The application of computer simulation to investigate drug absorption and bioavailability in disease states

by

May Khalifah Almukainzi

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In Pharmaceutical Sciences

Faculty of Pharmacy and Pharmaceutical Sciences
University of Alberta

Abstract

Oral drug absorption relies mainly on the physicochemical drug properties and the interaction with the physiological environment of the body. These factors could be altered significantly under abnormal conditions and disease states. Identifying these changes can optimize drug therapy under these conditions. Pain, obesity surgery and kidney failure are examples that can influence drug absorption through various mechanisms. Mechanistic tools can assist researchers to gain a better understanding of such effects. Computer simulations have promising applications in the recognition of alterations in drug absorption under disease conditions. The objectives of this thesis were to evaluate the utility of computer simulations to improve the understanding of such effects. This was done by investigating drug absorption and bioavailability in pain, gastric bypass surgery and renal impairments. The drug absorption of meloxicam, ibuprofen, and metformin were used as model drugs using advanced compartmental absorption and transit (ACAT) and physiologically based pharmacokinetics (PBPK) models in Gastroplus^{TM} (Simulations Plus, Inc.). In the pain model, published in vivo data of meloxicam and ibuprofen were used for the simulations using two formulations: a fast dissolving (FD) and regular release (RR). The oral bioavailability was compared between these formulations in vagally suppressed rats (gastric dysfunction) and a control group. Additionally, human data under pain induced by dental surgery was used. The in vivo drug release of all formulations was estimated for both drugs using the software's immediate or gastric release models. In gastric bypass surgery and renal failure models, we investigated the mechanistic background of the absorption of metformin using patient data with post gastric bypass surgery and moderate chronic renal failure. Assumptions to

explain the causes in the changes in drug absorption in these patients from their observed data were tested using the software. Using these models, I was able to explain the observed alteration in the drugs absorption that was induced by physiological changes in pain, gastric bypass, and renal failure. For meloxicam and ibuprofen, the software's built in immediate release (IR) model predicted the in vivo absorption in the control groups administered FD and IR. When gastric dysfunction was induced, the model did not predict absorption, while the gastric release model did so for both FD and IR formulations. Simulation of gastric bypass surgery on the absorption of metformin showed that the increase in the pore size and porosity of the last part of the small intestine successfully predicted the observed PK parameters. This indicates that the gut must have undergone an adaption process to compensate for the loss of parts of the small intestine. The renal model showed that the down regulations of the kidney and liver transporters, particularly, the multidrug and toxin extrusion (MATE1), can explain the observed data. This down regulation might be induced by the increased in uremic toxins. Creatinin, is one of the uremic toxins that are highly increased in renal dysfunction, our anticipation that creatinin, which is a substrate for the same transporters, can compete with metformin and inhibit the drug elimination. However, using in vitro cell line derived from human embryonic kidney (HEK293) showed that the presence of a high concentration of creatinine level has no effect on metformin elimination. The insights gained by these studies can be applied to other drugs that have similar physiochemical properties. In silico methods can be a potential tool to predict the influence of physiological changes induced by disease conditions. Using such software in disease conditions can help to understand the mechanism of drug absorption, predicting the pharmacokinetics (PK) of

administered drugs. This tool can be used in precision medicine in order to individualize patients' dose or treatment regimen and optimize dosage forms. This is a very important practice to maximize drug benefits and reduce possible side effects for these patents. This also can help the pharmaceutical industry in speeding up drug development cycles, reducing clinical testing, and reducing cost.

Preface

The presented thesis is original work prepared mainly by May Almukainzi. The following parts were published or submitted with co-authors:

Almukainzi,M; Jamali, F; Aghazadeh-Habashi,A; Löbenberg,R "Disease Specific Modelling: Simulation of the Pharmacokinetics of Meloxicam and Ibuprofen in Disease State vs. Healthy conditions. I was responsible for the simulation data collection and analysis as well as the manuscript composition. The original experimental data produced by: Jamali, F; Aghazadeh-Habashi,A . Löbenberg,R was the supervisory author and was involved with manuscript composition.

Chapter 3 of this thesis has been published as Almukainzi M, L. V., Löbenberg R (2014). "Modelling the Absorption of Metformin with Patients Post Gastric Bypass Surgery." Diabetes Metab 5: 353. I was responsible for the data collection and analysis as well as the manuscript composition. Lukacova , V assisted with the data collection and contributed to manuscript edits. Löbenberg R was the supervisory author and was involved with concept formation and manuscript composition.

Dedicated to my parents with all my love…

ACKNOWLEDGEMENTS

All the thanks are toward my God in first and last for the countless things that I am pleased with in this life. One of God blessings is the opportunity to meet so many amazing people along the way. I owe my deepest gratitude to my parents for their unconditional love, support, passion, and patience not only during my study, but also throughout the whole my life. I doubt that I will ever be able to express my thanks to them, but I devote to them all the success I had and I will have, God willing. The thanks extended to my dear brothers, sisters for their love, compassion and encouragement.

I thank my supervisor Dr. Raimar Löbenberg for his assistance, guidance and continuous help. My sincere thank not only for his supervision but also for his humanity in dealing with his students. Thanks are extended to Dr. Fakhreddin Jamali for his thoughtful comments and positive feedback throughout my program in addition of being my supervisory committee member. I also thank Dr. Jack Tuszynski for his help, guidance and advice as my supervisory committee member. Thanks Dr. El-Kadi and his lab members for their help in Western blotting procedure. Thanks to Dr. Marsh, Dr. Velazquez and Dr. Valizadeh for their kind in accepting to be my examining committee in such a short notice. Thanks to all my lab members and for the University of Alberta. Thanks to all members of Simulation Plus Inc. (USA) for making their software available. Lastly, I am grateful to Saudi Arabia Cultural Bureau and the ministry of higher education, particularly, to the princess Noura University in my lovely country Saudi Arabia for supporting me financially throughout my study period.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

ACAT	Advanced compartmental absorption and transit
API	Active pharmaceutical ingredient
App	Apparatus
An	Absorption number
BCS	Biopharmaceutical classification system
BDDCS	Biopharmaceutics drug disposition classification system
BE	Bioequivalence
BCRP	Breast Cancer Resistance Protein
BFaSSIF	Blank Fasted Simulated State Intestinal Fluid
BMI	Body mass index
CAT	Compartmental Absorption and Transit
CKD	Chronic kidney disease
CR	Controlled Release
Cl	Clearance
CLr	Renal clearance
CNT	Nucleoside transporter
Cs	Aqueous solubility
Cp	Plasma concentration
CYP	Cytochromes P450
D0	Dose number
Dn	Dissolution
EMA	European medicines agency
FD	Fast dissolving

FaSSIF	Fasted Simulated State Intestinal Fluid
FDA	Food and drug administration
FIP	International pharmaceutical federation
Fu	Unbound fraction in plasma
HEK293	Human embryonic kidney
Hr	Hours
IR	Immediate release
IS	Indoxyl sulfate
IV	Intravenous
IVIVC	In vitro/in vivo correlation
GF	Glomuler filtration
GFR	Glomerular filtration rate
GIT	Gastro intestinal tract
GSTs	Glutathione S-transferases
Kp	partientn cofecent
L	Litter
LogD	Distribution coefficient
Log P	Octanol-water partition coefficients
MATE	Multidrug and toxin extrusion
mg	Milligram
mg/dL	Milligram per deciliter
mg/mL	Milligram per milliliter
min	Minutes
mL	Milliliter
ml/min	Milliliter per minutes

mm	Millimeter
mM	Millimole
MRP	Multi-drug resistance associated protein
Mw	Molecular weight
Na	Sodium
NSAIDs	Nonsteroidal anti-inflammatory drugs
OAT	Organic Anion-Transporting Polypeptides
OCTs	Organic cation transporters
PBPK	Physiologically based pharmacokinetics
PD	Pharmacodynamics
PEAR	Population Estimates for Age-Related Physiology
Peff	Permeability
Pept1	Peptide transporter 1
pH	Acidity or alkalinity of measurement
Pgp	P-glycoprotein
PKa	Acid dissociation
PK	Pharmacokinetics
PMAT	Plasma Membrane Monoamine Transporter
PT	Parathyroid hormone
QbD	Quality by design
R	Radius of the intestinal
RR	Regular Release
RYGB	Roux-en-Y Gastric Bypass
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid

Tdiss	Time required for a drug particle to dissolve
UGTs	Glucuronosyltransferases
USP	United State Pharmacopeia
V0	Volume of water
WHO	World health organization
%	Percent
°C	Degree centigrade
BCRP	Breast Cancer Resistance Protein
Pept1	Peptide transporter 1
MCT1	Monocarboxylate transporter 1
OATP	Organic Anion-Transporting Polypeptides
ASBT	apical sodium dependent bile acids transporter
PEPT	the oligopeptide transporter
CNT	nucleoside transporter N
UGT	uridine diphosphate glucuronosyltransferase

Chapter 1: General Introduction

1.1 Oral Drug absorption:

Oral drug delivery is the most commonly used route of drug administration (Chow and Pang 2013). Dosage form disintegration followed by drug dissolution is required before a drug molecule can absorbed into the systemic circulation. Oral drug absorption is a complex overlapping process that relies on the administrated drug properties and the body's physiological characteristics (Almukainzi, Bou-Chacra et al. 2014). Drug properties include but are not limited to aqueous solubility, molecular size, and pKa. Physiological factors of the gastrointestinal tract (GIT) characters, such as gastric motility, intestinal emptying, pH, bile salt, and food can have an impact on the oral performance of a drug product (Valsami and Macheras 2011). An understanding of how these variables play together and affect drug absorption is extremely important to optimize drug delivery (Chow and Pang 2013).

1.1.1 The physiochemical/ biopharmaceutical drug characterizes and drug absorption:

A drug's physiochemical characteristics, the formulation method and the choice of dosage form control the GI absorption. This is because these properties impact the dissolution, precipitation, permeability and the overall drug bioavailability in the GIT. The solubility is one of the most important factors; without sufficient solubility, drug dissolution and absorption will be limited. This is because dissolution of poorly soluble drugs may take longer than the transit time past the absorptive sites, resulting in incomplete oral absorption. Active pharmaceutical ingredients are usually used as salt form, either weak acids or bases; this can form strong ionic interaction with water molecules (El-Kattan and Varma 2012). The pH of a solution is a logarithmic scale measurement of a drug in acidity or alkalinity on a scale of 0–14. As the number of protons in a solution increases, the pH value decreases, and as the amount of hydroxide, or basicity increases, the pH value increases (Moore, Derry et al. 2014). The dissociation constant (pKa) of a drug

substance is used to determine its charge state at a particular pH in solution (acidic, basic or neutral). By using the Henderson-Hasselbach equation, it is possible to calculate the amount of ionized to un-ionized drug species at any given pH (Po and Senozan 2001).

$$pH = pKa + \log [A]/ [HA]$$

where Ka is the dissociation constant of the weak acid, pKa = log Ka, and [HA] and [A] are the molarities of the weak acid and its conjugate base (Po and Senozan 2001).

The ionized compound is known to increase proportionally with the amount of the charged type as a value of pH (Manallack, Prankerd et al. 2013). This ionization state of compounds is a requirement for the drug solubility, absorption, and elimination (Bergström, Holm et al. 2014). Moreover, the charged state of a drug substance has an influence on the binding to transporters, enzymes, and the target receptors (Manallack, Prankerd et al. 2013). The drug lipophilicity is another important property that controls the drug permeability across the intestinal mucosa and, consequently, the pharmacokinetic (PK) profile of a drug. The lipophilicity of a drug substance represents its affinity for a water-immiscible organic phase in comparison to the aqueous surroundings (Camenisch, Alsenz et al. 1998). This property is used to measure the affinity of a molecule to penetrate the body membranes. (logP) and (logD) are the measurement values that represent this property. Log P is defined as the concentration ratio of the neutral species in octanol:water system, which is a constant value, whereas logD is the distribution coefficient that represents the drug concentration ratio of all charge states that differs as a consequence of pH variation (Comer and Tam 2001). Conversely to the requirement of ionization for a drug to be soluble, a highly charged a compound can limit the passive permeability across a membrane, as lipophilicity is inversely proportional to the amount of charged species (Comer and Tam 2001). In other words, a high value means the drug prefers to be in the hydrophobic environment, and

thus it can cross the intestinal membrane without the help of drug transporters; however, it will suffer from reduced solubility in water and may require emulsifying agents. Optimum absorption of a drug through the GIT occurs with log P values of 1–2 (Bergström, Holm et al. 2014). Therefore, the pKa, solubility and logD have to be evaluated to achieve the required partition coefficient into the systemic circulation. These characteristics can be identified through *in vitro* drug experiments or *in silico* predictions of molecular descriptors. One should note that lipophilicity is related to solubility and permeability, and consequently, absorption. This interconnection between the impact of the ionization ratio on the solubility and permeability led to propose the Biopharmaceutics Classification System (BCS) by Amidon et al in 1995 (Amidon, Lennernäs et al. 1995). This system is based on two fundamental parameters that control the oral drug absorption: The permeability and solubility. The dissolution number (Dn) is defined as the ratio of mean residence time to mean dissolution time.

Dn = tres / tDiss. (Amidon, Lennernäs et al. 1995)

tres is the mean residence time (≈180 min) and tdiss is the time required for a drug particle to dissolve.

The Dose number (Do) links the ratio of the drug solubility with the volume of intestinal fluids according to the following equation:

(Do = Mo / CsVo) (Amidon, Lennernäs et al. 1995)

where Mo is the dose of drug administered, Vo is the initial gastric volume (≈250 mL), Cs is the saturation solubility.

The Absorption number (An) represents the ratio of the mean residence time to the drug's mean absorption time.

An = Peff × tres / R; (Reddy and Karunakar 2011).

where Peff is the effective permeability, and R is the radius of the intestinal segment.

One should note that these values depend on a drug's physicochemical properties and stability of the drug in the GIT against acid or enzymatic degradations. Also, permeability into biological membranes and/or being a substrate for various uptake and efflux transporters can impact the apparent permeability. Based on this analysis, drug substances are classified into four drug classes based on their aqueous solubility and intestinal permeability: Highly soluble/high permeable (Class I), low soluble/high permeable (Class II), high soluble/low permeable (Class III), and low soluble/low permeable (Class IV) (Table 1.1). A drug is defined as highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1.0–7.5 , and is considered highly permeable when the extent of absorption in humans is determined to be > 90% of an administered dose (FDA 2000). In 2000, the United States Food and Drug Administration (FDA) applied the BCS system to allow waiver of *in vivo* bioavailability and bioequivalence (BE) studies of immediate-release (IR) solid oral dosage forms for Class 1 drugs when such drug products also exhibited rapid dissolution (FDA 2000) . In 2010, the European Medicine's Agency (EMA) revised its BE guideline stating that demonstration of complete absorption in humans is preferred for biowaiver of BCS Class 1 drug applications rather than measures of high permeability (EMA 2010) . The criteria for complete absorption in the EMA Guidline is ≥85% measured extent of absorption in humans based either upon absolute bioavailability or mass balance studies (EMA 2010). The pharmaceutical industry also has taken advantage of BCS as a guide throughout drug discovery and development to drive the manufacturing process.

The ability of the permeability the property to predict the extent of drug absorption by correlating the intestinal permeability with the rate and extent of drug metabolism led to the development of the Biopharmaceutics Drug Disposition Classification System (BDDCS) (Wu and Benet 2005). Benet and coworkers proposed this system in 2005 and suggested that the extent of drug metabolism may be used for characterizing high intestinal permeability drugs (Table 1.1) (Wu and Benet 2005; Benet 2010). The rationale behind this classification is that drugs displaying high intestinal permeability rates undergo metabolism, while drugs exhibiting poor intestinal permeability rates were eliminated as unchanged drugs in the urine and bile (Wu and Benet 2005). BDDCS extends the BCS approach to include drug elimination and the effects of efflux and transporters on oral drug absorption (Benet 2010).

They also suggest that drug classification according to BDDCS using elimination criteria, may expand the number of Class I biowaivers while it provides predictability of drug disposition profiles for drugs of Classes II, III and IV.

Table 1.1: BCS and BDDCS classifications

BCS class	**Water solubility**	**Membrane permeability**	**Metabolism**
Class 1	High	High	High
Class 2	Low	High	High
Class 3	High	Low	Low
Class 4	Low	Low	Low

1.1.2 The GIT physiological factors and drug absorption:

The human GIT consists of the stomach, small intestine (the duodenum, jejunum, and ileum) and large intestine (cecum, colon and rectum). The total length of the human GIT is 8.35m, 81% of this area is in the small intestine (DeSesso and Jacobson 2001).

The most important parameter that affects the drug absorption in the GIT is its pH. The stomach pH in fasted state is usually ≤ pH 2, but it can reach to 7.5 in some cases (Dressman, Berardi et al. 1990). In fed state, gastric pH increases to 4.5 and up to 6.7 for about 2–5 h after a meal ingestion (Dressman, Berardi et al. 1990; Kalantzi, Goumas et al. 2006). The fasted state pH of the small intestine is variable according to the location with a range from 5.6 to 7 (Dressman, Berardi et al. 1990). The duodenum pH have been found to be 5.6–7.0, whereas in the jejunum 6.5–7.8 (Kalantzi, Goumas et al. 2006; Moreno, Oth et al. 2006; Clarysse, Brouwers et al. 2011; Bergström, Holm et al. 2014). The pH drops in the terminal ileum to ascending colon to approximately pH 5.5–6.8 and increases in the distal colon to approximately 7-8 in the descending colon/rectum (Kararli 1995; NUGENT, KUMAR et al. 2001; Diakidou, Vertzoni et al. 2009; Bergström, Holm et al. 2014).

The transit time in the GIT significantly influences the extent and rate of drug absorption. The gastric emptying, however, depends on the particles size of the drug formulation (Davis, Stockwell et al. 1986). Larger solid objects such as tablets tend to have longer resident time in the stomach compared to solutions or pellets (Davis, Stockwell et al. 1986).

In contrast to gastric transit time, intestinal transit time is independent of the feeding conditions and the physical composition of the intestinal contents (El-Kattan and Varma 2012). The human small intestinal transit time is about 3 – 4 hours (hr) (DeSesso and Jacobson 2001),whereas, the

large intestine can vary in the range of 8 – 72 hr (DeSesso and Jacobson 2001). The small intestine, in contrast to the large intestine, is covered by villi that relatively increase its surface area (Kararli 1995).

Another factor that plays an important role in drugs absorption is the gut enzymes and transporters. The stomach enzymes, particularly pepsin and lipases, initiate fat digestion and lipid based drug formulations (Porter, Pouton et al. 2008). The human pancreas secrets three major types of enzymes into the small intestine: amylases, lipases, and proteases to degrade the carbohydrate and lipid content (Porter, Pouton et al. 2008).

Enzymes are distributed in large quantities in the intestinal epithelium. Intestinal metabolism usually takes place in enterocytes, but it also can occur within the apical membrane. The intestinal metabolism depends mainly on Phase I enzymes that belong to Cytochromes P450 (CYPs) 1, 2 and 3 families and epoxide hydrolases and Phase II enzymes (UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and glutathione S-transferases (GSTs). These enzymes in the intestinal epithelium may have a significant impact on oral bioavailability (Chow and Pang 2013).

Drug transporters in the gut can help in controlling the absorption of drugs into the systemic circulation; therefore, drug transporters are an important factor that influences the PK of orally dosed drugs. Some identified gut transporters include but are not limited to, P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP), Peptide transporter 1 (Pept1), Monocarboxylate transporter 1 (MCT1), Organic Anion-Transporting Polypeptides (OAT), apical sodium dependent bile acids transporter (ASBT), the oligopeptide transporter (PEPT), and nucleoside transporter N (CNT) (Chow and Pang 2013). Drug absorption in the intestine occurs through the influx transporters and efflux transporters that are distributed on the apical and basolateral

membranes of enterocytes to facilitate the transport of both hydrophilic and lipophilic molecules. Different drugs are subjected to different transporters depending on their chemical structure. For example, small cationic drugs such as, metformin are substrate for influx (OCT), which is distributed in the apical membrane, while in the basolateral membrane, multidrug and toxin extrusion (MATE) facilitates the efflux of the drug. According to the site and role of the transporter, expression of these transporters can lead to an increase of the oral bioavailability or can limit drug absorption. These transporters are subject to inhibition or induction by drugs or other endogenous substances (Chow and Pang 2013).

Bile acids are an essential element in intestinal absorption mainly for lipophilic drugs. Bile is produced by the liver and drained through the bile ducts into the small intestine (DeSesso and Jacobson 2001; El-Kattan and Varma 2012). Bile is composed of bile salts, such as taurocholic acid and deoxycholic acid, these salts combined with phospholipids can emulsify fats and aid in their absorption in the small intestine. Therefore, they play a vital role in the absorption of the lipophilic drugs such as fat-soluble vitamins and steroids (El-Kattan and Varma 2012). In the fasted state, intestinal chyme contains bile acids in the 2–6 mM concentration; whereas this concentration can increase to 15 mM under fed conditions (Kalantzi, Goumas et al. 2006; Bergström, Holm et al. 2014). Compared to the small intestine, the concentrations of bile salts in the large intestine are relatively low (Diakidou, Vertzoni et al. 2009; Bergström, Holm et al. 2014).

These are some of the main physiological features current known about the in the stomach, small intestine and proximal large intestine under normal conditions in adults. These factors, however, could be altered significantly under abnormal conditions such as disease state or even different age groups.

1.1.3 Examples of changes in drug absorption and PK in disease states:

Diseases, surgeries, abnormal conditions and symptoms or other "normal" conditions that are part of life, such as childhood, pregnancy, and aging, can significantly affect the *in vivo* drug performance directly or indirectly (Poggesi, Benedetti et al. 2009). Pain, kidney disease, and obesity treatment via surgery are some worldwide public health concerns that will be discussed in this dissertation.

1.1.3.1 Pain episode

Pain is a subjective feeling that involves multiple processes including sensory and emotional mechanisms (Kanda, Matsuhashi et al.). Pain could be the result of a stimuli (such as burning, cutting or sting), irregular symptoms (such as headache, dysmenorrhoea, or musculoskeletal conditions), or a consequence of other diseases (such as cancer, ischemia, and osteoarthritis) (Moore, Derry et al. 2014). Pain can also be caused by diagnostic and therapeutic treatments including chemotherapy, radiotherapy and surgeries (Urban, Cherny et al. 2010). Pain is often classified by its pathophysiology into nociceptive and neuropathic pain. Nociceptive pain is a normal neural processing that occurs when free nerve endings are triggered by tissue damage or inflammation (Kohan and Hamill-Ruth 2011). On the other hand, neuropathic pain is believed to be caused by abnormal processing of stimuli from the peripheral or central nervous systems. Nociception involves four processes: Transduction, transmission, perception, and modulation (Kohan and Hamill-Ruth 2011). First, an affected tissue releases chemical mediators, such as prostaglandins, bradykinin, serotonin, substance P or histamine. These substances then activate nociceptors to generate an electrical impulse (transduction). The action potential moves from the site of injury along afferent nerve fibers to nociceptors in the spinal cord and get transmitted to the thalamus and the midbrain (transmission). Finally, from the thalamus, nerves

send the nociceptive messages to the somatosensory cortex, parietal lobe, frontal lobe, and the limbic system (perception). Perception is where one can experience the sensory and emotional pain. Finally, descending fibres release substances such as endogenous opioids, serotonin, noradrenaline, gamma-aminobutyric acid, and neurotensin that exert inhibitory effects on pain transmission and produce analgesia (modulation phase) (Kohan and Hamill-Ruth 2011). The pathophysiology of pain is complex and not completely understood as the physiological changes induced by pain are not fully investigated (Urban, Cherny et al. 2010). The effect of the pain on the gastric motility is one of the physiological changes that are observed in pain episodes. The gastric emptying rate is found to be significantly delayed in patients with different kinds of pain (Riezzo, Chiloiro et al. 2000). Pain induced by migraine attacks is found to be associated with reduced stomach emptying time (Volans 1975; Volans 1978). Hypomotility in patients with migraine pain causes a reduced aspirin absorption during migraine attacks compared to headache-free periods in the same patients or control volunteers (Volans 1978). Patients in whom aspirin absorption was delayed were more likely to take longer to respond and to require additional treatment. Metoclopramide, which increases the gastric emptying rate, has been shown to improve the drug absorption rate during migraine and increase the rate of recovery (Volans 1975). The same observation was found in adult volunteers with pain induced by dental surgery. A study reported that ibuprofen absorption was clearly delayed by pain (Jamali and Kunz-Dober 1999; Jamali and Aghazadeh-Habashi 2008). Yomona et al investigated the relationship between "hunger pain" induced by dyspepsia and the alteration in the gastric emptying rates (Yomona-Hernández, Vicente-Ríos et al. 2003). The study was conducted in 40 patients presenting idiopathic dyspepsia, 20 with and 20 without "hunger pain", and 30 voluntary apparently normal control subjects. The patients and the controls ingested a gelatine capsule containing 10 radiopaque polyurethane markers with a standard breakfast, and the gastric

emptying of the markers was evaluated taking 3 x-ray films of the abdomen at 1.5, 3.0 and 4.5 hours after the breakfast. The gastric emptying rates of the markers were significantly longer in the patients with "hunger pain" than in the patients without "hunger pain" and the normal control subjects (Yomona-Hernández, Vicente-Ríos et al. 2003).

A recent study analysed pediatric gastric motility in pain attacks. The gastric motility rate of 102 children who had abdominal pain and 20 healthy controls were evaluated and compared (Devanarayana, Rajindrajith et al. 2013). The children with pain had significantly lower gastric emptying rates and motility contraction frequency than the healthy group. The study found the majority of children with pain had gastric emptying rates below the 10th percentile of that of the control group (Devanarayana, Rajindrajith et al. 2013). Motility of the stomach plays a pivotal role in driving gastric contents into the duodenum. A delay in the gastric motility rate is expected to affect drug absorption when administered in pain episodes, especially if a drug has low solubility such as BCS class 2 and 4 drugs. This would be the case particularly for Nonsteroidal anti-inflammatory drugs (NSAIDs) (Moore, Derry et al. 2014).

1.1.3.2 Obesity surgery treatment:

Obesity arise when one has a body mass index (BMI) $\geq$ 30 Kg/m^2 (Sawaya, Jaffe et al. 2012) . Chronic serious diseases such as diabetes mellitus, cardiovascular disease, dyslipidemia, hypertension, nonalcoholic fatty liver disease, cancers, musculoskeletal disorders, and other conditions have been repeatedly associated with obesity. To reduce the incidence of these sever diseases and increase the morbidity rate in these patients, a weight loss of total body weight is required (Mattar, Velcu et al. 2005). Traditional therapies such as dietary modifications and medical drug therapy are relatively ineffective to treat obesity in the long term (Smith, Henriksen et al. 2011). Surgery procedures to achieve weight loss are considered to be the most effective

treatment, especially in morbid obesity (Mattar, Velcu et al. 2005). Different surgical techniques such as intragastric balloons, sleeve gastrectomy, Roux-en-Y Gastric Bypass (RYGB) , gastroplasty, and gastric banding procedures have been used to control obesity (Buchwald and Buchwald 2002). Over the last 20 years the practice of obesity surgeries has been significantly increased; at least 220,000 operations were performed in USA alone (Sawaya, Jaffe et al. 2012) (ASMBS.org, accessed April 2015). The physiological abnormalities induced by these surgeries can strongly influence drug bioavailability and absorption (Tandra, Chalasani et al. 2013). The absorption might decrease, increase or does not change after these operations (Seaman, Bowers Sp Fau - Dixon et al. 2005; Tandra, Chalasani et al. 2013; Lloret-Linares, Hirt et al. 2014; Walsh and Volling 2014). No general rule is established in these conditions as there are many factors that influence this mechanism such as the drug's physiochemical properties, the type of procedure and the time period post operation. To clarify that, I will focus on the most advanced and most commonly currently used procedure: The RYGB procedure. In RYGB, a new proximal gastric pouch that holds 15–50 ml connects directly to the Roux limb of the jejunum, bypassing the rest of the stomach and the entire duodenum (Smith, Henriksen et al. 2011; Sawaya, Jaffe et al. 2012; Tandra, Chalasani et al. 2013). Patients who undergo RYGB lose about 100–200 cm of their proximal small bowel absorptive length. The remaining portion of the proximal jejunum is reconnected to the rest of the small intestine, which enables the passage of bile salts and pancreatic enzymes (Smith, Henriksen et al. 2011).

The creation of the small stomach pouch causes slowed gastric emptying, which may delay drug absorption. This small pouch formed after the surgery may also leads to increased stomach's pH in these patients (Rubio, Galrão et al. 2012). This effect is expected to influence the amount of ionized drug and the solubility of ingested drugs in the stomach and upper intestine of patients

who have undergone RYGB surgery. For example, most NSAIDs are weak acids with pKa values ranging from 3 to 5. As mentioned above, the normal stomach has a pH of 1–3, so the drugs remain un-ionized until they reach the small intestine. However, the physiological changes in patients with RYGB increase the stomach pH value, and thus make NSAIDs more ionized (Smith, Henriksen et al. 2011). This increases the solubility and accelerates the dissolution rate of NSAIDs in the stomach. Consequently, the absorption of these drugs will increase, and this could increase the risk of the side effects in these patients (Smith, Henriksen et al. 2011). On the other hand, the change in the pH after RYGB is not expected to change the solubility of drugs with high pKa values; therefore, it is less likely to be affected by the pH alteration (Smith, Henriksen et al. 2011). The intestinal transit time is found to be shortened in RYGB patients; therefore, the bioavailability of drugs with low solubility, or drugs in controlled release formulation could be limited as these drugs may not have adequate transit time for full dissolution and absorption (Seaman, Bowers Sp Fau - Dixon et al. 2005). The bioavailability of drugs with an intrinsically low bioavailability after oral administration could be lower in patients with an altered GI physiology, such as patients who have undergone RYGB.

The villi and microvilli responsible for the absorptive surface in the small intestine are of highest concentration in the duodenum and proximal jejunum. Therapy, bypassing this part of the intestine is expected to cause a decrease in the drug absorption (Smith, Henriksen et al. 2011).

The absorption of drugs subjected to transporters and metabolic enzymes can be significantly altered after surgery. Some of the key transporters and metabolic enzymes are not distributed equally, but they are expressed more in the proximal small intestine, duodenum and upper jejunum, which is the area being bypassed (Smith, Henriksen et al. 2011). The concentration and distribution of human intestinal cytochrome P-450 enzymes is altered after the surgery (Seaman,

Bowers Sp Fau - Dixon et al. 2005). Therefore, the drug substrate to these enzymes is expected to be affected (Seaman, Bowers Sp Fau - Dixon et al. 2005).

Drugs that rely on enterohepatic recycling may also have unpredictable alterations in the PK behavior due to a decreased contact with the proximal intestine or altered mesenteric blood flow (Lloret-Linares, Hirt et al. 2014) . For example, morphine undergoes a hepatic first pass as part of its total systemic clearance. P-gp and uridine diphosphate glucuronosyltransferase (UGT) isoforms are the key elements of morphine in the intestinal and hepatic first-pass effect (Lloret-Linares, Hirt et al. 2014). RYGB was found to dramatically increased the rate of morphine absorption and increase morphine exposure (Lloret-Linares, Hirt et al. 2014). The reduction in the morphine intestinal and/or liver first-pass effect and increased bioavailability with weight loss after surgery is supported by decrease in the UGT enzymes and P-gp transporter (Lloret-Linares, Hirt et al. 2014).

1.1.3.3 Renal dysfunction

Renal elimination is one of the main pathways of drug excretion. Therefore, renal impairment is expected to significantly affect drug's PK in patients who have this condition (Rowland Yeo, Aarabi et al. 2011). Generally, the degrees of renal impairment are categorized according to the kidney function and creatinine clearance values as mild, moderate, severe, and end-stage renal disease (Poggesi, Benedetti et al. 2009). The US Kidney Disease Outcomes Quality Initiatives of the National Kidney Foundation defines stages of Chronic kidney disease (CKD) based on the level of the Glomerular filtration rate (GFR) to 4 stages as presented in table 1.2 (Levey, Coresh et al. 2003)

Table 1.2: The stage of Kidney Disease based on the GFR levels

Stage	GFR (mL/min/1.73 $m^{2)}$
Stage 1: Normal	≥90
Stage 2: Mild	60–89
Stage 3: Moderate	30–59
Stage 4: Severe	15–29
Stage 5: Kidney failure	<15

Renal dysfunction will not only influence drugs that are normally excreted through kidney, but also drugs that are excreted by the liver or biliary (Rowland Yeo, Aarabi et al. 2011; Tsamandouras, Rostami-Hodjegan et al. 2015). In fact, renal dysfunction can induce changes in intestinal absorption and first pass metabolism, hepatic, renal and intestinal transport, plasma protein binding and tissue binding (Rowland, Peck et al. 2011; Rowland Yeo, Aarabi et al. 2011). This condition, therefore, can increase the risk of adverse events due to the accumulation of drugs and their active or toxic metabolites (Dowling 2002) . In 2010, the FDA issued a draft guidance highlighting the need to perform clinical studies on drugs that are eliminated by both non-renal and the renal route in patients with renal damage (FDA 2010).

The drug absorption changes in renal failure include a delay in gastric emptying that affects the time required to reach the maximum plasma concentration, especially if the administered drug has low solubility. The gastric pH is found to be higher in patients with renal failure (Watanabe, Kusuhara et al. 2009).

Drug metabolism and excretion are also altered in renal failure patients. Some of the identified mechanisms underlying this alteration are the accumulation of uremic toxins. Researchers have identified 90 uremic solutes in renal failure, such as indoxyl sulfate (IS), parathyroid hormone (PT), and cytokines (Vanholder, De Smet et al. 2003). Vanholder et al. has reviewed these toxin and their physicochemical characteristics in depth (Vanholder, De Smet et al. 2003). These toxins are found to cause variation in the enzymes and/or transporters (Dreisbach and Lertora 2008). For example, studies found that the high concentrations of PT in renal failure led to the down-regulation of hepatic P450 (CYP) enzymes (Leblond, Guevin et al. 2001; Guevin, Michaud et al. 2002; Dreisbach and Lertora 2008). Propranolol, which is metabolized in the liver, showed an increase in the bioavailability in renal failure animal models. The isolated perfused liver from control and renal failure animals showed a 50% reduction in hepatic CYP activity when perfused with uremic serum (Terao N Fau - Shen and Shen). Other drugs such as Nimodipine, Verapamil, Metoclopramide, Desmethyldiazepam, and Warfarin also showed ≥50% reduction of liver CYP activity in renal failure animal models (Dreisbach and Lertora 2008). Human studies in renal failure patients confirm these findings; Propranolol, Erythromycin, Propoxyphene and Oxprenolol show an increase by 100% or more in their bioavailability in renal failure patients (Bianchetti G Fau - Graziani, Graziani G Fau - Brancaccio et al. ; Dowling, Briglia Ae Fau - Fink et al. ; Wood, Vestal et al. 1980; Dreisbach and Lertora 2008). The activity of phase II enzymes; particularly N-acetyltransferase, was also shown to be inhibited in renal failures rats, and this inhibition was secondary to the increase in the PT hormone level (Kim, Shin et al. 1993). This observation was established in humans. The non-renal drug clearance of isoniazid, as an example of an N-acetyltransferase substrate, was remarkably decreased in renal failure patients. After kidney transplant and normalization of the kidney function, the drug

clearance (CL) returned to the normal level compared to the patients before renal transplantation (Kim, Shin et al. 1993; Simard, Naud et al. 2008).

In rat models, renal injury produces also showed a remarkable decrease in intestinal total CYP enzymes levels (Dreisbach and Lertora 2008). This effect leads to a decrease in first-pass metabolism which will likely increase the bioavailability of drugs that are subjected to this pathway (Dreisbach and Lertora 2008).

Drug transport and metabolism are closely connected. Renal failure affects the expression of drug transporters in the liver and intestine differently (Sun, Frassetto et al. 2006). Drug uptake across the hepatocytes mediated by influx transporters such as OAT and efflux transporters such as the multi-drug resistance associated protein (MRP) can be a rate-limiting step in the process of the hepatic elimination. Studies showed different types of alteration by increase, decrease, or unchanged transporter levels (Laouari, Yang et al. 2001). The down regulation of these transporters was explained by the increase of uremic toxin. Such observation was documented for OAT and organic cation transporters (OCT2) in a renal failure animal model. (Naud, Michaud et al. 2008). However, some cases showed an overexpression of the drug transporter as an adaption mechanism of the physiological changes in renal failure, enabling a shift in excretion from kidney to other tissues (Lalande, Charpiat et al. 2014) . The liver P-glycoprotein (Pgp) is an example of such a transporter which showed a 25% increase in the protein expression level and 45% of its activity in biliary excretion (Naud, Michaud et al. 2008). In contrast, another study by the same author showed a decrease in the intestinal Pgp expression transporter in renal dysfunction (Naud, Michaud et al. 2007). Incubation of rat enterocytes with rat uremic serum confirms this reduction in the intestinal Pgp expression and activity, which confirm the role of

circulating uremic factors (Naud, Michaud et al. 2007). On the other hand, the MRP transporter expression did not changed in renal failure patients (Laouari, Yang et al. 2001).

Renal failure can also lead to conformational change in the albumin, which can result in altered albumin binding affinity and consequently change the free unbound fraction (fu) of the administered drug (Dreisbach and Lertora 2008). Hypoalbuminemia in renal failure patients can decrease the plasma protein binding of acidic drugs, which increases the fu of these drugs. In the same manner the decrease in plasma levels of alpha 1-acid glycoprote reduces basic drugs binding in these patients (Sun, Frassetto et al. 2006; Dreisbach and Lertora 2008). This alteration will have a significant effect especially for drugs with a high extraction ratio and a narrow therapeutic window.

Pain, obesity surgery and kidney failure can influence drug absorption through various mechanisms. These complex mechanisms are difficult to evaluate or predict in clinical settings, especially with ethics restrictions in such cases. Mechanistic tools can assist researchers to gain a better understanding of such effects. Computer simulations are one of these tools. The next section will provide some background on the uses of computer simulations.

1.2 Computer simulation

Computational models currently are able to predict the properties discussed above and their effect on each other with a relatively high degree of accuracy. Using structural descriptors, many physiochemical and biopharmaceutical properties can be estimated (Bergström, Holm et al. 2014). Moreover, state-of-the art *in silico* tools can model physiological factors and help to predict the *in vivo* absorption. Some of these mechanistic gastrointestinal simulations are based on the Advanced Compartmental Absorption and Transit (ACAT) model. Such modeling offers advantages in modelling drug absorption; they account not only for the drug substance's

physicochemical, PK, and formulation characteristics but also for the physiological conditions of the GIT of the species of interest (Ilić, Đuriš et al. 2014). In more recent years, the knowledge of the important physiological factors on drug absorption lead to the development of physiologically based pharmacokinetic (PBPK) models. This development tool can assess clinical risks for new formulations (Kesisoglou 2014). These models will be discussed in more details.

The Compartmental Absorption and Transit (CAT) model is one of the earliest approaches to describe mechanistically the rate and extent of oral drug absorption (Ilić, Đuriš et al. 2014). The compartmental concept was adopted due to the variability in physiological factors in different segments of the gut. For example, as discussed earlier, the stomach transit time is different than the intestine, and transit time of the intestine varies according to anatomical location. This model was first created in the 1980s as a simple approach based on a series of mixing tank compartments (Goodacre and Murray 1981; Dressman, Fleisher et al. 1984; Yu, Lipka et al. 1996). The current CAT model structures is based on three theory categories: The first category is referred to as quasi-equilibrium models that include the pH-partition hypothesis and the absorption potential concept (Yu, Lipka et al. 1996). The second category is the steady-state model, which includes the film model and a mass balance approach. The last category is related to the dynamic models. The equations used in the CAT model are described in detail in the model review (Yu, Lipka et al. 1996). The CAT model consists of seven major absorptive compartments in the GIT: The stomach is the first compartment followed by five compartments within the small intestine and one in the colon. Using a set of differential equations and movement of the drug molecules across these compartments, the oral drug absorption can be predicted (Yu, Lipka et al. 1996).

The Advanced CAT (ACAT) model is an extended version of the CAT model that includes metabolism and colonic absorption (Yu and Amidon 1999). The ACAT model consists of nine compartments: the stomach, seven segments within the small intestine, and the colon (Yu and Amidon 1999) (Figure1.1). The advantage of the ACAT model is the ability to account for both linear and nonlinear transfers within three possible drug states: unreleased, undissolved, and dissolved. This model can be used to consider whether the drug remains within the formulation, or if it is dissolved or suspended in the intestinal fluids. Therefore, this model considers physicochemical properties, physiological properties, and dosage form aspects. This model can be used by pharmaceutical companies to examine formulation factors affecting drug absorption.

