

## **Potential and Challenges in Application of Physiologically Based Pharmacokinetic Modeling in Predicting Diarrheal Disease Impact on Oral Drug Pharmacokinetics**

Cindy X. Zhang and Samuel L. M. Arnold\*

Department of Pharmaceutics, University of Washington, Seattle, Washington, USA

### **\*Corresponding author:**

Samuel L. M. Arnold,

Department of Pharmaceutics, University of Washington, Seattle, Washington, USA

Phone: (206) 221-3441

Email: slarnold@uw.edu

## RUNNING TITLE PAGE

### Running title:

### PBPK model development for diarrheal diseases

Number of manuscript (text) pages: 23

Number of figures: 2

Number of tables: 4, including one supplemental table

Number of references: 161

Word count of the abstract: 173

Word count of the introduction: 751

Word count of the minireview body (excluding introduction and conclusion): 8222

Word count of the minireview conclusion: 83

### List of Non-standard Abbreviations:

A	amount of drug
$A_d$	dissolved mass of drug
$A_u$	undissolved mass of drug
ACAT	Advanced Compartment Absorption and Transit
ADAM	Advanced Dissolution Absorption Metabolism
ASF	absorption scale factor
AUC	area under the concentration-time curve
BAD	bile acid diarrhea
BCRP	Breast Cancer Resistance Protein
BCS	biopharmaceutics drug classes
$C_l$	luminal drug concentration in the gut
$C_{max}$	maximum concentration
$C_s$	solubility of drug

CAT	Compartmental Absorption and Transit
CD	Crohn's disease
CFTR	cystic fibrosis transmembrane conductance regulator
CsA	Cyclosporine A
CYP	cytochrome P450
D	diffusion coefficient for dissolved drug in a given solvent
DDI	drug-drug interaction
EMA	European Medicines Agency
ER	extended-release
F	bioavailability
$F_a$	the fraction of drug entering the gut wall from the gut lumen
$F_g$	the fraction of drug that escapes gut metabolism
$F_h$	the fraction of drug that escapes first pass hepatic elimination
FDA	Food and Drug Administration
GI	gastrointestinal
GVHD	graft-versus-host disease
$h_{eff}$	effective diffusion layer thickness
$k_a$	absorption rate constant
$k_t$	transit rate constant
OCTT	oro-caecal transit time
$\rho$	particle density
P-gp	P-glycoprotein
PBPK	physiologically based pharmacokinetic
PD	pharmacodynamics
PK	pharmacokinetics
PSA	parameter sensitivity analysis
$r_t$	spherical radius at time (t)
s	shape factor that accounts for non-spherical particle shapes
SEF	surface area enhancement factor
$T_{max}$	time at which the maximum concentration is achieved
UC	ulcerative colitis

## **Abstract**

Physiologically based pharmacokinetic (PBPK) modeling is a physiologically relevant approach that integrates drug-specific and system parameters to generate pharmacokinetic predictions for target populations. It has gained immense popularity for drug-drug interaction, organ impairment, and special population studies over the past two decades. However, an application of PBPK modeling with great potential remains rather overlooked – prediction of diarrheal disease impact on oral drug pharmacokinetics. Oral drug absorption is a complex process involving the interplay between physicochemical characteristics of the drug and physiological conditions in the gastrointestinal tract. Diarrhea, a condition common to numerous diseases impacting many worldwide, is associated with physiological changes in many processes critical to oral drug absorption. In this review, we outline key processes governing oral drug absorption, provide a high-level overview of key parameters for modeling oral drug absorption in PBPK models, examine how diarrheal diseases may impact these processes based on literature findings, illustrate the clinical relevance of diarrheal disease impact on oral drug absorption, and discuss the potential and challenges of applying PBPK modeling in predicting disease impacts.

**Significance Statement** Pathophysiological changes resulting from diarrheal diseases can alter important factors governing oral drug absorption, contributing to suboptimal drug exposure and treatment failure. Physiologically based pharmacokinetic (PBPK) modeling is an *in silico* approach that has been increasingly adopted for drug-drug interaction potential, organ impairment, and special population assessment. This

minireview highlights the potential and challenges of using PBPK modeling as a tool to improve our understanding of how diarrheal diseases impact oral drug pharmacokinetics.

## **1. Introduction**

Physiologically based pharmacokinetic (PBPK) modeling is a simulation and modeling approach that integrates drug-specific and physiological (system) parameters to generate pharmacokinetic (PK) predictions for therapeutics in target patient populations or preclinical model systems. PBPK models treat different tissues or organs as compartments and employ mathematical models to describe the transit, distribution, and elimination of therapeutics through the compartments, such that predictions of drug PK profiles can be made for both plasma and tissues (Rostami-Hodjegan, 2012; Rowland et al., 2011). Because of its design, PBPK models can be used to predict the impact on concentration versus time profiles due to changes in either drug properties (e.g., during the process of lead optimization) and/or system properties (e.g., differences between adult and pediatric populations). Its adoption by the pharmaceutical industry has increased drastically in recent years, evident by a 13-fold increase in the number of PBPK modeling publications supporting US Food and Drug Administration (FDA)-approved drug products from 2012 to 2018 (Perry et al., 2020). Regulatory agencies are also receptive to the use of PBPK models in model-informed drug development (Jean et al., 2021; Luzon et al., 2017; Manolis et al., 2023; X. Zhang et al., 2020); both the FDA (FDA, 2018) and European Medicines Agency (EMA) (EMA, 2018) have issued guidance documents on the proper use of PBPK modeling. Common uses for PBPK modeling include drug-drug interaction (DDI) potential assessment, pediatric dosing prediction, and organ impairment impact estimation (Abouir et al., 2021; Hariparsad et al., 2022; Heimbach et al., 2021; T. N. Johnson et al., 2021, 2022; Salerno et al., 2019; Taskar et al., 2020; Verscheijden et al., 2020; K. Wang et al., 2021). PBPK modeling has been applied across multiple therapeutic areas, including oncology, central nervous system disorders, infectious diseases, gastroenterology, and cardiovascular diseases (Jamei, 2016; Perry et al., 2020; X. Zhang et al., 2020) benefiting patient populations and the drug development process alike by allowing extrapolation to special populations (Chu et al., 2022), reduction in study size, and application for clinical trial waivers (Shebley et al., 2018). Theoretically, the potential uses of PBPK modeling can be extended to predicting the PK impact of any quantifiable changes in the drug-specific or system parameters.

Within PBPK modeling, oral drug absorption models are a key area of interest as oral administration is the most common and preferred route of drug administration due to its convenience, accessibility, and availability. An important consideration for oral drug administration is that oral drug absorption can be highly variable and the absorption process is prone to changes induced by disease states. For instance, diarrheal diseases are associated with physiological changes impacting gut motility, luminal pH, absorptive surface area, fluid volume, and permeability, all of which can impact oral drug dissolution and permeation in the gastrointestinal (GI) tract (Table 1) (Abuhelwa et al., 2017; Effinger et al., 2019; Jamei, Turner, et al., 2009; Kostewicz et al., 2014; Vinarov et al., 2021). Consequently, it is both challenging and crucial to understand how the multifold disease-associated physiological changes impact oral drug absorption and to be able to predict oral drug PK in a population with diarrheal disease.

A number of software programs are available for PBPK model development, such as GastroPlus® (Simulations Plus, Lancaster, CA), Simcyp® (Certara, Sheffield, UK), and PK-Sim®, and these programs utilize different strategies to model oral drug absorption. Popular absorption models include the Advanced Compartment Absorption and Transit (ACAT) model (Agoram et al., 2001) implemented in GastroPlus® and Advanced Dissolution Absorption Metabolism (ADAM) model (Jamei, Marciniak, et al., 2009) in Simcyp®. Another absorption model available in Simcyp® is the multi-layer gut wall (M-ADAM) model, which is an advanced form of the ADAM model that accounts for both apical and basolateral gut transporters (Dolton et al., 2020; Ezuruike et al., 2022). Both the ACAT and ADAM models are based on the Compartmental Absorption and Transit (CAT) model, which uses a series of compartments to represent different sections of the intestinal tract (L. X. Yu et al., 1996; L. X. Yu & Amidon, 1998, 1999). Drug absorption is dependent on dissolution, precipitation, luminal degradation, permeation, metabolism, transport, and transit in both ACAT and ADAM models (Fig. 1). As such, physiological changes in the GI tract that impact these processes may be reflected by changes in drug absorption and consequently the predicted PK profile. Here, we review the impact of diarrheal diseases on physiological parameters that have

key roles in oral drug absorption, the importance of predicting diarrhea-associated PK changes with PBPK models, and knowledge gaps to be addressed in the future.

## **2. Key processes governing oral drug absorption**

Oral bioavailability ( $F$ ), a parameter describing the fraction of orally administered dose reaching the systemic circulation, is a function of three separate terms reflective of drug penetration in the GI tract, GI metabolism, and hepatic elimination (Eq. 1).

$$F = F_a * F_g * F_h \quad (1)$$

$F_a$  is the fraction of drug entering the gut wall from the gut lumen,  $F_g$  is the fraction that escapes gut metabolism, and  $F_h$  is the fraction that escapes first pass hepatic elimination. While the processes summarized by these three terms can all be affected by diseases, this review mainly focuses on pathophysiological changes that appreciably impact the dissolution and permeation of drugs in the GI tract ( $F_a$ ) and discusses the potential application of PBPK modeling to capture the resulting oral drug PK changes.

### **2.1 Dissolution:**

Many dissolution models implemented within PBPK absorption models use the Nernst Brunner equation as a foundation which considers solute diffusion across a boundary of unstirred solvent surrounding the surface of each particle and the surface area of the particle (Brunner, 1904; Nernst, 1904). To improve drug dissolution simulations, additional factors have been incorporated into the Johnson and Wang-Flanagan models such as drug particle size, density, and shape (K. C. Johnson, 2003; J. Wang & Flanagan, 1999). The Johnson model (K. C. Johnson, 2003), the default dissolution model built into GastroPlus®, is shown below as an example (Eq. 2):

$$\frac{dA_d}{dt} = \frac{D}{p \cdot h_{eff} \cdot r_t} \frac{(1 + 2s)}{s} (C_s - C_l) A_{u,t} \quad (2)$$

in which  $dA_d$  is the total dissolved mass of the drug in the time interval  $dt$ ,  $D$  is the diffusion coefficient for the dissolved drug in a given solvent,  $p$  is the particle density,  $h_{eff}$  is the effective diffusion layer thickness,  $r_t$  is the spherical radius at time ( $t$ ),  $s$  is the



shape factor that accounts for non-spherical particle shapes,  $C_s$  is the solubility of the drug,  $C_l$  is the drug concentration in the lumen, and  $A_{u,t}$  is the undissolved drug mass at time (t). Since absorption models based on the CAT model separate the GI tract into multiple compartments, site specific changes in local solubility (due to differences in bile salts/pH) and/or drug diffusion are incorporated into the dissolution models, allowing dissolution rate predictions to vary among the GI compartments.

## 2.2 Permeation

Once drug molecules have dissolved in the fluid within the GI lumen, drug can permeate into the body by paracellular and/or transcellular diffusion (Dahlgren et al., 2015). With paracellular diffusion, the drug travels through intercellular space between intestinal epithelial cells. However, for many small molecules, the predominant route to the blood is transcellular diffusion which includes drug entry into the gut epithelium prior to entry into blood (Youhanna & Lauschke, 2021). Oral absorption models can simultaneously account for time varying drug levels in the GI lumen, gut wall, and blood when simulating both paracellular and transcellular drug diffusion. In addition to diffusion, the rate of change in drug concentration in enterocytes ( $\frac{dA_{ent}}{dt}$ ) can be affected by carrier mediated transport (apical and basolateral) and gut metabolism (Eq. 3):

$$\begin{aligned} \frac{dA_{ent}}{dt} = & \text{Apical diffusion rate} + \text{Net apical carrier mediated transport rate} \\ & - \text{Basolateral diffusion rate} \\ & + \text{Net basolateral carrier mediated transport rate} \\ & - \text{Gut metabolism rate} \end{aligned} \tag{3}$$

The incorporation of carrier mediated transport within absorption models varies. One common approach considers the difference between the influx and efflux rates (i.e., influx-efflux) at the apical and basolateral membranes to determine the net rate of carrier mediated drug transport at each site. If adequate data are available, the carrier mediated transport kinetics (e.g.,  $K_m$ ,  $V_{max}$ ) for each influx and efflux transporter can be included in the absorption model (Eq. 4):

$$\begin{aligned} & \text{Net carrier mediated transport rate} \\ & = DF_{influx} \left( \frac{V_{max,influx} \cdot C_i}{K_{m,influx} + C_i} \right) - DF_{efflux} \left( \frac{V_{max,efflux} \cdot C_{u,enterocyte}}{K_{m,efflux} + C_{u,enterocyte}} \right) \end{aligned} \quad (4)$$

in which DF is a distribution factor accounting for the difference in transporter expression/activity in the GI tract compared to the in vitro model used to determine  $V_{max}$ ,  $V_{max}$  is the maximum rate of drug transport,  $K_{m,influx}$  is the Michealis-Menten constant for the uptake transporter and substrate,  $K_{m,efflux}$  is the Michealis-Menten constant for the efflux transporter and substrate,  $C_i$  is the drug concentration in the GI lumen (apical) or blood (basolateral), and  $C_{u,enterocyte}$  is the unbound enterocyte concentration (Arnold & Isoherranen, 2022). In addition, for GI drug metabolism, a similar approach incorporating total or individual enzyme mediated drug metabolism can be incorporated into the absorption model. Since many GI PBPK absorption models separate the intestine into multiple compartments, the drug concentrations used to calculate rates of drug diffusion, carrier mediated transport, and drug metabolism will vary depending on the location in the GI tract. Thus, at a given time, carrier mediated transport and/or metabolism may be saturated in the proximal small intestine but not saturated in the distal intestine.

### **3. Diarrheal disease impacts on system components**

#### **3.1 pH**

GI pH is an important factor for oral drug PK because it can directly impact oral drug solubility which may lead to changes in dissolution rates (Eq. 2). The rate of drug permeation is also impacted because  $C_l$ , which depends on solubility and dissolution, determines the concentration gradient driving drug permeation. Drugs in class II of the biopharmaceutics classification system (BCS) have low solubility and high permeability, and their oral bioavailability can be heavily influenced by GI pH. For weakly acidic or basic drugs with solubility-limited absorption and a pKa close to physiologic pH of part(s) of the GI tract, even slight shifts in GI pH may affect the ionization state of a drug which in turn impacts the drug's solubility, protein binding, permeability, and dissolution rate,

potentially leading to changes in drug absorption (Charifson & Walters, 2014; Gaohua et al., 2021; Manallack, 2007). For instance, elevated gastric pH can reduce the ionization and solubility of weakly basic drugs thus decreasing their bioavailability. Reduced absorption of weakly basic drugs ketoconazole, itraconazole, dipyridamole, indinavir, enoxacin, dazatinib, cinnarizine, and cefpodoxime proxetil have been reported in association with elevated gastric pH (Abuhelwa et al., 2017). Furthermore, drug release from formulations can be impacted by GI pH since the disintegration of some coating polymers are pH dependent. This may result in unforeseen changes in the maximum concentration ( $C_{max}$ ) and the time at which  $C_{max}$  is achieved ( $T_{max}$ ) of impacted drugs. Unpredicted shifts in the release profile of extended or controlled release formulations may occur, potentially leading to suboptimal treatment effect, reduction in duration of therapeutic response, and/or undesired side effects.

The luminal pH differs among different sections of the GI tract. There is high between- and within-subject variability in GI pH due to factors such as fed/fasted state, meal caloric content, circadian rhythms, drug intake, and diseases (A. van Herwaarden, 1999; Abuhelwa et al., 2016; Fadda et al., 2022; Press et al., 1998). When building PBPK models, it is important to consider changes in GI pH relevant to the study population. For example, hypochlorhydria, a state of low stomach acid with fasting gastric pH greater than 4.0, was found to be significantly associated with HIV infection (Kelly et al., 2010) or *Helicobacter pylori* and HIV co-infection (Geraghty et al., 2015) (Table 1). Hence, when treatments studied in a different population are assigned to HIV-infected subjects, the impact of hypochlorhydria may need to be taken into consideration. Another common cause of GI pH change associated with GI diseases is changes in bile acid production or reabsorption. Bile acid diarrhea (BAD), a common cause of chronic diarrhea, is estimated to impact 1% of the population and up to 50% of patients with functional or diarrhea-predominant irritable bowel syndrome (Camilleri, 2015; Farrugia & Arasaradnam, 2021; Walters & Patti, 2010). BAD can be caused by either overproduction of bile acids due to defective feedback inhibition during synthesis or ileal malabsorption due to diseases or resection. Not only can BAD impact oral drug absorption by directly changing GI luminal pH, but it is also associated with modified

colonic motility through alterations in colonic neuromuscular activity (Bajor et al., 2010; Karlstrom et al., 1986; Kidd et al., 2008).

