

Journal Pre-proof



Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review

Zahari Vinarov , Mohammad Abdallah , José Agundez , Karel Allegaert , Abdul W. Basit , Marlies Braeckmans , Jens Ceulemans , Maura Corsetti , Brendan Griffin , Michael Grimm , Daniel Keszthelyi , Mirko Koziolk , Christine M. Madla , Christophe Matthys , Laura E. McCoubrey , Amitava Mitra , Christos Reppas , Jef Stappaerts , Nele Steenackers , Natalie L. Trevaskis , Tim Vanuytsel , Maria Vertzoni , Werner Weitschies , Clive Wilson , Patrick Augustijns

PII: S0928-0987(21)00114-7
DOI: <https://doi.org/10.1016/j.ejps.2021.105812>
Reference: PHASCI 105812

To appear in: *European Journal of Pharmaceutical Sciences*

Received date: 5 December 2020
Revised date: 19 February 2021
Accepted date: 16 March 2021

Please cite this article as: Zahari Vinarov , Mohammad Abdallah , José Agundez , Karel Allegaert , Abdul W. Basit , Marlies Braeckmans , Jens Ceulemans , Maura Corsetti , Brendan Griffin , Michael Grimm , Daniel Keszthelyi , Mirko Koziolk , Christine M. Madla , Christophe Matthys , Laura E. McCoubrey , Amitava Mitra , Christos Reppas , Jef Stappaerts , Nele Steenackers , Natalie L. Trevaskis , Tim Vanuytsel , Maria Vertzoni , Werner Weitschies , Clive Wilson , Patrick Augustijns , Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review, *European Journal of Pharmaceutical Sciences* (2021), doi: <https://doi.org/10.1016/j.ejps.2021.105812>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier B.V.

Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review

Zahari Vinarov^{a,b}, Mohammad Abdallah^c, José Agundez^d, Karel Allegaert^{e,f}, Abdul W. Basit^g, Marlies Braeckmans^a, Jens Ceulemans^h, Maura Corsetti^{i,j}, Brendan Griffin^k, Michael Grimm^l, Daniel Keszthelyi^m, Mirko Koziolokⁿ, Christine M. Madla^g, Christophe Matthys^{o,p}, Laura E. McCoubrey^g, Amitava Mitra^q, Christos Reppas^r, Jef Stappaerts^h, Nele Steenackers^o, Natalie L. Trevaskis^c, Tim Vanuytsel^s, Maria Vertzoni^r, Werner Weitschies^l, Clive Wilson^t, Patrick Augustijns^{a,u,*}

^aDrug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

^bDepartment of Chemical and Pharmaceutical Engineering, Sofia University, Sofia, Bulgaria

^cDrug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia

^dUniversity Institute of Molecular Pathology Biomarkers, UEx. ARADyAL, Instituto de Salud Carlos III, Cáceres, Spain

^eDepartment of Hospital Pharmacy, Erasmus MC, University medical center Rotterdam, the Netherlands

^fDepartment of Development and Regeneration and Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

^gUCL School of Pharmacy, University College London, United Kingdom

^hPharmaceutical Sciences, Janssen Research & Development, Beerse, Belgium

ⁱNIHR Nottingham Biomedical Research Centre (BRC), Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, United Kingdom

^jNottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, United Kingdom

^kSchool of Pharmacy, University College Cork, Cavanagh Pharmacy Building, Cork, Ireland

^lDepartment of Biopharmaceutics and Pharmaceutical Technology, Center of Drug Absorption and Transport, University of Greifswald, Germany

^mDivision of Gastroenterology-Hepatology, Department of Internal Medicine, NUTRIM, Maastricht University Medical Center, the Netherlands

ⁿNCE Formulation Sciences, AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

^oClinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, KU Leuven, Belgium

^pDepartment of Endocrinology, University Hospitals Leuven, Belgium

^qClinical Pharmacology and Pharmacometrics, Janssen Research & Development, Spring House, Pennsylvania, USA

^rDepartment of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

^sTranslational Research Centre for Gastrointestinal Disorders (TARGID), Department of Chronic Diseases, Metabolism and Ageing (ChroMeTa), KU Leuven, Belgium

^tStrathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, United Kingdom

^uChairperson of the UNGAP network, COST CA16205 (www.ungap.eu)

***Corresponding author:**

Patrick Augustijns

Patrick.augustijns@kuleuven.be

Drug Delivery and Disposition,

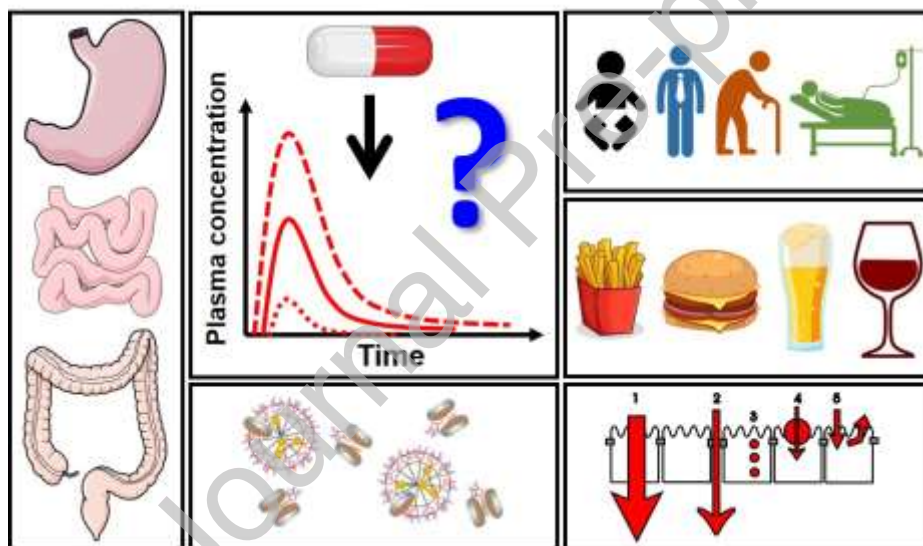
Department of Pharmaceutical and Pharmacological Sciences,

KU Leuven, Gasthuisberg O&N II,

Herestraat 49, Box 921,

3000 Leuven, Belgium

Graphical Abstract



ABSTRACT

The absorption of oral drugs is frequently plagued by significant variability with potentially serious therapeutic consequences. The source of variability can be traced back to interindividual variability in physiology, differences in special populations (age- and disease-dependent), drug and formulation properties, or food-drug interactions. Clinical evidence for the impact of some of these factors on drug pharmacokinetic variability is mounting: *e.g.* gastric pH and emptying time, small intestinal fluid properties, differences in pediatrics and the elderly, and surgical changes in gastrointestinal anatomy. However, the link of colonic factors variability (transit time, fluid composition, microbiome), sex differences (male *vs.* female) and gut-related diseases (chronic constipation, anorexia and cachexia) to drug absorption variability has not been firmly established yet. At the same time, a way to decrease oral drug pharmacokinetic variability is provided by the pharmaceutical industry: clinical evidence

suggests that formulation approaches employed during drug development can decrease the variability in oral exposure. This review outlines the main drivers of oral drug exposure variability and potential approaches to overcome them, while highlighting existing knowledge gaps and guiding future studies in this area.

Keywords (maximum of 6): variation, fasted and fed state, physiology, pediatrics and geriatrics, diseases, drug formulation

Table of contents

1	Introduction	8
2	Physiological inter- and intraindividual variability in the fasted and fed state.....	9
	2.1 Gastric conditions	9
	2.1.1 pH and buffer capacity	9
	2.1.2 Volumes	10
	2.1.3 Viscosity and osmolality	11
	2.1.4 Composition.....	11
	2.1.5 Gastric emptying of drugs.....	11
	2.2 Small intestinal phase	13
	2.2.1 Intestinal transit times	13
	2.2.2 Intestinal fluid volumes and pockets.....	17
	2.2.3 Chemical composition and colloidal aggregates in the small intestinal fluids ...	20
	2.2.4 Intestinal pH and buffer capacity	24
	2.2.5 Viscosity and osmolality of small intestinal contents	25
	2.2.6 Epithelial permeability.....	25
	2.2.7 Absorption into lymph and blood	27
	2.2.8 Enterohepatic circulation	29
	2.3 Colonic phase.....	32
	2.3.1 Colonic transit times	32
	2.3.2 Variability of luminal composition in distal ileum and proximal colon	34
	2.3.3 The microbiome	34
3	Effect of formulation on dosing and PK profile variability	38
	3.1 Solid state of the active ingredient.....	39
	3.2 Interplay pKa and gastro-intestinal pH variability.....	41
	3.3 Interplay between enteric polymers and gastro-intestinal pH variability	42
4	Variability in specific populations and patients with chronic conditions	43

4.1	Ageing and sex effects	43
4.1.1	Pediatrics	43
4.1.2	Geriatrics	45
4.1.3	Sex differences	45
4.2	Surgical conditions	48
4.2.1	Post-bariatric surgery changes	48
4.2.2	Effects of surgical resection	50
4.3	Chronic diseases	52
4.3.1	Effect of gut function variability in chronic constipation on clinical drug performance	52
4.3.2	Effects of small intestinal motility disorders on clinical performance of drugs ..	54
4.3.3	Patients on proton pump inhibitors	55
4.3.4	Effects of anorexia and cachexia on drug PK	57
5	Summary and future outlook.	59

Abbreviations list

5-FU	5-fluorouracil
ADME	Absorption, distribution, metabolism and excretion
AIDS	Acquired immunodeficiency syndrome
AUC	Area under the curve
BCS	Biopharmaceutics classification system
BMI	Body mass index
CD	Cyclodextrin
CETP	Cholesterylester transfer protein
CI	Confidence interval
CIPO	Chronic intestinal pseudoobstruction
C_{max}	Maximum plasma concentration
CRA	Cannabinoid receptor agonist
CTT	Colon transit times
CV	Coefficient of variance

CYP	Cytochrome P450
DAG	Diacylglycerides
DDI	Drug-drug interactions
D_{v50}	Median particle size by volume
EHC	Enterohepatic circulation
FaHIF	Fasted state human intestinal fluids
FaSSIF	Fasted state simulated intestinal fluids
FDA	U.S. Food and drug administration agency
FFA	Free fatty acids
GC	Glycocholate
GCDC	Glycochenodeoxycholate
GDC	Glycodeoxycholate
GET	Gastric emptying time
GIT	Gastrointestinal tract
HIV	Human immunodeficiency virus
HPMC	Hydroxypropyl methylcellulose
HPMC-AS	Hydroxypropyl methylcellulose acetate succinate
HPMCP	Hydroxypropyl methylcellulose phtalate
IBD	Inflammatory bowel disease
IMMC	Inter-digestive migrating motor complex
IR	Immediate release
L-DOPA	Levodopa
Lyso-PC	Lyso-phosphatidylcholine
MAG	Monoacylglycerides
MRI	Magnetic resonance imaging
NAPQI	N-acetyl- <i>p</i> -benzoquinone imine
NSAID	Nonsteroidal anti-inflammatory drug

OHM	1-hydroxy-midazolam
PBPK	Physiologically based pharmacokinetic modelling
PC	Phosphatidylcholine
PD	Pharmacodynamic(s)
PEG	Polyethylene glycol
PK	Pharmacokinetic(s)
PPI	Proton pump inhibitors
RYGB	Roux-en-Y gastric bypass
SBWC	Small bowel water content
SIBO	Small intestinal bacterial overgrowth
SITT	Small intestinal transit time
TAG	Triacylglycerides
TC	Taurocholate
TCDC	Taurochenodeoxycholate
TDC	Taurodeoxycholate
TKI	Tyrosine kinase inhibitors
t_{\max}	Time to reach maximum plasma concentration
UDC	Ursodeoxycholate
UNGAP	European network on understanding gastrointestinal absorption-related processes
VEGF-C	Vascular endothelial growth factor C

Introduction

The oral intake of drugs remains the preferred administration route because of its non-invasive character and convenience for the patient, which increases drug regimen compliance. However, ensuring sufficient and predictable systemic drug exposure when developing oral drug products is not straightforward (Basavaraj and Betageri, 2014; Korstanje, 2003; Li et al., 2016): issues with bioavailability and pharmacokinetics (PK) are among the top 3 reasons for attrition of oral small-molecule new drug candidates. In particular, the effectiveness of oral drug products in clinical practice can be plagued by significant variability in drug exposure with serious therapeutic consequences (Pasipanodya et al., 2012).

It is logical to expect that the factors, which control drug absorption and PK, are also responsible for the variability of drug exposure observed in the clinic. Hence, the impact of physiological differences (in special populations and gastrointestinal tract (GIT) regions), drug and formulation properties, and food-drug interactions on drug absorption was recognized and described by the European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP)(Boyd et al., 2019; Koziolok et al., 2019; Stillhart et al., 2020; Vertzoni et al., 2019). Considering the complexity and the lack of awareness about variability, the current review expands beyond the state-of-the-art to provide a focused description and analysis of the subject. Particular attention was paid to examples, which demonstrate the link between GIT variability and drug absorption/PK, in both fasted and post-prandial conditions.

All anatomical, physiological and pharmaceutical factors that were considered as a source of variability in the current paper are listed in Table 1.

Table 1. Anatomical, physiological and pharmaceutical factors discussed in the frame of oral absorption variability in the current paper.

Stomach	Small intestine	Colon	Special populations	Pharmaceutical factors
Gastric pH	Small intestinal transit time	Colonic transit times	Pediatrics	Drug properties
Volume of gastric fluids	Intestinal fluid volume	Colonic luminal composition	Geriatrics	Formulation effects
Viscosity and osmolality	Viscosity and osmolality	Microbiome	Sex differences	Fasted vs. fed state
Composition of gastric fluids	Intestinal fluid composition		Post-bariatric surgery changes	
Gastric emptying time	Intestinal pH		Surgical resection	
	Epithelial permeability		Chronic constipation	
	Absorption into blood and lymph		Small intestinal motility disorder	
	Enterohepatic circulation		Patients on proton pump inhibitors	
			Anorexia and cachexia patients	

The drug transit times (gastric emptying time (GET), intestinal transit time) and fluid volumes (including intestinal fluid pockets) in the GIT have been identified as considerable sources of variability and are the first factors discussed in the current review. The body of literature, which describes the compositional and physicochemical (*e.g.* pH and buffer capacity (buffer capacity)) variability of human gastric and intestinal fluids, will also be discussed from the angle of their contribution to oral absorption variability. Further down the GIT, the impact of the gut microbiota and bacterial drug degradation on variability will be addressed. The specific effect of formulation-related parameters are described in a separate section. Next, a number of additional factors, which are usually disregarded, but can play a huge role in the context of variability will be examined. These include the influence of age (pediatrics, geriatrics), sex and disease-specific differences. Finally, an industry

perspective on how drug product development takes into account the aforementioned challenges and succeeds to limit variability and provide drug exposure at therapeutic levels, will be presented.

In various sections, results obtained from different studies are reported. In order to compare the study results, the best option would be to perform a meta-analysis of the available data. However, as most results available in the literature are based on small scale explorative clinical studies, a statistical comparison is usually not justified. In addition, differences in the experimental protocols and instrumentation used to gather the data would undermine any attempts at such analysis. Therefore, results are reported in a descriptive way.

Furthermore, the impact of methodology, clinical study design and statistics on the measurement and quantification of variability is an issue on its own (Augustijns et al., 2020; Evans, 2010; Pocock et al., 2015) and will not be addressed in the current paper. The effect of drug absorption variability on clinical performance and therapeutic outcome is also not in the scope of the current review.

Physiological inter- and intraindividual variability in the fasted and fed state

Gastric conditions

This section focuses on the variability of data in healthy fasted and fed state adults. Fasted state is defined as an overnight fast, followed by a glass of water in the morning. The fed state data reported was generated by using the standard U.S. Food and drug administration agency (FDA) meal: a high-calorie (900-1000 kcal) breakfast with approximately 150, 250, and 500-600 calories originating from protein, carbohydrate and fat, respectively (EMA, 2010; FDA, 2002).

pH and buffer capacity

In the fasted state, median gastric pH values 10-20 min and 30-40 min after a glass of water have been reported to be 1.7-3.3 and 1.6-2.7, respectively (Kalantzi et al., 2006; Koziolok et al., 2014; Petrakis et al., 2015). The resistance of gastric fluids to increase with one pH unit when titrating with NaOH (buffer capacity) varies with gastric pH and becomes close to zero in adults treated with famotidine (Figure 1).

Oncology drugs constitute an important example of the impact of gastric pH in PK variability, as about half of oral cancer therapies are weak bases and display solubility-limited dissolution properties (Smelick et al., 2013). The implications of chronic use of PPI inhibitors on oral drug absorption are considerable and are examined in detail in section 4.3.3.

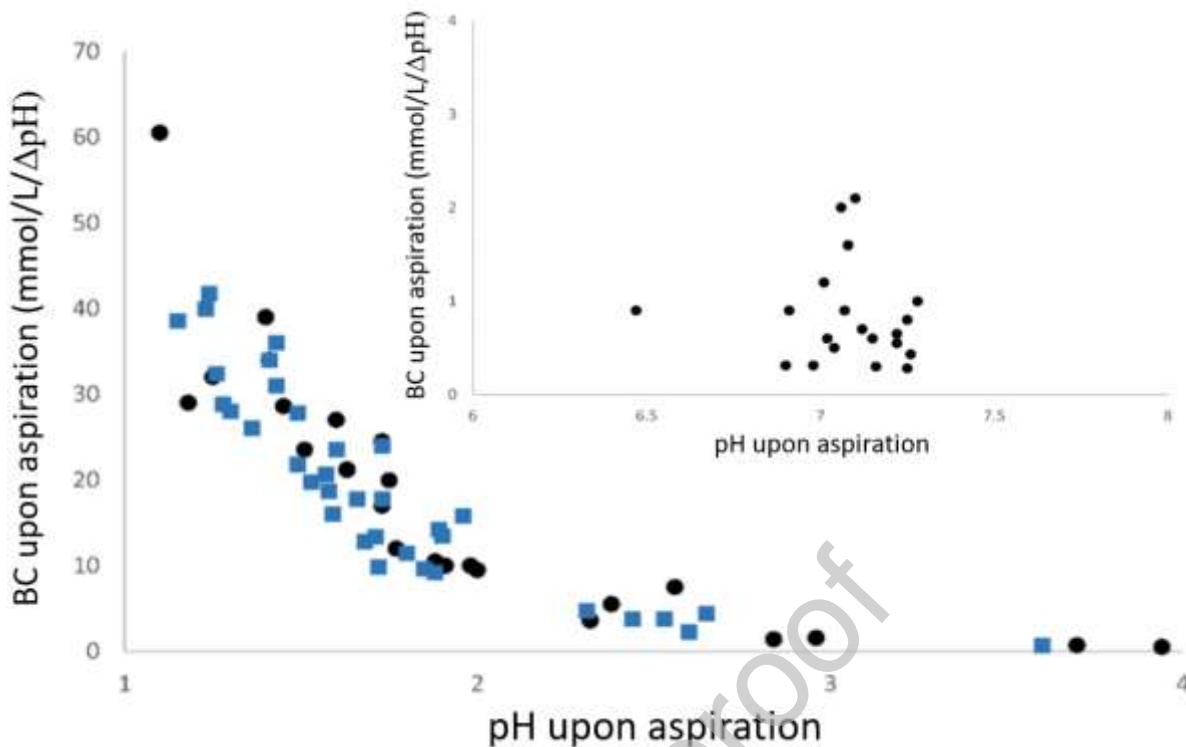


Figure 1. Buffer capacity (BC) of antral contents of healthy adults in the fasted state vs. the corresponding pH values estimated after titration with NaOH (data from (Kalantzi et al., 2006; Litou et al., 2016), squares and circles, respectively, $n=60$ individual samples measurements). The insert shows individual data estimated after titration of aspirates collected from healthy adults who had been treated with famotidine (data from (Litou et al., 2016), $n=16$ individual measurements) (modified from (Litou et al., 2020)).

In the fed state, the median pH values reported in literature at 30 min and 60 min after administration of the standard meal are around 3.5 (Dressman et al., 1990; Koziolok et al., 2015b) and 3 (Dressman et al., 1990; Koziolok et al., 2014; Pentafragka et al., 2020b), respectively. pH values return to baseline levels (median pH value lower than 2) at about 3 hours after the meal (Dressman et al., 1990; Koziolok et al., 2014). Interestingly, intraindividual variability in pH values is low. The average difference in pH values at specific time points during the first three hours after meal administration in a given individual ranges from -0.3 to 0.9 pH units (Pentafragka et al., 2020b). During the first four hours after initiation of meal administration, the average buffer capacity of antral contents is about 20 mmol/L/ Δ pH (Pentafragka et al., 2020b). In line with pH data, the average difference in buffer capacity values during the first three hours after meal administration in a given individual is low and ranges from -3.4% to 15% (Pentafragka et al., 2020b).

Volumes

Based on various magnetic resonance imaging (MRI) studies performed after at least 8 h of fasting, the volume of gastric contents is typically below 50 mL (Koziolok et al., 2016). Interestingly, interindividual and intraindividual variability is comparable, suggesting that the variability within the studies is mainly resulting from intraindividual day-to-day variations (Grimm et al., 2018a). On the other hand, direct aspiration of gastric contents in the fasted state indicates very limited resting gastric volumes (mean and median values less than 10 mL, $n = 15$) (Vertzoni et al., 2020a).

In the fed state, intragastric volumes are similar or slightly higher than the volume of the standard meal (slightly more than 500 mL) for more than two hours after administration of the standard meal (Koziolek et al., 2014; Pentafragka et al., 2020b), implying that gastric emptying of meal contents is balanced by intragastric secretions. Inter-individual variability in volumes 15 minutes after intake of the standard meal is low (579.6 ± 38.1 mL, $n=12$) (Koziolek et al., 2014).

Viscosity and osmolality

Both under fasting and under fed state conditions the gastric contents show pseudoplastic behavior, *i.e.* viscosity decreases with increasing shear rate. Especially after the standard meal, intragastric viscosity is highly variable (Pentafragka et al., 2020a). On average, the viscosity of gastric contents in the fasted state at 37 °C (1.4-6.4 mPa·s at a shear rate of 100 s^{-1} (Pedersen et al., 2013)) is 80-800 times lower than the viscosity at a shear rate of 100 s^{-1} , after the standard meal (Pentafragka et al., 2020a). Similar observations have been made at shear rates of 50 and 200 s^{-1} . A non-caloric hydroxy-propyl-methyl-cellulose aqueous meal, with a viscosity similar to intraluminal fed state values (Pentafragka et al. 2020b), has been shown to significantly decrease indinavir plasma concentrations after administration of Crixivan® capsules, as compared to the fasted state administration with an equal volume of water (Carver et al., 1999).

In the fasted state, contents are highly hypoosmotic (Pentafragka et al., 2019). After the standard meal, osmolality increases to reach iso-osmotic levels (Pentafragka et al., 2020a). The potential impact of such difference on gastric emptying rates in humans has not been investigated, however, hyperosmolarity delays the gastric emptying of liquid caloric meals (Paraskevopoulos et al., 1988; Vermeulen et al., 2011).

Composition

In the fasted state, bile salt levels are highly variable and concentrations are up to 0.15 mM on average (Pentafragka et al., 2019). In the fed state, no data after the standard meal have been reported.

There are no data on lipid levels in the fasted state (Pentafragka et al., 2019). After the standard meal, lipid concentrations are highly variable both between and within subjects (Pentafragka et al., 2020a). One reason may be the non-homogenous distribution of lipid components in the gastric contents (Koziolek et al., 2014). On average, triacylglycerides (TAG), diacylglycerides (DAG), free fatty acids (FFA) and phosphatidylcholine (PC) are comparatively the most abundant lipids (Pentafragka et al., 2020a). It has been shown that modelling of intragastric lipolysis is necessary for simulating felodipine release from extended release tablets in the fed stomach (Diakidou et al., 2009a), whereas simulation of colloidal species seems to be key for the prediction of intragastric apparent solubility of lipophilic molecules in the fed stomach (Diakidou et al., 2009b).

Gastric emptying of drugs

Fasted state

Gastric emptying of aqueous drug solutions administered in a total volume of about a glass of water (200-250 mL) is an apparent first-order process (Grimm et al., 2018b; Mudie et al., 2014). Interindividual and intraindividual variability of the process is comparable, suggesting that the variability within the studies was mainly resulting from intraindividual day-to-day variations (Grimm et al., 2018a). Based on published data, gastric emptying half-life can be estimated to be 11 min (85% of initial gastric volume is emptied after 30 min (Grimm et al., 2018a)) or slightly longer (15 min

(Kourentas et al., 2016b)). A slightly increased half-life of gastric emptying may be expected in case the administered aqueous solution is acidic or with increased caloric value (Cooke and Hunt, 1970).

Drug suspensions in aqueous media seem to empty with water unless intragastric dispersion of the particles is problematic. In the latter case, gastric emptying can be delayed substantially (Kourentas et al., 2016a).

Disintegrating solid dosage forms typically empty from stomach after disintegration. Rupture times of less than 10 min have been reported for hard gelatine capsules (Digenis et al., 2000). On average, disintegration times in the stomach after administration of the dosage form with a glass of water have been reported to be 10-20 min for tablets (Kelly et al. 2003) and little less than 30 min for the immediate release (IR) layer of a modified release product (Weitschies et al., 2008). It should be noted, however, that disintegration times depend to a significant extent not only on physiological conditions (liquid volumes, mixing), but also on the type of formulation and excipients used (Quodbach and Kleinebudde, 2016). As most of the water will be emptied until complete disintegration of tablets, emptying of disintegrating particles will be affected by the inter-digestive motility pattern and the density of the disintegrated particles. One of the first relevant studies in adults was performed by Aoyagi et al (Aoyagi et al., 1992). After an overnight fast, three adults received together with 200 mL water at two different occasions on a crossover basis: (A) 5 spherical enteric coated tablets of barium sulfate and (B) 50 cylindrical granules of barium sulfate coated with ethylcellulose. No food was consumed until 4 h after dosing. The number of tablets and granules remaining in the stomach were determined by periodic roentgenography. Data after administration of the tablets in one of the adults indicated 80 % gastric retention at 2 h. The same volunteer retained about 90 % of the granules in the stomach 1.5 h after dosing, with the granules appearing to lie along the gastric wall, as if trapped in the mucus layer. Authors concluded that the strength of the phasic contractions in addition to the time of occurrence of the phasic gastric contraction seemed to increase the variability of gastric emptying of non-digestible solids, at least of those with somewhat increased density.

Non-disintegrating dosage form (*e.g.* enteric coated or certain extended release tablets) are emptied from the stomach mainly during Phase III of the inter-digestive migrating motor complex (IMMC), *i.e.* their bolus emptying is difficult to manage, and can remain in the stomach up to 2 h or even longer (Koziolek et al., 2016). A significant consequence of this variability relates to the fact that safe and efficacious gastroretentive dosage forms remain an unmet goal, especially under fasting conditions, although they are considered as promising drug delivery systems (Lopes et al., 2016).

Fed state

Aqueous drug solutions administered after the standard meal are typically emptied as if they were administered in the fasted state (Grimm et al., 2017). For IR tablets or IR layers of modified release tablets, time for complete disintegration in the human stomach after a high-caloric, high-fat meal has been reported to range on average from slightly more than 10 min to about one hour (Kelly et al., 2003; Weitschies et al., 2008; Rubbens et al., 2019). For hard gelatine capsules, rupture time in the fed state is typically slightly longer than 10 min (Digenis et al., 2000). After disintegration of the dosage form, gastric emptying half-lives of Biopharmaceutics classification system (BCS) class 1 and class 2 drugs administered after the standard meal have been estimated to be around 40 min (Pentafraqka et al., 2020b). Data on disintegration times and subsequent gastric emptying half-lives are in line with earlier data: the half GET of paracetamol administered as Panadol IR tablets after the standard meal varies from 77 to 106 min (Kelly et al., 2003) with obvious consequences on the

variability in the onset of paracetamol absorption. The large variation could be explained by the length of the lag period prior to emptying, which is related to the principal motor reaction of the stomach after food ingestion, *i.e.* the accommodation reflex and the retroulsive antral contractions (Hasler, 2008). Non-disintegrating dosage forms will be retained in the stomach until the recurrence of the IMMC activity in the upper GIT, *i.e.* for more than 4 h after administration of the standard meal (Koziolek et al., 2016).

Small intestinal phase

Intestinal transit times

Although the small intestinal transit time (SITT) of oral drug products is often reasonably predictable, the presentation of the drug for absorption depends on various factors. These include the properties of the drug product (*e.g.* disintegration behavior), matrix characteristics as well as the individual gastrointestinal physiology (*e.g.* motility, dietary habits). In particular, for drugs with low permeability (BCS class 3 and 4 drugs) and those with limited absorption in the colon, the SITT may be of critical importance for drug absorption (Sugihara et al., 2015). Specifically, a drug product may have a SITT too short for dissolution or for absorption. Furthermore, there may also be a preferred region for drug absorption in the small intestine (absorption window). This is usually encountered in the upper small intestine, where the drug dissolved in the stomach remains supersaturated as it enters the intestine in chyme for a variable time before nucleation and precipitation. Moreover, the expression of uptake and efflux transporter as well as of drug-metabolizing enzymes also varies along the GIT, which can also lead to regional differences of drug absorption (Drozdziak et al., 2014; Fritz et al., 2018).

Various methods are available to assess the variability of intestinal transit times for liquids as well as solid objects. These include methods based on PK markers [*e.g.* combined use of paracetamol (gastric emptying marker) and sulfasalazine (colon arrival marker)], scintigraphy, magnetic marker monitoring (magnetic marker monitoring), magnetic resonance imaging as well as telemetric capsules (Hens et al., 2017). It should be noted that the *in vivo* technique itself as well as the study protocol can also contribute to the variability (see Table 2).

Typically, a range of 3–4 h is presented in the literature for SITT, being less variable as compared to gastric and colonic transit time. This value is in line with a meta-analysis on SITTs of single- and multiple-unit dosage forms that was recently published (Abuhelwa et al., 2016). In this work, the meta-mean SITT was 3.49 h and the meta-SD was 1.02 h. Thereby, the mean SITT was neither affected by the prandial state nor the type of dosage forms. Overall, similar values were observed for multiple and single-unit dosage forms. Further studies have also shown that SITT is not affected by the size of the object investigated, age and sex (Davis et al., 1986; Khosla et al., 1989).

Table 2. *In vivo* methods used to characterize SITTs.

Technique	Short description	Limitations
<i>PK markers</i>	Determination of SITT of solutions by calculating the difference between the onset of plasma concentrations for gastric emptying marker (<i>e.g.</i> paracetamol) and colon arrival marker (<i>e.g.</i> sulfasalazine)	Less suitable for solid objects as drug release occurs with a certain delay
<i>Scintigraphy</i>	Assessment of SITT based on image analysis. Useful for monitoring dispersion of drug formulation in the GIT	Lack of anatomical information in scintigraphic images
<i>Magnetic Marker Monitoring</i>	Assessment of SITT based on analyzing the tracking data of a magnetically labelled object	Limited availability of the equipment, lack of anatomical information, limited to single object
<i>Magnetic resonance imaging</i>	Assessment of SITT based on image analysis	Often limited by the frequency of imaging timepoints, contrasting agent typically needed
<i>Telemetric capsules</i>	Assessment of SITT based on characteristic pH changes in the GIT lumen	Colon arrival not always clearly detectable, no information about transit behavior within the small intestine as there are no pH 'landmarks'.

However, by having a closer look into different studies it is obvious that the SITT can also vary considerably and that certain aspects must be taken into deeper consideration. Published data from telemetric capsules as well as from scintigraphy have demonstrated that the SITT can vary between less than 1 h and more than 10 h (Aburub et al., 2018; Davis et al., 1986; Fallingborg et al., 1990; Koziolok et al., 2015b; Peh and Yuen, 1996). Solid objects larger than 5 mm are typically emptied from the stomach only by strong peristaltic waves occurring during phase III of the Migrating Motor Complex in the fasted state. Since these peristaltic waves can move down to the ileum, their presence may explain exceptionally short SITT as they have been observed in some studies. Additionally, the aforementioned meta-analysis revealed that an increased caloric content of the test meal results in a reduced variability in SITT of solid dosage forms. Moreover, Adkin *et al.* have shown that some excipients (*e.g.* mannitol) used in oral drug delivery can also affect the SITT of oral dosage forms (Adkin et al., 1995a). An excellent summary of the effects of various excipients on SITT was provided in a review by Yuen (Yuen, 2010). Moreover, certain drugs (*e.g.* opioids, metoclopramide erythromycin, laxatives) are known to affect GI motility and by this, they may also affect the SITT (Deane et al., 2009; Litou et al., 2019). Interestingly, some drugs such as erythromycin can accelerate gastric emptying, but seem to have little effect on small intestinal transit time (Deane et al., 2019). It can be expected that absolute variability will be reduced in case of prokinetic drugs, but conclusive literature on this question could not be identified.

An interesting study has been published by Ibekwe *et al.*, in which the transit behavior of the Bravo pH capsule and a tablet coated with Eudragit S was studied by scintigraphy (Figure 2) (Ibekwe *et al.*, 2008). As can be seen from Figure 2, the variability that was observed for the SITT in the aforementioned studies represents the sum of the variability of transit through the upper small intestine as well as the residence time at the ileocecal junction, an area serving as a reservoir. Both times can be variable and depend on further factors. The residence time at the ileocecal valve is mainly controlled by the gastroileal reflex, which represents a physiological mechanism that enables the transfer of small intestinal contents into the caecum upon intake of food and drinks (Deiteren *et al.*, 2010). Therefore, the dietary regime, in particular subsequent meals or drinks, can have dramatic consequences for the SITTs and orocecal transit times in clinical studies as it affects the transfer of material from the ileum to the cecum (Priebe *et al.*, 2006; Priebe *et al.*, 2004).

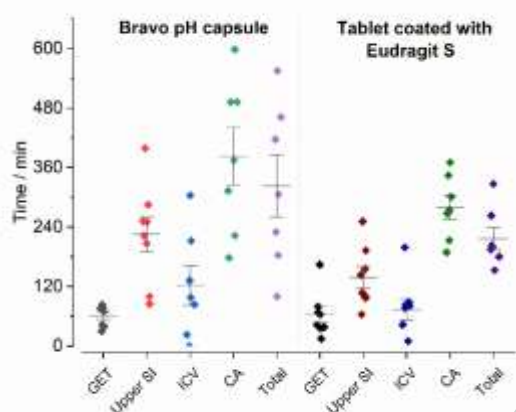


Figure 2. Variability of transit times of Bravo® pH capsule (left hand side) and a tablet (diameter: 8 mm; right hand side) coated with Eudragit® S in 8 fasted, healthy subjects. GET – gastric emptying time, Upper SI – upper small intestinal transit time, ICV - residence time at the ileocaecal valve, CA – caecal arrival time. The figure is based on data published by Ibekwe and colleagues (Ibekwe *et al.*, 2008).