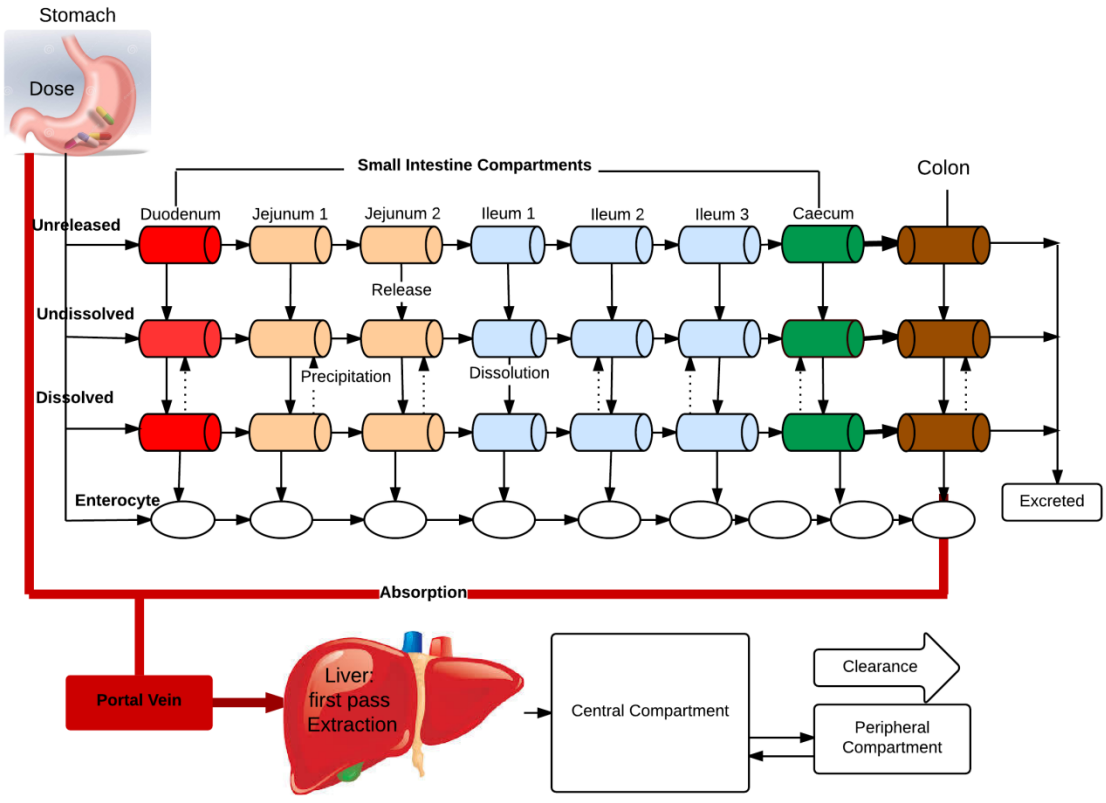


Figure1.1: ACAT model in GastroPlus™

The complex absorption behaviors such as regionally dependent absorption, pH-dependent solubility, influx and efflux transporters, and saturable systems and gut metabolism need to be considered in modeling. These demands lead to incorporate the mechanistic absorption physiologically based pharmacokinetic (PBPK) model. The PBPK approach was proposed in 1937 by a Swedish scientist: Teorell (Teorell 1937). He developed a five-compartment scheme to reflect the circulatory system, drug accumulation, fluid volume, kidney elimination and tissue inactivation (Teorell 1937; Edelmann 1959). A PBPK modeling is a collection of *in vitro* experiments of the target drug and the current knowledge physiology of the intended species (Kostewicz, Aarons et al. 2014). The PBPK model allows characterizing individual organ behaviors and accounting for separate inductive or inhibitory effects, such as transporters and enzymes, to the overall absorption (Chow and Pang 2013). PBPK models take into account volumes of distribution, fluid flow in the organ, and consider the tissue binding characteristics (Chow and Pang 2013). A schematic of the PBPK model is shown in Figure 1.2.

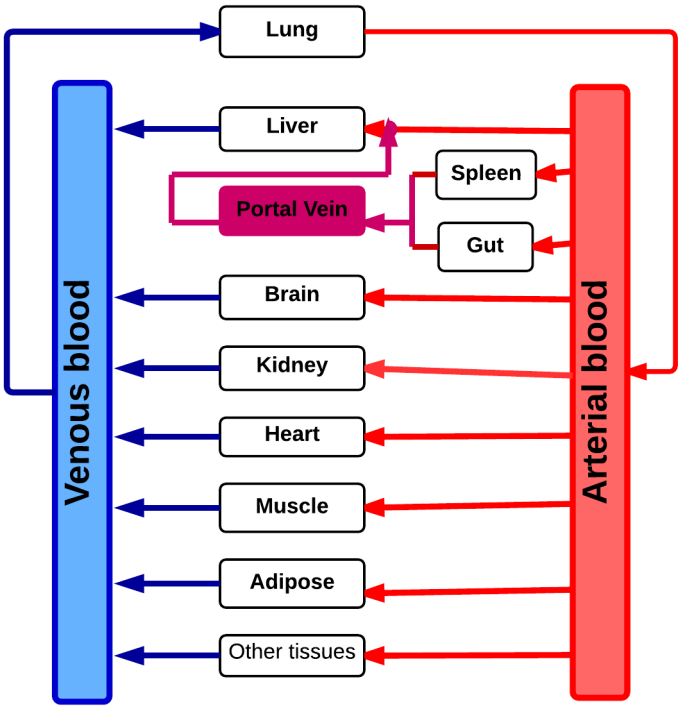


Figure 1.2: A schematic of a PBPK model

Each compartment is distinct by a tissue volume and a tissue blood flow rate which is particular for the species of interest. PBPK models should be able to predict human PK by scaling animal data to man. These physiological parameters are extracted from several sources in the literature (Jones, Parrott et al. 2006; Jones, Parrott et al. 2006; De Buck, Sinha et al. 2007; Jones, Gardner et al. 2009; Jones, Mayawala et al. 2013). According to the drug nature at the physiological barrier, a tissue can be described as either perfusion-rate-limited or permeability-rate-limited. Perfusion-rate-limited kinetics describes small lipophilic molecules that can passively crossing membranes, so a drug will be limited by the blood flow to the tissue. On the other hand, permeability-rate-limited kinetics occurs for polar larger drug molecules that have difficulty penetrating the tissue; in this case, the permeability across the cell membrane becomes the limiting factor (Jones, Gardner et al. 2009). Therefore, such models can provide mechanistic

insights in the PK of drugs in the GIT and the entire body. The mass balance equations used in the PBPK model generally described as follows:

Non-eliminating tissues mass: VT* dCT/dt=QT *CA- QT* CVT

Q is blood flow (L/h), C is concentration (mg/L), V is volume (L), T is tissues, A is arterial, V is venous, CVT = CT/Kp, where Kp is tissue to plasma partition coefficient of the compound (Jones, Gardner et al. 2009).

The equation of eliminating tissues (kidney and liver) is described as:

VT* dCT/dt=QT *CA -QT *CVT –Clint* CVT

where Clint is the intrinsic clearance of the compound (L/h) (Jones, Gardner et al. 2009).

The model requires compound-specific information such as clearance (CL), and the tissue to plasma partition coefficients (Kp values), to predict the plasma concentration time profiles of the compounds in a species of interest. Human CL can be predicted from *in vitro* systems (hepatocytes and microsomes) or from preclinical species using allometric scaling (Jones, Parrott et al. 2006; Jones, Gardner et al. 2009). Tissue Kp values can be calculated by different equations. These equations assume that a drug distributes homogenously into the tissue and plasma by passive diffusion estimated from drug lipophilicity data (LogP and LogD) (Jones, Dickins et al. 2011). The first set these equations was developed by Poulin and coworkers (Poulin and Theil 2000; Poulin, Schoenlein et al. 2001; Poulin and Theil 2002; Theil, Guentert et al. 2003) and later adjusted and extended by Berezhkovskiy (Berezhkovskiy 2004), Rodgers and the other coworkers (Rodgers, Leahy et al. 2005; Rodgers and Rowland 2006; Rodgers and Rowland 2007). According to the purpose of a simulation, this mechanistic model provides a physiological framework enabling the incorporation of active transport and metabolism when

such data are available.

As knowledge in this area grows, PBPK models are recognized and becoming more established. Therefore, scientists are seeking to develop sophisticated computer programs that can incorporate these models. Such software could predict the oral drug PK, facilitate drug candidate selection, establish formulation development strategy, and aid the progress of regulatory policies (Huang, Lee et al. 2009).

iDEA™ from (Lion Bioscience Inc.) was the first graphical based software that introduces the physiologic meaning in the model compartments (Grass 1997). iDEA™ is based on a physiological absorption model described by Norris et al and is now part of a system of simulation models called pK EXPRESS™ (Norris, Leesman et al. 2000; Schmitt and Willmann 2004). In 1998, more sophisticated commercial software in the context of PBPK models become available that provides a full description of the GIT model GastroPlus™. Other software packages are also currently available such as SAAM II, acslXtreme ®, ADAPT II, SAS ®, S-PLUS ®(Seattle, WA, USA), R, Xpose, NONMEM ®, Monolix, WinNonMix ®, WinBUGS, POPT, WinPOPT, WinNonLin®, Trial Simulator ®, Drug Model Explorer ®, PK-Sim ®, Cloe ®, MEDI CI-PK®, and Simcyp ® (Kostewicz, Aarons et al. 2014; Di Fenza et al., 2007) (Paixão, Gouveia et al. 2012). These simulations software uses a variety of *in vitro* and *in silico* input data with a series of built-in equations. Table 1.3 compares the main features of the most popular BPBK based model software (Bouzom, Ball et al. 2012). In this dissertation, the focus will be on GastroPlus™ as the most recognized available software used by the pharmaceutical industry and regulatory agencies.

Table 1.3: Comparison of the main features of selected PBPK based model software (Bouzom, Ball et al. 2012)

		Cloe ® Predict	GastroPlus ®	MEDI CI-PK®	PK-Sim ®	Simcyp ®
Species used in simulation	Humans	✓	✓		✓	✓
	rats		✓		✓	✓
	mice		✓			✓
	dogs		✓		✓	
	monkey		✓		✓	
	Beagle		✓			
	Cats		✓			
	Rabbit		✓			
	Minipig		✓			
	User define			✓		
Dosage form used in simulation	IV	✓	✓	✓	✓	✓
	Subcutaneous			✓	✓	
	PO	✓	✓		✓	✓
	pulmonary,		✓			✓
	Ocular		✓			
	Dermal		✓		✓	✓
	User define				✓	
Metabolism (Cyp-450 built-in database)			✓			✓
Transporters			✓	✓	✓	✓
DDI			✓	✓	✓	✓
IVIVC			✓		✓	✓
PBPK/PD			✓			
Paediatric module			✓		✓	✓
Sensitivity analysis		✓	✓	✓	✓	✓
Parameters estimation		✓	✓	✓	✓	✓
Virtual trials		✓	✓	✓	✓	✓

1.2.1 GastroPlus™:

This software is distributed by Simulations Plus, Inc., Lancaster, CA, USA. GastroPlus™ is periodically updated to match the current knowledge about the GIT in different species. Based on the diffusion equation of Fick's First Law (Fick 1855) the GastroPlus™ oral absorption simulation is a net of both, the secretion from enterocytes to the lumen as well as absorption from the lumen to the enterocytes **(**GastroPlus™ manual user).

The ACAT model in GastroPlus™ describes the drug release from the formulation, dissolution/precipitation rate, chemical stability, permeability, carrier mediated influx and efflux and gut wall metabolism. The ACAT model implemented in the GastroPlus™ is 18 compartments (stomach, six compartments for the small intestine, two colon and nine enterocyte compartments); three states of the material (unreleased, undissolved, and dissolved), and drug concentration in physiologically based organ compartments when tissue partition and flow rate parameters are available. The total amount of drug absorbed is summed over the integrated amounts being absorbed/secreted from each absorption/transit compartment (Figure1.3).

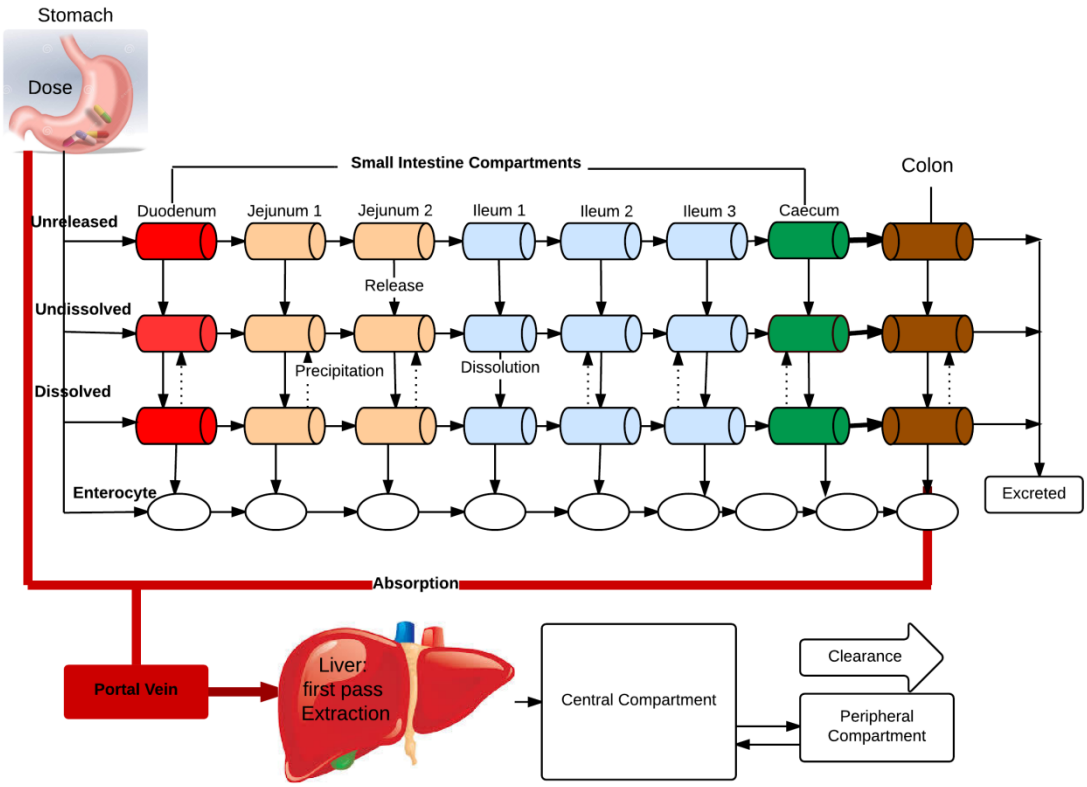


Figure1.3: The ACAT model in GastroPus™

The PK of both linear kinetics and nonlinear kinetics can be used. The nonlinear PK (Michaelis–Menten) can be selected to describe a saturable mechanism in metabolism and transport (Michaelis and Menten 1913).

GastroPlus™ can use the ACAT and PBPK mechanistic absorption models simultaneously (Figure 1.4). The input data required are a mix of the *in vitro*, *in vivo* or *in silico* estimation parameters according to the availability. When the input parameters required in GastroPlus™ are not available, the software alternatively can predict these characteristics from the drug structure using the optional ADMET Predictor™ module, which is another program from Simulations Plus, Inc., Lancaster, CA, USA. The accuracy of the prediction is improved through the availability of data input.

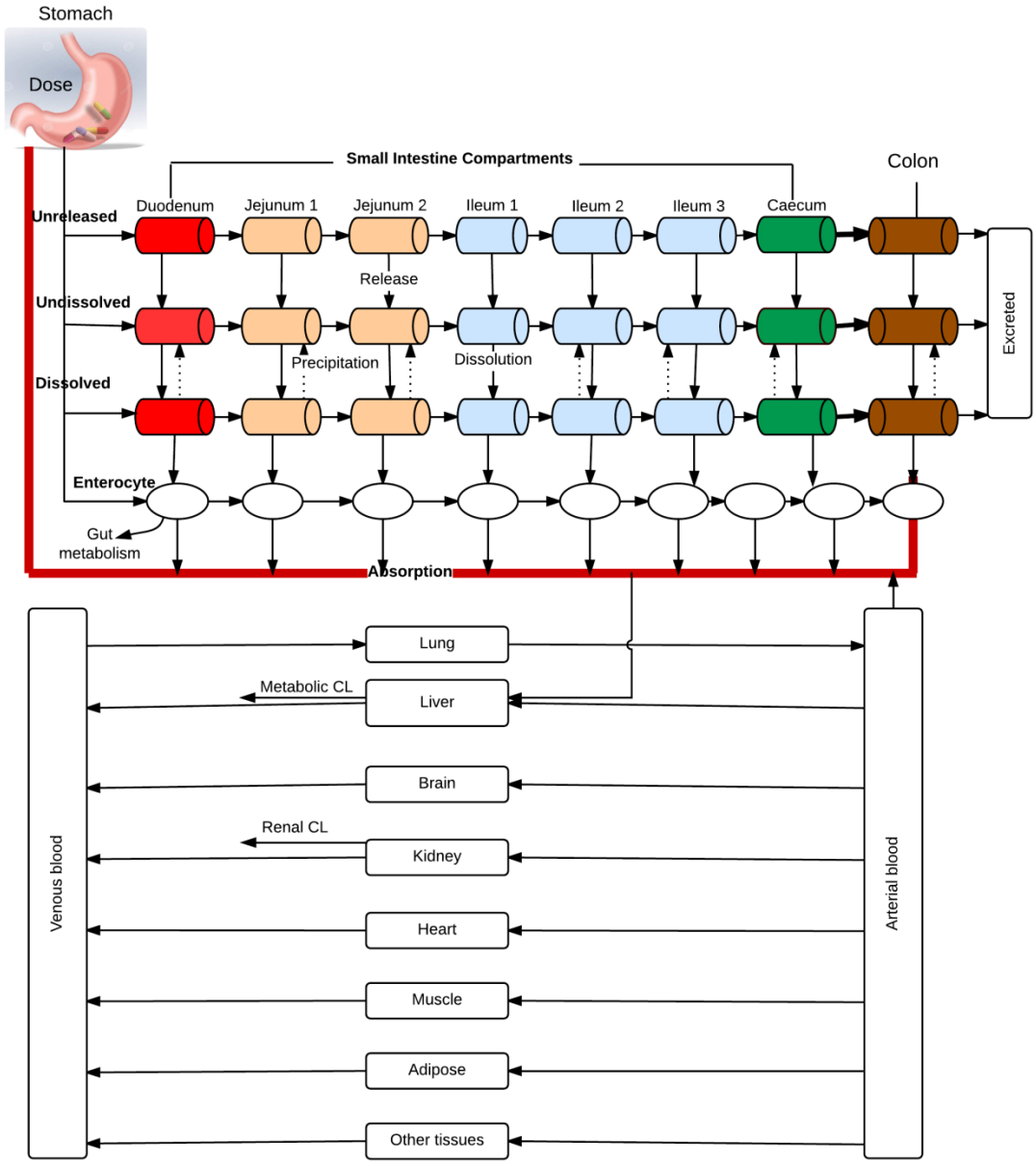


Figure 1.4: ACAT and PBPK model in Gastroplus™

The GastroPlus™ graphical user interface consists of five sections arranged as tabs on file folders. The compound tab contains a list of physicochemical properties. These formulation-related parameters include the drug aqueous solubility, pKa, permeability, dissolution profile, and formulation type. These values can be acquired from loading additional support files into the

GastroPlus™ File-Load menu such as the chemical structure, dissolution profile and solubility profile (Figure 1.5). The Physiology tab contains the physiological parameters for the selected species. In this tab, many settings can be chosen according to the predication goal. For example the species that can be selected (human, beagle, rats, mic, monkey, cats, or rabbits), fed or fasted state, tissue compartment characteristics (pH, transient time, volume and length). GastroPlus™ provides a logD model which scales regional permeability due to the changing environment for trans- and paracellular transport. Also, the changing surface area, the abundances of the villi and microvilli, and regional pH values are considered.

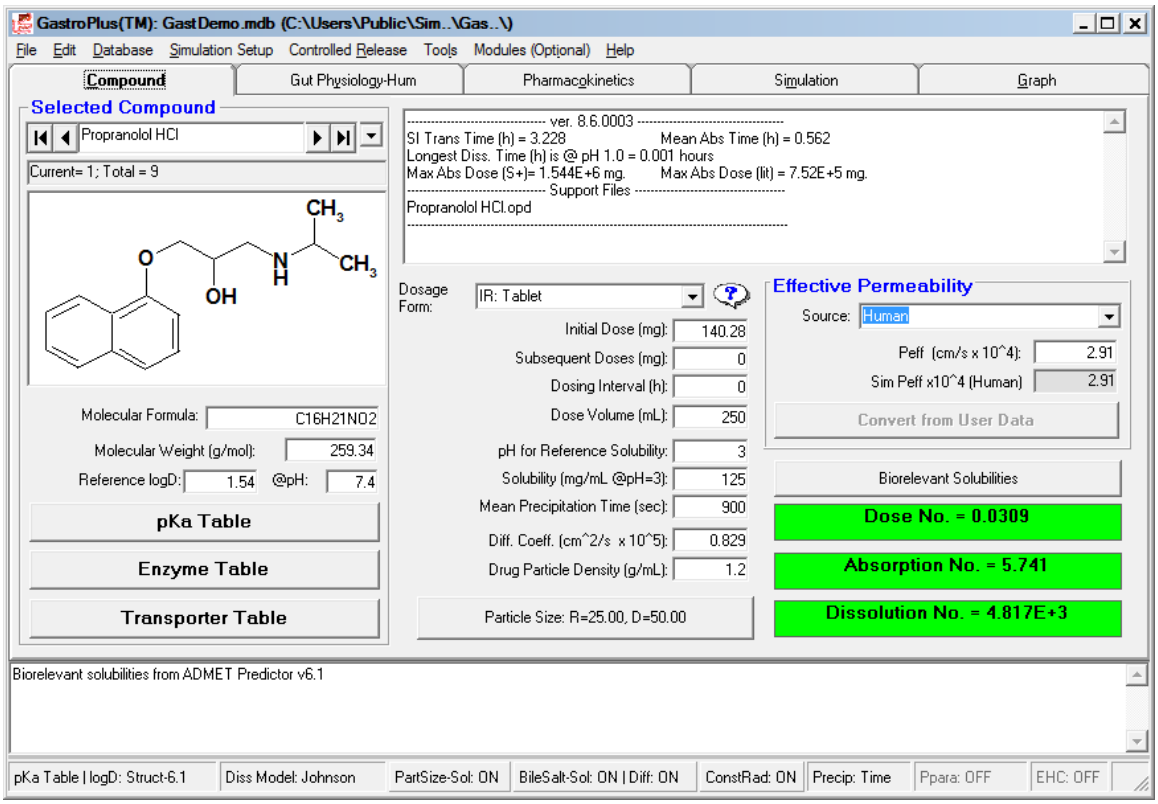


Figure 1.5: The compound tab in GastroPlus™

The Pharmacokinetics tab contains PK parameters. Certain observed values and scale factors for saturable processes in the ACAT model in the gut and the liver can be chosen when using

compartmental PK. The PK inputs required depend on the simulation objective. For example, to predict only the fraction absorbed, no PK inputs are necessary. In contrast, in order to predict plasma concentration (Cp)-time, some PK parameters from IV data would be necessary. The same applies when modeling influx and efflux transporters or enzymes. Here, the *in vitro* kinetics such as the Michaelis constant (K_m,), which represents the substrate concentration at which the rate of reaction is half-maximal, and the maximum rate of the reaction (V_{max}) as input parameters are required.

The last tab is the Simulation tab where the graph of plasma concentration vs time is simulated. Also the software has different optional tables for data inputs in order to increase the accuracy of the simulation. For example: Enzymes Table contains information on any enzymes responsible for metabolizing the drug *in vivo*, Transporters Table contains information on efflux and influx transporters for the drug, Pharmacodynamics Table contains pharmacodynamics (PD) models for each drug record in the database, and PBPK Table contains properties of each tissue.

In GastroPlus™ (version 9) different formulation type options can be used for the simulations. The formulations which can be selected are: Intravenous (IV*)* bolus, IV infusion, Immediate Release (IR) (tablet or capsule), solution, suspension, and Controlled Release (CR) formulations. The CR (dispersed, gastric, integral, or buccal patch) formulation dissolves according to the selected dissolution model and percent released vs. time table that can be specified. In the current version 9 there are additional other dosage forms, which are only available when having an optional license. For instance, ocular route: four of these are IR dosage forms and two are CR dosage forms (implants) available. Nasal-pulmonary drug delivery is another route that can be selected, which includes a number of different intranasal dosage forms according to the physiological state (liquid or solid) and delivery time (single bolus or over a certain period of

time). The dermal rout is also considered in GastroPlus ™: The Transdermal Compartmental Absorption & Transit (TCAT™) model, which represents the skin as a collection of the following compartments: stratum corneum, viable epidermis, dermis, subcutaneous tissue, sebum, hair lipid, and hair core. The model can simulate a variety of transdermal & subcutaneous dosage forms: Liquid formulations (solutions, lotions, and suspensions), semi-solid formations (gels, creams, lotions, pastes) and subcutaneous injections. Moreover, the software has an option to simulate mixed or multiple doses using different dosage forms at specified time intervals on the same data set.

Fitting is sometimes used in simulations in two ways: If IV plasma concentrations data is available, then a 1, 2 or 3 compartment model may be fitted to the data and used to simulate drug disposition. The chosen compartmental model links the data to the ACAT model. Using GastroPlus™, the first-pass effect due to metabolism can be estimated from *in vitro* data generated in intestinal microsomes or hepatocytes (Chow and Pang 2013). Improvements in the accuracy of simulations are based on the accuracy input parameters and *in vitro* methods which are used to generate them. The heterogeneous expression of transporters such as P-gp, OCTN1, and OAT1A2 - and physiological characteristics of the small and large intestines have been incorporated into the ACAT of GastroPlus™.

GastroPlus™ also takes into account the effects of age, genetics, and ethnic group. The GastroPlus™ implementation of PBPK offers an internal module called Population Estimates for Age-Related Physiology (PEAR) that have default settings calculation of the organ physiologies for Western and Asian human models for ages ranging from 0 to 85 years old (CDC 2010).

As discussed above, there are numerous variable factors that control oral drug absorption. This emphasizes the demand to use computer simulations in order to better understand the influence

of these factors, especially in disease conditions. Using such software in disease conditions can help to understand the mechanism of absorption, predict the PK of administered drugs, individualize patients' dose or treatment regimen and optimize dosage forms (Wagner 1981; Kostewicz, Aarons et al. 2014). However, these tools have to be evaluated and investigated for their accuracy.

The first disease modeling effort in this thesis represents pain, which can cause a direct effect on stomach leading to a manifest consequence on the drug absorption and bioavailability. The physiochemical characters and formulations play a rule in the drug absorption in a pain episode as will be discussed in details in Chapter 2. The changes in drug absorption induced by disease can be unpredictable as we see in obesity treatment surgery in Chapter 3. This can be even more complicated in disease conditions such as renal failure as discussed in Chapter 4.

1.3 Hypothesis:

In the previous sections, I have provided evidence from the literature on the potential for physiological changes induced by disease to affect drug absorption. I presented how the mechanisms of these changes are overlapping. Moreover, I have discussed the applications of computer simulation as modern tool to predict and evaluate the drug absorption. The hypothesis is that suitable computer software models can help to mechanistically understand the complex changes in drug absorption that are induced by disease conditions. This tool can be used to help researchers to gain insights in unexplained or unpredicted clinical observations under disease conditions.

1.4 Objectives:

Using GastroPlus™ as one of the most sophisticated software in its field, the follwing objectives were pursued:

- To investigate the ability of computer simulation to predict and understand the PK of drugs under normal and gastric dysfunction conditions using meloxicam and ibuprofen as model drugs.
- To test the assumption that the stomach controls the drug release under pain condition using meloxicam and ibuprofen.
- To investigate the formulation effect of meloxicam and ibuprofen in delayed gastric motility is induced by pain.
- To simulate metformin absorption in normal humans and in patients with post gastric bypass surgery.
- To investigate the mechanistic background of the changes in metformin absorption in patients with post gastric bypass surgery.
- To evaluate the utility of the software to gain knowledge about metformin absorption in renal impairment.
- To investigate the correlation between the effect in the increase in serum creatinine level and metformin elimination on the MATE1 transporter using *in vitro* experiments.

1.5 References:

Almukainzi, M., N. A. Bou-Chacra, et al. (2014). Biorelevant Dissolution Testing. Therapeutic Delivery Solutions, John Wiley & Sons, Inc.**:** 335-365.

Amidon, G., H. Lennernäs, et al. (1995). "A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability." Pharmaceutical Research **12**(3): 413-420.

Benet, L. Z. (2010). "Predicting Drug Disposition via Application of a Biopharmaceutics Drug Disposition Classification System." Basic & Clinical Pharmacology & Toxicology **106**(3): 162-167.

Berezhkovskiy, L. M. (2004). "Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination." Journal of Pharmaceutical Sciences **93**(6): 1628-1640.

Bergström, C. A. S., R. Holm, et al. (2014). "Early pharmaceutical profiling to predict oral drug absorption: Current status and unmet needs." European Journal of Pharmaceutical Sciences **57**(1): 173-199.

Bianchetti G Fau - Graziani, G., D. Graziani G Fau - Brancaccio, et al. "Pharmacokinetics and effects of propranolol in terminal uraemic patients and in patients undergoing regular dialysis treatment." (0312-5963 (Print)).

Bouzom, F., K. Ball, et al. (2012). "Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs?" Biopharmaceutics & drug disposition **33**(2): 55-71.

Buchwald, H. and J. Buchwald (2002). "Evolution of Operative Procedures for the Management of Morbid Obesity 1950-2000." Obesity Surgery **12**(5): 705-717.

Camenisch, G., J. Alsenz, et al. (1998). "Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight." European Journal of Pharmaceutical Sciences **6**(4): 313-319.

CDC. (2010). "National Center for Health Statistics (NCHS)."

Chow, E. C. Y. and K. S. Pang (2013). "Why we need proper PBPK models to examine intestine and liver oral drug absorption." Current Drug Metabolism **14**(1): 57-79.

Clarysse, S., J. Brouwers, et al. (2011). "Intestinal drug solubility estimation based on simulated intestinal fluids: Comparison with solubility in human intestinal fluids." European Journal of Pharmaceutical Sciences **43**(4): 260-269.

Comer, J. and K. Tam (2001). "Lipophilicity profiles: theory and measurement." Testa, B.; van de Waterbeemd, H.; Folkers, G: 275-304.

Davis, S., A. Stockwell, et al. (1986). "The effect of density on the gastric emptying of single- and multiple-unit dosage forms." Pharmaceutical Research **3**(4): 208-213.

De Buck, S. S., V. K. Sinha, et al. (2007). "Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs." Drug Metabolism and Disposition **35**(10): 1766-1780.

DeSesso, J. M. and C. F. Jacobson (2001). "Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats." Food and Chemical Toxicology **39**(3): 209-228.

Devanarayana, N. M., S. Rajindrajith, et al. (2013). "Ultrasonographic Assessment of Liquid Gastric Emptying and Antral Motility According to the Subtypes of Irritable Bowel Syndrome in Children." Journal of Pediatric Gastroenterology and Nutrition **56**(4): 443-448 410.1097/MPG.1090b1013e31827f31827a31823d.

Diakidou, A., M. Vertzoni, et al. (2009). "Characterization of the Contents of Ascending Colon to Which Drugs are Exposed After Oral Administration to Healthy Adults." Pharmaceutical Research **26**(9): 2141-2151.

Dowling, T. C. (2002). "Drug Metabolism Considerations in Patients With Chronic Kidney Disease." Journal of Pharmacy Practice **15**(5): 419-427.

Dowling, T. C., J. C. Briglia Ae Fau - Fink, et al. "Characterization of hepatic cytochrome p4503A activity in patients with end-stage renal disease." (0009-9236 (Print)).

Dreisbach, A. W. and J. J. Lertora (2008). "The effect of chronic renal failure on drug metabolism and transport." Expert Opinion on Drug Metabolism & Toxicology **4**(8): 1065-1074.

Dressman, J., R. Berardi, et al. (1990). "Upper Gastrointestinal (GI) pH in Young, Healthy Men and Women." Pharmaceutical Research **7**(7): 756-761.

Dressman, J. B., D. Fleisher, et al. (1984). "Physicochemical model for dose-dependent drug absorption." Journal of Pharmaceutical Sciences **73**(9): 1274-1279.

Edelmann, I. S., Liebmann, J. (1959). " Anatomy of body water and electrolytes." Am. J.

27: 256–277.

El-Kattan, A. and M. Varma (2012). "Oral absorption, intestinal metabolism and human oral bioavailability." Topics of Drugs Metabolism: 1-35.

EMA. (2010). " Guideline on the Investigation of Bioequivalence. ." Committee for Medicinal Products for Human Use, European Medicines Agency. Retrieved 2015, 2015.

FDA. (2000). "Guidance for Industry, Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. ." CDER/FDA., 2015.

FDA. (2010). "Guidance for Industry: Pharmacokinetics in patients with impaired renal function —study design, data analysis, and impact on dosing and labeling. 2010.", 2015.

Fick, Adolph (1855). "V. On liquid diffusion." The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science 10.63: 30-39.

Goodacre, B. C. and P. J. Murray (1981). "A mathematical model of drug absorption." Journal of Clinical and Hospital Pharmacy **6**(2): 117-133.

Grass, G. M. (1997). "Simulation models to predict oral drug absorption from in vitro data." Advanced Drug Delivery Reviews **23**(1–3): 199-219.

Guevin, C., J. Michaud, et al. (2002). "Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators." Br J Pharmacol **137**(7): 1039-1046.

Huang, W., S. Lee, et al. (2009). "Mechanistic Approaches to Predicting Oral Drug Absorption." The AAPS Journal **11**(2): 217-224.

Ilić, M., J. Đuriš, et al. (2014). "In vitro – in silico – in vivo drug absorption model development based on mechanistic gastrointestinal simulation and artificial neural networks: Nifedipine osmotic release tablets case study." European Journal of Pharmaceutical Sciences **62**(0): 212-218.

Jamali, F. and A. Aghazadeh-Habashi (2008). "Rapidly dissolving formulations for quick absorption during pain episodes: Ibuprofen." International Journal of Clinical Pharmacology and Therapeutics **46**(2): 55-63.

Jamali, F. and C. M. Kunz-Dober (1999). "Pain-mediated altered absorption and metabolism of ibuprofen: An explanation for decreased serum enantiomer concentration after dental surgery." British Journal of Clinical Pharmacology **47**(4): 391-396.

Jones, H., I. Gardner, et al. (2009). "Modelling and PBPK Simulation in Drug Discovery." The AAPS Journal **11**(1): 155-166.

Jones, H., N. Parrott, et al. (2006). "A Novel Strategy for Physiologically Based Predictions of Human Pharmacokinetics." Clinical Pharmacokinetics **45**(5): 511-542.

Jones, H. M., M. Dickins, et al. (2011). "Application of PBPK modelling in drug discovery and development at Pfizer." Xenobiotica **42**(1): 94-106.

Jones, H. M., I. B. Gardner, et al. (2009). "Modelling and PBPK Simulation in Drug Discovery." The AAPS Journal **11**(1): 155-166.

Jones, H. M., K. Mayawala, et al. (2013). "Dose selection based on physiologically based pharmacokinetic (PBPK) approaches." The AAPS Journal **15**(2): 377-387.

Jones, H. M., N. Parrott, et al. (2006). "Predicting Pharmacokinetic Food Effects Using Biorelevant Solubility Media and Physiologically Based Modelling." Clinical Pharmacokinetics **45**(12): 1213-1226.

Kalantzi, L., K. Goumas, et al. (2006). "Characterization of the Human Upper Gastrointestinal Contents Under Conditions Simulating Bioavailability/Bioequivalence Studies." Pharmaceutical Research **23**(1): 165-176.

Kanda, M., M. Matsuhashi, et al. "Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS)." Clinical Neurophysiology **113**(7): 1013-1024.

Kararli, T. T. (1995). "Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals." Biopharmaceutics & drug disposition **16**(5): 351-380.

Kesisoglou, F. (2014). "Use of preclinical dog studies and absorption modeling to facilitate late stage formulation bridging for a BCS II drug candidate." AAPS PharmSciTech **15**(1): 20-28.

Kim, Y.-G., J.-G. Shin, et al. (1993). "Decreased acetylation of isoniazid in chronic renal failure." Clinical Pharmacology & Therapeutics **54**(6): 612-620.

Kohan, L. and R. Hamill-Ruth (2011). "Pain Assessment and Pharmacologic Management." Critical Care Medicine **39**(9): 2209.

Kostewicz, E. S., L. Aarons, et al. (2014). "PBPK models for the prediction of in vivo performance of oral dosage forms." European Journal of Pharmaceutical Sciences **57**(0): 300-321.

Lalande, L., B. Charpiat, et al. (2014). "Consequences of Renal Failure on Non-Renal Clearance of Drugs." Clinical Pharmacokinetics **53**(6): 521-532.

Laouari, D., R. Yang, et al. (2001). Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure.

Leblond, F., C. Guevin, et al. (2001). "Downregulation of hepatic cytochrome P450 in chronic renal failure." J Am Soc Nephrol **12**(2): 326-332.

Levey, A. S., J. Coresh, et al. (2003). "National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification." Annals of Internal Medicine **139**(2): 137-147.

Lloret-Linares, C., D. Hirt, et al. (2014). "Effect of a Roux-en-Y Gastric Bypass on the Pharmacokinetics of Oral Morphine Using a Population Approach." Clinical Pharmacokinetics **53**(10): 919-930.

Manallack, D. T., R. J. Prankerd, et al. (2013). "The Significance of Acid/Base Properties in Drug Discovery." Chemical Society reviews **42**(2): 485-496.

Mattar, S. G., L. M. Velcu, et al. (2005). "Surgically-Induced Weight Loss Significantly Improves Nonalcoholic Fatty Liver Disease and the Metabolic Syndrome." Annals of surgery **242**(4): 610-620.

Michaelis, L. and M. M. L. Menten (1913). "The kinetics of invertin action." FEBS Letters 587(17): 2712-2720.

Moore, R. A., S. Derry, et al. (2014). "Faster, higher, stronger? Evidence for formulation and efficacy for ibuprofen in acute pain." Pain **155**(1): 14-21.

Moreno, M. P. d. l. C., M. Oth, et al. (2006). "Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum." Journal of Pharmacy and Pharmacology **58**(8): 1079-1089.

Naud, J., J. Michaud, et al. (2007). "Down-Regulation of Intestinal Drug Transporters in Chronic Renal Failure in Rats." Journal of Pharmacology and Experimental Therapeutics **320**(3): 978-985.

Naud, J., J. Michaud, et al. (2008). "Effects of Chronic Renal Failure on Liver Drug Transporters." Drug Metabolism and Disposition **36**(1): 124-128.

Norris, D. A., G. D. Leesman, et al. (2000). "Development of predictive pharmacokinetic simulation models for drug discovery." Journal of Controlled Release **65**(1–2): 55-62.

NUGENT, S. G., D. KUMAR, et al. (2001). "Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs." Gut **48**(4): 571-577.

Paixão, P., L. F. Gouveia, et al. (2012). "Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model." International Journal of Pharmaceutics **429**(1-2): 84-98.

Po, H. N. and N. M. Senozan (2001). "The Henderson-Hasselbalch Equation: Its History and Limitations." Journal of Chemical Education **78**(11): 1499.

Poggesi, I., M. S. Benedetti, et al. (2009). "Pharmacokinetics in special populations." Drug Metabolism Reviews **41**(3): 422-454.

Porter, C. J. H., C. W. Pouton, et al. (2008). "Enhancing intestinal drug solubilisation using lipid-based delivery systems." Advanced Drug Delivery Reviews **60**(6): 673-691.

Poulin, P., K. Schoenlein, et al. (2001). "Prediction of adipose tissue: Plasma partition coefficients for structurally unrelated drugs." Journal of Pharmaceutical Sciences **90**(4): 436-447.

Poulin, P. and F.-P. Theil (2000). "A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery." Journal of Pharmaceutical Sciences **89**(1): 16-35.

