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## Current challenges and future perspectives in oral absorption research: An opinion of the UNGAP network



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## ABSTRACT

Although oral drug delivery is the preferred administration route and has been used for centuries, modern drug discovery and development pipelines challenge conventional formulation approaches and highlight the insufficient mechanistic understanding of processes critical to oral drug absorption. This review presents the opinion of UNGAP scientists on four key themes across the oral absorption landscape: (1) specific patient populations, (2) regional differences in the gastrointestinal tract, (3) advanced formulations and (4) food-drug interactions. The differences of oral absorption in pediatric and geriatric populations, the specific issues in colonic absorption, the formulation approaches for poorly water-soluble (small molecules) and poorly permeable (peptides, RNA etc.) drugs, as well as the vast realm of food effects, are some of the topics discussed in detail. The identified controversies and gaps in the current understanding of gastrointestinal absorption-related processes are used to create a roadmap for the future of oral drug absorption research.

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## Contents

1.	Introduction . . . . .	291
2.	Specific patient populations . . . . .	291
2.1.	Introduction and scope . . . . .	291
2.2.	Pediatrics and geriatrics . . . . .	292
2.2.1.	The role of GIT fluid volumes in pediatric populations . . . . .	292
2.2.2.	Ontogeny of intestinal drug transport and metabolism in pediatric populations . . . . .	293
2.2.3.	Oral drug absorption in infants including food effects . . . . .	293
2.2.4.	Biorelevant dissolution testing and <i>in silico</i> modeling to predict pharmacokinetics in neonates and pediatrics . . . . .	294
2.2.5.	Oral drug absorption in older people and geriatric patients . . . . .	294
2.3.	Disease-specific populations . . . . .	295
2.3.1.	Cardiovascular, renal and metabolism diseases . . . . .	295
2.3.2.	Inflammatory bowel disease . . . . .	295
2.3.3.	COVID-19 . . . . .	296
3.	Regional differences . . . . .	296
3.1.	Introduction and scope . . . . .	296
3.2.	Targeting of oral drugs to the lower intestine and colonic drug absorption . . . . .	296
3.3.	Prediction of colonic absorption and physiologically based pharmacokinetic models . . . . .	297
3.4.	Intestinal drug-metabolizing enzymes . . . . .	298
3.5.	The role of lymphatic transport in oral drug delivery . . . . .	299
4.	Advanced formulations . . . . .	300
4.1.	Introduction and scope . . . . .	300
4.2.	Poorly water-soluble drugs . . . . .	300
4.2.1.	Understanding lipid-based drug delivery systems for increasing absorption and reducing food effects . . . . .	300
4.2.2.	Understanding the <i>in vivo</i> performance of amorphous solid dispersions . . . . .	301
4.3.	Biologicals . . . . .	303
4.3.1.	Introduction and scope . . . . .	303
4.3.2.	Oral delivery of therapeutic nucleic acids by non-viral methods . . . . .	303
4.3.3.	Oral peptide delivery . . . . .	304
5.	<i>In vitro</i> and <i>in silico</i> tools for exploring advanced drug formulations . . . . .	307
5.1.	Introduction and scope . . . . .	307
5.2.	Biorelevant dissolution testing . . . . .	307
5.3.	Permeation models . . . . .	309
5.4.	Combined dissolution-permeation models . . . . .	309
5.5.	Novel real-time analytics for supersaturating formulations . . . . .	310
5.6.	Combining <i>in vitro</i> , <i>in silico</i> and <i>in vivo</i> data: ambitions for prediction of oral product performance . . . . .	311