The variability of transit times through certain parts of the small intestine is also of interest, in particular for drugs with pronounced regional differences in absorption (*e.g.* levodopa (L-DOPA), furosemide). Unfortunately, little is known about regional transit time as the visualization of dosage forms along with exact localization within the small intestine is hampered by the complex anatomy and therefore, often not possible. Nonetheless, imaging techniques such as scintigraphy and magnetic marker monitoring have been used in the past for the purpose of determining regional transit times. For instance, magnetic marker monitoring data have shown that the transfer through the duodenum is typically fast (Weitschies *et al.*, 1999). In addition, the application of these techniques has also revealed that the transit of dosage forms through the small intestine is not a continuous process but characterized by alternating phases of low and high transit velocities. Thereby, dosage forms are in rest most of the time (Weitschies *et al.*, 2005). Multiple-unit formulations as well as formulations disintegrating in the stomach were further shown to spread within the small intestine before re-grouping again in the ileum (Khosla *et al.*, 1989). The dispersion within the small intestine was mainly affected by the kinetics of gastric emptying (Yuen, 2010).

Apart from SITTs of solid objects, pharmaceutical scientists are also interested in assessing the transit of solutions and suspensions. A meta-analysis of scintigraphic data in 1986 suggested that SITT

of solutions, multiple-unit and single-unit formulations are in the same range (Davis et al., 1986). In contrast, Kellow and colleagues administered a suspension containing 2 g of sulfasalazine directly into the duodenum of three healthy subjects. Interestingly, the time until sulfapyridine, a degradation product indicating colon arrival, could be detected varied between 90 and 140 min (Kellow et al., 1986). This data set suggests that liquids may be transferred quicker through the small intestine as compared to solid objects. However, further information is needed to confirm this hypothesis.

Although large variability in SITTs has been shown for different oral dosage forms, only a few studies have linked changes in SITT with changes in PK parameters. It seems obvious that for drugs with poor permeability longer exposure times would lead to higher drug absorption, but despite its relevance, this effect has not been well studied yet. Riley and co-workers have shown that for two poorly permeable drugs, atenolol (BCS class 3) and hydrochlorothiazide (BCS class 4), an increased osmotic load results in limited drug absorption (Riley et al., 1992). As compared to administration with water, the AUC of these drugs is clearly lower if they are co-administered with solutions containing high osmotic loads. However, it should be noted that high osmotic loads can also change the fluid volumes present in the small intestine and thus, may also result in changes of intraluminal drug concentration (Grimm et al., 2018b). The effects seen in this study may not only result from changed transit times and therefore, further studies are needed to investigate the link between transit time and drug absorption in humans. In addition, *in silico* investigations can also be applied to study the effect of variability in SITT on drug absorption (Willmann et al., 2009).

Intestinal fluid volumes and pockets

The described transit of dosage forms or drug substances always needs to be evaluated considering the available amount of dissolution medium and its physicochemical properties. It is very likely that not only different transit times, but also different media volumes and compositions contribute to PK variability.

The available fluid volume plays a key role in dissolution and absorption processes as it determines the concentration arising from a specific dose of a drug (Koziolok et al., 2016; Sutton, 2009; Yu et al., 2017). This role of fluid volume represents a general consent mainly based on physical laws like the Noyes-Whitney equation for dissolution rate and Fick's laws of diffusion. Depending on dosage form and physicochemical properties of the drug, a change in the available intestinal volume can lead to increased or decreased bioavailability (Grimm et al., 2018b; Karsdal et al., 2008; Koziolok et al., 2016; Sunesen et al., 2005).

Thus, the pronounced variability of freely available fluid in the small intestine is of high relevance and needs to be considered accordingly. After overnight fasting, very different average values of small bowel water content (SBWC) within representative young and healthy study populations were observed with values of 51 ± 34 mL (n=24), 91 ± 68 mL (n=16), 43 ± 14 mL (n=12) and 105 ± 72 mL (n=12), respectively (Grimm et al., 2018b; Marciani et al., 2010; Mudie et al., 2014; Schiller et al., 2005). Although all measurements were performed by T2 weighted MRI, differences might not only be attributed to interindividual variability or differences between subject groups, but also methodical differences in volume quantification. Thus, statistical comparison of these studies or meta-analysis are not meaningful. Moreover, individual data would be lacking. Repeated measurements of the same subject group of six individuals revealed no significant differences in resting SBWC in fasted state between four consecutive study days by use of Friedman test (Grimm et al., 2018b). Nonetheless, the ranges from minimum to maximum volume in the aforementioned studies were impressive. The ranges amounted to 45–319 mL (Schiller et al., 2005), 12–253 mL

(Marciani et al., 2010) and 5-159 mL (Mudie et al., 2014) at starting conditions of common clinical studies. The range might be a better predictor for variability than standard deviations, as it depends less on the sample size. The rapid but variable gastric emptying not only leads to an increase in SBWC after intake of water with the dosage form, but also in its variability. In a study by Grimm et al., the mean SBWC amounted to 54 ± 42 mL ($n=6$) with a range of 18-121 mL after overnight fasting. Nine minutes after intake of 240 mL of water, the variability was peaking with a range of 41-204 mL. This increase is only short lived, since, after 24 min, a mean SBWC of 45 mL with a range of 18-134 mL was observed (Grimm et al., 2018b). Due to the rapid distribution of fluid from the stomach through the jejunum and subsequent absorption, the available volume, its variability and its distribution are comparable to conditions before intake after approximately 45 min (Grimm et al., 2018b; Mudie et al., 2014). The SBWC and its variability in clinical trials under fasted conditions can be seen in Figure 3.

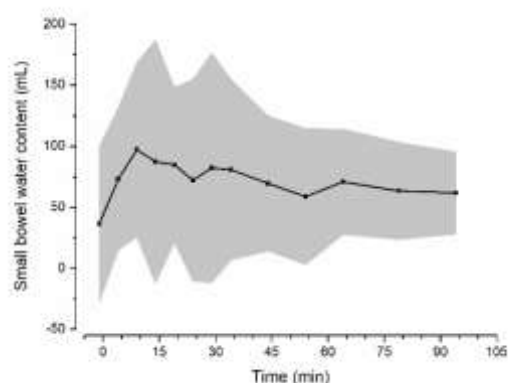


Figure 3. Median of SBWC (black) with interquartile range (the difference between 75th and 25th percentiles) (grey) before ($t = -1$ min) and after intake of 240 mL of water under fasted conditions ($n=6$)(Grimm et al., 2018b).

Data on intraindividual variability under clinically relevant conditions is lacking, but referring to data obtained for gastric volumes and gastric water emptying, it is very likely that also for SBWC, intraindividual variability is comparable to interindividual variability (Grimm et al., 2018a).

Besides the variability of cumulative small intestinal fluid volume, the small intestinal fluid distribution is characterized by variability. It is known that there is not one coherent volume in the small bowel, but that the volume is distributed in several discrete fluid pockets (Schiller et al., 2005). MRI studies revealed a highly variable and dynamic distribution of fluid pocket quantity and pocket volume, as illustrated in Figure 4. A study by Schiller et al. reported a median number of 4 fluid pockets with a median volume of 12 mL (Schiller et al., 2005). In contrast, Mudie et al. reported a mean of 8 ± 1 pockets with a mean volume of 4 ± 1 mL (Mudie et al., 2014). These highly variable but small coherent volumes will also have implications on dissolution rate, local drug concentrations and eventually on absorption.

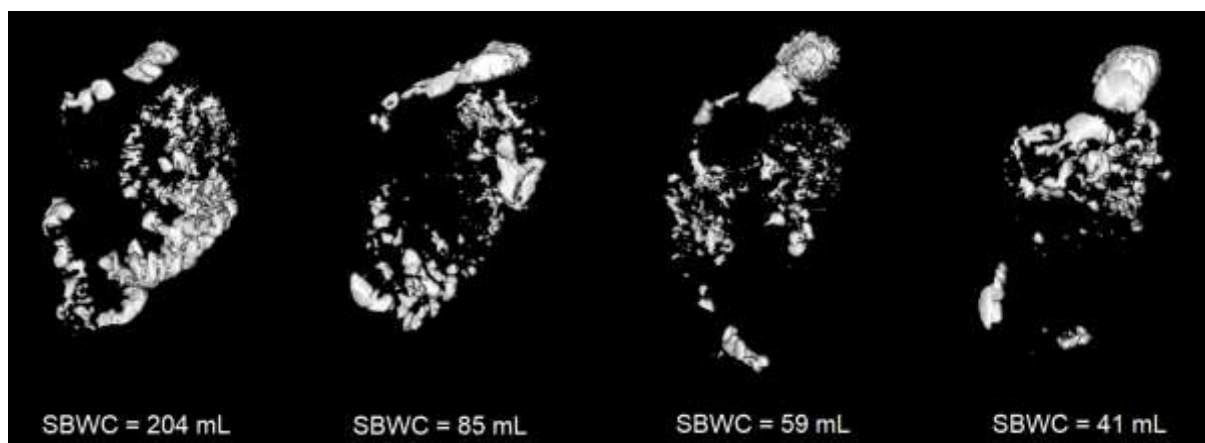


Figure 4. Clear differences in amount and distribution of fluids in 3D reconstructions of gastrointestinal water from four different subjects 9 min after intake of oral dosage form with 240 mL water in fasted state conditions.

Irrespective of the real number of pockets and their volume, these observations show that a dosage form in the small bowel might not necessarily have contact with a relevant amount of dissolution medium. It has been reported that, about 30 % of monolithic dosage forms had no contact and additional 20 % only had partial contact to intestinal fluids during transit after fasted administration (Schiller et al., 2005). Especially for enteric coated dosage forms, this variable contact with fluid pockets might explain the high variability of disintegration time after the gastric emptying of the dosage forms (Al-Gousous et al., 2017; Grimm et al., 2019; Wilding et al., 1992).

Regarding small intestinal volumes, the postprandial intake of dosage forms under conditions representing typical clinical studies is insufficiently studied until now. Nonetheless, particular food components were shown to have a specific effect not only on small intestinal volumes but also on its variability. For example, glucose is able to decrease variability of SBWC, whereas fructose can drastically increase it (Grimm et al., 2018b).

Moreover, a decrease in the volume of free fluid and high interindividual variability was observed after a meal, together with an increase in the number of fluid pockets (Schiller et al., 2005). But those evaluations of free intestinal fluids in fed state conditions need to be interpreted and used with care. Since the small intestine is filled with an inhomogeneous slurry of partially digested food components, the determination of freely available water in fluid pockets in this chyme and water bound to chyme is highly dependent on imaging and analysis procedures. It is to be expected that the variability of free small intestinal media is increased not only in terms of amount but also in distribution. On the other hand, the small bowel might be filled more quantitatively after a large meal, so that variations due to erratic fluid contact could be reduced.

The intake of oral dosage forms with or without food not only changes the volume available and its distribution, it can also cause dramatic changes in the physicochemical properties of the media.

Chemical composition and colloidal aggregates in the small intestinal fluids

The composition of human intestinal fluids changes constantly during the day, leading to great inter- and intraindividual variability. In addition, the intake of food leads to different biliary/pancreatic secretions, which further complicates the intestinal environment. Reported concentrations of the chemical composition of the GIT also depend on the study design (*e.g.* amount and timing of water intake, composition of the administered meal) and how the samples are handled and analyzed (Fuchs

and Dressman, 2014). An overview of the chemical composition of small intestinal fluids in the fasted and fed state (without drug co-administration) and the associated variability is summarized in Tables S1 and S2 (available in the Supporting information).

Bile salts

Bile salts are secreted into the upper GIT to aid in the solubilization of lipophilic compounds. In the fasted duodenum, the total bile salt concentration reported in the literature ranges from 0.03 to 36.18 mM (Table S1A in the Supporting information) (Riethorst et al., 2016b). However, bile salt levels are generally considered to be low (< 5 mM) in the fasted state, resulting in an overall mean of 3.3 mM in the duodenum and 3 mM in the proximal jejunum (Fuchs and Dressman, 2014). Reported high values are thought to be caused by sampling immediately after gall bladder emptying. In the fed state, a larger range in total bile salt levels was reported, namely between 0.74 and 86.14 mM (median 9.59 mM) (Riethorst et al., 2016b). This range is broader compared to Bergström et al., who reported a range of 3.6 to 24 mM (median 11.8 mM) (Bergstrom et al., 2014). In addition, the qualitative and quantitative composition of individual bile salts in the small intestine is highly variable (Table S1B in the Supporting information). Taurocholate (TC), glycocholate (GC) and glycochenodeoxycholate (GCDC) together represent about 70 – 75 % of the total bile salt concentration in the fasted state small intestine (Fuchs and Dressman, 2014; Persson et al., 2006). In both duodenum and jejunum, TC is most prevalent, followed by almost equivalent amounts of GC and GCDC (Fuchs and Dressman, 2014; Perez de la Cruz Moreno et al., 2006). In general, glyco-conjugated bile salts are more abundant than tauro-conjugated bile salts (70% vs. 30%, respectively) (Riethorst et al., 2016b). The relative abundance of individual bile salts was reported to remain nearly constant over time for all volunteers in both fasted and fed states (Riethorst et al., 2016b).

Phospholipids

Besides bile salts, phospholipids are also secreted with bile into the duodenum to aid in the solubilization of lipophilic compounds. The most prevalent phospholipids found in the GIT are PC and its hydrolysis product lyso-phosphatidylcholine (lyso-PC). PC is hydrolyzed in the small intestine to lyso-PC and a free fatty acid by phospholipase-A2 and nonspecific lipases secreted by the pancreas. Persson et al. showed that 98.4 % of the total phospholipid content consists of lyso-PC (Persson et al., 2005). In the fasted state, a range of total phospholipids between 0.01 and 6.33 mM is reported with higher concentrations found in the fasted duodenum compared to the fasted jejunum (Table S1C in the Supporting information) (Bergstrom et al., 2014; Fuchs and Dressman, 2014; Riethorst et al., 2016b). In postprandial conditions, higher levels of phospholipids can be detected as they can be present in food. The distribution of total phospholipids was reported to vary less after intake of a liquid meal but still, a large range from 0.16 to 14.39 mM was observed (Table S2C in the Supporting information) (Riethorst et al., 2016b). Phospholipids are known to influence the solubility of many compounds in a positive way. For example, the solubility of danazol, itraconazole, probucol, felodipine and fenofibrate increased with increasing phospholipid levels in simulated intestinal fluids (Madsen et al., 2021; Zhou et al., 2017). The ratio of bile salts to phospholipids is also highly variable, ranging from 4.5 to 39 in the fasted state and from 1.3 to 16 in the fed state (Kleberg et al., 2010), influencing the formation and structures of mixed micelles. The solubility of aprepitant was influenced positively by the bile salt to phospholipid ratio (Madsen et al., 2018), while that of fenofibrate, felodipine, zafirlukast and danazol was influenced negatively (Madsen et al., 2018; Madsen et al., 2021). The effect on permeability, however, was not investigated. In an Ussing chambers experiment with rat tissue, phospholipids decreased the permeation of propranolol and

indomethacin (Riethorst et al., 2018a). However, to the best of our knowledge, a direct influence of phospholipids on drug pharmacokinetics is not known.

Dietary lipids

In fasted state, cholesterol is only present in a low amount in the duodenum, ranging between 0 and 1.8 mM (Table S1C in the Supporting information) (Heikkila et al., 2011; Psachoulias et al., 2011; Riethorst et al., 2016b). In postprandial conditions, cholesterol concentrations increase due to bile secretions but also due to the presence of cholesterol in the meal. The range of cholesterol measured in the duodenum after intake of 400 mL of Ensure Plus (0.45 mM cholesterol content) was between 0 and 3.29 mM (Table S2C in the Supporting information) (Riethorst et al., 2016b).

In fasted state human intestinal fluids (FaHIF), only FFA (0-3.86 mg/mL) and monoacylglycerides (MAG; 0-1.09 mg/mL) were detected (Table S1C in the Supporting information). The FFA were probably derived from hydrolyzed phospholipids with C16/C18 chain lengths (Riethorst et al., 2016b). In the fed state, TAG and DAG originating from food degrade very rapidly and effectively to FFA and MAG in the small intestine. Hence, the majority of lipids present in duodenal fluids are degradation products. After intake of a liquid meal, the range of FFA, MAG, DAG and TAG were 0.53-15 mg/mL, 0-11.36 mg/mL, 0-3.64 mg/mL, and 0-6.76 mg/mL, respectively (Table S2C in the Supporting information). Intake of a meal with a higher lipid content does not result in higher duodenal lipid concentrations (Armand et al., 1996).

The combination of increased lipid, phospholipid and bile salt content in the fed state generally enhances the solubility of lipophilic drugs due to their poor aqueous solubility. In particular, the intake of a high fat meal (*e.g.* the FDA breakfast) can lead to drastically improved oral bioavailability in case of oral anticancer agents lapatinib (4-fold), vemurafenib (5-fold) (Koziolek et al., 2019) and abiraterone acetate (10-fold) (Stappaerts et al., 2015).

Enzymes

To aid in the digestion of various nutrients, the pancreas secretes enzymes, such as lipase, amylase, trypsin and chymotrypsin. Enzyme secretions peak 20-60 min postprandially, especially after a high fat meal as lipids are the strongest stimulants of pancreatic enzyme secretion (Keller and Layer, 2005). Fasted state pancreatic lipase concentrations ranged between 23 and 86 $\mu\text{g/mL}$ (corresponding to an activity between 184 and 690 IU/mL) in the duodenum, whereas in the fed state, they increased 5- to 10-fold (Riethorst et al., 2016b). Pancreatic lipase is responsible for 40 to 70 % of TAG hydrolysis (Armand, 2007). In addition, amylase and trypsin concentrations increase 3-6 fold after a meal (Keller and Layer, 2005). Duodenal phospholipase-A2 concentrations ranged between 3 and 6 ng/mL in the fasted state and increased 5-fold in the fed state (Riethorst et al., 2016b). In addition, an increase in esterases can be observed, influencing the conversion of ester prodrugs (Riethorst et al., 2016b). Although a considerable variation in the enzyme levels both in the fasted and the fed state is described in literature, studies that determine the enzyme activity in parallel with drug pharmacokinetics are required to reveal the clinical impact of enzyme variability.

Colloidal structures

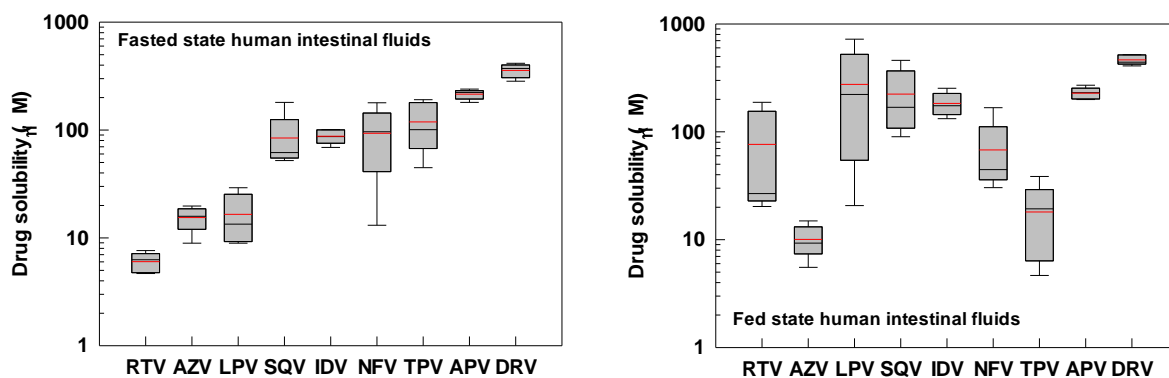
Besides the variable nature of the intestinal composition, the assembly of different colloidal structures in the GIT has a highly dynamic nature, influenced by variables such as motility, secretions, intestinal transfer and digestion. Bile salts are able to associate in aqueous media, forming micelles. In the presence of phospholipids, cholesterol and lipolytic hydrolysis products, mixed micelles are formed with higher solubilization capacity. PC generally forms vesicles, liposomes, bilayer sheets or

lamellar structures in the GIT due to the very low aqueous solubility. Lyso-PC, however, is able to form micelles, giving rise to more stable colloidal structures compared to PC.

In FaHIF, only simple (bile salt/phospholipid) micelles ranging between 10 and 50 nm in size could be detected but no vesicles (Riethorst et al., 2016a). In another study, few multi- and oligolamellar vesicles were observed 30 and 60 min after administration of an aqueous dipyridamole solution in the fasted state (Mullertz et al., 2015). The intraluminal environment gets more complex in the fed state, containing (mixed-)micelles (10 and 100 nm), large clusters of mixed-micelles and vesicles (100 and 500 nm), and lipid droplets ($\geq 2 \mu\text{m}$) (Riethorst et al., 2016a). Riethorst et al. found far less multilaminar vesicles than reported by Müllertz et al (Mullertz et al., 2012; Riethorst et al., 2016a). Multicompartmental vesicles, however, were common (Riethorst et al., 2016a). However, the intestinal composition and ultrastructure of intestinal aspirates vary over time and amongst individuals. In general, fed state human intestinal fluids (FeHIF) containing high concentrations of bile salts were found to display a more complex ultrastructure, including small mixed-micelles, whereas FeHIF containing high lipid concentrations was characterized by vesicles and lipid droplets (Elvang et al., 2019; Riethorst et al., 2018b). Nevertheless, composition does not directly translate into ultrastructure (Riethorst et al., 2016a).

Effect of chemical composition on solubility and ultrastructure

Data linking a specific factor of the chemical composition of intestinal fluids to drug release and dissolution in the human GIT are scarce. One way to investigate this is to aspirate intestinal fluids over time after drug intake or to look at specific disease states, which will be discussed later on (see section 4). For instance, the absence of intestinal bile flow in patients undergoing liver transplantation did not influence the absorption of tacrolimus (Böttiger et al., 2002). *In vitro* solubility and permeability studies using blank FaHIF and FeHIF are more abundant in literature, which provides an idea on how drugs will behave *in vivo*. However, the high variability in solubility of different compounds not only across volunteers and sampling sites but also in function of time in different nutritional states is reported (Clarysse et al., 2009a; de la Cruz-Moreno et al., 2017). This reported variability is the highest for highly lipophilic compounds and for compounds with a pKa value within the physiological pH range. Augustijns et al. summarized solubility data of 59 different compounds in FaHIF and 28 different compounds in FeHIF (Augustijns et al., 2014). Postprandial conditions increased the solubilizing capacity of 24 of the 28 compounds tested but no correlation could be found between the solubility and presence of bile acids and/or phospholipids. A more pronounced interindividual variability in solubility in the more complex fed state was also observed for different protease inhibitors, which suffer from a low bioavailability due to intraluminal solubility and dissolution issues and first-pass elimination (Wuyts et al., 2013). This interindividual variability in both fasted and fed state is visualized in Figure 5.



(A)

(B)

Figure 5. Interindividual variability of the solubility of human immunodeficiency virus (HIV) protease inhibitors in (A) fasted and (B) fed human intestinal fluids. Data from fluids aspirated from four individuals and pooled intestinal fluids (Wuyts et al., 2013).

The average coefficient of variance (CV) amounted to 40.4% and 60.6% in the fasted and fed state, respectively (Wuyts et al., 2013). The extent of drug solubilization is not only dependent on the chemical composition of intestinal fluids, but also on the ultrastructure (Riethorst et al., 2018b). The solubility of different lipophilic compounds increased when including the lipid layer of HIF, meaning that the structures in the lipid layer (*e.g.* vesicles, lipid droplets) play a significant role in solubilizing these compounds. It is, however, unclear what the fate of compounds solubilized in these larger colloidal structures is. Entrapment of lipophilic compounds in colloidal structures has been shown *in vitro* (Stappaerts et al., 2014; Wuyts et al., 2015a, b). The negative food effect observed *in vitro* for metoprolol and darunavir could be attributed to entrapment by lipid structures rather than influence by the bile salts and phospholipids present in SIF (Riethorst et al., 2018a). Using β -blockers with different physicochemical properties, Stappaerts et al. observed that micellar entrapment increased with increasing lipophilicity, causing a decrease in absorptive flux in the fed state compared to the fasted state in the *in situ* rat perfusion model (Stappaerts et al., 2014). It should be noted that the *in vivo* intestinal environment is highly dynamic and colloids change as digestion progresses, possibly resulting in higher absorptive flux. Nevertheless, predicting the *in vivo* behavior of drugs remains challenging due to the highly variable nature of both chemical composition and ultrastructure of intestinal fluids.

Intestinal pH and buffer capacity

As in the corresponding section of the gastric phase, this section focuses on the variability of data in fasted adults, after a glass of water (fasted state) and the variability of data in fasted adults after the standard meal (fed state).

In the fasted state, the average pH in the upper small intestine is near neutral with reported median values ranging from 6.1 to 7.0. Reported pH values seem to not follow normal or log-normal distributions (Pyper et al., 2020). However, during the first hour after water administration, variability is high and pH values as low as 3 could be occasionally observed (Vertzoni et al., 2019). The resistance of contents of the upper small intestine to decrease in one pH unit when titrating with HCl does not seem to be related to the pH and it is highly variable (Figure 6). *In vitro* data indicate that both the buffer capacity and the pH of bicarbonate solutions up to 30 mM are affected by subjecting the samples to a freeze-thaw cycle (Litou et al., 2020). Since subjecting aspirates to a freeze-thaw cycle does not significantly affect the pH of aspirates from the upper small intestine, it appears that species other than bicarbonates *e.g.* enzymes and/or mucin glycoproteins, may play an important role in regulating the intraluminal pH (Litou et al., 2020). This possibility is also supported by data concerning the importance of bicarbonates in biorelevant media simulating the conditions in the

stomach under elevated gastric pH conditions and in the upper small intestine in the fasted state (Litou et al., 2016; Litou et al., 2017).

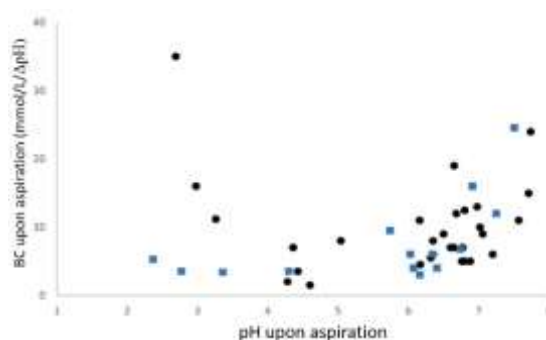


Figure 6. Buffer capacity (BC) of contents in the upper small intestine in the fasted state vs. the corresponding pH values estimated after titration with HCl (data from two studies, squares and circles, $n=45$ individual samples measurements) (modified from Litou et al. 2020).

In the fed state, the overall median pH value for the period between 60 and 240 min, after initiation of administration of the standard meal, has been reported to be 6.3 (Dressman et al. 1990) and 5.3 (Pentafragka et al., 2020b). Intraindividual variability in pH values has been reported to be low and similar to that in the stomach (Pentafragka et al., 2020b). During the first four hours after initiation of meal administration, buffer capacity values in the upper small intestine in the fed state are similar to those measured in the stomach in the fed state. Also, during the first four hours after meal administration, the average difference in buffer capacity values between two administrations in a given individual ranges from -50 % to 30 % in the upper small intestine, *i.e.* intraindividual variability of buffer capacity can be quite high.

Regardless of the dosing conditions, the pH in the distal ileum (20-30 cm from the ileocecal valve) is about pH = 8.0 and the buffer capacity is within the range of values reported for the upper small intestine in the fasted state (Reppas et al., 2015). However, data from more individuals are needed to confirm these findings.

Viscosity and osmolality of small intestinal contents

For the upper small intestine, viscosity data in the fasted state are limited, however, values seem to be similar or slightly higher than that of water (Pentafragka et al., 2020a). After the standard meal, rheological characteristics are pseudoplastic and viscosity is highly variable. Compared at a shear rate of 100 s^{-1} , the average viscosity is at least 100 times higher than in the fasted state (Pentafragka et al., 2020a). *In vitro* and *in silico* data suggest that concomitant food intake can diminish oral absorption of drugs with limited permeability and an absorption window in the proximal intestine, due to viscosity-mediated decrease in dosage form disintegration time and drug dissolution rates (Cvijic et al., 2014). In the distal ileum, the liquid fraction and the size of non-liquid particles has been measured in the fasted (glass of water) and fed (standard FDA meal) state, 5 h after liquid or food ingestion (Reppas et al., 2015). The liquid fraction was significantly lower in the fed state (69 %) compared with the fasted state (90 %). The volume mean diameter of non-liquid particles was slightly higher than $200 \mu\text{m}$, regardless of the dosing conditions.

Contents of the upper small intestine in the fasted state are almost iso-osmotic (Pentafragka et al., 2019). After the standard meal, contents become hyperosmotic (values are on average less than 400 mOsm/kg) between $t = 90$ and 180 min after food ingestion (Pentafragka et al., 2020a). Low

osmolality of a nutrient solution mediates an increase in water absorption from the small intestine and it lowers water flow along the upper small intestine (Pfeiffer et al., 1998).

In the distal ileum, contents are generally hypoosmotic with the mean value in the fasted state (60 mOsmol/kg) being significantly lower than the mean value in the fed state (252 mOsmol/kg) (Reppas et al., 2015).

When changes in osmolality are mediated primarily *via* changes in ionic strength, drug release characteristics from certain modified release products may (Mikac et al., 2010; Verhoeven et al., 2006) or may not (Li et al., 2013; Rahmouni et al., 2001) be significantly affected.

Epithelial permeability

The absorption of a drug following oral administration is a complex process that depends on the physicochemical properties of the drug, pharmaceutical formulation, and physiological and anatomical variables in the GIT (Koziolek et al., 2019; Williams et al., 2013). Once a drug is in solution in the gastrointestinal lumen, the absorption of the drug depends to a large extent on permeability of the epithelium of the small intestine since the small intestine is the major site of absorption of most drugs (Williams et al., 2013). Drugs can pass across the intestinal epithelial layer *via* either paracellular, transcellular, or carrier-mediated facilitated transport. Transport across the intestinal epithelial layer can be enhanced by the manipulation of drug physicochemical properties and structure (Laksitorini et al., 2014). For example, increasing lipophilicity and reducing ionization can enhance passive permeability (Williams et al., 2013) whereas conjugation of drugs with endogenous substrates for intestinal transporters can lead to facilitated transport (Zhang and Wu, 2014).

Any factor (physiological, pathological, or pharmacological) that alters intestinal epithelial permeability might affect the oral absorption of drugs. This is particularly true for drugs with permeability (rather than solubility) limited absorption (Williams et al., 2013). Many disease states are associated with altered intestinal epithelial permeability as further discussed in section 4, including intestinal inflammatory diseases (Buchman et al., 2005), infections (Allam et al., 2018), short bowel syndrome (Tappenden, 2014), critical illness (Fink, 2003) and Alzheimer's disease (Jin et al., 2020). Whilst pathological changes in intestinal epithelial permeability have been shown to alter nutrient absorption, their impact on the absorption of drugs has rarely been quantified (Jin et al., 2020).

In addition to disease states, many other factors can increase intestinal epithelial permeability including dietary components (Rohr et al., 2020), alcohol (Wang et al., 2014), microbial by-products (Guo et al., 2017), strenuous exercise (Dokladny et al., 2016), certain medications (Scarpignato and Bjarnason, 2019) and aging (Man et al., 2015). The impact of diet, supplements and pharmaceutical formulations on intestinal epithelial permeability has been most widely studied. Pharmaceutical permeability enhancers are purposely used to increase drug absorption through either paracellular or transcellular pathways, as reviewed (Aungst, 2012). Gut-microbiota modifiers (probiotics and prebiotics) (Guo et al., 2017) and modulators of tight junction function (*e.g.* zonulin antagonists) (Smyth, 2017) can decrease intestinal epithelial permeability although the effect on drug absorption has been rarely studied. Western style diets (high in fat and carbohydrate while low in fibers) are generally associated with increased paracellular permeability (Rohr et al., 2020). On the contrary, certain dietary components (*e.g.* polyphenols) can decrease intestinal epithelial permeability (Bernardi et al., 2019). In addition to diet type, malnutrition and fasting can cause duodenal atrophy and increased intestinal epithelial permeability (Ferraris and Carey, 2000). Alterations in epithelial permeability by diet factors are typically mediated by changes to the function of tight junctions between epithelial cells.

Diet and other factors (such as formulation components, ageing, genetics and disease states) can also affect drug passage across the intestinal epithelium through induction or inhibition of enterocyte-based metabolism by phase 1 and/or 2 enzymes, and influx or efflux between the enterocyte and intestinal lumen by transporters such as P-glycoprotein (Benet et al., 1999). For example, genetic variations in intestinal metabolism and/or transporters (influx and efflux) might lead to interindividual variation in drug response (Ahmed et al., 2016). However, the impact of genetic polymorphisms of intestinal efflux transporters and metabolic enzymes on drug absorption has so far found to be minimal (Tomalik-Scharte et al., 2008). In Crohn's disease, P-glycoprotein expression in the intestine is increased by 200% and this might explain the higher dose of tacrolimus needed in these patients (Buchman et al., 2005). In a familial Alzheimer's disease mouse model, the intestinal epithelial permeability of digoxin and valsartan was significantly reduced and this could be explained by enhanced expression of intestinal efflux transporters (Jin et al., 2020). It is thus evident that a range of factors may impact the intestinal permeability of drugs. The impact of disease states on oral drug absorption is further described in section 4.

Absorption into lymph and blood

After drugs permeate the intestinal epithelium, they are transported from the intestine by either blood or lymphatic capillaries in the underlying lamina propria (Trevaskis et al., 2015) (Figure 7).

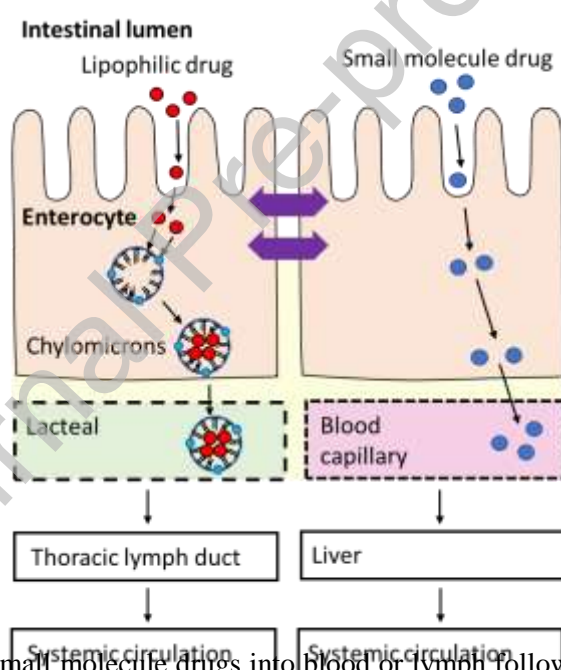


Figure 7. Distribution of small molecule drugs into blood or lymph following uptake into enterocytes from the intestinal lumen. Drugs transported *via* the lymphatic system bypass the liver and reach the systemic circulation *via* the thoracic lymph duct.

Most small molecule drugs distribute into blood capillaries. This has been suggested to result from the ~500 fold higher flow rate of blood compared with lymph flow from the intestine (Charman and Stella, 1986). Conditions that alter splanchnic blood flow have the potential to impact drug absorption into blood. For example, absorption of the model drugs sulfaethidole and haloperidol into blood after oral administration was reduced in dogs with reduced splanchnic blood flow (Crouthamel et al., 1975). Splanchnic blood flow is regulated by the autonomic nervous system and vasoactive mediators, and is impacted by many factors such as food intake, exercise, drugs and disease states. Splanchnic blood flow typically increases after meals depending on the type of food, while it

decreases with fasting and exercise (Koffert et al., 2017; Perko et al., 1998). The impact of food on oral drug absorption is, however, complicated as food affects many processes involved in oral drug absorption (Koziolok et al., 2019). Some drugs can affect splanchnic blood supply which might affect the absorption of other drugs. For example, digitalis is a potent vasoconstrictor and once absorbed can cause a 30-40 % decrease in splanchnic circulation (Crouthamel et al., 1975). Many disease states are associated with reduced splanchnic blood flow such as congestive heart failure, haemorrhage and critical illness (Fink, 2003). On the contrary, liver cirrhosis is typically associated with increased splanchnic blood flow (Bolognesi et al., 2014). Any alteration in splanchnic blood flow might affect drug absorption, however, this has not been evaluated for most factors.