Poulin, P. and F.-P. Theil (2002). "Prediction of pharmacokinetics prior to In Vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition." Journal of Pharmaceutical Sciences **91**(5): 1358-1370.

Reddy, B. B. K. and A. Karunakar (2011). "Biopharmaceutics classification system: a regulatory approach." Dissolution Technologies **18**(1): 31-37.

Riezzo, G., M. Chiloiro, et al. (2000). "Comparison of Gastric Electrical Activity and Gastric Emptying in Healthy and Dyspeptic Children." Digestive Diseases and Sciences **45**(3): 517-524.

Rodgers, T., D. Leahy, et al. (2005). "Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases." Journal of Pharmaceutical Sciences **94**(6): 1259-1276.

Rodgers, T. and M. Rowland (2006). "Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions." Journal of Pharmaceutical Sciences **95**(6): 1238-1257.

Rodgers, T. and M. Rowland (2007). "Mechanistic Approaches to Volume of Distribution Predictions: Understanding the Processes." Pharmaceutical Research **24**(5): 918-933.

Rowland, M., C. Peck, et al. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. **51:** 45-73.

Rowland Yeo, K., M. Aarabi, et al. (2011). "Modeling and predicting drug pharmacokinetics in patients with renal impairment." Expert Review of Clinical Pharmacology **4**(2): 261-274.

Rubio, I. G. S., A. L. Galrão, et al. (2012). "Levothyroxine absorption in morbidly obese patients before and after Roux-En-Y gastric bypass (RYGB) surgery." Obesity Surgery **22**(2): 253-258.

Sawaya, R. A., J. Jaffe, et al. (2012). "Vitamin, Mineral, and Drug Absorption Following Bariatric Surgery." Current Drug Metabolism **13**(9): 1345-1355.

Schmitt, W. and S. Willmann (2004). "Physiology-based pharmacokinetic modeling: ready to be used." Drug Discovery Today: Technologies **1**(4): 449-456.

Seaman, J. S., P. Bowers Sp Fau - Dixon, et al. (2005). "Dissolution of common psychiatric medications in a Roux-en-Y gastric bypass model." (0033-3182 (Print)).

Simard, E., J. Naud, et al. (2008). "Downregulation of Hepatic Acetylation of Drugs in Chronic Renal Failure." Journal of the American Society of Nephrology **19**(7): 1352-1359.

Smith, A., B. Henriksen, et al. (2011). "Pharmacokinetic considerations in Roux-en-Y gastric bypass patients." American Journal of Health-System Pharmacy **68**(23): 2241-2247.

Sun, H., L. Frassetto, et al. (2006). "Effects of renal failure on drug transport and metabolism." Pharmacology & Therapeutics **109**(1–2): 1-11.

Tandra, S., N. Chalasani, et al. (2013). "Pharmacokinetic and pharmacodynamic alterations in the Roux-en-Y gastric bypass recipients." Annals of surgery **258**(2): 262-269.

Teorell, T. (1937). "Kinetics of the Distribution of Substances Administered to the Body . II. The Extravascular Mode of Administration." Archives Internationales de Pharmacodynamie et de Thérapie (57): 205–240

Terao N Fau - Shen, D. D. and D. D. Shen "Reduced extraction of I-propranolol by perfused rat liver in the presence of uremic blood." (0022-3565 (Print)).

Theil, F.-P., T. W. Guentert, et al. (2003). "Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection." Toxicology letters **138**(1–2): 29-49.

Tsamandouras, N., A. Rostami-Hodjegan, et al. (2015). "Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: Fitting PBPK models to observed clinical data." British Journal of Clinical Pharmacology **79**(1): 48-55.

Urban, D., N. Cherny, et al. (2010). "The management of cancer pain in the elderly." Critical Reviews in Oncology/Hematology **73**(2): 176-183.

Valsami, G. and P. Macheras (2011). "Computational-regulatory developments in the rediction of oral drug absorption." Molecular Informatics **30**(2-3): 112-121.

Vanholder, R., R. De Smet, et al. (2003). "Review on uremic toxins: Classification, concentration, and interindividual variability." Kidney Int **63**(5): 1934-1943.

Volans, G. N. (1975). "The effect of metoclopramide on the absorption of effervescent aspirin in migraine." British Journal of Clinical Pharmacology **2**(1): 57-63.

Volans, G. N. (1978). "Migraine and Drug Absorption." Clinical Pharmacokinetics **3**(4): 313-318.

Wagner, J. G. (1981). "History of pharmacokinetics." Pharmacology & Therapeutics **12**(3): 537-562.

Walsh, K. and J. Volling (2014). "Lithium toxicity following Roux-en-Y gastric bypass." Bariatric Surgical Practice and Patient Care **9**(2): 77-80.

Watanabe, T., H. Kusuhara, et al. (2009). "Physiologically Based Pharmacokinetic Modeling to Predict Transporter-Mediated Clearance and Distribution of Pravastatin in Humans." Journal of Pharmacology and Experimental Therapeutics **328**(2): 652-662.

Wood, A. J., R. E. Vestal, et al. (1980). "Propranolol disposition in renal failure." British Journal of Clinical Pharmacology **10**(6): 561-566.

Wu, C.-Y. and L. Z. Benet (2005). "Predicting Drug Disposition via Application of BCS: Transport/Absorption/ Elimination Interplay and Development of a Biopharmaceutics Drug Disposition Classification System." Pharmaceutical Research **22**(1): 11-23.

Yomona-Hernández, J. L., P. Vicente-Ríos, et al. (2003). "Gastric emptying rates in idiopathic dyspepsia with and without "hunger pain"." Acta Gastroenterologica Latinoamericana **33**(3): 129-132.

Yu, L. X. and G. L. Amidon (1999). "A compartmental absorption and transit model for estimating oral drug absorption." International Journal of Pharmaceutics **186**(2): 119-125.

Yu, L. X., E. Lipka, et al. (1996). "Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption." Advanced Drug Delivery Reviews **19**(3): 359-376.

Chapter 2:
Disease Specific Modelling: Simulation of the Pharmacokinetics of Meloxicam and Ibuprofen in Pain vs. Healthy conditions

2.1 Introduction

Oral formulations are the most commonly administered dosage forms. They need to undergo disintegration and dissolution for the active ingredient to be absorbed. There are several factors that impact drug dissolution such as the physicochemical properties of the drug and the host's physiological environment. For instance, gastrointestinal (GI) motility, in general, and gastric emptying, in particular, has a significant role in the rate of drug absorption. This physiological factor could be altered significantly under abnormal conditions (Bar-Natan, Larson et al. 1996; Greene, Noorian et al. 2009). Delayed gastric emptying can be found in patients with post abdominal and dental surgery, which is usually associated with feeling pain (Marsh, Spencer et al. 1984; Bar-Natan, Larson et al. 1996; Jamali and Kunz-Dober 1999; Jamali and Aghazadeh-Habashi 2008). Studies have suggested that drug absorption patterns of the nonsteroidal anti-inflammatory drugs (NSAIDs) are altered in pain suffering patents (Aghazadeh-Habashi and Jamali 2008; Jamali and Aghazadeh-Habashi 2008). It has also been found that the drug absorption is less affected by gastric motility when a drug is administered as fast dissolving formulation (Aghazadeh-Habashi and Jamali 2008; Jamali and Aghazadeh-Habashi 2008; Moore, Derry et al. 2014).

Despite the complexity of the drug absorption process, computer simulations that incorporate physiologically based factors have proven to be useful in predicting pharmacokinetic (PK) patterns under different physiological conditions, such as fasted and fed state (Parrott and Lavé 2002; De Buck, Sinha et al. 2007; Okumu, DiMaso et al. 2009). The Compartmental Absorption and Transit (CAT) model is the first physiologically based absorption model used in a commercial software tool (Yu and Amidon 1999). The basic assumption of the CAT model is that there is simultaneous drug passing through the GI tract and absorption of the dissolved material from each compartment into the portal vein. This approach takes account of main three

important factors categories (Agoram, Woltosz et al. 2001). The first one represents the physicochemical factors such as a drug solubility and pKa. The second category is related to physiological characteristics, for example, the GI pH and gastric emptying. The last category is interrelates formulation factors such as surface area and drug particle size. Therefore, this model-based approach can be considered a powerful tool to simulate *in vivo* drug absorption. This model has been calibrated to accurately account for the observed human small intestine characteristics. GastroPlus™ is a simulation software product based on the (ACAT) model, which is an extension version of the CAT model (Figure 1.3) (Agoram, Woltosz et al. 2001). The ACAT in the GastroPlus™ assumes that a drug passes through of 18 compartments (stomach, seven compartments for the small intestine, colon and nine enterocyte compartments) and three states (unreleased, undissolved, and dissolved). The amount of absorbed material is a sum of the amounts being absorbed/unabsorbed from each compartment. The ACAT model in this software, include automatically default setting of regionally dependent absorption, pH-dependent solubility, precipitation, influx and efflux transport, and gut metabolism. Compartment properties are set according to published experimental databases for the pH, volume, and permeability characteristics of the corresponding intestinal region (Yu and Amidon 1999; Fu, Jeong et al. 2005; Jeong, Kimura et al. 2006; Yoshida, Lai et al. 2013; Ali 2014). Transit of a drug material between the compartments is modeled as a first order process that accounts for transit time in each compartment based on the physiological value for the corresponding region. In addition, linear and nonlinear rate equations were included in this software (Yoshida, Lai et al. 2013). The theoretical basis and mathematical description of the ACAT model is described in further details by Yu and Agoram et al. (Yu and Amidon 1999; Agoram, Woltosz et al. 2001)

Studies provided evidence that computer simulations are powerful tools to estimate drug absorption in healthy humans (Parrott and Lavé 2002; De Buck, Sinha et al. 2007). Many studies have demonstrated the application of computer simulations in establishing *in vivo/in vitro* correlations (IVIVC) (Okumu, DiMaso et al. 2008; Honório, Pinto et al. 2013). Therefore, regulatory agencies such FDA, and EMA recognized and utilized ACAT based *in silico* modeling in decision making (EMA 2010; FDA 2014). Recent studies have expanded the use of these simulations to disease states where physiological factors might have changed (Bolger, Irwin et al. 2012; Macwan 2013; R Das, P Gangwal et al. 2014). These attempts in diseases modeling are aimed to provide mechanistic insights to understand the physiological changes, and hence, the possible outcomes in different disease conditions. Pain is a very common state that happens to almost everyone at least once in his/her life. Ibuprofen and Meloxicam are NSAI drugs that are widely used to treat this condition.

We hypothesized that one can predict PK of drugs under normal and gastric dysfunction conditions using suitable computer software. We, therefore, used computer simulations to predict the PK of meloxicam in normal and gastric dysfunction conditions and ibuprofen in pain and pain-free states.

2.2 Methods

Published ibuprofen pharmacokinetic data under healthy and post dental surgery pain conditions as well as those in normal healthy rats and rats with gastric dysfunction were used (Jamali and Kunz-Dober 1999; Aghazadeh-Habashi and Jamali 2008; Jamali and Aghazadeh-Habashi 2008). For meloxicam, published data generated using normal and gastric dysfunctional rats were used (Aghazadeh-Habashi and Jamali 2008). In both, meloxicam and ibuprofen studies, two

formulations had been used: a fast dissolving and a regular release formulation. The pharmacokinetics of both drugs was simulated under both above conditions. All simulations were performed using Gastro Plus 7, a physiologically based simulation program (SimulationPlus, Inc. Lancaster,CA).

The program has different input tabs. The compound tab requires input of the drug's physicochemical properties including the dose, dosage form, solubility, permeability, molecular weight, particle density, particle size and pKa. Such data was taken from literatures as it is explained further. ADMET Predictor 7 (SimulationsPlus, Lancaster, CA) was used to estimate such parameters if they were unavailable in literature. All input parameters used in the simulation are presented in Table 2.1.

2.2.1 Meloxicam: The standard compartmental pharmacokinetic module in GastroPlus™ was used to fit intravenous data to a one, two or three compartment model. Meloxicam data fitted best to a two-compartmental model, as other reports have suggested (Shukla, Singh et al. 2007; Aghazadeh-Habashi and Jamali 2008). The simulations were performed using the default physiology parameters for fasted rats (Table 2.1). Since the formulations had slightly different PK profiles, dissolution differences were assumed to be the factor responsible for these differences. The FD formulation contains a carbonated effervescent agent that can enhance the gastric pH and improve the drug dissolution, whereas, the IR formulation would retained undissolved in the stomach for a longer time. The mean particle size used to simulate the fast dissolving formulation was 23 microns, and 30 microns fitted best for the FD and IR formulation, respectively.

2.2.2 Ibuprofen: An intravenous bolus of 20 mg/ml/kg was used to fit the data to a pharmacokinetic model for both enantiomers (Knihinicki, Day et al. 1990) to a two-compartment

model (Jamali and Aghazadeh-Habashi 2008). Studies have reported that R ibuprofen undergoes chiral inversion to S ibuprofen (Marsh, Spencer et al. 1984; Knihinicki, Day et al. 1990; Aiba, Tse et al. 1999; Landoni and Soraci 2001; Aghazadeh-Habashi and Jamali 2008). This tool can be combined with input from *in vitro* experimental methods to increase the accuracy in predicting the effect of changes in physiological condition changes on the in the overall dissolution (Bergström, Holm et al. 2014). Therefore, to simulate this metabolic pathway, the enzyme table in the compound tab was used with K_m= 1.5 mM, and V_{max} =3.4 nmol/min/mg protein. These two values were taken from a published study (Aiba, Tse et al. 1999). The simulations were performed using the default physiology parameters for fasted rats (Table 2.2) and fasted human in simulating human data (Table 2.3).

The software built-in immediate release model was used for the simulation of the pain free groups. A modified gastric release model in GastroPlus™ was used to simulate assumed altered drug release from the stomach caused by pain.

The regression coefficient between predicted vs. observed data was automatically calculated by the software. The mean absolute error (MAE) and the root mean squared error (RMSE) were also calculated according to the following formulas:

$$MAE = \frac{1}{N}\sum_{i=1}^{N}|x_{pred} - x_{obs}|$$ (Okumu, DiMaso et al. 2009)

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x}_i)^2}$$ (Okumu, DiMaso et al. 2009)

In vivo drug release curves were compared via Similarity Factor f2 using DDSolver (Zhang, Huo et al. 2010), a free Excel plugin software. A f2 value between 50 and 100 suggests

similarity between release profiles while any value under 50 shows that the profiles are not similar. Finally, we ran Parameter Sensitivity Analysis (PSA) simulation to determine the parameters that have an influence on our model.

Table 2.1: Input values which were used for the simulations

Input used in GastroPlus™	**Meloxicam**	**Ibuprofen**
Dose	0.216 mg (Aghazadeh-Habashi and Jamali 2008)	400 mg (human), 5.4 mg (rat) (Jamali and Kunz-Dober 1999; Jamali and Aghazadeh-Habashi 2008)
Molecular weight	351.4 [a]	206.29 [a]
Solubility	0.0465 mg/ml at pH 4.96[a]	0.0465 mg/ml at pH 1.2 (Landoni and Soraci 2001)
pka	2.5, 8.47, 5.98[a]	5.2[a]
Permeability	1.14 [a]	13.90 cm/s x 104 [a]
Log_p	2.32 [a]	3.9 (Mohammed, Weston et al. 2004)

[a] data were taken from ADMET predictor ™ 7

Table 2.2: ACAT model parameters for the physiology of fasted state rats.

Name	**pH**	**Volume(mL)**	**Length(cm)**	**Radius(cm)**	**Transit time (hrs)**
Oral Cavity	7.4	0.7	1	1	0.25
Stomach	3.9	3.36	1.07	1	0.25
Duodenum	5.89	0.57	9.5	0.22	0.19
Jejunum 1	6.13	2.49	45	0.21	0.83
Jejunum 2	6.13	2.26	45	0.2	0.75
Ileum 1	5.93	0.045	1	0.19	0.02
Ileum 2	5.93	0.04	1	0.18	0.01
Ileum 3	5.93	0.04	1	0.17	0.01
Caecum	6.58	0.88	5	0.75	4.29
Colon	6.23	0.30	9	0.33	7.71

Table 2.3: ACAT model parameter for the physiology of fasted state adult humans.

Name	pH	Volume(mL)	Length(cm)	Radius(cm)	Transit time (hrs)
Oral Cavity	7.4	0.7	1	1	0.25
Stomach	1.3	47.80	28.74	9.76	0.1
Duodenum	6	43	14.35	1.54	0.26
Jejunum 1	6.2	160.18	59.32	1.46	0.93
Jejunum 2	6.4	126.46	59.32	1.30	0.74
Ileum 1	6.6	97.97	59.32	1.146	0.58
Ileum 2	6.9	72.85	59.32	0.99	0.42
Ileum 3	7.4	51.63	59.32	0.83	0.29
Caecum	6.4	48.95	13.35	3.41	4.27
Colon	6.8	51.90	28	2.43	12.82

2.3 Results:

The observed and simulated C_{max}, T_{max}, and AUC_{0-t} for all groups are listed in Table 2.4.

2.3.1 Meloxicam: Using the immediate release function of the software we were able to closely predict the observed data generated using the control rats (Figure 2.1). For the gastric dysfunction cases, the fast dissolving and IR formulations, the regression coefficients were 0.7 and 0.9 for, respectively (table 2.5). The gastric release pattern, which cause the observed PK pattern observed for meloxicam (Figure 2.2). As seen the drug release under pain was differently delayed for the fast dissolving and the regular IR formulation (Figure 2.3). The similarity factor (f2) between the drugs released in pain and pain free condition was 75for the fast dissolving formulation and 18 for the IR formulation. The f2 between the release from the fast dissolving formulation and the IR formulation in pain free conditions was 41 and 20, respectively, when compared to the pain condition.

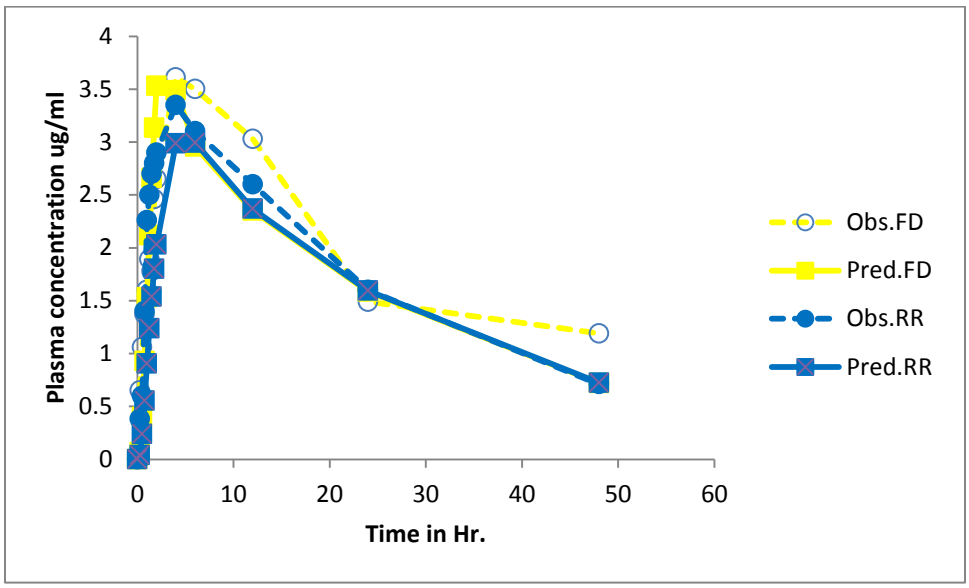


Figure 2.1: The meloxicam plasma concentration vs. time curves of observed (Obs) and predicted (Pre) of a fast dissolving formulation (FD) and regular release (RR) formulation in control rats.

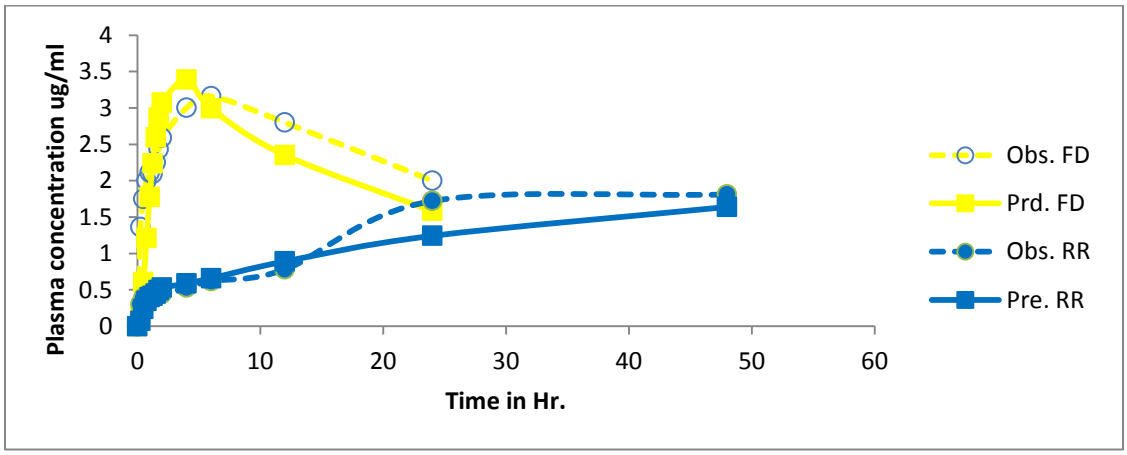
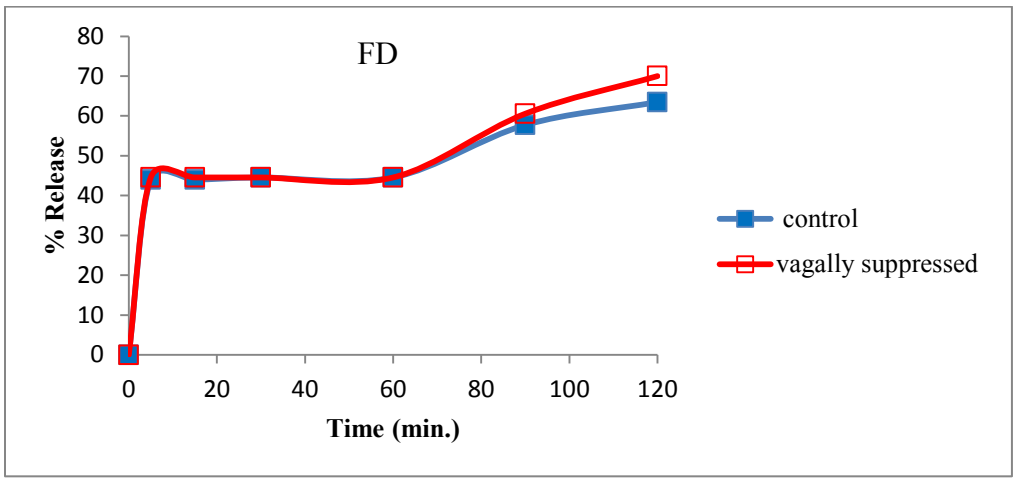
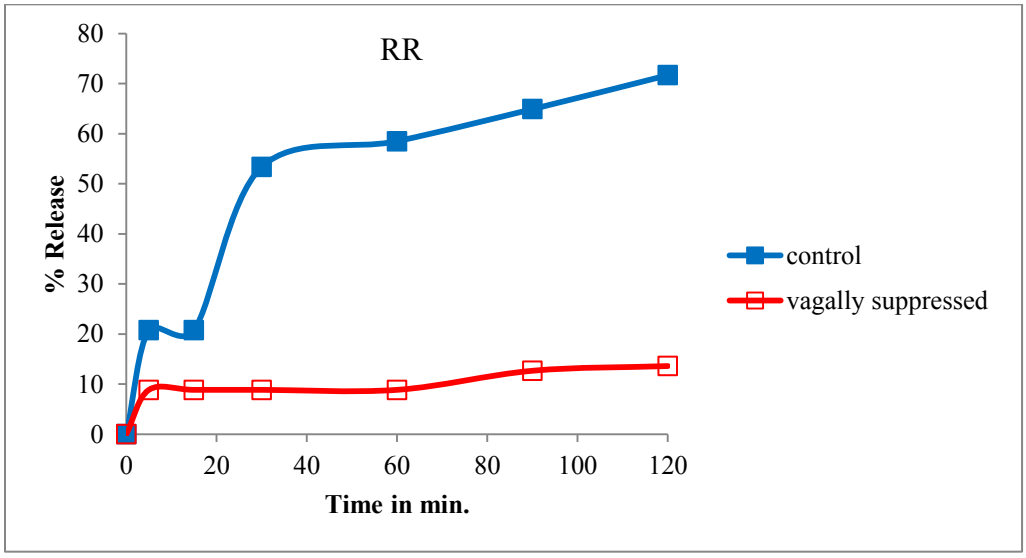


Figure 2.2: The meloxicam plasma concentration vs. time curves of observed and predicted of a fast dissolving formulation (FD) and regular release formulation (RR) in vagally suppressed rats.



(a)



(b)

Figure 2.3: The predicted *in vivo* release of meloxicam in control and vagally suppressed rats using a fast dissolving formulation (a) and a regular release formulation (b)

2.3.2 Ibuprofen: The simulated and observed data of the control rats had r^2 for the fast dissolving formulation of (S) enantiomer of 0.88, and 0.77 for the (R) enantiomer (Figure 2.4) (Table 2.5).

As seen for meloxicam, in vagally suppressed rats, the prediction fitted only if the modified gastric release mode was used (Figure 2.5). Figure 2.6 shows the calculated *in vivo* drug release for the fast dissolving formulation (A) and the IR formulation in control animals and vagally suppressed animals (B). There was no difference in drug release under control conditions between the formulations. However, gastric dysfunction in vagally suppressed rats clearly delayed drug release (Figure 2.5-2.6) and formulation differences between the fast dissolving and the IR tablet significantly impacted the *in vivo* drug release, and consequently, the bioavailability of the drug. The f2 between the control and the treated rats for the fast dissolving formulation for the S enantiomer is 58.8 and 63.49 for R enantiomer. The f2 between the control and the treated rats for the IR formulation is 16.61 for of S enantiomer and 25.17 for R enantiomer.

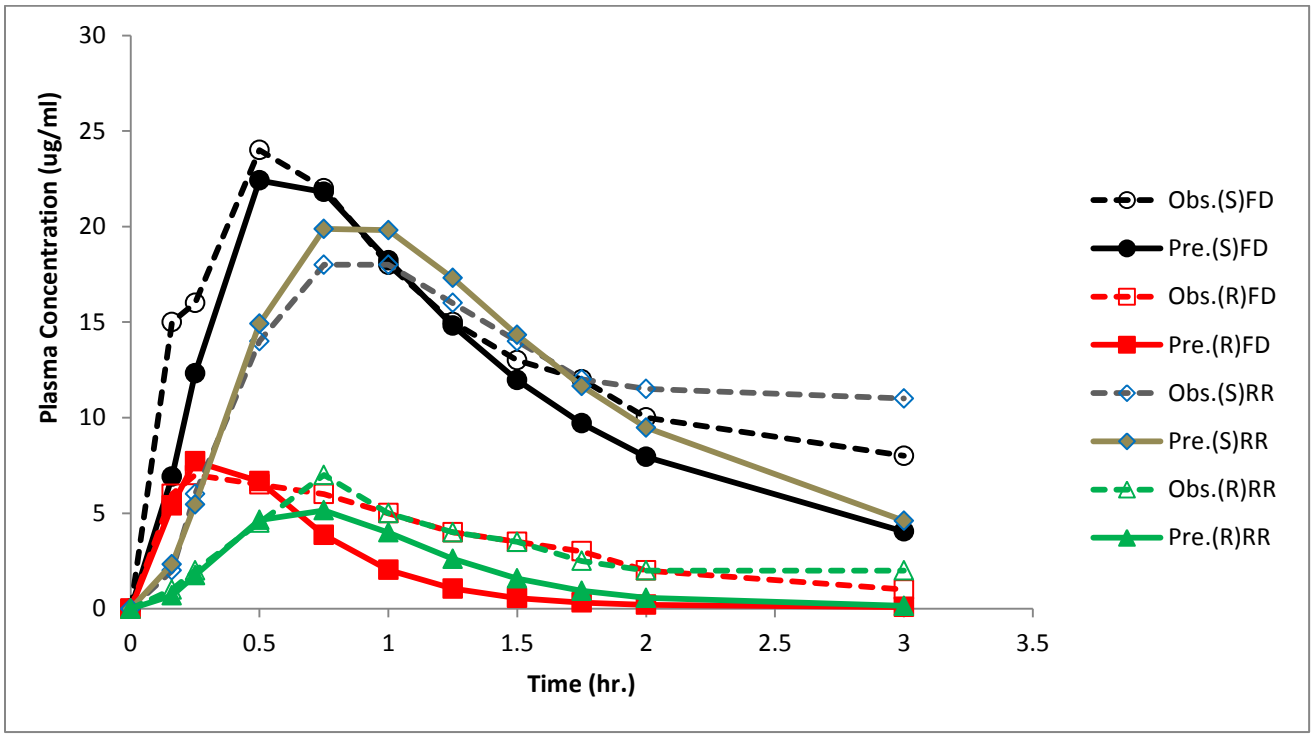


Figure 2.4: Ibuprofen (S) and (R) plasma concentration vs. time curves of observed (Obs) and predicted (Pre) of a fast dissolving (FD) and regular Release (RR) formulation in control rats.

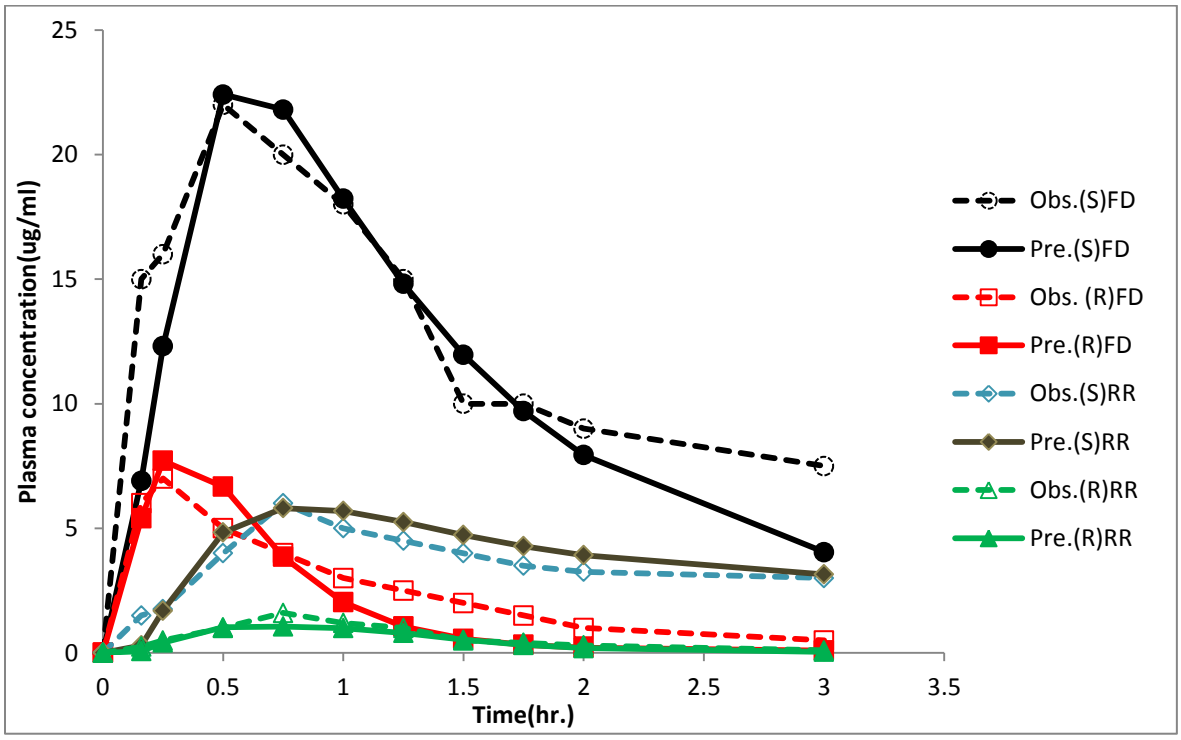
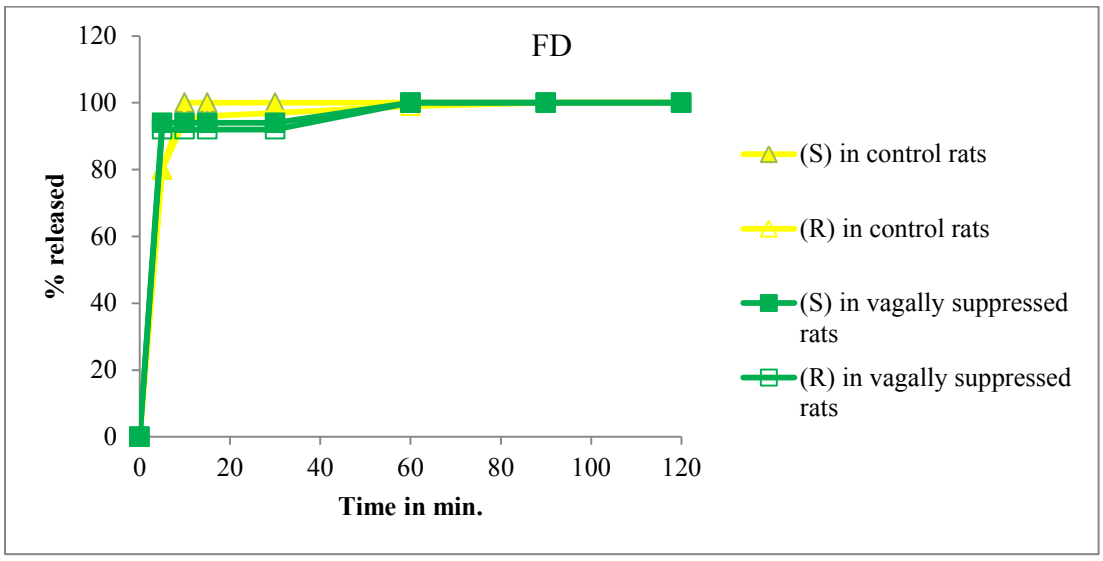
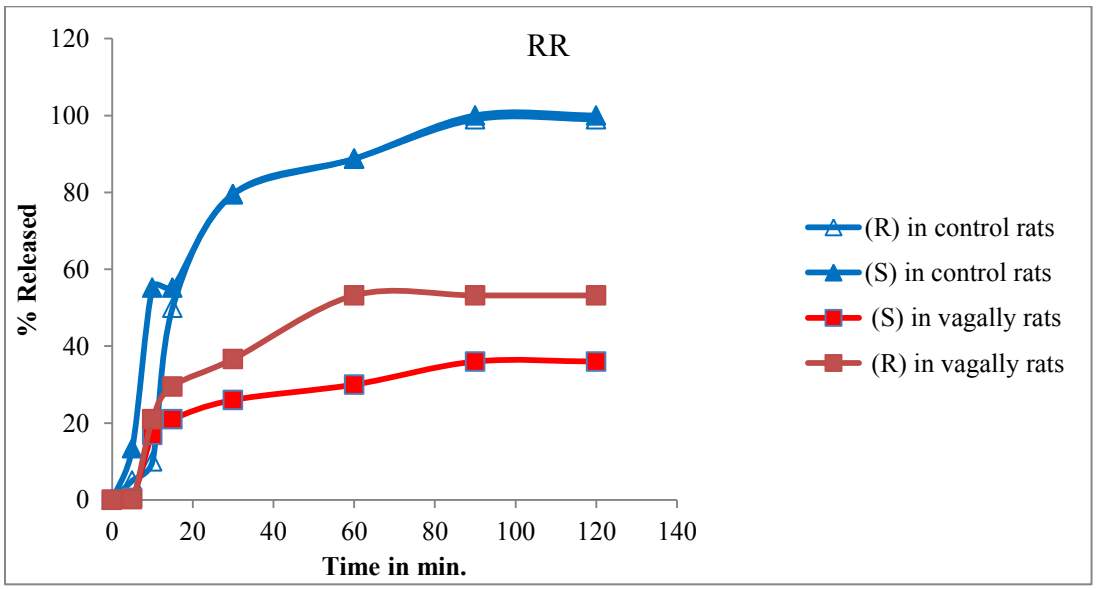


Figure 2.5: (S) and (R) ibuprofen plasma concentration vs. time curves of observed (Obs) and predicted (Pre) of a fast dissolving (FD) and regular release (RR) formulation in vagally suppressed rats.



(a)



(b)

Figure 2.6: *In vivo* release of a fast dissolving formulation (FD) (a) and a regular release (RR) (b) of ibuprofen for both (S) and (R) enantiomers in control (CT) and vagally suppressed (VS) rats

In humans, the software predicted the observed data very closely with an r^2= 0.9 for the fast dissolving and the IR formulation for both enantiomers (Figure 2.7). The *in vivo* releases of both enantiomers were almost identical since both enantiomers have the same physiochemical characteristics (Figure 2.9). However, the *in vivo* release of the formulations was significantly different. The IR formulation showed a much more delayed gastric release of almost one hour before it reached maximum drug release (Figure 2.9). The f2 between the fast dissolving formulation and the IR formulation in patients under pain was 24 and 22 for the S and R enantiomer, respectively. The PSA simulation showed that both formulations were sensitive to the changes in the drug solubility (data not shown). The pain models additionally were sensitive to stomach transit time for both formulations, which supported our hypothesis.

Table 2.4 Comparison between the actual and predicted values of C_{max} , T_{max} , and AUC 0-t of meloxicam and ibuprofen.

Drug	Subjects	Group	Formulations	Data	C_{max} (ug/ml)	% PE	T_{max} (hr)	% PE	AUC $_{0-t}$ Um-hr/ml	% PE
Meloxicam	Rats	Control	FD	Observed	3.80 ± 0.62	5.26	5.14 ± 3.24	21.40	113.6 ± 17.2	27.37
				Predicted	3.6		4.04		82.5	
			RR	Observed	3.35 ± 1.23	8.95	5.61 ± 4.89	12.65	86.3 ± 7.3	8.47
				Predicted	3.05		4.9		78.99	
		Gastric dysfunction	FD	Observed	3.16 ± 0.42	-9.17	12.3 ± 8.8	73.17	64.6 ± 8.9	14.24
				Predicted	3.45		3.3		55.4	
			RR	Observed	1.78 ± 0.87	10.11	30.9 ± 11.7	-55.33	69.7 ± 32.3	19.94
				Predicted	1.6		48		55.8	

Ibuprofen	Human (S) Enantiomer	Post dental surgery	FD	Observed	18.2 ± 2.8	-2.19	1.26± 0.49	17.46	49.7± 7.42	-28.77
				Predicted	18.6		1.04		64	
			RR	Observed	19.2 ±3.61	6.82	2.22 ±1.44	32.432	51.2±12.2	-22.3813
				Predicted	17.89		1.5		62.66	
	Human (R) Enantiomer		FD	Observed	24.9±4.32	21.28	1.26±0.49	11.11	63.8±9.88	-0.62
				Predicted	19.6		1.12		64.2	
			RR	Observed	24.92±4.32	24.55	2.19±1.45	26.94	60.8±21.2	-5.59
				Predicted	18.8		1.6		64.2	
	Rats (S) Enantiomer	Control	FD	Observed	23.14±8.34	-0.69	0.54±0.32	-7.40	41.48±10.61	16.82
				Predicted	23.3		0.58		34.5	
			RR	Observed	15.79±3.12	28.56	0.94±0.24	8.51	37.68±6.56	10.56
				Predicted	20.3		.86		33.7	
	Rats (R) Enantiomer		FD	Observed	9.04±3.44	9.29	0.23±0.12	-39.13	11.26±2.82	48.49
				Predicted	8.2		.32		5.8	
			RR	Observed	7.29±2.57	28.66	0.94±0.24	29.78	11.36±2.18	55.54
				Predicted	5.2		.66		5.05	
	Rats (S) Enantiomer	Gastric dysfunction	FD	Observed	24.03±17.56	10.94	.55±0.42	-7.27	31.56±24.91	-8.67
				Predicted	21.4		.59		34.3	
			RR	Observed	4.27±2.28	-35.83	0.95±45	13.68	5.96±1.92	-101.34
				Predicted	5.8		.82		12	
	Rats (R) Enantiomer		FD	Observed	9.26±6.10	13.60	.38±0.40	15.78	7.67±5.60	25.68
				Predicted	8		.32		5.7	
			RR	Observed	2.07±1.36	48.30	.88±0.63	26.13	2.26±1.27	38.05
				Predicted	1.07		.65		1.4	

AUC_{0-t}= AUC_{0-24} for Meloxicam , AUC_{0-t}= AUC_{0-3} for Ibuprpfen (rats) and AUC_{0-6} (humans), PE%= ((observed –predicted)/observed) X100

Table 2.5: Regression analysis output showing the coefficient of determination (r^2), the mean absolute error (MAE) and the root mean squared error (RMSE) of meloxicam and ibuprofen models.