**Abbreviations list:** A/V, Absorption surface to donor volume ratio; ACE2, Angiotensin converting enzyme 2; AhR, Aryl hydrocarbon receptor; AMI, Artificial membrane insert; ASD, Amorphous solid dispersions; BCS, Biopharmaceutics classification system; BE, Bioequivalence; CAR, Constitutive androstane receptor; CD, Crohn's disease;  $C_{max}$ , Maximum plasma concentration; COVID-19, Coronavirus disease 2019;  $C_p$ , Plasma concentration; CPP, Cell penetrating peptides; CVRM, Cardiovascular, renal and metabolism diseases; CYP, Cytochrome P450 enzymes; DDI, Drug-drug interactions; DGM, Dynamic gastric model; DLin-MC3-DMA, Heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate; DWS, Diffusing wave spectroscopy; EMA, European medicines agency; ER, Extended release; ESR, Early stage researcher; FDA, U.S. Food and drug administration; FTIR, Fourier transform infrared; GalNac, N-acetylgalactosamine; GIT, Gastrointestinal tract; GLP-1, Glucagon-like peptide 1; GRAS, Generally recognized as safe; HDV, Hepatic-directed vesicle; HHP, Hydrophobic hydrogen bond pairs; HIP, Hydrophobic ionic pairs; HIV, Human immunodeficiency virus; IBD, Inflammatory bowel disease; IVIVR, *In vitro-in vivo* relation; LbDDS, Lipid-based drug delivery systems; LLC-PK, Lilly laboratories culture porcine kidney cells; LNP, Lipid nanoparticles; LUMI, Luminal unfolding microneedle injector; MDCK, Madin-Darby Canine Kidney cells; MRI, Magnetic resonance imaging; NA, Nucleic acids; NLC, Nanostructured lipid carriers; NMR, Nuclear magnetic resonance; PAMPA, Parallel Artificial Membrane Permeability Assay; PAT-1, Proton-assisted small amino acid transporter; PBPK, Physiologically-based pharmacokinetic model; PD, Pharmacodynamics; PK, Pharmacokinetics; PSA, Parameter sensitivity analysis; PVDF, Polyvinylidene fluoride; PVPA, Phospholipid vesicle-based permeation assay; PWSDD, Poorly water-soluble drugs; PXR, Pregnane X receptor; RAS, Renin-angiotensin system; RBA, Relative bioavailability; RNA, Ribonucleic acid; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SAXS, Small-angle X-ray scattering; SLN, Solid lipid nanoparticles; SNAC, N-[8-(2-hydroxybenzoyl) amino] caprylate; SNEDDS, Self-nanoemulsifying drug delivery systems; SNP, Single nucleotide polymorphism; SOMA, Self-orienting millimeter-scale applicator; SULT, Sulfotransferase; TDC, Tyrosine decarboxylases; TIM, TNO (gastro-) intestinal model; TPE, Transient permeation enhancer; TPGS, Tocopheryl polyethylene glycol succinate; UC, Ulcerative colitis; UGT, Uridine 5'-diphospho-glucuronosyltransferase; UNGAP, European network on understanding gastrointestinal absorption-related processes; USP, United States Pharmacopoeia; UV, Ultraviolet; WG, Working group.

5.7.	Biopharmaceutics modeling and simulation: limitations and strengths . . . . .	312
5.7.1.	Limitations and threats in current PBPK modeling approaches . . . . .	312
5.7.2.	Progress in PBPK modeling for regulatory questions . . . . .	313
6.	Food-drug interactions . . . . .	313
6.1.	Introduction and scope . . . . .	313
6.2.	Administration of drugs with food as a strategy to increase oral bioavailability . . . . .	313
6.3.	Application of physiologically based models to predict food effects: current status, strengths and limitations, next steps . . . . .	315
6.4.	Opinions from other COST actions. . . . .	316
6.4.1.	Food-drug interactions: a perspective from the INFOGEST network. . . . .	316
6.4.2.	Absorption of carotenoids: a perspective from the Eurocaroten consortium . . . . .	316
6.5.	Exploring the impact of colon-fibre interactions on health and drug absorption: historical perspective and recent results . . . . .	317
6.6.	The role of the intestinal microbiome . . . . .	317
7.	Future outlook. . . . .	319
8.	Concluding remarks . . . . .	320
	Declaration of Competing Interest . . . . .	321
	Acknowledgements . . . . .	321
	References . . . . .	321

## 1. Introduction

Oral absorption is the cornerstone of oral drug delivery, which is the most convenient and widely used administration route. Decades of research have considerably advanced understanding of the critical anatomical, physiological, and drug formulation factors that control oral bioavailability, as recently reviewed [1–5]. This better understanding of physiological function and formulation functionality has been used in the design of better oral medicines: improved controlled-release drug delivery vehicles, enabling technologies for drugs with poor biopharmaceutical properties and tailored approaches for special populations have been developed.