Whilst most small molecule drugs are absorbed from the intestine into the blood, highly lipophilic drugs and prodrugs (typically with $\log P > 5$ and long chain lipid solubility >50 mg/g) can be transported from the intestine *via* lymphatic vessels (Trevaskis et al., 2015). This is mediated by drug association with the lipid-rich lipoproteins (primarily chylomicrons) that are assembled in enterocytes from dietary and endogenous lipids (Trevaskis et al., 2015)(Figure 7).

Chylomicrons are transported from the intestine *via* the lymphatic system as the blood vessel endothelium is less permeable than the lymphatic endothelium, precluding the access of chylomicrons that are 100-1000 nm in diameter. In contrast, the initial lymphatics (lacteals) in intestinal villi contain specialised openings between endothelial cells that facilitate the entry of chylomicrons (Zhang et al., 2018). However, some studies have also suggested a transcellular pathway into lacteals and that small chylomicrons might be able to distribute into blood capillaries (Dixon, 2010). Following entry into lacteals, chylomicrons (and associated drugs) distribute into mesenteric lymphatic vessels that flow into the thoracic duct, which joins the systemic circulation at the subclavian vein.

Many factors influence chylomicron production and transport through the mesenteric lymphatic system and may thus impact intestinal lymphatic drug transport. For example, drug association with chylomicrons is potentiated by administration with a lipid source such as food or a lipid-based formulation (Trevaskis et al., 2015). The type and dose of co-administered lipid can dramatically alter the extent of lymphatic drug transport (Caliph et al., 2000; Trevaskis et al., 2020). For example, long-chain lipids such as found in olive oil, peanut oil and soybean oil are assembled into chylomicrons and thus increase lymphatic transport of chylomicrons and lipophilic drugs. In contrast, short and medium-chain lipids (*e.g.* in coconut oil) are typically transported from the intestine *via* the draining blood capillaries and do not stimulate lymphatic transport of chylomicrons and drugs (Caliph et al., 2000). For model drugs halofantrine and methyltestosterone lymphatic transport increased with long-chain lipid doses up to ~ 130 mg/kg and then appeared to plateau at a maximum across several species (Trevaskis et al., 2020). The extent of lymphatic transport is thus highly variable depending on the co-administered lipid dose with maximum lymphatic drug transport seen in the fed state and minimal lymphatic transport seen in the fasted state (Trevaskis et al., 2015). Non-lipid based food components can also affect chylomicron production; chylomicron production is increased following acute oral intake of monosaccharides such as glucose and fructose (Xiao et al., 2013), while chylomicron production is reduced following acute ingestion of monosodium glutamate (Kohan et al., 2016). Ethanol consumption also modulates chylomicron production (Baraona and Lieber, 1975). A range of drugs modulate lymphatic lipid transport including pancreatic lipase inhibitors, glucagon like peptide-1 agonists, dipeptidyl peptidase-4 inhibitors, and modulators of lipoprotein metabolism such as fibrates and cholesterylester transfer protein (CETP) inhibitors (Dash et al., 2015; Trevaskis et al., 2010; Xiao et al., 2012). In addition, chylomicron production has been found to decrease after surgeries such as sleeve gastrectomy (Padilla et al., 2014). Many disease states affect chylomicron

production. For example, type 2 diabetes mellitus is associated with increased chylomicron production, while in abetalipoproteinemia (a rare genetic condition) chylomicron production is impaired (Dash et al., 2015). Any alteration in chylomicron synthesis and secretion has the potential to alter the absorption and/or lymphatic transport of lipophilic drugs and prodrugs, however, this has not been proven for all factors listed above.

Factors that regulate chylomicron uptake into and transport through lymphatic vessels following secretion from enterocytes are beginning to be elucidated and have the potential to impact lymphatic drug transport. As mentioned above, chylomicrons predominantly enter lacteals *via* paracellular transport such that any factor that closes lacteal junctions will preclude chylomicron uptake (Zhang et al., 2018). For example, vascular endothelial growth factor-C (VEGF-C) mediates the development and maintenance of intestinal lymphatics, including lacteals. Defects in *Vegfc* gene expression lead to atrophy of the lacteals and a substantial reduction in lipid absorption (Nurmi et al., 2015). The flow of lymph from the intestine and thus lymphatic lipid transport is also influenced by active pumping of the smooth muscle cells surrounding lacteals (Choe et al., 2015) and mesenteric collecting lymphatic vessels (Zawieja et al., 2012). In rodent models of metabolic syndrome, the contractility of the mesenteric collecting lymphatics is negatively affected, which affects lymph flow and absorption of chylomicrons (Zawieja et al., 2012). Similarly, in inflammatory bowel disease (IBD) there is significant lymphatic remodelling including lymphangiogenesis, lymphatic vessel dilation and leakiness, and impaired contractility which impairs lipid absorption (Stephens et al., 2019). Absorption of lipophilic drugs and prodrugs might be affected by conditions such as this that alter the transport of chylomicrons *via* the lymphatics.

Alterations to drug absorption *via* the blood versus the lymphatic system can in turn impact the oral bioavailability of drugs with high first pass hepatic metabolism as the mesenteric capillaries and veins join the portal vein which flows to the liver before reaching the systemic circulation. In contrast, the intestinal lymphatics join the thoracic lymph duct which empties into the systemic circulation directly without passing through the liver. For example, administration of the highly lipophilic drugs and prodrugs halofantrine, cannabinoid receptor agonist CRA13, testosterone undecanoate and methyl nortestosterone undecanoate with a lipid source (*e.g.* a meal) substantially increases oral bioavailability by promoting lymphatic uptake and avoiding passage through the liver (Khoo et al., 2001; Shackelford et al., 2003; Trevaskis et al., 2009; White et al., 2009). Overall, many anatomical and physiological factors modulate the intestinal absorption of lipids and transport of chylomicrons into the lymph, which could impact drug oral bioavailability.

Enterohepatic circulation

Excretion *via* the biliary and intestinal routes could be important for the elimination of drugs and metabolites from the body, thus creating an additional source of variability. It is common that drugs excreted *via* the biliary pathway have been metabolized by phase II enzymes within hepatocytes and then transported into bile, and for those which have been directly degraded by phase II enzymes, intestinal deconjugation and reabsorption as intact substance might occur (Roberts et al., 2002; Shou et al., 2005). The phenomenon when a compound is excreted *via* bile into the small intestine and then reabsorbed and excreted into bile again (wholly or partly) is called enterohepatic circulation (EHC) (Roberts et al., 2002). Biliary elimination is the most predominant non-renal elimination route of drugs and drug metabolites, which is affected by factors like compound characteristics (molecular weight, size, structure and polarity), species, age, sex differences, genetic factors, co-administered drugs, biotransformation, food and bile acid sequestrants, drug transport across different barriers (membranes

and transporters), disease conditions and diurnal variations (Malik et al., 2016). Diet in particular has been demonstrated to significantly impact bile pool size and turnover (Adlercreutz et al., 1987; Bisschop et al., 2004; Hepner, 1975). This can in turn have a significant effect on the PK and exposure variability of drugs, where EHC is known to contribute to its disposition. As a result, considerations need to be given to food intake during clinical studies, so as to not confound reasons for variability in the PK data. This was demonstrated for rifapentine, whose exposure can be substantially impacted by food: using population PK modeling with appropriate covariate analysis, the authors were able to prove that the double peaks observed in rifapentine PK profiles and exposure variability were not due to EHC (Zvada et al., 2010). The authors speculated that these could be due to a combination of absorption windows in the GIT, progressive solubilization along the GIT and variable gastric emptying.

Various endogenous as well as exogenous compounds are known to undergo EHC, which may serve a physiological function, for example in the recycling of bile acids. Maintenance of bile acids circulation is imperative for several liver and gastrointestinal functions including bile flow, clearance of toxins, solubilization and excretion of cholesterol, enhanced intestinal absorption of lipophilic nutrients, as well as metabolic and antimicrobial effects (Abu-Hayyen et al., 2013; Li and Chiang, 2015; Roberts et al., 2002).

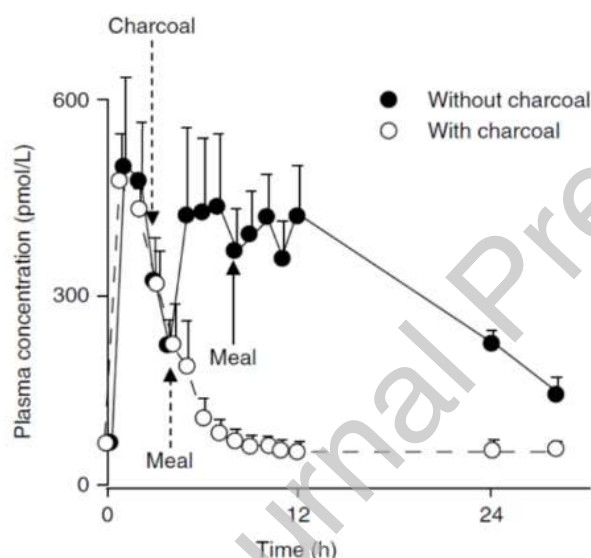


Figure 8. Plasma levels of estriol after oral administration of estriol 12 mg without and with 20 g of activated charcoal. Figure reproduced from Roberts *et al.*, 2002, with permission.

The EHC process can be divided into several components: (1) absorption of drug(s) from the GIT into the portal blood supply, (2) metabolism and efflux by the gut wall, (3) transport from the portal blood across the hepatocyte membranes, (4) transport from the hepatocyte membrane to metabolizing sites and to the bile canniculae, (5) biotransformation in the liver, (6) transport from the hepatocytes across the canalicular membrane into the bile, (7) active transport from the hepatocytes into the sinusoid, (8) bile transport into the duodenum.

Several drugs are secreted by the liver into bile and are therefore capable of undergoing EHC. These include antibiotics, nonsteroidal anti-inflammatory drug (NSAIDs), hormones, opioids, digoxin, and warfarin (Gao et al., 2014; Malik et al., 2016; Roberts et al., 2002). The impact that EHC has on the PK and pharmacodynamics (PD) of a drug depends on: (a) the importance of biliary excretion of the compound relative to renal and metabolic clearance processes; and (b) the efficiency of gastrointestinal absorption (*i.e.* permeability) of the drug. Of particular importance is the potential

amplifying effect of EHC on PK and exposure variability of a given compound (Roberts et al., 2002). High PK variability due to EHC has been reported for many compounds such as mycophenolate mofetil, ezetimibe, regorafenib, estrogen and steroids (Adlercreutz et al., 1979; Bullingham et al., 1998; Keunecke et al., 2020; Kosoglou et al., 2005; Sher and Rahman, 2000). The effect that EHC can have on PK variability was shown for regorafenib using population PK modeling (Keunecke et al., 2020). Only by incorporating EHC in the model as well as time and frequency of food intake was the model able to accurately describe the PK profile from clinical studies. Other covariates had only a minimal effect on describing the overall variability in the exposure. This model was useful in estimating the individual patient's exposure to regorafenib, which is becoming increasingly important in the growing application of precision medicine and flexible dosing to optimize clinical benefit. Ezetimibe is another drug known to undergo significant EHC (Kosoglou et al., 2005), which results in multiple peaks and significant interindividual variability (46% - 80%). It was conclusively demonstrated through population PK modeling that incorporation of EHC and food intake in the model accurately described the observed variability (Ezzet et al., 2001).

Biliary clearance is determined by the unbound fraction in blood, metabolic intrinsic clearance, flow rate and convection/mixing of liver blood, hepatocyte permeability and surface area, and drug concentration (Fagerholm, 2008). Biliary clearance is also determined by the stability of drugs and phase II metabolites in bile and intestinal fluids, permeability across the bile duct epithelium and intestinal wall, and flow rate of bile and intestinal contents. Additionally, highly permeable compounds excreted into the intestine *via* bile are expected to be more rapidly and extensively absorbed than low permeability compounds, and consequently will have more distinct EHC. Several other factors have also been shown to impact the extent of EHC, including the biological influences of species variation, sex differences, age and developmental stage, nutritional status, disease states, and drug-drug interactions (DDI). DDI can affect the production of polar metabolites by cytochrome P450 (CYP) enzymes, conjugation, transporters, intestinal transit time and bioavailability. Associated with such drug interactions is interindividual variability in the inhibition and induction of enzymes, with varying degrees of effect on EHC (Malik et al., 2016; Roberts et al., 2002).

For drugs undergoing biliary excretion, EHC represents a secondary absorption phase for the drug. From a PK perspective, therefore, EHC can prolong the elimination half-life ($t_{1/2}$), increase AUC (bioavailability), and may also produce multiple peaks in the plasma concentration-time profile of a drug (Gao et al., 2014; Malik et al., 2016; Roberts et al., 2002) (Figure 8). The typical multiple peak plasma concentration vs time profile is demonstrated for low metabolic clearance compounds, with efflux, moderate-to-high intestinal permeability and moderate-to-high fraction absorbed.

The clinical significance of EHC depends on the pharmacological and/or toxicological properties of the biliary excretory products, their availability for absorption, and whether the absorbed products are re-extracted by the liver or pass into the general circulation (Malik et al., 2016; Roberts et al., 2002). In some cases, EHC may be a therapeutic advantage due to sustained exposure achieved by the recirculation. On the other hand, it is also possible that EHC can lead to toxicity due to increased exposure and/or increase elimination half-life. In cases of acute drug toxicities, activated charcoal is a commonly used treatment because of its ability to adsorb materials with a high capacity. Although timely single-dose administration is effective in preventing drug absorption of orally ingested drugs, repeated doses of activated charcoal have been shown to increase drug clearance, resulting in reduced plasma exposure of drug. This is the result of decreased enterohepatic recycling and increased drug exsorption from the intestine (Figure 8).

Due to the potential significant impact on PK and PD of a drug, several efforts to accurately model EHC and its effect on PK has been explored. EHC has been described by classical compartmental models. These models include two or three compartments describing the transport of the drug from the central compartment to the gut and one compartment for the gall bladder. Recirculation loops of more than one compartment allowed a better simulation of the delay caused by the recirculation process. Such models have been successfully applied to describe EHC in preclinical species such as rats which do not have a gall bladder (Ouellet and Pollack, 1995; Pollack and Brouwer, 1991). They are not appropriate to describe the discontinuity in the enterohepatic cycling process caused by gallbladder emptying. EHC models that account for the effect of gall bladder emptying can further be classified in models where emptying is assumed to occur at regular intervals and models with irregular emptying times. Models based on irregular lag-time intervals are closer to physiological reality since gall bladder emptying starts when food begins to be digested. Furthermore, the extent of biliary emptying may depend on the quantity of fat in the meal. But these models are mathematically more complex (Plusquellec and Houin, 1992). Physiologically based PK modeling (PBPK) has also been explored in describing EHC. For example, PBPK has been used to successfully model the bile appearance of glycyrrhizic acid and its metabolites after intra-peritoneal administration (Ploeger et al., 2000). A similar whole-body PBPK model using *in vitro* hepatocytes was developed to characterize the hepatic transport of repaglinide and to predict its PK and DDI (Varma et al., 2013).

Colonic phase

Colonic transit times

The transfer of dissolved and undissolved drug material as well as of oral drug product through the colon depends on various factors, which are either formulation-related (*e.g.* size, shape and surface properties), related to the characteristics of the luminal contents (*e.g.* osmolarity and viscosity) or related to the individual's physiology (*e.g.* age, sex differences, body position, circadian rhythm) and dietary habits (time and composition of the last meal).

In a recent meta-analysis published by Abuhelwa and colleagues, meta-means of 20.28 h and 31.95 h were given for the colon transit times (CTT) of tablets and pellets, respectively (Abuhelwa et al., 2016). The longer CTT observed for multiple-unit dosage forms such as pellets as compared to monolithic objects is typically explained by trapping of smaller particles in the haustra of the colon. Therefore, larger objects typically move faster through the colon.

The same techniques used for measurement of SITT, can also be used to study CTT: scintigraphy, magnetic marker monitoring, MRI, telemetric capsules and radiopaque markers (Hens et al., 2017). In a SmartPill[®] study with 215 healthy volunteers, Wang and colleagues reported a mean CTT of 23.1 h, but the 5th and 95th percentiles had values of 3.4 h and 50.5 h, respectively. These data demonstrate that the colon transit time can be extremely variable. Similar results were obtained in a recent SmartPill[®] study with 19 young and healthy volunteers, where the colon transit time was found to range between 2 h and more than 40 h (Koziolek et al., 2015b). Since the object was the same for all volunteers, the variability could only be caused by the individual GI physiology as well as the dietary habits of the subjects.

Favorable conditions for drug release and absorption are mainly present in the ascending colon since sufficient volumes of free fluid can only be found in this part of the colon. Hence, pharmaceutical scientists are particularly interested in assessing regional transit times through ascending, transverse and descending colon (Abrahamsson et al., 1996). Several *in vivo* studies have

shown that there are large inter- and intraindividual variations in terms of transit times through the different segments of the colon. In Figure 9, the intraindividual variability in transit through the intestines is shown for one subject who repeated the same magnetic marker monitoring experiment on five occasions.

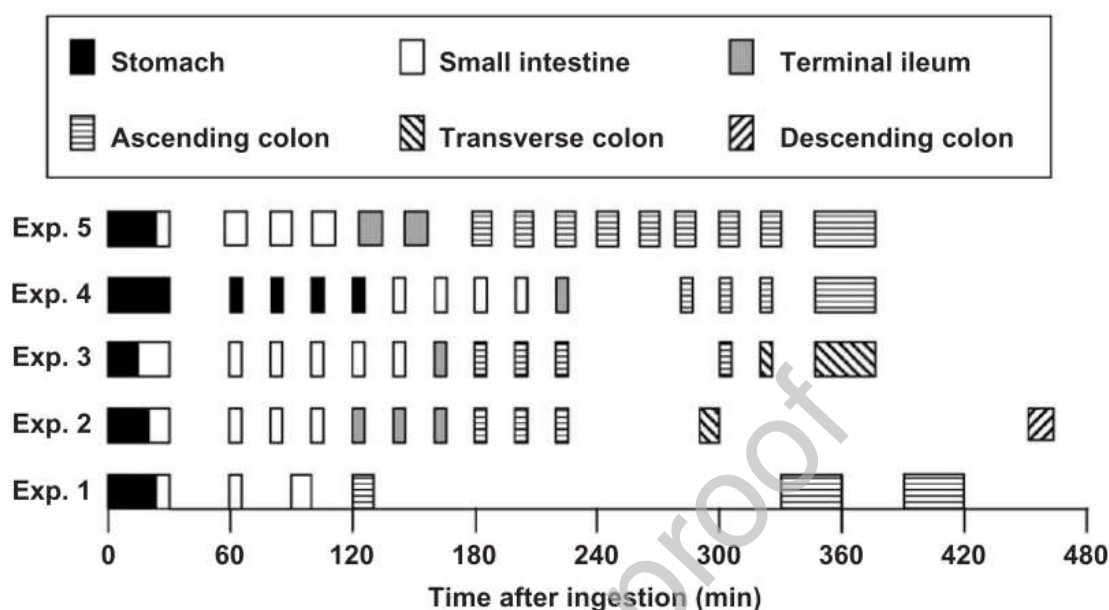


Figure 9. Gastrointestinal transit of magnetically marked non-disintegrating capsules in the same volunteer after ingestion with 150 mL of water. (Capsule intake after 8 h fasting, in the experiments 1–4 lunch was served 240 min after ingestion). Reprinted from Weitschies *et al.* (Weitschies *et al.*, 2005).

In a study by Watts and colleagues, mean residence times of 8.4 mm tablets in the ascending colon were in the range of 3.50 – 15.75 h. For small resin pellets, the same range was measured (Watts *et al.*, 1992). This large variation can be explained by the fact that the transfer through these regions is mainly controlled by propulsive mass movements. These occur infrequently several times a day (Bassotti *et al.*, 1995). Thereby, luminal material can be transported over distances of up to 50% of the colon length within short periods of time. By use of magnetic marker monitoring, it was nicely illustrated that it can take less than 2 min to push a small particle of 1 mm through the entire ascending and transverse colon (Figure 9) (Weitschies *et al.*, 2010). In case of an extended release formulation, for which colonic drug absorption plays an important role, an early mass movement event may limit drug absorption (Wilson and Washington, 1988; Xu *et al.*, 2018). Additionally, the mass movement in the colon initiates the entry of material from the ileum into the caecum. By this, it may terminate the absorption of drugs incompletely absorbed in the small intestine as has been noted for gefitinib (Wilson *et al.*, 2009).

Despite its relevance, the effect of variable CTT on drug absorption of orally administered drugs is not well studied. It is expected that short CTT have a negative impact on the processes of dissolution and/or absorption, particularly for modified-release formulations such as colon-targeted formulations or extended-release formulations. To assess how the variability in CTT affects the PK profile, combined imaging (*e.g.* MRI) and PK studies can provide better understanding.

Variability of luminal composition in distal ileum and proximal colon

Variability of lower intestinal contents can have an impact on the performance of advanced formulations (extended release or colon-targeting), or for drugs with incomplete small intestinal absorption.

Based on data from a limited number of individuals, in the distal ileum, pH values range from 7 to 8.7 (median pH 8.1), regardless of the dosing conditions (Reppas et al., 2015). In the fasted state, a median value of 7.8 and a range between 6.2-8.5 have been reported both for the cecum and the ascending colon (Diakidou et al., 2009c; Reppas et al., 2015). A similar degree of variability has been observed in the fed state but in this case pH values varied between 5.3-7.9 and the median pH was lower, about 6, presumably due to the increased bacterial fermentation activity, after meal consumption (Koziolek et al., 2015a). Variations of colonic pH could be expected to impact colon-targeted formulations based on pH-sensitive polymers.

A number of other characteristics of colonic contents (buffer capacity and osmolality; total short chain fatty acid, protein and carbohydrate concentrations) also show significant inter-individual variation, as recently described in an UNGAP review (Vertzoni et al., 2019).

The described variability of the composition of colonic contents results in a dramatic variability of drug solubility in colonic fluids, especially in the fed state (Figure S1 in the Supporting information). As solubility sets the upper limit of intracolonic drug concentration, it could in turn impact drug absorption from the lower intestine (Tannergren et al., 2009). Despite the high variability, higher solubility values were observed in the colonic aspirates than would be predicted from plain buffers at the equivalent pH values (Vertzoni et al., 2010). This observation has been attributed to the presence of bile acids, PC, and FFA at total concentration of about 1 mM (see Table S3 in the Supporting information) and, perhaps, peptides/proteins that are present in the ascending colon and could solubilize drugs (Vertzoni et al., 2010).

The microbiome

Trillions of bacteria, bacteriophages, fungi, protozoa and viruses known collectively as the microbiota inhabit the human GIT. Although commonly used interchangeably, the term 'microbiota' refers to a population of microbes, and 'microbiome' encompasses the genomes and functions of the microbes. The broad metabolic capacity of gut microbiota has been implicated in drug pharmacokinetics for several decades however the true scale of microbiota-drug interactions in the GIT has only emerged recently (Figure 10)(Clarke et al., 2019; Collins and Patterson, 2020; Scheline, 1968; Tian et al., 2020).

In the early 2000s Astra Zeneca developed an *in vitro* colonic model and found 19 out of 51 drugs analysed to be significantly altered in the presence of microbiota. A few years later just over 30 drugs were known to be metabolised by gut microbiota, however at the time this number was estimated as being just the tip of the iceberg (Sousa et al., 2008). Advances in genetic sequencing in following years facilitated the Human Microbiome Project in 2012, which for the first time characterised bacteria inhabiting the GIT at the genomic level (Huttenhower et al., 2012). Once the species of bacteria inhabiting the gut were known, then seminal work mapping reactions between bacteria and drugs followed (Javdan et al., 2020; Zimmermann et al., 2019a; Zimmermann et al., 2019b). Over 270 drugs are now recognised as being susceptible to direct metabolism by gut bacteria, yielding inactive, more active, or even toxic metabolites (Coombes et al., 2020; Enright et al., 2016; Yadav et al., 2013). As microbiome composition is as unique as one's fingerprint, it is highly likely

that microbiota drug metabolism will vary between individuals (Franzosa et al., 2015). Age, diet, medication use, and lifestyle are all important determinants of an individual's microbiome (Asnicar et al., 2021; Chaudhari et al., 2020; Keohane et al., 2020; Maier et al., 2018).

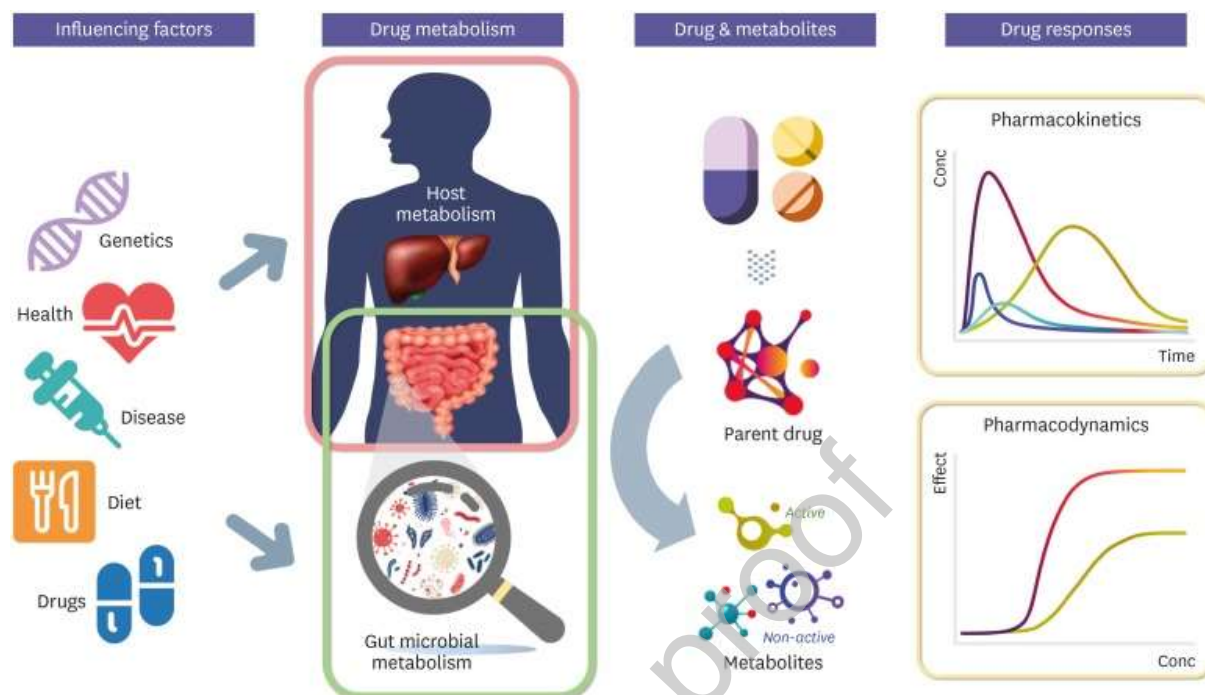


Figure 10. Overview of drug metabolism throughout the host and the gut microbiome [adapted from (Chae et al., 2020) and reproduced with permission from the publisher].

Intestinal microbiota are capable of altering pharmacokinetics no matter the route of drug administration. Orally administered drugs will encounter increasing concentrations of microbiota whilst passing down the GIT, making them vulnerable to reaction with microbial enzymes (James et al., 2020). Even drugs rapidly absorbed in the upper GIT, where microbial density is typically lower, are known to undergo microbiota metabolism (Maini Rekdal et al., 2019). Drugs administered parenterally may interact with intestinal microbiota through excretion in bile or contact with the GI epithelium during systemic circulation (Enright et al., 2018; Enright et al., 2017). Whilst direct alteration of drug structure by bacterial enzymes is the most characterised mode of microbiome-mediated drug metabolism, hepatic metabolism of drugs can also be indirectly altered by intestinal microbiota (Walsh et al., 2020). Metabolites produced by gut microbiota can diffuse over the GI epithelium and reach the liver via its portal vein. There, they can alter the hepatic transcriptome and thus the expression of CYP450 enzymes or drug transporters (Bjorkholm et al., 2009; Ishii et al., 2012; Kuno et al., 2016). Gut microbiota can also indirectly affect the absorption and pharmacokinetics of drugs through impacts on key GI parameters such as pH, bile acid concentration and composition, motility, and epithelial drug transporter expression (Ghyselinck et al., 2020; Mayeur et al., 2013; Pavlovic et al., 2018; Roager et al., 2016; Yuan et al., 2020).

Activation of drugs by the gut microbiome

The first identification of microbiota drug activation was in the 1930s when Prontosil, an early sulfonamide antibiotic, was observed to have no activity *in vitro* (Fuller, 1937). It was subsequently revealed that bacterial azoreductases in the gut are able to cleave Prontosil into its active form, sulfanilamide (Sharma et al., 2019). This discovery sparked the development of a number of other

prodrugs with reactive azo bonds, such as sulfasalazine, balsalazide, and olsalazine (Cooke, 1969; Sousa et al., 2014). These prodrugs are known to undergo a similar azo reduction as Prontosil, liberating the active anti-inflammatory 5-aminosalicylic acid for local treatment of colitis. Due to age-associated changes of the gut microbiota, elderly patients may have different biotransformation profiles of prodrugs such as sulfasalazine (Merchant et al., 2016). Indeed, one study has shown that the elimination half-life of sulfasalazine and steady state serum concentration of its metabolite N-acetyl-5-acetylsalicylic acid are greater in old age (Taggart et al., 1992). Lactulose, an osmotic laxative, is activated by colonic bacterial deglycosylation to lactic and acetic acids (Kim, 2015). These acids effectively trap ammonia in the colon, preventing its diffusion into circulation and lowering the risk of hepatic encephalopathy. A second laxative, sodium picosulfate, is transformed into its active form by caecal bacteria (Kim et al., 1992). Bacteria in the caecum are similarly involved in the activation of loperamide oxide through an N-oxide reduction reaction (Lavrijsen et al., 1995). Once activated, loperamide acts as a local opioid receptor agonist, helping to decrease gut motility and relieve diarrhoea symptoms. The presence of the right strains and concentrations of metabolising bacteria is vital for these prodrugs' activities. Enzymes produced by intestinal bacteria can be highly strain specific, and thus individual ability to activate prodrugs may vary due to differing microbiota compositions (Koppel et al., 2018).

Inactivation of drugs by the gut microbiome

Inactivation of a drug in the GIT can decrease diffusion of the active compound into systemic circulation, thus impairing therapeutic action. Significant variability of patient's gut microbiota is known to impact the pharmacokinetics of multiple drugs. The cardiac glycoside digoxin is a longstanding example of bacterial inactivation in the GIT. Certain strains of colonic actinobacterium *Eggerthella lenta* are known to reduce digoxin's lactone ring, and thus increase dose requirements of the drug (Saha et al., 1983). Recently, the metabolising enzyme produced by *E. lenta* has been identified as 'Cgr2', a novel flavoprotein reductase (Koppel et al., 2018). Strains of *E. lenta* capable of producing Cgr2 are thought to be present in the guts of over 40% of the global population in varying abundance (Koppel et al., 2018). Patients with higher concentrations of metabolising *E. lenta* may therefore be at risk of underdosing. To add another layer of variability, it has also been found that protein-rich diets can impair the actions of *E. lenta*, as the amino acid arginine is a natural inhibitor (Haiser et al., 2013). A second case of bacterial reduction resulting in drug inactivation can be exemplified by the histamine 2 receptor antagonists ranitidine and nizatidine. N-oxide reduction of these drugs in colonic conditions results in inactive metabolites; possibly explaining the poor bioavailability of ranitidine in the large intestine (Basit et al., 2002). Interestingly, cimetidine and famotidine, also histamine 2 receptor antagonists, are not seen to be bacterially degraded. The amino acid L-DOPA is a key component of Parkinson's disease management, and another prime example of gut bacteria inactivation. Premature conversion of L-DOPA to dopamine in the jejunum by the bacterial enzyme tyrosine decarboxylase is known to increase patients' L-DOPA dose requirements (van Kessel et al., 2019). Genes encoding tyrosine decarboxylase have been found in the genomes of several species of *Lactobacillus* and *Enterococcus* (Zhu et al., 2016). Variability in patient's disease severity and PK can often make finding the right dose of L-DOPA a difficult and fluctuating process. As conversion of L-DOPA to dopamine should ideally occur in the brain for therapeutic action, this intestinal inactivation has prompted an increased interest in identifying selective inhibitors of bacterial tyrosine decarboxylase (Lam et al., 2019).

Gut microbiome-mediated drug toxicity

Toxicity can be a grave outcome of microbiota-mediated drug metabolism. The most infamous example is undoubtedly sorivudine, which led to the death of 18 patients in Japan, only 40 days after its brief approval for market in 1993 (Okuda et al., 1998). The antiviral was unknown to interact with the 5-fluorouracil (5-FU) prodrug, tegafur, through a microbially-mediated mechanism. Intestinal microbiota hydrolyse sorivudine to bromovinyluracil in the caecum and colon. Bromovinyluracil is then metabolised by the host to an inhibitor of dihydropyrimidine dehydrogenase, which is necessary for breakdown of 5-FU (Nakayama et al., 1997). Tragically, patients coadministered sorivudine and tegafur experienced accumulation of 5-FU, which culminated in pancytopenia, bloody diarrhoea, severe anorexia, and ultimately, death (Okuda et al., 1998). Sorivudine was withdrawn from the market a few weeks later, and highlights the importance of studying drugs' microbial reactions prior to clinical use (McCoubrey et al., 2021). Bacterial metabolism of the antineoplastic agent irinotecan is another example of microbiome-mediated toxicity. β -glucuronidases produced by colonic bacteria deconjugate glucuronide-conjugated irinotecan in bile, leading to severe dose-limiting diarrhoea (Wilson and Nicholson, 2017). Administration of antibiotics in rats concurrently dosed with irinotecan has shown significantly decreased β -glucuronidase activity and reduced intestinal damage (Takasuna et al., 1996). As broad-spectrum antibiotics are not an ideal solution to this problem, there has been much focus on developing specific β -glucuronidase inhibitors; this has been relatively complex owing to nuances in β -glucuronidases produced by different bacterial strains (Wilson and Nicholson, 2017).

Impact of microbiome variability on bacterial drug degradation in the lower intestine

Gut microbiome variability on bacterial drug degradation in the lower intestine has been studied *ex vivo* by using two chemically stable compounds, metronidazole (a nitroreductase substrate) and olsalazine (an azoreductase substrate) (Karatza et al., 2017). Interindividual variability of drug degradation rates was very high in all cases, with lowest variability observed for the data for olsalazine in the contents of distal ileum collected in the fed state (Figures S2 and S3 in the Supporting information). While olsalazine was found to be practically stable in the contents of distal ileum, its degradation characteristics in the cecum were similar to those in the entire ascending colon (Vertzoni et al., 2011).