Drug	Subjects	Group	Formulations	R^2	MAE	RMSE
Meloxicam	Rats	Control	FD	0.9	0.45	0.53
			RR	0.89	0.56	0.75
		Gastric dysfunction	FD	0.7	0.48	0.6
			RR	0.9	0.1	0.16
Ibuprofen	Human (S) Enantiomer	Post dental surgery	FD	.96	1.1	1.4
			RR	.98	.92	1.2-
	Human (R) Enantiomer		FD	.99	0.5	.68
			RR	.95	1.2	-1.4
	Rats (S) Enantiomer	Control	FD	0.88	2.1	3.1
			RR	0.9	1.4	2.2
	Rats (R)		FD	0.77	1.6	1.7

	Enantiomer		RR	.84	1	1.2
	Rats (S) Enantiomer	Gastric dysfunction	FD	0.83	1.9	3
			RR	0.93	0.85	0.66-

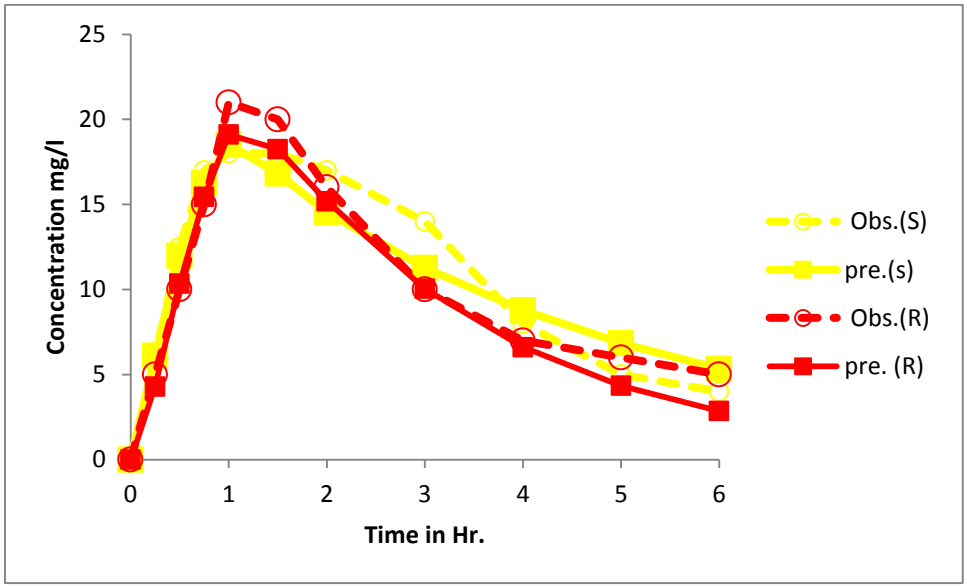


Figure 2.7: (S) and (R) ibuprofen plasma concentration vs. time curves of observed (Obs) and predicted (Pre) of a fast dissolving formulation in humans after dental surgery.

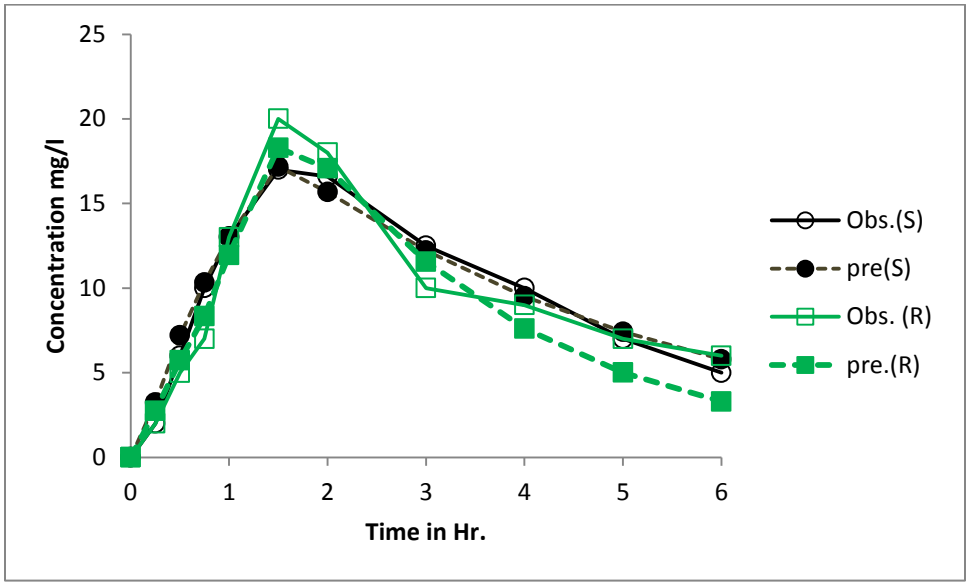
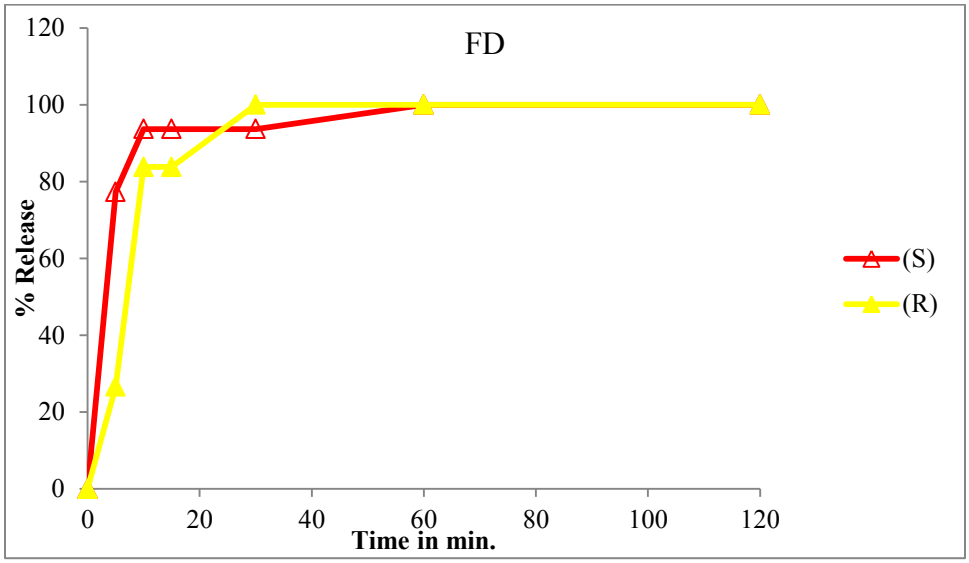
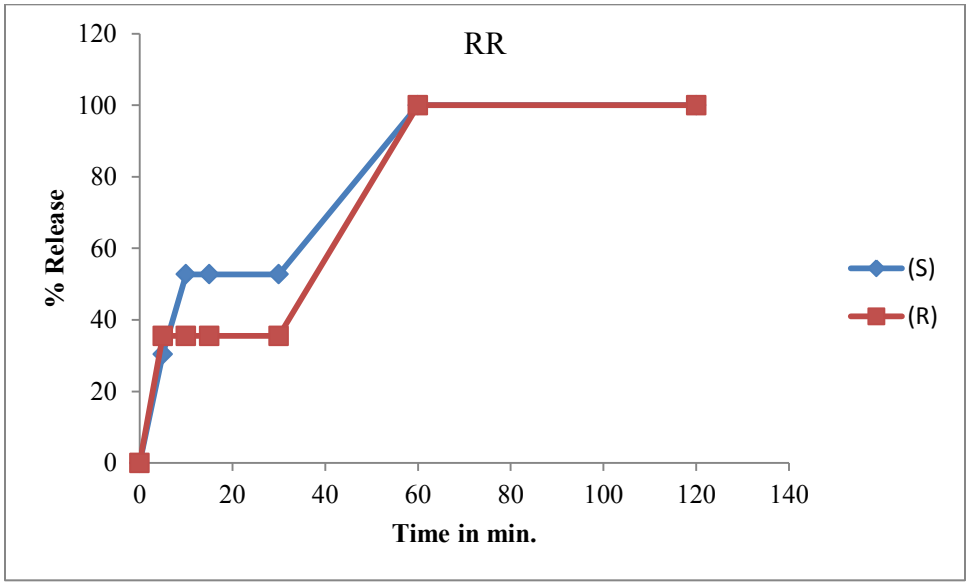


Figure 2.8: (S) and (R) ibuprofen plasma concentration vs. time curves of observed (Obs) and predicted (Pre) of a regular release formulation in humans after dental surgery.



(a)



(b)

Figure 2.9: *In vivo* predicted release of a Fast dissolving (FD) (A) and regular release (RR) (B) formulation of (S) and (R) ibuprofen in post dental surgery patients.

2.4 Discussion

Pain, or its associated trauma, is known to cause delayed absorption of orally administered drugs that is attributed to gastric dysfunction (Jamali and Kunz-Dober 1999; Aghazadeh-Habashi and Jamali 2008; Jamali and Aghazadeh-Habashi 2008; Padwal, Gabr et al. 2011). Recently, using ultrasound approach, Devanarayana et al. (Devanarayana, Rajindrajith et al. 2013) have tested the effect of abdominal pain on the gastric motility in children. They have found a significant delay in the gastric motility rate during the pain episodes, which correlated with the severity of the abdominal pain (Devanarayana, Rajindrajith et al. 2013).

The use of animal models to understand the pathogenesis of human disease is a common practice (Lieschke and Currie 2007). Mammalian models, particularly rats, have been widely used in modeling human diseases from cancer models through the tissue-specific expression. Many examples of disease induced and transgenic rats have been used to study the pathology of human. This is mainly because of the many similarities in anatomy, cell biology and physiology between rats and humans (Lieschke and Currie 2007). The *in vivo* release of many drugs showed similarity profiles in human and rats Chiou et al studies 64 drugs that have different physiochemical characters in human and rats (Chiou and Barve 1998). The study showed a linear correlation in the drug exposure between human and rats. Therefore, rats are considered a safe alternative to represent the drug release in human, especially in disease conditions.

Using commercially available simulation software we were able to predict plasma-concentration-time patterns of two NSAIDs under both healthy and pathophysiological altered conditions. In our hands, the prediction of the PK pattern under either gastric dysfunction or pain was possible by assuming a delayed gastric emptying rate. The effect of gastric emptying was substantially greater on the absorption pattern of the regular release as compared with that of the

fast dissolving formulation (Figures 2.3, 2.6,2.9). This is very likely due to a faster dissolution of the formulation as has been suggested earlier (Aghazadeh-Habashi and Jamali 2008). However, the possibility of faster gastric emptying caused by the effervescent nature of the formulation and the more alkaline environment produced by the latter cannot be ruled out (Aghazadeh-Habashi and Jamali 2008). The gastric emptying rate is a vital factor affecting the absorption profile of orally administered drugs (Tucker, Casey et al. 1981; Abuasal, Bolger et al. 2012). In our pain model we assumed that the stomach controls the release of the drug, so the dose stays in the stomach and releases the drug in a manner similar to a control release tablet would do.

Factors that influence gastric emptying rate are expected to impact drug absorption. However, this seems to be also affected by the formulation as well as , in our hand, the absorption of a fast dissolving formulation was less influenced by the both gastric dysfunction and pain that did that of the IR formulation. Moreover, this observation is more pronounced for drugs that belongs to biopharmaceutical classification system (BCS) class II, which have very limited stomach drug solubility, such as meloxicam (Aghazadeh-Habashi and Jamali 2008) or ibuprofen (Jamali and Aghazadeh-Habashi 2008).

Studies have shown many applications of using simulations thought drugs discovery and developments cycle. The use of computational simulation has expanded from modeling the *in vitro* release of drugs (Almukainzi, Okumu et al. 2014) to predict the *in vivo* drug absorption (Zhao, Vieira et al. 2012; Almukainzi M 2014; Maharaj and Edginton 2014). Recently, in *silico* models have been introduced to predict the drug absorption under disease conditions (Johnson, Rostami-Hodjegan et al. 2006; Gaohua, Abduljalil et al. 2012; Ghosal and Said 2012; Ke, Nallani et al. 2012; Zhao, Vieira et al. 2012; Almukainzi M 2014; Maharaj and Edginton 2014). These studies showed the validity in predicted drug absorption in different sub-group

populations such as in patients with post gastric bypass surgery (Almukainzi M 2014), renal impairment (Zhao, Vieira et al. 2012; Maharaj and Edginton 2014), children (Johnson, Rostami-Hodjegan et al. 2006), and pregnancy (Gaohua, Abduljalil et al. 2012; Ke, Nallani et al. 2012). These tools can assists in understanding, addressing and predicting many factors that conventionally human studies may reveal. This is especially crucial in disease conditions, where the drug absorption cannot be predicted straightforward.

In the present study, we successfully mimic the effect of gastric dysfunction, which is associated with pain, on the absorption of meloxicam and ibuprofen considering a formulation effect. This study proves, via simulations, that the stomach controls the drug release in pain episode (Jamali and Aghazadeh-Habashi 2008; Wilson, Clarke et al. 2011). The computer simulations suggest that the *in vivo* dissolution of different formulations that appeared to be influenced by the pathophysiological changes can have a significant impact on the PK pattern under pain conditions Drug release from a tablet is a function of tablet disintegration and drug dissolution. The software only calculates the released drug rate, which is a hybrid metric consisting of both processes. The simulations suggest that the tested formulations differ in the release properties under pain condition. Since the release of the drug from the fast-dissolving formulation commences immediately after swallowing and exposure to water, gastric dysfunction appears to have minimal influence on the absorption process (Almukainzi, Bou-Chacra et al. 2014). Hence, absorption starts shortly after administration, in the stomach and subsequently in the small intestine, if gastric emptying is stimulated by the formulation. On the other hand, the absorption of regular release, particularly capsules and coated tablet, formulations requires agitation for disintegration and fluid for dissolution (Almukainzi 2011; Almukainzi, Bou-Chacra et al. 2014). Clinical studies showed the maximum ibuprofen concentration of fast acting formulations was

achieved in 20–50 min compared to standard formulations that took about 90–120 min (Moore, Derry et al. 2014). The *in vivo* prediction in our study confirm this observation that a fast formulation reached the maximum (100%) in about 20 min after dosing; whereas the regular formulation reached the maximum after one hour (Figure 2.9).

In developing fast acting formulations, pharmacokinetics of drugs is studied in healthy subjects assuming similar PK patterns between healthy and pathophysiologically altered conditions. Such an approach has been suggested to be flawed due to changes expected under some disease conditions (Rowland, Peck et al. 2011). An understanding of these changes and their incorporation into computer simulation, as presented herein, can facilitate prediction of the influence of the changes on the PK outcome and save a great deal of resources and time.

2.5 Conclusion

Computer simulations were able to predict the drug plasma time curves of two analgesics under pathophysiologically altered conditions. A gastric modified drug release model best estimated the PK under pain conditions. The computer model indicated significant differences in the *in vivo* dissolution of the tested formulations when the gastric modified model was used. This disease modeling approach can be used in designing and assessing dosage forms and their ability to deliver a drug efficiently in healthy and disease conditions. Disease specific modelling can improve the therapeutic outcomes and establish interchangeability between products by assessing bioequivalence between disease free and disease conditions.

2.6 References

Abuasal, B. S., M. B. Bolger, et al. (2012). "In silico modeling for the nonlinear absorption kinetics of UK-343,664: A P-gp and CYP3A4 substrate." Molecular Pharmaceutics **9**(3): 492-504.

Aghazadeh-Habashi, A. and F. Jamali (2008). "Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: Influence of gastrointestinal dysfunction on the relative bioavailability of two formulations." European Journal of Pharmaceutics and Biopharmaceutics **70**(3): 889-894.

Agoram, B., W. S. Woltosz, et al. (2001). "Predicting the impact of physiological and biochemical processes on oral drug bioavailability." Advanced Drug Delivery Reviews **50, Supplement 1**(0): S41-S67.

Aiba, T., M. M. Tse, et al. (1999). "Effect of dosage form on stereoisomeric inversion of ibuprofen in volunteers." Biological and Pharmaceutical Bulletin **22**(6): 616-622.

Ali, S. K., K (2014). "A Recent Advancement in Orally Disintegrating Formulations." American Pharmaceutical review **17**(4): 6.

Almukainzi, M. (2011). Evaluation of performance testing and computer simulations for quality by design approaches of oral dosage forms. Master Evaluation of performance testing and computer simulations for quality by design approaches of oral dosage forms University of alberta.

Almukainzi, M., N. A. Bou-Chacra, et al. (2014). Biorelevant Dissolution Testing. Therapeutic Delivery Solutions, John Wiley & Sons, Inc.**:** 335-365.

Almukainzi M, L. V., Löbenberg R (2014). "Modelling the Absorption of Metformin with Patients Post Gastric Bypass Surgery." Diabetes Metab **5**: 353.

Almukainzi, M., A. Okumu, et al. (2014). "Simulation of In Vitro Dissolution Behavior Using DDDPlus™." AAPS PharmSciTech: 1-5.

Bar-Natan, M., G. M. Larson, et al. (1996). "Delayed gastric emptying after gastric surgery." The American Journal of Surgery **172**(1): 24-28.

Bergström, C. A. S., R. Holm, et al. (2014). "Early pharmaceutical profiling to predict oral drug absorption: Current status and unmet needs." European Journal of Pharmaceutical Sciences **57**(1): 173-199.

Bolger, M. B., R. Irwin, et al. (2012). "GastroPlus PBPK/PD Model Applied to Estimating Dose for an Elderly Population in an Alzheimer's Disease Clinical Trial."

Chiou, W. and A. Barve (1998). "Linear Correlation of the Fraction of Oral Dose Absorbed of 64 Drugs Between Humans and Rats." Pharmaceutical Research **15**(11): 1792-1795.

De Buck, S. S., V. K. Sinha, et al. (2007). "Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs." Drug Metabolism and Disposition **35**(10): 1766-1780.

Devanarayana, N. M., S. Rajindrajith, et al. (2013). "Ultrasonographic Assessment of Liquid Gastric Emptying and Antral Motility According to the Subtypes of Irritable Bowel Syndrome in Children." Journal of Pediatric Gastroenterology and Nutrition **56**(4): 443-448 410.1097/MPG.1090b1013e31827f31827a31823d.

EMA. (2010). " Guideline on the Investigation of Bioequivalence. ." Committee for Medicinal Products for Human Use, European Medicines Agency. Retrieved 2015, 2015.

FDA. (2014). "Program of physiologically-based pharmacokinetic and pharmacodynamic modeling (PBPK Program)." FDA, 2015, from

http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm365118.htm.
Fu, Y., S. H. Jeong, et al. (2005). "Fast-melting tablets based on highly plastic granules." Journal of Controlled Release **109**(1–3): 203-210.
Gaohua, L., K. Abduljalil, et al. (2012). "A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4." British Journal of Clinical Pharmacology **74**(5): 873-885.
Gaohua, L., K. Abduljalil, et al. (2012). "A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4." British Journal of Clinical Pharmacology **74**(5): 873-885.
Ghosal, A. and H. M. Said (2012). "Cys(294) is essential for the function of the human sodium-dependent multivitamin transporter." Biochim Biophys Acta **1818**(1): 97-102.
Greene, J. G., A. R. Noorian, et al. (2009). "Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease." Experimental Neurology **218**(1): 154-161.
Grillo, J. A., P. Zhao, et al. (2012). "Utility of a physiologically–based pharmacokinetic (PBPK) modeling approach to quantitatively predict a complex drug–drug–disease interaction scenario for rivaroxaban during the drug review process: implications for clinical practice." Biopharmaceutics & Drug Disposition **33**(2): 99-110.
Honório, T. d., E. Pinto, et al. (2013). "In Vitro–In Vivo Correlation of Efavirenz Tablets Using GastroPlus®." AAPS PharmSciTech **14**(3): 1244-1254.
Jamali, F. and A. Aghazadeh-Habashi (2008). "Rapidly dissolving formulations for quick absorption during pain episodes: Ibuprofen." International Journal of Clinical Pharmacology and Therapeutics **46**(2): 55-63.
Jamali, F. and C. M. Kunz-Dober (1999). "Pain-mediated altered absorption and metabolism of ibuprofen: An explanation for decreased serum enantiomer concentration after dental surgery." British Journal of Clinical Pharmacology **47**(4): 391-396.
Jeong, S., S. Kimura, et al. (2006). Fast-melting tablets having taste-masking and sustained release properties, Google Patents.
Johnson, T. N., A. Rostami-Hodjegan, et al. (2006). "Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children." Clinical Pharmacokinetics **45**(9): 931-956.
Ke, A. B., S. C. Nallani, et al. (2012). "A PBPK Model to Predict Disposition of CYP3A-Metabolized Drugs in Pregnant Women: Verification and Discerning the Site of CYP3A Induction." CPT: pharmacomet. syst. pharmacol. **1**: e3.
Knihinicki, R. D., R. O. Day, et al. (1990). "Stereoselective disposition of ibuprofen and flurbiprofen in rats." Chirality **2**(3): 134-140.
Landoni, M. F. and A. Soraci (2001). "Pharmacology of chiral compounds: 2-Arylpropionic acid derivatives." Current Drug Metabolism **2**(1): 37-51.
Lieschke, G. J. and P. D. Currie (2007). "Animal models of human disease: zebrafish swim into view." Nat Rev Genet **8**(5): 353-367.
Macwan, J. (2013). "Model-Based Approaches to Characterize Clinical Pharmacokinetics of Atorvastatin and Rosuvastatin in Disease State." Open Access Dissertations: 28.
Maharaj, A. R. and A. N. Edginton (2014). "Physiologically Based Pharmacokinetic Modeling and Simulation in Pediatric Drug Development." CPT: pharmacomet. syst. pharmacol. **3**: e148.

Marsh, R. H. K., R. Spencer, et al. (1984). "Gastric emptying and drug absorption before surgery." British Journal of Anaesthesia **56**(2): 161-164.
Mohammed, A. R., N. Weston, et al. (2004). "Liposome formulation of poorly water soluble drugs: Optimisation of drug loading and ESEM analysis of stability." International Journal of Pharmaceutics **285**(1-2): 23-34.
Moore, R. A., S. Derry, et al. (2014). "Faster, higher, stronger? Evidence for formulation and efficacy for ibuprofen in acute pain." Pain **155**(1): 14-21.
Okumu, A., M. DiMaso, et al. (2008). "Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug." Pharmaceutical Research **25**(12): 2778-2785.
Okumu, A., M. DiMaso, et al. (2009). "Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib solid oral drug products." European Journal of Pharmaceutics and Biopharmaceutics **72**(1): 91-98.
Padwal, R. S., R. Q. Gabr, et al. (2011). "Effect of gastric bypass surgery on the absorption and bioavailability of metformin." Diabetes Care **34**(6): 1295-1300.
Parrott, N. and T. Lavé (2002). "Prediction of intestinal absorption: Comparative assessment of GASTROPLUS™ and IDEA™." European Journal of Pharmaceutical Sciences **17**(1-2): 51-61.
R Das, N., R. P Gangwal, et al. (2014). "A PPAR-β/δ Agonist is Neuroprotective and Decreases Cognitive Impairment in a Rodent Model of Parkinson's Disease." Current Neurovascular Research **11**(2): 114-124.
Rowland, M., C. Peck, et al. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. **51:** 45-73.
Shukla, M., G. Singh, et al. (2007). "Comparative plasma pharmacokinetics of meloxicam in sheep and goats following intravenous administration." Comparative Biochemistry and Physiology - C Toxicology and Pharmacology **145**(4): 528-532.
Tucker, G. T., C. Casey, et al. (1981). "Metformin kinetics in healthy subjects and in patients with diabetes mellitus." British Journal of Clinical Pharmacology **12**(2): 235-246.
Wilson, C. G., C. P. Clarke, et al. (2011). "Comparison of a novel fast-dissolving acetaminophen tablet formulation (FD-APAP) and standard acetaminophen tablets using gamma scintigraphy and pharmacokinetic studies." Drug Dev Ind Pharm **37**(7): 747-753.
Yoshida, T., T. C. Lai, et al. (2013). "pH- and ion-sensitive polymers for drug delivery." Expert Opinion on Drug Delivery **10**(11): 1497-1513.
Yu, L. X. and G. L. Amidon (1999). "A compartmental absorption and transit model for estimating oral drug absorption." International Journal of Pharmaceutics **186**(2): 119-125.
Zhang, Y., M. Huo, et al. (2010). "DDSolver: An add-in program for modeling and comparison of drug dissolution profiles." AAPS Journal **12**(3): 263-271.
Zhao, P., M. d. L. T. Vieira, et al. (2012). "Evaluation of Exposure Change of Nonrenally Eliminated Drugs in Patients With Chronic Kidney Disease Using Physiologically Based Pharmacokinetic Modeling and Simulation." The Journal of Clinical Pharmacology **52**(S1): 91S-108S.

Chapter 3: Modelling the Absorption of Metformin with Patients Post Gastric Bypass Surgery

3.1 Introduction:

Phase one clinical drug development and bioequivalence studies are usually performed using healthy volunteers. However, some conditions and diseases can change the physiology in a patient. These changes can have a significant impact on the ability of a dosage form to deliver the desired drug dose. One of these conditions that cause changes in physiological properties is weight loss surgery. Gastric bypass surgery is an obesity treatment that shortens the length of the small intestine, which can have a major impact on drug absorption.

Obesity surgery is a treatment procedure that aims to decrease the body weight in obese patients by, for example, shortening the intestine and/or shrinking the stomach. The operations are effective treatment options for patients with body mass index BMI $\geq 30 kg/m^2$ (Sarwer DB 2011). In 2008, almost 350,000 bariatric procedures were performed around the world and 220,500 procedures were performed in the US/Canada alone (Padwal, Klarenbach et al. 2011). There are many types of obesity surgery: purely restrictive (gastric banding, gastroplasty), primarily malabsorptive (jejunoileal bypass), and combined restrictive and malabsorptive (gastric bypass Roux-En-Y), and biliopancreatic diversion with Duodenal Switch. Roux-en-Y gastric bypass (RYGB) is a popular operation as it claimed to be the most effective and safest procedure to decrease the body weight in obese patients (Smith, Henriksen et al. 2011) (Figure 3.1). Currently there are no specific guidelines for adjusting the dose of drugs administered to post gastric bypass surgery patients, and the effect of the surgery on a drugs' bioavailability is not very clear (Darwich, Henderson et al. 2012). Literature reports that the observed drug absorption patterns after gastric bypass surgery are sometimes unexpected. One report states that the absorption of metformin was higher after gastric bypass surgery (Padwal, Gabr et al. 2011).

The published study compared the absorption of metformin in 16 non-diabetics after ≥3 months gastric bypass and 16 -sex and BMI matched- control subjects, who were administered 1000 mg metformin. The study hypothesized that gastric bypass would significantly reduce the amount of absorbed drug in RYGB subjects compared to control subjects (Padwal, Gabr et al. 2011). Contrary to their hypothesis, the metformin bioavailability in RYGB subjects was increased (Padwal, Gabr et al. 2011). This study has discussed several potential mechanisms that could explain the observation, and concluded by suggesting performing further studies to explain the presented assumption (Padwal, Gabr et al. 2011). Understanding the mechanistic reasons behind the observation in drug absorption is essential to avoid unwanted outcomes and might help to address shortfalls in drug delivery. *In silico* simulations can be used for this purpose as these tools can save time, experiments and, therefore, money.

The importance of modeling disease states is to mimic the true situation and to identify variables that explain the observed data, and this will help in understanding the relationship between these variables and the observations. Physiologically based pharmacokinetic (PBPK) computer models are commercially available and can be used to investigate these variables. GastroPlus™ is one of these computer programs that used PBPK. GastroPlus™ includes ACAT model. This model is an extension of the original Compartmental Absorption and Transit (CAT) model which was developed by Yu and Amidon (Yu and Amidon 1999) to predict the drug absorption from the Gastrointestinal tract (GIT). ACAT model can be used to predict absorption-based properties from preclinical and *in vitro* data. Theses models accounts not only for physiological properties in GIT, but also for physicochemical parameters of the compounds, which impact the drug bioavailability and absorption (Yu, Lipka et al. 1996; Yu and Amidon 1999).

The purpose of this study was to investigate the mechanistic background of the reported metformin absorption in patients post gastric bypass surgery using GastroPlus™

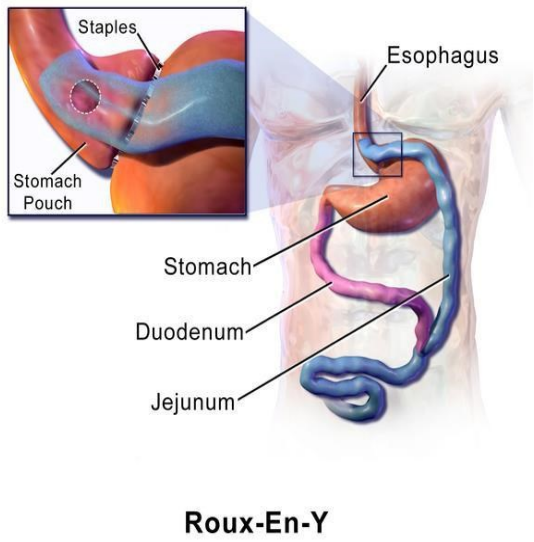


Figure 3.1: Roux-en-Y gastric bypass (RYGB)

3.2 Methods

3.2.1 Control Group:

In order to validate the simulation model, IV data was first used for the simulation to obtain (fit) compartmental PK parameters. The IV data was for healthy individuals receiving a 500 mg dose of metformin as IV bolus (Pentikainen, Neuvonen et al. 1979). The simulations were performed on a Dell Laptop with Intel core i7 (2.4 GHz) using GastroPlus™ version 8.5 software (Simulation Plus, Inc., Lancaster, CA). Input parameters required to run the prediction were extracted from literature or predicted from the drug structure using ADMET Predictor™ version 6 (Simulations Plus, Inc., Lancaster, CA) and are presented in Table 3.1. The default PBPK setting of a 30 years old human American male was used. The body weight was changed to 114 kg, which is the average body weight of the subjects in the experimental study.

All tissues in the PBPK modules were set to be limited by their permeability because Metformin has low permeability. Metformin is reported to be a substrate for influx organic cation transporters (OCTs). OCT1 is the first member of the OCTs family, and it is mainly located in the human liver, whereas OCT2 is expressed highly in kidney(Wang, Jonker et al. 2002; Proctor, Bourdet et al. 2008; Wang, Agenor et al. 2012). OCT3, is reported to be localized on the apical membrane of human small intestine (Proctor, Bourdet et al. 2008) However, OCT3 has low affinity to metformin. Therefore, OCT3 was not included in the simulation. Recently, studies suggest that metformin is a substrate to a new transporter: Plasma Membrane Monoamine Transporter (PMAT) (Zhou, Xia et al. 2007). PMAT is expressed in human small intestine, and play a role in the intestinal uptake of metformin (Xia, Engel et al. 2007; Zhou, Xia et al. 2007). Studies have also showed that Metformin is a substrate for the efflux transporters multidrug and toxin extrusions (MATE1 and MATE2-K) (Tanihara, Masuda et al. 2007; Ito, Kusuhara et al. 2012). MATE1 is located in the apical membrane of the liver and kidney, and MATE 2 is expressed mainly in the kidney (Tanihara, Masuda et al. 2007).

GastroPlus™ has the option to use and specified the location of such transporters by adding some extra inputs in to the software. The values of the *K*m and *V*max of transporters were extracted from *in vitro* experimental data in the literatures and used as input in to the software (Table 3.2). Metformin absorption in the control group was simulated using default human fasted intestinal physiology in GastroPlus™. The physiological factors and parameters including the GI transit time, physiological pH, and small intestine is presented in Table 3.3.

In the intestine, 90% of Metformin is absorbed paracellularly (Proctor, Bourdet et al. 2008) GastroPlus™ was used to estimate and account for the paracellular permeability mechanism of Metformin. To validate the model, the pharmacokinetic parameters were compared with the experimental data.

3.2.2 POST RYGB group

Once a suitable absorption and pharmacokinetic model was established and evaluated for the control group, the physiology input parameters were changed to mimic the RYGB. These conditions included: gastric volume, pH, small intestine length and small intestine transit time. These inputs were taken from literature that identified the changes in physiology after RYGB. Surgery of RYGB reduces the stomach volume by creating a 15 to 20 mL gastric pouch. The jejunum is divided at 30 to 40 cm distal to the ligament of Treitz. Then the distal jejunal limb is reconstructed to the new gastric pouch to form the Roux limb. The length of the Roux limb is approximately 75 to 150 cm in long, depending on the patient's weight (Smith, Henriksen et al. 2011; Johnson and DeMaria 2006) (Figure 3.1). The main changes that were made in the GIT physiology to build the RYGB physiology in GastroPlus^{TM} were as the following:

- **GI pH:** Normal gastric pH in fasted state in humans is in the range 1 to 3 (Smith, Henriksen et al. 2011).The small gastric pouch surface area is decreased in patients after RYGB. As a result, the stomach pH is expected to increase in these patients (Malone 2003; Miller and Smith 2006; Padwal, Klarenbach et al. 2011). To mimic this change, the pH was changed from the default 1.3 to 2 in the RYGB physiology model.
- **GI Volume:** To simulate the smaller gastric pouch, the stomach volume in the ACAT model was changed to 30 ml.
- **GI transit time:** Literature has reported an acceleration in gastric emptying after gastric bypass (Horner, Byrne et al. 2011; Chambers, Wilson-Perez et al. 2012); Others found that the gastric emptying time after RYGB is reduced (Suzuki, Ramos et al. 2005) and some concluded that these changes are variable Padwal, Klarenbach et al. 2011). Therefore, no changes were made for the gastric emptying**.** As discussed above, in the RYGB the intestinal duodenum and jejunum is bypassed (Smith, Henriksen et al. 2011).

To mimic this condition, the transit times and the length of duodenum and jejunum compartments in the ACAT model were set to zero. Data suggest that the transit time of small intestine is increased (Darwich, Pade et al. 2013; Tappenden 2014). Quigley et al found that the small intestinal transit time is increased by 35% following 50% resections of dogs small intestine and by 29% following 75% of the resections (Quigley and Thompson 1993). Therefore, the transit time of the ileum was increased by 32% especially because the RYGB resections, to some extent, are variable.

To mechanistically understand the reasons behind the observed data, every assumption that was presented the article (Padwal, Gabr et al. 2011) was examined by changing the related parameters.

- The first mechanism that was discussed in the article was that RYGB was expected to delay the gastric emptying and the increased gastrointestinal transit time may increase the overall absorption of metformin. The article mentioned that this process is working in a manner similar to slow-release formulations of metformin, which remained in the stomach and released drug gradually into the small intestine. To examine this assumption, a modified gastric release formulation model was used to simulate this effect. The modified Gastric Release option allows retaining drug in the stomach and releasing it over time.
- The second assumption was that there is an alteration in the transporters. To test this assumption, using Gastroplus^{TM}, the expression of the transporters was doubled in the remaining part of small intestine.
- The last assumption was that the small-intestine undergoes adaptation from villous hyperplasia. To test this assumption, the pore size, pore density and the absorptive surface area were changed to the values given in Table 3.4 for the remaining part of the small intestine.

Table 3.1: Input parameters used in control and post gastric bypass in the compound window of GastroPlus™

Parameter	Input	Reference
Dose	1000	(Padwal, Gabr et al. 2011)
Dosage form	immediate release	(Padwal, Gabr et al. 2011)
Molecular weight	129.17	ADMET Predictor 6
LogD(7.4)	-3.37	(Crison, Timmins et al. 2012; Mathias and Crison 2012)
Solubility	134.87 mg/mL @ pH=12.9	ADMET Predictor 6
Permeability	P_{app}, total = 0.05x 10^{-5} cm/s Paracellular = .4467 x 10^{-4} cm/s Transcellular = 0.0001 x 10^{-4} cm/s	ADMET Predictor 6, (Zhou, Xia et al. 2007)
pKa	10.17, 7.14	ADMET Predictor 6

Table 3.2: Input parameters used in control and post gastric bypass in the transporters window of GastroPlus™

Transporter	Location	Transporter kinetics	Reference
PMAT	Intestine	Km= 1.32 mM	(Zhou, Xia et al. 2007)
OCT1	Liver	Km= 1.47mM *V*max =396pmol/ min/mg protein	(Kimura, Masuda et al. 2005)
OCT2	Kidney	Km= 0.99 mM, Vmax = 1444pmol/ min/mg protein	(Kimura, Masuda et al. 2005)
MATE1	Liver and Kidney	Km= 0.78 mM, Vmax = 4.46nmol/ min/mg protein	(Tanihara, Masuda et al. 2007)
MATE-K2	Kidney	Km= 1.98mM, Vmax = 1.69nmol/ min/mg protein	(Tanihara, Masuda et al. 2007)

Table 3.3: ACAT Model Parameters for the Human fasted physiology

Compartment	pH	Transit time (h)	Volume (ml)	Length (cm)	Radius (cm)	SEF[a]	Pore Radius (A)	Porosity/Pore Length (cm^-1)
Stomach	1.3	0.25	50	30	10	1.00	2.20	2.580
Duodenum	6	0.26	48.25	15	1.60	4.23	10.41	48.64
Jejunum 1	6.20	0.95	175.3	62	1.50	3.94	9.64	38.90
Jejunum 2	6.40	0.76	139.9	62	1.34	3.48	8.40	26.09
Ileum 1	6.60	0.59	108.5	62	1.18	3.02	7.16	16.46
Ileum 2	6.90	0.43	79.48	62	1.01	2.56	5.92	9.54
Ileum 3	7.40	0.31	56	62	0.85	2.11	4.68	4.89
Caecum	6.40	4.50	52.92	13.75	3.50	1.79	3.92	2.91
Ascending colon	6.80	13.50	56.98	29.02	2.50	2.48	3.50	3.22

[a] – SEF represent Surface area Enhancement Factors which are a measure of absorptive surface area in individual compartments.

Table 3.4: The pore size, pore density and the absorptive surface area in post gastric model.

Compartment	SEF	Pore Radius (A)	Porosity/Pore Length (cm^-1)	Transit time (h)
Stomach	1.00	2.20	2.58	0.25
Duodenum	4.23	10.41	48.64	0.26
Jejunum 1	3.95	9.640	38.90	0.95
Jejunum 2	3.49	8.40	26.09	0.76
Ileum 1	5.25	12.53	29.00	0.78
Ileum 2	4.49	10.36	16.60	0.57
Ileum 3	8.19	5.68	8.50	0.41
Caecum	1.79	3.92	2.92	4.50
Ascending colon	2.48	3.50	3.220	13.50

3.3 Results:

3.3.1 Control Group

Using default Human Physiological Fasted intestinal physiology, GastroPlus™ estimated the plasma concentration curve very close to observed data with an R^2=0.9, (Figure 3.2). The values of C_{max}, T_{max} and AUC of the predicted data were closely matched to the observed ones for the control group (Table 3.5).