However, the simultaneous impact of several drivers has changed the oral absorption landscape considerably in the last years. The realization that peptides, proteins and nucleic acids can be potent medicinal agents has now split modern drug discovery pipelines in two separate streams: classical, small-molecule therapeutics [5] and high molecular weight biologicals [4,6,7]. These two directions in the development of therapeutic entities have strikingly different (even completely opposite) physicochemical and biopharmaceutical properties, which pose specific challenges to their oral delivery.

The selection of highly potent small molecules during drug discovery has been seen as a cause of the extremely poor solubility of many new drug candidates that emerge from modern drug development pipelines [8–10]. As a result, the oral absorption of such compounds is frequently solubility or dissolution rate-limited.

In contrast, biologicals are usually characterized by sufficient solubility, but very poor intestinal permeability [4,6,7]. In addition, they face considerable stability challenges in the enzyme-rich intestinal environment. This paradigm shift in drug development has created a strong driving force for the development of biopredictive *in silico* and *in vitro* tools to support both academia and the pharmaceutical industry, as well as aid regulatory decisions [11–17]. At the same time, new discoveries in human physiology (e.g. intestinal fluid pockets, extent of variability) illustrate the possible complexities of oral absorption, and raise questions about the validity of the *in vitro* methods currently used in the pharmaceutical community [18–22].

The outlined challenges in oral drug delivery inspired the creation of the European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP, [www.ungap.eu](http://www.ungap.eu)), which aims to improve the understanding of intestinal drug absorption by creating a multidisciplinary network of researchers from academia and industry. The network consists of 500+ members from 32 countries and four working groups (WGs) across key themes of oral drug absorption: special populations (WG1), regional differences (WG2), advanced formulations (WG3) and food-drug interactions (WG4).

Therefore, the aim of this paper is not to provide a comprehensive review of the state-of-the-art, but instead to present the opinion of UNGAP scientists on specific controversial topics, identify critical knowledge gaps and provide an outlook on future research activities. The paper is largely structured according to the four main WGs in UNGAP, with the addition of a special methodology section focused on *in vitro* and *in silico* tools.

## 2. Specific patient populations

### 2.1. Introduction and scope

The rate and extent of intestinal absorption are key properties to determine the bioavailability of orally dosed drugs. In pharmaceutical development, the prediction of these properties plays an important role in the design and testing of new drugs and a combination of *in vitro* and *in silico* tools is typically used for this purpose. Biorelevant tools, including artificial gastrointestinal tract (GIT) fluids [23–27] and physiologically-based biopharmaceutical models [17,28], have been developed and validated based on the extensively characterized GIT physiology in healthy adults. First-in-human trials are also usually conducted in healthy adult volunteers to provide information about the pharmacokinetic (PK) properties of a compound. However, these conditions are often not representative for the GIT in a specific patient population, in which oral drug absorption (in addition to other PK properties) is often significantly different to the “average” patient [1,29–31].

Changes in GIT physiology and oral drug absorption can be triggered by local diseases in the GIT (e.g., inflammatory bowel disease (IBD), infectious diseases, celiac disease, cancer) [1,29,32–34] or systemic diseases (e.g., Parkinson's disease, diabetes, HIV, critical illness) [1,31,35–37]. In addition to the disease-dependent factors, differences in GIT physiology have been described for non-disease dependent conditions such as age, ethnicity, sex, or diet [1,38–41]. These population-specific GIT conditions and their impact on absorption are still poorly characterized [1]. Amongst the main reasons are the ethical constraints associated with the conduct of research in populations including children or critically-ill patients. Secondly, specific patient populations are often very heterogeneous in terms of severity of the disease, co-medications, co-morbidities, or ontogeny. The characterization of a “standard patient population” for specific diseases thus becomes very challenging. Finally, there is poor availability of GIT physiology and clinical PK data for specific patient populations. These knowledge gaps result in substantial limitations in the development and validation of *in vitro* and *in silico* tools representative of special populations, which is a critical gap in the development of new medicines [42,43]. It may

lead to over- or under-estimation of oral drug absorption in these patients resulting in potential safety and/or efficacy issues for a given drug.