Based on metronidazole and olsalazine data (Figures S2 and S3 in the Supporting information), the average nitroreductase and azoreductase activity in the fasted state appears to increase when switching from the distal ileum to the cecum. This is in line with observations showing that nitroreductase activity increases from the proximal to the distal colon (McBain and Macfarlane, 1998). In comparison with the fasted state, regional (*e.g.* distal ileum vs. caecum) differences in degradation appear to be small or negligible in the fed state, presumably because food residues decrease bacterial degradation *via* a competitive inhibition mechanism. For both metronidazole and olsalazine, the degradation rate constant in the fasted state was higher in the cecum than in the distal ileum (Karatza et al., 2017).

Effect of formulation on dosing and PK profile variability

In general, in an industrial setting where patient-centric development is applied, formulation-induced variability of PK parameters (C_{max} , t_{max} , AUC) will be kept to an absolute minimum by design. More specifically, *via* formulation screening and robustness studies, the impact of the formulation on the release kinetics will be characterized and kept within a predefined formulation

design space in which no impact on the *in vivo* release and PK profile is expected. This approach should also guarantee a similar pharmacological response across the entire patient population. Especially for drugs that have a narrow therapeutic margin, excessive inter- and intra-individual variability in exposure can directly lead to safety or efficacy issues. This implicates that formulations should be robust enough to cope with the numerous potential sources of variable absorption as described in previous paragraphs.

Already in 1996, Hellriegel et al. highlighted the inverse relationship between absolute bioavailability and interindividual variability (Hellriegel et al., 1996). This relationship is one of the important drivers for development efforts to increase the bioavailability and as such decrease the variability in exposure. This principle has major consequences in the current drug development landscape where a significant proportion of the newly discovered drugs are poorly soluble and hence prone to limited absorption and bioavailability, significant food effects and high variability. The continuous effort to enhance bioavailability comprises a multitude of formulation techniques that have been reviewed extensively in the past (Williams et al., 2013). Finally, all development efforts need to result in a drug product that is compliant with the target product profile listing the required product characteristics to assure efficacy, safety and compliance towards the targeted patient population (*e.g.* acceptable pill burden, food label and restrictions, dosage strength range).

In this section, typical formulation characteristics are described that could contribute to *in vivo* variability, as well as potential mitigations to reduce the variability. A few cases are included to illustrate the impact of formulation approaches on variability of exposure, observed with highly lipophilic drugs. Some of the physicochemical and biopharmaceutical properties of the example drugs, including their solubility in biorelevant media such as fasted state simulated gastric fluid (FaSSGF), fasted state simulated intestinal fluids (FaSSIF-v1) and fed state simulated intestinal fluids (FeSSIF-v1) are presented in Table 3.

Table 3. Physicochemical and biopharmaceutical characteristics of compounds A, B and C.

Characteristic	Compound A	Compound B	Compound C
pKa (moiety)	4.8 (basic pyridine)	8.0 (acidic thiazolidinone)	4.4 (carboxylic acid)
Lipophilicity	LogP = 2.95	LogD _{7.4} = 3.59	LogP = 5.20
Biorelevant solubility (mg/ml)			
FaSSGF	2.17	0.001	BLoQ
FaSSIF-v1	0.002	0.002	0.008
FeSSIF-v1	0.021	0.028	0.019
Aqueous solubility (mg/ml)			
	0.298 (pH = 2)	0.001 (pH = 1)	BLoQ (pH = 3)
	0.003 (pH = 4)	0.001 (pH = 4)	0.003 (pH = 5)

	<0.001 (pH = 8)	0.001 (pH = 7.4)	3.22 (pH = 7)
--	-----------------	------------------	---------------

BLoQ = Below Limit of Quantification

Solid state of the active ingredient

As the *in vivo* release and exposure is related to the gastrointestinal dissolution rate, the administration of a compound as a solution (containing the compound without its crystal lattice) could eliminate dissolution as a potential variability factor, provided that gastrointestinal precipitation after intake does not occur or can be controlled (*e.g. via* a precipitation inhibitor added to the solution). Therefore, a solution can be considered during drug product development as a low PK variability reference compared to a solid including the crystalline form of the compound. An additional option to pursue lower PK variability *via* a solid state change is the administration of the amorphous form of the active ingredient as a solid dispersion. Similar to a solution, auxiliary agents in the form of polymers that improve supersaturation and inhibit precipitation could be needed (*e.g. various types of hydroxypropyl methylcellulose (HPMC)*). In general, the improvement in dissolution and solubility characteristics associated with amorphous solid dispersions goes hand in hand with enhanced bioavailability and a lower variability of PK parameters.

A case (Compound A) is discussed in which the variability in exposure between an oral solid formulation containing crystalline drug and a solution is compared. Compound A is a BCS class 2 compound and a weak base for which the solubility in simulated gastric fluids is more than 1000-fold higher than in simulated intestinal fluids of the fasted state (Table 3). To evaluate the absorption potential of a microsuspension of compound A, a relative bioavailability study was performed in which the microsuspension was studied in cross-over comparison with a cyclodextrin (CD)-based solution. Despite its high solubility in the stomach and good permeability properties, the overall exposure when using the microsuspension under fasted state conditions was low (more than 10-fold lower compared to the CD solution). Moreover, variability both in AUC and C_{max} was 2-fold and 3-fold higher, respectively, than for the CD solution (Table 4). CDs have been reported to inhibit supersaturation (Brewster et al., 2008) and this might explain why the supersaturation which is induced upon GI transfer and which is inherently metastable in nature does not result in larger variability in the PK for the CD solutions. The PK profile obtained with the CD solution was subsequently used as a target profile for new formulation candidates.

This case is well in accordance with the observation by Hellriegel et al. that improvement of oral bioavailability usually goes hand in hand with an improvement in the inter- and intraindividual variability (Hellriegel et al., 1996).

Table 4. Coefficient of variation on the geometric means of C_{max} and AUC_{inf} for formulations of Compound A.

Formulation type	CV % on C_{max}	CV % on AUC_{inf}
microsuspension	52.5	59.6
CD solution	18.4	33

Particle size distribution of the active ingredient

Next to the solid state, another factor that could impact the dissolution rate is the particle size distribution of the active ingredient. Especially for BCS class 2 or 4 compounds, the formulation

should be sufficiently robust to keep the particle size range within predefined limits. Therefore, typically, specifications will be defined to monitor the particle size distribution of the active ingredient during and after completion of manufacturing, as well as upon stability. Discriminative *in vitro* dissolution techniques are often applied as an indirect control for potential particle size changes of the active ingredient in the drug product. The following case study (compound B) illustrates the impact of particle size on variability in exposure.

Compound B is a neutral weakly acidic BCS class 2 compound with a very low aqueous solubility of 1 µg/mL and a high permeability (Table 3). In support of the development of an oral solid dosage form, the impact of particle size on dissolution rate and exposure was evaluated. The drug was milled to subfractions with a median particle size by volume (D_{v50}) of 6 and 25 µm and the dissolution behaviour of capsules containing the milled drug was evaluated in fasted state simulated intestinal fluids (FaSSIF-v1, pH 6.5), see Figure S4 in the Supporting information. The capsules containing drug milled to $D_{v50} = 6$ µm showed a significantly faster dissolution rate as compared to the coarser material with $D_{v50} = 25$ µm. To evaluate the *in vivo* impact of this observed *in vitro* difference in dissolution rate, the capsules were dosed to dogs and the plasma concentrations were followed up to 24 hours after dosing. As can be observed in Table 5 the average exposure was not affected by the difference in particle size.

Table 5. Pharmacokinetic parameters derived from the plasma profiles of dogs that were dosed Compound B milled to $D_{v50} = 6$ µm or 25 µm.

	Capsule, 2 mg, $D_{v50} = 6$ µm	Capsule, 2 mg, $D_{v50} = 25$ µm
C_{max} (ng/mL)	24.7 ± 3.96	18.7 ± 8.97
t_{max} (h)	2.00 (2.00 - 2.00)	7.00 (2.00 - 7.00)
AUC ₀₋₂₄ (ng•h/mL)	129 ± 11.8	123 ± 55.9

The variability in exposure, however, was much higher for the capsules containing the larger particles than for the capsules containing the smaller particles. The CV % on AUC and C_{max} upon dosing the coarser particles were almost 5-fold and 3-fold higher, respectively than observed for the smaller particles. The more variable absorption is also reflected by the t_{max} , which is consistent for the drug milled to $D_{v50} = 6$ µm and highly variable for the material milled to $D_{v50} = 25$ µm.

Interplay pKa and gastro-intestinal pH variability

A parameter affecting the *in vivo* solubilization of the compound in the GIT is the dissociation constant, usually expressed as pK_a . As illustrated by the Henderson-Hasselbalch equation, weak bases will typically show higher solubility in the acidic environment of the fasted stomach and can precipitate upon transfer to the duodenum and lower intestine with typically higher pH values. Weak acids generally demonstrate the opposite behaviour with lower solubility in the acidic stomach and higher solubility in intestinal environment. For these compounds, (weak bases or weak acids), variations in stomach pH and/or co-medication with gastroprotective drugs such as PPIs or histamine 2 receptor antagonists could contribute to the overall PK variability (both inter- and intraindividual). For example, upon administration of a weak base, an acidic stomach contributes to solubilization in the stomach and supersaturation upon GI transfer, driving a higher compound concentration gradient, absorption and exposure throughout the GIT. However, an increase of the stomach pH (as a natural

physiological variation or caused by PPI intake) will lower stomach solubilization and supersaturation upon GI transfer and cause lower downstream absorption. Both drug substance and drug product related aspects including the intestinal permeability of the compound, the dose and the presence of a precipitation inhibitor, can impact supersaturation/precipitation behaviour of a weak base upon GI transfer.

Also for weak acids, the reported intra- and interindividual variations in GI pH can result in variable exposure (Abuhelwa et al., 2016; Clarysse et al., 2009b). Formulation approaches that lower the sensitivity of the solubility to the pH for a certain dose can therefore positively impact the variability in PK as exemplified in the below case study (Compound C).

For compound C, a weak acid with a pKa of 4.4, the solubility of the most stable crystalline form is low and highly sensitive to pH changes (Table 3). Figure 11 shows the dissolution profiles of the crystalline material in FaSSIF at pH values ranging from 5 to 7. The fraction of the dose dissolved was low in FaSSIF at all three pH values tested and the relative difference in the dissolution plateau was significant along the pH range tested. A 6-fold increase in dissolution plateau was observed between pH 5 and pH 7 and between pH 6.5 and 7, still a significant increase in the dissolution plateau of around 50% was measured. In contrast, when the drug was introduced as amorphous material, close to complete dissolution of the dose was observed in FaSSIF with a pH ranging from 5.7 to 6.8 resulting in dissolution behaviour that is much less sensitive to the inter and intraindividual pH variability that is inherent to the GI environment.

Other specific cases of compounds with PK profiles that are sensitive to PPI intake are described in section 4 (patients on PPI).

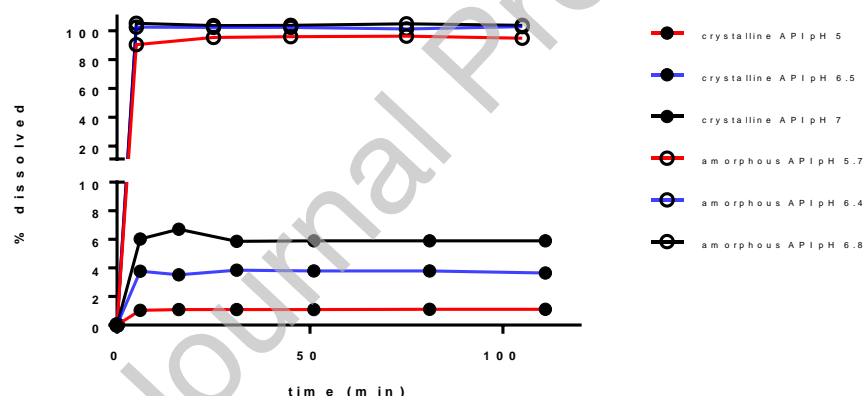


Figure 11. Dissolution of crystalline and amorphous drug in fasted state simulated intestinal fluids, buffered at different pH values. Mass to volume ratio of the dissolution experiment was 333 $\mu\text{g}/\text{ml}$.

Interplay between enteric polymers and gastro-intestinal pH variability

Polymers that are added to the formulation as an auxiliary agent to reduce *in vivo* precipitation and/or stabilize amorphous dispersion (*e.g.* hydroxypropyl methylcellulose phthalate (HPMCP) or hydroxypropyl methylcellulose acetate succinate (HPMC-AS)) may equally cause variation in release and absorption if they contain chemical groups with a pKa in the pH range that's relevant for the GIT. For example, hydrated tablets containing HPMCP will swell between pH 4 and 6 (Xu et al., 2003) and release compound in this range. Also for different grades of HPMC-AS (referring to differences in substitution extents of mainly acetyl and succinoyl groups), differences in dissolution properties have been reported in function of pH (Sakuma et al., 2009).

Therefore, variation in pH along the GIT could cause variation in release in specific intestinal parts linked to the absorption window. Figure 12 shows the meta-means and the meta-standard deviations of the pH along the GIT as reported by Abuhelwa et al. (Abuhelwa et al., 2016). A comparison of the GI pH ranges with the pH values at which the different HPMC-AS grades start to dissolve exemplifies how variability in the GIT can lead to variation in release profile of drugs from formulations containing these types of polymer.

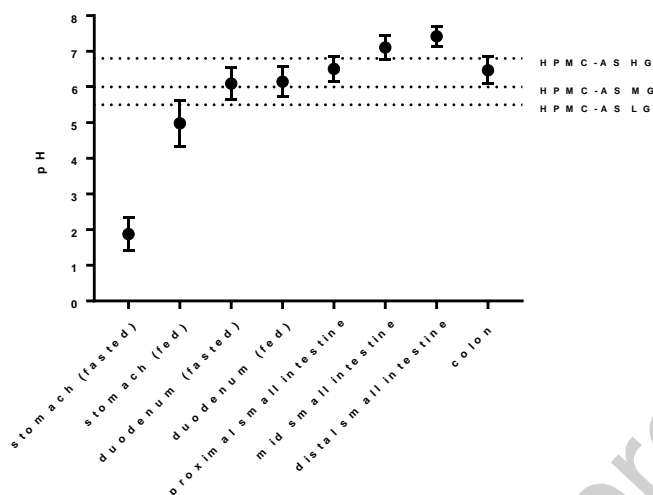


Figure 12. Illustration of the pH at which the different HPMC-AS grades start to dissolve (dotted lines) in view of the reported meta-means and meta-standard deviations of the pH in the different parts of the gastrointestinal tract (filled circles).

Variability in specific populations and patients with chronic conditions

Even in a healthy population, there exists a significant variation in GIT physiology related to age (pediatrics, geriatrics), or sex (male vs. female). On top of that, a variety of diseases impact the GIT, which can also cause significant variability in oral drug absorption and, consequently, compromise drug safety and efficacy. These aspects of variability and their implicit or explicit effect on drug absorption are reviewed in the current section.

However, it should be noted that due to limited (in most cases) evidence, the question whether the observed variability is even stronger due to the underlying physiological or pathological differences, remains unclear. Still, the presented information can be used to initiate a discussion on this unexplored topic and can serve as a starting point to conceive much needed clinical studies that are required to gather data with good statistical quality: an indispensable tool in the study of the variability problem.

Ageing and sex effects

Pediatrics

Children commonly need flexible formulations, with a shift from liquids to solid forms (mini-tablets, multiparticulates, orodispersibles) (Thabet et al., 2018). This further adds to the maturation driven variability of absorption, as maturation is the key source of variability in pediatrics, most

pronounced in early infancy (van den Anker et al., 2018). During maturation, body weight, organ size and function alter, as does body composition, protein expression and cellular functions (Stillhart et al., 2020). These maturational changes also relate to GI physiology.

Over the first years of life, there are changes in gastric pH by maturational parietal cell density and function (Kelly and Newell, 1994). A neutral gastric pH observed at birth is consistently reported. In contrast, there is debate on the maturational changes in gastric acid production and pH throughout childhood (van den Anker et al., 2018). Some suggest that this pattern starts with a neutral gastric pH in the first days of life, followed by a progressive decrease over weeks to years to reach adult values. Others suggest that an acidic gastric pH is present from neonatal life onwards (Batchelor et al., 2014; Mooij et al., 2012). A neutral pH at birth facilitates absorption of macromolecules from colostrum (Smits et al., 2013). Furthermore, the gastric pH in early infancy is likely affected by the frequency and volume of milk ingested, as this also displays maturational changes (Yeung et al., 2020). The gastric fluid composition (osmolarity, bile salts) also displays age-dependent changes (Van Den Abeele et al., 2018).

The gastric pH matters for drug absorption: a higher pH results in increased oral bioavailability of acid-labile drugs (like beta-lactam antibiotics) due to decreased degradation. For weak organic acids with a narrow therapeutic index (like phenytoin), developmental pH may result in frequent dose adjustments to maintain target exposure (Smits et al., 2013).

Gastric emptying is another example of the merged effects of maturation, feeding practices or diet. Gastric emptying is slower in (pre)term infants, reflected by *e.g.* paracetamol absorption (Bonner et al., 2015). In a meta-analysis, meal type (aqueous>breast milk>formula milk>semi-solid>solid) was the main driver of gastric emptying (Bonner et al., 2015). The formula type (extensively hydrolysed>partially hydrolysed>intact protein formula) further affects gastric emptying (Staelens et al., 2008). The SITT in healthy children is likely not affected by age, although PBPK modeling was only explored with theophylline as model compound in children (8-14 years) but is slower in neonates and infants (Bonner et al., 2015; Maharaj and Edginton, 2016). For the colonic transit time, a mean value of 40 hours (4-15 years) is suggested, compared to adult values of 20 h and 32 h for tablets and pellets (Abuhelwa et al., 2016; Wagener et al., 2004).

Pancreatic enzymes like lipase are low at birth to evolve over infancy (McClellan and Weaver, 1993). However, this is somewhat compensated by lipase activity in human milk (Li et al., 2007). The bile acid pool size, bile flow and its ileal reabsorption also display maturation, resulting in lower duodenal bile acid concentrations (Nicolas et al., 2017). Such data, when combined with volumes, are relevant covariates of age-related changes in solubility (Maharaj et al., 2016).

Maturation of intestinal drug-metabolizing enzymes and transporters determine age-dependent bioavailability (Fakhoury et al., 2005; Mooij et al., 2014). However, maturational changes are commonly enzyme-specific, and can be organ specific. Based on midazolam and 1-hydroxy-midazolam disposition following oral or intravenous midazolam administration, intrinsic gut wall and liver clearance were very low (0.0196 and 6.7 L/h, respectively). This results in a highly variable and high total oral bioavailability of 92.1% (range 67-95 %) in preterms and 66 (range 25-85 %) in stable, critically ill children, compared to 30 % in adults (Brussee et al., 2018b; van Groen et al., 2020). The exposure of 1-hydroxy-midazolam and its glucuronide was highest in the youngest, decreasing significantly with postnatal age (van Groen et al., 2020).

Non-maturational covariates, like diet or disease characteristics also matter. To illustrate this, human versus formula milk affects caffeine and dextromethorphan metabolism (Blake et al., 2006). Similarly, critical illness significantly reduces (-90 %) midazolam metabolism (Brussee et al., 2018a).

Geriatrics

Today, the advanced age population (> 65 years) represents more than 20 % of the global population. About 50 % of this population suffers from at least three chronic diseases, resulting in a significant and chronic use of medications (Moore et al., 2018). Despite being the main end-users of drugs, geriatric patients are underrepresented in clinical trials due to advanced age, multimorbidity or polypharmacy (Ruiter et al., 2019).

Ageing is assumed to alter the physiological characteristics of the GIT, thus affecting oral drug absorption. The physiology of the gastrointestinal lumen of older people has not been yet elucidated in detail. Besides alterations in gastric pH values and gastric emptying, other luminal characteristics have been poorly investigated or poorly understood in older people and geriatric patients (Russell et al., 1993; Russell et al., 1994; Vertzoni et al., 2020b). Russell et al reported that the incidence of subjects with an elevated gastric pH in both the fasted and the fed state is greater in the older people and in 10% of the older people who participated in that clinical study, gastric pH was also elevated in the fasted state (Russell et al., 1993). It has also been reported that in 50% of the elderly subjects, gastric pH decreases more slowly than in young subjects after the consumption of a large meal (Russell et al., 1993). Elevated pH in older people influences gastric emptying as it has been shown that gastric emptying of nutrient liquids is slower in the elderly with an elevated pH (Russell et al., 1994).

Although concrete data are lacking, variability is expected to increase with ageing, due to multimorbidity or polypharmacy. However, data can be conflicting. For example, GETs in adults vs. older people have been published for the fed state, but they are conflicting, and/or not relevant for orally administered drug dosage forms (Mojaverian et al., 1988; Moore et al., 1983; Stillhart et al., 2020).

Sex differences

Males and females differ in physiology, disease manifestations and importantly, how they respond to drugs. There are significant sex-specific differences in terms of drug bioavailability and PK (PK) which can, in turn, differentially affect drug efficacy and safety. Underlying reasons for sex-related variations in drug performance include obvious differences in physiological parameters such as body fat content and hormonal control (Soldin et al., 2011). Fundamental differences at the level of the GIT, liver and kidneys can further influence drug absorption, metabolism and elimination, and consequently lead to variability in drug therapy and potential toxicity (Valodara and Sr, 2019).

For example, during post-market drug surveillance it became clear that females were more susceptible to next-day effects following the administration of the sedative zolpidem as drug elimination was slower than in males. The FDA subsequently recommended the dose of immediate-release and extended-release products for females to be reduced from 10 to 5 mg and from 12.5 to 6.25 mg, respectively (Norman et al., 2017). In addition, a number of studies have reported that females are at a greater risk of experiencing adverse side effects by 50 – 70% (Bots et al., 2019). It is difficult to identify whether differences in drug performance and adverse effects is linked to a single PK parameter and governed by a single organ. Rather, such sex differences may result from an interplay of the complete system following oral drug administration. Herein, we focus on drug variability directly linked to GI physiological differences between the sexes.

Bioavailability of orally administered drugs depends on gastric fluid pH and volumes, GET and SITT, competition and/or regulation of intestinal transporters and drug metabolising enzymes, and the potential interactions of sex steroids on drug PK (Hatton et al., 2015) (Figure 13). In terms of the stomach, males have been reported to have higher gastric fluid volumes than females (Gotch et al., 1957) which may affect the extent of drug dissolution. Average fasted gastric pH is significantly higher in females (2.79 ± 0.18 ; $n = 133$) than in males (2.16 ± 0.09 ; $n = 252$) ($p < 0.05$) which may be attributed to reduced acid secretion and the smaller stomach size seen in females (Feldman and Barnett, 1991). Lowered gastric acid secretion may influence drug ionisation and solubility of pH-sensitive drugs, thereby impairing absorption in the small intestine and consequently, oral drug bioavailability.

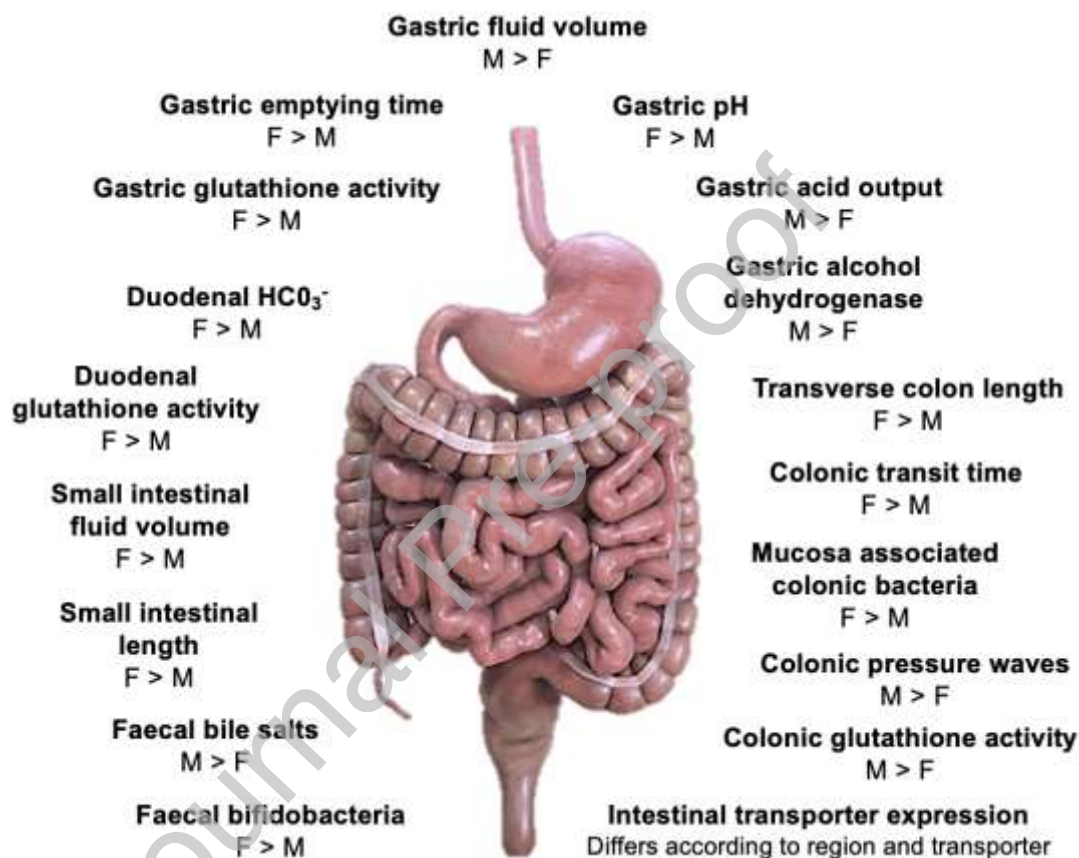


Figure 13. Key sex differences at the level of the GIT that impact oral drug delivery and bioavailability, (M = Male; F = Female). Adapted from (Freire et al., 2011).

With regards to motility, females have a significantly longer half-GET for solids and calorific liquids (118.0 ± 8.1 min) than in males (91.4 ± 7.5 min) however, GET decreases in post-menopausal females (97.9 ± 7.6 min) similar to that in males (Hutson et al., 1989). Variabilities in drug PK can be attributed to differences in GET; for example, peak plasma concentration of orally administered carbidopa was achieved 22 min later in females than in males due to longer GET (Senek et al., 2018). Sex differences in the oral bioavailability of a gastroresistant ketoprofen formulation have also been demonstrated. Males showed a higher C_{\max}/AUC than females (0.468 ± 0.094 vs $0.361 \pm 0.087 \text{ h}^{-1}$) and a significantly lower t_{\max} (3 – 5 h versus 5 – 10 h) respectively. Such differences were attributed to the shorter GET in males allowing for ketoprofen to reach the appropriate intestinal environment for dissolution and absorption to occur more rapidly (Magallanes et al., 2016).

In terms of CTT, longer transverse and descending CTT, but shorter rectosigmoid transit time were observed in females than males (Nandhra et al., 2020). The longer GI residence time for sustained-release dosage forms may facilitate enhanced drug absorption in females, as demonstrated with diltiazem which is sensitive to GI transit time (Zimmermann et al., 1999). This, however, may be further complicated by the regulation of intestinal membrane transporters and metabolising enzymes located in the GI mucosa.

CYP enzymes are responsible for the metabolism of a number of drug substrates of which CYP2C and 3A are most commonly expressed in the small intestine. In terms of sex differences in clinical drug performance, the oral bioavailability of verapamil (a CYP3A and P-gp substrate) was observed to be higher in females than males, possibly from lower hepatic female CYP3A4 expression (Krecic-Shepard et al., 2000; Tamargo et al., 2017), although the differing levels of intestinal CYP3A4 and P-gp expression between males and females may also be responsible for this sex difference. Drugs may also compete for intestinal membrane transporters into cells which affect the downstream metabolism or availability of the drug at its target site. For example, the OATP1B1 transporters are responsible for the transport of oestrogens including oestone-3-sulfate and oestradiol 17-beta-D-glucuronide. Statins, however, are also transported by OATP1B1. As such, competitive inhibition can occur if multiple substrates are present. Several studies have found sex-specific effects of SLCO1B1 genetic variants which compromise the efficacy of statin treatment; female SLCO1B1 521TT subjects had a significantly higher C_{max} and AUC of pitavastatin lactone when compared with male 521TT subjects (Zhou et al., 2013).

In addition, there is an increasing body of literature evidence supporting the inherent sex-specific expression of a number of uptake and efflux intestinal transporters (Smirnova, 2012) that elicit differential treatment outcomes. For example, a recent study showed that plasma concentrations of cimetidine, a drug substrate of the intestinal efflux transporter P-gp were significantly higher in females than males due to the innately higher expression of P-gp in the proximal small intestine of males (Mai et al., 2020). Adding further to the complexity in formulation response in males and females is the sex-specific influence of excipients. In the presence of the solubilising excipient polyethylene glycol (PEG) 400, cimetidine bioavailability significantly increased, but only in males, not in females (Mai et al., 2020). In addition, when co-formulated with PEG 400, the bioavailability of ranitidine significantly increased in male subjects but decreased in females when orally administered with the same formulations (Ashiru et al., 2008). In a rat model, other solubilising excipients including PEG 2000, Cremophor RH 40, Poloxamer 188 and Tween 80 significantly enhanced ranitidine bioavailability in males but not females. Span 20 was also studied: although this excipient was able to increase oral drug bioavailability, such effects were not affected by sex. Sex-specific effects may be attributed to the presence of a polyoxyethylated group in PEG 2000, Cremophor RH 40, Poloxamer 188 and Tween 80, but not for Span 20 (Mai et al., 2019).

Distinct sex differences in drug performance have been further demonstrated in treatments for GI syndromes. For example, alosetron, a 5-hydroxytryptamine receptor 3 antagonist, is a drug that is effective in females but has low performance in males (Koch et al., 2002). At identical plasma concentrations, alosetron achieves therapeutic levels only in females due to the fundamental difference in serotonergic receptors in the colon (Viramontes et al., 2001). Alosetron, however, was withdrawn from the market in 2000 due to significant side effects such as ischemic colitis, but was reintroduced in 2002 in the US under restrictive conditions of use only for females suffering from severe diarrhoea-related irritable bowel syndrome (Farkouh et al., 2020).

The gut microbiota adds further to the complexity to GI physiology and varying drug responses in males and females. For example, L-DOPA undergoes increased metabolism in the presence of *Helicobacter pylori* consequently decreasing drug bioavailability. The eradication of *Helicobacter pylori* infection, however, improved L-DOPA action and clinical symptoms. The prevalence of *Helicobacter pylori* infection, however, is more prevalent in male than female individuals (Cabal, 2018) and as such, may lead to differences in L-DOPA PK between the sexes.

Research that aims to understand differences in drug metabolism, including its different clinical performance in both sexes, continues to be very limited. It is clear that males and females respond differently to drugs due to the dynamic interplay of GI physiology, drug PK itself and contributions from other associated organs. A single PK parameter cannot be considered as the only rate limiting step as this may occur in a drug-by-drug basis. For a better understanding of the basic mechanisms of sex differences, future studies should be designed with this primary focus in mind to determine the importance of these differences in clinical management.

Surgical conditions

Post-bariatric surgery changes

Obesity has become a major public health problem with 650 million adults having a body mass index (BMI) ≥ 30 kg/m² (World Health, 2016 Fact Sheet No 311). With the rising numbers of obesity, the prevalence of bariatric surgery is increasing as it is considered the most effective long-term weight loss treatment (Jakobsen et al., 2018). Next to sustained weight-loss, bariatric surgery can improve or lead to the resolution of obesity-associated comorbidities (Jakobsen et al., 2018). However, bariatric surgery can be associated with short- and long-term gastrointestinal and nutritional complications (Decker et al., 2007). The post-operative incidence of complications depends on the patient characteristics and the type of bariatric surgery that is performed (Courcoulas et al., 2020). Different bariatric procedures are available, but one out of three performed procedures is a Roux-en-Y Gastric Bypass (RYGB) (Ramos et al., 2019). The RYGB is performed by constructing a small gastric pouch (± 30 mL). Next, the small intestine is segmented ± 30 to 50 cm below the ligament of Treitz and the distal part is anastomosed to the gastric pouch with the formation of the alimentary or Roux-limb. The proximal part of the divided small intestine is anastomosed 75-150 cm distally through a jejunojunal anastomosis into a Y-configuration (Nguyen and Varela, 2017b). The anatomical alterations have a profound effect on gastrointestinal physiology including reduced gastric mixing capacity, increased gastric pH, accelerated gastric emptying, reduced surface area for absorption, reduced intestinal first pass metabolism and a delayed inlet of bile acids and pancreatic juice (Stillhart et al., 2020). Together with the solubility of a drug and intestinal permeability, these gastrointestinal changes determine postoperative drug bioavailability. Subtherapeutic and toxic drug levels have been observed after bariatric surgery for drugs that are essential to treat postoperative complications or remaining obesity-associated complications. In the following section, we discuss the postoperative changes in disposition of drugs that belong to different BCS classes (Cder/Fda, 2017).

Metoprolol is a commonly used cardiovascular drug that belongs to BCS class 1 (high solubility and high permeability). Gesquiere *et al.* investigated the disposition of an immediate and controlled release formulation containing 200 mg metoprolol tartrate in 14 subjects before and after RYGB. No statistically significant differences were observed in the extent of oral exposure of metoprolol before or after surgery (AUC), C_{\max} or the time to reach C_{\max} (t_{\max}). However, there was a tendency towards an increased oral exposure of metoprolol following the intake of an immediate release formulation (+32.4% (95% confidence interval (CI): 1.36; 63.5); $P=0.07$) and the controlled release formulation

after surgery (+55.9% (95% CI: 5.73; 106); $P=0.30$). After surgery, postoperative weight loss might compensate for the reduced area of absorption by reducing distribution volume. In addition, the tendency of increased exposure might originate from the bypassed area that contains the highest expression of metabolizing CYP enzymes. Consequently, decreased presystemic biotransformation might contribute to the increased trend of metoprolol exposure (Gesquiere et al., 2015).

From BCS class 2 (low solubility and high permeability), the disposition of 67 mg fenofibrate (hypolipidemic agent) and 400 mg posaconazole (anti-fungal agent) was investigated in two groups of 12 subjects before and after RYGB (Gesquiere et al., 2016).

For fenofibrate, no significant differences were observed in any of the PK parameters (AUC_{0-48h} , C_{max} , t_{max}). Interestingly, substantial inter-individual differences were observed. Two subjects showed a decrease of more than 25% in AUC_{0-48h} after surgery. In contrast, for four subjects an increase of more than 25% in AUC_{0-48h} was observed after surgery. The intraluminal solubility of fenofibrate depends on bile salts concentration, of which the inlet is delayed due to the altered intestinal anatomy after RYGB (Gesquiere et al., 2016). However, a two-fold increase in serum bile acid concentrations has been observed after surgery that could explain the comparable pre- and postoperative exposure (Steinert et al., 2013). In addition, a similar time to reach plasma concentration was observed before and after surgery. The delayed inlet of bile acids might be compensated by an accelerated gastric emptying, resulting in a similar timeframe between oral drug ingestion and bile acid contact before and after surgery. The variable effect of surgery on the extent of exposure might depend on interindividual differences in bile acid secretion (Gesquiere et al., 2016).