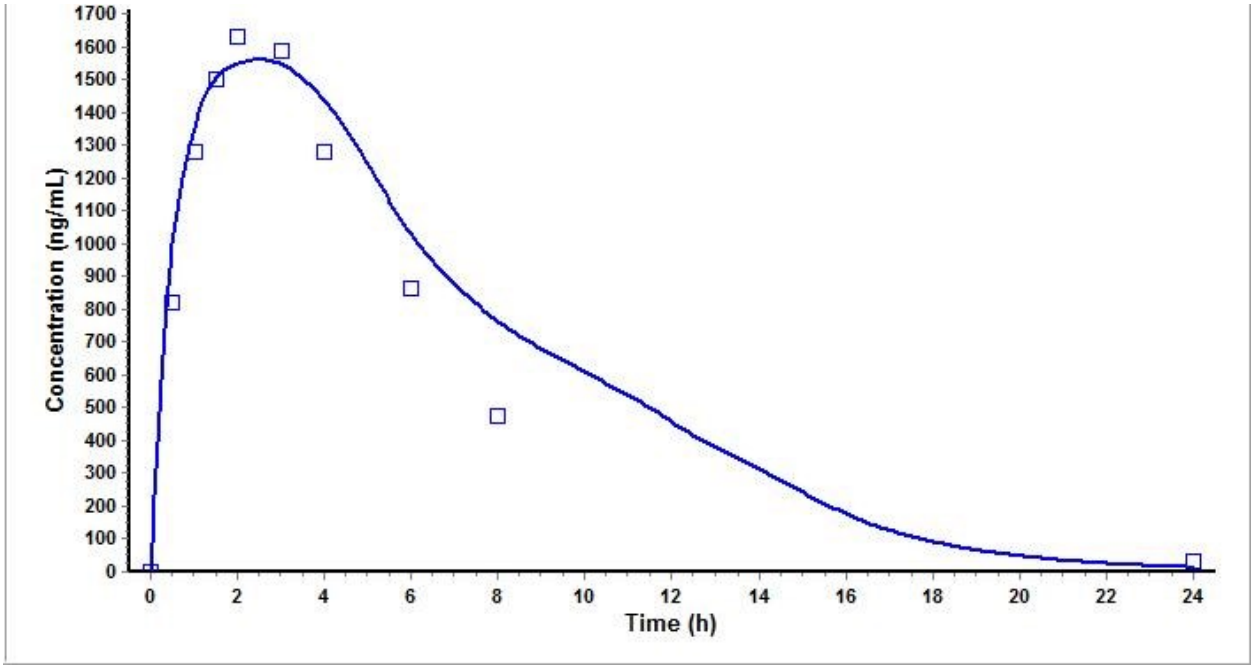


Figure 3.2: The observed (dots) and the predicted (solid line) plasma concentration profile after oral administration of 1000mg of metformin in the control group.

Table 3.5: The observed and predicted C_{max}, T_{max} and $AUC_{0-24\,h}$ after oral administration of 1000mg of metformin in controls and post RYGB (simulation resulted from model with an increased pore size, porosity and absorptive surface area).

Condition	Parmeters	Predicted mean	Observed mean
Control	C_{max} (ug/h)	1.59	1.8 ± (0.61)
	T_{max} (hr)	2.49	3.0 ± (1.5)
	AUC0–inf h(ug/h/mL)	13.70	11.4 ± (3.6)
	AUC0–24 h(ug/h/mL)	13.59	11.1 ± (3.6)
Post RYGB	C_{max} (ug/h)	2.3617	2.0 ± (0.86)
	T_{max} (hr)	1.4067	3.0 ± (1.5)
	AUC0–inf h(ug/h/mL):	15.82	13.7 ± (6.0)
	AUC0–24 h(ug/h/mL)	15.75	13.4± (5.7)

Data are means (SD) based on the experimental sample size 15.

3.3.2 Post gastric bypass

As expected, the adjustment of intestinal physiology based on simple removal of the bypassed portions of small intestine from the model, underestimated the exposure of metformin in subjects after RYGB. Figure 3.3 presents the observed and predicted absorption of metformin after RYGB.

Using the modified Gastric Release option did not predict observed data (data not shown). Increasing the distribution of the transporters in Gastroplus™ in the remaining part of small intestine also did not improve the prediction of the observed data (data not shown). However, increasing the pore size, porosity and the absorptive surface area in the remaining part of the small

intestine resulted in a close match of plasma drug concentration vs time of simulation to the observed data (Figure 3.4). The result also showed a comparable result between the pharmacokinetics parameters of the experimental and predicted values (Table 3.5)

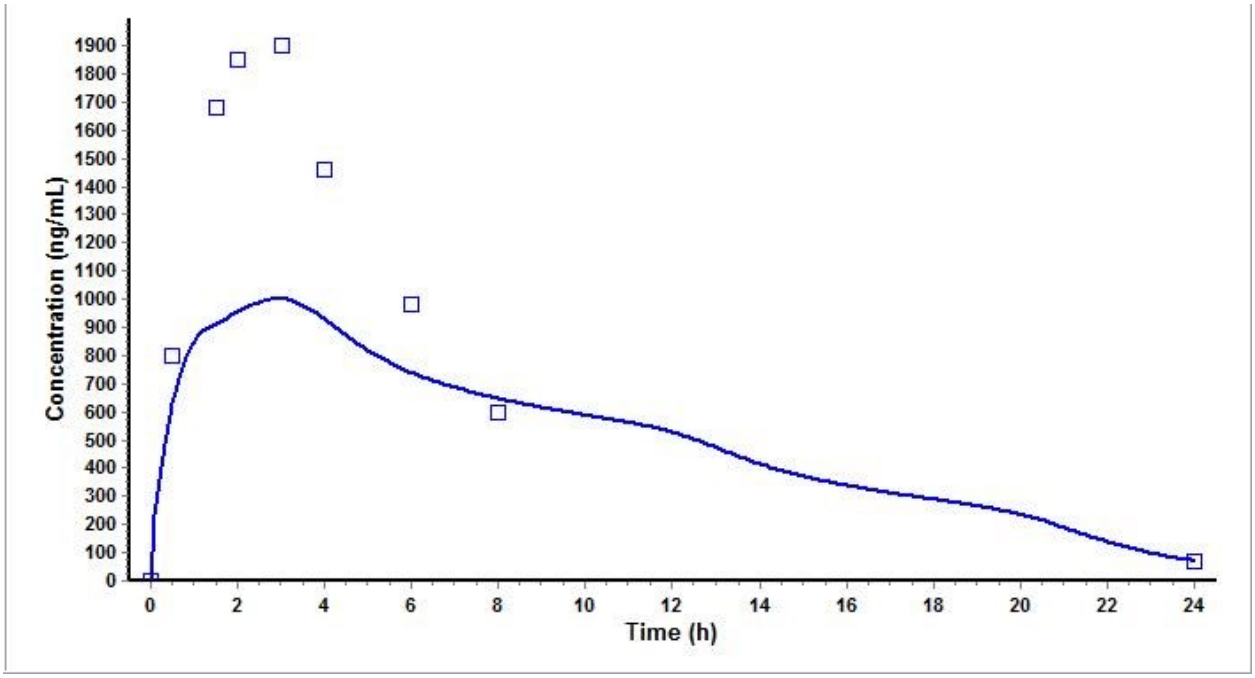


Figure 3.3: The observed (dots) and the predicted (solid line) plasma concentration profile after oral administration of 1000mg metformin in patients post RYGB. Simulation is based on intestinal model with simple removal of bypassed portions of small intestine.

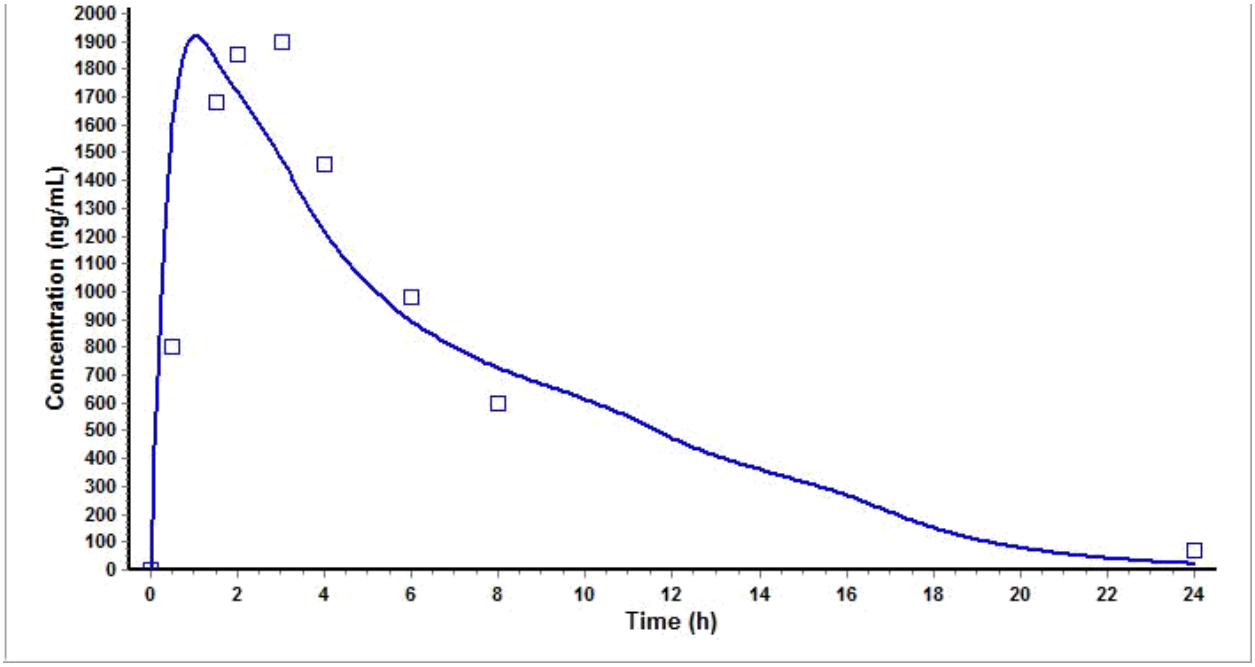


Figure 3.4: The observed (dots) and the predicted (solid line) plasma concentration profile after oral administration of 1000mg metformin in patients post RYGB. Simulation resulted from a model with an increased in pore size, porosity and absorptive surface area.

3.4 Discussion and conclusions:

Over the past years, studies have shown many applications of simulations in drug discovery, particularly in predicting drug absorption. The use of *in silico* modeling helped in understanding, identifying, and addressing many factors that affect the drug absorption and causes of observed absorption patterns. These factors range from the *in vitro* drug properties such as physiochemical characters of drugs to *in vivo* characteristics such as physiological properties of the GIT. Obesity surgery treatment changes the human GIT physiology, which is expected to have an influence on drug absorption. In this study, we have examined the application of simulations to understand the mechanistic changes in drug absorption in post RYGB subjects using metformin as a model

drug. Metformin is an interesting model drug because the drug is highly soluble, but it is limited by its permeability. Studies reported that, in normal physiology, metformin is absorbed mainly in duodenum (Proctor, Bourdet et al. 2008). RYGB surgery is required to bypass the entire duodenum and a small portion of the proximal jejunum, therefore, reduced metformin absorption is anticipated. However, the observed published study showed the opposite: an increase in drug absorption (Padwal, Gabr et al. 2011). In this study, computer simulations were used to test the ability of computer program to simulate the absorption after gastric bypass surgery and to test the theoretical assumptions that were reported to explain the observed drug absorption in such patients. This was done by altering the related values in the program inputs and comparing the predicted data with the observed ones. Then, to find out the most probable cause of the observed data, the assumption that could not predict the observed data was ruled out.

Applying only the controlled gastric release formulation in GastroPlus^{TM} could not predict the observed data, though this was actually not surprising. The Biopharmaceutical Classification System (BCS) classifies drugs according to their solubility and permeability characteristics have classified Metformin as a BCS class 3 drug (Crison, Timmins et al. 2012). Metformin is a highly soluble drug; therefore, retaining the drug in the stomach and releasing it slowly would not be expected to significantly affect the drug absorption. However, such effects seem to have a significant effect for poorly soluble drugs as shown by Jamali et al for meloxicam (Aghazadeh-Habashi and Jamali 2008). This finding was supported by a study conducted by Marathe et al, who studied the effect of prolonged gastric emptying and the gastrointestinal transient time induced by metcloprmide and propantheline on the metformin absorption in healthy volunteers (Marathe, Wen et al. 2000). The study was conducted in 11 healthy volunteers, who received 550 mg metformin hydrochloride in solution alone; 5 min after a 10 mg IV dose of

metoclopramide; and 30 min after a 30 mg oral dose of propantheline. Metformin solution was radiolabeled and then the gastrointestinal transit was monitored by gamma scintigraphy (Marathe, Wen et al. 2000). The study showed that the increase in the small intestinal transit times induced by propantheline causes a decline in metformin plasma concentrations associated with the arrived in the colon (Marathe, Wen et al. 2000). The study concluded that the first part of the small intestine is the main site of absorption of metformin. Therefore, the prolonged in the GI transient time by RYGB could not explain the observation as the first part of small intestine is removed by surgery.

The results also showed that increasing the transporters expression in the last part of the small intestine did not predict the observed data. As mentioned above, metformin is limited by its permeability. Studies showed that 90% of metformin is absorbed paracellularly, which occurs via facilitated diffusion, and only 10 % of the drug is absorbed transcellularly, which is mediated by transporters (Proctor, Bourdet et al. 2008). Therefore, increase in the transporters expression is not expected to significantly increase the drug absorption. Moreover, studies showed that PMAT transporter, which is a substrate for metformin absorption, is sensitive to pH change. Studies showed a significant influence of acidic pH on PMAT in mediating metformin (Zhou, Xia et al. 2007). The study found that PMAT-mediated metformin uptake at pH 6.6 is 4 fold higher than at pH 7.4 (Xia, Engel et al. 2007). However, the pH of the last part of the small intestine, which is the part lifted after the surgery, is higher than 6.6. In other words, PMAT activity is expected to be decrease after RYGB surgery. These findings support the inability of the simulation of the up regulation the transporter in prediction of the observed metformin bioavailability after RYGB.

The last assumption that was used to explain the observation was an adaptation mechanism in the intestinal drug absorption. This assumption is supported by many studies in man and animals (Miskowiak and Andersen 1983; Stock-Damge, Aprahamian et al. 1986; Doldi 1991). Studies have reported that obese patients, who had bypass surgery, were losing body weight after the surgery, but later the body weight will stabilize.

Therefore, studies were conducted to explain this phenomenon. Interestingly, researchers found that patients who had obesity bypass surgery showed a clear evidence of adaptation. Studies have observed an increase in the intestinal epithelial surface area with an increase of the functional capacity of the intestine (Doldi 1991). Miskowiak et al has measured the length of the small intestine in thirty-two patients after 6 to77 months of jejunoileal bypass surgery (Miskowiak and Andersen 1983). The lengths were recorded 3 times by the same surgeon. Intestinal wall thickness was also measured for some patients. Miskowiak et al found that the length of the small intestine was increased in patients with an association of unsatisfactory weight losts (Miskowiak and Andersen 1983) . Stock-Damge et al also supported this observation. In his study, intestinal biopsy samples were obtained surgically from 41 obese patients subjected to a biliopancreatic bypass (Stock-Damge, Aprahamian et al. 1986). The specimens were collected from the proximal and distal ileum, and colon. An image analyzer determined the height of the mucosa, including villi and crypts. The study showed that the mucosa of the proximal and distal ileum showed a marked lengthening of the villi after biliopancreatic bypass. In the colon the biliopancreatic bypass induced 40% of the patients' focal changes in the mucosa, characterized by the presence of true villi with mucous cells and epithelial cells (Stock-Damge, Aprahamian et al. 1986). In all other patients, the colonic mucosa was slightly thicker, but its morphologic aspect remained normal. The same observation was documented after RYGB in rat models (Shaw, Gohil et al. 2012). The intestine showed an

increase in villus height and crypt cell proliferation adaptation after 6–8 months of RYGB surgery (Spak, Björklund et al. 2010; Shaw, Gohil et al. 2012). These results suggest that this observation was not limited to the type of bypass surgery. Studies showed that there is a correlation between the length of intestine removed and the subsequent change in villus height in humans. Studies have referred these adaptations to a systemic hormonal stimulus (Shaw, Gohil et al. 2012). Gastrin and enteroglucagon, particularly glucagon-like peptide 2 (GLP-2) have been proposed as primary cause of the adaptation (Barry, Barisch et al. 1977; Stock-Damge, Aprahamian et al. 1986). Le Roux et al determined the changes in GLP-2 levels and crypt cell proliferation in rodents and in man after RYGB and the association between increased GLP-2 concentrations and crypt cell proliferation in rodents after RYGB (le Roux, Borg et al. 2010). The study showed that in humans GLP-2 levels rise substantially after RYGB, peaking at 6 to 12 months, before returning to normal levels. This increase appears to be sufficient to maintain an increased small bowel mass following RYBG (le Roux, Borg et al. 2010). An interesting recently published study has investigated the impact of RYGB on the permeability of the small intestine (Shaw, Gohil et al. 2012; Savassi-Rocha, Diniz et al. 2014). The study evaluated the intestine permeability by measuring the renal excretion rate of orally administered lactulose and mannitol and assessed the lactulose/mannitol ratio excretion rate in patients in three periods: before RYGB, one month after RYGB and 6 months after RYGB (Savassi-Rocha, Diniz et al. 2014). The study found a significant increase in mannitol excretion after RYGB surgery in addition to an increase in lactulose/mannitol ratio excretion (Savassi-Rocha, Diniz et al. 2014). The study concludes that patients after RYGB have an increase in the intestinal permeability by an adaption mechanism (Savassi-Rocha, Diniz et al. 2014). As a matter of fact, this study substantiates our result because mannitol is absorbed *in vivo* in a manner similar to metformin, through paracellular passive diffusion.

All of these studies supported the assumption behind the observed absorption of metformin. This mechanism corresponds with the stabilizing in the weight loss and the decrease in surgery side effects such as diarrhea for a time period after the operation. However, one should remember that this does not mean that all drug absorption would be the same after bypass surgery. As mentioned above drug absorption is influenced by both the GIT physiology and the drug physiochemical properties. Therefore, if a drug is limited by its permeability i.e. a class 3 drug and is absorbed by passive diffusion with no active metabolites, the overall drug absorption is expected to be improving or showing no change after RYGB due to the adaption mechanism. Based on these data, the increased bioavailability of the drug can improve clinical outcomes and especially the pharmacodynamics action of Metformin in diabetic patients who undergo RYGB, for more than 6 months. However, if the drug is limited by its solubility, i.e a class 2 drug, a change in the fraction dose absorbed is expected as the GI, pH, and the transit time after bypass surgery have changed.

Computer simulations can be used to confirm this finding. Rowland et al. has reviewed the application of a PBPK modele to understand the variations in the observation of drug absorption (Rowland, Peck et al. 2011). He reviewed many studies, which used PBPK module, to realize how physiological factors such as age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ impairments influence the PK of drugs that were administered to each populations in these conditions (Rowland, Peck et al. 2011). One recent study has also evaluated PBPK modeling, using the Simcyp^{TM} software as a tool for examining the impact of physiological changes after obesity surgery (Darwich, Pade et al. 2013). The study used cyclosporine and atorvastatin as model drugs to predict drug absorption after RYGB and biliopancreatic diversion with duodenal switch, respectively. The study concluded that PBPK has a potential to predict the oral drug bioavailability in the absence of clinical data (Darwich,

Pade et al. 2013). The current study supports this finding and showed the importance of using computer simulations as a valuable tool to simulate the impact and mechanistic background of the physiological changes on drugs absorption. The insights gained by this study can be used to help to predict the absorption and the clinical outcomes of other drugs that have similar physiochemical properties like metformin after RYBG.

3.5 References:

Aghazadeh-Habashi, A. and F. Jamali (2008). "Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: Influence of gastrointestinal dysfunction on the relative bioavailability of two formulations." European Journal of Pharmaceutics and Biopharmaceutics **70**(3): 889-894.

Barry, R. E., J. Barisch, et al. (1977). "Intestinal adaptation after jejunoileal bypass in man." The American Journal of Clinical Nutrition **30**(1): 32-42.

Chambers, A. P., H. E. Wilson-Perez, et al. (2012). "Effect of vertical sleeve gastrectomy on food selection and satiation in rats." American Journal of Physiology - Endocrinology and Metabolism **303**(8): E1076-E1084.

Crison, J. R., P. Timmins, et al. (2012). "Biowaiver approach for biopharmaceutics classification system class 3 compound metformin hydrochloride using In Silico modeling." Journal of Pharmaceutical Sciences **101**(5): 1773-1782.

Darwich, A. S., K. Henderson, et al. (2012). "Trends in oral drug bioavailability following bariatric surgery: Examining the variable extent of impact on exposure of different drug classes." British Journal of Clinical Pharmacology **74**(5): 774-787.

Darwich, A. S., D. Pade, et al. (2013). "Evaluation of an In Silico PBPK Post-Bariatric Surgery Model through Simulating Oral Drug Bioavailability of Atorvastatin and Cyclosporine." CPT: pharmacometrics & systems pharmacology **2**(6): 1-9.

Doldi, S. B. (1991). "Intestinal adaptation following jejuno-ileal bypass." Clinical Nutrition **10**(3): 138-145.

Horner, K. M., N. M. Byrne, et al. (2011). "The effects of weight loss strategies on gastric emptying and appetite control." Obesity Reviews **12**(11): 935-951.

Ito, S., H. Kusuhara, et al. (2012). "Competitive Inhibition of the Luminal Efflux by Multidrug and Toxin Extrusions, but Not Basolateral Uptake by Organic Cation Transporter 2, Is the Likely Mechanism Underlying the Pharmacokinetic Drug-Drug Interactions Caused by Cimetidine in the Kidney." Journal of Pharmacology and Experimental Therapeutics **340**(2): 393-403.

Johnson, W. and E. DeMaria (2006). "Surgical treatment of obesity." Curr Treat Options Gastroenterol **9**(2): 167-174.

Kimura, N., S. Masuda, et al. (2005). "Metformin is a Superior Substrate for Renal Organic Cation Transporter OCT2 rather than Hepatic OCT1." Drug Metabolism and Pharmacokinetics **20**(5): 379-386.

le Roux, C. W., C. Borg, et al. (2010). "Gut hypertrophy after gastric bypass is associated with increased glucagon-like peptide 2 and intestinal crypt cell proliferation." Annals of surgery **252**(1): 50-56.

Malone, M. (2003). "Altered drug disposition in obesity and after bariatric surgery." Nutrition in clinical practice **18**(2): 131-135.

Marathe, P. H., Y. Wen, et al. (2000). "Effect of altered gastric emptying and gastrointestinal motility on metformin absorption." British Journal of Clinical Pharmacology **50**(4): 325-332.

Mathias, N. R. and J. Crison (2012). "The Use of Modeling Tools to Drive Efficient Oral Product Design." The AAPS Journal **14**(3): 591-600.

Miller, A. D. and K. M. Smith (2006). "Medication and nutrient administration considerations after bariatric surgery." American Journal of Health-System Pharmacy **63**(19): 1852-1857.

Miskowiak, J. and B. Andersen (1983). "Intestinal adaptation after jejunoileal bypass for morbid obesity: a possible explanation for inadequate weight loss." The British journal of surgery **70**(1): 27-28.
Padwal, R., S. Klarenbach, et al. (2011). "Bariatric surgery: A systematic review and network meta-analysis of randomized trials." Obesity Reviews **12**(8): 602-621.
Padwal, R. S., R. Q. Gabr, et al. (2011). "Effect of gastric bypass surgery on the absorption and bioavailability of metformin." Diabetes Care **34**(6): 1295-1300.
Pentikainen, P. J., P. J. Neuvonen, et al. (1979). "Pharmacokinetics of metformin after intravenous and oral administration to man." European Journal of Clinical Pharmacology **16**(3): 195-202.
Proctor, W. R., D. L. Bourdet, et al. (2008). "Mechanisms Underlying Saturable Intestinal Absorption of Metformin." Drug Metabolism and Disposition **36**(8): 1650-1658.
Quigley, E. M. and J. S. Thompson (1993). "The motor response to intestinal resection: motor activity in the canine small intestine following distal resection." Gastroenterology **105**(3): 791-798.
Rowland, M., C. Peck, et al. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. **51:** 45-73.
Sarwer DB, F. L., Steffen KJ, Roerig JL, Mitchell JE. (2011) "Managing patients after surgery: Changes in the gastrointestinal tract and body weight can alter drug absorption." Current Psychiatry **10**, 18-30.
Savassi-Rocha, A., M. Diniz, et al. (2014). "Changes in Intestinal Permeability after Roux-en-Y Gastric Bypass." Obesity Surgery **24**(2): 184-190.
Shaw, D., K. Gohil, et al. (2012). "Intestinal mucosal atrophy and adaptation." World Journal of Gastroenterology **18**(44): 6357-6375.
Smith, A., B. Henriksen, et al. (2011). "Pharmacokinetic considerations in Roux-en-Y gastric bypass patients." American Journal of Health-System Pharmacy **68**(23): 2241-2247.
Spak, E., P. Björklund, et al. (2010). "Changes in the mucosa of the Roux-limb after gastric bypass surgery." Histopathology **57**(5): 680-688.
Stock-Damge, C., M. Aprahamian, et al. (1986). "Small-intestinal and colonic changes after biliopancreatic bypass for morbid obesity." Scand J Gastroenterol **21**(9): 1115-1123.
Suzuki, S., E. J. B. Ramos, et al. (2005). "Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model." Surgery **138**(2): 283-290.
Tanihara, Y., S. Masuda, et al. (2007). "Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H+-organic cation antiporters." Biochemical Pharmacology **74**(2): 359-371.
Tappenden, K. A. (2014). "Intestinal Adaptation Following Resection." Journal of Parenteral and Enteral Nutrition.
Wang, D.-S., J. W. Jonker, et al. (2002). "Involvement of Organic Cation Transporter 1 in Hepatic and Intestinal Distribution of Metformin." Journal of Pharmacology and Experimental Therapeutics **302**(2): 510-515.
Wang, G., K. Agenor, et al. (2012). "Accelerated Gastric Emptying but No Carbohydrate Malabsorption 1 Year After Gastric Bypass Surgery (GBP)." Obesity Surgery **22**(8): 1263-1267.
Xia, L., K. Engel, et al. (2007). Membrane localization and pH-dependent transport of a newly cloned organic cation transporter (PMAT) in kidney cells.

Yu, L. X. and G. L. Amidon (1999). "A compartmental absorption and transit model for estimating oral drug absorption." International Journal of Pharmaceutics **186**(2): 119-125.
Yu, L. X., E. Lipka, et al. (1996). "Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption." Advanced Drug Delivery Reviews **19**(3): 359-376.
Zhou, M., L. Xia, et al. (2007). "Metformin Transport by a Newly Cloned Proton-Stimulated Organic Cation Transporter (Plasma Membrane Monoamine Transporter) Expressed in Human Intestine." Drug Metabolism and Disposition **35**(10): 1956-1962.

Chapter 4: Mechanistic Understanding of the Effect of Renal Impairment on Metformin Oral Absorption Using Computer Simulation

4.1 Introduction:

Diabetes is one of the most common worldwide diseases. Almost 350 million people worldwide have diabetes (Danaei, Finucane et al. 2011). Diabetes can increase the risk of other concomitant factors related to the disease, such as obesity, cardiovascular disease, high cholesterol, and hypertension. Diabetes and the incidences of these factors cause an increase in the manifestation of renal impairment and chronic kidney disease (CKD) (Naud, Nolin et al. 2012). Approximately 1 of 3 adults with diabetes has CKD (CDC 2015).

Metformin has been prescribed as first line treatment for type II diabetics for more than 50 years (Rojas and Gomes 2013). Metformin is an inexpensive drug and has many advantages over other anti-diabetics drugs. This drug has an effect in reducing the body weight and protecting against heart disease, cancer, polycystic ovarian disease, and osteopenia (Lalau 2010; Lalau, Arnouts et al. 2014). This drug also has fewer side effects compared to all other oral anti-diabetic drugs (Lalau 2010, Asensio-López,2011). The most serious side effect of the drug is lactic acidosis. Though it is seriousness, lactic acidosis induced by metformin is a rare side effect. Lactic acidosis induced by metformin is observed in 9.7 events per 100 000 persons (Lalau 2010). Metformin is contraindicated in patients with kidney disease due to the increase risk of this side effect. The U.S. Food and Drug Administration does not recommend the use of metformin with serum creatinine concentrations of 1.4 mg/dL (Lipska, Bailey et al. 2011). However, despite these contraindications practitioners are still prescribing Metformin for these patients because of all the advantages (Lipska, Bailey et al. 2011). Metformin blood concentrations are high in renal impaired patients (Sambol, Chiang et al. 1995). This is believed to be due to insufficient renal clearance in these patients. (Pentikainen, Neuvonen et al. 1979; Tucker, Casey et al. 1981). Metformin undergoes glomuler filtration (GF) and 80% of the drug

is eliminated by active tubular secretion (Zamek-Gliszczynski, Lee et al. 2013). The main transporters that control metformin excretion are the influx organic cations transporters (OCTs) and the efflux multidrug and toxin extrusion (MATEs) transporters (Zolk 2011). OCT1 uptake transport is expressed mainly in basolateral membrane of hepatocytes; OCT2 transporter is expressed highly in the basolateral of renal tubular cells (Zolk 2011). The efflux transporter MATE1 is expressed in several tissues such as skeletal muscles, heart, and mainly in the apical tubular cells in the kidney and hepatocytes in the liver. MATE2 efflux transporters are highly distributed in tubular kidney cells (Figure 4.1) (Hilgendorf, Ahlin et al. 2007). However, the physiological parameters that describe the reasons behind the drug accumulation in renal failure patients are not fully understood. Because of the seriousness of this side effect in these patients, very limited in vivo studies have been conducted.

Computer simulations can be an alternative safe method to better understand clinical observations by building a suitable model to investigate how a drug behaves in healthy and disease states (Almukainzi M 2014). Physiologically Based Pharmacokinetic (PBPK) model is a tool that can be used for this purpose (Clewell, Reddy et al. 2010). PBPK models integrate drug physiochemical characterizes and physiological information to mimic a drug's absorption and disposition in a biological system (FDA 2014). This tool links pharmacokinetics (PK) concepts to whole-body processes, thus, each organ of a species represents a compartment. Each compartment is characterized by a blood flow rate, a volume and a tissue partition coefficient in addition to an intrinsic clearance value for the eliminated organ (Peters, Ungell et al. 2009). This model is described in detail in the literature (Peters, Ungell et al. 2009; Rostami-Hodjegan, Tamai et al. 2012; Rowland 2013)

The objectives of this work were (1) to describe and evaluate the utility of PBPK to predict the plasma levels of metformin in healthy and renal impairment individuals. (2) recognize the most important PBPK parameters that explain the reason behind the observation in renal impairment and (3) confirm these findings using in vitro experiments.

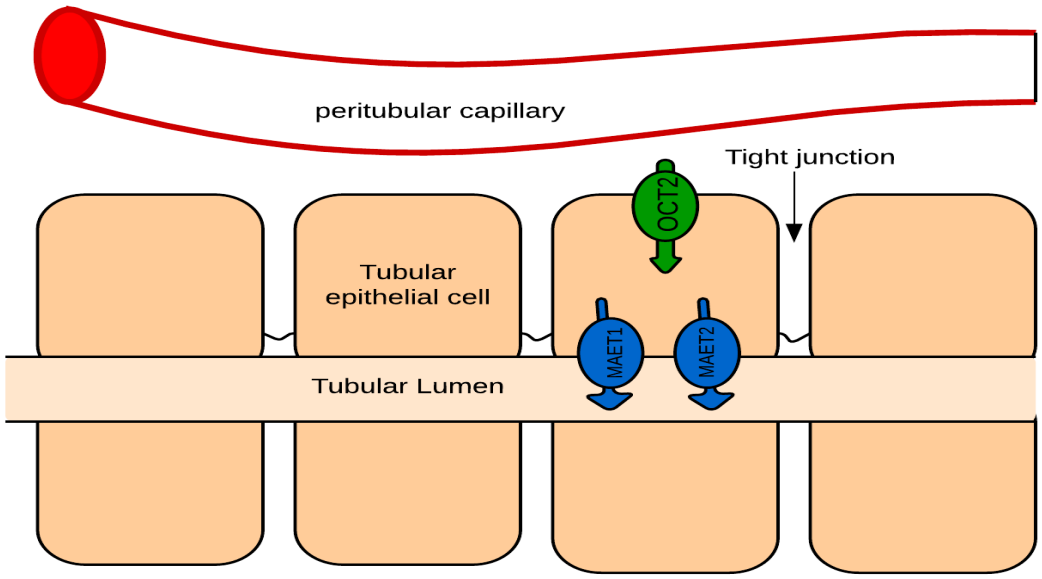


Figure 4.1: Transporter that mediated the renal clearance of Metformin. OCT: organic cation transporters, MATE: multidrug and toxin extrusion.

4.2 Methods:

4.2.1 Simulation of renal failure on metformin bioavailability

All simulations were performed on a Dell Laptop computer with Intel core i7 (2.4 GHz) using GastroPlusTM version 8.6 software (Simulation Plus, Inc., Lancaster, CA). GastroPlus™ uses the Advanced Compartmental Absorption Transit (ACAT) model. The ACAT model is a nine-compartment physiologically based absorption model that mimics the transport of compounds along the gastrointestinal tract (GIT). The mass balance equations used by the software describe

six different states a drug substance can be in: unreleased, undissolved, dissolved, degraded, metabolized, and absorbed in the different areas of the GIT. The kinetics associated with the dissolution and absorption processes are coupled with linear and nonlinear rate equations (Li, Wang et al. 2012). In PBPK each compartment represents a tissue, with a specific volume, blood perfusion rate, and partition coefficient for each tissue. The (PBPK) Modeling in GastroplusTM is composed of 14 tissues: heart, lung, brain, adipose, muscle, skin, spleen, gastrointestinal tract, liver, kidney, yellow marrow, red marrow, and rest of the body.

To model kidney renal failure states, we started with modeling the absorption in healthy individuals. Once a good simulation was achieved for the experimental data for the healthy state, the same physiochemical inputs were used and possible relevant physiological parameters were changed to mimic the effect of renal failure on the drug absorption, distribution and elimination.. Due to the polarity of metformin, all tissues in the PBPK module were set to be limited by their permeability so the actual tissue concentration is determined by the permeability and surface area exposed to the plasma (Almukainzi M 2014). Therefore, the metformin tissue concentration will be less than the concentration in the blood. Using this setting in GastroPlusTM, the rate of drug transfer into or out of the tissue was calculated by multiplying the permeability value for each tissue times the surface area.

4.2.1.1 Modeling the drug absorption for healthy individuals

The input profile of the intravenous metformin data for healthy individuals was used to predict the oral data for healthy individuals. These steps are discussed in detail by Almukainzi et al. (Almukainzi M 2014). The simulated data was compared to experimental published data by Nancy et. al (Nancy C. Sambol ,1995). The dosage form used was an immediate release tablet. The dose was 663 mg of metformin, which is the equivalent dose to 850 mg in metformin HCL

as used in the experimental study. GastroPlus^{TM} has embedded a Population Estimates for Age-Related Physiology (PEAR) differences, which calculates the human physiology on the basis of an ethnic population database for males and females aged 1 to 85 years old (SimulationPlus 2015). The age and gender related population database was extracted from the National Health and Nutrition Examination Survey database based on 11039 Americans and 4667 Japanese (CDC 2010; Li, Wang et al. 2012). Similar age and weight for healthy individuals in the experimental study was used (Table 4.1).

4.2.2 Modeling drug absorption under kidney failure conditions

The same set of physiochemical and pharmacokinetic inputs were used to predict drug absorption under these conditions. To model the disease condition, the PBPK model was altered with kidney related physiological deviations to mimic dysfunction expected in renal failure relevant to metformin pharmacokinetics. For example, studies showed a decrease in hematocrit (Hsu, McCulloch et al. 2002), and renal blood flow by 20% to 60% (Bellomo, Ronco et al. 2004; Hoang Thi, Morel et al. 2012; Philips, Lane et al. 2014). On the other hand, gastric emptying time and gastric pH is increased in renal dysfunction as a result of excess urea in the saliva, which is transformed to ammonia by gastric urease enzymes (Rinaldo Bellomo,2004.). Such changes were included in the model. In normal cases, the renal clearance (CLr) of metformin is about 507 ml/min, so the drug is subject to tubular secretion by transporters (Graham, Punt et al. 2011). The renal filtration estimation method used in this study was filtration fup*GFR. In the control group, the GFR was set to 120 ml/min.

Renal impairment was found when the GFR value was ≤ 60ml/min/1.73 m2 (Naud, Nolin et al. 2012). For moderate renal chronic failure data, which is the data that we tried to simulate, GFR was found to be between 30 and 60 ml/min (Bellomo, Ronco et al. 2004). Therefore, the GFR

was set to 60 ml/min in the program. Acute kidney injury and chronic renal failure conditions showed a decrease in the level of the renal transporters, in particular, OCT2, MATE1 and MATE 2 in the kidney (Ji, Masuda et al. 2002; Yonezawa, Masuda et al. 2005; Nishihara, Masuda et al. 2007; Matsuzaki, Morisaki et al. 2008; Yonezawa and Inui 2011). Therefore, OCT2, MATE1, and MATE2 expression in kidney tissue were decreased by almost 30% (Komazawa, Yamaguchi et al. 2013).

When the model could not predict the absorption, and excretion of metformin in renal disfuction other assumptions were tested. Renal impairment may also impact transporter expression in the liver as reported in some studies.(Dreisbach and Lertora 2008). To check this assumption, the expression of OCT1, and MATE2 in the liver was changed. Now the model was able to predict the observed data. The main changes in renal dysfunction that are relevant to metformin are summarized in table 4.1.

Table 4.1: The physiological changes used to simulate the renal impairment

parameter	**Control group**	**kidney failure**
hematocrit %	0.45	0.39
renal blood flow	17.16 ml/s	12.68 ml/s
gastric pH	1.30	2.00
GFR value	2.06 ml/s	1.00 ml/s

4.2.2 Experimental cell line verification:

The simulation showed that not only a decrease in the metformin kidney transporters (OCT2, MATE1 and MATE2) causes the observed data, but also there seems to be a decrease MATE1 expression in the liver. Therefore, our second assumption was that the high plasma concentration of metformin in renal failure is explained by the down regulation of MATE1 expression level in both kidney and liver. This decrease in the expression can be explained by the increase in the serum creatinine in these patients. To test this hypothesis, an *in vitro* cell line using human embryonic kidney (HEK293) cells was chosen. Researchers have used this cell line in many experimental studies as a tool to study kidney transporters, particularly, OCTs and MATs (Urakami, Kimura et al. 2004; J. Liu, Lu et al. 2012).

4.2.2.1 Confirmation of MATE1 expression in HEK293

The HEK293 cells were cultured on 10 cm culture dishes. The cells were maintained in complete medium consisting of Dulbecco's modified Eagle's medium supplemented with 0.45% glucose, 0.15% sodium bicarbonate, 0.11% sodium pyruvate, 10% fetal bovine serum, 20 μM L-glutamine, 50 μg/ml gentamicin sulfate, 100 IU/ml penicillin, 10 μg/ml streptomycin at 37 °C in a 5% CO2:95% air humidified incubator. Culture medium was changed every 2 days. When the cells were 60-80% confluent, they were harvested by scraping them off using 200 μl lysing buffer and then transferred into 500 ul Eppendorf tubes. The protein concentration was measured by Lowry method 1951 (Lowry, Rosebrough et al. 1951). Then the remaining cell solutions were then used in western plotting to detect the transporter expression. The proteins were incubated in the human MATE1 primary antibody (1:100) for overnight and subsequently exposed to goat anti-rabbit secondary antibody following the Western blotting protocol.

4.2.2.2 Protein extraction and Western blot analysis

Western blot analysis was performed following the Sambrook et al. method (Sambrook J. 1989). Briefly, cells were collected in lysis buffer as described previously. Protein samples (50 μg) were resolved by denaturing electrophoresis. The cell homogenates were dissolved in 1X sample buffer, boiled for 5 min, separated by 10% SDS–PAGE and electrophoretically transferred to a nitrocellulose membrane. Protein blots were blocked for 24 h at 4 °C in blocking buffer containing 5% skim milk powder, 2% bovine serum albumin and 0.05% (v/v) Tween 20 in Tris-buffered saline solution (0.15 M sodium chloride, 3 mM potassium chloride and 25 mM Tris base). After blocking, the membrane were incubated with MATE-1 human primary antibody for 24 h at 4 °C in Tris-buffered saline containing 0.05% (v/v) Tween 20 and 0.02% sodium azide. Incubation with a peroxidase-conjugated goat anti-rabbit IgG secondary antibody was carried out in blocking buffer for 2 h at room temperature. The bands were visualized with the enhanced chemiluminescence method according to the manufacturer's instructions (GE Healthcare, Piscataway, NJ). The intensity of protein bands was quantified relative to the signals obtained for β-actin protein, using ImageJ (National Institutes of Health, Bethesda, MD) image processing program (ImageJ)

4.2.2.3 Time dependence and transporter uptake

For uptake experiments, the cells were seeded into poly-D-lysine-coated 24-well plates. At 60 to 80% confluence (about 1–2 days later), the cell monolayers in each well waere treated with 10 μM metformin HCl dissolved in BPS. This concentration is equivalent to Metformin concentrations in the kidneys at steady state in healthy individuals (Christensen, Brasch-Andersen et al. 2011). After each 1, 2, 5, and 10 minutes time points the drug was withdrawn. Then the drug concentration was analyzed by HPLC using a published method (Gabr, Padwal et

al. 2010). The mobile phase consisted of 0.01 M phosphate buffer (pH 6.5)/acetonitrile (65:35) at a flow rate of 0.7 ml/min and 236 nm for UV detection. The injection volume was 100 µl.