### **3.2 Absorptive surface area/epithelial integrity**

GI disorders often cause changes to the availability of absorptive surface area, which may lead to impaired drug absorption, further complicating the prediction of oral drug PK for impacted populations. For example, celiac disease is commonly associated with villous atrophy leading to a reduction in absorptive surface area (Table 1) (Effinger et al., 2019). Villous blunting has also been reported for Common Variable Immunodeficiency (Uzzan et al., 2016; Yan & Sundaram, 2013), HIV infection (Batman et al., 2007; Sakai et al., 2017), and Crohn's disease (CD) (Vyhlidal et al., 2021).

In addition to absorptive surface area changes, altered intestinal permeability has been previously reported for various GI disorders. The symptoms of celiac disease, an autoimmune disorder where an immune response in the small intestine is triggered upon the ingestion of gluten, often include diarrhea, abdominal pain, nausea, bloating, and malabsorption. Studies suggest that the barrier function of the small intestine is compromised in celiac disease due to modifications to tight junctions, resulting in increased ionic permeability (Table 1) (J. D. Schulzke et al., 1995; J.-D. Schulzke et al., 1998; Schumann et al., 2012; Vanuytsel et al., 2021). CD, a form of inflammatory bowel disease that causes chronic inflammation of the GI tract, is associated with increased paracellular permeability as well due to a reduction in tight junction integrity in the GI tract (Effinger et al., 2019).

### **3.3 Transit times**

The transit time of a drug within the GI tract is an important system parameter affecting oral absorption predictions. The CAT model, from which both the ADAM model and the ACAT model expanded, divides the small intestine into seven compartments with rate constants describing therapeutic movement between the compartments as shown in Equation 5 (L. X. Yu et al., 1996; L. X. Yu & Amidon, 1998, 1999):

$$\frac{dA_n}{dt} = k_t * A_{n-1} - k_t * A_n - k_a * A_n \quad (5)$$

where  $A_n$  is the amount of a drug in the  $n^{\text{th}}$  compartment,  $A_{n-1}$  is the amount of drug in the previous compartment,  $k_t$  is the transit rate constant, and  $k_a$  is the absorption rate constant for the  $n^{\text{th}}$  compartment. The ACAT model adopted the general structure of the CAT model while incorporating different states of drug component, the stomach and the colon compartments, different states of excreted material, and additional physicochemical and physiological factors that were not accounted for in the CAT model (Agoram et al., 2001). Similarly, the ADAM model was expanded upon the CAT model to account for additional factors such as the dissolution process, intestinal metabolism, active transport, and gut wall permeation (Jamei, Turner, et al., 2009). Regardless of the compartmental model structure for the GI tract, accurate predictions of oral drug absorption heavily depend on correct knowledge of transit times through the GI tract. Alterations in any compartment's transit time would result in changes in the  $k_t$  of that compartment, consequently impacting absorption in the respective compartment and contributing to changes in overall drug absorption and the PK profile. The transit time of a drug through the GI tract may depend on a variety of factors including formulation (liquid vs. solid), fed/fasted state, composition of food, age, sex, and disease status (Davis et al., 1986; Grybäck et al., 2000).

Ulcerative colitis (UC) is a form of inflammatory bowel disease that is associated with inflammation of the mucosa and submucosa of the colon and rectum. Common symptoms of active UC include diarrhea, rectal bleeding, abdominal pain, rectal pain, weight loss, and fever (Gajendran et al., 2019). GI transit time is reported to be prolonged and more variable in UC patients, compared with healthy adults (Table 1) (Effinger et al., 2019; Fireman et al., 2007; Fischer et al., 2017; Haase et al., 2016; Hardy et al., 1988; Nugent et al., 2000; Rana et al., 2013; Rao & Read, 1990). Therefore, a PBPK model adopting transit times representative of UC conditions would incorporate reduced  $k_t$  values compared to a healthy population model (Eq. 5), potentially leading to changes in PK predictions.

Altered GI pathology is commonly observed in human immunodeficiency virus (HIV)-infected patients because the GI tract is a major site of HIV replication. Moreover,

co-infections in HIV-infected patients can lead to diarrhea as well as other GI disorders. Previous studies on GI transit times in HIV-infected subjects revealed delayed gastric emptying and accelerated small bowel transit, especially in patients with protozoal diarrhea (Table 1) (Sharpstone et al., 1999). Similar GI transit time changes have been reported in children with severely symptomatic acquired immunodeficiency syndrome (AIDS) (Densupsoontorn et al., 2009). Accelerated whole-GI transit time in HIV-infected children was found to be significantly associated with malnutrition. The prevalence of GI transit time changes in various disease populations highlights the importance of having a tool to quantitatively evaluate the impact of these changes on oral drug PK.

### **3.4 Luminal fluid volume**

Not only does luminal fluid volume have a direct role in drug dissolution and permeation predictions, but it also impacts many of the previously discussed physiological parameters, including drug motility and luminal pH. Fluctuations in luminal pH due to changes in fluid volume can impact the ionization states of weakly acidic or weakly basic drugs, leading to changes in their solubility and ultimately their absorption. Altered bile acid concentrations in the luminal content may have a direct impact on the dissolution of lipophilic drugs. Furthermore, digestive enzyme concentrations depend on fluid volume as well, adding another layer of complexity to understanding the stability and dissolution of drugs in an altered GI environment. In addition, fluid volume affects the luminal drug concentration, thus impacting the drug concentration gradient driving diffusion between the luminal content and the enterocytes/blood. Lastly, changes in fluid secretion can affect the thickness and composition of the mucus layer lining the luminal surface, potentially compromising the barrier function of the mucus layer which may impact drug permeability (Johansson et al., 2013).

Changes in intestinal fluid secretion or absorption are common for diarrheal diseases. For instance, cholera is characterized by extensive fluid loss due to decreased absorption and increased secretion of fluid in the GI tract (Lee & Silverberg, 1972; Love, 1969). Therefore, luminal fluid volume is another important system parameter to consider when building PBPK models for diarrheal diseases. To capture the dynamic nature of luminal fluid volume for GI compartments, which can be both site-

specific and time-varying, a dynamic fluid model can be adopted when building PBPK models in GastroPlus® (Hens & Bolger, 2019).

### ***3.5 Transport and metabolism***

In addition to compromising epithelial integrity as discussed earlier, diarrheal diseases are also associated with alterations in the expression or activity of proteins involved in active transport and/or gut metabolism. For example, decreased P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) expression were observed for the colon and the rectum in patients with active UC (Blokzijl et al., 2007; Effinger et al., 2019; Englund et al., 2007). Altered intestinal expression of P-gp and cytochrome P450 (CYP) 3A4 have been reported for CD patients (Alrubia, Al-Majdoub, et al., 2022; Blokzijl et al., 2007; Plewka et al., 2014; Wilson et al., 2017, 2019). A reduction in CYP3A4 activity has also been observed in celiac disease patients in a clinical setting (Morón et al., 2013). While the importance of active transport and metabolism on oral drug PK cannot be overlooked or dismissed, the breadth of their respective research areas warrants separate discussions of their own. In this review, we focus on the potential of utilizing a PBPK modeling approach to capture the impact of the other aforementioned system parameters while acknowledging that PBPK models can be expanded to include additional disease-specific changes impacting drug metabolism and transport.

## **4. Examples of clinically significant diarrheal disease impact on PK**

With a basic appreciation of the numerous ways diarrheal diseases can impact oral drug PK, the question becomes whether it is clinically relevant to predict oral drug PK for patient populations coping with diarrhea. Are PK changes associated with diarrheal diseases remarkable enough to warrant separate PK predictions for these populations or are the impacts too miniscule to influence clinical practice? Learnings from a few published studies demonstrate the pressing need for making accurate PK predictions in populations affected by diarrhea.

#### **4.1 Oral cyclosporine A absorption is impaired in bone marrow transplant patients with diarrhea**

Cyclosporine A (CsA) was first approved in 1983 and is still used as a treatment to prevent organ rejection, graft-versus-host disease (GVHD) post-transplant, and for patients with autoimmune diseases (Galeazzi et al., 2006). The many years of use in the clinic is testimony to CsA's success, yet the first-generation oral formulation, Sandimmune®, demonstrated highly variable oral PK with oral bioavailability ranging from 2 to 89% (Beauchesne et al., 2007). Atkinson et al.'s study in 1984 examined CsA PK in relation to diarrhea of various causes for recipients of bone marrow transplants (Atkinson et al., 1984). The study observed that subjects with diarrhea had very minimal increases in serum CsA levels, regardless of the cause of diarrhea, following an oral dose. The area under the concentration-time curve (AUC) for CsA was  $1757 \pm 909$  ng\*h/mL for subjects with >500 mL of diarrhea in 72 hours compared to  $4616 \pm 3362$  ng\*h/mL for those with <500 mL of diarrhea in 72 hours and the difference in exposure was significant (p-value < 0.002). Due to impaired absorption in diarrhea subjects, it was challenging to achieve the target minimum trough concentration. Therefore, the authors recommended intravenous infusion of CsA for patients experiencing gut dysfunction. Another study in pediatric patients also reported low blood CsA concentration following diarrhea which required a dose increase to overcome this issue (Burckart et al., 1985).

As a narrow therapeutic index drug, it is crucial to dose CsA precisely to prevent transplant failure while minimizing toxicity. To improve the highly variable bioavailability of the original formulation, a microemulsion formulation, Neoral®, was developed to provide a rapid release of CsA that would improve CsA absorption and reduce the variability in oral bioavailability (Beauchesne et al., 2007; Singh & Narsipur, 2011). Novel delivery systems involving micelles, nanoparticles, or liposomes have also been developed to address issues with CsA absorption (Beauchesne et al., 2007; Goyal et al., 2015; Jain et al., 2011; H. Yu et al., 2013; C. Zhang et al., 2019).

As shown by the example of CsA, the substantial and potentially dangerous impacts of diarrhea symptoms on oral drug PK can drive changes in clinical practice and drug development. PBPK modeling can effectively inform and improve clinical



decisions and drug development efforts by incorporating formulation changes as drug-specific parameters and diarrhea physiology as system parameters to model the complex interplay of these factors. Gertz et al.'s PBPK model for the evaluation of CsA DDI potential successfully predicted CsA concentration-time profiles following oral administrations of different CsA formulations (Sandimmune® and Neoral®), providing an excellent example of how formulation knowledge can be utilized and illustrating the versatility of a PBPK modeling approach (Gertz et al., 2013). This model framework can be expanded to evaluate other CsA formulations and to make tailored predictions for various diarrheal disease populations.

#### ***4.2 Subtherapeutic antiretroviral plasma levels associated with diarrhea in AIDS patients***

HIV infection is often complicated by diarrhea and a compromised GI tract, and there is clinical evidence that these complications can impact plasma levels of antiretrovirals used to control HIV infection and prevent comorbidities. A clinical study in northeast Brazil compared stavudine and didanosine plasma concentrations between AIDS patients with (n=12) or without (n=7) diarrhea (Brantley et al., 2003). Between the six subjects with diarrhea and three controls without diarrhea all of whom had received at least 4 dosing intervals of stavudine at doses prescribed by their physicians, plasma stavudine levels were significantly lower for the group presenting with diarrhea. Alarmingly, subtherapeutic (<0.3 µg/mL) stavudine plasma levels were observed in five out of the six subjects with diarrhea, whereas all three control subjects without diarrhea had stavudine plasma levels of ~0.8 µg/mL. Similarly, plasma didanosine levels were lower in subjects with diarrhea. Three out of the four subjects with diarrhea who took didanosine had plasma levels below the limit of detection (<0.1 µg/mL). Although the only control subject who took didanosine also had a subtherapeutic plasma level of 0.5 µg/mL, it was substantially higher than those of the subjects with diarrhea.

While there were limitations to this study such as that study participants received variable antiretroviral regimens at different doses as prescribed by their physicians and that significant BMI differences existed between patient groups, the findings nonetheless highlight the potential impact diarrheal diseases may have on oral drug PK.

The observation that antiretroviral plasma levels could fall below therapeutic levels in diarrhea patients is alarming and possibly contributed to the significant difference observed in HIV viral load between the diarrhea group and the non-diarrhea group, where the median viral load was  $230 \times 10^3$  copies/mL for the diarrhea group and  $81 \times 10^3$  copies/mL for the non-diarrhea group. Thereafter, a randomized, double-blinded, placebo-controlled trial was conducted by the same group to evaluate the effect of glutamine and alanyl-glutamine for treating diarrhea and for improving antiretroviral drug PK in patients with AIDS (Bushen et al., 2004). It was found that glutamine and alanyl-glutamine treatment significantly improved diarrheal symptoms and antiretroviral drug levels, supporting a link between diarrhea and compromised antiretroviral drug absorption in HIV patients. Another study of antiretroviral zidovudine bioavailability in HIV-infected patients also reported a significant correlation between mild diarrhea and reduced zidovudine bioavailability (Macnab et al., 1993). However, conflicting evidence exists where a lack of significant association was found between zidovudine PK and the presence of diarrhea such that the story remains inconclusive (Macnab et al., 1996; Zorza et al., 1993).

Since diarrhea is a common co-infection in HIV-infected individuals (Colebunders et al., 1987; Knox et al., 2000; MacArthur & DuPont, 2012; Moreira Júnior et al., 1993), antiretroviral medications are often administered in the presence of diarrhea. Failure in achieving therapeutic antiretroviral levels due to the influence of diarrhea may result in worsening HIV infection status, further aggravating the GI complications forming a vicious cycle propagating treatment failure. Therefore, a mechanistic approach such as PBPK modeling that can be employed to inform dose adjustment for a diarrhea co-infected population may considerably increase the probability of treatment success.

#### **4.3 iOWH032 PK in cholera patients experiencing severe diarrhea**

iOWH032 is an inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel and was developed to treat cholera toxin-induced secretory diarrhea. A study compared the PK of iOWH032 following a single oral 300 mg dose in Bangladeshi cholera patients (n=14) and healthy Bangladeshi volunteers (n=8) (Kumar et al., 2014). When the study populations were compared, the

investigators observed that the mean  $AUC_{inf}$  was only 6,250 ng\*h/mL for cholera patients compared with 22,700 ng\*h/mL for healthy Bangladeshi volunteers. The mean  $C_{max}$  was also markedly lower for cholera patients at 482 ng/mL, in contrast to 1,280 ng/mL for healthy Bangladeshi volunteers (Fig. 2A). Moreover,  $T_{max}$  was earlier at 3.8 h in Bangladeshi cholera patients in comparison to 4.8 h in healthy Bangladeshi volunteers.

## **5. Applications of PBPK modeling for predicting the impact of pathophysiological changes in the GI on oral drug PK**

There are a few examples of PBPK models built for populations with GI disorders to improve PK prediction. Using budesonide (Crohn's disease) and levodopa extended-release (Parkinson's disease) as examples, we will illustrate how disease-associated pathophysiological changes for the GI were incorporated into the models and how these changes improved PK predictions for each drug.

### ***5.1 Budesonide PBPK model in healthy patients versus patients with Crohn's disease***

CD, a type of inflammatory bowel disease, is a non-curable disease impacting large numbers globally, with reported prevalence of up to 318.5 per 100,000 in North America and 262.0 per 100,000 in Northern Europe (Ng et al., 2017). As oral drug administration is common for treating CD as well as concomitant conditions, understanding oral drug PK in the CD patient population is very important. To assess oral drug PK differences between healthy subjects and CD patients, Effinger et al. chose budesonide as a model drug and constructed a PBPK model for a controlled-release budesonide formulation. The PBPK model incorporated drug release data collected with in vitro studies conducted under conditions reflecting CD (Effinger et al., 2021). To examine the impact of CD on the release of budesonide from the Entocort® formulation, the controlled-release formulation used in Effinger et al.'s study, biorelevant media representative of healthy and CD conditions accounting for differences in bile salt/lecithin concentration, stomach pH, colonic osmolality for both fasted and fed states were developed. Drug release was simulated with USP IV dissolution apparatuses with

adjusted flow rates and transit times to simulate CD conditions. The release tests demonstrated a similar extent of drug release in the fasted state and slightly lower release under CD conditions in the fed state.

Next, a minimal PBPK model with a single non-physiological adjusting compartment was developed, incorporating physicochemical properties available in literature. The CD population was developed using the Simcyp® healthy volunteer population as its basis with modifications reflecting pathophysiological changes in CYP3A4 abundance, human serum albumin concentration, gastric pH, GI transit time, and ileal absorptive surface area according to existing literature. The release study results were integrated into the PBPK model for the respective populations and conditions. The ratio of model-predicted versus observed  $C_{max}$  and  $AUC_{0-inf}$  was used for external validation of the final model, using a 2-fold criterion.

Parameter sensitivity analysis (PSA) revealed that reduction of hepatic CYP3A4 abundance would result in a substantial increase in budesonide  $C_{max}$  and AUC and reduced human serum albumin concentration would result in reduced budesonide  $C_{max}$  and AUC, highlighting the significance of these system parameters on budesonide PK. Changes in gastric pH, ileal absorptive surface area, and transit times, on the other hand, had limited effects on drug  $C_{max}$  and AUC. These findings illustrate the utility of PSA as a tool for assessing the magnitude of impact that various system parameter changes can potentially have on oral drug PK.