For posaconazole, a significant decrease was observed in the extent of exposure (3.11 ± 0.78 $\mu\text{g/mL}\cdot\text{h}$ vs 1.81 ± 0.20 $\mu\text{g/mL}\cdot\text{h}$; $P=0.03$) and C_{max} (0.12 ± 0.04 $\mu\text{g/mL}$ vs 0.06 ± 0.01 $\mu\text{g/mL}$; $P=0.03$) after surgery. The solubility of posaconazole in the stomach depends on gastric residence time and pH, which are both affected by the formation of a gastric pouch. An elevated gastric pH and accelerated gastric emptying might reduce the capacity and the timeframe for posaconazole to dissolve, resulting in lower gastric concentrations (Gesquiere et al., 2016). For poorly soluble and weakly basic drugs, the physiological change in pH after gastric emptying is normally associated with a drop in solubility and the induction of supersaturation (Hens et al., 2016; Walravens et al., 2011). After RYGB, the decreased dissolution of posaconazole may limit the creation of supersaturation in the Roux-limb and thus result in the lower systemic exposure of posaconazole after RYGB.

From BCS class 3 (high solubility and low permeability), metformin was selected. The disposition of 1000 mg metformin was investigated in 16 RYGB-patients and 16 gender- and BMI-matched control subjects. Metformin is predominantly absorbed in the proximal small intestine through organic cation transporters (transcellular transport) and facilitated diffusion (paracellular transport), which is bypassed after RYGB. Contrary to expectations, a tendency towards a higher systemic exposure (21 %) was observed in the RYGB-patients compared to the control subjects (13.7 ± 6.0 $\mu\text{g/mL}\cdot\text{h}$ vs 11.4 ± 3.6 ; $P = 0.2$). In addition, a 50% higher bioavailability was observed in the RYGB-patients compared to control subjects (41.8 ± 16.2 % vs 27.8 ± 10.4 %; $P = 0.007$). Potential mechanisms, that unravel these findings, are undiscovered. But considering the limited absorption window, it is possible that trans- and paracellular metformin absorption might change after surgery due to intestinal morphological and/or functional adaptation (Padwal et al., 2011).

Furosemide is a BCS class 4 drug (low solubility and low permeability). The disposition of 40 mg furosemide was investigated in 13 RYGB-patients and 14 controls (age-, gender-, race- and BMI-matched). Compared with controls, significantly higher serum levels were observed in RYGB patients one hour (38 ± 30 ng/mL; 347 ± 253 ng/mL; $P = 0.01$) and two hours (127 ± 116 ng/mL; 591 ± 418

ng/mL; $P = 0.02$) after oral administration. A significantly shorter time to reach peak plasma concentration was observed in the RYGB-patients (1.8 ± 0.3 h; 4.2 ± 1.2 h; $P = 0.001$). The aqueous solubility of furosemide is pH-dependent, with a higher solubility at an increased pH. After RYGB, the increased gastric pH and the accelerated gastric emptying might explain the higher serum furosemide concentration and faster time to reach the peak plasma concentration (Tandra et al., 2013).

In conclusion, these observations emphasize the fact that the PK of a drug can change after RYGB. Drug absorption can increase, decrease or remain unaltered after surgery. To date, the available PK investigations in bariatric patients are limited. But the available data provides already some rationale for the presence of interindividual variability in drug bioavailability in post-bariatric patients. Interindividual differences in gastrointestinal physiology may explain the variability to some extent, but the overall degree of variability and the underlying causes are largely unknown.

Effects of surgical resection

The normal small bowel length is highly variable and ranges between 285 and 1049 cm based on surgical series (Tacchino, 2015; Teitelbaum et al., 2013). Short bowel syndrome is generally defined as a residual small bowel length less than two meters, measured from the duodeno-jejunal flexure (Pironi et al., 2015). Surgical resections as a life-saving intervention during mesenteric ischemia or – often repeated – enterectomies in patients with IBD are the two most common conditions leading to short bowel syndrome. In the case the preserved intestinal length does not suffice to absorb sufficient fluids, electrolytes and nutrients to sustain life without malnutrition or growth in case of children, this is defined as chronic intestinal failure or type 3 intestinal failure (Pironi et al., 2015). However, more than just the remaining intestinal length, also the anatomy plays an important role. If the ileocecal valve and the entire colon can be salvaged, for example, a small bowel length of 35 cm can be sufficient to avoid the need for parenteral support. However, at the other end of the spectrum, in the case the entire colon is removed and the small bowel ends in a jejunostomy, at least 100-115 cm of the remaining intestine is needed to avoid parenteral nutrition, fluids and/or electrolytes (Messing et al., 1999). Moreover, resections of the small intestine also influence gastric motility. Indeed, the GET is accelerated in patients with short bowel syndrome, most likely because of the lack of a hormonal ileocolonic brake (Nightingale et al., 1993).

Self-evidently, a reduced length of small bowel has important implications for drug disposition. For example, absorption of paracetamol and L-thyroxine was reduced in patients with short bowel syndrome, both of which are absorbed distally to the duodenojejunal flexure (Stone et al., 1984; Ueno et al., 1995). Unfortunately, the literature on oral drug disposition in short bowel syndrome is limited, but it can be assumed that most of the (better documented) alterations in drug absorption after bariatric surgery (see the previous section) are similar, but most likely more pronounced in case of short bowel syndrome-related intestinal failure. One important difference between both situations is that in most patients with short bowel syndrome, the biliopancreatic juices will be in contact with the ingested luminal contents and drugs more proximally, *i.e.* after emptying from the stomach, in contrast to only in the common limb after gastric bypass surgery, which can change drug solubility and absorption (Nguyen and Varela, 2017a). Nevertheless, the absence of the terminal ileum in most patients with short bowel syndrome interrupts the normal EHC, resulting in bile acid malabsorption and impaired micelle formation. This could have an important impact on drug oral bioavailability, due to the altered drug solubilization capacity of the intestinal fluids (Riethorst et al., 2018b). Massive loss of bile acids, combined with the short transit times will lead to steatorrhea in many patients, which is especially problematic for lipid-soluble drugs, *e.g.* cyclosporin and azoles.

Nevertheless, oral drug therapy is still possible in this patient population, even if they are fully dependent on parenteral nutrition. Indeed, early case reports documented therapeutic drug concentrations with nortriptyline and digoxin and therapeutic anticoagulation levels with warfarin, with as little as 12-15 cm of jejunum remaining (McFadden et al., 1993). However, many patients receiving parenteral nutrition are also treated with intermittent doses of intravenous vitamin K which can sometimes explain an apparent resistance to vitamin K antagonists or unstable anticoagulation levels (Pironi et al., 2016). If therapeutic drug monitoring is possible and readily available, oral drug therapy can be attempted even in the case of limited small bowel length. Also for drugs for which monitoring is not recommended in the general population, *e.g.* the non-vitamin K oral anticoagulant drugs, therapeutic drug monitoring can be a useful tool in guiding oral drug therapy after surgical resection. Indeed, rivaroxaban and dabigatran reached lower peak levels in a recent study including six short bowel syndrome patients (Cheung et al., 2017). This is of importance since patients with short bowel syndrome are more likely to suffer from atrial fibrillation as a cause of mesenteric ischemia or to develop thromboses as a consequence of the indwelling central intravenous line, necessitating long-term anticoagulation. Subcutaneous administration of low-molecular weight heparins is an alternative in this situation but less convenient for the patients.

If available, soluble drug formulations, including effervescent tablets, buccal administration forms or liquid solutions, are preferred to bypass the dissolution phase and leave more time for drug absorption (Ward, 2010). However, oral drug solutions are often hyperosmolar and can stimulate luminal secretion, further increasing intestinal fluid and electrolyte losses (Dickerson and Melnik, 1988). In general, modified release formulations should be avoided in patients with short bowel syndrome because of the unpredictable and often incomplete release and absorption (Ward, 2010). An exhaustive description of the drug disposition of individual drugs after surgical resections, including short bowel syndrome, is beyond the scope of the current review, but has recently been covered elsewhere (Santamaria et al., 2018).

Guidelines recommend the treatment with a double dose proton-pump inhibitor (PPI) in patients with short bowel syndrome, thereby reducing gastric acid secretion and as a consequence stomal output, especially in case of end-jejunostomy (Jeppesen et al., 1998; Pironi et al., 2016). Changing gastric acidity by a high-dose PPI will alter drug solubility and absorption, although no specific data are available in short bowel syndrome (Gubbins and Bertch, 1991; Rubbens et al., 2016). High-dose loperamide, codeine and sometimes octreotide are used to slow down gastrointestinal motility to allow more time for nutrient, fluid and drug absorption, although the effect of motility-inhibitors on drug absorption in short bowel syndrome has not been evaluated. More recently, glucagon-like peptide 2 analogues such as teduglutide are used to improve absorption through their intestinotrophic effects, *i.e.* increase of the absorptive surface (Wauters and Vanuytsel, 2018). In clinical practice, an increased absorption of drugs of which the levels are monitored, including tacrolimus (personal experience, *Tim Vanuytsel*), are observed during the administration of glucagon-like peptide 2 analogues, but studies evaluating oral drug absorption in this setting are lacking.

Chronic diseases

Effect of gut function variability in chronic constipation on clinical drug performance

Chronic constipation is a disorder characterised by the presence of alteration of bowel habit in terms of reduced frequency, increased stool consistency, straining, presence of feeling of incomplete evacuation and/or need of manual manoeuvres to complete evacuation (Bharucha et al., 2013). Almost everyone experiences an episode of constipation at some point in their life but fortunately most

episodes of constipation are temporary. However, for some individuals the problem with constipation can become chronic. Chronic constipation affects about 16% of the population worldwide.

Chronic constipation is considered the result of alterations of gut secretory and motor function (Bharucha et al., 2013). The role of altered secretion is still unclear as until the recent application of the MRI to study *in vivo* in humans the intraluminal content, the only way to assess secretion *in vivo* was through invasive intraluminal sampling (Camilleri et al., 2017). Constipation is therefore normally classified as constipation with normal or delayed colon transit and/or with defecation disorder (Bharucha et al., 2013). In large studies conducted in tertiary care centres it has been demonstrated that about 20% of patients with chronic constipation present with slow transit (Camilleri et al., 2008; Simren et al., 2019). Patients with chronic constipation can also manifest alterations of the transit time of other parts of the gut. Previous studies have reported that the presence of delayed gastric emptying and/or small bowel transit time is frequent in patients with chronic constipation (Kuo et al., 2011; Shahid et al., 2012).

It should be noted that gut transit is an indirect measure of motility. It has indeed become clear that the same colonic transit time can result from quite different colonic motor patterns. Studies conducted by means of the magnetic capsule have demonstrated that in healthy individuals, same colonic transit time could be the result of the capsule remaining for about 21 hours in the right colon and then travelling the entire colon to be expelled by the rectum by a single high amplitude propagating contraction or alternatively resulting from the capsule slowly transiting the entire colon during the same amount of time (Mark et al., 2019). Preliminary data in patients with slow transit constipation suggest that the same entity of delayed colonic transit can be the result of reduced propulsive motor activity or from an increase of non-propulsive motor activity (Corsetti et al., 2016). This different motor response of the colon in health and disease is likely to affect the clinical performance of the drug. Longer retention of a drug in the right colon where normally the intraluminal content is expected to be different from other regions of the colon, in terms of viscosity, pH, bile acids, microbiota is expected to influence its bioavailability (Koziolek et al., 2019).

The situation is made more complex by the fact that different techniques have been applied in the literature to measure gut transit and even for what is considered the gold standard (scintigraphy) there is no standardization across centres (Corsetti et al., 2019). This of course needs to be considered when evaluating or performing studies assessing the variability of gut function.

In the literature no studies have formally evaluated the influence of chronic constipation and of its different subtypes (normal or delayed colon transit and/or with defecation disorder) on drug clinical performance. However, some information can be collected by the studies in Parkinson's disease where the alteration of gut transit has been reported to affect bioavailability of drugs used to treat this condition (Mukherjee et al., 2016). In these patients, chronic constipation and alteration of gut function have been demonstrated to precede neurological symptoms (Leclair-Visonneau et al., 2020). A systematic review has estimated the prevalence of delayed gastric emptying is between 70 % and 100 % in patients with Parkinson's disease (Heetun and Quigley, 2012). Recent studies applying the magnetic capsule in a small group of patients with Parkinson's have found that 79 % percent of Parkinson's disease patients displayed prolonged colonic transit time (Knudsen et al., 2017). In these patients a significant relationship has been found between L-DOPA PK and gastric emptying (Marrinan et al., 2014).

All these data suggest that future studies should clarify whether the alterations of gut function associated with chronic constipation influence the clinical performance of drugs. These studies should be conducted standardising the technique to study gut function and considering the factors that have

been already demonstrated to affect gut function in health. Ideally, a non-invasive technique allowing the simultaneous evaluation of the transit of the different part of the gut (stomach, small bowel and colon), such as scintigraphy or MRI would be preferable. In the case of drugs that are expected to behave like undigestible food techniques such as the wireless capsule or the magnetic capsule would add the advantage of evaluating the retention time in specific areas of the gut.

Effects of small intestinal motility disorders on clinical performance of drugs

For most drugs, the principal site of absorption is the proximal small intestine. Absorption primarily depends on the overall absorptive capacity, but drug absorption is also influenced by small intestinal motility. In humans, SITT is reasonably constant (for the case of chronic constipation, see section 4.3.1): at around three hours for a drug formulation (or for a meal) to pass from the stomach to the ileo-caecal junction (Davis et al., 1986).

Consequently, the bioavailability of a drug, which is largely or exclusively absorbed from the proximal small intestine, will be affected by factors that change GI transit. Increases in small intestinal transit are one of the key factors impacting drug absorption, as has been elegantly shown using mannitol for cimetidine, a polar drug that is almost exclusively absorbed from the small intestine (Adkin et al., 1995b). Such phenomena can also explain the impact of certain laxatives on drug absorption, although reports on this subject are scarce (Altree and Galletly, 2013).

Drugs most affected by changes to small intestinal transit are generally those with narrow absorption windows in the small intestine, the most important ones being acyclovir, atenolol, bisphosphonates, captopril, cimetidine, furosemide, metformin, gabapentin, L-DOPA, baclofen, ciprofloxacin and verapamil.

Several pathological conditions of the GIT are known to affect SITT, see Table 6. It is noteworthy, however, that drug absorption has generally not been systematically assessed in these conditions as other mechanisms can also influence bioavailability in these disorders. For lactose intolerance and celiac disease (both causes for malabsorption syndromes), Crohn's disease, Parkinson's disease and infectious enterocolitis, we refer here to a recent UNGAP review specifically discussing these conditions (Stillhart et al., 2020). The impact of spinal cord injury on drug PK is extensively discussed elsewhere (Mestre et al., 2011).

Table 6. Pathological conditions of the GIT known to affect SITT.

Disorders resulting in increased small intestinal transit	Disorders resulting in decreased intestinal transit
Malabsorption syndromes (osmotic effect)	Chronic intestinal pseudoobstruction (CIPO)
Infectious enterocolitis	Systemic sclerosis
Crohn's disease (active)	Postoperative ileus
Radiation enteritis	Spinal cord injury
Hyperthyroidism	Hypothyroidism
Neuroendocrine tumor	Parkinson's disease

A particular case of alteration in small intestinal motility is postoperative ileus, a condition seen often in clinical practice. Postoperative ileus is an iatrogenic condition that occurs following

abdominal surgery, characterized by transient cessation of coordinated propulsive motility (Boeckxstaens and de Jonge, 2009).

The inability to restore normal intestinal motility in the postoperative phase will also impact oral drug bioavailability. In these clinical situations, it is important to realize that the motility of the proximal small intestine is perturbed in the immediate postoperative phase and that normal motor patterns including phasic motor activity only normalizes after approximately 50-70 hours. (Benson et al., 1994) As for the impact of these changes on drug bioavailability, a study examining differences between pre- and postoperative PK of paracetamol administered to the duodenum found peak plasma concentration from the small bowel to be decreased postoperatively but the overall amount of paracetamol absorbed over time was unaffected (Kennedy and van Rij, 2006). One of the postulated mechanisms for the observed effect was related to an alteration in the spread of the paracetamol mixture throughout the GIT or reflux back into the stomach. The question still remains to which extent postoperative motility disorders contribute to drug bioavailability and whether this is also drug-specific.

Patients on proton pump inhibitors

PPIs inhibit the H^+/K^+ -ATPase in gastric parietal cells, which brings about a reduction of acid secretion and hence an increase in gastric pH. One direct effect of PPIs is the increase of the gastric pH (hypochlorhydria) which might reduce the absorption of weakly basic drugs that are more soluble in acidic environments. Also, PPIs cause reduced gastric osmolality, surface tension, and reduced buffer capacity and these factors are major modifiers of drug absorption. It is of note that the increase in pH also affects the first portion of the small intestine (Litou et al., 2016) and therefore intestinal drug absorption is also modified by PPIs. Also, PPIs influence drug absorption because of their effect in the CYP system affecting the intestinal first-pass metabolism (Blume et al., 2006).

PPIs are considered as drugs with a good safety profile, because they provoke mild and infrequent adverse drug events, and are widely used because of their low cost and, in most countries, their availability over the counter. PPIs are used as a single dose or as a short-term treatment, to treat unspecific gastrointestinal symptoms. Besides, these drugs are frequently used in the long-term for diseases such as gastroesophageal reflux, treatment of peptic ulcer, or prevention of gastrointestinal adverse events during NSAID therapy. It is in the context of chronic treatment where the effect of PPIs in drug absorption becomes of particular relevance.

The influence of PPIs in anticancer therapy is of special concern because many recently approved oral anticancer drugs have pH-dependent solubility. Of particular clinical relevance is that PPIs negatively affect the absorption of oral tyrosine kinase inhibitors (TKIs). Interactions of PPIs with several TKIs such as dacomitinib, dasatinib, erlotinib, gefitinib, ibrutinib, neratinib, osimertinib, pazopanib, or ponatinib have been described. PK exposure to TKIs was reduced upon co-administration of PPIs such as omeprazole, lansoprazole, esomeprazole, and rabeprazole, in all cases due to the increased gastric pH. For example, the maximum plasma concentration (C_{max}) and AUC of dasatinib decreased by 63 % and 61 %, respectively, even when administered 10 h after a single dose of PPI (FDA, 2011). Besides TKIs, the area under the curve for other kinase inhibitors such as acalabrutinib, ceritinib, palbociclib, pictilisib, and ribociclib is also reduced when co-administered with PPIs. For recent reviews, see (Gay et al., 2017; Keller et al., 2018; van Leeuwen et al., 2017). The co-administration of acidic drinks (*e.g.* cola drinks), can be expected to increase significantly the bioavailability of these kinase inhibitors in patients receiving PPIs. So far, this has been shown for the combined use of erlotinib plus esomeprazole (van Leeuwen et al., 2016). Also, re-acidification of the

gastric pH with betaine hydrochloride restores absorption, as it has been shown in the absorption of dasatinib in patients with hypochlorhydria induced with rabeprazole (Yago et al., 2014). The absorption of oral anticancer drugs classified as Hedgehog signaling inhibitors such as glasdegib and sonidegib is modestly affected by PPIs such as rabeprazole or esomeprazole (Giri et al., 2017; Zhou et al., 2016). Another oral anticancer drug that is affected by co-administration of PPIs is capecitabine (Chu et al., 2017), and a decrease in progression-free survival has been reported for patients receiving capecitabine and PPIs (Wong et al., 2019). Therefore, the effects of acid reducing agents on cancer therapeutics are challenging not only for oncologists but also for pharmaceutical companies developing new drugs and regulators that aim to achieve safe and efficacious products for patients (Smelick et al., 2013).

Another group of drugs affected by PPIs, also due to changes in gastric and intestinal pH, are antivirals such as atazanavir or glecaprevir. Atazanavir is an antiretroviral used in the treatment of HIV and glecaprevir is used in the treatment of Hepatitis C virus. Co-administration of PPIs cause a significant decrease in the bioavailability of these drugs (Faber et al., 2017; Flamm et al., 2019), and it is widely accepted that the use of PPIs should be carefully considered in patients receiving these antivirals.

The absorption of oral antifungal drugs such as itraconazole, ketoconazole, or posaconazole is also affected by PPIs, which cause a decrease in their bioavailability due to the pH-dependent solubility of these drugs (Abuhelwa et al., 2019; Dolton et al., 2014; Ogawa and Echizen, 2010; Walravens et al., 2011).

Regarding hormones, PPIs affect the absorption of ulipristal, a selective progesterone receptor modulator that has a pH-dependent solubility. Co-administration with esomeprazole causes a significant decrease in ulipristal plasma concentrations (Pohl et al., 2013). Also, the dissolution of levothyroxine tablets is pH-dependent and it has been shown that PPIs impair absorption and decrease exposure to this synthetic hormone (Vita et al., 2014).

Another drug that has its PK profile affected due to the effect of PPIs is mycophenolic acid, an immunosuppressant drug, and an increase in the risk of organ rejection has been reported in some patients when receiving concomitantly PPIs (Knorr et al., 2014).

Clinically relevant interaction of PPIs with cardiovascular drugs lies in the fact that the absorption and the bioavailability of the cardiotoxic drug digoxin, and the calcium channel blocker nifedipine, is increased when gastric pH is increased (Fashner and Gitu, 2013).

Besides drugs, PPIs modify the absorption of iron, magnesium, and calcium. Dietary iron absorption is affected by PPIs because iron absorption is more efficient in the reduced form (Fe^{2+}) than in the dietary form (Fe^{3+}), and this reduction is facilitated by the acidic gastric fluids. It has been reported an increased risk of anemia, or of having altered anemia-related analytical values in PPI users as compared to non-PPI users (Sarzynski et al., 2011). Magnesium absorption is also affected by PPIs, due to reduced magnesium active transport and reduced absorption due to increased pH. This is especially relevant in elderly patients and patients with polypharmacy (Yucel et al., 2016). Calcium absorption is reduced too in PPI users as a consequence of hypochlorhydria, and increased risk of fractures has been reported in patients receiving PPIs (Kim et al., 2020). Vitamin B12 deficiency has been reported to occur in PPI users and the proposed mechanism to explain such association is a reduction of protein-bound vitamin B12 absorption due to a decrease of gastric proteolytic digestion, which is affected by gastric pH (Eusebi et al., 2017).

PPIs can also alter drug absorption and exposure indirectly because they alter the gastric microbiome. After one year of treatment with PPIs, the number of colony-forming units in gastric juices and in the duodenum grows dramatically (Fisher and Fisher, 2017), and persistent changes toward a less healthy gut microbiome are observed in PPI users (Imhann et al., 2016) (for more information on the role of the gut microbiome in drug PK, see the relevant section in this manuscript). Also, PPIs reduce steatorrhea after treatment with pancreatic enzymes (Proesmans and De Boeck, 2003). In sum, PPIs cause frequent and clinically relevant drug interactions due to hypochlorhydria, which take special importance in elderly patients, in patients with susceptibility or additional risk factors for bone fractures or gastrointestinal infections, and patients under polypharmacy.

Effects of anorexia and cachexia on drug PK

Anorexia is defined as a loss of normal appetite. Anorexia can have different causes, including eating disorders such as anorexia nervosa. A related term is cachexia, which refers to the situation when anorexia is associated with nutritional deficiencies and involuntary weight loss, as a result of an underlying chronic disease. Cachexia is characterized by progressive nutritional changes, weakness, and wasting, is often debilitating and potentially life-threatening over a lengthy period (Bruera, 1997). It is a hypercatabolic state that is defined by an accelerated loss of skeletal muscle in the context of a chronic inflammatory response. Anorexia and cachexia are most frequently seen in the later stages of the acquired immunodeficiency syndrome (AIDS), dementia, cancer, heart failure and renal failure. Cachexia-induced changes in bodily functions may alter the PK of various drugs. These changes include the following mechanisms, according to an absorption, distribution, metabolism and excretion (ADME) concept (see Table 7 for a summary). Changes in the gut wall such as increased bowel wall thickness (due to edema and increased collagen deposition), increased intestinal permeability, changes in intestinal motility, decreased splanchnic perfusion and impaired function of transport proteins are associated with weight loss regardless of the underlying chronic disease (Barry, 1974). Higher intestinal permeability can also affect drug bioavailability, which has been discussed earlier in this paper (2.2).

As for specific changes in patients with cachexia, a limited number of studies (Mouly et al., 2000; Trout et al., 2004) investigated the impact of diarrhea in combination with weight loss in patients with HIV-infected patients. Increased intestinal permeability is a key factor in drug absorption in these patients (Keating et al., 1995). It was observed that these patients showed higher intestinal absorption of ganciclovir and saquinavir as demonstrated by higher AUC (area under concentration-time curve) and oral clearance. On the contrary, lower bioavailability was observed in AIDS-associated diarrhea and wasting for stavudine and didanosine (Brantley et al., 2003). This discrepancy is probably explained by the difference in PK properties of the specific drugs: drugs with otherwise low bioavailability (ganciclovir, saquinavir) have an increased absorption due to higher intestinal permeability; drugs with otherwise good intestinal absorption (stavudine, didanosine) are more influenced by faster elimination of the drug from the GIT, which results in lower bioavailability. Therefore, it was suggested that doses of low bioavailability drugs should be lowered and doses of drugs with high bioavailability should be increased in cachectic patients with wasting and diarrhea (Trobec et al., 2013). It remains to be established whether such recommendations can be extrapolated to other populations affected by cachexia.

Due to the fact that we have an incomplete understanding how cachexia can impact oral and subcutaneous drug bioavailability and drug metabolism, in current clinical practice, the medication dose is usually guided by experience and clinical effect, resulting in adaptation of a universal starting dose rather than defining a personalized dose beforehand based on solid PK characteristics (Franken et

al., 2016). To overcome these shortcomings, initiatives have been taken to predict PK profiles and the effect of intrinsic and extrinsic factors using population-based PBPK modeling approach in an oncology population (Cheeti et al., 2013). Until such prediction models can be fine-tuned, alternative routes of drug administration should be preferred when there is an evident discrepancy between administered dose and clinical effect, or when this situation is to be expected a priori based on the clinical condition of the patients. Nonetheless, as most drugs for chronic diseases are administered orally, studies focusing on drug absorption in cachexia are highly necessary to optimize PK predictive models. Obtaining such data can however easily run into practical issues of execution due to ethical considerations, particularly in the terminally ill. Modeling studies using limited sampling strategies may therefore provide a solution and may eventually lead to individualized dosing guidelines (Franken et al., 2016).

Table 7. Mechanisms by which oral drug disposition can be influenced by cachexia

Absorption	Distribution	Metabolism	Elimination
Gut wall thickening	Decreased fat tissue	Decreased hepatic function	Impaired renal and hepatic function
Increased permeability	Decreased muscle volume	Decreased liver blood flow	
Decreased splanchnic perfusion	Changes in plasma binding capacity	Alterations due to inflammation	
Changes in GI transit	Fluid deficit (dehydration)		
Impaired function of transport proteins	Third spacing (ascites, pleural effusion)		

Of note is that other causes of anorexia, such as anorexia nervosa, are not associated with a substantial systemic inflammatory reaction (Dalton et al., 2018) and therefore impact drug bioavailability to a different extent. In fact, a study investigating the bioavailability of oral ergocalciferol showed that bioavailability was similar in young women with anorexia nervosa with severe malnutrition compared to healthy-weighted controls (Divasta et al., 2011).

Summary and future outlook.

Variability of drug exposure after oral administration has been known as long as pharmacokinetics itself, yet it still poses a formidable challenge in modern drug development. While the parameters influencing oral drug absorption and PK are as diverse as any aspect of human physiology, the issue is further complicated by the various enabling formulation technologies available. The purpose of the current paper was to facilitate the exploration of this difficult problem by collating literature data on the various aspects of variability and examining the evidence for impact on oral drug absorption and/or PK (Table 8).

While GI physiology is currently studied relatively well in the healthy adult, this is not the case for pediatric, geriatric and various other patient populations, which hinders any attempt to understand the reasons behind drug absorption differences in case studies. Hence, considerable future efforts should be dedicated to studying these populations that are frequently underrepresented (if present at all) in the academic and industry-driven clinical trials.

Table 8. Role of physiological, pathophysiological and pharmaceutical factors on drug absorption variability.

Variability factor	Details
Stomach	
Gastric pH and patients on PPI	Significant variability of gastric pH in both fasted and fed state human fluids has been determined. Clinical evidence for reduced peak plasma concentrations and AUC of weakly basic drugs for patients on protein pump inhibitors is available.
Gastric emptying time	Several clinical studies demonstrate the impact of gastric emptying time on drug absorption.
Composition of gastric fluids	The level of bile salts and lipids in the gastric contents is highly variable, the concentrations are low, but no link to drug pharmacokinetics has been established yet.
Small intestine	
Small intestinal fluids composition	Significant inter- and intraindividual variability of human small intestinal fluids is documented and impact on drug solubility and permeability has been established. Data for impact on drug pharmacokinetics still scarce.
Intestinal fluid volume	The variability of human intestinal fluid volumes and distribution is high, with some evidence for impact on drug absorption in clinical studies.
Small intestinal transit time and motility disorders	Small intestinal transit time variability is relatively low but can be affected significantly by pathological conditions. Evidence for clinical impact is still scarce.
Epithelial permeability	
Absorption into blood and lymph	Various factors that can impact epithelial permeability, absorption into blood and lymph, and enterohepatic circulation have been identified, but their impact on drug pharmacokinetics remains to be proved.
Enterohepatic circulation	
Colon	
Colonic transit times and chronic constipation	Dramatic variability in colonic transit times is evident (especially in the case of chronic constipation), however, its implications on drug pharmacokinetics are still unclear.
Colonic luminal composition	High variability of colonic fluids composition has been shown to result in a significant variation of solubility for a few drugs. The potential clinical impact has not been systematically investigated.
Microbiome	The high interindividual variability of the intestinal microbiome has been associated with changes in drug metabolism that can influence drug absorption.

Special populations	
Pediatrics	The high variability in pediatric populations is related to maturational differences in gastrointestinal physiology, with confirmed impact on drug pharmacokinetics.
Older people and geriatrics	Gastrointestinal physiology in the elderly is underexplored yet but is expected to have significant impact on drug absorption.
Sex differences	Various physiological parameters have been found to be affected by the sex of an individual. Effect on drug pharmacokinetics has been demonstrated in a few cases.
Anorexia and cachexia patients	Anorexia and cachexia impact several parameters related to intestinal absorption, but more studies are required to show the effect on drug pharmacokinetics.
Post-bariatric surgery changes	Significant variability in drug absorption after surgery is observed in the clinic, but more studies are required to establish general trends.
Surgical resection	
Pharmaceutical factors	
Drug formulation	The drug pharmacokinetic variability can be significantly affected by the formulation type and properties.

At the same time, clinical evidence for the impact of well-characterized GIT variables on drug absorption is also scarce. Hence, only a handful of factors are relatively well characterized, and their impact on variable drug absorption has been illustrated by clinical studies: intestinal fluid volume, drug formulation type, PPIs, age and sex differences. Most of the other factors considered either require more in-depth characterization, or dedicated clinical studies, in order to clarify the implications on drug absorption.

Looking ahead, the opportunity to minimize variability is probably only truly possible in a clinical academic setting. Do we therefore have to live with much greater variability in the patient setting? To control the release of oral drugs *via* robust physicochemical mechanisms, we will need to quantify the impact of genetic influences, societal factors and physiological variables (*e.g.* gastrointestinal transit), which in turn are affected by diet, energy output and drug effects. Thus, understanding our patient is key and variability is multi-factorial.

So how could the industry respond to this challenge, and to the demand for personalized medicine? Ultimately, we should aim to accurately model the ranges of drug exposure and peak concentrations that will be experienced by patients. This will then assure that our medicines possess an efficacy and safety profile tailored to the heterogenous population that we seek to treat. In that respect, society has to make choices, because more information about individuals and specifically those who need to be treated, needs to be securely shared across healthcare providers.

Credit author statement

All authors contributed equally to this review.

In addition, Zahari Vinarov and Patrick Augustijns were responsible for putting the individual parts together and revising the manuscript.

References

- Abrahamsson, B., Alpsten, M., Jonsson, U.E., Lundberg, P.J., Sandberg, A., Sundgren, M., Svenheden, A., Tölli, J., 1996. Gastro-intestinal transit of a multiple-unit formulation (metoprolol CR/ZOK) and a non-disintegrating tablet with the emphasis on colon. *Int. J. Pharm.* 140, 229-235.
- Abu-Hayyeh, S., Papacleovoulou, G., Williamson, C., 2013. Nuclear receptors, bile acids and cholesterol homeostasis series - bile acids and pregnancy. *Mol Cell Endocrinol* 368, 120-128.
- 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 II: Gastrointestinal Transit Time. *AAPS J* 18, 1322-1333.
- Abuhelwa, A.Y., Mudge, S., Upton, R.N., Foster, D.J.R., 2019. Mechanistic Assessment of the Effect of Omeprazole on the In Vivo Pharmacokinetics of Itraconazole in Healthy Volunteers. *European journal of drug metabolism and pharmacokinetics* 44, 201-215.
- Aburub, A., Fischer, M., Camilleri, M., Semler, J.R., Fadda, H.M., 2018. Comparison of pH and motility of the small intestine of healthy subjects and patients with symptomatic constipation using the wireless motility capsule. *Int J Pharm* 544, 158-164.
- Adkin, D.A., Davis, S.S., Sparrow, R.A., Huckle, P.D., Phillips, A.J., Wilding, I.R., 1995a. The effects of pharmaceutical excipients on small intestinal transit. *Br J Clin Pharmacol* 39, 381-387.
- Adkin, D.A., Davis, S.S., Sparrow, R.A., Huckle, P.D., Wilding, I.R., 1995b. The effect of mannitol on the oral bioavailability of cimetidine. *J Pharm Sci* 84, 1405-1409.
- Adlercreutz, H., Hockerstedt, K., Bannwart, C., Bloigu, S., Hamalainen, E., Fotsis, T., Ollus, A., 1987. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem* 27, 1135-1144.
- Adlercreutz, H., Martin, F., Jarvenpaa, P., Fotsis, T., 1979. Steroid absorption and enterohepatic recycling. *Contraception* 20, 201-223.
- Ahmed, S., Zhou, Z., Zhou, J., Chen, S.-Q., 2016. Pharmacogenomics of drug metabolizing enzymes and transporters: relevance to precision medicine. *Genomics, proteomics & bioinformatics* 14, 298-313.
- Al-Gousous, J., Tsume, Y., Fu, M., Salem, I.I., Langguth, P., 2017. Unpredictable Performance of pH-Dependent Coatings Accentuates the Need for Improved Predictive in Vitro Test Systems. *Mol. Pharm.* 14, 4209-4219.
- Allam, O., Samarani, S., Mehraj, V., Jenabian, M.-A., Tremblay, C., Routy, J.-P., Amre, D., Ahmad, A., 2018. HIV induces production of IL-18 from intestinal epithelial cells that increases intestinal permeability and microbial translocation. *PloS one* 13.
- Altree, T.J., Galletly, C., 2013. Laxative use and altered drug absorption. *Aust N Z J Psychiatry* 47, 686.
- Aoyagi, N., Ogata, H., Kaniwa, N., Uchiyama, M., Yasuda, Y., Tanioka, Y., 1992. Gastric emptying of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled rabbits. *J Pharm Sci* 81, 1170-1174.
- Armand, M., 2007. Lipases and lipolysis in the human digestive tract: where do we stand? *Current opinion in clinical nutrition and metabolic care* 10, 156-164.
- Armand, M., Borel, P., Pasquier, B., Dubois, C., Senft, M., Andre, M., Peyrot, J., Salducci, J., Lairon, D., 1996. Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum.
- Ashiru, D.A., Patel, R., Basit, A.W., 2008. Polyethylene glycol 400 enhances the bioavailability of a BCS class III drug (ranitidine) in male subjects but not females. *Pharm Res* 25, 2327-2333.