4.2.2.4 Metformin and creatinine in regular HEK cell and in gene overexpressed cells

To examine metformin effects in the presence of creatinine on MATE1 transport, both regular HEK293 cell and MATE1 overexpressed HEK293 cell were used. The day before the transfection, HEK293 cells were seeded onto poly-D-lysine-coated 24-well plates. When they were 70% confluent, the HEK293 cells were transfected according to the transfection protocol methods by the manufacture using Plasmid vector DNA containing cDNA SLC47A1, MATE1 Clone (MyBioSource, Inc., San Diego, CA, USA), in each well with LipoD293 DNA In Vitro Transfection Reagent (SignaGen Laboratories,Rockville, MD). At 24 h after transfection, both regular HEK293 cell and MATE1 overexpressed HEK293 were treated with 10 µM metformin with different concentration of creatinine concomitantly. The creatinine concentration used were 0.35, 0.5, 1, 2, and 5 mg/dl , which is equivalent to the increase in serum creatinine under renal dysfunction (Levey, Coresh et al. 2003; Mehta and Chertow 2003; Ciarimboli, Lancaster et al. 2012).

4.2.2.5 Cell viability assay

The effect of Metformin and creatinine on cell viability was determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This method measures mitochondrial activity based on the reductive cleavage of yellow tetrazolium salt to a purple formazan compound by the dehydrogenase activity of intact mitochondria. Cells were grown on 96-well plates and exposed to metformin conc. (10 µM) for 24 h. The culture medium was replaced by a fresh medium containing 0.5 mg/mL MTT and cells were incubated for an

additional 2 h. The medium was removed and the formazan product was dissolved in 200 µL isopropyl alcohol for 60 min under gentle agitation. The plates were read with a 570-nm filter in a Synergy H1 Hybrid Multi-Mode Microplate Reader (Biotek Instruments Inc., Vermont, USA).

4.3 Results:

4.3.1 Modeling Metformin absorption in healthy and renal failure

Gastroplus™ was able to predict the drug absorption in heathy individuals as presented in figure 4.2(A). For renal Failure patients figure 4.2 (B), we were able to capture the observed absorption by applying the estimated changes in renal failure in addition to decreasing MATE1 expression in liver and decreasing OCT2, and MATE2 expression in kidney.

4.3.2 MATE1 transporter in HEK293

Western blotting showed the MATE1 expression band at 62 KD, which confirm the expresses of MATE1 in HEK293 cell (data not shown)

4.3.3 Transporter uptake control time

The uptake of Metformin was linear for up to 2 min. (Figure 4.3). This result is agreed with uptake experiments documented by other studies (Kusuhara, Ito et al. 2011).

4.3.4 The creatinine concentration and Metformin

Figure 4.4 shows the effect of the increase in the creatinine concentration in the metformin excretions in regular and transfected cells.

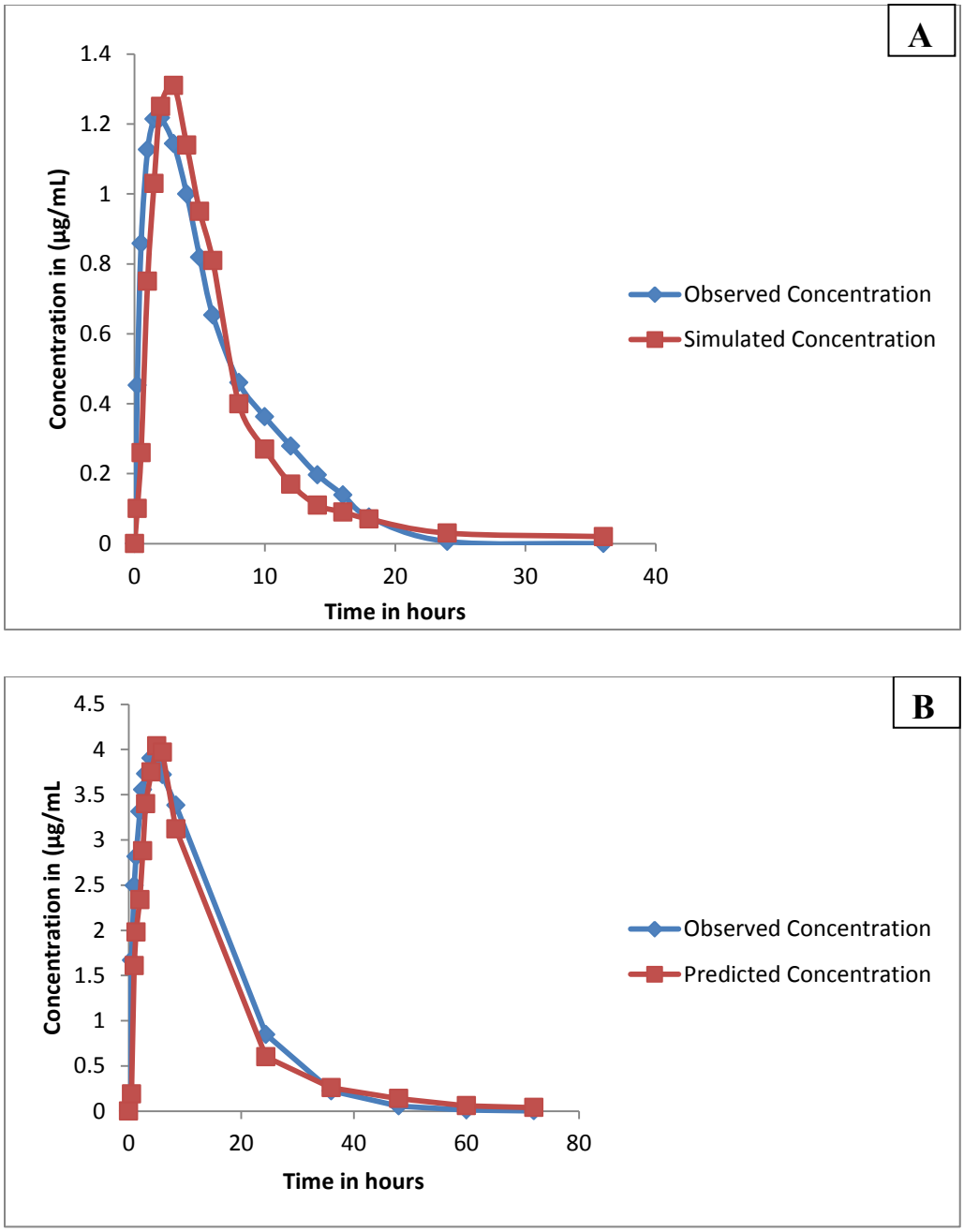


Figure 4.2: Plasma concentration versus time curve after 850 mg oral dose of Metformin HCL in healthy individual (A) and chronic renal failure (B).

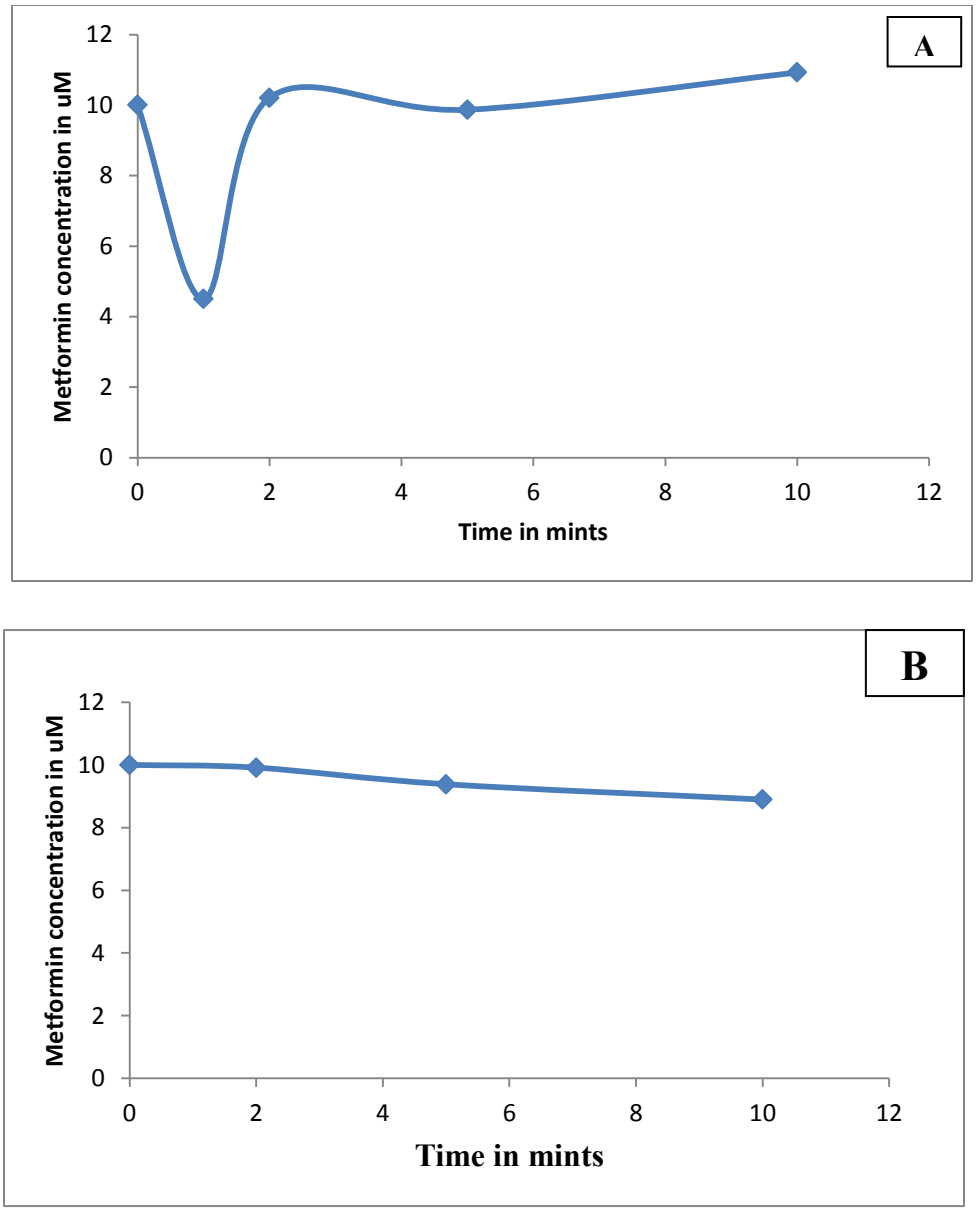


Figure 4.3: The concentration-time dependence of metformin uptake in regular (A) and overexpressed hMATE1 HEK293 cells (B).

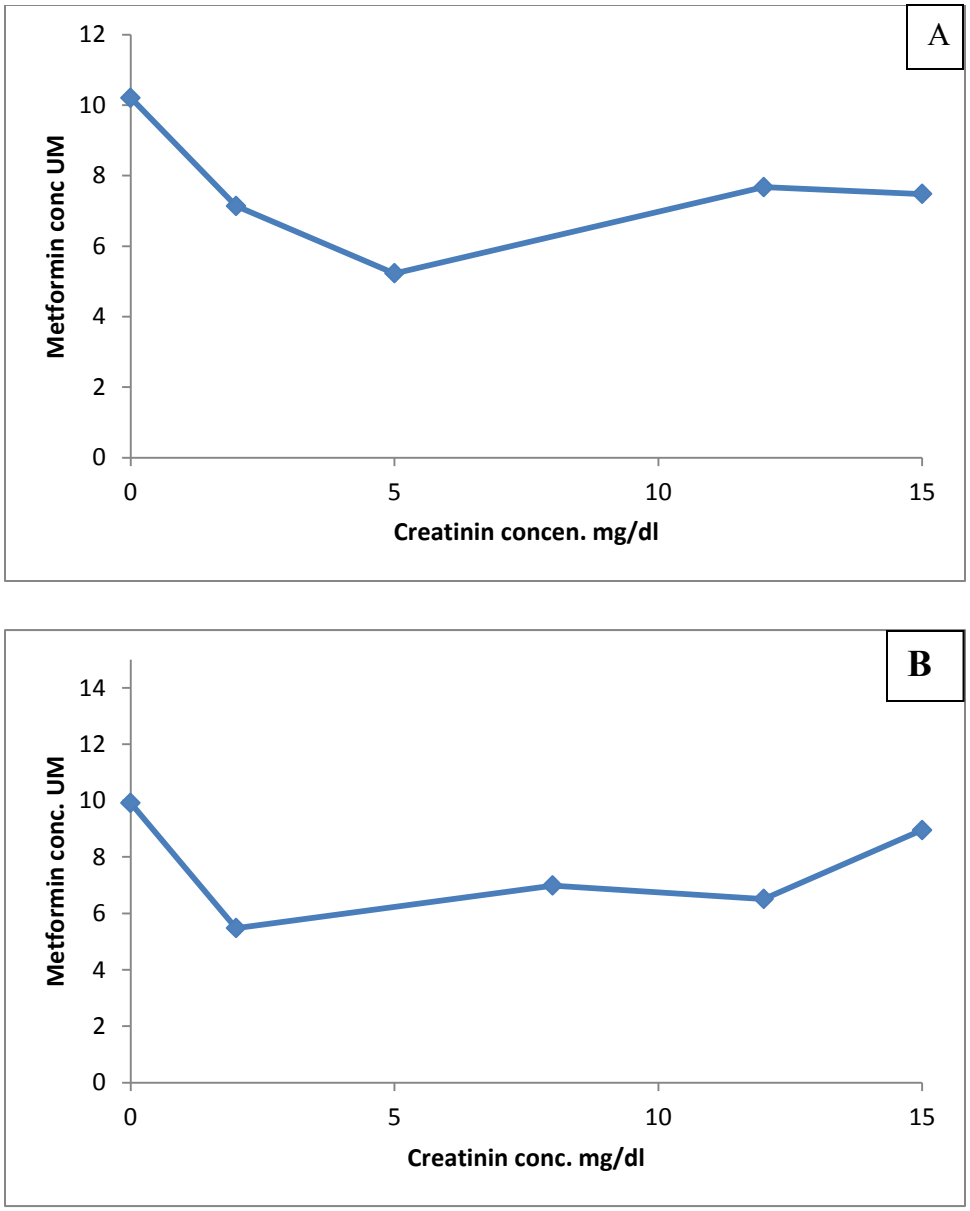


Figure 4.4: the effect of increasing the creatinine concentration on the Metformin excretion in the regular (A) and overexpressed hMATE1 HEK293 cells (B).

4.4 Discussion

The PBPK models proposed in 1937 by Teorell et al based on drug distribution and concentration over a period of time in the blood and tissue of organs (Teorell 1937). Today, this system is developed to become more sophisticated using the available knowledge of the body

fluid dynamics, tissue size and the distribution of drugs' transporters and enzymes in various organ compartments (Micaela B. Reddy 2013). PBPK modeling is a safe method to model drug distribution and disposition in body tissues taking into account the rate of absorption, distribution, metabolism, and excretion of the administered drug from the body. Many intrinsic factors, such as age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction, on the drug bioavailability can be evaluated using such modeling (Micaela B. Reddy 2013). The most common software programs that implement PBPK modeling are GastroplusTM, PK-SimTM and SimcypTM (Micaela B. Reddy 2013).

Here robust models can be created by incorporating the knowledge of physiological parameters that influence the drug behavior with the drug absorption, distribution, and metabolism and elimination pathways. The PBPK allows the modification of physiological parameters which can describe disease states (Edginton and Willmann 2008; Almukainzi M 2014).

Renal impairment is a complication and/or a concomitant disease with diabetes that affects almost 26 million adults in the United States alone (Naud, Nolin et al. 2012). Prediction the drug absorption in renal impairment patients is not always an easy task due to the lack of the defined mechanisms of the physiological changes in these patients (Philips, Lane et al. 2014). Though metformin has been available for over half-a-century, it is surprising to note that there is very limited scientific literature on using metformin in renal impairment patients (Zhao, Vieira et al. 2012; Lalau, Arnouts et al. 2014). Metformin pharmacokinetics has an high volume of distribution with almost 723 L (Sambol, Chiang et al. 1995). Compartmental analysis suggests that metformin pharmacokinetics is best described by a multi-compartmental model, with a rapid elimination from plasma and a slow elimination from deep tissue such as liver and erythrocytes (Wilcock, Wyre et al. 1991; Robert, Fendri et al. 2003). Metformin elimination occurs mainly

through the kidneys (Pentikainen, Neuvonen et al. 1979). In normal subjects, metformin elimination is a fast process with a half-life of 1.5-6 hr (Robert, Fendri et al. 2003), and the terminal half-life excretion phase of metformin is between 8-48 hr but represents only ≤ 5% of the administration dose (Pentikainen, Neuvonen et al. 1979; Robert, Fendri et al. 2003). This is because the peak concentration is much higher in the central compartment than that in the deep tissues. However, impairment in renal function affects the drug excretion and leads to an increase of this percent of the fraction of the dose administered in the deep tissues. Therefore, the drug is expected slower and remains longer in the blood and this may lead to an accumulation of the drug in the body, an increase in the drug uptake into deep tissue, and eventually, increase in the risk of lactic acidosis (Lalau, Westeel et al. 1987).

In this study, we evaluated using a PBPK model to predict the effect of renal impairment of metformin absorption. The aim was to bridge knowledge gaps in understanding the mechanism behind the drug behavior in these patients. Our simulation showed that down regulation of the influx and efflux renal transporters in addition to down regulating the MATE1 transporter in the liver could be the key factors that can explain the observed drug plasma levels.. This means that not only renal clearances might be reduced in renal failure (Figure 4.6). Studies have linked the changes in the drug pharmacokinetics in renal failure patients to the accumulation of uremic toxins. Their concentration is increased due to renal dysfunction. There are more than 110 uremic toxins identified until now (Toyohara, Suzuki et al. 2009; Naud, Nolin et al. 2012). Kynuric acid , indoxy sulphate (IS), guanidine succinate (GSA), asymmetric dimethylarginin (ADMA), indoleacetate, and hippurate (HA) , and 3-Carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF) are some examples of these toxins (Toyohara, Suzuki et al. 2009; Naud, Nolin et al. 2012). Studies showed that these toxins are competitively inhibiting related

transporters. Organic anion-transporting polypeptide (OAT), which functions as an efflux pump in the renal tubules and liver, is one of known transporters that is affected by these toxins (Sun, Huang et al. 2004; Enomoto and Endou 2005; Matsuzaki, Morisaki et al. 2008)). In particular OAT1 and OAT3 transporters are significantly reduced in renal dysfunction. This observation was directly linked to the toxin accumulation that is formed in renal dysfunction (Kasliwal, Negi et al. 2011; Maeda, Shinoda et al. 2011).

Komazawa et al examined the activity of the OAT transporters expressed in the renal tubulars in kidney impairment using adenine-induced renal failure rats. Additionally, he investigated the effect on the in vivo kidney uptake clearances of metformin (Komazawa, Yamaguchi et al. 2013) . Komazawa et al. found that the renal impairment significantly decreased the mRNA levels and the protein expression of OCT1, OCT2, OATP1, OATP3 and MATE1. Moreover, the plasma concentration of metformin was significantly increased in renal dysfunction groups compared to the control one. Komazawa et al. also tested the effect of incubating serum samples from rats with renal impairment on the metformin uptake in hepatocytes in vitro. A significant decrease in the metformin uptake was found, and this was linked to the decrease in transporter activity in the renal impaired rats (Komazawa, Yamaguchi et al. 2013). A study showed that erythromycin elimination, which is mainly metabolized and eliminated by liver, was significantly decreased in renal failure (Sun, Huang et al. 2004). Uremic toxins, specifically CMPF, were the main factor that inhibit the uptake of erythromycin in the liver though inhibiting OATP2, in addition to its effect on their enzymatic metabolism of erythromycin (Sun, Huang et al. 2004). Therefore, our simulations were able to predict the experimental data when we decreased the expression of the transporter, especially, MATE1 not only in the kidney but also in liver.

In renal failure, serum creatinine, which is another toxic waste product, is known to accumulate significantly in the body of these patients. In normal patients, creatinine is excreted mainly by glomerular filtration (GF); therefore, it is used as an indication of kidney function (Urakami, Kimura et al. 2004; Urakami, Kimura et al. 2005). Studies showed that creatinine, just like metformin, is a substrate to the basolaterally expressed OCT2; and the apically expressed (MATE1 and 2) transporters. Therefore, we anticipated that the accumulation of creatinine in renal patients competitively inhibits the MATE1 transporter in liver and kidney. This competition was expected to be noticeable in renal failure since there is already a down regulation in the kidney transporters. This theory was applied to other drugs, such as cimetidine, rilpivirine, dolutegravir, and quinolone in other studies. These drugs inhibited the tubular secretion and elevate the serum creatinine levels (Sano, Iwao et al. 2011). This is because these drugs and creatinine are eliminated by OCT2 and MATE1, which makes them to compete for the same transporters. The HEK293 cell line was used to test this theory because MATE1 is highly expressed in the kidney (Otsuka et al., 2005; Hiasa et al., 2006). We confirmed that these in vitro cells expressed the MATE1 transporter. However, metformin uptake did not change with the investigated creatinine concentration. This could be due to the low affinity and high capacity of MATEs transporters toward creatinine (Lepist, Zhang et al. 2014). Or the down regulation in MATE1 could be induced by other uremic toxins such as the IS. Morisaki, et al. investigated the renal expression of MATE1 in the rats with kidney failure that was induced by cisplatin (Matsuzaki, Morisaki et al. 2008). The renal dysfunction rats showed a significantly reduced mRNA and protein levels compared to control groups. Morisaki et al. showed that this reduction could be restored by the administration of AST-120 (Matsuzaki, Morisaki et al. 2008). AST-120

or Kremezin is a spherical adsorptive carbon preparation, which absorbs uremic toxins, mainly IS (Enomoto and Endou 2005) .

Therefore, it was suggested that the down regulation in MATE1 expression is due to the accumulation of IS (Matsuzaki, Morisaki et al. 2008). The significant effect of MATE1 in metformin elimination is supported by a study that investigated the genetic differences of MATE1 distribution on metformin pharmacokinetics. Wild-type [Mate1(+/+)] and MATE1 knockout [Mate1(-/-)] mice were used for this purpose. After a single IV administration of metformin (5 mg/kg), a 2-fold increase in the AUC0-60 and a significant decrease in the urinary excretion of metformin was observed in Mate1(-/-) mice (Tsuda, Terada et al. 2009).

Interestingly, studies linked metformin induced lactic acidosis to MATE1 dysfunction and this observation was demonstrated even when plasma concentrations of metformin were within the therapeutic range (Tymms and Leatherdale 1988; Misbin, Green et al. 1998; Stades, Heikens et al. 2004; Toyama, Yonezawa et al. 2012). This confirms that the MATE1 transporters play a key role not only in the drug elimination but also the disposition into deep tissues.

Quantifying a transporter in a human organ is difficult to achieve. The PBPK model is a useful tool to investigate such effects, and hence, it helps in understanding the effect of drug-drug interactions and genetic variations when drugs are exposed to the blood and organs. Using the PBPK model, Watanabe et al was able to predict the transporter effect and genetic variations on the plasma concentration profiles of pravastatin (Watanabe, Kusuhara et al. 2009). Using computer software that embedded PBPK modeling allows us to predict, learn, and interpret the clinical observations; thus, using this approach untested clinical outcomes might be predicted

(FDA 2014). Understanding the reasons for the increase in metformin blood concentrations can help in reducing the incidents of side effects in these patients

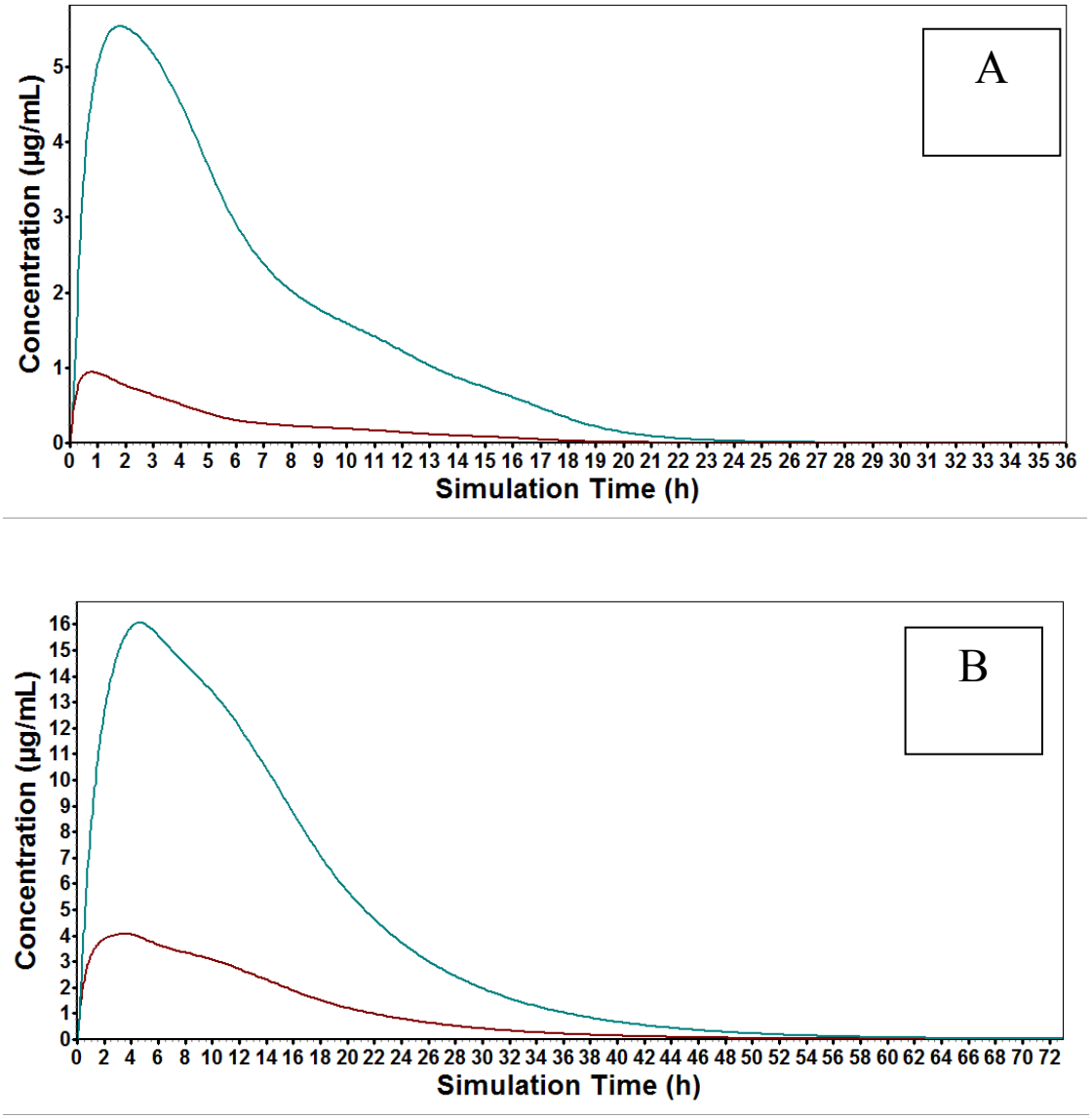


Figure 4.6: The predicted kidney concentration (blue line) and liver concentration (red line) of metformin in normal subjects (A), and renal failure patients (B).

4.5 Conclusion:

This study showed the ability of our PBPK model to simulate metformin absorption in control and renal dysfunction patients. The renal dysfunction model showed that down-regulation of the MATE1 transporter not only in kidney but also in liver explained the increase in drug concentration in these patients. However, the accumulation of creatinine in renal impairment patients has no effect on the metformin elimination. PBPK modeling has the potential to explain the mechanistically complex changes in drug absorption/disposition induced by disease conditions, and to predict the drug distribution and concentration in different organs and tissues. This might help to reduce the need for clinical studies in these patients and increases drug therapy outcomes by adjusting drug doses in such patients. PBPK models have the potential to play an important role in precision medicine in the future.

4.6 Refrences:

Almukainzi M, L. V., Löbenberg R (2014). "Modelling the Absorption of Metformin with Patients Post Gastric Bypass Surgery." Diabetes Metab **5**: 353.

Bellomo, R., C. Ronco, et al. (2004). "Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group." Critical Care **8**(4): R204-R212.

CDC. (2010). "National Center for Health Statistics (NCHS)."

CDC. (2015). "Chronic Kidney Disease." Retrieved 2014, 2014, from (http://www.cdc.gov/diabetes/pubs/factsheets/kidney.htm.

Christensen, M. M. H., C. Brasch-Andersen, et al. (2011). "The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c." Pharmacogenetics and Genomics **21**(12): 837-850.

Ciarimboli, G., C. S. Lancaster, et al. (2012). "Proximal Tubular Secretion of Creatinine by Organic Cation Transporter OCT2 in Cancer Patients." Clinical Cancer Research **18**(4): 1101-1108.

Clewell, H. J., M. B. Reddy, et al. (2010). Physiologically Based Pharmacokinetic Modeling. Pharmaceutical Sciences Encyclopedia, John Wiley & Sons, Inc.

Danaei, G., M. M. Finucane, et al. (2011). "National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants." The Lancet **378**(9785): 31-40.

Dreisbach, A. W. and J. J. Lertora (2008). "The effect of chronic renal failure on drug metabolism and transport." Expert Opinion on Drug Metabolism & Toxicology **4**(8): 1065-1074.

Edginton, A. and S. Willmann (2008). "Physiology-Based Simulations of a Pathological Condition." Clinical Pharmacokinetics **47**(11): 743-752.

Enomoto, A. and H. Endou (2005). "Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease." Clinical and Experimental Nephrology **9**(3): 195-205.

FDA. (2014). "Program of physiologically-based pharmacokinetic and pharmacodynamic modeling (PBPK Program)." FDA, 2015, from http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm365118.htm.

Gabr, R. Q., R. S. Padwal, et al. (2010). "Determination of metformin in human plasma and urine by high-performance liquid chromatography using small sample volume and conventional octadecyl silane column." Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques **13**(4): 486-494.

Graham, G., J. Punt, et al. (2011). "Clinical Pharmacokinetics of Metformin." Clinical Pharmacokinetics **50**(2): 81-98.

Hilgendorf, C., G. Ahlin, et al. (2007). "Expression of Thirty-six Drug Transporter Genes in Human Intestine, Liver, Kidney, and Organotypic Cell Lines." Drug Metabolism and Disposition **35**(8): 1333-1340.

Hoang Thi, T. H., S. Morel, et al. (2012). "Development and evaluation of taste-masked drug for paediatric medicines – Application to acetaminophen." International Journal of Pharmaceutics **434**(1–2): 235-242.
Hsu, C.-y., C. E. McCulloch, et al. (2002). "Iron Status and Hemoglobin Level in Chronic Renal Insufficiency." Journal of the American Society of Nephrology **13**(11): 2783-2786.
ImageJ.
J. Liu, J., J. Lu, et al. (2012). "Membrane Transporters as Determinants of the Pharmacology of Platinum Anticancer Drugs." Current Neuropharmacology **12**(8): 962-986.
Ji, L., S. Masuda, et al. (2002). "Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy." Kidney Int **62**(2): 514-524.
Kasliwal, N., J. Negi, et al. (2011). "Formulation, development, and performance evaluation of metoclopramide HCl oro-dispersible sustained release tablet." Archives of Pharmacal Research **34**(10): 1691-1700.
Komazawa, H., H. Yamaguchi, et al. (2013). "Renal uptake of substrates for organic anion transporters Oat1 and Oat3 and organic cation transporters Oct1 and Oct2 is altered in rats with adenine-induced chronic renal failure." Journal of Pharmaceutical Sciences **102**(3): 1086-1094.
Kusuhara, H., S. Ito, et al. (2011). "Effects of a MATE Protein Inhibitor, Pyrimethamine, on the Renal Elimination of Metformin at Oral Microdose and at Therapeutic Dose in Healthy Subjects." Clinical Pharmacology & Therapeutics **89**(6): 837-844.
Lalau, J.-D. (2010). "Lactic Acidosis Induced by Metformin." Drug Safety **33**(9): 727-740.
Lalau, J. D., P. Arnouts, et al. (2014). "Metformin and other antidiabetic agents in renal failure patients." Kidney international.
Lalau, J. D., P. F. Westeel, et al. (1987). "Bicarbonate haemodialysis: an adequate treatment for lactic acidosis in diabetics treated by metformin." Intensive Care Medicine **13**(6): 383-387.
Lepist, E.-I., X. Zhang, et al. (2014). "Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat." Kidney Int **86**(2): 350-357.
Levey, A. S., J. Coresh, et al. (2003). "National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification." Annals of Internal Medicine **139**(2): 137-147.
Li, G.-f., K. Wang, et al. (2012). "Simulation of the pharmacokinetics of bisoprolol in healthy adults and patients with impaired renal function using whole-body physiologically based pharmacokinetic modeling." Acta Pharmacol Sin **33**(11): 1359-1371.
Lipska, K. J., C. J. Bailey, et al. (2011). "Use of Metformin in the Setting of Mild-to-Moderate Renal Insufficiency." Diabetes Care **34**(6): 1431-1437.
Lowry, O. H., N. J. Rosebrough, et al. (1951). "PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT." Journal of Biological Chemistry **193**(1): 265-275.
Maeda, A., T. Shinoda, et al. (2011). "Evaluating tamsulosin hydrochloride-released microparticles prepared using single-step matrix coating." International Journal of Pharmaceutics **408**(1–2): 84-90.
Matsuzaki, T., T. Morisaki, et al. (2008). "Altered Pharmacokinetics of Cationic Drugs Caused by Down-Regulation of Renal Rat Organic Cation Transporter 2 (Slc22a2) and Rat Multidrug and Toxin Extrusion 1 (Slc47a1) in Ischemia/Reperfusion-Induced Acute Kidney Injury." Drug Metabolism and Disposition **36**(4): 649-654.

Mehta, R. L. and G. M. Chertow (2003). "Acute Renal Failure Definitions and Classification: Time for Change?" Journal of the American Society of Nephrology **14**(8): 2178-2187.
Micaela B. Reddy, H. J. C. I., Thierry Lave and Melvin E. Andersen (2013). Physiologically Based Pharmacokinetic Modeling: A Tool for Understanding ADMET Properties and Extrapolating to Human. New Insights into Toxicity and Drug Testing. D. S. Gowder.
Misbin, R. I., L. Green, et al. (1998). "Lactic Acidosis in Patients with Diabetes Treated with Metformin." New England Journal of Medicine **338**(4): 265-266.
Naud, J., T. D. Nolin, et al. (2012). "Current Understanding of Drug Disposition in Kidney Disease." The Journal of Clinical Pharmacology **52**(S1): 10S-22S.
Nishihara, K., S. Masuda, et al. (2007). "Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats." Biochemical Pharmacology **73**(9): 1482-1490.
Pentikainen, P. J., P. J. Neuvonen, et al. (1979). "Pharmacokinetics of metformin after intravenous and oral administration to man." European Journal of Clinical Pharmacology **16**(3): 195-202.
Peters, S. A., A. L. Ungell, et al. (2009). "Physiologically based pharmacokinetic (PBPK) modeling and simulation: applications in lead optimization." Current opinion in drug discovery & development **12**(4): 509-518.
Philips, B. J., K. Lane, et al. (2014). "The effects of acute renal failure on drug metabolism." Expert Opinion on Drug Metabolism & Toxicology **10**(1): 11-23.
Robert, F., S. Fendri, et al. (2003). "Kinetics of plasma and erythrocyte metformin after acute administration in healthy subjects." Diabetes & Metabolism **29**(3): 279-283.
Rojas, L. B. A. and M. B. Gomes (2013). "Metformin: an old but still the best treatment for type 2 diabetes." Diabetology & Metabolic Syndrome **5**(1): 6.
Rostami-Hodjegan, A., I. Tamai, et al. (2012). "Physiologically based pharmacokinetic (PBPK) modeling: it is here to stay!" Biopharmaceutics & drug disposition **33**(2): 47-50.
Rowland, M. (2013). "Physiologically-Based Pharmacokinetic (PBPK) Modeling and Simulations Principles, Methods, and Applications in the Pharmaceutical Industry." CPT: pharmacometrics & systems pharmacology **2**: e55.
Sambol, N. C., J. Chiang, et al. (1995). "Kidney Function and Age Are Both Predictors of Pharmacokinetics of Metformin." The Journal of Clinical Pharmacology **35**(11): 1094-1102.
Sambrook J., F. E. F., Maniatis T (1989). Molecular cloning: A laboratory Manual., Cold Spring HarborNY: Cold Spring Harbor Laboratory Press.
Sano, S., Y. Iwao, et al. (2011). "Preparation and evaluation of swelling induced-orally disintegrating tablets by microwave irradiation." International Journal of Pharmaceutics **416**(1): 252-259.
SimulationPlus. (2015). "GastrPlus manual."
Stades, A. M. E., J. T. Heikens, et al. (2004). "Metformin and lactic acidosis: cause or coincidence? A review of case reports." Journal of Internal Medicine **255**(2): 179-187.
Sun, H., Y. Huang, et al. (2004). "EFFECTS OF UREMIC TOXINS ON HEPATIC UPTAKE AND METABOLISM OF ERYTHROMYCIN." Drug Metabolism and Disposition **32**(11): 1239-1246.
Teorell, T. (1937). "Kinetics of the Distribution of Substances Administered to the Body . II. The Extravascular Mode of Administration." Archives Internationales de Pharmacodynamie et de Thérapie (57): 205–240

Toyama, K., A. Yonezawa, et al. (2012). "Loss of multidrug and toxin extrusion 1 (MATE1) is associated with metformin-induced lactic acidosis." British Journal of Pharmacology **166**(3): 1183-1191.
Toyohara, T., T. Suzuki, et al. (2009). "SLCO4C1 Transporter Eliminates Uremic Toxins and Attenuates Hypertension and Renal Inflammation." Journal of the American Society of Nephrology **20**(12): 2546-2555.
Tsuda, M., T. Terada, et al. (2009). "Targeted Disruption of the Multidrug and Toxin Extrusion 1 (Mate1) Gene in Mice Reduces Renal Secretion of Metformin." Molecular Pharmacology **75**(6): 1280-1286.
Tucker, G. T., C. Casey, et al. (1981). "Metformin kinetics in healthy subjects and in patients with diabetes mellitus." British Journal of Clinical Pharmacology **12**(2): 235-246.
Tymms, D. J. and B. A. Leatherdale (1988). "Lactic acidosis due to metformin therapy in a low risk patient." Postgraduate Medical Journal **64**(749): 230-231.
Urakami, Y., N. Kimura, et al. (2004). "Creatinine Transport by Basolateral Organic Cation Transporter hOCT2 in the Human Kidney." Pharmaceutical Research **21**(6): 976-981.
Urakami, Y., N. Kimura, et al. (2005). "Transcellular Transport of Creatinine in Renal Tubular Epithelial Cell Line LLC-PK₁." Drug Metabolism and Pharmacokinetics **20**(3): 200-205.
Watanabe, T., H. Kusuhara, et al. (2009). "Physiologically Based Pharmacokinetic Modeling to Predict Transporter-Mediated Clearance and Distribution of Pravastatin in Humans." Journal of Pharmacology and Experimental Therapeutics **328**(2): 652-662.
Wilcock, C., N. D. Wyre, et al. (1991). "Subcellular distribution of metformin in rat liver." Journal of Pharmacy and Pharmacology **43**(6): 442-444.
Yonezawa, A. and K.-i. Inui (2011). "Organic cation transporter OCT/SLC22A and H+/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents." Biochemical Pharmacology **81**(5): 563-568.
Yonezawa, A., S. Masuda, et al. (2005). "Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat." Biochemical Pharmacology **70**(12): 1823-1831.
Zamek-Gliszczynski, M. J., C. A. Lee, et al. (2013). "ITC Recommendations for Transporter Kinetic Parameter Estimation and Translational Modeling of Transport-Mediated PK and DDIs in Humans." Clinical pharmacology and therapeutics **94**(1): 64-79.
Zhao, P., M. d. L. T. Vieira, et al. (2012). "Evaluation of Exposure Change of Nonrenally Eliminated Drugs in Patients With Chronic Kidney Disease Using Physiologically Based Pharmacokinetic Modeling and Simulation." The Journal of Clinical Pharmacology **52**(S1): 91S-108S.
Zolk, O. (2011). "Disposition of metformin: Variability due to polymorphisms of organic cation transporters." Annals of Medicine **44**(2): 119-129.