The generation and availability of physiology reference data will underpin the development of advanced tools representative of specific populations. Such innovative tools will require extensive validation to build confidence in their predictive power and, for this purpose, the availability of clinical PK data in healthy vs. diseased patients is critical. Such research at the interface between (bio-)pharmaceutics and clinical practice requires close cross-functional collaboration between pharmaceutical scientists, clinical pharmacologists, and medical practitioners from academia, hospitals and the pharmaceutical industry to leverage the potential of diagnostic or medical procedures (e.g. magnetic resonance imaging (MRI), surgical interventions) to gain insights into the physiology of specific patient populations whilst minimizing the burden to individual patients.

The opinion of the UNGAP network about the current state, challenges and opportunities in selected hot-topics across the broad theme of specific patient populations is presented in the following sections, starting with age-segregated populations (pediatrics and geriatrics) and populations suffering from specific diseases (cardiovascular, renal and metabolism diseases; IBD and COVID19). The main gaps and the proposed way forward in the oral absorption research for age-segregated and disease-specific populations are summarized in Figs. 1 and 2, respectively.

## 2.2. Pediatrics and geriatrics

The following paragraphs will focus on specific aspects of oral absorption in pediatric populations, such as the role of GIT fluid volumes, intestinal drug transport and metabolism, food effects, as well as the current state of pediatric *in vitro* and *in silico* tools. At the end of the section, the largely unexplored field of oral absorption in geriatric patients will be introduced.

### 2.2.1. The role of GIT fluid volumes in pediatric populations

Disintegration and dissolution of orally administered solid dosage forms is critical prior to absorption of a drug. The volume and localization of fluid within the GIT will influence the relative rates of disintegration, dissolution and ultimately absorption. Thus knowledge of the relevant volumes (and their distribution) in pediatric populations is important to predict the absorption and design appropriate *in vitro* dissolution apparatus; assign age-appropriate biopharmaceutics classification system (BCS) to drugs and to better understand some of the variability associated with PK profiles.

A very useful, non-invasive technique to visualize and measure the small bowel water content, which has been established in the clinical

management of enteric disorders and bowel motility, is MRI [44]. Existing MRI data suggests that gastric and small intestinal fluid volumes in children are much lower than in adults [21].

The colon is another attractive site for drug delivery of both locally acting drugs and modified release formulations. It is widely appreciated that fluid volumes there are low, as the function of the colon is to recover water, whereby the luminal contents become progressively drier as they move through the colon. The data on available fluid in the colon is scarce, with only two studies having been reported for adults [20,45] and there are no data reported on fluid within the colon of pediatric patients. A better understanding of the colonic environment, particularly the fluid volume, location and motion, would facilitate the design of appropriate *in vitro* and *in silico* tests to predict the performance and variability associated with local or modified release formulations in pediatric populations.

Unfortunately, there are many knowledge gaps in the area of pediatric GIT volumes. Fundamentally, the very limited data currently available has come from routine clinical MRI scans and thus might not represent the norms for a “healthy” population. The data has come from the extremes of fasted and fluid fed children, which are not representative of the typical patient [21] and are likely to show extensive variability. The existing data on GIT fluid volumes in children is insufficiently robust to fully represent the variability associated with fluid volumes that contributes to the overall variability observed in PK bioequivalence (BE) studies.

In addition, MRI methodology is limited to visualization of bulk fluids and the resolution of images does not currently allow fluid volumes at the absorptive surface to be quantified. Thus despite fluid being evident in MRI as present in temporary pockets, there is likely to be a continuous fluid layer at the absorptive surface and the mixing between the bulk and this layer is unknown.

Advances in MRI including the use of dynamic and filmed measurements will facilitate non-invasive measurement of intestinal motility, GIT transit times, as well as volumes present within the stomach, small intestine and colon [46]. Application of these minimally invasive tools to a pediatric population would enable the design of predictive *in vitro* and *in silico* tools, whilst minimizing the burden of clinical testing in pediatric populations. However, due to the ethical burdens associated with the conduct of clinical testing in pediatric populations, there is a need to mine existing data sets and to use advanced *in silico* models to elucidate the likely variability and consequences of diverse fluid volumes. If these were judged sufficient to justify a clinical investigation, then the rationale for the development of predictive pediatric absorption tools is provided, ultimately reducing clinical testing whilst improving therapy.