In the fasted state, the CD population developed by Effinger et al. successfully improved the  $C_{max}$  prediction up to 25% and AUC up to 32% compared to the healthy volunteer population. In the fed state, the AUC prediction was improved up to 24% with the CD population while  $C_{max}$  prediction accuracy was similar among CD and healthy simulations. Higher budesonide exposure was predicted for CD patients, in agreement with existing literature. This study demonstrates that adaptations made to PBPK models to reflect disease physiology could effectively capture changes in drug PK in a GI disease population. It also presents a workflow that can be adopted by future studies for making PK predictions in patients with other GI diseases.

More recently, a meta-analysis studying oral drug bioavailability changes in the presence of CD summarized available knowledge (up to October 2021) on CD-

associated physiological changes, including changes in drug metabolizing enzyme and transporter expression, GI motility, intestinal pH, blood flow, and protein binding (Alrubia, Mao, et al., 2022). Physiological changes as revealed by the meta-analysis were used to build a CD population in Simcyp® that incorporated the M-ADAM absorption model option. PBPK model performance was verified using available published budesonide and midazolam data for virtual healthy volunteer, active CD, and inactive CD populations. For both budesonide and midazolam, simulations for the inactive CD population produced greater than twofold prediction error. The active CD model assuming normal albumin levels captured the observed budesonide data within twofold but was inadequate for the midazolam data. While the PBPK model outcomes were unable to accurately predict the observed budesonide and midazolam PK in some if not all CD patients, this study sets a great example of how PBPK modeling can be implemented. Moreover, the shortcoming of the final PBPK models reveals many challenges that need to be overcome to successfully build a PBPK model to estimate the impact on oral drug PK due to CD as well as other diseases with diarrheal symptoms. Namely, thorough knowledge associated with disease physiology is necessary for model building. In addition, abundant and adequately designed clinical study data are also essential for model verification.

## ***5.2 Extended-release (ER) levodopa PBPK model in Parkinson's disease patients***

While Parkinson's disease is a progressive neurodegenerative disease normally associated with motor impairments such as tremor and rigid muscles, it is commonly accompanied by GI symptoms such as abdominal pain, gastroparesis, and constipation affecting nearly all regions of the GI tract (Fasano et al., 2015; Poirier et al., 2016; Wollmer & Klein, 2017). Constipation has been reported to be present in 7-71% of patients with Parkinson's disease across studies (Fasano et al., 2015). Similar to diarrheal diseases, pathophysiological changes in the GI tract associated with Parkinson's disease may also lead to oral drug PK changes. Levodopa, a dopamine precursor, is often prescribed for the management of Parkinson's disease. However, unpredictable and highly variable PK has been reported for levodopa, necessitating better PK predictions for oral levodopa formulations to improve treatment efficacy and

safety (LeWitt et al., 2009; Nyholm & Lennernäs, 2008; Stocchi, 2006). To further the understanding of levodopa controlled-release formulation dissolution and absorption in Parkinson's disease-altered GI environment, Wollmer and Klein developed in vitro Parkinson's disease-specific release models and integrated the obtained release data into a Parkinson's disease PBPK model (Wollmer & Klein, 2022).

Three different oral ER levodopa formulations, Nacom® ER, Madopar® Depot, and Rytary®, were studied by Wollmer and Klein. Instead of building a Parkinson's disease physiology within a PBPK model to predict drug dissolution, Wollmer and Klein experimentally determined the impact of disease-associated physiological changes in GI motility, residence times, and fluid volumes on drug release through measuring release profiles of various levodopa formulations using in vitro systems that mimic GI passage in Parkinson's disease patients. The in vitro release data of each drug formulation under disease conditions was subsequently incorporated into a whole-body PBPK model developed in PK-Sim®, along with other drug and system parameters from model predictions or existing literature. Default physiology was adopted apart from salivary flow rate and GI transit.

Remarkable improvements in PBPK model simulations were obtained after integrating the in vitro Parkinson's disease condition release data, as opposed to using the healthy adult condition release profiles. For both Rytary® and Nacom® ER formulations, the predicted-to-observed  $C_{max}$  ratio improved from approximately 2 using average healthy adult dissolution data to less than 1.10 using Parkinson's disease patient-specific dissolution data, as shown by external validation against published in vivo study data. Similarly, massive improvements in both  $C_{max}$  and AUC prediction accuracy were observed for Madopar® Depot. Model validation using observed data from two available studies yielded  $C_{max}$  predicted-to-observed ratios of 1.00 and 0.87 with the disease model, compared to those of 2.83 and 2.03 using the healthy model. AUC ratios were 0.92 and 1.03 with the disease model compared with 2.12 and 2.69 using the healthy model. These findings verified that disease-associated changes in GI motility, transit times, and the extent of drug contact with GI fluids can impact drug dissolution profiles and that PBPK models developed to reflect these changes had superior predictive power than a general model based on an average healthy population.

The significance of this study extends beyond Parkinson's disease; it showcases the viability and value of applying a disease-specific PBPK modeling approach. The methodology can be borrowed and adapted to studying diarrheal disease impact on oral drug PK, which also involves altered dissolution processes as discussed in previous sections of this review.

## **6. Case study: using PBPK modeling to capture the impact of cholera-associated pathophysiological changes on iOWH032 pharmacokinetics**

As mentioned in Section 4.3, iOWH032 PK was notably different between healthy volunteers and cholera patients (Fig. 2A). Therefore, using iOWH032 as an example, we aimed to demonstrate the impact incorporating cholera-associated physiological changes can have on PBPK model predictions.

Drug-specific parameters for the iOWH032 PBPK model were predicted with MedChem Designer™ (version 6.3.0.4, Simulations Plus, Lancaster, CA) (Table 2). PK parameters were obtained by fitting a 1-compartment model to digitized healthy Bangladeshi volunteer data from Kumar et al.'s iOWH032 PK study (Kumar et al., 2014), using the PKPlus™ module in GastroPlus® (version 9.8.2) (Table 2). The GastroPlus® healthy fasted physiology was first used to simulate a trial of 100 participants (Table S1). The simulation results were overlaid with observed median iOWH032 plasma concentration data from healthy Bangladeshi volunteer, extracted from Kumar et al.'s study (Fig. 2B) (Kumar et al., 2014). The PBPK model with the healthy fasted physiology reasonably captured the observed PK in a healthy population, as the simulated profile is closely aligned with the observed data. However, when PBPK model simulations using the healthy physiology were compared with observed data in Bangladeshi cholera patients, the model clearly overpredicted iOWH032 exposure with simulated-to-observed ratios of 2.68 for  $C_{max}$  and 3.93 for  $AUC_{0-inf}$  (Fig. 2C; Table 3). Therefore, it was evident that the PBPK model with a healthy physiology was not applicable to making PK predictions in cholera patients. Consequently, several different cholera models were attempted to improve PBPK model performance by introducing changes in system parameters based on available literature.

During acute cholera infection, observed changes in the GI tract include pH increase, transit time reduction, villous blunting, and fluid volume increase (Dunmire et al., 2022; C. T. L. Huang et al., 1982; Molla et al., 1983; Sack et al., 2004). Hence, separate cholera physiology models were constructed to incorporate each of these changes or combinations of two or more (Table 3, S1). For pH changes, the pH of the small intestine compartments was increased to 7.6 to match the observed median pH in vomit samples from cholera patients (Dunmire et al., 2022). Likewise, the pH of the ascending colon was increased to 8.5 to match the observed median pH in stool samples from cholera patients (Dunmire et al., 2022). A previous study suggested that whole-gut transit time during acute diarrhea can be reduced to ~5.5 h (Molla et al., 1983). Therefore, when transit time reduction was applied to a cholera model, the transit times for GI compartments starting from the duodenum to the ascending colon were reduced by the same proportion such that the total transit time from the stomach to the ascending colon was 5.5 h. Lastly, the fluid output in cholera patients was reported to be approximately 1 L/h (Lippi et al., 2016; Sack et al., 2004). To reflect this increase in fluid volume in a cholera model, the luminal volume of the ascending colon was increased in relation to the transit time through increasing the radius of the lumen to produce an output of 1 L/h. The luminal volumes of the other compartments were increased in a first-order fashion starting from the duodenum to mimic the gradual fold increase in fluid volume through the GI tract due to altered secretion and/or absorption processes. One caveat for increasing GI luminal radius to account for luminal volume increase is that the model-estimated available absorptive surface area for each compartment would also be increased proportionally to the increase in luminal radius. This is unlikely to be reflective of true disease physiology. Hence, the absorption scale factor (ASF) of each compartment was adjusted by the inverse of the fold increase in radius to keep the absorptive surface area unchanged. Percent fluid in luminal content was set at 100% for both the small and the large intestine. Bile salt concentration was reduced to account for change in volume for each compartment because the total amount of excreted bile salts does not change with cholera diarrhea (C. T. L. Huang et al., 1982).



Villous blunting, a pathological change where intestinal villi become shortened, is a phenomenon that has been observed with many diarrheal diseases including cholera (Martins et al., 2016; Rosenberg, 2003; Saha et al., 2006; Slavik & Lauwers, 2018; Uzzan et al., 2016; Yan & Sundaram, 2013). Although the degree of blunting may be highly variable, as seen in other GI diseases (Martins et al., 2016; Slavik & Lauwers, 2018), and quantitative knowledge of villous blunting is lacking for cholera, its potential impact was explored by reducing the surface area enhancement factor (SEF) by up to 4-fold (bounded by a lower limit of 1) in cholera disease models (Table 3, S1).

For each cholera model, iOWH032 concentration versus time profiles were simulated in 100 subjects. The  $AUC_{0-inf}$ ,  $C_{max}$ , and  $T_{max}$  values were calculated and compared to the observed mean values reported by Kumar et al. (Table 3) (Kumar et al., 2014). The cholera model with changes in transit time, luminal volume, and SEF produced the best PBPK model predictions (Table 3; Fig. 2D). The cholera model incorporating changes in transit time, luminal volume, and SEF greatly reduced the  $C_{max}$  and  $AUC_{0-inf}$  simulated-to-observed ratios to 1.47 and 1.82, respectively, predicting an approximately 54% reduction in iOWH032 exposure compared with the healthy fasted model and thus illustrating the impact these parameters likely have on iOWH032 PK (Table 3; Fig. 2D). These results demonstrate that when pathophysiological changes were applied to building disease specific PBPK models, the predictive power can be substantially improved.

Although the cholera models did not fully capture the observed PK, we only tested changes to four parameters and many assumptions had to be made due to the limited availability of both drug specific and cholera pathophysiology data in the literature. For instance, the GI luminal pH in the cholera physiology models could only be inferred from available vomit and stool sample pH data of cholera patients because direct measurements were not available/feasible (Dunmire et al., 2022). Similar to the budesonide example described in Section 5.1, in vitro dissolution studies with simulated intestinal fluid reflecting conditions associated with cholera infection may improve the predictive power of the model. Moreover, the remaining unexplained reduction in iOWH032 exposure suggests that there may be other physiological processes accountable for the observed PK changes and further highlights that thorough disease

knowledge is essential to inform an accurate model. For example, transport and metabolism are key processes that may impact a drug's PK profile. However, it is unclear how transporter and metabolic enzyme expression and activity change with cholera. Hence, the performance of the current model is restrained by the inadequacy of disease physiology knowledge. Alrubia et al., who recently took up the challenge to build a PBPK model in CD for oral drug PK prediction also experienced similar challenges where their CD model performance was curbed by the limited availability of clinical and proteomic data (Alrubia, Mao, et al., 2022). This calls for future in vitro and in vivo studies to quantitatively determine disease-specific system parameters for improving the predictive performance and confidence in disease PBPK models.

## **7. Current challenges, knowledge gaps, and future directions**

### ***7.1 Characterizing pathophysiology of diarrheal diseases***

Although diarrhea is a common symptom for many GI disorders, its etiology, severity, duration, and pathophysiology can vary greatly. For instance, diarrhea can be categorized as inflammatory, fatty, or watery (Sweetser, 2012). Inflammatory diarrhea is marked by the presence of leukocytes or leukocyte proteins in the stool and is associated with inflamed mucosa, such as that caused by ischemic colitis. Fatty diarrhea is due to malabsorption and maldigestion, such as that caused by celiac disease and cirrhosis. Watery diarrhea can be further classified as either osmotic or secretory (Sweetser, 2012; Whyte & Jenkins, 2012). Osmotic diarrhea is caused by the presence of poorly absorbable yet osmotically active solutes in the GI lumen, resulting in fluid movement into the GI lumen in response to the osmotic gradient. Secretory diarrhea, on the other hand, involves increased secretion and/or impaired absorption of water and electrolytes. The region of the GI tract impacted by diarrheal diseases can be limited or diffused. For example, while CD and UC are both types of inflammatory bowel disease, CD can affect any region along the GI tract and result in inflammation that penetrates all intestinal layers whereas UC is localized to the colon (including the rectum and anus) and affects only the mucosal layer of the colon (Saeid Seyedian et al.,

2019). The diversity and complexity of diarrhea precludes building a uniform “diarrhea population” for PBPK modeling of all diarrheal diseases.

Since physiological changes associated with any diarrheal disease are manifold, a thorough understanding of each disease is critical to accurately capture all relevant changes for PBPK modeling. However, these studies will likely be resource-intensive, time-consuming, and sometimes impractical. To best allocate available resources, PSA can be performed to screen the most impactful system parameters for further evaluation, as illustrated by the case examples presented in Sections 5 and 6 (McNally et al., 2011). As many of the input parameters for PBPK models are obtained experimentally, variability and uncertainty associated with experimental measurements would be introduced into PBPK models. The sensitive model parameters identified by PSA that cannot be accurately and precisely measured should be further assessed with an uncertainty analysis, in order to estimate the associated uncertainty quantitatively (Fàbrega et al., 2016; Peters & Dolgos, 2019). Nevertheless, uncertainty cannot be eliminated from the PBPK modeling approach. The more specific and highly parametric the modeled population is, the more uncertainty there may be around model predictions resulting in a greater range of model outputs, as errors could have been introduced with every disease-specific system parameter integrated into the model.

## **7.2 Model validation**

While plasma concentration data are routinely used for PBPK model validation, human tissue concentration data are rarely available. Not only does the lack of tissue concentration data complicate oral absorption model validation, but it also complicates pharmacokinetic-pharmacodynamic (PK/PD) model development because the effect sites for drugs targeting diarrheal diseases are often in the GI tissue. For example, budesonide, a corticosteroid for the treatment of CD, has high topical anti-inflammatory activity but low systemic activity due to extensive first-pass metabolism (Greenberg et al., 1994). To make this already challenging task more daunting, diarrheal diseases can impact different regions and layers of the GI tract so that the sites of action for drugs targeting GI conditions are disease-specific and cannot be simply bundled as a single tissue type. Although the commonly adopted absorption models divide the GI tract into

multiple sections allowing modifications to be made to each section independently, the task of validating model predictions for different sections of the GI tract is extremely challenging if not impossible.

Although the feasibility of using plasma data for PBPK model validation is tempting, solely relying on plasma concentration data may be misleading and dangerous in the case of diarrheal diseases. Because the tissue volume for the gut is relatively small, typically set at 4.5% of total body volume for human PBPK models (Clewell & Clewell, 2008), the gut's impact on overall volume of distribution and plasma drug PK can be very small unless the drug of interest extensively partitions into the GI tissue. Therefore, inaccuracies in GI tissue PK predictions may not be reflected by plasma PK or the magnitude of prediction error may be mistakenly deemed negligible. Acceptable model prediction as determined by external validation using plasma data may result in false optimism and overconfidence about the model. Furthermore, diarrheal disease impact on oral drug PK at the tissue level, which is potentially directly correlated with a drug's efficacy and safety, may be underestimated without accurate tissue PK predictions.

Alternatively, fecal concentration data is often suggested as a surrogate of drug PK in the GI tract. The major advantage of this approach is that fecal sample collection is non-invasive and can be performed either in clinical settings or in the comfort of one's home. However, there are many confounding factors and limitations to using fecal concentration data. Drugs detected in the feces could be from multiple sources, including unabsorbed, undissolved or precipitated drugs in the GI lumen. In addition, drug levels in the feces may be products of enterohepatic circulation or impacted by efflux transporters (Roberts et al., 2002). In recent years, studies on gut microbiota have revealed that the diversity and abundance of gut microbiome are correlated with drug metabolism and can affect fecal drug concentration (Jarmusch et al., 2020; Tsunoda et al., 2021). Therefore, fecal drug concentration is not an accurate measure of GI luminal or tissue drug concentration and is prone to the influence of a plethora of factors.