Chapter 5: General Discussion and Conclusion

5.1 Discussion

An innovation in the health sciences and drug development is a valuable investment since it increases the ability to treat diseases and improve the quality of life. The discovery and development of new drug substances is a very lengthy and costly process (Di Maio and Carrier 2011) Figure 5.1. Application of computational technology during drug discovery and development has the potential to decrease development time and save resources. The (ACAT) and (PBPK) modeling have become essential parts of such *in silico* tools. They can account for the main aspects of drug absorption like the physicochemical drug properties and physiological factors, to yield more robust and successful simulations (Bergström, Holm et al. 2014). Previously, the usage of drug modeling was limited to predict drug exposure or focusing on toxicological aspects and safety with risk assessment studies (Jones, Dickins et al. 2011). Currently, the usage of computational tools is rapidly growing to become part of almost every stage in the drug discovery and development process.

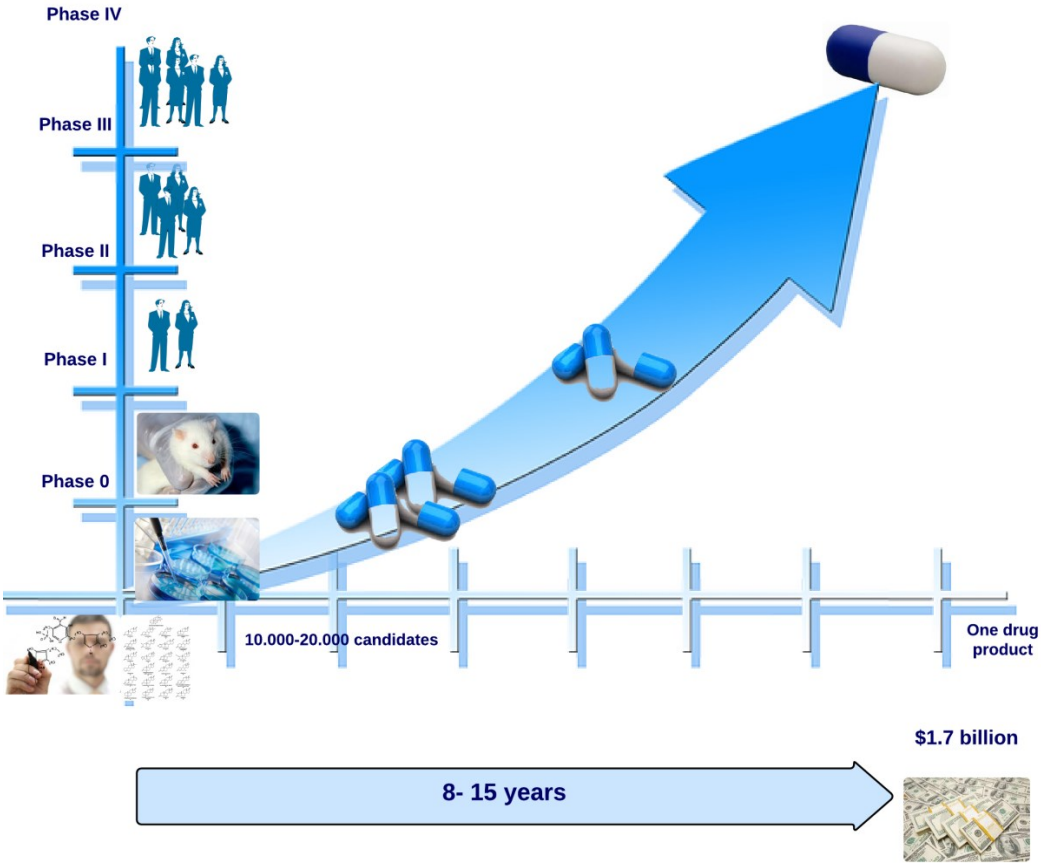


Figure 5.1: Drug discovery and development: a lengthy and costly process

In the discovery stage, modeling approaches provide descriptor properties e.g. predict solubility, permeability, and absorption (Valsami and Macheras 2011; Almukainzi, Okumu et al. 2015). These parameters can be further applied to classify drugs according to the BCSC and BDDCS using appropriate software approaches (Waldmann, Almukainzi et al. 2012). Moreover, in this stage drug modeling can predict animal PK studies prior to conducting actual human studies. This can help to prioritize compounds for clinical studies and guide clinical studies strategies with the aim to reduce study time and resources. Many pharmaceutical companies have also adopted this approach in building better formulation strategies and avoiding reformulation

(Kesisoglou 2014). For example, the impact of excipients on dosage form and the proper *in vitro* method to evaluate dissolution rate and solubility can be estimated at early stages of drug development (Bergström, Holm et al. 2014; Almukainzi, Okumu et al. 2015). In a book chapter that was published in 2014 and titled by (Biorelevant Dissolution Testing), I have reviewed most common dissolution test methods that can be used for this purpose (Almukainzi, Bou-Chacra et al. 2014). The chapter discussed in detail the dissolution testing and types of apparatus commonly used to set biorelevant product specifications. These specifications differ according to the dosage forms used. For example, orally disintegrating tablets (ODT) are one of the dosage forms that are not recommended to be evaluated using conventional dissolution testing. This is because these tests do not reflect the *in vivo* condition; I have discussed this novel dosage form in more depth in another book chapter (Orally disintegrating dosage forms, in press).

The early stage of drug discovery and development relies mainly on the use of various physicochemical properties extracted from *in silico* data using molecular structures and/or from *in vitro* data. The values of these measurements are usually used to categorize drug substances into a high or low solubility or permeability class (Bergström, Holm et al. 2014). In later stages of the drug development, the determination of accuracy of these values may be increased to build robust *in vitro* models and estimate *in vivo* PK parameters. Therefore, the information from *in vitro* experiments and preclinical species can be used as input parameters into *in silico* modeling. Additionally, the impact of other factors that are related to GIT physiology, such as solubility under fasted and fed conditions, or individual drug properties can be explored using these tool (Jones, Dickins et al. 2011; Bergström, Holm et al. 2014; Jones, Chen et al. 2015). In the post-marketing stage, drug modelling can be used to grant biowaivers using the *in vitro–in vivo* correlations (IVIVC) approaches. *In vitro– in vivo* extrapolation (IVIVE) approaches can be

performed to predict hepatic metabolism, tissue distribution, drug PK, and pharmacodynamics (PD) simulation in special populations (Lukacova, Woltosz et al. 2009; Jones, Dickins et al. 2011; R. Das, P. Gangwal et al. 2014). The importance of utilizing computer simulations in combination with these dissolution protocols to establish meaningful (IVIVC) in modern product development was also discussed with examples in my book chapter (Almukainzi, Bou-Chacra et al. 2014).

Successful applications of drug modeling can reduce the number of experimental procedures required in the drug development cycle. The drug modeling during drug discovery and development can be built on two approaches: Top down approach and bottom up approach (Tsamandouras, Rostami-Hodjegan et al. 2015). In the early stages of drug discovery only limited data is available about the drug; hence, researchers collect the pieces of the available information together with a broader understanding of the human body to build a bottom-up approach (Figure 5.2). On the other hand, in in the later stages of drug development more observed clinical data are available for a top down approach (Tsamandouras, Rostami-Hodjegan et al. 2015). Over 90% of drug candidates that are taken through the drug development cycle fail to reach the market, and many of these failures were related to the performance of the drug candidates in subgroup populations and not in an 'average' person (Jamei, Marciniak et al. 2009). Therefore, efforts to identify disease variables that can induce changes in the performance of the drug absorption in early stages is critical. This can help in optimizing molecular features to get the desired performance in diseases conditions. Normally, lack of the information in disease states is related to the physiological changes induced by the investigated condition. Computational models greatly help in combining these pieces of information to permit predicting and describing as much of the behavioral and physiological evidences as possible (Bergström,

Holm et al. 2014). However, one of the most challenging things when it comes to oral drug absorption is the fact that the GIT is not a static environment, instead, the physiological states alter differently between each disease condition (Bergström, Holm et al. 2014). The changes in drug behavior induced by disease conditions can be related to many known and unknown mechanisms. Other 'average individual' data values cannot explain the observation in disease states and special populations. Optimization (fitting) parameters based on special considerations can help in such cases. This can be done based upon *in vivo* data and subsequently through multiple runs and adjusting the physiological parameters values to match the tissue concentration/ time profile of the investigated drug (Theil, Guentert et al. 2003). A good fit to plasma concentration profiles is not necessarily sufficient to build a conclusion, so each estimated parameter should be checked carefully to ensure they are within the physiological acceptable range. Therefore, in order to rationalize the proposed model, it is recommended firstly to evaluate the model's predictive performance in a control group, then assess if the optimized parameters will work for disease conditions and are physiologically plausible (Tsamandouras, Rostami-Hodjegan et al. 2015). As presented in Chapters 2, 3 and 4, one way to control the uncertainty in modeling is to fix most of the known values from previous *in vitro* or/and *in vivo* experimental parameters, then optimize only a few anticipated unknown model parameters at a time (Peters and Hultin 2008; Peters, Ungell et al. 2009). This can be achieved either by a trial and error visual calibration to the observed concentration profiles or by built-in statistical approaches such as regression, non-linear least squares and maximum likelihood methods (Tsamandouras, Rostami-Hodjegan et al. 2015). Following such approaches, we were able to mechanistically investigate the influence of pain, RYFB, and renal impairment conditions on meloxicam, ibuprofen and metformin PK.

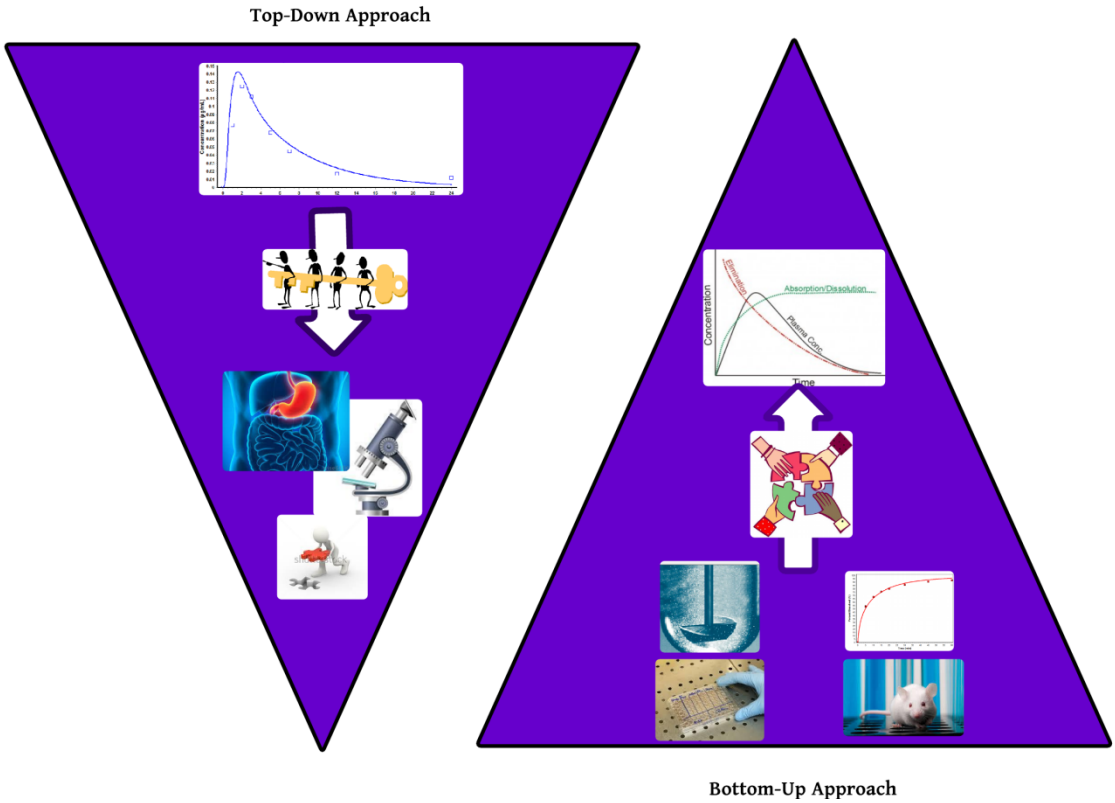


Figure 5.2: Top-down vs bottom up approaches

In chapter 2, the most common NSAIDs that are administered to treat pain, ibuprofen and meloxicam, were used as model drugs to build a pain model. Both drugs belong to BCS class II with a low solubility profile. To model pain episode effects on drug absorption we assumed a delayed gastric emptying rate as the main factor of the observed drug behavior under pain. The software was able to capture the drug absorption in control as well as in the pain condition. Our study found that in a pain episode, the stomach behaves like a reservoir and controls the release of the drug; the dosage form stays in the stomach and released the drug like a control release tablet would do it. The study emphasized the fact that the drug nature and formulation factors are the key elements to determine the magnitude of this retention effect in pain episodes. Generally speaking, NSAIDs have a low solubility at low pH in the stomach; therefore, physiology is a solubility-limiting step in the absorption of these drugs. A controlled release pharmaceutical

formulation can practically overcome the stomach's control induced by pain (Jamali and Aghazadeh-Habashi 2008; Cattaneo and Clementi 2010; Moore, Derry et al. 2014). The simulation of the pain condition confirms that a fast dissolving formulation was not affected that much by the delay in gastric motility induced by pain. This finding was confirmed by multiple clinical studies in patients under pain. Moore et al. has published a detailed review of 30 studies in 787 subjects and 11 unpublished clinical trial reports in 228 subjects about the formulation effect on ibuprofen absorption under pain (Moore, Derry et al. 2014). Interestingly, in each case of these studies the absorption of fast-acting ibuprofen formulations was significantly superior. Using a 'bottom-up' modelling approach, our software was able to predict the *in vivo* drug dissolution from the plasma drug concentration curve. The *in vivo* prediction in our study was very representative to the clinical studies. In pain, the fast formulation release of ibuprofen reached the maximum in about 20- 50 min after dosing; whereas the regular formulation reached the maximum after almost an hour.(Moore, Derry et al. 2014). Using a fast acting formulation in pain episodes offers other valuable advantages. Studies found that the efficacy of a 200-mg fast-acting ibuprofen dose was equal to the efficacy of the double dose given as standard ibuprofen formulation in patients under pain (Moore, Derry et al. 2014). This means that using such formulations in pain episodes can reduce the side effects of the administered drug. Using a fast dissolving formulation showed not only fast onset of action, but also better pain relief over the duration of 6hr (Derry, Derry et al. 2009). This can be explained by the predicted *in vivo* dissolution profile using Gastroplus^{TM}. The software shows a complete dissolution of the fast acting formulation, thus better absorption and pain reduction throughout the length of action. In contrast, the regular formulation did not yield a sufficient dissolution to create the full pharmacological action. Therefore, using computer simulations especially in early stages of drug

development can have the potential not only to predict the performance of NSAI drugs in pain episodes, but also to develop an optimized drug formulation to increase the pharmacological action in patients.

In Chapter 3, another example of the application of drug modeling in disease condition was presented. In this study, we evaluated the use of computer modeling and simulations to explain the observed metformin absorption behavior in patients after gastric bypass surgery. As was discussed, the basic outlining concepts of the drugs absorption after RYGB are not sufficiently identified. In normal subjects, metformin has limited permeability, so the absorption is the rate-limiting step, thus, the drug undergoes flip flop phenomena (Pentikainen, Neuvonen et al. 1979). The absorption after gut shortening by surgery was theoretically expected to be decreased; surprisingly, the observed data showed the total opposite. Another speculation was that metformin as a very basic drug might precipitate out in the small intestine fluids once the drug dissolved in the stomach. Therefore, the increase in stomach pH induced by the surgery can worsen the drug dissolution and absorption, which was not the case. Thus, the lack of understanding of the drug absorption after RYGB emphasizes the importance of using other tools to predict and understand the mechanism of the drug absorption in these patients. Metformin was a very interesting model drug to use in such conditions. Metformin is almost completely ionized at the physiological pH; therefore, the dug has a low permeability. Metformin is absorbed primarily by a paracellular route in which the drug molecule crosses the enterocyte by passing through the tight water-filled junctions between the cells (Proctor, Bourdet et al. 2008). This is possible because it is a small hydrophilic molecule with a molecular weight [MW] =129.16364 g/mol. Our simulation was only successful in predicting the observed data if the paracellular permeability was increased. Increasing active transporters in the gut could not simulate the

observed data. The amount of the absorbed drug was increased post-surgery, and only the increase in the permeability explained the observed data. As discussed earlier, the absorption rate in the ACAT model in Gastroplus™ depends on the effective permeability of the drug (transcellular and/or paracellular) and a physiological Absorption Scale Factor (ASF) for each compartment. Using optimization features, we were able to confirm our conclusion since the ACAT model of the software accounts for changes in surface area to volume ratio along the GI tract, changes in regional permeability due to changes in pH, and changes in paracellular pore size and porosity. The (BCS) and BDDCS classification also supports our finding. As discussed earlier, metformin is a BCS and BDDCS class 3 i.e. low permeable, high soluble and poorly metabolized drug. Therefore, the main factor that would significantly increase the drug bioavailability in these patients is the increase in drug permeability due to the adaptation mechanism that was also seen in other kind of surgeries. Metformin induced lactic acidosis is rare event as it appears in 3 per 100 000 patient, but this side effect can be fatal with a mortality up to 50% (Toyama, Yonezawa et al. 2012) (Misbin, Green et al. 1998). Understanding factors that may lead to increased bioavailability and aggravate the risk of this side effect is critical to avoid unwanted side effects in these patients.

Chapter 4 discussed another disease case that can increase metformin concentrations, which is kidney dysfunction. The PK of metformin in experimental studies showed a significant difference in metformin clearance between control and renal failure patients', when central and deep compartments were investigated. The experimental study showed that the time of peak extraction rate in a control group was 2.67 hr compared to 6hr in a renal failure group; however, the study did not show a significant differences in the terminal half-life of metformin in plasma and blood (Sambol, Chiang et al. 1995). This could be due to the short period of the time in

which data were collected and the sensitivity of the analysis. The study collected data points up to 72 hr. A prolonged collection period may show a slow-down in plasma concentration decline since the drug concentration was much higher in the plasma and blood of these patients. This is especially true with the down-regulation in the kidney and liver transporters, which leads to drug saturation. The same observation was documented for procainamide, which shares some characteristics with metformin. Both drugs are excreted mainly by the kidney GF and active tubular secretion. Both are a substrates for the influx OCT2 and efflux MATE1 transporters (Connolly Sj Fau - Kates and Kates 1982; Yonezawa and Inui 2011). Procainamide showed much longer terminal half-life (8.5 hr) when 24 hour data points were collected compared to 12 hour data points (3.5 hr) in heathy subjects (Jamali, Alballa Rs Fau - Mehvar et al. 1988). The anticipated pathways most likely to be responsible for altering drug elimination in kidney failure patients are transporters. The simulation showed that liver transporters would also be affected in renal kidney disease, which makes the drug a particular risk due to accumulation in patients with kidney disease. Studies have shown that kidney failure can influence non-kidney clearance of drugs by altering metabolic enzymes, transport proteins, or both (Sun, Huang et al. 2004; Lalande, Charpiat et al. 2014). Our model predicts an alteration in the liver MATE1 transporter in these patients. Consequently, polymorphism in this transporter in renal failure patients is assumed to increase the toxicity of metformin (Tsuda, Terada et al. 2009; Zolk 2011; Toyama, Yonezawa et al. 2012). Therefore, practitioners need to be alerted to the importance of such effects. Specific evaluation of pharmacogenomics variation in renal failure is highly suggested for these patients.

Uremic toxins in renal failure are assumed to be the main cause of the alteration in drug efflux of metformin (Lalande, Charpiat et al. 2014). This has led us to design an *in vitro* experiment to

define this mechanism more precisely. This promises to be a step forward in the understanding the observed data and validates or improves the quality of our model. Our result made us exclude creatinine as a toxin that induced a down regulation in the MATE1 transporter in renal failure patients. However, other toxins could be the reasons behind this mechanism as discussed in Chapter 4. Sun et al. (2006) discussed the alterations in drug transporters in the kidney, liver, and intestine in patients with renal failure (Sun, Frassetto et al. 2006). He presented the potential mechanisms and the contributions of uremic-toxin–induced alterations in drug transporters, which eventually affected the drug clearance in patients with renal failure. When no clinical data are available, computer simulations and the BDDCS classification can be very helpful. Researchers applied PBPK based software modeling to predict drug absorption in renal dysfunction conditions. Using the top-down approach, Rowland et al. evaluated the ability of such modelling to predict the PK of paroxetine, diltiazem, and repaglinide, which are eliminated predominantly by non-renal routes, in renal failure patients using Simcyp^{TM} software (Rowland Yeo, Aarabi et al. 2011). The study shows the capability to build IVIVE to predict the PK of these drugs in patients with renal impairment. GastroPlus^{TM} using a PBPK model was also used to predict systemic exposure of two statin drugs, atorvastatin acid and rosuvastatin, following an oral administration in kidney transplant recipients with diabetes mellitus (Macwan 2013). In that study the *in vitro* Km and Vmax values of metabolic clearance of the drugs were determined *in vitro* using diabetic human liver microsomal cells. The values were used as an input to build the disease model. The proposed disease model was adequately able to define the observed mean plasma concentration-time curves for both statins and their metabolites in these patients (Macwan 2013). Validation of such models and using additional *in vitro* research to understand

physiological changes induced by kidney failure are required to refine the performance of these models.

BCS have been clearly recognized and appreciated by regulatory agencies (EMA 2010; FDA 2014). The ACAT based *in silico* modeling is utilized by the FDA, EMA and the Ministry of Health, Labour, and Welfare in Japan (MHLW) in decision making to grant waivers of *in vivo* BE studies for new and generic drugs that fulfill the criteria for high solubility and permeability. BCS enables the use of *in vitro* solubility and permeability data to waive expensive BE for high solubility-high permeability (Class I) drugs. The FDA Guidance recommends possible methods not involving human subjects including *in vivo* or *in situ* intestinal perfusion in a suitable animal model; therefore, *in silico* modellings are considered a perfect substitution for this purpose. The regulatory agencies have also used such modelling and simulations in their guidelines to determine for need of conducting specific clinical pharmacology studies and recommending specific study designs (Jones, Chen et al. 2015). The FDA has reviewed numerous investigational new drug (IND) and new drug application (NDA) submissions containing PBPK modeling and simulations conducted by the sponsors, which should include applications in sub populations (Zhao, Zhang et al. 2011). Specially, GastroPlus™ was used to support research by several divisions in the FDA such as the Office of Generic Drugs, the Center for Food Safety and Applied Nutrition, and the Center for Veterinary Medicine. Several licenses of GastroPlus™ are available for FDA scientists in the Office of Clinical Pharmacology (OCP). This division is responsible for the analysis of clinical data, not only in healthy individuals, but also in patients groups.

As discussed above, there are many different valuable applications of using computer modelling especially in disease conditions. However, there are still some challenges in using

such tools. Our incomplete understanding of the correlation between different diseases can be the main issue in drug modeling (Lalande, Charpiat et al. 2014). For example, when there are multiple diseases in a person, such as obesity with diabetes or renal failure with sepsis; there is an intrinsic connection between the influences of these diseases on the drug absorption. Therefore, one of the diseases can influence enzyme and transporter activity independently of whether another disease is present or not; whereas other diseases might interplay with each other or may also induce compensatory effects (Lalande, Charpiat et al. 2014). Another challenge is that some factors may be ignored when building these models. For instance, an important consideration is the influence of circadian rhythm on GI transit. At night time a relatively longer gastric residence time is observed compared to the day time (Baraldo 2008). However, given the ethics restrictions, time consumed, resources needed to conduct a full PK study in such patients, drug modelling is still recommended (Lalande, Charpiat et al. 2014).

Modeling and simulation of oral drug absorption has increasingly been used in drug discovery, development, and regulation decision-making because of their capabilities for data integration and their superior predictive power (Jiang et al., 2011; Rowland et al., 2011; Zhao et al., 2011). This dissertation showed some examples of using these tools to provide insights into drug absorption under disease conditions. Utilizing both the drug properties and the body's physiology parameters in computer simulation can give more in-depth knowledge of drug absorption mechanisms in the disease of interest. This approach can be clinically useful for studying oral drug performance in other conditions such as depression, arrhythmias, infections, convulsion, hypertension, diabetes, and hyperlipidemia which can influence drug absorption even for drugs that are not intended to treat these conditions (Smith, Henriksen et al. 2011). Drug researchers expect that by 2020, computer-based technologies will be used extensively to create

a greater understanding of the biology of diseases, allowing the evolution of a 'Virtual Man' to enhance predictions of the effects of new drug candidates before they are administered to humans (Jamei, Marciniak et al. 2009). This can help the pharmaceutical industry in speeding up drug development cycles and reducing cost (Valsami and Macheras 2011). Understanding and rationalizing the physiological variables that can influence drug absorption in disease conditions can help to increase pharmaceutical product performance. Evaluating these models is essential in ensuring the pre-defined quality by design (QbD) characters in drugs development. Modeling and simulation efforts promote the development of the QbD approach and encourage innovation in drug development and regulatory sciences.

5.2 Conclusion

In this dissertation, we showed the feasibility to develop mechanistically sound models using 'bottom up' modelling strategies that are based on clinical relevance to simulate pain, gastric bypass, and renal failure effects on drug absorption. A gastric modified drug release model is able to predict the PK of meloxicam and ibuprofen under pain conditions. The computer model indicated significant differences in the *in vivo* dissolution of meloxicam and ibuprofen formulations in the pain condition. The formulation differences, which were clinically not relevant in healthy subjects, became relevant because of the changed motility pattern of the stomach. In pain conditions the stomach tightly controls drug release, which is different to normal conditions. The different formulations result in significantly different *in vivo* dissolution. The stomach then controls the release of the dissolved drug into the small intestine.

The studies in this thesis have shown the ability of PBPK models to simulate metformin absorption in control, patients after RYBG surgery, and under renal dysfunction conditions. These disease modeling has the potential to unveil the mechanistic background of the physiological changes, which impact metformin absorption under these disease conditions. The RYBG surgery model showed that adaptation in the paracellular permeability explained the absorption of metformin after surgery. The renal dysfunction model showed that inhibition of MATE1 transporter in the kidney and liver was the reason for the increase in drug concentration in renal failure patients. However, the *in vitro* study showed that the high level of creatinine in renal failure patients had no effect on metformin excretion by this transporter in such patients.

Computer based disease modeling can be used to mechanistically understand the changes in drug absorption induced by disease conditions. Integration the prior knowledge about the

disease and drugs characteristics is very important to fill the knowledge gap of the influence of the disease on drug performance. The insights gained by this study can be used to help to predict the absorption and the clinical outcomes of other drugs that share similar physiochemical characters as these drugs under similar conditions. This can enable to design drug products according to quality by design approaches. For researchers, regulatory agency and pharmaceutical industries, the quantitative prediction of the impact of disease conditions on drug absorption is very important not only for the optimal design and dosage adjustment, but also to have maximum benefits and avoid possible side effects. This will ultimately improve drug performance and therapeutic out comes. Computer simulations can assist health care professionals and pharmaceutical industry to utilize and develop better drug products and apply them in precision medicine.

5.3 Future direction:

In this thesis, metformin advantages over other anti-diabetics drugs were discussed. The presented work showed that the significant increase in metformin bioavailability in renal failure patients was due to the down regulation of the drug transporter in these patients. Moreover, creatinine has no influence on metformin excretion through the MATE1 transporter. However, this effect could be due to other uremic toxins such as IS. Investigating of this theory can provide an explanation for this observation. If this was the case, the next step would be providing a solution for this problem. In the market, AST-120, a spherical adsorptive carbon preparation, that absorbs IS uremic toxins is available. Administration of this product to renal failure patients could prevent the accumulation of uremic toxins and prevent down-regulation of the metformin transporter. However, this agent needs to be evaluated in term of its interaction with metformin

Pharmaceutical preparations can provide another way to tolerate metformin in renal patients. A specific dosage form designed for to these patient could optimize therapy, particularly, gastric floating control release dosage forms that can be retained in the stomach for several hours and release the drug content in a predefined controlled time (Arora, Ali et al. 2005). Therefore, such dosage forms could overcome the saturation of metformin at the transporter; hence, the drug can be excreted regularly and maintain the pharmacological concentration without causing toxicity. These dosage forms need to be evaluated *in vitro* by evaluating formulation parameter such as the floating duration, dissolution, profiles, content uniformity, hardness, and friability in the case of solid dosage forms. Moreover, the *in vivo* assessment of their release, PK and PD would also be required.

5.4 References:

Almukainzi, M., A. Okumu, et al. (2015). "Simulation of In Vitro Dissolution Behavior Using DDDPlus™." AAPS PharmSciTech **16**(1): 217-221.

Almukainzi, M., N. A. Bou-Chacra, et al. (2014). Biorelevant Dissolution Testing. Therapeutic Delivery Solutions, John Wiley & Sons, Inc.: 335-365.

Arora, S., J. Ali, et al. (2005). "Floating drug delivery systems: A review." AAPS PharmSciTech 6(3): E372-E390.

Baraldo, M. (2008). "The influence of circadian rhythms on the kinetics of drugs in humans." Expert Opinion on Drug Metabolism & Toxicology **4**(2): 175-192.

Bergström, C. A. S., R. Holm, et al. (2014). "Early pharmaceutical profiling to predict oral drug absorption: Current status and unmet needs." European Journal of Pharmaceutical Sciences **57**(1): 173-199.

Cattaneo, D. and E. Clementi (2010). "Clinical pharmacokinetics of ibuprofen arginine." (2212-3938 (Electronic)).

Chow, E. C. Y. and K. S. Pang (2013). "Why we need proper PBPK models to examine intestine and liver oral drug absorption." Current Drug Metabolism **14**(1): 57-79.

Connolly Sj Fau - Kates, R. E. and R. E. Kates (1982). "Clinical pharmacokinetics of N-acetylprocainamide." (0312-5963 (Print)).

Derry, C. J., S. Derry, et al. (2009). "Single dose oral ibuprofen for acute postoperative pain in adults." The Cochrane database of systematic reviews(3): CD001548-CD001548.

Di Maio, S. and R. L. Carrier (2011). "Gastrointestinal contents in fasted state and post-lipid ingestion: In vivo measurements and in vitro models for studying oral drug delivery." Journal of Controlled Release **151**(2): 110-122.

Jamali, F. and A. Aghazadeh-Habashi (2008). "Rapidly dissolving formulations for quick absorption during pain episodes: Ibuprofen." International Journal of Clinical Pharmacology and Therapeutics **46**(2): 55-63.

Jamali, F., R. Alballa Rs Fau - Mehvar, et al. (1988). "Longer plasma half-life for procainamide utilizing a very sensitive high performance liquid chromatography assay." (0163-4356 (Print)).

Jamei, M., S. Marciniak, et al. (2009). "The Simcyp® Population-based ADME Simulator." Expert Opinion on Drug Metabolism & Toxicology **5**(2): 211-223.

Jones, H. M., Y. Chen, et al. (2015). "Physiologically based pharmacokinetic modeling in drug discovery and development: A pharmaceutical industry perspective." Clinical Pharmacology & Therapeutics **97**(3): 247-262.

Jones, H. M., M. Dickins, et al. (2011). "Application of PBPK modelling in drug discovery and development at Pfizer." Xenobiotica **42**(1): 94-106.

Jones, H. M., I. B. Gardner, et al. (2009). "Modelling and PBPK simulation in drug discovery." AAPS Journal **11**(1): 155-166.

Kesisoglou, F. (2014). "Use of preclinical dog studies and absorption modeling to facilitate late stage formulation bridging for a BCS II drug candidate." AAPS PharmSciTech **15**(1): 20-28.

Lalande, L., B. Charpiat, et al. (2014). "Consequences of Renal Failure on Non-Renal Clearance of Drugs." Clinical Pharmacokinetics **53**(6): 521-532.

Lukacova, V., W. Woltosz, et al. (2009). "Prediction of Modified Release Pharmacokinetics and Pharmacodynamics from In Vitro, Immediate Release, and Intravenous Data." The AAPS Journal **11**(2): 323-334.
Macwan, J. (2013). "Model-Based Approaches to Characterize Clinical Pharmacokinetics of Atorvastatin and Rosuvastatin in Disease State." Open Access Dissertations: 28.
Misbin, R. I., L. Green, et al. (1998). "Lactic Acidosis in Patients with Diabetes Treated with Metformin." New England Journal of Medicine **338**(4): 265-266.
Moore, R. A., S. Derry, et al. (2014). "Faster, higher, stronger? Evidence for formulation and efficacy for ibuprofen in acute pain." Pain **155**(1): 14-21.
Paixão, P., L. F. Gouveia, et al. (2012). "Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model." International Journal of Pharmaceutics **429**(1-2): 84-98.
Pentikainen, P. J., P. J. Neuvonen, et al. (1979). "Pharmacokinetics of metformin after intravenous and oral administration to man." European Journal of Clinical Pharmacology **16**(3): 195-202.
Peters, S. A. and L. Hultin (2008). "Early identification of drug-induced impairment of gastric emptying through physiologically based pharmacokinetic (PBPK) simulation of plasma concentration-time profiles in rat." Journal of pharmacokinetics and pharmacodynamics **35**(1): 1-30.
Peters, S. A., A. L. Ungell, et al. (2009). "Physiologically based pharmacokinetic (PBPK) modeling and simulation: applications in lead optimization." Current opinion in drug discovery & development **12**(4): 509-518.
Proctor, W. R., D. L. Bourdet, et al. (2008). "Mechanisms Underlying Saturable Intestinal Absorption of Metformin." Drug Metabolism and Disposition **36**(8): 1650-1658.
R. Das, N., R. P. Gangwal, et al. (2014). "A PPAR-β/δ Agonist is Neuroprotective and Decreases Cognitive Impairment in a Rodent Model of Parkinson’s Disease." Current Neurovascular Research **11**(2): 114-124.
Rowland Yeo, K., M. Aarabi, et al. (2011). "Modeling and predicting drug pharmacokinetics in patients with renal impairment." Expert Review of Clinical Pharmacology **4**(2): 261-274.
Sambol, N. C., J. Chiang, et al. (1995). "Kidney Function and Age Are Both Predictors of Pharmacokinetics of Metformin." The Journal of Clinical Pharmacology **35**(11): 1094-1102.
Smith, A., B. Henriksen, et al. (2011). "Pharmacokinetic considerations in Roux-en-Y gastric bypass patients." American Journal of Health-System Pharmacy **68**(23): 2241-2247.
Sun, H., L. Frassetto, et al. (2006). "Effects of renal failure on drug transport and metabolism." Pharmacology & Therapeutics **109**(1–2): 1-11.
Sun, H., Y. Huang, et al. (2004). "EFFECTS OF UREMIC TOXINS ON HEPATIC UPTAKE AND METABOLISM OF ERYTHROMYCIN." Drug Metabolism and Disposition **32**(11): 1239-1246.
Theil, F.-P., T. W. Guentert, et al. (2003). "Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection." Toxicology letters **138**(1–2): 29-49.
Toyama, K., A. Yonezawa, et al. (2012). "Loss of multidrug and toxin extrusion 1 (MATE1) is associated with metformin-induced lactic acidosis." British Journal of Pharmacology **166**(3): 1183-1191.

Tsamandouras, N., A. Rostami-Hodjegan, et al. (2015). "Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: Fitting PBPK models to observed clinical data." British Journal of Clinical Pharmacology **79**(1): 48-55.

Tsuda, M., T. Terada, et al. (2009). "Targeted Disruption of the Multidrug and Toxin Extrusion 1 (Mate1) Gene in Mice Reduces Renal Secretion of Metformin." Molecular Pharmacology **75**(6): 1280-1286.

Valsami, G. and P. Macheras (2011). "Computational-regulatory developments in the rediction of oral drug absorption." Molecular Informatics **30**(2-3): 112-121.

Waldmann, S., M. Almukainzi, et al. (2012). "Provisional Biopharmaceutical Classification of Some Common Herbs Used in Western Medicine." Molecular Pharmaceutics **9**(4): 815-822.

Yonezawa, A. and K.-i. Inui (2011). "Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics." British Journal of Pharmacology **164**(7): 1817-1825.

Zhao, P., L. Zhang, et al. (2011). "Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review." Clinical pharmacology and therapeutics **89**(2): 259-267.

Zolk, O. (2011). "Disposition of metformin: Variability due to polymorphisms of organic cation transporters." Annals of Medici

Biblography:

Abuasal, B. S., M. B. Bolger, et al. (2012). "In silico modeling for the nonlinear absorption kinetics of UK-343,664: A P-gp and CYP3A4 substrate." Molecular Pharmaceutics **9**(3): 492-504.

Aghazadeh-Habashi, A. and F. Jamali (2008). "Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: Influence of gastrointestinal dysfunction on the relative bioavailability of two formulations." European Journal of Pharmaceutics and Biopharmaceutics **70**(3): 889-894.

Agoram, B., W. S. Woltosz, et al. (2001). "Predicting the impact of physiological and biochemical processes on oral drug bioavailability." Advanced Drug Delivery Reviews 50, Supplement 1(0): S41-S67.

Aiba, T., M. M. Tse, et al. (1999). "Effect of dosage form on stereoisomeric inversion of ibuprofen in volunteers." Biological and Pharmaceutical Bulletin **22**(6): 616-622.

Ali, S. K., K (2014). "A Recent Advancement in Orally Disintegrating Formulations." American Pharmaceutical review **17**(4): 6.

Almukainzi, M. (2011). Evaluation of performance testing and computer simulations for quality by design approaches of oral dosage forms. Master Evaluation of performance testing and computer simulations for quality by design approaches of oral dosage forms

University of alberta.Almukainzi, M., N. A. Bou-Chacra, et al. (2014). Biorelevant Dissolution Testing. Therapeutic Delivery Solutions, John Wiley & Sons, Inc.**:** 335-365.

Almukainzi M, L. V., Löbenberg R (2014). "Modelling the Absorption of Metformin with Patients Post Gastric Bypass Surgery." Diabetes Metab **5**: 353.

Almukainzi, M., A. Okumu, et al. (2014). "Simulation of In Vitro Dissolution Behavior Using DDDPlus™." AAPS PharmSciTech: 1-5.

Amidon, G., H. Lennernäs, et al. (1995). "A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability." Pharmaceutical Research **12**(3): 413-420.

Bar-Natan, M., G. M. Larson, et al. (1996). "Delayed gastric emptying after gastric surgery." The American Journal of Surgery **172**(1): 24-28.

Baraldo, M. (2008). "The influence of circadian rhythms on the kinetics of drugs in humans." Expert Opinion on Drug Metabolism & Toxicology **4**(2): 175-192.

Barry, R. E., J. Barisch, et al. (1977). "Intestinal adaptation after jejunoileal bypass in man." The American Journal of Clinical Nutrition **30**(1): 32-42.

Bellomo, R., C. Ronco, et al. (2004). "Acute renal failure – definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group." Critical Care **8**(4): R204-R212.

Benet, L. Z. (2010). "Predicting Drug Disposition via Application of a Biopharmaceutics Drug Disposition Classification System." Basic & Clinical Pharmacology & Toxicology **106**(3): 162-167.