Asnicar, F., Berry, S.E., Valdes, A.M., Nguyen, L.H., Piccinno, G., Drew, D.A., Leeming, E., Gibson, R., Le Roy, C., Khatib, H.A., Francis, L., Mazidi, M., Mompeo, O., Valles-Colomer, M., Tett, A., Beghini, F., Dubois, L., Bazzani, D., Thomas, A.M., Mirzayi, C., Khleborodova, A., Oh, S., Hine, R., Bonnett, C., Capdevila, J., Danzanvilliers, S., Giordano, F., Geistlinger, L., Waldron, L., Davies, R., Hadjigeorgiou, G., Wolf, J., Ordovas, J.M., Gardner, C., Franks, P.W., Chan, A.T., Huttenhower, C., Spector, T.D., Segata, N., 2021. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nat Med*.

Augustijns, P., Vertzoni, M., Reppas, C., Langguth, P., Lennernas, H., Abrahamsson, B., Hasler, W.L., Baker, J.R., Vanuytsel, T., Tack, J., Corsetti, M., Bermejo, M., Paixao, P., Amidon, G.L., Hens, B., 2020. Unraveling the behavior of oral drug products inside the human gastrointestinal tract using the aspiration technique: History, methodology and applications. *Eur J Pharm Sci* 155, 105517.

Augustijns, P., Wuyts, B., Hens, B., Annaert, P., Butler, J., Brouwers, J., 2014. A review of drug solubility in human intestinal fluids: implications for the prediction of oral absorption. *Eur J Pharm Sci* 57, 322-332.

Aungst, B.J., 2012. Absorption enhancers: applications and advances. *The AAPS journal* 14, 10-18.

Baraona, E., Lieber, C.S., 1975. Intestinal lymph formation and fat absorption stimulation by acute ethanol administration and inhibition by chronic ethanol feeding. *Gastroenterology* 68, 495-502.

Barry, R.E., 1974. Malignancy, weight loss, and the small intestinal mucosa. *Gut* 15, 562-570.

Basavaraj, S., Betageri, G.V., 2014. Can formulation and drug delivery reduce attrition during drug discovery and development-review of feasibility, benefits and challenges. *Acta Pharm Sin B* 4, 3-17.

Basit, A.W., Newton, J.M., Lacey, L.F., 2002. Susceptibility of the H₂-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora. *Int. J. Pharm.* 237, 23-33.

Bassotti, G., Germani, U., Morelli, A., 1995. Human colonic motility: physiological aspects. *Int J Colorectal Dis* 10, 173-180.

Batchelor, H.K., Fotaki, N., Klein, S., 2014. Paediatric oral biopharmaceutics: key considerations and current challenges. *Adv Drug Deliv Rev* 73, 102-126.

Benet, L.Z., Izumi, T., Zhang, Y., Silverman, J.A., Wacher, V.J., 1999. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *Journal of controlled release* 62, 25-31.

Benson, M.J., Roberts, J.P., Wingate, D.L., Rogers, J., Deeks, J.J., Castillo, F.D., Williams, N.S., 1994. Small bowel motility following major intra-abdominal surgery: the effects of opiates and rectal cisapride. *Gastroenterology* 106, 924-936.

Bergstrom, C.A., Holm, R., Jorgensen, S.A., Andersson, S.B., Artursson, P., Beato, S., Borde, A., Box, K., Brewster, M., Dressman, J., Feng, K.I., Halbert, G., Kostewicz, E., McAllister, M., Muenster, U., Thinner, J., Taylor, R., Mullertz, A., 2014. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. *Eur J Pharm Sci* 57, 173-199.

Bernardi, S., Del Bo', C., Marino, M., Gargari, G., Cherubini, A., Andrés-Lacueva, C., Hidalgo-Liberona, N., Peron, G., González-Dominguez, R.I., Kroon, P., 2019. Polyphenols and intestinal permeability: rationale and future perspectives. *J. Agric. Food Chem.* 68, 1816-1829.

Bharucha, A.E., Pemberton, J.H., Locke, G.R., 3rd, 2013. American Gastroenterological Association technical review on constipation. *Gastroenterology* 144, 218-238.

Bisschop, P.H., Bandsma, R.H., Stellaard, F., ter Harmsel, A., Meijer, A.J., Sauerwein, H.P., Kuipers, F., Romijn, J.A., 2004. Low-fat, high-carbohydrate and high-fat, low-carbohydrate diets decrease primary bile acid synthesis in humans. *Am J Clin Nutr* 79, 570-576.

Bjorkholm, B., Bok, C.M., Lundin, A., Raftar, J., Hibberd, M.L., Pettersson, S., 2009. Intestinal microbiota regulate xenobiotic metabolism in the liver. *PLoS One* 4, e6958.

Blake, M.J., Abdel-Rahman, S.M., Pearce, R.E., Leeder, J.S., Kearns, G.L., 2006. Effect of diet on the development of drug metabolism by cytochrome P-450 enzymes in healthy infants. *Pediatr Res* 60, 717-723.

Blume, H., Donath, F., Warnke, A., Schug, B.S., 2006. Pharmacokinetic drug interaction profiles of proton pump inhibitors. *Drug safety* 29, 769-784.

Boeckxstaens, G.E., de Jonge, W.J., 2009. Neuroimmune mechanisms in postoperative ileus. *Gut* 58, 1300-1311.

- Bolognesi, M., Di Pascoli, M., Verardo, A., Gatta, A., 2014. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J. Gastroenterol.* 20, 2555-2563.
- Bonner, J.J., Vajjah, P., Abduljalil, K., Jamei, M., Rostami-Hodjegan, A., Tucker, G.T., Johnson, T.N., 2015. Does age affect gastric emptying time? A model-based meta-analysis of data from premature neonates through to adults. *Biopharm Drug Dispos* 36, 245-257.
- Bots, S.H., Groepenhoff, F., Eikendal, A.L.M., Tannenbaum, C., Rochon, P.A., Regitz-Zagrosek, V., Miller, V.M., Day, D., Asselbergs, F.W., den Ruijter, H.M., 2019. Adverse Drug Reactions to Guideline-Recommended Heart Failure Drugs in Women: A Systematic Review of the Literature. *JACC Heart Fail* 7, 258-266.
- Böttiger, Y., Undre, N.A., Säwe, J., Stevenson, P.J., Ericzon, B.G., 2002. Effect of bile flow on the absorption of tacrolimus in liver allograft transplantation. *Transplantation Proceedings* 34, 1544-1545.
- Boyd, B.J., Bergstrom, C.A.S., Vinarov, Z., Kuentz, M., Brouwers, J., Augustijns, P., Brandl, M., Bernkop-Schnurch, A., Shrestha, N., Preat, V., Mullertz, A., Bauer-Brandl, A., Jannin, V., 2019. Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. *Eur J Pharm Sci* 137, 104967.
- Brantley, R.K., Williams, K.R., Silva, T.M., Sistro, M., Thielman, N.M., Ward, H., Lima, A.A., 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*. *Braz J Infect Dis* 7, 16-22.
- Brewster, M.E., Vandecruys, R., Verreck, G., Peeters, J., 2008. Supersaturating drug delivery systems: effect of hydrophilic cyclodextrins and other excipients on the formation and stabilization of supersaturated drug solutions. *Pharmazie* 63, 217-220.
- Bruera, E., 1997. ABC of palliative care. Anorexia, cachexia, and nutrition. *BMJ* 315, 1219-1222.
- Brussee, J.M., Vet, N.J., Krekels, E.H.J., Valkenburg, A.J., Jacqz-Aigrain, E., van Gerven, J.M.A., Swart, E.L., van den Anker, J.N., Tibboel, D., de Hoog, M., de Wildt, S.N., Knibbe, C.A.J., 2018a. Predicting CYP3A-mediated midazolam metabolism in critically ill neonates, infants, children and adults with inflammation and organ failure. *Br J Clin Pharmacol* 84, 358-368.
- Brussee, J.M., Yu, H., Krekels, E.H.J., de Roos, B., Brill, M.J.E., van den Anker, J.N., Rostami-Hodjegan, A., de Wildt, S.N., Knibbe, C.A.J., 2018b. First-Pass CYP3A-Mediated Metabolism of Midazolam in the Gut Wall and Liver in Preterm Neonates. *CPT Pharmacometrics Syst Pharmacol* 7, 374-383.
- Buchman, A.L., Paine, M.F., Wallin, A., Ludington, S.S., 2005. A higher dose requirement of tacrolimus in active Crohn's disease may be related to a high intestinal P-glycoprotein content. *Digestive diseases and sciences* 50, 2312-2315.
- Bullingham, R.E., Nicholls, A.J., Kamm, B.R., 1998. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 34, 429-455.
- Cabal, A., Wassenaar, T.M. and Ussery, D.W., 2018. Gender Differences in the Gut Microbiome and how these affect Cardiovascular Diseases, in: Mehta, J.L.a.M., J. (Ed.), *Gender Differences in the Pathogenesis and Management of Heart Disease*. Springer, Switzerland.
- Caliph, S.M., Charman, W.N., Porter, C.J., 2000. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J. Pharm. Sci.* 89, 1073-1084.
- Camilleri, M., Ford, A.C., Mawe, G.M., Dinning, P.G., Rao, S.S., Chey, W.D., Simren, M., Lembo, A., Young-Fadok, T.M., Chang, L., 2017. Chronic constipation. *Nature Reviews Disease Primers* 3, 19.
- Camilleri, M., McKinzie, S., Busciglio, I., Low, P.A., Sweetser, S., Burtow, D., Baxter, K., Ryks, M., Zinsmeister, A.R., 2008. Prospective study of motor, sensory, psychologic, and autonomic functions in patients with irritable bowel syndrome. *Clin. Gastroenterol. Hepatol.* 6, 772-781.
- Carver, P.L., Fleisher, D., Zhou, S.Y., Kaul, D., Kazanjian, P., Li, C., 1999. Meal composition effects on the oral bioavailability of indinavir in HIV-infected patients. *Pharm Res* 16, 718-724.
- Chae, S., Kim, D.J., Cho, J.Y., 2020. Complex influences of gut microbiome metabolism on various drug responses. *Transl Clin Pharmacol* 28, 7-16.
- Charman, W., Stella, V., 1986. Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules. *International journal of pharmaceutics* 34, 175-178.

- Chaudhari, D.S., Dhotre, D.P., Agarwal, D.M., Gaike, A.H., Bhalerao, D., Jadhav, P., Mongad, D., Lubree, H., Sinkar, V.P., Patil, U.K., Salvi, S., Bavdekar, A., Juvekar, S.K., Shouche, Y.S., 2020. Gut, oral and skin microbiome of Indian patrilineal families reveal perceptible association with age. *Sci. Rep.* 10, 5685.
- Cheeti, S., Budha, N.R., Rajan, S., Dresser, M.J., Jin, J.Y., 2013. A physiologically based pharmacokinetic (PBPK) approach to evaluate pharmacokinetics in patients with cancer. *Biopharm Drug Dispos* 34, 141-154.
- Cheung, Y.W., Barco, S., Mathot, R.A.A., van den Dool, E.J., Stroobants, A.K., Serlie, M.J., Middeldorp, S., Coppens, M., 2017. Pharmacokinetics of dabigatran etexilate and rivaroxaban in patients with short bowel syndrome requiring parenteral nutrition: The PDER PAN study. *Thromb Res* 160, 76-82.
- Choe, K., Jang, J.Y., Park, I., Kim, Y., Ahn, S., Park, D.-Y., Hong, Y.-K., Alitalo, K., Koh, G.Y., Kim, P., 2015. Intravital imaging of intestinal lacteals unveils lipid drainage through contractility. *The Journal of clinical investigation* 125, 4042-4052.
- Chu, M.P., Hecht, J.R., Slamon, D., Wainberg, Z.A., Bang, Y.J., Hoff, P.M., Sobrero, A., Qin, S., Afenjar, K., Houe, V., King, K., Koski, S., Mulder, K., Hiller, J.P., Scarfe, A., Spratlin, J., Huang, Y.J., Khan-Wasti, S., Chua, N., Sawyer, M.B., 2017. Association of Proton Pump Inhibitors and Capecitabine Efficacy in Advanced Gastroesophageal Cancer: Secondary Analysis of the TRIO-013/LOGiC Randomized Clinical Trial. *JAMA oncology* 3, 767-773.
- Clarke, G., Sandhu, K.V., Griffin, B.T., Dinan, T.G., Cryan, J.F., Hyland, N.P., 2019. Gut Reactions: Breaking Down Xenobiotic-Microbiome Interactions. *Pharmacol Rev* 71, 198-224.
- Clarysse, S., Psachoulias, D., Brouwers, J., Tack, J., Annaert, P., Duchateau, G., Reppas, C., Augustijns, P., 2009a. Postprandial changes in solubilizing capacity of human intestinal fluids for BCS class II drugs. *Pharm Res* 26, 1456-1466.
- Clarysse, S., Tack, J., Lammert, F., Duchateau, G., Reppas, C., Augustijns, P., 2009b. Postprandial Evolution in Composition and Characteristics of Human Duodenal Fluids in Different Nutritional States. *J. Pharm. Sci.* 98, 1177-1192.
- Collins, S.L., Patterson, A.D., 2020. The gut microbiome: an orchestrator of xenobiotic metabolism. *Acta Pharm Sin B* 10, 19-32.
- Cooke, A.R., Hunt, J.N., 1970. Absorption of acetylsalicylic acid from unbuffered and buffered gastric contents. *Am J Dig Dis* 15, 95-102.
- Cooke, E.M., 1969. Faecal flora of patients with ulcerative colitis during treatment with salicylazosulphapyridine. *Gut* 10, 565-568.
- Coombes, Z., Yadav, V., McCoubrey, L.E., Freire, C., Basit, A.W., Conlan, R.S., Gonzalez, D., 2020. Progestogens Are Metabolized by the Gut Microbiota: Implications for Colonic Drug Delivery. *Pharmaceutics* 12.
- Corsetti, M., Costa, M., Bassotti, G., Bharucha, A.E., Borrelli, O., Dinning, P., Di Lorenzo, C., Huizinga, J.D., Jimenez, M., Rao, S., Spiller, R., Spencer, N.J., Lentle, R., Pannemans, J., Thys, A., Benninga, M., Tack, J., 2019. First translational consensus on terminology and definitions of colonic motility in animals and humans studied by manometric and other techniques. *Nature Reviews Gastroenterology & Hepatology* 16, 559-579.
- Corsetti, M., Thys, A., Vanuytsel, T., Demedts, I., Wolthuis, A., D'Hoore, A., Tack, J.F., 2016. Colonic Motor Responses to a Meal and to Bisacodyl, Evaluated by High-Resolution Manometry (HRM), Differ Between Laxative-Refractory Slow Transit Constipation With or Without Pain. *Gastroenterology* 150, S533-S534.
- Courcoulas, A., Coley, R.Y., Clark, J.M., McBride, C.L., Cirelli, E., McTigue, K., Arterburn, D., Coleman, K.J., Wellman, R., Anau, J., Toh, S., Janning, C.D., Cook, A.J., Williams, N., Sturtevant, J.L., Horgan, C., Tavakkoli, A., 2020. Interventions and Operations 5 Years after Bariatric Surgery in a Cohort from the US National Patient-Centered Clinical Research Network Bariatric Study. *JAMA Surgery*.
- Crouthamel, W.G., Diamond, L., Dittert, L.W., Doluisio, J.T., 1975. Drug absorption VII: Influence of mesenteric blood flow on intestinal drug absorption in dogs. *J. Pharm. Sci.* 64, 664-671.
- Cvijic, S., Parojcic, J., Langguth, P., 2014. Viscosity-mediated negative food effect on oral absorption of poorly-permeable drugs with an absorption window in the proximal intestine: In vitro experimental simulation and computational verification. *Eur J Pharm Sci* 61, 40-53.
- Dalton, B., Campbell, I.C., Chung, R., Breen, G., Schmidt, U., Himmerich, H., 2018. Inflammatory Markers in Anorexia Nervosa: An Exploratory Study. *Nutrients* 10.
- Dash, S., Xiao, C., Morgantini, C., Lewis, G.F., 2015. New insights into the regulation of chylomicron production. *Annual review of nutrition* 35, 265-294.

- Davis, S.S., Hardy, J.G., Fara, J.W., 1986. Transit of pharmaceutical dosage forms through the small intestine. *Gut* 27, 886-892.
- de la Cruz-Moreno, M.P., Montejó, C., Aguilar-Ros, A., Dewe, W., Beck, B., Stappaerts, J., Tack, J., Augustijns, P., 2017. Exploring drug solubility in fasted human intestinal fluid aspirates: Impact of inter-individual variability, sampling site and dilution. *Int J Pharm* 528, 471-484.
- Deane, A.M., Chapman, M.J., Reintam Blaser, A., McClave, S.A., Emmanuel, A., 2019. Pathophysiology and Treatment of Gastrointestinal Motility Disorders in the Acutely Ill. *Nutr Clin Pract* 34, 23-36.
- Deane, A.M., Fraser, R.J., Chapman, M.J., 2009. Prokinetic drugs for feed intolerance in critical illness: current and potential therapies. *Crit Care Resusc* 11, 132-143.
- Decker, G.A., Swain, J.M., Crowell, M.D., Scolapio, J.S., 2007. Gastrointestinal and nutritional complications after bariatric surgery. *Am. J. Gastroenterol.* 102, 2571-2580.
- Deiteren, A., Camilleri, M., Burton, D., McKinzie, S., Rao, A., Zinsmeister, A.R., 2010. Effect of meal ingestion on ileocolonic and colonic transit in health and irritable bowel syndrome. *Dig Dis Sci* 55, 384-391.
- Diakidou, A., Vertzoni, M., Abrahamsson, B., Dressman, J., Reppas, C., 2009a. Simulation of gastric lipolysis and prediction of felodipine release from a matrix tablet in the fed stomach. *Eur J Pharm Sci* 37, 133-140.
- Diakidou, A., Vertzoni, M., Dressman, J., Reppas, C., 2009b. Estimation of intragastric drug solubility in the fed state: comparison of various media with data in aspirates. *Biopharm Drug Dispos* 30, 318-325.
- Diakidou, A., Vertzoni, M., Goumas, K., Soderlind, E., Abrahamsson, B., Dressman, J., Reppas, C., 2009c. Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults. *Pharm Res* 26, 2141-2151.
- Dickerson, R.N., Melnik, G., 1988. Osmolality of oral drug solutions and suspensions. *Am J Hosp Pharm* 45, 832-834.
- Digenis, G.A., Sandefer, E.P., Page, R.C., Doll, W.J., Gold, T.B., Darwazeh, N.B., 2000. Bioequivalence study of stressed and nonstressed hard gelatin capsules using amoxicillin as a drug marker and gamma scintigraphy to confirm time and GI location of in vivo capsule rupture. *Pharm Res* 17, 572-582.
- Divasta, A.D., Feldman, H.A., Brown, J.N., Giancaterino, C., Holick, M.F., Gordon, C.M., 2011. Bioavailability of vitamin D in malnourished adolescents with anorexia nervosa. *J Clin Endocrinol Metab* 96, 2575-2580.
- Dixon, J.B., 2010. Mechanisms of chylomicron uptake into lacteals. *Annals of the New York Academy of Sciences* 1207, E52-E57.
- Dokladny, K., Zuhl, M.N., Moseley, P.L., 2016. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. *J. Appl. Physiol.* 120, 692-701.
- Dolton, M.J., Bruggemann, R.J., Burger, D.M., McLachlan, A.J., 2014. Understanding variability in posaconazole exposure using an integrated population pharmacokinetic analysis. *Antimicrob Agents Chemother* 58, 6879-6885.
- Dressman, J.B., Berardi, R.R., Dermentzoglou, L.C., Russell, T.L., Schmaltz, S.P., Barnett, J.L., Jarvenpaa, K.M., 1990. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm Res* 7, 756-761.
- Drozdik, M., Groer, C., Penski, J., Lapczuk, J., Ostrowski, M., Lai, Y., Prasad, B., Unadkat, J.D., Siegmund, W., Oswald, S., 2014. Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine. *Mol Pharm* 11, 3547-3555.
- Elvang, P.A., Bohsen, M.S., Stein, P.C., Bauer-Brandl, A., Riethorst, D., Brouwers, J., Augustijns, P., Brandl, M., 2019. Co-existing colloidal phases of human duodenal aspirates: Intraindividual fluctuations and interindividual variability in relation to molecular composition. *Journal of pharmaceutical and biomedical analysis* 170, 22-29.
- EMA, 2010. Guideline on the investigation of bioequivalence. European Medicines Agency.
- Enright, E.F., Gahan, C.G., Joyce, S.A., Griffin, B.T., 2016. The Impact of the Gut Microbiota on Drug Metabolism and Clinical Outcome. *Yale J Biol Med* 89, 375-382.
- Enright, E.F., Griffin, B.T., Gahan, C.G.M., Joyce, S.A., 2018. Microbiome-mediated bile acid modification: Role in intestinal drug absorption and metabolism. *Pharmacol Res* 133, 170-186.

- Enright, E.F., Joyce, S.A., Gahan, C.G., Griffin, B.T., 2017. Impact of Gut Microbiota-Mediated Bile Acid Metabolism on the Solubilization Capacity of Bile Salt Micelles and Drug Solubility. *Mol Pharm* 14, 1251-1263.
- Eusebi, L.H., Rabitti, S., Artesiani, M.L., Gelli, D., Montagnani, M., Zagari, R.M., Bazzoli, F., 2017. Proton pump inhibitors: Risks of long-term use. *Journal of gastroenterology and hepatology* 32, 1295-1302.
- Evans, S.R., 2010. Common statistical concerns in clinical trials. *J Exp Stroke Transl Med* 3, 1-7.
- Ezzet, F., Krishna, G., Wexler, D.B., Statkevich, P., Kosoglou, T., Batra, V.K., 2001. A population pharmacokinetic model that describes multiple peaks due to enterohepatic recirculation of ezetimibe. *Clin Ther* 23, 871-885.
- Faber, K.P., Wu, H.F., Yago, M.R., Xu, X., Kadiyala, P., Frassetto, L.A., Benet, L.Z., 2017. Meal Effects Confound Attempts to Counteract Rabeprazole-Induced Hypochlorhydria Decreases in Atazanavir Absorption. *Pharm Res* 34, 619-628.
- Fagerholm, U., 2008. Prediction of human pharmacokinetics-biliary and intestinal clearance and enterohepatic circulation. *J Pharm Pharmacol* 60, 535-542.
- Fakhoury, M., Litalien, C., Medard, Y., Cave, H., Ezzahir, N., Peuchmaur, M., Jacqz-Aigrain, E., 2005. Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age. *Drug Metab Dispos* 33, 1603-1607.
- Fallingborg, J., Christensen, L.A., Ingeman-Nielsen, M., Jacobsen, B.A., Abildgaard, K., Rasmussen, H.H., Rasmussen, S.N., 1990. Gastrointestinal pH and transit times in healthy subjects with ileostomy. *Aliment Pharmacol Ther* 4, 247-253.
- Farkouh, A., Riedl, T., Gottardi, R., Czejka, M., Kautzky-Willer, A., 2020. Sex-Related Differences in Pharmacokinetics and Pharmacodynamics of Frequently Prescribed Drugs: A Review of the Literature. *Adv Ther* 37, 644-655.
- Fashner, J., Gitu, A.C., 2013. Common gastrointestinal symptoms: risks of long-term proton pump inhibitor therapy. *FP essentials* 413, 29-39.
- FDA, 2002. Guidance for industry: food-effect bioavailability and fed bioequivalence studies, in: FDA, C. (Ed.).
- FDA, 2011. Dasatinib (Sprycel) Prescribing Information.
- FDA, 2017. Guidance for Industry, Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system. Center for Drug Evaluation and Research.
- Feldman, M., Barnett, C., 1991. Fasting gastric pH and its relationship to true hypochlorhydria in humans. *Dig Dis Sci* 36, 866-869.
- Ferraris, R.P., Carey, H.V., 2000. Intestinal transport during fasting and malnutrition. *Annu. Rev. Nutr.* 20, 195-219.
- Fink, M.P., 2003. Intestinal epithelial hyperpermeability: update on the pathogenesis of gut mucosal barrier dysfunction in critical illness. *Curr. Opin. Crit. Care* 9, 143-151.
- Fisher, L., Fisher, A., 2017. Acid-Suppressive Therapy and Risk of Infections: Pros and Cons. *Clinical drug investigation* 37, 587-624.
- Flamm, S., Reddy, K.R., Zadeikis, N., Hassanein, T., Bacon, B.R., Maieron, A., Zeuzem, S., Bourliere, M., Calleja, J.L., Kosloski, M.P., Oberoi, R.K., Lin, C.W., Yu, Y., Lovell, S., Semizarov, D., Mensa, F.J., 2019. Efficacy and Pharmacokinetics of Glecaprevir and Pibrentasvir With Concurrent Use of Acid-Reducing Agents in Patients With Chronic HCV Infection. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 17, 527-535 e526.
- Franken, L.G., de Winter, B.C., van Esch, H.J., van Zuylen, L., Baar, F.P., Tibboel, D., Mathot, R.A., van Gelder, T., Koch, B.C., 2016. Pharmacokinetic considerations and recommendations in palliative care, with focus on morphine, midazolam and haloperidol. *Expert Opin Drug Metab Toxicol* 12, 669-680.
- Franzosa, E.A., Huang, K., Meadow, J.F., Gevers, D., Lemon, K.P., Bohannon, B.J., Huttenhower, C., 2015. Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci U S A* 112, E2930-2938.
- Freire, A.C., Basit, A.W., Choudhary, R., Piong, C.W., Merchant, H.A., 2011. Does sex matter? The influence of gender on gastrointestinal physiology and drug delivery. *Int. J. Pharm.* 415, 15-28.

- Fritz, A., Busch, D., Lapczuk, J., Ostrowski, M., Drozdziak, M., Oswald, S., 2018. Expression of clinically relevant drug-metabolizing enzymes along the human intestine and their correlation to drug transporters and nuclear receptors: An intra-subject analysis. *Basic Clin. Pharmacol. Toxicol.*
- Fuchs, A., Dressman, J.B., 2014. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a critical evaluation of the available data. *J Pharm Sci* 103, 3398-3411.
- Fuller, A.T., 1937. IS p-AMINO BENZENESULPHONAMIDE THE ACTIVE AGENT IN PRONTOSIL THERAPY ? *The Lancet* 229, 194-198.
- Gao, Y., Shao, J., Jiang, Z., Chen, J., Gu, S., Yu, S., Zheng, K., Jia, L., 2014. Drug enterohepatic circulation and disposition: constituents of systems pharmacokinetics. *Drug Discov Today* 19, 326-340.
- Gay, C., Toulet, D., Le Corre, P., 2017. Pharmacokinetic drug-drug interactions of tyrosine kinase inhibitors: A focus on cytochrome P450, transporters, and acid suppression therapy. *Hematological oncology* 35, 259-280.
- Gesquiere, I., Darwich, A.S., Van Der Schueren, B., De Hoon, J., Lannoo, M., Matthys, C., Rostami, A., Foulon, V., Augustijns, P., 2015. Drug disposition and modelling before and after gastric bypass: Immediate and controlled-release metoprolol formulations. *Br. J. Clin. Pharmacol.*
- Gesquiere, I., Hens, B., Van der Schueren, B., Mols, R., de Hoon, J., Lannoo, M., Matthys, C., Foulon, V., Augustijns, P., 2016. Drug disposition before and after gastric bypass: fenofibrate and posaconazole. *Br. J. Clin. Pharmacol.*
- Ghyselinck, J., Verstrepen, L., Moens, F., Van den Abbeele, P., Said, J., Smith, B., Bjarnason, I., Basit, A.W., Gaisford, S., 2020. A 4-strain probiotic supplement influences gut microbiota composition and gut wall function in patients with ulcerative colitis. *Int J Pharm* 587, 119648.
- Giri, N., Lam, L.H., LaBadie, R.R., Krzyzaniak, J.F., Jiang, H., Hee, B., Liang, Y., Shaik, M.N., 2017. Evaluation of the effect of new formulation, food, or a proton pump inhibitor on the relative bioavailability of the smoothened inhibitor glasdegib (PF-04449913) in healthy volunteers. *Cancer chemotherapy and pharmacology* 80, 1249-1260.
- Gotch, F., Nadell, J., Edelman, I.S., 1957. Gastrointestinal water and electrolytes. IV. The equilibration of deuterium oxide (D₂O) in gastrointestinal contents and the proportion of total body water (T.B.W.) in the gastrointestinal tract. *J Clin Invest* 36, 289-296.
- Grimm, M., Ball, K., Scholz, E., Schneider, F., Sivert, A., Benameur, H., Kromrey, M.-L., Kühn, J.-P., Weitschies, W., 2019. Characterization of the gastrointestinal transit and disintegration behavior of floating and sinking acid-resistant capsules using a novel MRI labeling technique. *Eur. J. Pharm. Sci.* 129, 163-172.
- Grimm, M., Koziolok, M., Kuhn, J.P., Weitschies, W., 2018a. Interindividual and intraindividual variability of fasted state gastric fluid volume and gastric emptying of water. *Eur J Pharm Biopharm* 127, 309-317.
- Grimm, M., Koziolok, M., Saleh, M., Schneider, F., Garbacz, G., Kuhn, J.P., Weitschies, W., 2018b. Gastric Emptying and Small Bowel Water Content after Administration of Grapefruit Juice Compared to Water and Isocaloric Solutions of Glucose and Fructose: A Four-Way Crossover MRI Pilot Study in Healthy Subjects. *Mol Pharm* 15, 548-559.
- Grimm, M., Scholz, E., Koziolok, M., Kuhn, J.P., Weitschies, W., 2017. Gastric Water Emptying under Fed State Clinical Trial Conditions Is as Fast as under Fasted Conditions. *Mol Pharm* 14, 4262-4271.
- Gubbins, P.O., Bertch, K.E., 1991. Drug absorption in gastrointestinal disease and surgery. *Clinical pharmacokinetic and therapeutic implications. Clin Pharmacokinet* 21, 431-447.
- Guo, S., Gillingham, T., Guo, Y., Meng, D., Zhu, W., Walker, W.A., Ganguli, K., 2017. Secretions of *Bifidobacterium infantis* and *Lactobacillus acidophilus* protect intestinal epithelial barrier function. *J. Pediatr. Gastroenterol. Nutr.* 64, 404-412.
- Haiser, H.J., Gootenberg, D.B., Chatman, K., Sirasani, G., Balskus, E.P., Turnbaugh, P.J., 2013. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science (New York, N.Y.)* 341, 295-298.
- Hasler, W.L., 2008. The Physiology of Gastric Motility and Gastric Emptying, in: Yamada, T. (Ed.), *Textbook of Gastroenterology*, pp. 207-230.
- Hatton, G.B., Yadav, V., Basit, A.W., Merchant, H.A., 2015. Animal Farm: Considerations in Animal Gastrointestinal Physiology and Relevance to Drug Delivery in Humans. *J Pharm Sci* 104, 2747-2776.

- Heetun, Z.S., Quigley, E.M.M., 2012. Gastroparesis and Parkinson's disease: A systematic review. *Parkinsonism Relat. Disord.* 18, 433-440.
- Heikkilä, T., Karjalainen, M., Ojala, K., Partola, K., Lammert, F., Augustijns, P., Urtti, A., Yliperttula, M., Peltonen, L., Hirvonen, J., 2011. Equilibrium drug solubility measurements in 96-well plates reveal similar drug solubilities in phosphate buffer pH 6.8 and human intestinal fluid. *Int J Pharm* 405, 132-136.
- Hellriegel, E.T., Björnsson, T.D., Hauck, W.W., 1996. Interpatient variability in bioavailability is related to the extent of absorption: Implications for bioavailability and bioequivalence studies. *Clin. Pharmacol. Ther.* 60, 601-607.
- Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2016. Supersaturation and Precipitation of Posaconazole Upon Entry in the Upper Small Intestine in Humans. *J Pharm Sci* 105, 2677-2684.
- Hens, B., Corsetti, M., Spiller, R., Marciani, L., Vanuytsel, T., Tack, J., Talatoff, A., Amidon, G.L., Koziolk, M., Weitschies, W., Wilson, C.G., Bennink, R.J., Brouwers, J., Augustijns, P., 2017. Exploring gastrointestinal variables affecting drug and formulation behavior: Methodologies, challenges and opportunities. *Int J Pharm* 519, 79-97.
- Hepner, G.W., 1975. Effect of decreased gallbladder stimulation on enterohepatic cycling and kinetics of bile acids. *Gastroenterology* 68, 1574-1581.
- Hutson, W.R., Roehrkasse, R.L., Wald, A., 1989. Influence of gender and menopause on gastric emptying and motility. *Gastroenterology* 96, 11-17.
- Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., Chinwalla, A.T., Creasy, H.H., Earl, A.M., FitzGerald, M.G., Fulton, R.S., Giglio, M.G., Hallsworth-Pepin, K., Lobos, E.A., Madupu, R., Magrini, V., Martin, J.C., Mitreva, M., Muzny, D.M., Sodergren, E.J., Versalovic, J., Wollam, A.M., Worley, K.C., Wortman, J.R., Young, S.K., Zeng, Q., Aagaard, K.M., Abolude, O.O., Allen-Vercoe, E., Alm, E.J., Alvarado, L., Andersen, G.L., Anderson, S., Appelbaum, E., Arachchi, H.M., Armitage, G., Arze, C.A., Ayvaz, T., Baker, C.C., Begg, L., Belachew, T., Bhonagiri, V., Bihan, M., Blaser, M.J., Bloom, T., Bonazzi, V., Paul Brooks, J., Buck, G.A., Buhay, C.J., Busam, D.A., Campbell, J.L., Canon, S.R., Cantarel, B.L., Chain, P.S.G., Chen, I.M.A., Chen, L., Chhibba, S., Chu, K., Ciulla, D.M., Clemente, J.C., Clifton, S.W., Conlan, S., Crabtree, J., Cutting, M.A., Davidovics, N.J., Davis, C.C., DeSantis, T.Z., Deal, C., Delehaunty, K.D., Dewhirst, F.E., Deych, E., Ding, Y., Dooling, D.J., Dugan, S.P., Michael Dunne, W., Scott Durkin, A., Edgar, R.C., Erlich, R.L., Farmer, C.N., Farrell, R.M., Faust, K., Feldgarden, M., Felix, V.M., Fisher, S., Fodor, A.A., Forney, L.J., Foster, L., Di Francesco, V., Friedman, J., Friedrich, D.C., Fronick, C.C., Fulton, L.L., Gao, H., Garcia, N., Giannoukos, G., Giblin, C., Giovanni, M.Y., Goldberg, J.M., Goll, J., Gonzalez, A., Griggs, A., Gujja, S., Kinder Haake, S., Haas, B.J., Hamilton, H.A., Harris, E.L., Hepburn, T.A., Herter, B., Hoffmann, D.E., Holder, M.E., Howarth, C., Huang, K.H., Huse, S.M., Izard, J., Jansson, J.K., Jiang, H., Jordan, C., Joshi, V., Katancik, J.A., Keitel, W.A., Kelley, S.T., Kells, C., King, N.B., Knights, D., Kong, H.H., Koren, O., Koren, S., Kota, K.C., Kovar, C.L., Kyrpides, N.C., La Rosa, P.S., Lee, S.L., Lemon, K.P., Lennon, N., Lewis, C.M., Lewis, L., Ley, R.E., Li, K., Liolios, K., Liu, B., Liu, Y., Lo, C.-C., Lozupone, C.A., Dwayne Lunsford, R., Madden, T., Mahurkar, A.A., Mannon, P.J., Mardis, E.R., Markowitz, V.M., Mavromatis, K., McCorrison, J.M., McDonald, D., McEwen, J., McGuire, A.L., McInnes, P., Mehta, T., Mihindukulasuriya, K.A., Miller, J.R., Minx, P.J., Newsham, I., Nusbaum, C., O'Laughlin, M., Orvis, J., Pagani, I., Palaniappan, K., Patel, S.M., Pearson, M., Peterson, J., Podar, M., Pohl, C., Pollard, K.S., Pop, M., Priest, M.E., Proctor, L.M., Qin, X., Raes, J., Ravel, J., Reid, J.G., Rho, M., Rhodes, R., Riehle, K.P., Rivera, M.C., Rodriguez-Mueller, B., Rogers, Y.-H., Ross, M.C., Russ, C., Sanka, R.K., Sankar, P., Fah Sathirapongsasuti, J., Schloss, J.A., Schloss, P.D., Schmidt, T.M., Scholz, M., Schriml, L., Schubert, A.M., Segata, N., Segre, J.A., Shannon, W.D., Sharp, R.R., Sharpton, T.J., Shenoy, N., Sheth, N.U., Simone, G.A., Singh, I., Smillie, C.S., Sobel, J.D., Sommer, D.D., Spicer, P., Sutton, G.G., Sykes, S.M., Tabbaa, D.G., Thiagarajan, M., Tomlinson, C.M., Torralba, M., Treangen, T.J., Truty, R.M., Vishnivetskaya, T.A., Walker, J., Wang, L., Wang, Z., Ward, D.V., Warren, W., Watson, M.A., Wellington, C., Wettersrand, K.A., White, J.R., Wilczek-Boney, K., Wu, Y., Wylie, K.M., Wylie, T., Yandava, C., Ye, L., Ye, Y., Yoosheph, S., Youmans, B.P., Zhang, L., Zhou, Y., Zhu, Y., Zoloth, L., Zucker, J.D., Birren, B.W., Gibbs, R.A., Highlander, S.K., Methé, B.A., Nelson, K.E., Petrosino, J.F., Weinstock, G.M., Wilson, R.K., White, O., The Human Microbiome Project, C., 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207-214.
- Ibekwe, V.C., Fadda, H.M., McConnell, E.L., Khela, M.K., Evans, D.F., Basit, A.W., 2008. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. *Pharm Res* 25, 1828-1835.
- Imhann, F., Bonder, M.J., Vich Vila, A., Fu, J., Mujagic, Z., Vork, L., Tigchelaar, E.F., Jankipersadsing, S.A., Cenit, M.C., Harmsen, H.J., Dijkstra, G., Franke, L., Xavier, R.J., Jonkers, D., Wijmenga, C., Weersma, R.K., Zhernakova, A., 2016. Proton pump inhibitors affect the gut microbiome. *Gut* 65, 740-748.
- Ishii, M., Toda, T., Ikarashi, N., Ochiai, W., Sugiyama, K., 2012. [Effects of intestinal flora on the expression of cytochrome P450 3A in the liver]. *Yakugaku Zasshi* 132, 301-310.
- Jakobsen, G.S., Småstuen, M.C., Sandbu, R., Nordstrand, N., Hofso, D., Lindberg, M., Hertel, J.K., Hjeltnes, J., 2018. Association of Bariatric Surgery vs Medical Obesity Treatment With Long-term Medical Complications and Obesity-Related Comorbidities. *JAMA* 319, 291-301.