### ***7.3 Implication and uses of model predictions***

Given the limitations and challenges of applying PBPK modeling for oral drug PK predictions in populations with diarrheal diseases, one should be cautious how model predictions are interpreted and the extent to which they can be relied on. More specifically, for a model that is only validated with plasma data while the effect site is in a GI tissue, its tissue level predictions may not be accurate, and thus predictions of efficacy or safety associated with a certain dose may deviate substantially from the true value. This can be particularly dangerous for narrow therapeutic index drugs. Furthermore, since plasma samples are usually collected for therapeutic drug monitoring, subtherapeutic or elevated tissue drug levels may not be detected. This may result in valuable time and resource being lost, an effective (at the correct dosage) treatment option mistakenly determined as ineffective, and adverse effects that could have been avoided had an accurate dose been given.

## **8. Conclusions**

PBPK modeling is a powerful tool for understanding and predicting the impact diarrheal diseases may have on oral drug PK, which have been largely overlooked to date. When used appropriately, PBPK modeling can support candidate selection, inform dose optimization, save resources, and maximize the chance of success for relevant drugs. While there are major hurdles that require substantial effort and resources to address in this area, we hope this review inspires future PBPK modeling work to improve treatments for populations with diarrheal diseases.

## **Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Zhang, C. X., Arnold, S. L. M.

## References:

- A. van Herwaarden, M. S. A. J., M. (1999). 24-h Recording of Intra-gastric pH: Technical Aspects and Clinical Relevance. *Scandinavian Journal of Gastroenterology*, 34(230), 9–16. <https://doi.org/10.1080/003655299750025219>
- Abouir, K., Samer, C. F., Gloor, Y., Desmeules, J. A., & Daali, Y. (2021). Reviewing Data Integrated for PBPK Model Development to Predict Metabolic Drug-Drug Interactions: Shifting Perspectives and Emerging Trends. *Frontiers in Pharmacology*, 12. <https://doi.org/10.3389/fphar.2021.708299>
- Abuhelwa, A. Y., Foster, D. J. R., & Upton, R. N. (2016). A Quantitative Review and Meta-Models of the Variability and Factors Affecting Oral Drug Absorption—Part I: Gastrointestinal pH. *The AAPS Journal*, 18(5), 1309–1321. <https://doi.org/10.1208/s12248-016-9952-8>
- Abuhelwa, A. Y., Williams, D. B., Upton, R. N., & Foster, D. J. R. (2017). Food, gastrointestinal pH, and models of oral drug absorption. *European Journal of Pharmaceutics and Biopharmaceutics*, 112, 234–248. <https://doi.org/10.1016/j.ejpb.2016.11.034>
- Agoram, B., Woltosz, W. S., & Bolger, M. B. (2001). Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Advanced Drug Delivery Reviews*, 50, S41–S67. [https://doi.org/10.1016/S0169-409X\(01\)00179-X](https://doi.org/10.1016/S0169-409X(01)00179-X)
- Alrubia, S., Al-Majdoub, Z. M., Achour, B., Rostami-Hodjegan, A., & Barber, J. (2022). Quantitative Assessment of the Impact of Crohn's Disease on Protein Abundance of Human Intestinal Drug-Metabolising Enzymes and Transporters. *Journal of Pharmaceutical Sciences*, 111(10), 2917–2929. <https://doi.org/10.1016/j.xphs.2022.07.012>
- Alrubia, S., Mao, J., Chen, Y., Barber, J., & Rostami-Hodjegan, A. (2022). Altered Bioavailability and Pharmacokinetics in Crohn's Disease: Capturing Systems Parameters for PBPK to Assist with Predicting the Fate of Orally Administered Drugs. *Clinical Pharmacokinetics*, 61(10), 1365–1392. <https://doi.org/10.1007/s40262-022-01169-4>
- Arnold, S. L. M., & Isoherranen, N. (2022). Role of Pharmacokinetics and Pharmacokinetic Modeling in Drug Development. In *Comprehensive Pharmacology* (pp. 743–768). Elsevier. <https://doi.org/10.1016/B978-0-12-820472-6.00066-9>
- Atkinson, K., Biggs, J. C., Britton, K., Short, R., Mrongovius, R., and, A. C., & Dodds, A. (1984). Oral administration of cyclosporin A for recipients of allogeneic marrow transplants: implications of clinical gut dysfunction. *British Journal of Haematology*, 56(2), 223–231. <https://doi.org/10.1111/j.1365-2141.1984.tb03950.x>
- Bajor, A., Gillberg, P.-G., & Abrahamsson, H. (2010). Bile acids: short and long term effects in the intestine. *Scandinavian Journal of Gastroenterology*, 45(6), 645–664. <https://doi.org/10.3109/00365521003702734>
- Batman, P. A., Kotler, D. P., Kapembwa, M. S., Booth, D., Potten, C. S., Orenstein, J. M., Scally, A. J., & Griffin, G. E. (2007). HIV enteropathy: crypt stem and transit cell hyperproliferation induces villous atrophy in HIV/Microsporidia-infected jejunal mucosa. *AIDS*, 21(4), 433–439. <https://doi.org/10.1097/QAD.0b013e3280142ee8>

- Beauchesne, P. R., Chung, N. S. C., & Wasan, K. M. (2007). Cyclosporine A: A Review of Current Oral and Intravenous Delivery Systems. *Drug Development and Industrial Pharmacy*, 33(3), 211–220. <https://doi.org/10.1080/03639040601155665>
- Blokzijl, H., Borght, S. Vander, Bok, L. I. H., Libbrecht, L., Geuken, M., van den Heuvel, F. A. J., Dijkstra, G., Roskams, T. A. D., Moshage, H., Jansen, P. L. M., & Faber, K. N. (2007). Decreased P-glycoprotein (P-gp/MDR1) expression in inflamed human intestinal epithelium is independent of PXR protein levels. *Inflammatory Bowel Diseases*, 13(6), 710–720. <https://doi.org/10.1002/ibd.20088>
- Brantley, R. K., Williams, K. R., Silva, T. M. J., Siström, M., Thielman, N. M., Ward, H., Lima, A. A. M., & Guerrant, R. L. (2003). AIDS-associated diarrhea and wasting in northeast Brazil is associated with subtherapeutic plasma levels of antiretroviral medications and with both bovine and human subtypes of *Cryptosporidium parvum*. *Brazilian Journal of Infectious Diseases*, 7(1). <https://doi.org/10.1590/S1413-86702003000100003>
- Brunner, E. (1904). Reaktionsgeschwindigkeit in heterogenen Systemen. *Zeitschrift Für Physikalische Chemie*, 47U(1), 56–102. <https://doi.org/10.1515/zpch-1904-4705>
- Burckart, G., Starzl, T., Williams, L., Sanghvi, A., Gartner, C., Venkataramanan, R., Zitelli, B., Malatack, J., Urbach, A., Diven, W., Ptachcinski, R., Shaw, B., & Iwatsuki, S. (1985). Cyclosporine Monitoring and Pharmacokinetics in Pediatric Liver Transplant Patients. *Transplantation Proceedings*, 17(1), 1172–1175.
- Bushen, O. Y., Davenport, J. A., Lima, A. B., Piscitelli, S. C., Uzgiris, A. J., Silva, T. M. J., Leite, R., Kosek, M., Dillingham, R. A., Girao, A., Lima, A. A. M., & Guerrant, R. L. (2004). Diarrhea and Reduced Levels of Antiretroviral Drugs: Improvement with Glutamine or Alanyl-Glutamine in a Randomized Controlled Trial in Northeast Brazil. *Clinical Infectious Diseases*, 38(12), 1764–1770. <https://doi.org/10.1086/421394>
- Camilleri, M. (2015). Bile Acid Diarrhea: Prevalence, Pathogenesis, and Therapy. *Gut and Liver*, 9(3). <https://doi.org/10.5009/gnl14397>
- Castiglione, F., Del Vecchio Blanco, G., Rispo, A., Petrelli, G., Amalfi, G., Cozzolino, A., Cuccaro, I., & Mazzacca, G. (2000). Orocecal Transit Time and Bacterial Overgrowth in Patients with Crohn's Disease. *Journal of Clinical Gastroenterology*, 31(1), 63–66. <https://doi.org/10.1097/00004836-200007000-00015>
- Caviglia, G. P., Dughera, F., Ribaldone, D. G., Rosso, C., Abate, M. L., Pellicano, R., Bresso, F., Smedile, A., Saracco, G. M., & Astegiano, M. (2019). Serum zonulin in patients with inflammatory bowel disease: a pilot study. *Minerva Medica*, 110(2). <https://doi.org/10.23736/S0026-4806.18.05787-7>
- Charifson, P. S., & Walters, W. P. (2014). Acidic and Basic Drugs in Medicinal Chemistry: A Perspective. *Journal of Medicinal Chemistry*, 57(23), 9701–9717. <https://doi.org/10.1021/jm501000a>
- Chiarioni, G., Bassotti, G., Germani, U., Battaglia, E., Brentegani, M. T., Morelli, A., & Vantini, I. (1997). Gluten-free diet normalizes mouth-to-cecum transit of a caloric meal in adult patients with celiac disease. *Digestive Diseases and Sciences*, 42(10), 2100–2105. <https://doi.org/10.1023/a:1018878703699>
- Chu, X., Prasad, B., Neuhoff, S., Yoshida, K., Leeder, J. S., Mukherjee, D., Taskar, K., Varma, M. V. S., Zhang, X., Yang, X., & Galetin, A. (2022). Clinical Implications of Altered Drug Transporter Abundance/Function and PBPK Modeling in Specific

- Populations: An ITC Perspective. *Clinical Pharmacology & Therapeutics*, 112(3), 501–526. <https://doi.org/10.1002/cpt.2643>
- Clewell, R. A., & Clewell, H. J. (2008). Development and specification of physiologically based pharmacokinetic models for use in risk assessment. *Regulatory Toxicology and Pharmacology*, 50(1), 129–143. <https://doi.org/10.1016/j.yrtph.2007.10.012>
- Colebunders, R., Francis, H., Mann, J. M., Bila, K. M., Izaley, L., Kimputu, L., Behets, F., Van der Groen, G., Quinn, T. C., & Curran, J. W. (1987). Persistent diarrhea, strongly associated with HIV infection in Kinshasa, Zaire. *The American Journal of Gastroenterology*, 82(9), 859–864.
- Conlon, C. P., Pinching, A. J., Perera, C. U., Moody, A., Luo, N. P., & Lucas, S. B. (1990). HIV-related enteropathy in Zambia: a clinical, microbiological, and histological study. *The American Journal of Tropical Medicine and Hygiene*, 42(1), 83–88. <https://doi.org/10.4269/ajtmh.1990.42.83>
- Dahlgren, D., Roos, C., Sjögren, E., & Lennernäs, H. (2015). Direct In Vivo Human Intestinal Permeability (Peff) Determined with Different Clinical Perfusion and Intubation Methods. *Journal of Pharmaceutical Sciences*, 104(9), 2702–2726. <https://doi.org/10.1002/jps.24258>
- Davis, S. S., Hardy, J. G., & Fara, J. W. (1986). Transit of pharmaceutical dosage forms through the small intestine. *Gut*, 27(8), 886–892. <https://doi.org/10.1136/gut.27.8.886>
- Densupsoontorn, N., Issaragraiseel, P., Thamonsiri, N., Wongarn, R., & Jirapinyo, P. (2009). Whole gastrointestinal transit time is associated with clinical severity and nutritional status of HIV-infected children. *Journal of the Medical Association of Thailand = Chotmaihet Thangphaet*, 92(7), 914–919.
- Dolton, M. J., Chiang, P.-C., Ma, F., Jin, J. Y., & Chen, Y. (2020). A Physiologically Based Pharmacokinetic Model of Vismodegib: Deconvoluting the Impact of Saturable Plasma Protein Binding, pH-Dependent Solubility and Nonsink Permeation. *The AAPS Journal*, 22(5), 117. <https://doi.org/10.1208/s12248-020-00503-7>
- Dunmire, C. N., Chac, D., Chowdhury, F., Khan, A. I., Bhuiyan, T. R., LaRocque, R. C., Akter, A., Amin, M. A., Ryan, E. T., Qadri, F., & Weil, A. A. (2022). *Vibrio cholerae* Isolation from Frozen Vomitus and Stool Samples. *Journal of Clinical Microbiology*, 60(10). <https://doi.org/10.1128/jcm.01084-22>
- Edsbäcker, S., Bengtsson, B., Larsson, P., Lundin, P., Nilsson, Å., Ulmius, J., & Wollmer, P. (2003). A pharmacoscintigraphic evaluation of oral budesonide given as controlled-release (Entocort) capsules. *Alimentary Pharmacology & Therapeutics*, 17(4), 525–536. <https://doi.org/10.1046/j.1365-2036.2003.01426.x>
- Effinger, A., O'Driscoll, C. M., McAllister, M., & Fotaki, N. (2019). Impact of gastrointestinal disease states on oral drug absorption – implications for formulation design – a PEARRL review. *Journal of Pharmacy and Pharmacology*, 71(4), 674–698. <https://doi.org/10.1111/jphp.12928>
- Effinger, A., O'Driscoll, C. M., McAllister, M., & Fotaki, N. (2021). Predicting budesonide performance in healthy subjects and patients with Crohn's disease using biorelevant in vitro dissolution testing and PBPK modeling. *European Journal of Pharmaceutical Sciences*, 157, 105617. <https://doi.org/10.1016/j.ejps.2020.105617>



- EMA. (2018, December 13). *Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation*. European Medicines Agency. [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-physiologically-based-pharmacokinetic-pbpbk-modelling-simulation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-physiologically-based-pharmacokinetic-pbpbk-modelling-simulation_en.pdf)
- Englund, G., Jacobson, A., Rorsman, F., Artursson, P., Kindmark, A., & Rönnblom, A. (2007). Efflux transporters in ulcerative colitis. *Inflammatory Bowel Diseases*, 13(3), 291–297. <https://doi.org/10.1002/ibd.20030>
- Ewe, K., Schwartz, S., Petersen, S., & Press, A. G. (1999). Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. *Digestive Diseases and Sciences*, 44(7), 1434–1439. <https://doi.org/10.1023/A:1026664105112>
- Ezuruike, U., Zhang, M., Pansari, A., De Sousa Mendes, M., Pan, X., Neuhoff, S., & Gardner, I. (2022). Guide to development of compound files for PBPK modeling in the Simcyp population-based simulator. *CPT: Pharmacometrics & Systems Pharmacology*, 11(7), 805–821. <https://doi.org/10.1002/psp4.12791>
- Fàbrega, F., Nadal, M., Schuhmacher, M., Domingo, J. L., & Kumar, V. (2016). Influence of the uncertainty in the validation of PBPK models: A case-study for PFOS and PFOA. *Regulatory Toxicology and Pharmacology*, 77, 230–239. <https://doi.org/10.1016/j.yrtph.2016.03.009>
- Fadda, H. M., Hellström, P. M., & Webb, D.-L. (2022). Intra- and inter-subject variability in gastric pH following a low-fat, low-calorie meal. *International Journal of Pharmaceutics*, 625, 122069. <https://doi.org/10.1016/j.ijpharm.2022.122069>
- Fallingborg, J., Christensen, L. A., Jacobsen, B. A., & Rasmussen, S. N. (1993). Very low intraluminal colonic pH in patients with active ulcerative colitis. *Digestive Diseases and Sciences*, 38(11), 1989–1993. <https://doi.org/10.1007/BF01297074>
- Fallingborg, J., Pedersen, P., & Jacobsen, B. A. (1998). Small Intestinal Transit Time and Intraluminal pH in Ileocecal Resected Patients with Crohn's Disease. *Digestive Diseases and Sciences*, 43(4), 702–705. <https://doi.org/10.1023/A:1018893409596>
- Farrugia, A., & Arasaradnam, R. (2021). Bile acid diarrhoea: pathophysiology, diagnosis and management. *Frontline Gastroenterology*, 12(6), 500–507. <https://doi.org/10.1136/flgastro-2020-101436>
- Fasano, A., Visanji, N. P., Liu, L. W. C., Lang, A. E., & Pfeiffer, R. F. (2015). Gastrointestinal dysfunction in Parkinson's disease. *The Lancet Neurology*, 14(6), 625–639. [https://doi.org/10.1016/S1474-4422\(15\)00007-1](https://doi.org/10.1016/S1474-4422(15)00007-1)
- FDA. (2018, September). *Physiologically Based Pharmacokinetic Analyses — Format and Content Guidance for Industry*. U.S. Food & Drug Administration. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/physiologically-based-pharmacokinetic-analyses-format-and-content-guidance-industry>
- Fireman, Z., Kopelman, Y., Friedman, S., Ephrath, H., Choman, E., Debby, H., & Eliakim, R. (2007). Age and Indication for Referral to Capsule Endoscopy Significantly Affect Small Bowel Transit Times: The Given Database. *Digestive Diseases and Sciences*, 52(10), 2884–2887. <https://doi.org/10.1007/s10620-007-9789-1>
- Fischer, M., Siva, S., Wo, J. M., & Fadda, H. M. (2017). Assessment of Small Intestinal Transit Times in Ulcerative Colitis and Crohn's Disease Patients with Different