Berezhkovskiy, L. M. (2004). "Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination." Journal of Pharmaceutical Sciences **93**(6): 1628-1640.
Bergström, C. A. S., R. Holm, et al. (2014). "Early pharmaceutical profiling to predict oral drug absorption: Current status and unmet needs." European Journal of Pharmaceutical Sciences **57**(1): 173-199.
Bianchetti G Fau - Graziani, G., D. Graziani G Fau - Brancaccio, et al. "Pharmacokinetics and effects of propranolol in terminal uraemic patients and in patients undergoing regular dialysis treatment." (0312-5963 (Print)).
Bolger, M. B., R. Irwin, et al. (2012). "GastroPlus PBPK/PD Model Applied to Estimating Dose for an Elderly Population in an Alzheimer's Disease Clinical Trial."
Bouzom, F., K. Ball, et al. (2012). "Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs?" Biopharmaceutics & drug disposition **33**(2): 55-71.
Buchwald, H. and J. Buchwald (2002). "Evolution of Operative Procedures for the Management of Morbid Obesity 1950-2000." Obesity Surgery **12**(5): 705-717.
Camenisch, G., J. Alsenz, et al. (1998). "Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight." European Journal of Pharmaceutical Sciences **6**(4): 313-319.
Cattaneo, D. and E. Clementi (2010). "Clinical pharmacokinetics of ibuprofen arginine." (2212-3938 (Electronic)).
CDC. (2010). "National Center for Health Statistics (NCHS)."
CDC. (2015). "Chronic Kidney Disease." Retrieved 2014, 2014, from (http://www.cdc.gov/diabetes/pubs/factsheets/kidney.htm.
Chambers, A. P., H. E. Wilson-Perez, et al. (2012). "Effect of vertical sleeve gastrectomy on food selection and satiation in rats." American Journal of Physiology - Endocrinology and Metabolism **303**(8): E1076-E1084.
Chiou, W. and A. Barve (1998). "Linear Correlation of the Fraction of Oral Dose Absorbed of 64 Drugs Between Humans and Rats." Pharmaceutical Research **15**(11): 1792-1795.
Chow, E. C. Y. and K. S. Pang (2013). "Why we need proper PBPK models to examine intestine and liver oral drug absorption." Current Drug Metabolism **14**(1): 57-79.
Christensen, M. M. H., C. Brasch-Andersen, et al. (2011). "The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c." Pharmacogenetics and Genomics **21**(12): 837-850.
Ciarimboli, G., C. S. Lancaster, et al. (2012). "Proximal Tubular Secretion of Creatinine by Organic Cation Transporter OCT2 in Cancer Patients." Clinical Cancer Research **18**(4): 1101-1108.
Clarysse, S., J. Brouwers, et al. (2011). "Intestinal drug solubility estimation based on simulated intestinal fluids: Comparison with solubility in human intestinal fluids." European Journal of Pharmaceutical Sciences **43**(4): 260-269.
Clewell, H. J., M. B. Reddy, et al. (2010). Physiologically Based Pharmacokinetic Modeling. Pharmaceutical Sciences Encyclopedia, John Wiley & Sons, Inc.
Comer, J. and K. Tam (2001). "Lipophilicity profiles: theory and measurement." Testa, B.; van de Waterbeemd, H.; Folkers, G: 275-304.
Connolly Sj Fau - Kates, R. E. and R. E. Kates (1982). "Clinical pharmacokinetics of N-acetylprocainamide." (0312-5963 (Print)).

Crison, J. R., P. Timmins, et al. (2012). "Biowaiver approach for biopharmaceutics classification system class 3 compound metformin hydrochloride using In Silico modeling." Journal of Pharmaceutical Sciences **101**(5): 1773-1782.
Danaei, G., M. M. Finucane, et al. (2011). "National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants." The Lancet **378**(9785): 31-40.
Darwich, A. S., K. Henderson, et al. (2012). "Trends in oral drug bioavailability following bariatric surgery: Examining the variable extent of impact on exposure of different drug classes." British Journal of Clinical Pharmacology **74**(5): 774-787.
Darwich, A. S., D. Pade, et al. (2013). "Evaluation of an In Silico PBPK Post-Bariatric Surgery Model through Simulating Oral Drug Bioavailability of Atorvastatin and Cyclosporine." CPT: pharmacometrics & systems pharmacology **2**(6): 1-9.
Davis, S., A. Stockwell, et al. (1986). "The effect of density on the gastric emptying of single- and multiple-unit dosage forms." Pharmaceutical Research **3**(4): 208-213.
De Buck, S. S., V. K. Sinha, et al. (2007). "Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs." Drug Metabolism and Disposition **35**(10): 1766-1780.
Derry, C. J., S. Derry, et al. (2009). "Single dose oral ibuprofen for acute postoperative pain in adults." The Cochrane database of systematic reviews(3): CD001548-CD001548.
DeSesso, J. M. and C. F. Jacobson (2001). "Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats." Food and Chemical Toxicology **39**(3): 209-228.
Devanarayana, N. M., S. Rajindrajith, et al. (2013). "Ultrasonographic Assessment of Liquid Gastric Emptying and Antral Motility According to the Subtypes of Irritable Bowel Syndrome in Children." Journal of Pediatric Gastroenterology and Nutrition **56**(4): 443-448 410.1097/MPG.1090b1013e31827f31827a31823d.
Di Maio, S. and R. L. Carrier (2011). "Gastrointestinal contents in fasted state and post-lipid ingestion: In vivo measurements and in vitro models for studying oral drug delivery." Journal of Controlled Release **151**(2): 110-122.
Diakidou, A., M. Vertzoni, et al. (2009). "Characterization of the Contents of Ascending Colon to Which Drugs are Exposed After Oral Administration to Healthy Adults." Pharmaceutical Research **26**(9): 2141-2151.
Doldi, S. B. (1991). "Intestinal adaptation following jejuno-ileal bypass." Clinical Nutrition **10**(3): 138-145.
Dowling, T. C. (2002). "Drug Metabolism Considerations in Patients With Chronic Kidney Disease." Journal of Pharmacy Practice **15**(5): 419-427.
Dowling, T. C., J. C. Briglia Ae Fau - Fink, et al. "Characterization of hepatic cytochrome p4503A activity in patients with end-stage renal disease." (0009-9236 (Print)).
Dreisbach, A. W. and J. J. Lertora (2008). "The effect of chronic renal failure on drug metabolism and transport." Expert Opinion on Drug Metabolism & Toxicology **4**(8): 1065-1074.
Dressman, J., R. Berardi, et al. (1990). "Upper Gastrointestinal (GI) pH in Young, Healthy Men and Women." Pharmaceutical Research **7**(7): 756-761.
Dressman, J. B., D. Fleisher, et al. (1984). "Physicochemical model for dose-dependent drug absorption." Journal of Pharmaceutical Sciences **73**(9): 1274-1279.

Edelmann, I. S., Liebmann, J. (1959). " Anatomy of body water and electrolytes." Am. J.

27: 256–277.
Edginton, A. and S. Willmann (2008). "Physiology-Based Simulations of a Pathological Condition." Clinical Pharmacokinetics **47**(11): 743-752.
El-Kattan, A. and M. Varma (2012). "Oral absorption, intestinal metabolism and human oral bioavailability." Topics of Drugs Metabolism: 1-35.
EMA. (2010). " Guideline on the Investigation of Bioequivalence. ." Committee for Medicinal Products for Human Use, European Medicines Agency. Retrieved 2015, 2015.
Enomoto, A. and H. Endou (2005). "Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease." Clinical and Experimental Nephrology **9**(3): 195-205.
FDA. (2000). "Guidance for Industry, Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. ." CDER/FDA., 2015.
FDA. (2010). "Guidance for Industry: Pharmacokinetics in patients with impaired renal function —study design, data analysis, and impact on dosing and labeling. 2010.", 2015.
FDA. (2014). "Program of physiologically-based pharmacokinetic and pharmacodynamic modeling (PBPK Program)." FDA, 2015, from http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm365118.htm.
Fu, Y., S. H. Jeong, et al. (2005). "Fast-melting tablets based on highly plastic granules." Journal of Controlled Release **109**(1–3): 203-210.
Gabr, R. Q., R. S. Padwal, et al. (2010). "Determination of metformin in human plasma and urine by high-performance liquid chromatography using small sample volume and conventional octadecyl silane column." Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques **13**(4): 486-494.
Gaohua, L., K. Abduljalil, et al. (2012). "A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4." British Journal of Clinical Pharmacology **74**(5): 873-885.
Gaohua, L., K. Abduljalil, et al. (2012). "A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4." British Journal of Clinical Pharmacology **74**(5): 873-885.
Ghosal, A. and H. M. Said (2012). "Cys(294) is essential for the function of the human sodium-dependent multivitamin transporter." Biochim Biophys Acta **1818**(1): 97-102.
Goodacre, B. C. and P. J. Murray (1981). "A mathematical model of drug absorption." Journal of Clinical and Hospital Pharmacy **6**(2): 117-133.
Graham, G., J. Punt, et al. (2011). "Clinical Pharmacokinetics of Metformin." Clinical Pharmacokinetics **50**(2): 81-98.
Grass, G. M. (1997). "Simulation models to predict oral drug absorption from in vitro data." Advanced Drug Delivery Reviews **23**(1–3): 199-219.
Greene, J. G., A. R. Noorian, et al. (2009). "Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease." Experimental Neurology **218**(1): 154-161.
Grillo, J. A., P. Zhao, et al. (2012). "Utility of a physiologically–based pharmacokinetic (PBPK) modeling approach to quantitatively predict a complex drug–drug–disease interaction

scenario for rivaroxaban during the drug review process: implications for clinical practice." Biopharmaceutics & Drug Disposition **33**(2): 99-110.
Guevin, C., J. Michaud, et al. (2002). "Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators." Br J Pharmacol **137**(7): 1039-1046.
Hilgendorf, C., G. Ahlin, et al. (2007). "Expression of Thirty-six Drug Transporter Genes in Human Intestine, Liver, Kidney, and Organotypic Cell Lines." Drug Metabolism and Disposition **35**(8): 1333-1340.
Hoang Thi, T. H., S. Morel, et al. (2012). "Development and evaluation of taste-masked drug for paediatric medicines – Application to acetaminophen." International Journal of Pharmaceutics **434**(1–2): 235-242.
Honório, T. d., E. Pinto, et al. (2013). "In Vitro–In Vivo Correlation of Efavirenz Tablets Using GastroPlus®." AAPS PharmSciTech **14**(3): 1244-1254.
Horner, K. M., N. M. Byrne, et al. (2011). "The effects of weight loss strategies on gastric emptying and appetite control." Obesity Reviews **12**(11): 935-951.
Hsu, C.-y., C. E. McCulloch, et al. (2002). "Iron Status and Hemoglobin Level in Chronic Renal Insufficiency." Journal of the American Society of Nephrology **13**(11): 2783-2786.
Huang, W., S. Lee, et al. (2009). "Mechanistic Approaches to Predicting Oral Drug Absorption." The AAPS Journal **11**(2): 217-224.
Ilić, M., J. Đuriš, et al. (2014). "In vitro – in silico – in vivo drug absorption model development based on mechanistic gastrointestinal simulation and artificial neural networks: Nifedipine osmotic release tablets case study." European Journal of Pharmaceutical Sciences **62**(0): 212-218.
ImageJ.
Ito, S., H. Kusuhara, et al. (2012). "Competitive Inhibition of the Luminal Efflux by Multidrug and Toxin Extrusions, but Not Basolateral Uptake by Organic Cation Transporter 2, Is the Likely Mechanism Underlying the Pharmacokinetic Drug-Drug Interactions Caused by Cimetidine in the Kidney." Journal of Pharmacology and Experimental Therapeutics **340**(2): 393-403.
J. Liu, J., J. Lu, et al. (2012). "Membrane Transporters as Determinants of the Pharmacology of Platinum Anticancer Drugs." Current Neuropharmacology **12**(8): 962-986.
Jamali, F. and A. Aghazadeh-Habashi (2008). "Rapidly dissolving formulations for quick absorption during pain episodes: Ibuprofen." International Journal of Clinical Pharmacology and Therapeutics **46**(2): 55-63.
Jamali, F., R. Alballa Rs Fau - Mehvar, et al. (1988). "Longer plasma half-life for procainamide utilizing a very sensitive high performance liquid chromatography assay." (0163-4356 (Print)).
Jamali, F. and C. M. Kunz-Dober (1999). "Pain-mediated altered absorption and metabolism of ibuprofen: An explanation for decreased serum enantiomer concentration after dental surgery." British Journal of Clinical Pharmacology **47**(4): 391-396.
Jamei, M., S. Marciniak, et al. (2009). "The Simcyp® Population-based ADME Simulator." Expert Opinion on Drug Metabolism & Toxicology **5**(2): 211-223.
Jeong, S., S. Kimura, et al. (2006). Fast-melting tablets having taste-masking and sustained release properties, Google Patents.
Ji, L., S. Masuda, et al. (2002). "Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy." Kidney Int **62**(2): 514-524.

Johnson, T. N., A. Rostami-Hodjegan, et al. (2006). "Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children." Clinical Pharmacokinetics **45**(9): 931-956.
Johnson, W. and E. DeMaria (2006). "Surgical treatment of obesity." Curr Treat Options Gastroenterol **9**(2): 167-174.
Jones, H., I. Gardner, et al. (2009). "Modelling and PBPK Simulation in Drug Discovery." The AAPS Journal **11**(1): 155-166.
Jones, H., N. Parrott, et al. (2006). "A Novel Strategy for Physiologically Based Predictions of Human Pharmacokinetics." Clinical Pharmacokinetics **45**(5): 511-542.
Jones, H. M., Y. Chen, et al. (2015). "Physiologically based pharmacokinetic modeling in drug discovery and development: A pharmaceutical industry perspective." Clinical Pharmacology & Therapeutics **97**(3): 247-262.
Jones, H. M., M. Dickins, et al. (2011). "Application of PBPK modelling in drug discovery and development at Pfizer." Xenobiotica **42**(1): 94-106.
Jones, H. M., I. B. Gardner, et al. (2009). "Modelling and PBPK Simulation in Drug Discovery." The AAPS Journal **11**(1): 155-166.
Jones, H. M., K. Mayawala, et al. (2013). "Dose selection based on physiologically based pharmacokinetic (PBPK) approaches." The AAPS Journal **15**(2): 377-387.
Jones, H. M., N. Parrott, et al. (2006). "Predicting Pharmacokinetic Food Effects Using Biorelevant Solubility Media and Physiologically Based Modelling." Clinical Pharmacokinetics **45**(12): 1213-1226.
Kalantzi, L., K. Goumas, et al. (2006). "Characterization of the Human Upper Gastrointestinal Contents Under Conditions Simulating Bioavailability/Bioequivalence Studies." Pharmaceutical Research **23**(1): 165-176.
Kanda, M., M. Matsuhashi, et al. "Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS)." Clinical Neurophysiology **113**(7): 1013-1024.
Kararli, T. T. (1995). "Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals." Biopharmaceutics & drug disposition **16**(5): 351-380.
Kasliwal, N., J. Negi, et al. (2011). "Formulation, development, and performance evaluation of metoclopramide HCl oro-dispersible sustained release tablet." Archives of Pharmacal Research **34**(10): 1691-1700.
Ke, A. B., S. C. Nallani, et al. (2012). "A PBPK Model to Predict Disposition of CYP3A-Metabolized Drugs in Pregnant Women: Verification and Discerning the Site of CYP3A Induction." CPT: pharmacomet. syst. pharmacol. **1**: e3.
Kesisoglou, F. (2014). "Use of preclinical dog studies and absorption modeling to facilitate late stage formulation bridging for a BCS II drug candidate." AAPS PharmSciTech **15**(1): 20-28.
Kim, Y.-G., J.-G. Shin, et al. (1993). "Decreased acetylation of isoniazid in chronic renal failure." Clinical Pharmacology & Therapeutics **54**(6): 612-620.
Kimura, N., S. Masuda, et al. (2005). "Metformin is a Superior Substrate for Renal Organic Cation Transporter OCT2 rather than Hepatic OCT1." Drug Metabolism and Pharmacokinetics **20**(5): 379-386.
Knihinicki, R. D., R. O. Day, et al. (1990). "Stereoselective disposition of ibuprofen and flurbiprofen in rats." Chirality **2**(3): 134-140.

Kohan, L. and R. Hamill-Ruth (2011). "Pain Assessment and Pharmacologic Management." Critical Care Medicine **39**(9): 2209.
Komazawa, H., H. Yamaguchi, et al. (2013). "Renal uptake of substrates for organic anion transporters Oat1 and Oat3 and organic cation transporters Oct1 and Oct2 is altered in rats with adenine-induced chronic renal failure." Journal of Pharmaceutical Sciences **102**(3): 1086-1094.
Kostewicz, E. S., L. Aarons, et al. (2014). "PBPK models for the prediction of in vivo performance of oral dosage forms." European Journal of Pharmaceutical Sciences **57**(0): 300-321.
Kusuhara, H., S. Ito, et al. (2011). "Effects of a MATE Protein Inhibitor, Pyrimethamine, on the Renal Elimination of Metformin at Oral Microdose and at Therapeutic Dose in Healthy Subjects." Clinical Pharmacology & Therapeutics **89**(6): 837-844.
Lalande, L., B. Charpiat, et al. (2014). "Consequences of Renal Failure on Non-Renal Clearance of Drugs." Clinical Pharmacokinetics **53**(6): 521-532.
Lalau, J.-D. (2010). "Lactic Acidosis Induced by Metformin." Drug Safety **33**(9): 727-740.
Lalau, J. D., P. Arnouts, et al. (2014). "Metformin and other antidiabetic agents in renal failure patients." Kidney international.
Lalau, J. D., P. F. Westeel, et al. (1987). "Bicarbonate haemodialysis: an adequate treatment for lactic acidosis in diabetics treated by metformin." Intensive Care Medicine **13**(6): 383-387.
Landoni, M. F. and A. Soraci (2001). "Pharmacology of chiral compounds: 2-Arylpropionic acid derivatives." Current Drug Metabolism **2**(1): 37-51.
Laouari, D., R. Yang, et al. (2001). Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure.
le Roux, C. W., C. Borg, et al. (2010). "Gut hypertrophy after gastric bypass is associated with increased glucagon-like peptide 2 and intestinal crypt cell proliferation." Annals of surgery **252**(1): 50-56.
Leblond, F., C. Guevin, et al. (2001). "Downregulation of hepatic cytochrome P450 in chronic renal failure." J Am Soc Nephrol **12**(2): 326-332.
Lepist, E.-I., X. Zhang, et al. (2014). "Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat." Kidney Int **86**(2): 350-357.
Levey, A. S., J. Coresh, et al. (2003). "National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification." Annals of Internal Medicine **139**(2): 137-147.
Li, G.-f., K. Wang, et al. (2012). "Simulation of the pharmacokinetics of bisoprolol in healthy adults and patients with impaired renal function using whole-body physiologically based pharmacokinetic modeling." Acta Pharmacol Sin **33**(11): 1359-1371.
Lieschke, G. J. and P. D. Currie (2007). "Animal models of human disease: zebrafish swim into view." Nat Rev Genet **8**(5): 353-367.
Lipska, K. J., C. J. Bailey, et al. (2011). "Use of Metformin in the Setting of Mild-to-Moderate Renal Insufficiency." Diabetes Care **34**(6): 1431-1437.
Lloret-Linares, C., D. Hirt, et al. (2014). "Effect of a Roux-en-Y Gastric Bypass on the Pharmacokinetics of Oral Morphine Using a Population Approach." Clinical Pharmacokinetics **53**(10): 919-930.

Lowry, O. H., N. J. Rosebrough, et al. (1951). "PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT." Journal of Biological Chemistry **193**(1): 265-275.
Lukacova, V., W. Woltosz, et al. (2009). "Prediction of Modified Release Pharmacokinetics and Pharmacodynamics from In Vitro, Immediate Release, and Intravenous Data." The AAPS Journal **11**(2): 323-334.
Macwan, J. (2013). "Model-Based Approaches to Characterize Clinical Pharmacokinetics of Atorvastatin and Rosuvastatin in Disease State." Open Access Dissertations: 28.
Maeda, A., T. Shinoda, et al. (2011). "Evaluating tamsulosin hydrochloride-released microparticles prepared using single-step matrix coating." International Journal of Pharmaceutics **408**(1–2): 84-90.
Maharaj, A. R. and A. N. Edginton (2014). "Physiologically Based Pharmacokinetic Modeling and Simulation in Pediatric Drug Development." CPT: pharmacomet. syst. pharmacol. **3**: e148.
Malone, M. (2003). "Altered drug disposition in obesity and after bariatric surgery." Nutrition in clinical practice **18**(2): 131-135.
Manallack, D. T., R. J. Prankerd, et al. (2013). "The Significance of Acid/Base Properties in Drug Discovery." Chemical Society reviews **42**(2): 485-496.
Marathe, P. H., Y. Wen, et al. (2000). "Effect of altered gastric emptying and gastrointestinal motility on metformin absorption." British Journal of Clinical Pharmacology **50**(4): 325-332.
Marsh, R. H. K., R. Spencer, et al. (1984). "Gastric emptying and drug absorption before surgery." British Journal of Anaesthesia **56**(2): 161-164.
Mathias, N. R. and J. Crison (2012). "The Use of Modeling Tools to Drive Efficient Oral Product Design." The AAPS Journal **14**(3): 591-600.
Matsuzaki, T., T. Morisaki, et al. (2008). "Altered Pharmacokinetics of Cationic Drugs Caused by Down-Regulation of Renal Rat Organic Cation Transporter 2 (Slc22a2) and Rat Multidrug and Toxin Extrusion 1 (Slc47a1) in Ischemia/Reperfusion-Induced Acute Kidney Injury." Drug Metabolism and Disposition **36**(4): 649-654.
Mattar, S. G., L. M. Velcu, et al. (2005). "Surgically-Induced Weight Loss Significantly Improves Nonalcoholic Fatty Liver Disease and the Metabolic Syndrome." Annals of surgery **242**(4): 610-620.
Mehta, R. L. and G. M. Chertow (2003). "Acute Renal Failure Definitions and Classification: Time for Change?" Journal of the American Society of Nephrology **14**(8): 2178-2187.
Micaela B. Reddy, H. J. C. I., Thierry Lave and Melvin E. Andersen (2013). Physiologically Based Pharmacokinetic Modeling: A Tool for Understanding ADMET Properties and Extrapolating to Human. New Insights into Toxicity and Drug Testing. D. S. Gowder.
Miller, A. D. and K. M. Smith (2006). "Medication and nutrient administration considerations after bariatric surgery." American Journal of Health-System Pharmacy **63**(19): 1852-1857.
Misbin, R. I., L. Green, et al. (1998). "Lactic Acidosis in Patients with Diabetes Treated with Metformin." New England Journal of Medicine **338**(4): 265-266.
Miskowiak, J. and B. Andersen (1983). "Intestinal adaptation after jejunoileal bypass for morbid obesity: a possible explanation for inadequate weight loss." The British journal of surgery **70**(1): 27-28.

Mohammed, A. R., N. Weston, et al. (2004). "Liposome formulation of poorly water soluble drugs: Optimisation of drug loading and ESEM analysis of stability." International Journal of Pharmaceutics **285**(1-2): 23-34.
Moore, R. A., S. Derry, et al. (2014). "Faster, higher, stronger? Evidence for formulation and efficacy for ibuprofen in acute pain." Pain **155**(1): 14-21.
Moreno, M. P. d. l. C., M. Oth, et al. (2006). "Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum." Journal of Pharmacy and Pharmacology **58**(8): 1079-1089.
Naud, J., J. Michaud, et al. (2007). "Down-Regulation of Intestinal Drug Transporters in Chronic Renal Failure in Rats." Journal of Pharmacology and Experimental Therapeutics **320**(3): 978-985.
Naud, J., J. Michaud, et al. (2008). "Effects of Chronic Renal Failure on Liver Drug Transporters." Drug Metabolism and Disposition **36**(1): 124-128.
Naud, J., T. D. Nolin, et al. (2012). "Current Understanding of Drug Disposition in Kidney Disease." The Journal of Clinical Pharmacology **52**(S1): 10S-22S.
Nishihara, K., S. Masuda, et al. (2007). "Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats." Biochemical Pharmacology **73**(9): 1482-1490.
Norris, D. A., G. D. Leesman, et al. (2000). "Development of predictive pharmacokinetic simulation models for drug discovery." Journal of Controlled Release **65**(1–2): 55-62.
NUGENT, S. G., D. KUMAR, et al. (2001). "Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs." Gut **48**(4): 571-577.
Okumu, A., M. DiMaso, et al. (2008). "Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug." Pharmaceutical Research **25**(12): 2778-2785.
Okumu, A., M. DiMaso, et al. (2009). "Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib solid oral drug products." European Journal of Pharmaceutics and Biopharmaceutics **72**(1): 91-98.
Padwal, R., S. Klarenbach, et al. (2011). "Bariatric surgery: A systematic review and network meta-analysis of randomized trials." Obesity Reviews **12**(8): 602-621.
Padwal, R. S., R. Q. Gabr, et al. (2011). "Effect of gastric bypass surgery on the absorption and bioavailability of metformin." Diabetes Care **34**(6): 1295-1300.
Paixão, P., L. F. Gouveia, et al. (2012). "Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model." International Journal of Pharmaceutics **429**(1-2): 84-98.
Parrott, N. and T. Lavé (2002). "Prediction of intestinal absorption: Comparative assessment of GASTROPLUS™ and IDEA™." European Journal of Pharmaceutical Sciences **17**(1-2): 51-61.
Pentikainen, P. J., P. J. Neuvonen, et al. (1979). "Pharmacokinetics of metformin after intravenous and oral administration to man." European Journal of Clinical Pharmacology **16**(3): 195-202.
Peters, S. A. and L. Hultin (2008). "Early identification of drug-induced impairment of gastric emptying through physiologically based pharmacokinetic (PBPK) simulation of plasma concentration-time profiles in rat." Journal of pharmacokinetics and pharmacodynamics **35**(1): 1-30.

Peters, S. A., A. L. Ungell, et al. (2009). "Physiologically based pharmacokinetic (PBPK) modeling and simulation: applications in lead optimization." Current opinion in drug discovery & development **12**(4): 509-518.
Philips, B. J., K. Lane, et al. (2014). "The effects of acute renal failure on drug metabolism." Expert Opinion on Drug Metabolism & Toxicology **10**(1): 11-23.
Po, H. N. and N. M. Senozan (2001). "The Henderson-Hasselbalch Equation: Its History and Limitations." Journal of Chemical Education **78**(11): 1499.
Poggesi, I., M. S. Benedetti, et al. (2009). "Pharmacokinetics in special populations." Drug Metabolism Reviews **41**(3): 422-454.
Porter, C. J. H., C. W. Pouton, et al. (2008). "Enhancing intestinal drug solubilisation using lipid-based delivery systems." Advanced Drug Delivery Reviews **60**(6): 673-691.
Poulin, P., K. Schoenlein, et al. (2001). "Prediction of adipose tissue: Plasma partition coefficients for structurally unrelated drugs." Journal of Pharmaceutical Sciences **90**(4): 436-447.
Poulin, P. and F.-P. Theil (2000). "A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery." Journal of Pharmaceutical Sciences **89**(1): 16-35.
Poulin, P. and F.-P. Theil (2002). "Prediction of pharmacokinetics prior to In Vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition." Journal of Pharmaceutical Sciences **91**(5): 1358-1370.
Proctor, W. R., D. L. Bourdet, et al. (2008). "Mechanisms Underlying Saturable Intestinal Absorption of Metformin." Drug Metabolism and Disposition **36**(8): 1650-1658.
Quigley, E. M. and J. S. Thompson (1993). "The motor response to intestinal resection: motor activity in the canine small intestine following distal resection." Gastroenterology **105**(3): 791-798.
R Das, N., R. P Gangwal, et al. (2014). "A PPAR-β/δ Agonist is Neuroprotective and Decreases Cognitive Impairment in a Rodent Model of Parkinson's Disease." Current Neurovascular Research **11**(2): 114-124.
R. Das, N., R. P. Gangwal, et al. (2014). "A PPAR-β/δ Agonist is Neuroprotective and Decreases Cognitive Impairment in a Rodent Model of Parkinson’s Disease." Current Neurovascular Research **11**(2): 114-124.
Reddy, B. B. K. and A. Karunakar (2011). "Biopharmaceutics classification system: a regulatory approach." Dissolution Technologies **18**(1): 31-37.
Riezzo, G., M. Chiloiro, et al. (2000). "Comparison of Gastric Electrical Activity and Gastric Emptying in Healthy and Dyspeptic Children." Digestive Diseases and Sciences **45**(3): 517-524.
Robert, F., S. Fendri, et al. (2003). "Kinetics of plasma and erythrocyte metformin after acute administration in healthy subjects." Diabetes & Metabolism **29**(3): 279-283.
Rodgers, T., D. Leahy, et al. (2005). "Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases." Journal of Pharmaceutical Sciences **94**(6): 1259-1276.
Rodgers, T. and M. Rowland (2006). "Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions." Journal of Pharmaceutical Sciences **95**(6): 1238-1257.
Rodgers, T. and M. Rowland (2007). "Mechanistic Approaches to Volume of Distribution Predictions: Understanding the Processes." Pharmaceutical Research **24**(5): 918-933.

Rojas, L. B. A. and M. B. Gomes (2013). "Metformin: an old but still the best treatment for type 2 diabetes." Diabetology & Metabolic Syndrome **5**(1): 6.
Rostami-Hodjegan, A., I. Tamai, et al. (2012). "Physiologically based pharmacokinetic (PBPK) modeling: it is here to stay!" Biopharmaceutics & drug disposition **33**(2): 47-50.
Rowland, M. (2013). "Physiologically-Based Pharmacokinetic (PBPK) Modeling and Simulations Principles, Methods, and Applications in the Pharmaceutical Industry." CPT: pharmacometrics & systems pharmacology **2**: e55.
Rowland, M., C. Peck, et al. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. **51:** 45-73.
Rowland Yeo, K., M. Aarabi, et al. (2011). "Modeling and predicting drug pharmacokinetics in patients with renal impairment." Expert Review of Clinical Pharmacology **4**(2): 261-274.
Rubio, I. G. S., A. L. Galrão, et al. (2012). "Levothyroxine absorption in morbidly obese patients before and after Roux-En-Y gastric bypass (RYGB) surgery." Obesity Surgery **22**(2): 253-258.
Sambol, N. C., J. Chiang, et al. (1995). "Kidney Function and Age Are Both Predictors of Pharmacokinetics of Metformin." The Journal of Clinical Pharmacology **35**(11): 1094-1102.
Sambrook J., F. E. F., Maniatis T (1989). Molecular cloning: A laboratory Manual., Cold Spring HarborNY: Cold Spring Harbor Laboratory Press.
Sano, S., Y. Iwao, et al. (2011). "Preparation and evaluation of swelling induced-orally disintegrating tablets by microwave irradiation." International Journal of Pharmaceutics **416**(1): 252-259.
Sarwer DB, F. L., Steffen KJ, Roerig JL, Mitchell JE. (2011) "Managing patients after surgery: Changes in the gastrointestinal tract and body weight can alter drug absorption." Current Psychiatry **10**, 18-30.
Savassi-Rocha, A., M. Diniz, et al. (2014). "Changes in Intestinal Permeability after Roux-en-Y Gastric Bypass." Obesity Surgery **24**(2): 184-190.
Sawaya, R. A., J. Jaffe, et al. (2012). "Vitamin, Mineral, and Drug Absorption Following Bariatric Surgery." Current Drug Metabolism **13**(9): 1345-1355.
Schmitt, W. and S. Willmann (2004). "Physiology-based pharmacokinetic modeling: ready to be used." Drug Discovery Today: Technologies **1**(4): 449-456.
Seaman, J. S., P. Bowers Sp Fau - Dixon, et al. (2005). "Dissolution of common psychiatric medications in a Roux-en-Y gastric bypass model." (0033-3182 (Print)).
Shaw, D., K. Gohil, et al. (2012). "Intestinal mucosal atrophy and adaptation." World Journal of Gastroenterology **18**(44): 6357-6375.
Shukla, M., G. Singh, et al. (2007). "Comparative plasma pharmacokinetics of meloxicam in sheep and goats following intravenous administration." Comparative Biochemistry and Physiology - C Toxicology and Pharmacology **145**(4): 528-532.
Simard, E., J. Naud, et al. (2008). "Downregulation of Hepatic Acetylation of Drugs in Chronic Renal Failure." Journal of the American Society of Nephrology **19**(7): 1352-1359.
SimulationPlus. (2015). "GastrPlus manual."
Smith, A., B. Henriksen, et al. (2011). "Pharmacokinetic considerations in Roux-en-Y gastric bypass patients." American Journal of Health-System Pharmacy **68**(23): 2241-2247.
Spak, E., P. Björklund, et al. (2010). "Changes in the mucosa of the Roux-limb after gastric bypass surgery." Histopathology **57**(5): 680-688.

Stades, A. M. E., J. T. Heikens, et al. (2004). "Metformin and lactic acidosis: cause or coincidence? A review of case reports." Journal of Internal Medicine **255**(2): 179-187.
Stock-Damge, C., M. Aprahamian, et al. (1986). "Small-intestinal and colonic changes after biliopancreatic bypass for morbid obesity." Scand J Gastroenterol **21**(9): 1115-1123.
Sun, H., L. Frassetto, et al. (2006). "Effects of renal failure on drug transport and metabolism." Pharmacology & Therapeutics **109**(1–2): 1-11.
Sun, H., Y. Huang, et al. (2004). "EFFECTS OF UREMIC TOXINS ON HEPATIC UPTAKE AND METABOLISM OF ERYTHROMYCIN." Drug Metabolism and Disposition **32**(11): 1239-1246.
Suzuki, S., E. J. B. Ramos, et al. (2005). "Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model." Surgery **138**(2): 283-290.
Tandra, S., N. Chalasani, et al. (2013). "Pharmacokinetic and pharmacodynamic alterations in the Roux-en-Y gastric bypass recipients." Annals of surgery **258**(2): 262-269.
Tanihara, Y., S. Masuda, et al. (2007). "Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H+-organic cation antiporters." Biochemical Pharmacology **74**(2): 359-371.
Tappenden, K. A. (2014). "Intestinal Adaptation Following Resection." Journal of Parenteral and Enteral Nutrition.
Teorell, T. (1937). "Kinetics of the Distribution of Substances Administered to the Body . II. The Extravascular Mode of Administration." Archives Internationales de Pharmacodynamie et de Thérapie (57): 205–240
Terao N Fau - Shen, D. D. and D. D. Shen "Reduced extraction of I-propranolol by perfused rat liver in the presence of uremic blood." (0022-3565 (Print)).
Theil, F.-P., T. W. Guentert, et al. (2003). "Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection." Toxicology letters **138**(1–2): 29-49.
Toyama, K., A. Yonezawa, et al. (2012). "Loss of multidrug and toxin extrusion 1 (MATE1) is associated with metformin-induced lactic acidosis." British Journal of Pharmacology **166**(3): 1183-1191.
Toyohara, T., T. Suzuki, et al. (2009). "SLCO4C1 Transporter Eliminates Uremic Toxins and Attenuates Hypertension and Renal Inflammation." Journal of the American Society of Nephrology **20**(12): 2546-2555.
Tsamandouras, N., A. Rostami-Hodjegan, et al. (2015). "Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: Fitting PBPK models to observed clinical data." British Journal of Clinical Pharmacology **79**(1): 48-55.
Tsuda, M., T. Terada, et al. (2009). "Targeted Disruption of the Multidrug and Toxin Extrusion 1 (Mate1) Gene in Mice Reduces Renal Secretion of Metformin." Molecular Pharmacology **75**(6): 1280-1286.
Tucker, G. T., C. Casey, et al. (1981). "Metformin kinetics in healthy subjects and in patients with diabetes mellitus." British Journal of Clinical Pharmacology **12**(2): 235-246.
Tymms, D. J. and B. A. Leatherdale (1988). "Lactic acidosis due to metformin therapy in a low risk patient." Postgraduate Medical Journal **64**(749): 230-231.
Urakami, Y., N. Kimura, et al. (2004). "Creatinine Transport by Basolateral Organic Cation Transporter hOCT2 in the Human Kidney." Pharmaceutical Research **21**(6): 976-981.

Urakami, Y., N. Kimura, et al. (2005). "Transcellular Transport of Creatinine in Renal Tubular Epithelial Cell Line LLC-PK₁." Drug Metabolism and Pharmacokinetics **20**(3): 200-205.
Urban, D., N. Cherny, et al. (2010). "The management of cancer pain in the elderly." Critical Reviews in Oncology/Hematology **73**(2): 176-183.
Valsami, G. and P. Macheras (2011). "Computational-regulatory developments in the rediction of oral drug absorption." Molecular Informatics **30**(2-3): 112-121.
Vanholder, R., R. De Smet, et al. (2003). "Review on uremic toxins: Classification, concentration, and interindividual variability." Kidney Int **63**(5): 1934-1943.
Volans, G. N. (1975). "The effect of metoclopramide on the absorption of effervescent aspirin in migraine." British Journal of Clinical Pharmacology **2**(1): 57-63.
Volans, G. N. (1978). "Migraine and Drug Absorption." Clinical Pharmacokinetics **3**(4): 313-318.
Wagner, J. G. (1981). "History of pharmacokinetics." Pharmacology & Therapeutics **12**(3): 537-562.
Waldmann, S., M. Almukainzi, et al. (2012). "Provisional Biopharmaceutical Classification of Some Common Herbs Used in Western Medicine." Molecular Pharmaceutics **9**(4): 815-822.
Walsh, K. and J. Volling (2014). "Lithium toxicity following Roux-en-Y gastric bypass." Bariatric Surgical Practice and Patient Care **9**(2): 77-80.
Wang, D.-S., J. W. Jonker, et al. (2002). "Involvement of Organic Cation Transporter 1 in Hepatic and Intestinal Distribution of Metformin." Journal of Pharmacology and Experimental Therapeutics **302**(2): 510-515.
Wang, G., K. Agenor, et al. (2012). "Accelerated Gastric Emptying but No Carbohydrate Malabsorption 1 Year After Gastric Bypass Surgery (GBP)." Obesity Surgery **22**(8): 1263-1267.
Watanabe, T., H. Kusuhara, et al. (2009). "Physiologically Based Pharmacokinetic Modeling to Predict Transporter-Mediated Clearance and Distribution of Pravastatin in Humans." Journal of Pharmacology and Experimental Therapeutics **328**(2): 652-662.
Wilcock, C., N. D. Wyre, et al. (1991). "Subcellular distribution of metformin in rat liver." Journal of Pharmacy and Pharmacology **43**(6): 442-444.
Wilson, C. G., C. P. Clarke, et al. (2011). "Comparison of a novel fast-dissolving acetaminophen tablet formulation (FD-APAP) and standard acetaminophen tablets using gamma scintigraphy and pharmacokinetic studies." Drug Dev Ind Pharm **37**(7): 747-753.
Wood, A. J., R. E. Vestal, et al. (1980). "Propranolol disposition in renal failure." British Journal of Clinical Pharmacology **10**(6): 561-566.
Wu, C.-Y. and L. Z. Benet (2005). "Predicting Drug Disposition via Application of BCS: Transport/Absorption/ Elimination Interplay and Development of a Biopharmaceutics Drug Disposition Classification System." Pharmaceutical Research **22**(1): 11-23.
Xia, L., K. Engel, et al. (2007). Membrane localization and pH-dependent transport of a newly cloned organic cation transporter (PMAT) in kidney cells.
Yomona-Hernández, J. L., P. Vicente-Ríos, et al. (2003). "Gastric emptying rates in idiopathic dyspepsia with and without "hunger pain"." Acta Gastroenterologica Latinoamericana **33**(3): 129-132.

Yonezawa, A. and K.-i. Inui (2011). "Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics." British Journal of Pharmacology **164**(7): 1817-1825.
Yonezawa, A. and K.-i. Inui (2011). "Organic cation transporter OCT/SLC22A and H+/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents." Biochemical Pharmacology **81**(5): 563-568.
Yonezawa, A., S. Masuda, et al. (2005). "Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat." Biochemical Pharmacology **70**(12): 1823-1831.
Yoshida, T., T. C. Lai, et al. (2013). "pH- and ion-sensitive polymers for drug delivery." Expert Opinion on Drug Delivery **10**(11): 1497-1513.
Yu, L. X. and G. L. Amidon (1999). "A compartmental absorption and transit model for estimating oral drug absorption." International Journal of Pharmaceutics **186**(2): 119-125.
Yu, L. X., E. Lipka, et al. (1996). "Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption." Advanced Drug Delivery Reviews **19**(3): 359-376.
Zamek-Gliszczynski, M. J., C. A. Lee, et al. (2013). "ITC Recommendations for Transporter Kinetic Parameter Estimation and Translational Modeling of Transport-Mediated PK and DDIs in Humans." Clinical pharmacology and therapeutics **94**(1): 64-79.
Zhang, Y., M. Huo, et al. (2010). "DDSolver: An add-in program for modeling and comparison of drug dissolution profiles." AAPS Journal **12**(3): 263-271.
Zhao, P., M. d. L. T. Vieira, et al. (2012). "Evaluation of Exposure Change of Nonrenally Eliminated Drugs in Patients With Chronic Kidney Disease Using Physiologically Based Pharmacokinetic Modeling and Simulation." The Journal of Clinical Pharmacology **52**(S1): 91S-108S.
Zhao, P., L. Zhang, et al. (2011). "Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review." Clinical pharmacology and therapeutics **89**(2): 259-267.
Zhou, M., L. Xia, et al. (2007). "Metformin Transport by a Newly Cloned Proton-Stimulated Organic Cation Transporter (Plasma Membrane Monoamine Transporter) Expressed in Human Intestine." Drug Metabolism and Disposition **35**(10): 1956-1962.
Zolk, O. (2011). "Disposition of metformin: Variability due to polymorphisms of organic cation transporters." Annals of Medicine **44**(2): 119-129.