))

K20=CL/V2

; SCALE CONCENTRATIONS
S2 = V2

$DES
DADT(1) = -KA*A(1)
DADT(2) = KA*A(1)-K20*A(2)

$ERROR
IPRED = F
   W = SQRT(THETA(5)**2*IPRED**2+THETA(6)**2)
   Y = IPRED+W*EPS(1)
 IRES = DV-IPRED
IWRES = IRES/W

$THETA
(0, 25)         ; CL
(0, 100)        ; V2
(0, 0.5)        ; KA
0.55 FIX        ; F1
(0, 0.1)        ; Prop
(0, 0.05)       ; Add
```

```
$OMEGA BLOCK(3)
0.1                    ; IIV CL
0.01     0.1             ; IIV V2
0.01     0.01     0.1     ; IIV KA

$OMEGA
0 FIX           ; IIV F1

$SIGMA
1 FIX ; Proportional PK S1

$EST METHOD=COND INTER MAXEVAL=9999 NOABORT SIGL=9
NSIG=3 PRINT=10
$COV

; Xpose
$TABLE STUDY ID TIME DV MDV EVID CMT1 CMT2 CMT3 BLQ IPRED
 IWRES CWRES ONEHEADER NOPRINT FILE=sdtab4e
$TABLE STUDY ID CL V2 KA F1 ETA1 ETA2 ETA3 ONEHEADER
NOPRINT FILE=patab4e
```

A6.4.2. Example NONMEM control file for a partially constrained model

```
$PROBLEM METFORMIN PK

$INPUT C STUDY ID STUDYID TIME TAD UVOL DV AMT CMT=CMT1
CMT2 CMT3 EVID DVID MDV BLQ AGE SEX HTCM WTKG BMI FFM
 GENTCL CR CG MDRD CKDEPI DWTKG BLACT BBICAR DSTART
DSTOP DRETURN BFR DFR SA DIAL

$DATA metformin_singledose3.csv
    IGNORE = C
    IGNORE = (BLQ.EQ.1)
    IGNORE = (STUDY.EQ.3)
    IGNORE = (STUDY.EQ.4)
    IGNORE = (CMT1.EQ.3)
    IGNORE = (CMT1.EQ.-3)

$SUBROUTINES ADVAN13 TOL=6

$MODEL
COMP(DEPOT)
COMP(CENTRAL, DEFOBS)

$PK
; STRUCTURAL MODEL
; model structure is based on cl =v*k, where k>ka | k<ka

TVCL=THETA(1)
TVV2=THETA(2)

IF(CG.GE.30)THEN
TVKA = (TVCL/TVV2)/(1+THETA(3)); absorption rate limited (ka<k)
```

```
ELSE
TVKA = (TVCL/TVV2)*(1+THETA(4)) ; elimination rate limited (ka>k)
ENDIF

TVF1 = THETA(5)

; BETWEEN SUBJECT VARIABILITY
CL = TVCL * EXP(ETA(1))
V2 = TVV2 * EXP(ETA(2))
KA = TVKA * EXP(ETA(3))

K20 = CL / V2

; SCALE CONCENTRATIONS
S2 = V2

$DES
DADT(1) = -KA*A(1)
DADT(2) = KA*A(1)-K20*A(2)

$ERROR
IPRED = F
   W = SQRT(THETA(6)**2*IPRED**2+THETA(7)**2)
   Y = IPRED+W*EPS(1)
 IRES = DV-IPRED
IWRES = IRES/W

$THETA
(0, 44.7)       ; CL
(0, 157)        ; V2
(0, 0.0001)     ; KA_SMALL
```

```
(0, 0.349)      ; KA_BIG
0.55 FIX        ; F1
(0, 0.258)      ; Prop
(0, 0.018)      ; Add

$OMEGA BLOCK(3)
0.10                    ; IIV CL
0.01    0.10            ; IIV V2
0.01    0.01    0.10    ; IIV KA

$SIGMA
1 FIX ; Proportional PK S1

$EST METHOD=COND INTER MAXEVAL=9999 NOABORT SIGL=6
NSIG=3 PRINT=10
$COV

; Xpose
$TABLE STUDY ID TIME DV MDV EVID CMT1 CMT2 CMT3 BLQ IPRED
IWRES CWRES ONEHEADER NOPRINT FILE=sdtab4
$TABLE STUDY ID CL V2 K20 KA ETA1 ETA2 ETA3 ONEHEADER
NOPRINT FILE=patab4
```

A6.4.3. Example NONMEM control file for a fully constrained model

```
$PROBLEM METFORMIN PK

$INPUT C STUDY ID STUDYID TIME TAD UVOL DV AMT CMT=CMT1
CMT2 CMT3 EVID DVID MDV BLQAGE SEX HTCM WTKG BMI FFM
GENTCL CR CG MDRD CKDEPI DWTKG BLACT BBICAR DSTART
 DSTOP DRETURN BFR DFR SA DIAL