- Disease Activity Using Video Capsule Endoscopy. *AAPS PharmSciTech*, 18(2), 404–409. <https://doi.org/10.1208/s12249-016-0521-3>
- Gajendran, M., Loganathan, P., Jimenez, G., Catinella, A. P., Ng, N., Umapathy, C., Ziade, N., & Hashash, J. G. (2019). A comprehensive review and update on ulcerative colitis. *Disease-a-Month*, 65(12), 100851. <https://doi.org/10.1016/j.disamonth.2019.02.004>
- Galeazzi, M., Bellisai, F., Manganelli, S., Morozzi, G., & Sebastiani, G. D. (2006). Cyclosporine A for the treatment of autoimmune disorders in HCV infected patients. *Autoimmunity Reviews*, 5(7), 493–498. <https://doi.org/10.1016/j.autrev.2006.01.002>
- Gaohua, L., Miao, X., & Dou, L. (2021). Crosstalk of physiological pH and chemical pKa under the umbrella of physiologically based pharmacokinetic modeling of drug absorption, distribution, metabolism, excretion, and toxicity. *Expert Opinion on Drug Metabolism & Toxicology*, 17(9), 1103–1124. <https://doi.org/10.1080/17425255.2021.1951223>
- Geraghty, J., Thumbs, A., Kankwatira, A., Andrews, T., Moore, A., Malamba, R., Mtunthama, N., Hellberg, K., Kalongolera, L., O'Toole, P., Varro, A., Pritchard, D. M., & Gordon, M. (2015). Helicobacter pylori, HIV and Gastric Hypochlorhydria in the Malawian Population. *PLOS ONE*, 10(8), e0132043. <https://doi.org/10.1371/journal.pone.0132043>
- Gertz, M., Cartwright, C. M., Hobbs, M. J., Kenworthy, K. E., Rowland, M., Houston, J. B., & Galetin, A. (2013). Cyclosporine Inhibition of Hepatic and Intestinal CYP3A4, Uptake and Efflux Transporters: Application of PBPK Modeling in the Assessment of Drug-Drug Interaction Potential. *Pharmaceutical Research*, 30(3), 761–780. <https://doi.org/10.1007/s11095-012-0918-y>
- Goyal, R., Macri, L., & Kohn, J. (2015). Formulation Strategy for the Delivery of Cyclosporine A: Comparison of Two Polymeric Nanospheres. *Scientific Reports*, 5(1), 13065. <https://doi.org/10.1038/srep13065>
- Greenberg, G. R., Feagan, B. G., Martin, F., Sutherland, L. R., Thomson, A., Williams, C. N., Nilsson, L.-G., & Persson, T. (1994). Oral Budesonide for Active Crohn's Disease. *New England Journal of Medicine*, 331(13), 836–841. <https://doi.org/10.1056/NEJM199409293311303>
- Grybäck, P., Hermansson, G., Lyrenäs, E., Beckman, K.-W., Jacobsson, H., & Hellström, P. M. (2000). Nationwide standardisation and evaluation of scintigraphic gastric emptying: reference values and comparisons between subgroups in a multicentre trial. *European Journal of Nuclear Medicine and Molecular Imaging*, 27(6), 647–655. <https://doi.org/10.1007/s002590050558>
- Haase, A. M., Gregersen, T., Christensen, L. A., Agnholt, J., Dahlerup, J. F., Schlageter, V., & Krogh, K. (2016). Regional gastrointestinal transit times in severe ulcerative colitis. *Neurogastroenterology & Motility*, 28(2), 217–224. <https://doi.org/10.1111/nmo.12713>
- Hardy, J., Davis, S., Khosla, R., & Robertson, C. (1988). Gastrointestinal transit of small tablets in patients with ulcerative colitis. *International Journal of Pharmaceutics*, 48(1–3), 79–82. [https://doi.org/10.1016/0378-5173\(88\)90249-9](https://doi.org/10.1016/0378-5173(88)90249-9)
- Hariparsad, N., Ramsden, D., Taskar, K., Badée, J., Venkatakrisnan, K., Reddy, M. B., Cabalu, T., Mukherjee, D., Rehmel, J., Bolleddula, J., Emami Riedmaier, A., Prakash, C., Chanteux, H., Mao, J., Umehara, K., Shah, K., De Zwart, L., Dowty,

- M., Kotsuma, M., ... Parrott, N. (2022). Current Practices, Gap Analysis, and Proposed Workflows for PBPK Modeling of Cytochrome P450 Induction: An Industry Perspective. *Clinical Pharmacology & Therapeutics*, 112(4), 770–781. <https://doi.org/10.1002/cpt.2503>
- Heimbach, T., Chen, Y., Chen, J., Dixit, V., Parrott, N., Peters, S. A., Poggesi, I., Sharma, P., Snoeys, J., Shebley, M., Tai, G., Tse, S., Upreti, V. V., Wang, Y., Tsai, A., Xia, B., Zheng, M., Zhu, A. Z. X., & Hall, S. (2021). Physiologically-Based Pharmacokinetic Modeling in Renal and Hepatic Impairment Populations: A Pharmaceutical Industry Perspective. *Clinical Pharmacology & Therapeutics*, 110(2), 297–310. <https://doi.org/10.1002/cpt.2125>
- Hens, B., & Bolger, M. B. (2019). Application of a Dynamic Fluid and pH Model to Simulate Intraluminal and Systemic Concentrations of a Weak Base in GastroPlus™. *Journal of Pharmaceutical Sciences*, 108(1), 305–315. <https://doi.org/10.1016/j.xphs.2018.10.041>
- Holt, S., Heading, R. C., Clements, J. A., Tothill, P., & Prescott, L. F. (1981). Acetaminophen absorption and metabolism in celiac disease and Crohn's disease. *Clinical Pharmacology and Therapeutics*, 30(2), 232–238. <https://doi.org/10.1038/clpt.1981.153>
- Huang, C. T. L., Levine, M. M., Daoud, G. S., Nalin, D. R., & Nichols, B. L. (1982). Fecal steroids in diarrhea: IV. Cholera. *Lipids*, 17(9), 612–616. <https://doi.org/10.1007/BF02535367>
- Huang, W., Lee, S. L., & Yu, L. X. (2009). Mechanistic Approaches to Predicting Oral Drug Absorption. *The AAPS Journal*, 11(2), 217–224. <https://doi.org/10.1208/s12248-009-9098-z>
- Jain, S., Mittal, A., & K. Jain, A. (2011). Enhanced Topical Delivery of Cyclosporin-A Using PLGA Nanoparticles as Carrier. *Current Nanoscience*, 7(4), 524–530. <https://doi.org/10.2174/157341311796196835>
- Jamei, M. (2016). Recent Advances in Development and Application of Physiologically-Based Pharmacokinetic (PBPK) Models: a Transition from Academic Curiosity to Regulatory Acceptance. *Current Pharmacology Reports*, 2(3), 161–169. <https://doi.org/10.1007/s40495-016-0059-9>
- Jamei, M., Marciniak, S., Feng, K., Barnett, A., Tucker, G., & Rostami-Hodjegan, A. (2009). The Simcyp Population-based ADME Simulator. *Expert Opinion on Drug Metabolism & Toxicology*, 5(2), 211–223. <https://doi.org/10.1517/17425250802691074>
- Jamei, M., Turner, D., Yang, J., Neuhoff, S., Polak, S., Rostami-Hodjegan, A., & Tucker, G. (2009). Population-Based Mechanistic Prediction of Oral Drug Absorption. *The AAPS Journal*, 11(2), 225–237. <https://doi.org/10.1208/s12248-009-9099-y>
- Jarmusch, A. K., Vrbanac, A., Momper, J. D., Ma, J. D., Alhaja, M., Liyanage, M., Knight, R., Dorrestein, P. C., & Tsunoda, S. M. (2020). Enhanced Characterization of Drug Metabolism and the Influence of the Intestinal Microbiome: A Pharmacokinetic, Microbiome, and Untargeted Metabolomics Study. *Clinical and Translational Science*, 13(5), 972–984. <https://doi.org/10.1111/cts.12785>
- Jean, D., Naik, K., Milligan, L., Hall, S., Mei Huang, S., Isoherranen, N., Kuemmel, C., Seo, P., Tegenge, M. A., Wang, Y., Yang, Y., Zhang, X., Zhao, L., Zhao, P., Benjamin, J., Bergman, K., Grillo, J., Madabushi, R., Wu, F., ... Zineh, I. (2021).

- Development of best practices in physiologically based pharmacokinetic modeling to support clinical pharmacology regulatory decision-making—A workshop summary. *CPT: Pharmacometrics & Systems Pharmacology*, 10(11), 1271–1275. <https://doi.org/10.1002/psp4.12706>
- Jenkins, R. T., Ramage, J. K., Jones, D. B., Collins, S. M., Goodacre, R. L., & Hunt, R. H. (1988). Small bowel and colonic permeability to 51Cr-EDTA in patients with active inflammatory bowel disease. *Clinical and Investigative Medicine. Medecine Clinique et Experimentale*, 11(2), 151–155.
- Johansson, M. E. v., Sjövall, H., & Hansson, G. C. (2013). The gastrointestinal mucus system in health and disease. *Nature Reviews Gastroenterology & Hepatology*, 10(6), 352–361. <https://doi.org/10.1038/nrgastro.2013.35>
- Johnson, K. C. (2003). Dissolution and Absorption Modeling: Model Expansion to Simulate the Effects of Precipitation, Water Absorption, Longitudinally Changing Intestinal Permeability, and Controlled Release on Drug Absorption. *Drug Development and Industrial Pharmacy*, 29(8), 833–842. <https://doi.org/10.1081/DDC-120024179>
- Johnson, T. N., Small, B. G., Berglund, E. G., & Rowland Yeo, K. (2021). A best practice framework for applying physiologically-based pharmacokinetic modeling to pediatric drug development. *CPT: Pharmacometrics & Systems Pharmacology*, 10(9), 967–972. <https://doi.org/10.1002/psp4.12678>
- Johnson, T. N., Small, B. G., & Rowland Yeo, K. (2022). Increasing application of pediatric physiologically based pharmacokinetic models across academic and industry organizations. *CPT: Pharmacometrics & Systems Pharmacology*, 11(3), 373–383. <https://doi.org/10.1002/psp4.12764>
- Karlstrom, L., Cassuto, J., Jodal, M., & Lundgren, O. (1986). Involvement of the Enteric Nervous System in the Intestinal Secretion Induced by Sodium Deoxycholate and Sodium Ricinoleate. *Scandinavian Journal of Gastroenterology*, 21(3), 331–340. <https://doi.org/10.3109/00365528609003083>
- Kelly, P., Shawa, T., Mwanamakondo, S., Soko, R., Smith, G., Barclay, G. R., & Sanderson, I. R. (2010). Gastric and intestinal barrier impairment in tropical enteropathy and HIV: limited impact of micronutrient supplementation during a randomised controlled trial. *BMC Gastroenterology*, 10, 72. <https://doi.org/10.1186/1471-230X-10-72>
- Kidd, M., Modlin, I. M., Gustafsson, B. I., Drozdov, I., Hauso, O., & Pfragner, R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 295(2), G260–G272. <https://doi.org/10.1152/ajpgi.00056.2008>
- Kitis, G., Lucas, M. L., Bishop, H., Sargent, A., Schneider, R. E., Blair, J. A., & Allan, R. N. (1982). Altered Jejunal Surface pH in Coeliac Disease: Its Effect on Propranolol and Folic Acid Absorption. *Clinical Science*, 63(4), 373–380. <https://doi.org/10.1042/cs0630373>
- Knox, T. A., Spiegelman, D., Skinner, S. C., & Gorbach, S. (2000). Diarrhea and Abnormalities of Gastrointestinal Function in A Cohort of Men and Women With Hiv Infection. *American Journal of Gastroenterology*, 95(12), 3482–3489. <https://doi.org/10.1111/j.1572-0241.2000.03365.x>

- Konturek, J. W., Fischer, H., Van Der Voort, I. R., & Domschke, W. (1997). Disturbed Gastric Motor Activity in Patients with Human Immunodeficiency Virus Infection. *Scandinavian Journal of Gastroenterology*, 32(3), 221–225. <https://doi.org/10.3109/00365529709000198>
- Kostewicz, E. S., Aarons, L., Bergstrand, M., Bolger, M. B., Galetin, A., Hatley, O., Jamei, M., Lloyd, R., Pepin, X., Rostami-Hodjegan, A., Sjögren, E., Tannergren, C., Turner, D. B., Wagner, C., Weitschies, W., & Dressman, J. (2014). PBPK models for the prediction of in vivo performance of oral dosage forms. *European Journal of Pharmaceutical Sciences*, 57, 300–321. <https://doi.org/10.1016/j.ejps.2013.09.008>
- Kumar, A., Kochhar, S., Ings, R., Ji, Y., de Hostos, E., Choy, R., Salam, M., Khan, W., Lin, P., & Schwertschlag, U. (2014). Pharmacokinetics and tolerability of iOWH032, an inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, in normal volunteers and cholera patients. In *www.path.org*.
- Lebwohl, B., Murray, J. A., Rubio-Tapia, A., Green, P. H. R., & Ludvigsson, J. F. (2014). Predictors of persistent villous atrophy in coeliac disease: a population-based study. *Alimentary Pharmacology & Therapeutics*, 39(5), 488–495. <https://doi.org/10.1111/apt.12621>
- Lee, J. S., & Silverberg, J. W. (1972). Effect of Cholera Toxin on Fluid Absorption and Villus Lymph Pressure in Dog Jejunal Mucosa. *Gastroenterology*, 62(5), 993–1000. [https://doi.org/10.1016/S0016-5085\(72\)80116-1](https://doi.org/10.1016/S0016-5085(72)80116-1)
- LeWitt, P. A., Jennings, D., Lyons, K. E., Pahwa, R., Rabinowicz, A. L., Wang, J., Guarnieri, M., Hubble, J. P., & Murck, H. (2009). Pharmacokinetic-pharmacodynamic crossover comparison of two levodopa extension strategies. *Movement Disorders*, 24(9), 1319–1324. <https://doi.org/10.1002/mds.22587>
- Lin, L., & Wong, H. (2017). Predicting Oral Drug Absorption: Mini Review on Physiologically-Based Pharmacokinetic Models. *Pharmaceutics*, 9(4), 41. <https://doi.org/10.3390/pharmaceutics9040041>
- Lippi, D., Gotuzzo, E., & Caini, S. (2016). Cholera. *Microbiology Spectrum*, 4(4). <https://doi.org/10.1128/microbiolspec.PoH-0012-2015>
- Love, A. H. (1969). Water and sodium absorption by the intestine in cholera. *Gut*, 10(1), 63–67. <https://doi.org/10.1136/gut.10.1.63>
- Luzon, E., Blake, K., Cole, S., Nordmark, A., Versantvoort, C., & Berglund, E. G. (2017). Physiologically based pharmacokinetic modeling in regulatory decision-making at the European Medicines Agency. *Clinical Pharmacology & Therapeutics*, 102(1), 98–105. <https://doi.org/10.1002/cpt.539>
- MacArthur, R. D., & DuPont, H. L. (2012). Etiology and pharmacologic management of noninfectious diarrhea in HIV-infected individuals in the highly active antiretroviral therapy era. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 55(6), 860–867. <https://doi.org/10.1093/cid/cis544>
- Macnab, K. A., Gill, M. J., Sutherland, L. R., & Brant, R. (1996). Zidovudine absorption and small intestinal function in HIV seropositive patients. *Journal of Antimicrobial Chemotherapy*, 37(4), 825–829. <https://doi.org/10.1093/jac/37.4.825>
- Macnab, K. A., Gill, M. J., Sutherland, L. R., De Boer Visser, N., & Church, D. (1993). Erratic zidovudine bioavailability in HIV seropositive patients. *The Journal of Antimicrobial Chemotherapy*, 31(3), 421–428. <https://doi.org/10.1093/jac/31.3.421>