- James, K.R., Gomes, T., Elmentaite, R., Kumar, N., Gulliver, E.L., King, H.W., Stares, M.D., Bareham, B.R., Ferdinand, J.R., Petrova, V.N., Polanski, K., Forster, S.C., Jarvis, L.B., Suchanek, O., Howlett, S., James, L.K., Jones, J.L., Meyer, K.B., Clatworthy, M.R., Saeb-Parsy, K., Lawley, T.D., Teichmann, S.A., 2020. Distinct microbial and immune niches of the human colon. *Nat Immunol* 21, 343-353.
- Javdan, B., Lopez, J.G., Chankhamjon, P., Lee, Y.J., Hull, R., Wu, Q., Wang, X., Chatterjee, S., Donia, M.S., 2020. Personalized Mapping of Drug Metabolism by the Human Gut Microbiome. *Cell* 181, 1661-1679 e1622.
- Jeppesen, P.B., Staun, M., Tjellesen, L., Mortensen, P.B., 1998. Effect of intravenous ranitidine and omeprazole on intestinal absorption of water, sodium, and macronutrients in patients with intestinal resection. *Gut* 43, 763-769.
- Jin, L., Pan, Y., Tran, N.L.L., Polychronopoulos, L.N., Warriar, A., Brouwer, K.L., Nicolazzo, J.A., 2020. Intestinal permeability and oral absorption of selected drugs are reduced in a mouse model of familial Alzheimer's disease. *Molecular Pharmaceutics* 17, 1527-1537.
- Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J.B., Reppas, C., 2006. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm Res* 23, 165-176.
- Karatza, E., Goumas, C., Muenster, U., Reppas, C., Vertzoni, M., 2017. Ex vivo evaluation of degradation rates of metronidazole and olsalazine in distal ileum and in cecum: The impact of prandial state. *Int J Pharm* 534, 237-241.
- Karsdal, M.A., Byrjalsen, I., Riis, B.J., Christiansen, C., 2008. Optimizing bioavailability of oral administration of small peptides through pharmacokinetic and pharmacodynamic parameters: The effect of water and timing of meal intake on oral delivery of Salmon Calcitonin. *BMC Clin. Pharmacol.* 8, 1-10.
- Keating, J., Bjarnason, I., Somasundaram, S., Macpherson, A., Francis, N., Price, A.B., Sharpstone, D., Smithson, J., Menzies, I.S., Gazzard, B.G., 1995. Intestinal absorptive capacity, intestinal permeability and jejunal histology in HIV and their relation to diarrhoea. *Gut* 37, 623-629.
- Keller, J., Layer, P., 2005. Human pancreatic exocrine response to nutrients in health and disease. *Gut* 54 Suppl 6, vi1-28.
- Keller, K.L., Franquiz, M.J., Duffy, A.P., Trovato, J.A., 2018. Drug-drug interactions in patients receiving tyrosine kinase inhibitors. *Journal of oncology pharmacy practice : official publication of the International Society of Oncology Pharmacy Practitioners* 24, 110-115.
- Kellow, J.E., Borody, T.J., Phillips, S.F., Haddad, A.C., Brown, M.L., 1986. Sulfapyridine appearance in plasma after salicylazosulfapyridine. *Gastroenterology* 91, 396-400.
- Kelly, E.J., Newell, S.J., 1994. Gastric ontogeny: clinical implications. *Arch Dis Child Fetal Neonatal Ed* 71, F136-141.
- Kelly, K., O'Mahony, B., Lindsay, B., Jones, T., Grattan, T.J., Rostami-Hodjegan, A., Stevens, H.N., Wilson, C.G., 2003. Comparison of the rates of disintegration, gastric emptying, and drug absorption following administration of a new and a conventional paracetamol formulation, using gamma scintigraphy. *Pharm Res* 20, 1668-1673.
- Kennedy, J.M., van Rij, A.M., 2006. Drug absorption from the small intestine in immediate postoperative patients. *Br J Anaesth* 97, 171-180.
- Keohane, D.M., Ghosh, T.S., Jeffery, I.B., Molloy, M.G., O'Toole, P.W., Shanahan, F., 2020. Microbiome and health implications for ethnic minorities after enforced lifestyle changes. *Nat Med* 26, 1089-1095.
- Keunecke, A., Hoefman, S., Drenth, H.J., Zisowsky, J., Cleton, A., Ploeger, B.A., 2020. Population pharmacokinetics of regorafenib in solid tumours: Exposure in clinical practice considering enterohepatic circulation and food intake. *Br. J. Clin. Pharmacol.*
- Khoo, S.M., Edwards, G.A., Porter, C.J., Charman, W.N., 2001. A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. *J. Pharm. Sci.* 90, 1599-1607.
- Khosla, R., Feely, L.C., Davis, S.S., 1989. Gastrointestinal transit of non-disintegrating tablets in fed subjects. *Int. J. Pharm.* 53, 107-117.
- Kim, D.-H., 2015. Gut Microbiota-Mediated Drug-Antibiotic Interactions. *Drug Metabolism and Disposition* 43, 1581.
- Kim, D.-H., Hyun, S.-H., Shim, S.-B., Kobashi, K., 1992. The Role of Intestinal Bacteria in the Transformation of Sodium Picosulfate. *The Japanese Journal of Pharmacology* 59, 1-5.
- Kim, J.J., Jang, E.J., Park, J., Sohn, H.S., 2020. Association between proton pump inhibitor use and risk of fracture: A population-based case-control study. *PLoS One* 15, e0235163.

- Kleberg, K., Jacobsen, J., Mullertz, A., 2010. Characterising the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies. *J Pharm Pharmacol* 62, 1656-1668.
- Knorr, J.P., Sjeime, M., Braitman, L.E., Jawa, P., Zaki, R., Ortiz, J., 2014. Concomitant proton pump inhibitors with mycophenolate mofetil and the risk of rejection in kidney transplant recipients. *Transplantation* 97, 518-524.
- Knudsen, K., Fedorova, T.D., Bekker, A.C., Iversen, P., Ostergaard, K., Krogh, K., Borghammer, P., 2017. Objective Colonic Dysfunction is Far more Prevalent than Subjective Constipation in Parkinson's Disease: A Colon Transit and Volume Study. *Journal of Parkinsons Disease* 7, 359-367.
- Koch, K.M., Palmer, J.L., Noordin, N., Tomlinson, J.J., Baidoo, C., 2002. Sex and age differences in the pharmacokinetics of alosetron. *Br J Clin Pharmacol* 53, 238-242.
- Koffert, J., Honka, H., Teuho, J., Kauhanen, S., Hurme, S., Parkkola, R., Oikonen, V., Mari, A., Lindqvist, A., Wierup, N., 2017. Effects of meal and incretins in the regulation of splanchnic blood flow. *Endocrine connections* 6, 179-187.
- Kohan, A.B., Yang, Q., Xu, M., Lee, D., Tso, P., 2016. Monosodium glutamate inhibits the lymphatic transport of lipids in the rat. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 311, G648-G654.
- Koppel, N., Bisanz, J.E., Pandelia, M.E., Tumbaugh, P.J., Balskus, E.P., 2018. Discovery and characterization of a prevalent human gut bacterial enzyme sufficient for the inactivation of a family of plant toxins. *Elife* 7.
- Korstanje, C., 2003. Integrated assessment of preclinical data: shifting high attrition rates to earlier phase drug development. *Curr Opin Investig Drugs* 4, 519-521.
- Kosoglou, T., Statkevich, P., Johnson-Levonas, A.O., Paolini, J.F., Bergman, A.J., Alton, K.B., 2005. Ezetimibe: a review of its metabolism, pharmacokinetics and drug interactions. *Clin Pharmacokinet* 44, 467-494.
- Kourentas, A., Vertzoni, M., Khadra, I., Symillides, M., Clark, H., Halbert, G., Butler, J., Reppas, C., 2016a. Evaluation of the Impact of Excipients and an Albendazole Salt on Albendazole Concentrations in Upper Small Intestine Using an In Vitro Biorelevant Gastrointestinal Transfer (BioGIT) System. *J Pharm Sci* 105, 2896-2903.
- Kourentas, A., Vertzoni, M., Stavrinoudakis, N., Symillidis, A., Brouwers, J., Augustijns, P., Reppas, C., Symillides, M., 2016b. An in vitro biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: Design, implementation, and evaluation. *Eur J Pharm Sci* 82, 106-114.
- Koziolek, M., Alcaro, S., Augustijns, P., Basit, A.W., Grimm, M., Hens, B., Hoad, C.L., Jedamzik, P., Madla, C.M., Maliepaard, M., Marciani, L., Maruca, A., Parrott, N., Pavsek, P., Porter, C.J.H., Reppas, C., van Riet-Nales, D., Rubbens, J., Stelova, M., Trevaskis, N.L., Valentova, K., Vertzoni, M., Cepo, D.V., Corsetti, M., 2019. The mechanisms of pharmacokinetic food-drug interactions - A perspective from the UNGAP group. *Eur J Pharm Sci* 134, 31-59.
- Koziolek, M., Grimm, M., Becker, D., Jordanov, V., Zou, H., Shimizu, J., Wanke, C., Garbacz, G., Weitschies, W., 2015a. Investigation of pH and Temperature Profiles in the GI Tract of Fasted Human Subjects Using the Intellicap((R)) System. *J Pharm Sci* 104, 2855-2863.
- Koziolek, M., Grimm, M., Garbacz, G., Kuhn, J.P., Weitschies, W., 2014. Intra-gastric volume changes after intake of a high-caloric, high-fat standard breakfast in healthy human subjects investigated by MRI. *Mol Pharm* 11, 1632-1639.
- Koziolek, M., Grimm, M., Schneider, F., Jedamzik, P., Sager, M., Kuhn, J.P., Siegmund, W., Weitschies, W., 2016. Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers. *Adv Drug Deliv Rev* 101, 75-88.
- Koziolek, M., Schneider, F., Grimm, M., Modebeta, C., Seekamp, A., Roustom, T., Siegmund, W., Weitschies, W., 2015b. Intra-gastric pH and pressure profiles after intake of the high-caloric, high-fat meal as used for food effect studies. *J Control Release* 220, 71-78.
- Krecic-Shepard, M.E., Barnas, C.R., Slimko, J., Jones, M.P., Schwartz, J.B., 2000. Gender-specific effects on verapamil pharmacokinetics and pharmacodynamics in humans. *J Clin Pharmacol* 40, 219-230.
- Kuno, T., Hirayama-Kurogi, M., Ito, S., Ohtsuki, S., 2016. Effect of Intestinal Flora on Protein Expression of Drug-Metabolizing Enzymes and Transporters in the Liver and Kidney of Germ-Free and Antibiotics-Treated Mice. *Mol Pharm* 13, 2691-2701.
- Kuo, B., Maneerattanaporn, M., Lee, A.A., Baker, J.R., Wiener, S.M., Chey, W.D., Wilding, G.E., Hasler, W.L., 2011. Generalized Transit Delay on Wireless Motility Capsule Testing in Patients with Clinical Suspicion of Gastroparesis, Small Intestinal Dysmotility, or Slow Transit Constipation. *Dig. Dis. Sci.* 56, 2928-2938.

- Laksitorini, M., Prasasty, V.D., Kiptoo, P.K., Siahaan, T.J., 2014. Pathways and progress in improving drug delivery through the intestinal mucosa and blood–brain barriers. *Therapeutic delivery* 5, 1143-1163.
- Lam, K.N., Alexander, M., Turnbaugh, P.J., 2019. Precision Medicine Goes Microscopic: Engineering the Microbiome to Improve Drug Outcomes. *Cell Host & Microbe* 26, 22-34.
- Lavrijsen, K., van Dyck, D., van Houdt, J., Hendrickx, J., Monbaliu, J., Woestenborghs, R., Meuldermans, W., Heykants, J., 1995. Reduction of the prodrug loperamide oxide to its active drug loperamide in the gut of rats, dogs, and humans. *Drug Metabolism and Disposition* 23, 354.
- Leclair-Visonneau, L., Neunlist, M., Derkinderen, P., Lebouvier, T., 2020. The gut in Parkinson's disease: Bottom-up, top-down, or neither? *Neurogastroenterol. Motil.* 32, 6.
- Li, H., He, J., Jia, W., 2016. The influence of gut microbiota on drug metabolism and toxicity. *Expert Opin Drug Metab Toxicol* 12, 31-40.
- Li, L., Wang, L., Shao, Y., Ni, R., Zhang, T., Mao, S., 2013. Drug release characteristics from chitosan-alginate matrix tablets based on the theory of self-assembled film. *Int J Pharm* 450, 197-207.
- Li, T., Chiang, J.Y.L., 2015. Bile acids as metabolic regulators. *Current opinion in gastroenterology* 31, 159-165.
- Li, X., Lindquist, S., Lowe, M., Noppa, L., Hernell, O., 2007. Bile salt-stimulated lipase and pancreatic lipase-related protein 2 are the dominating lipases in neonatal fat digestion in mice and rats. *Pediatr Res* 62, 537-541.
- Litou, C., Effinger, A., Kostewicz, E.S., Box, K.J., Fotaki, N., Dressman, J.B., 2019. Effects of medicines used to treat gastrointestinal diseases on the pharmacokinetics of coadministered drugs: a PEARRL Review. *J Pharm Pharmacol* 71, 643-673.
- Litou, C., Psachoulas, D., Vertzoni, M., Dressman, J., Reppas, C., 2020. Measuring pH and Buffer Capacity in Fluids Aspirated from the Fasted Upper Gastrointestinal Tract of Healthy Adults. *Pharm Res* 37, 42.
- Litou, C., Vertzoni, M., Goumas, C., Vasdekis, V., Xu, W., Kesiosoglou, F., Reppas, C., 2016. Characteristics of the Human Upper Gastrointestinal Contents in the Fasted State Under Hypo- and A-chlorhydric Gastric Conditions Under Conditions of Typical Drug - Drug Interaction Studies. *Pharm Res* 33, 1399-1412.
- Litou, C., Vertzoni, M., Xu, W., Kesiosoglou, F., Reppas, C., 2017. The impact of reduced gastric acid secretion on dissolution of salts of weak bases in the fasted upper gastrointestinal lumen: Data in biorelevant media and in human aspirates. *Eur J Pharm Biopharm* 115, 94-101.
- Lopes, C.M., Bettencourt, C., Rossi, A., Buttini, F., Barata, P., 2016. Overview on gastroretentive drug delivery systems for improving drug bioavailability. *Int J Pharm* 510, 144-158.
- Madsen, C.M., Feng, K.I., Leithead, A., Canfield, N., Jorgensen, S.A., Mullertz, A., Rades, T., 2018. Effect of composition of simulated intestinal media on the solubility of poorly soluble compounds investigated by design of experiments. *Eur J Pharm Sci* 111, 311-319.
- Madsen, C.M., Plum, J., Hens, B., Augustijns, P., Mullertz, A., Rades, T., 2021. Exploring the Impact of Intestinal Fluid Components on the Solubility and Supersaturation of Danazol. *J Pharm Sci.*
- Magallanes, L., Lorier, M., Ibarra, M., Guevara, N., Vazquez, M., Fagiolino, P., 2016. Sex and Food Influence on Intestinal Absorption of Ketoprofen Gastroresistant Formulation. *Clin Pharmacol Drug Dev* 5, 196-200.
- Maharaj, A.R., Edginton, A.N., 2016. Examining Small Intestinal Transit Time as a Function of Age: Is There Evidence to Support Age-Dependent Differences among Children? *Drug Metab Dispos* 44, 1080-1089.
- Maharaj, A.R., Edginton, A.N., Fotaki, N., 2016. Assessment of Age-Related Changes in Pediatric Gastrointestinal Solubility. *Pharm Res* 33, 52-71.
- Mai, Y., Ashiru-Oredope, D.A.I., Yao, Z., Dou, L., Madla, C.M., Taherali, F., Murdan, S., Basit, A.W., 2020. Boosting drug bioavailability in men but not women through the action of an excipient. *Int J Pharm* 587, 119678.
- Mai, Y., Dou, L., Madla, C.M., Murdan, S., Basit, A.W., 2019. Sex-Dependence in the Effect of Pharmaceutical Excipients: Polyoxyethylated Solubilising Excipients Increase Oral Drug Bioavailability in Male but not Female Rats. *Pharmaceutics* 11.
- Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E.E., Brochado, A.R., Fernandez, K.C., Dose, H., Mori, H., Patil, K.R., Bork, P., Typas, A., 2018. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 555, 623-628.

- Maini Rekdal, V., Bess, E.N., Bisanz, J.E., Turnbaugh, P.J., Balskus, E.P., 2019. Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science* 364.
- Malik, M.Y., Jaiswal, S., Sharma, A., Shukla, M., Lal, J., 2016. Role of enterohepatic recirculation in drug disposition: cooperation and complications. *Drug Metab Rev* 48, 281-327.
- Man, A.L., Bertelli, E., Rentini, S., Regoli, M., Briars, G., Marini, M., Watson, A.J., Nicoletti, C., 2015. Age-associated modifications of intestinal permeability and innate immunity in human small intestine. *Clin. Sci.* 129, 515-527.
- Marciani, L., Cox, E.F., Hoad, C.L., Pritchard, S., Totman, J.J., Foley, S., Mistry, A., Evans, S., Gowland, P.A., Spiller, R.C., 2010. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology* 138, 469-477, 477 e461.
- Mark, E.B., Poulsen, J.L., Haase, A.M., Espersen, M., Gregersen, T., Schlageter, V., Scott, S.M., Krogh, K., Drewes, A.M., 2019. Ambulatory assessment of colonic motility using the electromagnetic capsule tracking system. *Neurogastroenterol. Motil.* 31, 11.
- Marrinan, S., Emmanuel, A.V., Burn, D.J., 2014. Delayed Gastric Emptying in Parkinson's Disease. *Mov. Disord.* 29, 23-32.
- Mayeur, C., Gratadoux, J.J., Bridonneau, C., Chegdani, F., Larroque, B., Kapel, N., Corcos, O., Thomas, M., Joly, F., 2013. Faecal D/L lactate ratio is a metabolic signature of microbiota imbalance in patients with short bowel syndrome. *PLoS One* 8, e54335.
- McBain, A.J., Macfarlane, G.T., 1998. Ecological and physiological studies on large intestinal bacteria in relation to production of hydrolytic and reductive enzymes involved in formation of genotoxic metabolites. *Journal of medical microbiology* 47, 407-416.
- McClellan, P., Weaver, L.T., 1993. Ontogeny of human pancreatic exocrine function. *Arch Dis Child* 68, 62-65.
- McCoubrey, L.E., Elbadawi, M., Orlu, M., Gaisford, S., Basit, A.W., 2021. Harnessing machine learning for development of microbiome therapeutics. *Gut Microbes* 13, 1-20.
- McFadden, M.A., DeLegge, M.H., Kirby, D.F., 1993. Medication delivery in the short-bowel syndrome. *JPEN J Parenter Enteral Nutr* 17, 180-186.
- Merchant, H.A., Liu, F., Orlu Gul, M., Basit, A.W., 2016. Age-mediated changes in the gastrointestinal tract. *Int. J. Pharm.* 512, 382-395.
- Messing, B., Crenn, P., Beau, P., Boutron-Ruault, M.C., Rambaud, J.C., Matuchansky, C., 1999. Long-term survival and parenteral nutrition dependence in adult patients with the short bowel syndrome. *Gastroenterology* 117, 1043-1050.
- Mestre, H., Alkon, T., Salazar, S., Ibarra, A., 2011. Spinal cord injury sequelae alter drug pharmacokinetics: an overview. *Spinal Cord* 49, 955-960.
- Mikac, U., Sepe, A., Kristl, J., Baumgartner, S., 2010. A new approach combining different MRI methods to provide detailed view on swelling dynamics of xanthan tablets influencing drug release at different pH and ionic strength. *J Control Release* 145, 247-256.
- Mojaverian, P., Vlases, P.H., Kellner, P.E., Rocci, M.L., Jr., 1988. Effects of gender, posture, and age on gastric residence time of an indigestible solid: pharmaceutical considerations. *Pharm Res* 5, 639-644.
- Mooij, M.G., de Koning, B.A., Huijsman, M.L., de Wildt, S.N., 2012. Ontogeny of oral drug absorption processes in children. *Expert Opin Drug Metab Toxicol* 8, 1293-1303.
- Mooij, M.G., Schwarz, U.I., de Koning, B.A., Leeder, J.S., Gaedigk, R., Samsom, J.N., Spaans, E., van Goudoever, J.B., Tibboel, D., Kim, R.B., de Wildt, S.N., 2014. Ontogeny of human hepatic and intestinal transporter gene expression during childhood: age matters. *Drug Metab Dispos* 42, 1268-1274.
- Moore, J.G., Tweedy, C., Christian, P.E., Datz, F.L., 1983. Effect of age on gastric emptying of liquid--solid meals in man. *Dig Dis Sci* 28, 340-344.
- Moore, K.L., Patel, K., Boscardin, W.J., Steinman, M.A., Ritchie, C., Schwartz, J.B., 2018. Medication burden attributable to chronic comorbid conditions in the very old and vulnerable. *PLoS One* 13, e0196109.
- Mouly, S., Aymard, G., Diquet, B., Caulin, C., Bergmann, J.F., 2000. Oral ganciclovir systemic exposure is enhanced in HIV-infected patients with diarrhea and weight loss. *J Acquir Immune Defic Syndr* 24, 344-351.

- Mudie, D.M., Murray, K., Hoad, C.L., Pritchard, S.E., Garnett, M.C., Amidon, G.E., Gowland, P.A., Spiller, R.C., Amidon, G.L., Marciani, L., 2014. Quantification of Gastrointestinal Liquid Volumes and Distribution Following a 240 mL Dose of Water in the Fasted State. *Mol. Pharm.* 11, 3039-3047.
- Mukherjee, A., Biswas, A., Das, S.K., 2016. Gut dysfunction in Parkinson's disease. *World J. Gastroenterol.* 22, 5742-5752.
- Mullertz, A., Fatouros, D.G., Smith, J.R., Vertzoni, M., Reppas, C., 2012. Insights into intermediate phases of human intestinal fluids visualized by atomic force microscopy and cryo-transmission electron microscopy ex vivo. *Mol Pharm* 9, 237-247.
- Mullertz, A., Reppas, C., Psachoulas, D., Vertzoni, M., Fatouros, D.G., 2015. Structural features of colloidal species in the human fasted upper small intestine. *J Pharm Pharmacol* 67, 486-492.
- Nakayama, H., Kinouchi, T., Kataoka, K., Akimoto, S., Matsuda, Y., Ohnishi, Y., 1997. Intestinal anaerobic bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(E)-(2-bromovinyl)uracil that increases the level and toxicity of 5-fluorouracil. *Pharmacogenetics and Genomics* 7.
- Nandhra, G.K., Mark, E.B., Di Tanna, G.L., Haase, A.M., Poulsen, J., Christodoulides, S., Kung, V., Klinge, M.W., Knudsen, K., Borghammer, P., Andersen, K.O., Fynne, L., Sutter, N., Schlageter, V., Krogh, K., Drewes, A.M., Birch, M., Scott, S.M., 2020. Normative values for region-specific colonic and gastrointestinal transit times in 111 healthy volunteers using the 3D-Transit electromagnet tracking system: Influence of age, gender, and body mass index. *Neurogastroenterol Motil* 32, e13734.
- Nguyen, N.T., Varela, J.E., 2017a. Bariatric surgery for obesity and metabolic disorders: state of the art. *Nat Rev Gastroenterol Hepatol* 14, 160-169.
- Nguyen, N.T., Varela, J.E., 2017b. Bariatric surgery for obesity and metabolic disorders: State of the art, pp. 160-169.
- Nicolas, J.M., Bouzom, F., Hugues, C., Ungell, A.L., 2017. Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions. *Biopharm Drug Dispos* 38, 209-230.
- Nightingale, J.M., Kamm, M.A., van der Sijp, J.R., Morris, G.P., Walker, E.R., Mather, S.J., Britton, K.E., Lennard-Jones, J.E., 1993. Disturbed gastric emptying in the short bowel syndrome. Evidence for a 'colonic brake'. *Gut* 34, 1171-1176.
- Norman, J.L., Fixen, D.R., Saseen, J.J., Saba, L.M., Linnebur, S.A., 2017. Zolpidem prescribing practices before and after Food and Drug Administration required product labeling changes. *SAGE Open Med* 5, 2050312117707687.
- Nurmi, H., Saharinen, P., Zarkada, G., Zheng, W., Robciuc, M.R., Alitalo, K., 2015. VEGF-C is required for intestinal lymphatic vessel maintenance and lipid absorption. *EMBO molecular medicine* 7, 1418-1425.
- Ogawa, R., Echizen, H., 2010. Drug-drug interaction profiles of proton pump inhibitors. *Clin Pharmacokinet* 49, 509-533.
- Okuda, H., Ogura, K., Kato, A., Takubo, H., Watabe, T., 1998. A possible mechanism of eighteen patient deaths caused by interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs. *J. Pharmacol. Exp. Ther.* 287, 791-799.
- Ouellet, D.M., Pollack, G.M., 1995. Biliary excretion and enterohepatic recirculation of morphine-3-glucuronide in rats. *Drug Metab Dispos* 23, 478-484.
- Padilla, N., Maraninchi, M., Béliard, S., Berthet, B., Nogueira, J.-P., Wolff, E., Nicolay, A., Bégu, A., Dubois, N., Grangeot, R., 2014. Effects of bariatric surgery on hepatic and intestinal lipoprotein particle metabolism in obese, nondiabetic humans. *Arterioscler. Thromb. Vasc. Biol.* 34, 2330-2337.
- Padwal, R.S., Gabr, R.Q., Sharma, A.M., Langkaas, L.A., Birch, D.W., Karmali, S., Brocks, D.R., 2011. Effect of gastric bypass surgery on the absorption and bioavailability of metformin. *Diabetes Care* 34, 1295-1300.
- Paraskevopoulos, J.A., Houghton, L.A., Eyre-Brooke, I., Johnson, A.G., Read, N.W., 1988. Effect of composition of gastric contents on resistance to emptying of liquids from stomach in humans. *Dig Dis Sci* 33, 914-918.
- Pasipanodya, J.G., Srivastava, S., Gumbo, T., 2012. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clin Infect Dis* 55, 169-177.
- Pavlovic, N., Golocorbin-Kon, S., Ethanic, M., Stanimirov, B., Al-Salami, H., Stankov, K., Mikov, M., 2018. Bile Acids and Their Derivatives as Potential Modifiers of Drug Release and Pharmacokinetic Profiles. *Front Pharmacol* 9, 1283.

- Pedersen, P.B., Vilmann, P., Bar-Shalom, D., Mullertz, A., Baldursdottir, S., 2013. Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities. *Eur J Pharm Biopharm* 85, 958-965.
- Peh, K.K., Yuen, K.H., 1996. Indirect gastrointestinal transit monitoring and absorption of theophylline. *Int. J. Pharm.* 139, 95-103.
- Pentafragka, C., Symillides, M., McAllister, M., Dressman, J., Vertzoni, M., Reppas, C., 2019. The impact of food intake on the luminal environment and performance of oral drug products with a view to in vitro and in silico simulations: a PEARRL review. *J Pharm Pharmacol* 71, 557-580.
- Pentafragka, C., Vertzoni, M., Dressman, J., Symillides, M., Goumas, K., C., R., 2020a. Characteristics of contents in the upper gastrointestinal lumen after a standard high-calorie high-fat meal and implications for the in vitro drug product performance testing conditions. *Eur J Pharm Sci.* 155, 105535.
- Pentafragka, C., Vertzoni, M., Symillides, M., Goumas, K., Reppas, C., 2020b. Disposition of two highly permeable drugs in the upper gastrointestinal lumen of healthy adults after a standard high-calorie, high-fat meal. *Eur J Pharm Sci* 149, 105351.
- Perez de la Cruz Moreno, M., Oth, M., Deferme, S., Lammert, F., Tack, J., Dressman, J., Augustijns, P., 2006. Characterization of fasted-state human intestinal fluids collected from duodenum and jejunum. *J Pharm Pharmacol* 58, 1079-1089.
- Perko, M., Nielsen, H., Skak, C., Clemmesen, J., Schroeder, T., Secher, N., 1998. Mesenteric, coeliac and splanchnic blood flow in humans during exercise. *The Journal of Physiology* 513, 907-913.
- Persson, E.M., Gustafsson, A.S., Carlsson, A.S., Nilsson, R.G., Knutson, L., Forsell, P., Hanisch, G., Lennernas, H., Abrahamsson, B., 2005. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharm Res* 22, 2141-2151.
- Persson, E.M., Nilsson, R.G., Hansson, G.I., Lofgren, L.J., Liback, F., Knutson, L., Abrahamsson, B., Lennernas, H., 2006. A clinical single-pass perfusion investigation of the dynamic in vivo secretory response to a dietary meal in human proximal small intestine. *Pharm Res* 23, 742-751.
- Petrakis, O., Vertzoni, M., Angelou, A., Kesisoglou, F., Bentz, K., Goumas, K., Reppas, C., 2015. Identification of key factors affecting the oral absorption of salts of lipophilic weak acids: a case example. *J Pharm Pharmacol* 67, 56-67.
- Pfeiffer, A., Schmidt, T., Kaess, H., 1998. The role of osmolality in the absorption of a nutrient solution. *Aliment Pharmacol Ther* 12, 281-286.
- Pironi, L., Arends, J., Baxter, J., Bozzetti, F., Pelaez, R.B., Cuerda, C., Forbes, A., Gabe, S., Gillanders, L., Holst, M., Jeppesen, P.B., Joly, F., Kelly, D., Klek, S., Irtun, O., Olde Damink, S.W., Panisic, M., Rasmussen, H.H., Staun, M., Szczepanek, K., Van Gossum, A., Wanten, G., Schneider, S.M., Shaffer, J., Home Artificial, N., Chronic Intestinal Failure Special Interest Groups of, E., 2015. ESPEN endorsed recommendations. Definition and classification of intestinal failure in adults. *Clin Nutr* 34, 171-180.
- Pironi, L., Arends, J., Bozzetti, F., Cuerda, C., Gillanders, L., Jeppesen, P.B., Joly, F., Kelly, D., Lal, S., Staun, M., Szczepanek, K., Van Gossum, A., Wanten, G., Schneider, S.M., Home Artificial, N., Chronic Intestinal Failure Special Interest Group of, E., 2016. ESPEN guidelines on chronic intestinal failure in adults. *Clin Nutr* 35, 247-307.
- Ploeger, B.A., Meulenbelt, J., DeJongh, J., 2000. Physiologically Based Pharmacokinetic Modeling of Glycyrrhizic Acid, a Compound Subject to Presystemic Metabolism and Enterohepatic Cycling. *Toxicol. Appl. Pharmacol.* 162, 177-188.
- Plusquellec, Y., Houin, G., 1992. Drug recirculation model with multiple cycles occurring at unequal time intervals. *Journal of biomedical engineering* 14, 521-526.
- Pocock, S.J., McMurray, J.J., Collier, T.J., 2015. Making Sense of Statistics in Clinical Trial Reports: Part 1 of a 4-Part Series on Statistics for Clinical Trials. *J Am Coll Cardiol* 66, 2536-2549.
- Pohl, O., Osterloh, I., Lecomte, V., Gotteland, J.P., 2013. Changes in gastric pH and in pharmacokinetics of ulipristal acetate - a drug-drug interaction study using the proton pump inhibitor esomeprazole. *International journal of clinical pharmacology and therapeutics* 51, 26-33.
- Pollack, G.M., Brouwer, K.L., 1991. Physiologic and metabolic influences on enterohepatic recirculation: simulations based upon the disposition of valproic acid in the rat. *J Pharmacokinetic Biopharm* 19, 189-225.
- Priebe, M.G., Wachters-Hagedoorn, R.E., Landman, K., Heimweg, J., Elzinga, H., Vonk, R.J., 2006. Influence of a subsequent meal on the oro-cecal transit time of a solid test meal. *Eur J Clin Invest* 36, 123-126.