$DATA metformin_singledose3.csv
     IGNORE = C
     IGNORE = (BLQ.EQ.1)
     IGNORE = (STUDY.EQ.3)
     IGNORE = (STUDY.EQ.4)
     IGNORE = (CMT1.EQ.3)
     IGNORE = (CMT1.EQ.-3)

$SUBROUTINES ADVAN13 TOL=6

$MODEL
COMP(DEPOT)
COMP(CENTRAL, DEFOBS)

$PK
; STRUCTURAL MODEL

TVCL=THETA(1)
TVV2=THETA(2)
TVF1=THETA(3)

; BETWEEN SUBJECT VARIABILITY
;model is based on individually constraining cl=v*k, where k>ka | k<ka
```

```
CL = TVCL*EXP(ETA(1))
V2 = TVV2*EXP(ETA(2))

IF(CG.GE.30)THEN
 KA=(CL/V2)/(1+THETA(4)*EXP(ETA(3))); absorption rate limited (ka<k)
ELSE
 KA=(CL/V2)*(1+THETA(5)*EXP(ETA(3))); elimination rate limited (ka>k)
ENDIF

K20 = CL/V2

; SCALE CONCENTRATIONS
S2 = V2

$DES
DADT(1) = -KA*A(1)
DADT(2) = KA*A(1)-K20*A(2)

$ERROR
IPRED = F
   W = SQRT(THETA(6)**2*IPRED**2+THETA(7)**2)
   Y = IPRED+W*EPS(1)
 IRES = DV-IPRED
IWRES = IRES/W

$THETA
(0, 41.1)       ; CL
(0, 175)        ; V2
0.55 FIX        ; F1
(0, 0.00001)    ; KA_SMALL
```

```
(0, 0.74)       ; KA_BIG
(0, 0.28)       ; Prop
(0, 0.0204)     ; Add

$OMEGA BLOCK(3)
0.10                    ; IIV CL
0.01    0.10            ; IIV V2
0.01    0.01    0.10    ; IIV KA

$SIGMA
1 FIX ; Proportional PK S1

$EST METHOD=COND INTER MAXEVAL=9999 NOABORT SIGL=6
NSIG=3 PRINT=10
$COV

; Xpose
$TABLE STUDY ID TIME DV MDV EVID CMT1 CMT2 CMT3 BLQ IPRED
IWRES CWRES ONEHEADER NOPRINT FILE=sdtab4
$TABLE STUDY ID CL V2 K20 KA ETA3 ONEHEADER NOPRINT
FILE=patab4
```

A6.5. Identification of a cut-off to address local identifiability

A creatinine clearance ($CLcr_{CG}$) value calculated using the Cockcroft and Gault equation was determined to identify when the elimination and absorption rate constants were expected to be equal. The transition point was identified from subjects with both oral and intravenous metformin concentration data. A one-compartment model with first-order absorption and $CLcr_{CG}$ as a covariate on clearance (CL) was developed. Model estimates of clearance (CL), volume of distribution (V) and the absorption rate constant (k_a) were used to determine the $CLcr_{CG}$ value at which k is expected to equal k_a calculated as follows:

$$k_a = k$$

$$k_a = CL/V$$

$$k_a = \frac{CL\left(\frac{CLcr_{CG}}{100}\right)}{V}$$

Rearranged

$$CLcr_{CG} = 100 \cdot \left(\frac{k_a \cdot V}{CL}\right)$$

Here, k_a is the absorption rate constant (h^{-1}), k is the elimination rate constant (h^{-1}), CL is clearance (L/h) and V is volume of distribution (L). The model parameter estimates used to theoretically derive the transition point for metformin are shown in Table A6.2.

Table A6.2 *Parameter estimates for the population pharmacokinetic model*

Parameter	Estimate (%RSE)
θ_{CL} ($L \cdot h^{-1}$)	27.85 (16)
θ_V (L)	24.13 (8)
θ_{KA} (h^{-1})	0.28 (13)
θ_{F1}	0.50 (8)
θ_{CG_EFF}	1 FIX
Between subject variability	
ω_{KA} (CV%)	31.4 (13)
Residual error	
σ_{add} (mg/L)	0.154 (44)
σ_{prop} (CV%)	0.331(10)

θ_{CL} is the mean population value for clearance, θ_V is the mean population value for the volume of distribution, θ_{k_a} is the mean population value for the absorption rate constant, θ_{F1} is the mean population value for bioavailability, θ_{CG_EFF} is the estimated exponent on the covariate effect for creatinine clearance calculated using the Cockcroft and Gault equation [80]. ω_{k_a} is between subject variability for the absorption rate constant, σ_{add} is additive residual error (mg/L) and σ_{prop} is proportional residual error (CV%).

The calculations to derive the cut-off are as follows:

$$0.282 = \frac{27.9\left(\frac{CLcr_{CG}}{100}\right)}{24.1}$$

$$6.7962 = 27.9\left(\frac{CLcr_{CG}}{100}\right)$$

$$\frac{CLcr_{CG}}{100} = 0.2436$$

$$CLcr_{CG} = 24.36$$

Here, a creatinine clearance value of 24.36 mL/min was calculated as the flip-flop transition point for when k_a is anticipated to equal k.

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