- Magnusson, K.-E., Kihlström, E., & Sundqvist, T. (1985). Effect of cholera toxin on rat intestinal permeability assessed with fluorescent dextran 3000. *FEMS Microbiology Letters*, 29(1–2), 15–18. <https://doi.org/10.1111/j.1574-6968.1985.tb00827.x>
- Manallack, D. T. (2007). The pK(a) Distribution of Drugs: Application to Drug Discovery. *Perspectives in Medicinal Chemistry*, 1, 25–38.
- Manolis, E., García-Arieta, A., Lindahl, A., Kotzagiorgis, E., Limberg, J., Holte, Ø., Paixao, P., Versantvoort, C., Tshinanu, F. M., Blake, K., & Van Den Heuvel, M. (2023). Using mechanistic models to support development of complex generic drug products: European Medicines Agency perspective. *CPT: Pharmacometrics & Systems Pharmacology*. <https://doi.org/10.1002/psp4.12906>
- Martins, C., Teixeira, C., Ribeiro, S., Trabulo, D., Cardoso, C., Mangualde, J., Freire, R., Alves, A. L., Gamito, É., Cremers, I., & Oliveira, A. P. (2016). Seronegative Intestinal Villous Atrophy: A Diagnostic Challenge. *Case Reports in Gastrointestinal Medicine*, 2016, 1–4. <https://doi.org/10.1155/2016/6392028>
- McNally, K., Cotton, R., & Loizou, G. D. (2011). A Workflow for Global Sensitivity Analysis of PBPK Models. *Frontiers in Pharmacology*, 2. <https://doi.org/10.3389/fphar.2011.00031>
- Molla, A., Molla, A. M., Sarker, S. A., & Khatun, M. (1983). Whole-Gut Transit Time and Its Relationship to Absorption of Macronutrients during Diarrhoea and after Recovery. *Scandinavian Journal of Gastroenterology*, 18(4), 537–543. <https://doi.org/10.3109/00365528309181634>
- Moreira Júnior, E. D., Silva, N., Brites, C., Carvalho, E. M., Bina, J. C., Badaro, R., & Johnson, W. D. (1993). Characteristics of the acquired immunodeficiency syndrome in Brazil. *The American Journal of Tropical Medicine and Hygiene*, 48(5), 687–692. <https://doi.org/10.4269/ajtmh.1993.48.687>
- Morón, B., Verma, A. K., Das, P., Taavela, J., Dafik, L., DiRaimondo, T. R., Albertelli, M. A., Kraemer, T., Mäki, M., Khosla, C., Rogler, G., & Makharia, G. K. (2013). CYP3A4-Catalyzed Simvastatin Metabolism as a Non-Invasive Marker of Small Intestinal Health in Celiac Disease. *American Journal of Gastroenterology*, 108(8), 1344–1351. <https://doi.org/10.1038/ajg.2013.151>
- Murphy, B., Taylor, C., Crane, R., Okong, P., & Bjarnason, I. (1999). Comparison of intestinal function in human immunodeficiency virus-seropositive patients in Kampala and London. *Scandinavian Journal of Gastroenterology*, 34(5), 491–495. <https://doi.org/10.1080/003655299750026227>
- Nernst, W. (1904). Theorie der Reaktionsgeschwindigkeit in heterogenen Systemen. *Zeitschrift Für Physikalische Chemie*, 47U(1), 52–55. <https://doi.org/10.1515/zpch-1904-4704>
- Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., Panaccione, R., Ghosh, S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., & Kaplan, G. G. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *The Lancet*, 390(10114), 2769–2778. [https://doi.org/10.1016/S0140-6736\(17\)32448-0](https://doi.org/10.1016/S0140-6736(17)32448-0)
- Niv, E., Fishman, S., Kachman, H., Arnon, R., & Dotan, I. (2014). Sequential capsule endoscopy of the small bowel for follow-up of patients with known Crohn's disease. *Journal of Crohn's and Colitis*, 8(12), 1616–1623. <https://doi.org/10.1016/j.crohns.2014.03.003>

- Nóbrega, A. C. M., Ferreira, B. R. S., Oliveira, G. J., Sales, K. M. O., Santos, A. A., Nobre e Souza, M. Â., Braga, L. L. B. C., de Almeida Troncon, L. A. E., & Souza, M. H. L. P. (2012). Dyspeptic symptoms and delayed gastric emptying of solids in patients with inactive Crohn's disease. *BMC Gastroenterology*, *12*(1), 175. <https://doi.org/10.1186/1471-230X-12-175>
- Nugent, S. G., Rampton, D. S., Kumar, D., Yazaki, E., & Evans, D. F. (2000). Gut pH and transit time in ulcerative colitis appear sufficient for complete dissolution of pH-dependent 5-ASA-containing capsules. *Digestive and Liver Disease*, *32*, A45. [https://doi.org/10.1016/S1590-8658\(00\)80222-4](https://doi.org/10.1016/S1590-8658(00)80222-4)
- Nyholm, D., & Lennernäs, H. (2008). Irregular gastrointestinal drug absorption in Parkinson's disease. *Expert Opinion on Drug Metabolism & Toxicology*, *4*(2), 193–203. <https://doi.org/10.1517/17425255.4.2.193>
- Perry, C., Davis, G., Conner, T. M., & Zhang, T. (2020). Utilization of Physiologically Based Pharmacokinetic Modeling in Clinical Pharmacology and Therapeutics: an Overview. *Current Pharmacology Reports*, *6*(3), 71–84. <https://doi.org/10.1007/s40495-020-00212-x>
- Peters, S. A., & Dolgos, H. (2019). Requirements to Establishing Confidence in Physiologically Based Pharmacokinetic (PBPK) Models and Overcoming Some of the Challenges to Meeting Them. *Clinical Pharmacokinetics*, *58*(11), 1355–1371. <https://doi.org/10.1007/s40262-019-00790-0>
- Plewka, D., Plewka, A., Szczepanik, T., Morek, M., Bogunia, E., Wittek, P., & Kijonka, C. (2014). Expression of selected cytochrome P450 isoforms and of cooperating enzymes in colorectal tissues in selected pathological conditions. *Pathology - Research and Practice*, *210*(4), 242–249. <https://doi.org/10.1016/j.prp.2013.12.010>
- Poirier, A.-A., Aubé, B., Côté, M., Morin, N., di Paolo, T., & Soulet, D. (2016). Gastrointestinal Dysfunctions in Parkinson's Disease: Symptoms and Treatments. *Parkinson's Disease*, *2016*, 1–23. <https://doi.org/10.1155/2016/6762528>
- Press, Hauptmann, Hauptmann, Fuchs, Fuchs, Ewe, & Ramadori. (1998). Gastrointestinal pH profiles in patients with inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*, *12*(7), 673–678. <https://doi.org/10.1046/j.1365-2036.1998.00358.x>
- Rana, S. V., Sharma, S., Malik, A., Kaur, J., Prasad, K. K., Sinha, S. K., & Singh, K. (2013). Small Intestinal Bacterial Overgrowth and Orocecal Transit Time in Patients of Inflammatory Bowel Disease. *Digestive Diseases and Sciences*, *58*(9), 2594–2598. <https://doi.org/10.1007/s10620-013-2694-x>
- Rao, S. S. C., & Read, N. W. (1990). Gastrointestinal Motility in Patients with Ulcerative Colitis. *Scandinavian Journal of Gastroenterology*, *25*(sup172), 22–28. <https://doi.org/10.3109/00365529009091905>
- Resnick, R. H., Royal, H., Marshall, W., Barron, R., & Werth, T. (1990). Intestinal permeability in gastrointestinal disorders. *Digestive Diseases and Sciences*, *35*(2), 205–211. <https://doi.org/10.1007/BF01536764>
- Roberts, M. S., Magnusson, B. M., Burczynski, F. J., & Weiss, M. (2002). Enterohepatic Circulation. *Clinical Pharmacokinetics*, *41*(10), 751–790. <https://doi.org/10.2165/00003088-200241100-00005>

- Rosenberg, I. H. (2003). Tropical Enteritis: Nutritional Consequences and Connections with the Riddle of Cholera. *The Journal of Nutrition*, 133(1), 333S-335S. <https://doi.org/10.1093/jn/133.1.333S>
- Rostami-Hodjegan, A. (2012). Physiologically Based Pharmacokinetics Joined With In Vitro–In Vivo Extrapolation of ADME: A Marriage Under the Arch of Systems Pharmacology. *Clinical Pharmacology & Therapeutics*, 92(1), 50–61. <https://doi.org/10.1038/clpt.2012.65>
- Rowland, M., Peck, C., & Tucker, G. (2011). Physiologically-Based Pharmacokinetics in Drug Development and Regulatory Science. *Annual Review of Pharmacology and Toxicology*, 51(1), 45–73. <https://doi.org/10.1146/annurev-pharmtox-010510-100540>
- Sack, D. A., Sack, R. B., Nair, G. B., & Siddique, A. (2004). Cholera. *The Lancet*, 363(9404), 223–233. [https://doi.org/10.1016/S0140-6736\(03\)15328-7](https://doi.org/10.1016/S0140-6736(03)15328-7)
- Saeid Seyedian, S., Nokhostin, F., & Dargahi Malamir, M. (2019). A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. *Journal of Medicine and Life*, 12(2), 113–122. <https://doi.org/10.25122/jml-2018-0075>
- Saha, D. R., Pal, A., Rajendran, K., & Dutta, P. (2006). Histopathological changes in experimental cholera with a non toxigenic non- O1 non-O139 Vibrio cholerae strain isolated from Kolkata, India. *Indian Journal of Experimental Biology*, 44(3), 221–227.
- Sakai, E., Higurashi, T., Ohkubo, H., Hosono, K., Ueda, A., Matsuhashi, N., & Nakajima, A. (2017). Investigation of Small Bowel Abnormalities in HIV-Infected Patients Using Capsule Endoscopy. *Gastroenterology Research and Practice*, 2017, 1–7. <https://doi.org/10.1155/2017/1932647>
- Salerno, S. N., Burckart, G. J., Huang, S., & Gonzalez, D. (2019). Pediatric Drug–Drug Interaction Studies: Barriers and Opportunities. *Clinical Pharmacology & Therapeutics*, 105(5), 1067–1070. <https://doi.org/10.1002/cpt.1234>
- Schulzke, J. D., Schulzke, I., Fromm, M., & Riecken, E. O. (1995). Epithelial barrier and ion transport in coeliac sprue: electrical measurements on intestinal aspiration biopsy specimens. *Gut*, 37(6), 777–782. <https://doi.org/10.1136/gut.37.6.777>
- Schulzke, J.-D., Bentzel, C. J., Schulzke, I., Riecken, E.-O., & Fromm, M. (1998). Epithelial Tight Junction Structure in the Jejunum of Children with Acute and Treated Celiac Sprue. *Pediatric Research*, 43(4), 435–441. <https://doi.org/10.1203/00006450-199804000-00001>
- Schumann, M., Günzel, D., Buergel, N., Richter, J. F., Troeger, H., May, C., Fromm, A., Sorgenfrei, D., Daum, S., Bojarski, C., Heyman, M., Zeitz, M., Fromm, M., & Schulzke, J.-D. (2012). Cell polarity-determining proteins Par-3 and PP-1 are involved in epithelial tight junction defects in coeliac disease. *Gut*, 61(2), 220–228. <https://doi.org/10.1136/gutjnl-2011-300123>
- Sharpstone, D., Neild, P., Crane, R., Taylor, C., Hodgson, C., Sherwood, R., Gazzard, B., & Bjarnason, I. (1999). Small intestinal transit, absorption, and permeability in patients with AIDS with and without diarrhoea. *Gut*, 45(1), 70–76. <https://doi.org/10.1136/gut.45.1.70>
- Shebley, M., Sandhu, P., Emami Riedmaier, A., Jamei, M., Narayanan, R., Patel, A., Peters, S. A., Reddy, V. P., Zheng, M., de Zwart, L., Beneton, M., Bouzom, F.,



- Chen, J., Chen, Y., Cleary, Y., Collins, C., Dickinson, G. L., Djebli, N., Einolf, H. J., ... Rowland, M. (2018). Physiologically Based Pharmacokinetic Model Qualification and Reporting Procedures for Regulatory Submissions: A Consortium Perspective. *Clinical Pharmacology & Therapeutics*, *104*(1), 88–110. <https://doi.org/10.1002/cpt.1013>
- Singh, A. K., & Narsipur, S. S. (2011). Cyclosporine: A Commentary on Brand versus Generic Formulation Exchange. *Journal of Transplantation*, *2011*, 1–6. <https://doi.org/10.1155/2011/480642>
- Slavik, T., & Lauwers, G. Y. (2018). Navigating the jungles of tropical infectious gastrointestinal pathology: a pattern-based approach to the endoscopic biopsy. *Virchows Archiv*, *472*(1), 135–147. <https://doi.org/10.1007/s00428-017-2166-3>
- Stocchi, F. (2006). The levodopa wearing-off phenomenon in Parkinson's disease: pharmacokinetic considerations. *Expert Opinion on Pharmacotherapy*, *7*(10), 1399–1407. <https://doi.org/10.1517/14656566.7.10.1399>
- Sweetser, S. (2012). Evaluating the Patient With Diarrhea: A Case-Based Approach. *Mayo Clinic Proceedings*, *87*(6), 596–602. <https://doi.org/10.1016/j.mayocp.2012.02.015>
- Taskar, K. S., Pilla Reddy, V., Burt, H., Posada, M. M., Varma, M., Zheng, M., Ullah, M., Emami Riedmaier, A., Umehara, K., Snoeys, J., Nakakariya, M., Chu, X., Beneton, M., Chen, Y., Huth, F., Narayanan, R., Mukherjee, D., Dixit, V., Sugiyama, Y., & Neuhoff, S. (2020). Physiologically-Based Pharmacokinetic Models for Evaluating Membrane Transporter Mediated Drug–Drug Interactions: Current Capabilities, Case Studies, Future Opportunities, and Recommendations. *Clinical Pharmacology & Therapeutics*, *107*(5), 1082–1115. <https://doi.org/10.1002/cpt.1693>
- Tsunoda, S. M., Gonzales, C., Jarmusch, A. K., Momper, J. D., & Ma, J. D. (2021). Contribution of the Gut Microbiome to Drug Disposition, Pharmacokinetic and Pharmacodynamic Variability. *Clinical Pharmacokinetics*, *60*(8), 971–984. <https://doi.org/10.1007/s40262-021-01032-y>
- Tursi, A., Brandimarte, G., Giorgetti, G., & Nasi, G. (2003). Assessment of oro-caecal transit time in different localization of Crohn's disease and its possible influence on clinical response to therapy. *European Journal of Gastroenterology & Hepatology*, *15*(1), 69–74. <https://doi.org/10.1097/00042737-200301000-00012>
- Urgesi, R., Cianci, R., Bizzotto, A., Costamagna, G., & Riccioni, M. E. (2013). Evaluation of gastric and small bowel transit times in coeliac disease with the small bowel PillCam®: a single centre study in a non gluten-free diet adult Italian population with coeliac disease. *European Review for Medical and Pharmacological Sciences*, *17*(9), 1167–1173.
- Uzzan, M., Ko, H. M., Mehandru, S., & Cunningham-Rundles, C. (2016). Gastrointestinal Disorders Associated with Common Variable Immune Deficiency (CVID) and Chronic Granulomatous Disease (CGD). *Current Gastroenterology Reports*, *18*(4), 17. <https://doi.org/10.1007/s11894-016-0491-3>
- Vanuytsel, T., Tack, J., & Farre, R. (2021). The Role of Intestinal Permeability in Gastrointestinal Disorders and Current Methods of Evaluation. *Frontiers in Nutrition*, *8*. <https://doi.org/10.3389/fnut.2021.717925>
- Verscheijden, L. F. M., Koenderink, J. B., Johnson, T. N., de Wildt, S. N., & Russel, F. G. M. (2020). Physiologically-based pharmacokinetic models for children: Starting to

- reach maturation? *Pharmacology & Therapeutics*, 211, 107541.  
<https://doi.org/10.1016/j.pharmthera.2020.107541>
- Vinarov, Z., Abdallah, M., Agundez, J. A. G., Allegaert, K., Basit, A. W., Braeckmans, M., Ceulemans, J., Corsetti, M., Griffin, B. T., Grimm, M., Keszthelyi, D., Koziolok, M., Madla, C. M., Matthys, C., McCoubrey, L. E., Mitra, A., Reppas, C., Stappaerts, J., Steenackers, N., ... Augustijns, P. (2021). Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. *European Journal of Pharmaceutical Sciences*, 162, 105812.  
<https://doi.org/10.1016/j.ejps.2021.105812>
- Vyhlidal, C. A., Chapron, B. D., Ahmed, A., Singh, V., Casini, R., & Shakhnovich, V. (2021). Effect of Crohn's Disease on Villous Length and CYP3A4 Expression in the Pediatric Small Intestine. *Clinical and Translational Science*, 14(2), 729–736.  
<https://doi.org/10.1111/cts.12938>
- Walters, J. R. F., & Pattni, S. S. (2010). Managing bile acid diarrhoea. *Therapeutic Advances in Gastroenterology*, 3(6), 349–357.  
<https://doi.org/10.1177/1756283X10377126>
- Wang, J., & Flanagan, D. R. (1999). General solution for diffusion-controlled dissolution of spherical particles. 1. Theory. *Journal of Pharmaceutical Sciences*, 88(7), 731–738. <https://doi.org/10.1021/js980236p>
- Wang, K., Jiang, K., Wei, X., Li, Y., Wang, T., & Song, Y. (2021). Physiologically Based Pharmacokinetic Models Are Effective Support for Pediatric Drug Development. *AAPS PharmSciTech*, 22(6), 208. <https://doi.org/10.1208/s12249-021-02076-w>
- Welage, L. S., Carver, P. L., Revankar, S., Pierson, C., & Kauffman, C. A. (1995). Alterations in Gastric Acidity in Patients Infected with Human Immunodeficiency Virus. *Clinical Infectious Diseases*, 21(6), 1431–1438.  
<https://doi.org/10.1093/clinids/21.6.1431>
- Whyte, L. A., & Jenkins, H. R. (2012). Pathophysiology of diarrhoea. *Paediatrics and Child Health*, 22(10), 443–447. <https://doi.org/10.1016/j.paed.2012.05.006>
- Wilson, A., Tirona, R. G., & Kim, R. B. (2017). CYP3A4 Activity is Markedly Lower in Patients with Crohn's Disease. *Inflammatory Bowel Diseases*, 23(5), 804–813.  
<https://doi.org/10.1097/MIB.0000000000001062>
- Wilson, A., Urquhart, B. L., Ponich, T., Chande, N., Gregor, J. C., Beaton, M., & Kim, R. B. (2019). Crohn's Disease Is Associated with Decreased CYP3A4 and P-Glycoprotein Protein Expression. *Molecular Pharmaceutics*, 16(9), 4059–4064.  
<https://doi.org/10.1021/acs.molpharmaceut.9b00459>
- Wollmer, E., & Klein, S. (2017). A review of patient-specific gastrointestinal parameters as a platform for developing in vitro models for predicting the in vivo performance of oral dosage forms in patients with Parkinson's disease. *International Journal of Pharmaceutics*, 533(1), 298–314. <https://doi.org/10.1016/j.ijpharm.2017.08.126>
- Wollmer, E., & Klein, S. (2022). Patient-specific in vitro drug release testing coupled with in silico PBPK modeling to forecast the in vivo performance of oral extended-release levodopa formulations in Parkinson's disease patients. *European Journal of Pharmaceutics and Biopharmaceutics*, 180, 101–118.  
<https://doi.org/10.1016/j.ejpb.2022.09.015>
- Wyatt, J., Oberhuber, G., Pongratz, S., Püspök, A., Moser, G., Novacek, G., Lochs, H., & Vogelsang, H. (1997). Increased gastric and intestinal permeability in patients