The impact of disease in the pediatric population should also be considered. A large number of children suffer at some point with GIT issues,

## Pediatrics and geriatrics

### Gaps

*In vitro* and *in silico* tools predominantly based on adult physiology data

Compendial methods are not biorelevant

GI aspects specific to paediatrics and the elderly (e.g. physiology, polypharmacy) are overlooked

### Way forward

GI fluid volumes and composition (fasted vs. fed state) need to be determined

Intestinal drug-metabolizing enzymes and drug transporters must be further characterized

Quality clinical PK data must be generated

*In vitro* and *in silico* models must be further developed and verified (based on above)

Fig. 1. Pediatrics and geriatrics summary.

such as diarrhea and constipation, yet the impact of these GIT disorders on GIT fluid volumes and subsequent oral drug absorption is currently unknown. A better understanding of the difference in GIT fluid present in children with these disorders would provide understanding on the conditions under which to administer medicine. For instance, should there be greater emphasis on administration with water in a constipated child? Furthermore, differences in malnourished compared to healthy pediatric populations have been reviewed [47] where there are likely to be large differences in drug absorption; thus knowledge of fluid volumes and localization can provide insights into administration or dosing adjustments.

An ideal dataset would provide real time visualization of fluid volumes paired with PK data; an example of such a dataset has only recently been generated for adults [48], so it is unlikely to be available from a pediatric population. In spite of this limitation, we should not simply exclude children from clinical testing due to ethical complexities, as it is essential that pediatric populations gain access to appropriate medicines. Therefore, excluding them from trials is ethically questionable.

### 2.2.2. Ontogeny of intestinal drug transport and metabolism in pediatric populations

A survey of knowledge on intestinal drug metabolism and transport reveals large information gaps for children as patients compared to adults [1]. Consequently, dose predictions of orally administered drugs, based on system information of oral absorption processes are met with uncertainty. This was illustrated by a recently developed pediatric absorption physiologically-based pharmacokinetic model (PBPK) [41]. At the same time, limited data from *ex vivo* studies on intestinal and hepatic CYP3A, as well as oral bioavailability PK data of midazolam (as marker of CYP3A) suggest a different intestinal vs hepatic maturation pattern than suggested by a physiology-based population PK model [49,50].

Moreover, the newest methodology to determine protein abundance, *i.e.* LC-MS proteomics, shows large inter-lab variability and hence, results from pediatric proteomics studies addressing drug-metabolizing enzymes and drug transporter ontogeny should be used carefully, and/or validated in other cohorts and laboratories [51].

Therefore, while some ontogeny data from drug-metabolizing enzymes and drug transport studies is available, future studies covering the whole pediatric age range, elucidating protein abundance and activity, using proteomics and Ussing methodology are highly desirable [52].

As discussed in a recent White Paper, variability in proteomics results can potentially be explained by differences in sample collection *e.g.* fresh, snap-frozen or formalin-fixed tissue, postsurgical or postmortem tissue [51]. Moreover, as drug-metabolizing enzymes and drug transport expression changes along the intestinal tract and different surgical techniques are used to collect tissue (*e.g.* biopsy vs. whole tissue) this further contributes to variation. On a more analytical level cell fractions used (*e.g.* membrane fractions vs cell lysates) and measurement techniques further introduce variability (*e.g.* global vs. targeted proteomics). Most of these issues are not specific for pediatrics, but apply to proteomic studies in general [51]. However, differences in underlying disease, medication use, access to specific intestinal areas and sample size limitations in neonates/young infants may contribute to variability in proteomics results between children and adults, which reflect 'age-related' differences in methodology, rather than true age-related variation in protein expression.

Despite the development of innovative techniques like proteomics, one of the main roadblocks remains the scarcity of pediatric intestinal tissue. Innovative methodologies should be developed to overcome this limitation, *e.g.* intestinal derived organoids [53]. A method for growing functional intestinal organoids (enteroids) from human intestine has recently been developed [54].