- Priebe, M.G., Wachters-Hagedoorn, R.E., Stellaard, F., Heiner, A.M., Elzinga, H., Vonk, R.J., 2004. Oro-cecal transit time: influence of a subsequent meal. *Eur J Clin Invest* 34, 417-421.
- Proesmans, M., De Boeck, K., 2003. Omeprazole, a proton pump inhibitor, improves residual steatorrhea in cystic fibrosis patients treated with high dose pancreatic enzymes. *Eur J Pediatr* 162, 760-763.
- Psachoulas, D., Vertzoni, M., Goumas, K., Kalioras, V., Beato, S., Butler, J., Reppas, C., 2011. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. *Pharm Res* 28, 3145-3158.
- Pyper, K., Brouwers, J., Augustijns, P., Khadra, I., Dunn, C., Wilson, C.G., Halbert, G.W., 2020. Multidimensional analysis of human intestinal fluid composition. *Eur J Pharm Biopharm* 153, 226-240.
- Quodbach, J., Kleinebudde, P., 2016. A critical review on tablet disintegration. *Pharm Dev Technol* 21, 763-774.
- Rahmouni, M., Chouinard, F., Nekka, F., Lenaerts, V., Leroux, J.C., 2001. Enzymatic degradation of cross-linked high amylose starch tablets and its effect on in vitro release of sodium diclofenac. *Eur J Pharm Biopharm* 51, 191-198.
- Ramos, A., Kow, L., Brown, W., Welbourn, R., Dixon, J., Kinsman, R., Walton, P., 2019. The IFSO Global Registry: 5th IFSO Global Registry Report, pp. 1-100.
- Reppas, C., Karatza, E., Goumas, C., Markopoulos, C., Vertzoni, M., 2015. Characterization of Contents of Distal Ileum and Cecum to Which Drugs/Drug Products are Exposed During Bioavailability/Bioequivalence Studies in Healthy Adults. *Pharm Res* 32, 3338-3349.
- Riethorst, D., Baatsen, P., Remijn, C., Mitra, A., Tack, J., Brouwers, J., Augustijns, P., 2016a. An In-Depth View into Human Intestinal Fluid Colloids: Intersubject Variability in Relation to Composition. *Mol Pharm* 13, 3484-3493.
- Riethorst, D., Brouwers, J., Motmans, J., Augustijns, P., 2018a. Human intestinal fluid factors affecting intestinal drug permeation in vitro. *Eur J Pharm Sci* 121, 338-346.
- Riethorst, D., Mitra, A., Kesisoglou, F., Xu, W., Tack, J., Brouwers, J., Augustijns, P., 2018b. Human intestinal fluid layer separation: The effect on colloidal structures & solubility of lipophilic compounds. *Eur J Pharm Biopharm* 129, 104-110.
- Riethorst, D., Mols, R., Duchateau, G., Tack, J., Brouwers, J., Augustijns, P., 2016b. Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions. *J Pharm Sci* 105, 673-681.
- Riley, S.A., Sutcliffe, F., Kim, M., Kapas, M., Rowland, M., Turnberg, L.A., 1992. The influence of gastrointestinal transit on drug absorption in healthy volunteers. *Br J Clin Pharmacol* 34, 32-39.
- Roager, H.M., Hansen, L.B., Bahl, M.L., Frandsen, H.L., Carvalho, V., Gobel, R.J., Dalgaard, M.D., Plichta, D.R., Sparholt, M.H., Vestergaard, H., Hansen, T., Sicheritz-Ponten, T., Nielsen, H.B., Pedersen, O., Lauritzen, L., Kristensen, M., Gupta, R., Licht, T.R., 2016. Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* 1, 16093.
- Roberts, M.S., Magnusson, B.M., Burczynski, F.J., Weiss, M., 2002. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. *Clin Pharmacokinet* 41, 751-790.
- Rohr, M.W., Narasimulu, C.A., Rudeski-Rohr, T.A., Parthasarathy, S., 2020. Negative effects of a high-fat diet on intestinal permeability: a review. *Adv. Nutr.* 11, 77-91.
- Rubbens, J., Brouwers, J., Tack, J., Augustijns, P., 2016. Gastrointestinal dissolution, supersaturation and precipitation of the weak base indinavir in healthy volunteers. *Eur J Pharm Biopharm* 109, 122-129.
- Ruiter, R., Burggraaf, J., Rissmann, R., 2019. Under-representation of elderly in clinical trials: An analysis of the initial approval documents in the Food and Drug Administration database. *Br J Clin Pharmacol* 85, 838-844.
- Russell, T.L., Berardi, R.R., Barnett, J.L., Dermentzoglou, L.C., Jarvenpaa, K.M., Schmaltz, S.P., Dressman, J.B., 1993. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. *Pharm Res* 10, 187-196.
- Russell, T.L., Berardi, R.R., Barnett, J.L., O'Sullivan, T.L., Wagner, J.G., Dressman, J.B., 1994. pH-related changes in the absorption of dipyridamole in the elderly. *Pharm Res* 11, 136-143.
- Saha, Butler, V.P., Neu, H.C., Lindenbaum, J., 1983. Digoxin-inactivating bacteria: identification in human gut flora. *Science* 220, 325.

- Sakuma, S., Ogura, R., Masaoka, Y., Kataoka, M., Tanno, F.K., Kokubo, H., Yamashita, S., 2009. Correlation between In Vitro Dissolution Profiles from Enteric-Coated Dosage Forms and In Vivo Absorption in Rats for High-Solubility and High-Permeability Model Drugs. *J. Pharm. Sci.* 98, 4141-4152.
- Santamaria, M.M., Villafranca, J.J.A., Abiles, J., Lopez, A.F., Rodas, L.V., Goitia, B.T., Navarro, P.U., 2018. Systematic review of drug bioavailability following gastrointestinal surgery. *Eur J Clin Pharmacol* 74, 1531-1545.
- Sarzynski, E., Puttarajappa, C., Xie, Y., Grover, M., Laird-Fick, H., 2011. Association between proton pump inhibitor use and anemia: a retrospective cohort study. *Dig Dis Sci* 56, 2349-2353.
- Scarpignato, C., Bjamason, I., 2019. Drug-Induced Small Bowel Injury: a Challenging and Often Forgotten Clinical Condition. *Current gastroenterology reports* 21, 55-55.
- Scheline, R.R., 1968. Drug Metabolism by Intestinal Microorganisms. *J. Pharm. Sci.* 57, 2021-2037.
- Schiller, C., Frohlich, C.P., Giessmann, T., Siegmund, W., Monnikes, H., Hosten, N., Weitschies, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment Pharmacol Ther* 22, 971-979.
- Senek, M., Nyholm, D., Nielsen, E.I., 2018. Population pharmacokinetics of levodopa/carbidopa microtablets in healthy subjects and Parkinson's disease patients. *Eur J Clin Pharmacol* 74, 1299-1307.
- Shackleford, D.M., Faassen, W.F., Houwing, N., Lass, H., Edwards, G.A., Porter, C.J., Charman, W.N., 2003. Contribution of lymphatically transported testosterone undecanoate to the systemic exposure of testosterone after oral administration of two andriol formulations in conscious lymph duct-cannulated dogs. *J. Pharmacol. Exp. Ther.* 306, 925-933.
- Shahid, S., Ramzan, Z., Maurer, A.H., Parkman, H.P., Fisher, R.S., 2012. Chronic Idiopathic Constipation More Than A Simple Colonic Transit Disorder. *J. Clin. Gastroenterol.* 46, 150-154.
- Sharma, A., Buschmann, M.M., Gilbert, J.A., 2019. Pharmacomicrobiomics: The Holy Grail to Variability in Drug Response? *Clin Pharmacol Ther* 106, 317-328.
- Sher, A., Rahman, M.A., 2000. Enterohepatic recycling of estrogen and its relevance with female fertility. *Arch Pharm Res* 23, 513-517.
- Shou, M., Lu, W., Kari, P.H., Xiang, C., Liang, Y., Lu, P., Cui, D., Emary, W.B., Michel, K.B., Adelsberger, J.K., Brunner, J.E., Rodrigues, A.D., 2005. Population pharmacokinetic modeling for enterohepatic recirculation in Rhesus monkey. *Eur J Pharm Sci* 26, 151-161.
- Simren, M., Tornblom, H., Palsson, O.S., Van Oudenhove, L., Whitehead, W.E., Tack, J., 2019. Cumulative Effects of Psychologic Distress, Visceral Hypersensitivity, and Abnormal Transit on Patient-reported Outcomes in Irritable Bowel Syndrome. *Gastroenterology* 157, 391-402 e392.
- Smelick, G.S., Heffron, T.P., Chu, L., Dean, B., West, D.A., Duvall, S.L., Lum, B.L., Budha, N., Holden, S.N., Benet, L.Z., Frymoyer, A., Dresser, M.J., Ware, J.A., 2013. Prevalence of acid-reducing agents (ARA) in cancer populations and ARA drug-drug interaction potential for molecular targeted agents in clinical development. *Mol Pharm* 10, 4055-4062.
- Smirnova, O.V., 2012. [Sex differences in drug action: the role of multidrug-resistance proteins (MRPs)]. *Fiziol Cheloveka* 38, 124-136.
- Smits, A., Annaert, P., Allegaert, K., 2013. Drug disposition and clinical practice in neonates: cross talk between developmental physiology and pharmacology. *Int J Pharm* 452, 8-13.
- Smyth, M.C., 2017. Intestinal permeability and autoimmune diseases. *Bioscience Horizons: The International Journal of Student Research* 10, hzx015.
- Soldin, O.P., Chung, S.H., Mattison, D.R., 2011. Sex differences in drug disposition. *J Biomed Biotechnol* 2011, 187103.
- Sousa, T., Paterson, R., Moore, V., Carlsson, A., Abrahamsson, B., Basit, A.W., 2008. The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int J Pharm* 363, 1-25.
- Sousa, T., Yadav, V., Zann, V., Borde, A., Abrahamsson, B., Basit, A.W., 2014. On the colonic bacterial metabolism of azo-bonded prodrugs of 5-aminosalicylic acid. *J Pharm Sci* 103, 3171-3175.

- Staelens, S., Van den Driessche, M., Barclay, D., Carrie-Faessler, A.L., Haschke, F., Verbeke, K., Vandebroek, H., Allegaert, K., Van Overmeire, B., Van Damme, M., Veereman-Wauters, G., 2008. Gastric emptying in healthy newborns fed an intact protein formula, a partially and an extensively hydrolysed formula. *Clin Nutr* 27, 264-268.
- Stappaerts, J., Geboers, S., Snoeys, J., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2015. Rapid conversion of the ester prodrug abiraterone acetate results in intestinal supersaturation and enhanced absorption of abiraterone: in vitro, rat in situ and human in vivo studies. *Eur J Pharm Biopharm* 90, 1-7.
- Stappaerts, J., Wuyts, B., Tack, J., Annaert, P., Augustijns, P., 2014. Human and simulated intestinal fluids as solvent systems to explore food effects on intestinal solubility and permeability. *Eur J Pharm Sci* 63, 178-186.
- Steinert, R.E., Peterli, R., Keller, S., Meyer-Gerspach, A.C., Drewe, J., Peters, T., Beglinger, C., 2013. Bile acids and gut peptide secretion after bariatric surgery: a 1-year prospective randomized pilot trial. *Obesity (Silver Spring)* 21, E660-668.
- Stephens, M., Liao, S., von der Weid, P.Y., 2019. Mesenteric Lymphatic Alterations Observed During DSS Induced Intestinal Inflammation Are Driven in a TLR4-PAMP/DAMP Discriminative Manner. *Front Immunol* 10, 557.
- Stillhart, C., Vucicevic, K., Augustijns, P., Basit, A.W., Batchelor, H., Flanagan, T.R., Gesquiere, I., Greupink, R., Keszthelyi, D., Koskinen, M., Madla, C.M., Matthys, C., Miljus, G., Mooij, M.G., Parrott, N., Ungell, A.L., de Wildt, S.N., Orlu, M., Klein, S., Mullertz, A., 2020. Impact of gastrointestinal physiology on drug absorption in special populations--An UNGAP review. *Eur J Pharm Sci* 147, 105280.
- Stone, E., Leiter, L.A., Lambert, J.R., Silverberg, J.D., Jeejeebhoy, K.N., Burrow, G.N., 1984. L-thyroxine absorption in patients with short bowel. *J Clin Endocrinol Metab* 59, 139-141.
- Sugihara, M., Takeuchi, S., Sugita, M., Higaki, K., Kataoka, M., Yamashita, S., 2015. Analysis of Intra- and Intersubject Variability in Oral Drug Absorption in Human Bioequivalence Studies of 113 Generic Products. *Mol Pharm* 12, 4405-4413.
- Sunesen, V.H., Vedelsdal, R., Kristensen, H.G., Christrup, L., Müllertz, A., 2005. Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug. *Eur. J. Pharm. Sci.* 24, 297-303.
- Sutton, S.C., 2009. Role of Physiological Intestinal Water in Oral Absorption. *The AAPS Journal* 11, 277-285.
- Tacchino, R.M., 2015. Bowel length: measurement, predictors, and impact on bariatric and metabolic surgery. *Surg Obes Relat Dis* 11, 328-334.
- Taggart, A.J., McDermott, B.J., Roberts, S.D., 1992. The Effect of Age and Acetylator Phenotype on the Pharmacokinetics of Sulfasalazine in Patients with Rheumatoid Arthritis. *Clin. Pharmacokinet.* 23, 311-320.
- Takasuna, K., Hagiwara, T., Hirohashi, M., Kato, M., Nomura, M., Nagai, E., Yokoi, T., Kamataki, T., 1996. Involvement of β -Glucuronidase in Intestinal Microflora in the Intestinal Toxicity of the Antitumor Camptothecin Derivative Irinotecan Hydrochloride (CPT-11) in Rats. *Cancer Res.* 56, 3752.
- Tamargo, J., Rosano, G., Walther, T., Duarte, J., Niessner, A., Kaski, J.C., Ceconi, C., Drexel, H., Kjeldsen, K., Savarese, G., Torp-Pedersen, C., Atar, D., Lewis, B.S., Agewall, S., 2017. Gender differences in the effects of cardiovascular drugs. *Eur Heart J Cardiovasc Pharmacother* 3, 163-182.
- Tandra, S., Chalasani, N., Jones, D.R., Mattar, S., Hall, S.D., Vuppalanchi, R., 2013. Pharmacokinetic and pharmacodynamic alterations in the Roux-en-Y gastric bypass recipients. *Ann. Surg.*
- Tannergren, C., Bergendal, A., Lennernas, H., Abrahamsson, B., 2009. Toward an increased understanding of the barriers to colonic drug absorption in humans: implications for early controlled release candidate assessment. *Mol Pharm* 6, 60-73.
- Tappenden, K.A., 2014. Intestinal adaptation following resection. *Journal of Parenteral and Enteral Nutrition* 38, 23S-31S.
- Teitelbaum, E.N., Vaziri, K., Zettervall, S., Amdur, R.L., Orkin, B.A., 2013. Intraoperative small bowel length measurements and analysis of demographic predictors of increased length. *Clin Anat* 26, 827-832.
- Thabet, Y., Klingmann, V., Breitkreutz, J., 2018. Drug Formulations: Standards and Novel Strategies for Drug Administration in Pediatrics. *J Clin Pharmacol* 58 Suppl 10, S26-S35.
- Tian, L., Wang, X.W., Wu, A.K., Fan, Y., Friedman, J., Dahlin, A., Waldor, M.K., Weinstock, G.M., Weiss, S.T., Liu, Y.Y., 2020. Deciphering functional redundancy in the human microbiome. *Nat Commun* 11, 6217.

- Tomalik-Scharte, D., Doroshyenko, O., Kirchheiner, J., Jetter, A., Lazar, A., Klaassen, T., Frank, D., Wyen, C., Fätkenheuer, G., Fuhr, U., 2008. No role for the CYP3A5*3 polymorphism in intestinal and hepatic metabolism of midazolam. *European journal of clinical pharmacology* 64, 1033-1035.
- Trevaskis, N.L., Kaminskas, L.M., Porter, C.J., 2015. From sewer to saviour—targeting the lymphatic system to promote drug exposure and activity. *Nature Reviews Drug Discovery* 14, 781-803.
- Trevaskis, N.L., Lee, G., Escott, A., Phang, K.L., Hong, J., Cao, E., Katneni, K., Charman, S.A., Han, S., Charman, W.N., Phillips, A.R.J., Windsor, J.A., Porter, C.J.H., 2020. Intestinal Lymph Flow, and Lipid and Drug Transport Scale Allometrically From Pre-clinical Species to Humans. *Front Physiol* 11, 458.
- Trevaskis, N.L., Shackelford, D.M., Charman, W.N., Edwards, G.A., Gardin, A., Appel-Dingemanse, S., Kretz, O., Galli, B., Porter, C.J., 2009. Intestinal lymphatic transport enhances the post-prandial oral bioavailability of a novel cannabinoid receptor agonist via avoidance of first-pass metabolism. *Pharm. Res.* 26, 1486-1495.
- Trevaskis, N.L., Shanker, R.M., Charman, W.N., Porter, C.J., 2010. The mechanism of lymphatic access of two cholesteryl ester transfer protein inhibitors (CP524, 515 and CP532, 623) and evaluation of their impact on lymph lipoprotein profiles. *Pharmaceutical research* 27, 1949-1964.
- Trobec, K., Kerec Kos, M., von Haehling, S., Springer, J., Anker, S.D., Lainscak, M., 2013. Pharmacokinetics of drugs in cachectic patients: a systematic review. *PLoS One* 8, e79603.
- Trout, H., Mentre, F., Panhard, X., Kodjo, A., Escaut, L., Pernet, P., Gobert, J.G., Vittecoq, D., Knellwolf, A.L., Caulin, C., Bergmann, J.F., 2004. Enhanced saquinavir exposure in human immunodeficiency virus type 1-infected patients with diarrhea and/or wasting syndrome. *Antimicrob Agents Chemother* 48, 538-545.
- Ueno, T., Tanaka, A., Hamanaka, Y., Suzuki, T., 1995. Serum drug concentrations after oral administration of paracetamol to patients with surgical resection of the gastrointestinal tract. *Br J Clin Pharmacol* 39, 330-332.
- Valodara, A.M., Sr, K.J., 2019. Sexual Dimorphism in Drug Metabolism and Pharmacokinetics. *Curr Drug Metab* 20, 1154-1166.
- Van Den Abeele, J., Rayyan, M., Hoffman, I., Van de Vijver, E., Zhu, W., Augustijns, P., 2018. Gastric fluid composition in a paediatric population: Age-dependent changes relevant for gastrointestinal drug disposition. *Eur J Pharm Sci* 123, 301-311.
- van den Anker, J., Reed, M.D., Allegaert, K., Kearns, G.L., 2018. Developmental Changes in Pharmacokinetics and Pharmacodynamics. *J Clin Pharmacol* 58 Suppl 10, S10-S25.
- van Groen, B.D., Krekels, E.H.J., Mooij, M.G., van Duijn, E., Vaes, W.H.J., Windhorst, A.D., van Rosmalen, J., Hartman, S.J.F., Hendrikse, N.H., Koch, B.C.P., Allegaert, K., Tibboel, D., Knibbe, C.A.J., de Wildt, S.N., 2020. The Oral Bioavailability and Metabolism of Midazolam in Stable Critically Ill Children: A Pharmacokinetic Microtracing Study. *Clin Pharmacol Ther.*
- van Kessel, S.P., Frye, A.K., El-Gendy, A.O., Castejon, M., Keshavarzian, A., van Dijk, G., El Aidy, S., 2019. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nature communications* 10, 310-310.
- van Leeuwen, R.W., Peric, R., Hussaarts, K.G., Kienhuis, E., NS, I.J., de Bruijn, P., van der Leest, C., Codrington, H., Kloover, J.S., van der Holt, B., Aerts, J.G., van Gelder, T., Mathijssen, R.H., 2016. Influence of the Acidic Beverage Cola on the Absorption of Erlotinib in Patients With Non-Small-Cell Lung Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 34, 1309-1314.
- van Leeuwen, R.W.F., Jansman, F.G.A., Hunfeld, N.G., Peric, R., Reyners, A.K.L., Imholz, A.L.T., Brouwers, J., Aerts, J.G., van Gelder, T., Mathijssen, R.H.J., 2017. Tyrosine Kinase Inhibitors and Proton Pump Inhibitors: An Evaluation of Treatment Options. *Clin Pharmacokinet* 56, 683-688.
- Varma, M.V.S., Lai, Y., Kimoto, E., Goosen, T.C., El-Kattan, A.F., Kumar, V., 2013. Mechanistic Modeling to Predict the Transporter- and Enzyme-Mediated Drug-Drug Interactions of Repaglinide. *Pharm. Res.* 30, 1188-1199.
- Verhoeven, E., Vervaet, C., Remon, J.P., 2006. Xanthan gum to tailor drug release of sustained-release ethylcellulose mini-matrices prepared via hot-melt extrusion: in vitro and in vivo evaluation. *Eur J Pharm Biopharm* 63, 320-330.
- Vermeulen, M.A., Richir, M.C., Garretsen, M.K., van Schie, A., Ghatei, M.A., Holst, J.J., Heijboer, A.C., Uitdehaag, B.M., Diamant, M., Eekhoff, E.M., van Leeuwen, P.A., Ligthart-Melis, G.C., 2011. Gastric emptying, glucose metabolism and gut hormones: evaluation of a common preoperative carbohydrate beverage. *Nutrition* 27, 897-903.

- Vertzoni, M., Augustijns, P., Grimm, M., Koziolok, M., Lemmens, G., Parrott, N., Pentafragka, C., Reppas, C., Rubbens, J., Van Den Alphabeel, J., Vanuytsel, T., Weitschies, W., Wilson, C.G., 2019. Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: An UNGAP review. *Eur J Pharm Sci* 134, 153-175.
- Vertzoni, M., Carlsson, A., Abrahamsson, B., Goumas, K., Reppas, C., 2011. Degradation kinetics of metronidazole and olsalazine by bacteria in ascending colon and in feces of healthy adults. *Int J Pharm* 413, 81-86.
- Vertzoni, M., Diakidou, A., Chatziliadis, M., Soderlind, E., Abrahamsson, B., Dressman, J.B., Reppas, C., 2010. Biorelevant media to simulate fluids in the ascending colon of humans and their usefulness in predicting intracolonic drug solubility. *Pharm Res* 27, 2187-2196.
- Vertzoni, M., Koulouri, C., Poulou, A., Goumas, K., Reppas, C., 2020a. Exploring the impact of Crohn's disease on the intragastric environment of fasted adults. *ADMET and DMPK* 8, 122-128.
- Vertzoni, M., Sulaiman, S., Goumas, K., Kersten, E., Anlahr, J., Muenster, U., Reppas, C., 2020b. Characteristics of Contents of Lower intestine in the 65-74 Years of Age Range Could Impact the Performance of Safe and Efficacious Modified Release Products. *J. Pharm. Sci.*
- Viramontes, B.E., Camilleri, M., McKinzie, S., Pardi, D.S., Burton, D., Thomforde, G.M., 2001. Gender-related differences in slowing colonic transit by a 5-HT₃ antagonist in subjects with diarrhea-predominant irritable bowel syndrome. *Am J Gastroenterol* 96, 2671-2676.
- Vita, R., Saraceno, G., Trimarchi, F., Benvenega, S., 2014. Switching levothyroxine from the tablet to the oral solution formulation corrects the impaired absorption of levothyroxine induced by proton-pump inhibitors. *J Clin Endocrinol Metab* 99, 4481-4486.
- Wagener, S., Shankar, K.R., Turnock, R.R., Lamont, G.L., Baillie, C.T., 2004. Colonic transit time--what is normal? *J Pediatr Surg* 39, 166-169; discussion 166-169.
- Walravens, J., Brouwers, J., Spriet, I., Tack, J., Annaert, P., Augustijns, P., 2011. Effect of pH and comedication on gastrointestinal absorption of posaconazole: monitoring of intraluminal and plasma drug concentrations. *Clin Pharmacokinet* 50, 725-734.
- Walsh, J., Gheorghe, C.E., Lyte, J.M., van de Wouw, M., Boehme, M., Dinan, T.G., Cryan, J.F., Griffin, B.T., Clarke, G., Hyland, N.P., 2020. Gut microbiome-mediated modulation of hepatic cytochrome P450 and P-glycoprotein: impact of butyrate and fructo-oligosaccharide-inulin. *J. Pharm. Pharmacol.* n/a.
- Wang, Y., Tong, J., Chang, B., Wang, B., Zhang, D., Wang, B., 2014. Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. *Mol. Med. Report.* 9, 2352-2356.
- Ward, N., 2010. The impact of intestinal failure on oral drug absorption: a review. *J Gastrointest Surg* 14, 1045-1051.
- Watts, P.J., Barrow, L., Steed, K.P., Wilson, C.G., Spiller, R.C., Melia, C.D., Davies, M.C., 1992. The transit rate of different-sized model dosage forms through the human colon and the effects of a lactulose-induced catharsis. *Int. J. Pharm.* 87, 215-221.
- Wauters, L., Vanuytsel, T., 2018. Applications of peptide hormone ligands for the treatment of dumping and short bowel syndrome. *Curr Opin Pharmacol* 43, 118-123.
- Weitschies, W., Blume, H., Monnikes, H., 2010. Magnetic marker monitoring: high resolution real-time tracking of oral solid dosage forms in the gastrointestinal tract. *Eur J Pharm Biopharm* 74, 93-101.
- Weitschies, W., Cardini, D., Karaus, M., Trahms, L., Semmler, W., 1999. Magnetic marker monitoring of esophageal, gastric and duodenal transit of non-disintegrating capsules. *Pharmazie* 54, 426-430.
- Weitschies, W., Friedrich, C., Wedemeyer, R.S., Schmidtman, M., Kosch, O., Kinzig, M., Trahms, L., Sorgel, F., Siegmund, W., Horkovics-Kovats, S., Schwarz, F., Raneburger, J., Monnikes, H., 2008. Bioavailability of amoxicillin and clavulanic acid from extended release tablets depends on intragastric tablet deposition and gastric emptying. *Eur J Pharm Biopharm* 70, 641-648.
- Weitschies, W., Kosch, O., Monnikes, H., Trahms, L., 2005. Magnetic Marker Monitoring: An application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behavior of magnetically marked solid dosage forms. *Adv Drug Deliv Rev* 57, 1210-1222.
- White, K.L., Nguyen, G., Charman, W.N., Edwards, G.A., Faassen, W.F., Porter, C.J., 2009. Lymphatic transport of methyltestosterone undecanoate (MU) and the bioavailability of methyltestosterone are highly sensitive to the mass of coadministered lipid after oral administration of MU. *J. Pharmacol. Exp. Ther.* 331, 700-709.

- Wilding, I.R., Hardy, J.G., Sparrow, R.A., Davis, S.S., Daly, P.B., English, J.R., 1992. In Vivo Evaluation of Enteric-Coated Naproxen Tablets Using Gamma Scintigraphy. *Pharm. Res.* 9, 1436-1441.
- Williams, H.D., Trevaskis, N.L., Charman, S.A., Shanker, R.M., Charman, W.N., Pouton, C.W., Porter, C.J., 2013. Strategies to address low drug solubility in discovery and development. *Pharmacol Rev* 65, 315-499.
- Willmann, S., Edginton, A.N., Kleine-Besten, M., Jantrtid, E., Thelen, K., Dressman, J.B., 2009. Whole-body physiologically based pharmacokinetic population modelling of oral drug administration: inter-individual variability of cimetidine absorption. *J Pharm Pharmacol* 61, 891-899.
- Wilson, C.G., O'Mahony, B., Connolly, S.M., Cantarini, M.V., Farmer, M.R., Dickinson, P.A., Smith, R.P., Swaisland, H.C., 2009. Do gastrointestinal transit parameters influence the pharmacokinetics of gefitinib? *Int J Pharm* 376, 7-12.
- Wilson, C.G., Washington, N., 1988. Assessment of Disintegration and Dissolution of Dosage Forms In Vivo Using Gamma Scintigraphy. *Drug Dev. Ind. Pharm.* 14, 211-281.
- Wilson, I.D., Nicholson, J.K., 2017. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. *Transl Res* 179, 204-222.
- Wong, G.G., Ha, V., Chu, M.P., Dersch-Mills, D., Ghosh, S., Chambers, C.R., Sawyer, M.B., 2019. Effects of Proton Pump Inhibitors on FOLFOX and CapeOx Regimens in Colorectal Cancer. *Clinical colorectal cancer* 18, 72-79.
- World Health, O., 2016. WHO Obesity and Overweight, Fact Sheet No 311. Obesity and Overweight Fact Sheet, 4--4.
- Wuyts, B., Brouwers, J., Mols, R., Tack, J., Annaert, P., Augustijns, P., 2013. Solubility profiling of HIV protease inhibitors in human intestinal fluids. *J Pharm Sci* 102, 3800-3807.
- Wuyts, B., Riethorst, D., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2015a. Evaluation of fasted and fed state simulated and human intestinal fluids as solvent system in the Ussing chambers model to explore food effects on intestinal permeability. *Int J Pharm* 478, 736-744.
- Wuyts, B., Riethorst, D., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2015b. Evaluation of fasted state human intestinal fluid as apical solvent system in the Caco-2 absorption model and comparison with FaSSiF. *Eur J Pharm Sci* 67, 126-135.
- Xiao, C., Bandsma, R.H., Dash, S., Szeto, L., Lewis, G.F., 2012. Exenatide, a glucagon-like peptide-1 receptor agonist, acutely inhibits intestinal lipoprotein production in healthy humans. *Arterioscler. Thromb. Vasc. Biol.* 32, 1513-1519.
- Xiao, C., Dash, S., Morgantini, C., Lewis, G.F., 2013. Novel role of enteral monosaccharides in intestinal lipoprotein production in healthy humans. *Arterioscler. Thromb. Vasc. Biol.* 33, 1056-1062.
- Xu, J., Lin, Y., Boulas, P., Peterson, M.L., 2018. Low colonic absorption drugs: risks and opportunities in the development of oral extended release products. *Exp Opin Drug Deliv* 15, 197-211.
- Xu, L., Nagasawa, N., Yoshii, F., Kume, T., 2003. Syntheses of hydroxypropyl methylcellulose phthalate hydrogels in Na₂CO₃ aqueous solutions with electron-beam irradiation. *Journal of Applied Polymer Science* 89, 2123-2130.
- Yadav, V., Gaisford, S., Merchant, H.A., Basit, A.W., 2013. Colonic bacterial metabolism of corticosteroids. *Int J Pharm* 457, 268-274.
- Yago, M.R., Frymoyer, A., Benet, L.Z., Smelick, G.S., Frassetto, L.A., Ding, X., Dean, B., Salphati, L., Budha, N., Jin, J.Y., Dresser, M.J., Ware, J.A., 2014. The use of betaine HCl to enhance dasatinib absorption in healthy volunteers with rabeprazole-induced hypochlorhydria. *AAPS J* 16, 1358-1365.
- Yeung, C.H.T., Fong, S., Malik, P.R.V., Edginton, A.N., 2020. Quantifying breast milk intake by term and preterm infants for input into paediatric physiologically based pharmacokinetic models. *Matern Child Nutr* 16, e12938.
- Yu, A., Jackson, T., Tsume, Y., Koenigsnecht, M., Wysocki, J., Marciani, L., Amidon, G.L., Frances, A., Baker, J.R., Hasler, W., Wen, B., Pai, A., Sun, D., 2017. Mechanistic Fluid Transport Model to Estimate Gastrointestinal Fluid Volume and Its Dynamic Change Over Time. *The AAPS Journal* 19, 1682-1690.
- Yuan, T., Wang, J., Chen, L., Shan, J., Di, L., 2020. Lactobacillus murinus Improved the Bioavailability of Orally Administered Glycyrrhizic Acid in Rats. *Front Microbiol* 11, 597.
- Yucel, E., Sancar, M., Yucel, A., Okuyan, B., 2016. Adverse drug reactions due to drug-drug interactions with proton pump inhibitors: assessment of systematic reviews with AMSTAR method. *Expert opinion on drug safety* 15, 223-236.

- Yuen, K.H., 2010. The transit of dosage forms through the small intestine. *Int J Pharm* 395, 9-16.
- Zawieja, S.D., Wang, W., Wu, X., Nepiyushchikh, Z.V., Zawieja, D.C., Muthuchamy, M., 2012. Impairments in the intrinsic contractility of mesenteric collecting lymphatics in a rat model of metabolic syndrome. *Am J Physiol Heart Circ Physiol* 302, H643-653.
- Zhang, F., Zarkada, G., Han, J., Li, J., Dubrac, A., Ola, R., Genet, G., Boye, K., Michon, P., Kunzel, S.E., Camporez, J.P., Singh, A.K., Fong, G.H., Simons, M., Tso, P., Fernandez-Hernando, C., Shulman, G.I., Sessa, W.C., Eichmann, A., 2018. Lacteal junction zippering protects against diet-induced obesity. *Science* 361, 599-603.
- Zhang, X., Wu, W., 2014. Ligand-mediated active targeting for enhanced oral absorption. *Drug Discov. Today* 19, 898-904.
- Zhou, J., Quinlan, M., Glenn, K., Boss, H., Picard, F., Castro, H., Sellami, D., 2016. Effect of esomeprazole, a proton pump inhibitor on the pharmacokinetics of sonidegib in healthy volunteers. *Br J Clin Pharmacol* 82, 1022-1029.
- Zhou, Q., Chen, Q.X., Ruan, Z.R., Yuan, H., Xu, H.M., Zeng, S., 2013. CYP2C9*3(1075A > C), ABCB1 and SLCO1B1 genetic polymorphisms and gender are determinants of inter-subject variability in pitavastatin pharmacokinetics. *Pharmazie* 68, 187-194.
- Zhou, Z., Dunn, C., Khadra, I., Wilson, C.G., Halbert, G.W., 2017. Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs. *Eur J Pharm Sci* 99, 95-104.
- Zhu, H., Xu, G., Zhang, K., Kong, X., Han, R., Zhou, J., Ni, Y., 2016. Crystal structure of tyrosine decarboxylase and identification of key residues involved in conformational swing and substrate binding. *Sci. Rep.* 6, 27779-27779.
- Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., Goodman, A.L., 2019a. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature* 570, 462-467.
- Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., Goodman, A.L., 2019b. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. *Science* 363, eaat9931.
- Zimmermann, T., Laufen, H., Yeates, R., Scharpf, F., Riedel, K.D., Schumacher, T., 1999. The pharmacokinetics of extended-release formulations of calcium antagonists and of amlodipine in subjects with different gastrointestinal transit times. *J Clin Pharmacol* 39, 1021-1031.
- Zvada, S.P., Van Der Walt, J.S., Smith, P.J., Fourie, P.B., Roscigno, G., Mitchison, D., Simonsson, U.S., McIlleron, H.M., 2010. Effects of four different meal types on the population pharmacokinetics of single-dose rifapentine in healthy male volunteers. *Antimicrob Agents Chemother* 54, 3390-3394.