- with Crohn's disease. *The American Journal of Gastroenterology*, 92(10), 1891–1896.
- Yan, W., & Sundaram, U. (2013). Chronic Diarrhea, Malabsorption, Villous Blunting, and Microscopy Colitis due to Common Variable Immunodeficiency Syndrome. *American Journal of Gastroenterology*, 108, S287. <https://doi.org/10.14309/00000434-201310001-00960>
- Youhanna, S., & Lauschke, V. M. (2021). The Past, Present and Future of Intestinal In Vitro Cell Systems for Drug Absorption Studies. *Journal of Pharmaceutical Sciences*, 110(1), 50–65. <https://doi.org/10.1016/j.xphs.2020.07.001>
- Yu, H., Xia, D., Zhu, Q., Zhu, C., Chen, D., & Gan, Y. (2013). Supersaturated polymeric micelles for oral cyclosporine A delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 85(3), 1325–1336. <https://doi.org/10.1016/j.ejpb.2013.08.003>
- Yu, L. X., & Amidon, G. L. (1998). Characterization of small intestinal transit time distribution in humans. *International Journal of Pharmaceutics*, 171(2), 157–163. [https://doi.org/10.1016/S0378-5173\(98\)00174-4](https://doi.org/10.1016/S0378-5173(98)00174-4)
- Yu, L. X., & Amidon, G. L. (1999). A compartmental absorption and transit model for estimating oral drug absorption. *International Journal of Pharmaceutics*, 186(2), 119–125. [https://doi.org/10.1016/S0378-5173\(99\)00147-7](https://doi.org/10.1016/S0378-5173(99)00147-7)
- Yu, L. X., Crison, J. R., & Amidon, G. L. (1996). Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. *International Journal of Pharmaceutics*, 140(1), 111–118. [https://doi.org/10.1016/0378-5173\(96\)04592-9](https://doi.org/10.1016/0378-5173(96)04592-9)
- Zhang, C., Cheng, Y., Liu, D., Liu, M., Cui, H., Zhang, B., Mei, Q., & Zhou, S. (2019). Mitochondria-targeted cyclosporin A delivery system to treat myocardial ischemia reperfusion injury of rats. *Journal of Nanobiotechnology*, 17(1), 18. <https://doi.org/10.1186/s12951-019-0451-9>
- Zhang, X., Yang, Y., Grimstein, M., Fan, J., Grillo, J. A., Huang, S., Zhu, H., & Wang, Y. (2020). Application of PBPK Modeling and Simulation for Regulatory Decision Making and Its Impact on US Prescribing Information: An Update on the 2018-2019 Submissions to the US FDA's Office of Clinical Pharmacology. *The Journal of Clinical Pharmacology*, 60(S1). <https://doi.org/10.1002/jcph.1767>
- Zorza, G., Beaugerie, L., Taburet, A.-M., Le Quintrec, Y., & Singlas, E. (1993). Absorption of zidovudine in patients with diarrhoea. *European Journal of Clinical Pharmacology*, 44(5), 501–503. <https://doi.org/10.1007/BF00315553>

## Footnotes

Funding: This work was supported in part by funding from the National Institutes of Health, R01AI170784.

Conflicts of interest: None

## Figure Legends:

**Figure 1. Major diarrhea-associated physiological changes in the gastrointestinal tract and their potential impact on oral drug absorption.**

**Figure 2. iOWH032 PBPK model development for cholera population.** Observed median iOWH032 plasma concentration in cholera patients following oral administration of a 300 mg fast-release tablet to Bangladeshi cholera patients (n=14) with severe acute diarrhea compared to median iOWH032 plasma concentration in healthy Bangladeshi volunteers (n=6) receiving the same dose (A). PBPK model simulation of iOWH032 plasma concentration in a healthy population after an oral dose of 300 mg iOWH032 under fasted conditions overlaid with observed median iOWH032 plasma concentration in healthy Bangladeshi volunteers (B) or observed median iOWH032 plasma concentration in Bangladeshi cholera patients (C). PBPK model simulation of iOWH032 plasma concentration in a cholera population with decreased GI transit time, increased luminal volume, and decreased surface area enhancement factor (SEF), compared with observed median iOWH032 plasma concentration in Bangladeshi cholera patients (D). Black solid circle represents the median observed iOWH032 plasma concentration in Bangladeshi cholera patients. White diamond shape represents the median observed iOWH032 plasma concentration in healthy Bangladeshi volunteers. Solid black line represents the median plasma-time profile of PBPK model simulation. Dashed black lines mark the 95<sup>th</sup> and 5<sup>th</sup> percentile of PBPK model simulation. Grey shaded areas indicate the 90% PBPK model prediction interval.

**Table 1.** Summary of physiological changes in the gastrointestinal tract associated with ulcerative colitis, Crohn’s disease, celiac disease, HIV infection, and cholera.

Disease	Parameter	Region	Study Population	Physiological Change	References
Ulcerative Colitis (UC)	pH	Stomach, small intestine, colon	UC patients (n=11), healthy controls (n=12)	Median gastric pH for UC patients (1.95) was significantly higher than that of the healthy controls (1.55). UC patients had significantly higher pH values in the terminal ileum, the caecum and the right colon.	(Press et al., 1998)
	pH	Stomach, small intestine, colon	Active UC (n=6)	Normal pH values in the stomach and small intestine. Three patients had very low pH levels (2.3, 2.9, 3.4) in the proximal colon.	(Fallingborg et al., 1993)
	pH	Rectum	Moderate UC patient (n=1), severe UC patients (n=4), healthy subjects (n=15)	Median pH in UC patients was 7.8, compared to 7.2 in healthy controls.	(Ewe et al., 1999)
	pH	Small intestine, colon	Active UC patients (n=8), normal controls (n=4)	Mean colonic pH was not different. Small intestinal pH was similar between UC patients and controls.	(Nugent et al., 2000)
	Permeability	Small and large intestine	Healthy controls (n=11), UC patients (n=21)	Intestinal permeability was increased for UC patients.	(Resnick et al., 1990)
	Permeability	GI	Crohn's disease patients (n=86), UC patients (n=32), healthy controls (n=23)	Serum zonulin concentration, reflective of paracellular permeability, was higher in UC patients (40.3 ng/mL) compared to healthy controls (8.6 ng/mL).	(Caviglia et al., 2019)
	Transit time	Total GI	Severe UC patients (n=20), healthy subjects (n=20)	Significantly longer total GI transit time in severe UC patients (median 44.5 h) compared with healthy subjects (median 27.6 h).	(Haase et al., 2016)

Transit time	Small intestine, colon	Active UC patients (n=8), normal controls (n=4)	Mean transit time in small intestine was similar for UC patients (7 h) and for normal controls (6 h). Transit time through the distal colon was longer for UC patients (12 h) compared to the controls (7 h). Transit time in the proximal colon were comparable for UC patients (7 h) and for controls (8 h).	(Nugent et al., 2000)
Transit time	Mouth to caecum, gastric	UC patients (n=62), healthy controls (n=20)	Orocaecal transit was significantly slower in UC patients. Gastric emptying was similar between UC patients and healthy controls, except for a slight increase in those with quiescent distal colitis. Total GI transit time was not significantly different among UC patient groups and the healthy controls.	(Rao & Read, 1990)
Transit time	Orocaecal transit time (OCTT)	UC patients (n=95), healthy controls (n=115)	Mean OCTT in UC patients (2.0 h) was longer than in the controls (1.5 h).	(Rana et al., 2013)
Transit time	Small intestine	UC patients (n=23), non-IBD patients (n=125)	Median small intestinal transit time in UC patients (4.4 h) was significantly longer than in non-IBD patients (3.6 h).	(Fischer et al., 2017)
Transit time	Stomach, small intestine	Active UC patients (n=2), quiescent UC patients (n=4)	Mean gastric emptying time of 1.6 h and mean small intestinal transit time of 3.4 h were similar to previously reported values for healthy subjects.	(Hardy et al., 1988)
Transit time	Stomach, small intestine	Familial adenomatous polyposis, intestinal lymphoma, and ulcerative colitis patients (n=116), healthy volunteers (n=87)	Mean gastric transit time for the combined pathology patient group (48 min) and that for healthy volunteers (39 min) were not significantly different. Mean small intestinal transit time in the patient group was 4 h 27 min, whereas that for healthy volunteers was 3 h 56 min.	(Fireman et al., 2007)

Crohn's Disease (CD)	pH	Terminal ileum	CD patients (n=15), healthy subjects (n=15)	Median pH in CD patients was 7.5, compared to 7.2 in healthy controls.	(Ewe et al., 1999)
	pH	Stomach, small intestine, colon	CD patients (n=12), healthy controls (n=12)	Median gastric pH for CD patients (2.4) was significantly higher than that of healthy controls (1.55). Small intestinal and colonic pH were similar between CD patients and healthy controls.	(Press et al., 1998)
	pH	Small intestine, right colon	Ileocecal-resected CD patients (n=9), healthy volunteers (n=13)	Small intestinal pH was not significantly different in CD patients vs. controls. Median right colon pH was 0.9 pH units higher in resected CD patients (6.7) compared to controls (5.8).	(Fallingborg et al., 1998)
	Absorptive surface area	Duodenum and terminal ileum	Biopsies from children 1-19 years with/out CD (n=107)	Villous length was reduced by 2-fold in inflamed duodena and ilia.	(Vyhlidal et al., 2021)
	Permeability	Small and large intestine	CD of the small intestine (n=15) and CD/UC of the colon (n=19)	CD patients had increased intestinal permeability, particularly in the colon.	(Jenkins et al., 1988)
	Permeability	Small and large intestine	Healthy controls (n=11), CD patients (n=35)	Intestinal permeability was increased in CD patients.	(Resnick et al., 1990)
	Permeability	Stomach, small and large intestine	CD patients (n=50), healthy controls (n=30)	CD patients showed higher gastric and intestinal permeability.	(Wyatt et al., 1997)
	Permeability	GI	CD patients (n=86), UC patients (n=32), healthy controls (n=23)	Serum zonulin concentration, reflective of paracellular permeability, was significantly higher in CD patients (33.5 ng/mL) compared to healthy controls (8.6 ng/mL).	(Caviglia et al., 2019)



Transit time	Stomach	CD patients (n=12), controls (n=13)	Mean gastric emptying $t_{1/2}$ was significantly increased in CD subjects (38.5 min) compared with controls (23.6 min)	(Holt et al., 1981)
Transit time	Orocaecal transit time (OCTT)	CD patients (n=45), healthy subjects (n=20)	OCTT was delayed in 67% CD patients (120 min) compared to the control (88.2 min). OCTT was more prolonged in ileal localization CD (182.2 min), and less in patients with ileocolonic (122 min) or colonic (106 min) localization.	(Tursi et al., 2003)
Transit time	Orocaecal transit time (OCTT)	CD patients (n=57), healthy controls (n=4)	Longer OCTT was observed in patients (154 min) compared to controls (136 min) but the difference was not significant. OCTT was significantly longer in 14 CD patients with ileocolic resection (180 min) compared to controls.	(Castiglione et al., 2000)
Transit time	Orocaecal transit time (OCTT)	CD patients (n=42), healthy controls (n=115)	Mean OCTT in UC patients (2.3 h) was higher than that in the controls (1.5 h).	(Rana et al., 2013)
Transit time	Small intestine	Active CD patients (n=33), inactive CD patients (n=22), non-IBD patients (n=125)	Median small intestinal transit time was significantly longer in active CD patients (4.2 h) than in non-IBD patients (3.6 h) and inactive CD patients (3.1 h).	(Fischer et al., 2017)

	Transit time	Stomach, small intestine, colon	CD patients (n=6), healthy controls (n=8)	Mean gastric emptying was significantly longer for CD patients (4.0 h) compared to healthy controls (2.7 h). Small intestinal transit time was not significantly different between CD patients (2.4 h) and controls (3.0 h). Transit through the ascending colon tended to be shorter in CD patients (8.1 h) than in healthy controls (15.5 h), but the difference was not significant.	(Edsbäcker et al., 2003)
	Transit time	Stomach, small intestine	CD patients (n=19), non-CD patient controls (n=178)	Mean gastric emptying time was comparable between CD patients (36.4 min) and the controls (34.9 min). Small intestinal transit time was significantly longer for the CD patients (337 min) than for the controls (243.6 min).	(Niv et al., 2014)
	Transit time	Stomach	Inactive CD patients (n=26), age- and sex-matched healthy controls (n=19)	CD patients had significantly delayed and prolonged gastric emptying compared to the controls.	(Nóbrega et al., 2012)
	Transit time	Small intestine	Ileocecal-resected CD patients (n=9), healthy volunteers (n=13)	Median small intestinal transit time was significantly shorter in ileocecal-resected patients (5.2 h) compared to controls (8.0 h).	(Fallingborg et al., 1998)
	Transit time	Stomach, small intestine	Suspected CD patients (n=96), healthy volunteers (n=87)	Mean gastric transit time for CD patients (42 min) and that for healthy volunteers (39 min) were not significantly different. Mean small intestinal transit time was 3 h 58 min in CD patients and 3 h 56 min in healthy volunteers.	(Fireman et al., 2007)
Celiac Disease	pH	Jejunum	Treated and untreated celiac disease patients (n=19), healthy controls (n=10)	Jejunum surface pH was significantly higher in celiac disease patients compared with healthy controls.	(Kitis et al., 1982)

Absorptive surface area	Duodenum	Celiac disease patients who underwent follow-up biopsy (n=7648)	Persistent villous atrophy was found in 43% of patients.	(Lebwohl et al., 2014)
Permeability	Jejunum	Biopsy specimens from celiac patients and healthy controls	Epithelial barrier function was reduced in acute celiac disease, compared with the controls.	(J. D. Schulzke et al., 1995)
Permeability	Jejunum	Children with treated and untreated celiac disease	Epithelial barrier function was reduced by structural modifications of the tight junction in acute celiac disease. Children with a gluten-free diet had partially recovered barrier function.	(J.-D. Schulzke et al., 1998)
Permeability	Duodenum	Healthy controls and celiac disease patients receiving a gluten-containing diet	Celiac disease patients had increased paracellular leakage associated with alteration to tight junction protein expression and assembly.	(Schumann et al., 2012)
Transit time	Stomach	Treated (n=9) and untreated (n=7) celiac disease subjects, controls (n=13)	Mean gastric emptying $t_{1/2}$ was significantly increased in untreated celiac disease patients (35.1 min) and treated celiac disease patients (45.2 min) compared with controls (23.6 min).	(Holt et al., 1981)
Transit time	Stomach, small intestine	Untreated celiac disease patients (n=30), age-, sex-, and BMI-matched healthy controls (n=30)	No statistically significant differences in gastric emptying and small intestinal transit times were found between the two groups.	(Urgesi et al., 2013)
Transit time	Orocaecal transit time (OCTT)	Celiac disease patients (n=16), healthy volunteers (n=20)	Before gluten-free diet, mean OCTT was significantly prolonged in celiac disease patients (243 min) compared to controls (117 min). After a gluten-free diet, OCTT in celiac patients (134 min) was not significantly different from that of the controls.	(Chiarioni et al., 1997)

	Transit time	Stomach, small intestine	Celiac disease patients (n=65), healthy volunteers (n=87)	Mean gastric transit time for celiac disease patients (33 min) and that for healthy volunteers (39 min) were not significantly different. Mean small intestinal transit time in celiac disease patients was 4 h 43 min, whereas that for healthy volunteers was 3 h 56 min.	(Fireman et al., 2007)
Cholera	pH	Vomitus	Patients with acute, severe cholera (n=18 samples)	Median pH in vomit samples was ~7.6	(Dunmire et al., 2022)
	pH	Stool	Patients with acute, severe cholera (n=18 samples)	Median pH in stool samples was ~8.5	(Dunmire et al., 2022)
	Permeability	Ileum	Female Sprague-Dawley rats treated with cholera toxin perorally via a gastric tube (n=2) or locally (n=4), control animals (n=4)	Cholera toxin significantly increased permeability to large molecules. The increase was the greater for local administration of cholera toxin compared to peroral treatment.	(Magnusson et al., 1985)
	Transit time	Whole gut	Children ≤ 5 years (n=68; including 29 cholera, 17 rotavirus, 13 enterotoxigenic Escherichia coli, and 9 Shigella cases) during acute stages of diarrhea	Mean whole gut transit time ranges from 5.5 to 7.3 h.	(Molla et al., 1983)
	Luminal fluid volume	Jejunum	Dog jejunal mucosa exposed to cholera toxin	In vitro fluid absorption rate was reduced by ~50% of the control value. Fluid secretion rate was increased.	(Lee & Silverberg, 1972)
	Luminal fluid volume	Fluid output	Adults with cholera	1 L/h fluid output	(Lippi et al., 2016; Sack et al., 2004)

HIV Infection	pH	Stomach	Fasting adults with/out HIV infection (n=203)	HIV infection was significantly associated with hypochlorhydria. Digitized median gastric pH was ~1.5 for HIV-negative subjects, ~4.9 for HIV+ subjects with CD4 cell count >200 cells/ $\mu$ L, ~3.5 for HIV+ subjects with CD4 <200 cells/ $\mu$ L, and ~1.5 for HIV+ subjects on highly active antiretroviral therapy.	(Kelly et al., 2010)
	pH	Stomach	HIV-positive (n=17), HIV-negative (n=78) outpatients symptomatic for hypochlorhydria and referred for gastroscopy	Mean gastric pH was 4.6 for HIV-negative patients and 5.6 for HIV+ patients. HIV infections was not independently associated with hypochlorhydria. Co-infection with HIV and <i>Helicobacter pylori</i> was significantly associated with hypochlorhydria.	(Geraghty et al., 2015)
	pH	Stomach	HIV-infected subjects (n=19)	60% of HIV+ patients and 67% of AIDS patients had persistently elevated gastric pH during the baseline period.	(Welage et al., 1995)
	Absorptive surface area	Jejunum	HIV-infected subjects with chronic diarrhea (n=21), controls (n=14)	Significant difference was found in mean surface area/volume ratio: 41.6 in HIV+ subjects and 56.5 in controls. Mean crypt length was significantly different: 45.9 in HIV+ subjects and 31.4 in controls.	(Batman et al., 2007)
	Absorptive surface area	Duodenum	HIV-infected patients (n=24), controls (n=21)	Villous atrophy was found in 54% of HIV-infected patients, compared to 0% in control subjects.	(Sakai et al., 2017)
	Absorptive surface area	Duodenum	HIV-positive patients (n=63), HIV-negative controls (n=36)	Based on duodenal biopsies, HIV+ patients had more inflamed duodenum than the controls. Those with chronic diarrhea and parasitic infection had more blunting than those with diarrhea but not parasitic infection.	(Conlon et al., 1990)

Permeability	Small intestine	HIV-positive subjects (n=60), HIV-negative controls (n=20)	Intestinal permeability was significantly increased with AIDS.	(Sharpstone et al., 1999)
Permeability	Small intestine	Black (n=39) and white (n=39) HIV-infected patients, black (n=40) and white (n=57) healthy controls	Both black and white HIV-infected patients with diarrhea had increased intestinal permeability and malabsorption compared to their respective healthy controls. Only white HIV-positive patients without diarrhea had increased intestinal permeability as compared with white healthy controls.	(Murphy et al., 1999)
Transit time	Stomach, jejunum to caecum	HIV-positive subjects (n=60), HIV-negative controls (n=20)	Gastric emptying was significantly delayed in AIDS patients. Mean jejunal to caecal transit time was not significantly different between controls (246 min) and patients without diarrhea or patients with diarrhea of causes other than cryptosporidiosis, although a trend of reduced transit time was seen for patients with pathogen-negative diarrhea or microsporidiosis. Patients with cryptosporidiosis and diarrhea had significantly reduced jejunal to caecal transit time (135 min) compared to the controls.	(Sharpstone et al., 1999)
Transit time	Whole GI	HIV-positive children (n=40) between 1 month and 3 years old, HIV-negative age-matched controls (n=30)	Whole GI transit time was shortest in severely symptomatic AIDS patients.	(Densupsoontorn et al., 2009)
Transit time	Stomach	HIV-positive patients (n=20), healthy volunteers (n=20 for liquid emptying rate, n=54 for solid emptying rate)	Mean gastric emptying was significantly faster in HIV patients (18 min) than in healthy controls (28 min). Mean solid emptying rate was significantly delayed in HIV patients ( $t_{1/2}$ = 88 min) compared to in healthy volunteers ( $t_{1/2}$ = 47 min).	(Konturek et al., 1997)

**Table 2.** Input parameters for iOWH032 PBPK model

Parameter	Unit	Value	Source
Molecular weight	g/mol	545.18	MedChem Designer™ version 6.3.0.4
log P		5.766	
Basic pKa <sub>1</sub> /pKa <sub>2</sub>		0.12 / -5.45	
Acidic pKa <sub>1</sub> /pKa <sub>2</sub>		6.19 / 11.2	
P <sub>eff, man</sub>	10 <sup>-4</sup> cm/s	2.703	
Diffusion coefficient	cm <sup>2</sup> /s * 10 <sup>5</sup>	0.627	
Solubility			
pH=7.4	mg/mL	0.162	
FaSSGF	mg/mL	0.001	
FaSSIF	mg/mL	0.007	
FeSSIF	mg/mL	0.029	
Fraction unbound	%	2.937	
B/P		0.667	
CL	L/h/kg	0.234	PKPlus™ module in GastroPlus®
V	L/kg	2.949	
t <sub>1/2</sub>	h	8.72	

CL: clearance; V: volume of distribution; t<sub>1/2</sub>: half-life

**Table 3.** Summary of observed and predicted values for iOWH032  $C_{max}$ ,  $AUC_{0-inf}$  and  $T_{max}$  in cholera patients.

	Population	$C_{max}$ (ng/mL)	$AUC_{0-inf}$ (ng*h/mL)	$T_{max}$ (h)	$C_{max}$ Ratio sim/obs <sup>b</sup>	$AUC_{0-inf}$ Ratio sim/obs <sup>b</sup>	$T_{max}$ Ratio sim/obs <sup>b</sup>
Observed*	Bangladeshi cholera patients	482	6250	3.8			
Predicted	Healthy fasted	1294	24587	7.6	2.68	3.93	2.00
	pH changes <sup>a</sup>	1394	24810	5.1	2.89	3.97	1.33
	TT changes <sup>a</sup>	1060	17920	5.9	2.20	2.87	1.56
	V changes <sup>a</sup>	749	16860	11.7	1.55	2.70	3.07
	pH + TT changes <sup>a</sup>	1435	24350	4.4	2.98	3.90	1.16
	pH + V changes <sup>a</sup>	681	16080	11.0	1.41	2.57	2.91
	TT + V changes <sup>a</sup>	703	11480	5.6	1.46	1.84	1.47
	pH + TT + V changes <sup>a</sup>	923	15380	5.5	1.91	2.46	1.44
	TT + V + SEF changes <sup>a</sup>	709	11390	5.6	1.47	1.82	1.47
	pH + TT + V + SEF changes <sup>a</sup>	889	14790	5.6	1.84	2.37	1.47

TT: transit time; V: luminal volume (including bile salt concentration adjusted based on changes in luminal volume); SEF: surface area enhancement factor; sim: simulated; obs: observed

\* Observed data were mean PK parameter values for Bangladeshi cholera patients with diarrhea reported by Kumar et al. previously (Kumar et al., 2014)

<sup>a</sup> Changes made to the healthy fasted population to create various cholera populations

<sup>b</sup> Ratio of simulated to observed value for  $AUC_{0-inf}$  and  $C_{max}$  in Bangladeshi cholera patients (n=14)



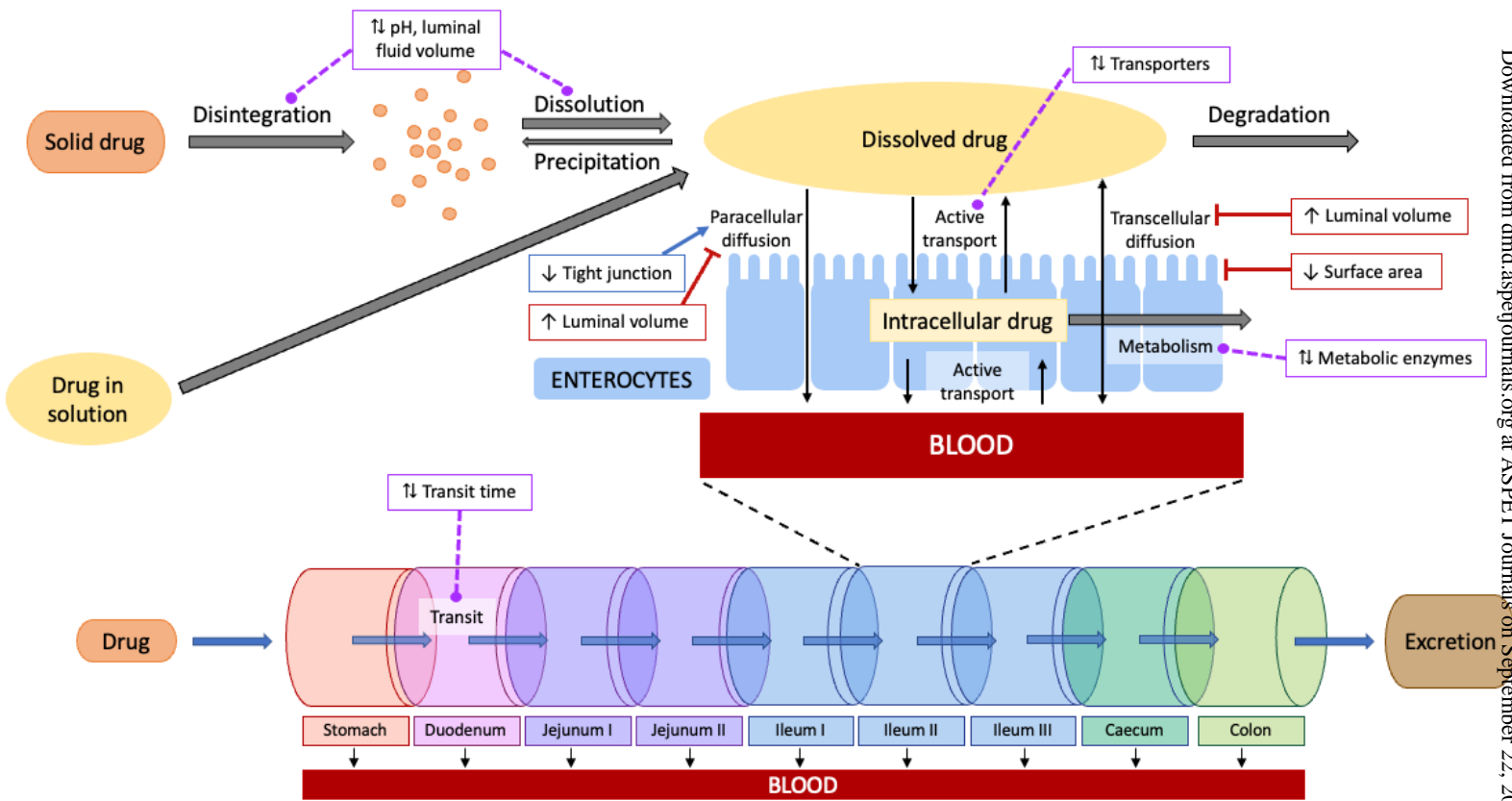
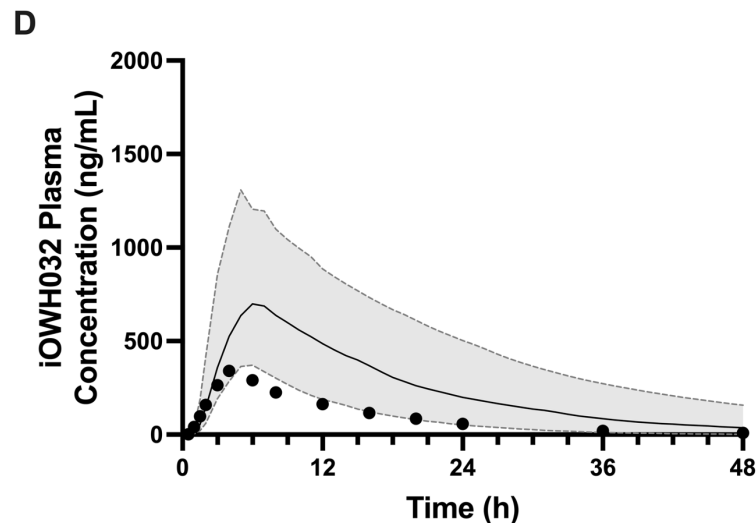
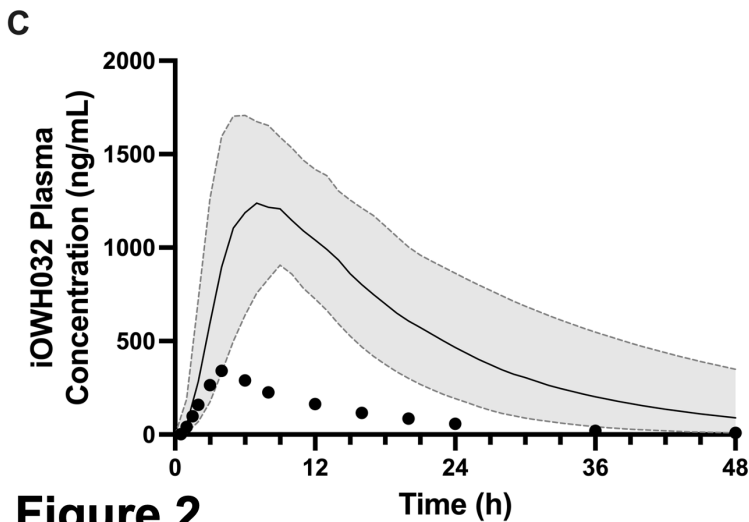
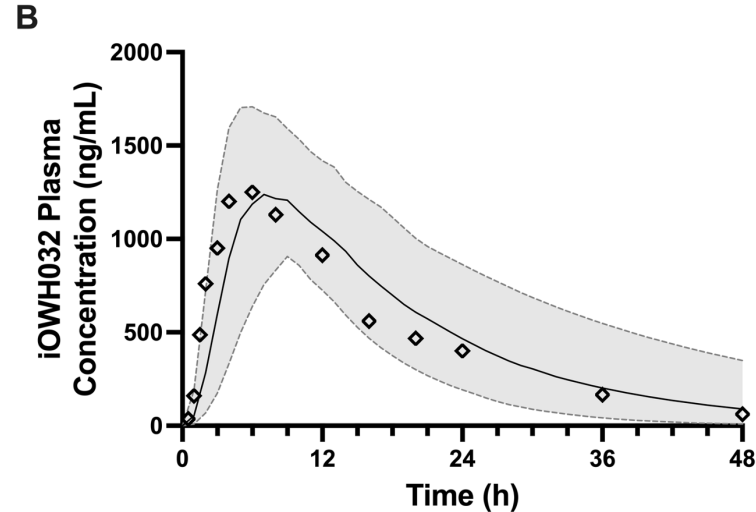
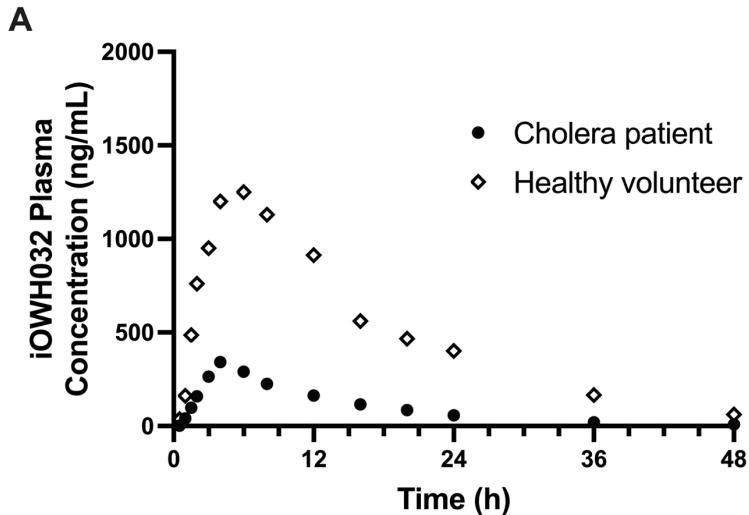


Figure 1

**Figure 2**