Even biopsy size tissue samples can be used to grow these enteroids, which can not only be used over many weeks, but can also be cryopreserved for use at a later stage. Whilst the original intestinal enteroids grow in 3D conformation with the intestinal lumen inside, they can be converted to 2D tissue layers, which can be used to study drug transport and metabolism [55]. To be able to use organoids to elucidate maturation of drug-metabolizing enzymes and drug transporters, it is of paramount importance to understand if and how these organoids mature with increasing passages.

Finally, while the combination of *ex vivo* methodology with PBPK modeling is a strong tool to predict oral drug disposition in children, validation of these models using PK or phenotyping studies is needed.

Collaborative research is required to close the information gap on drug-metabolizing enzymes and drug transporters protein expression and activity, potentially combining, usually limited sample sets, to cover the neonatal (0–30 days of age) and the rest of the pediatric life age range. When using proteomics to determine drug-metabolizing enzymes and drug transporters expression, the challenges associated with inter-laboratory variability should be acknowledged and reporting should reflect how challenges were addressed. The lack of pediatric tissue may be overcome by the use of intestinal epitheloid organoids, provided their age-specific properties are elucidated. Validation of results from *ex vivo* studies *in vivo*, including PBPK models based on this information, should be performed, potentially using innovative methodology for phenotyping such as liquid biopsies for drug-metabolizing enzymes and drug transporter genes, as well as microdosing [56,57].

### 2.2.3. Oral drug absorption in infants including food effects

Oral delivery is the usual route for drug administration to infants (1 month–2 years). Understanding drug and drug formulation performance in relation to the prandial conditions is essential for ensuring safety and efficacy of products to be administered to pediatric patients, especially infants whose diet has limited variation (100% milk in infants < 5 months). Whether differences in the formulation design and/or dosing (including the co-administered food) substantially impacts pediatric PK is likely drug and formulation dependent, with some drugs/formulations being at a higher risk of significant changes than others [58,59].

Based on a recent draft guidance issued by the U.S. Food and Drug Administration (FDA), when the same to-be-marketed formulation that is approved for use in adults is approved for use in a pediatric population, a separate food effect study is not necessary and the same may also apply in case the pediatric formulation is very similar to the adult formulation and has been approved based on *in vitro* dissolution tests [60]. Although this may be a practical approach, based on nine datasets from food effect studies (seven drugs) in adults vs infants and young children reported in the literature [61,62], only one study highlighted a similar food effect in infants and in adults [62], as highlighted by Stelova et al. [40].

The same draft guidance suggests that when the pediatric formulation is not similar to the adult formulation, food effects could be evaluated in adults. This would be achieved by using foods commonly consumed with drugs by pediatric patients (*e.g.* formula for infants, apple sauce for toddlers) and extrapolating the results to the pediatric population [60].

However, it has been shown by Stelova and colleagues that food effects on drug absorption in infants may not be adequately evaluated from the data collected as suggested by regulatory agencies for adult drug products, even for drugs with non-problematic absorption, no intestinal permeability limitations, highly soluble in the small intestine, no documented intraluminal interactions with food components and administered in simple dosage forms [63]. The investigated dosing conditions included administration of pediatric drug formulations under fasted conditions, fed conditions as proposed by current regulatory guidelines for adults (30 min after the start of the consumption of the

reference meal) and conditions mimicking dosing in infants where the drug formulation was administered during infant formula consumption, *i.e.* infant-formula fed conditions. That study revealed reduced early exposure of paracetamol and ibuprofen after administration under infant fed conditions, compared to the administration under the fasted or reference meal fed conditions in adults. In addition, using PBPK modeling, successful predictions of observed paracetamol plasma concentration ( $C_p$ ) levels in infants were achieved when extrapolating from fasted conditions and infant-fed conditions adult data whereas data collected following the consumption of the FDA breakfast appeared to be less useful [63].

Clinical studies (especially, well-controlled clinical studies) in infants are limited, due to ethical concerns. Difficulties in recruitment are reflected by the limited number of food effect studies in infants published to date. The determination of gastric emptying rates and luminal composition (including the pH) in infants is challenging, due to frequent meal administration, therefore, food-related physiological responses in infants are difficult to evaluate [40,64]. Collaboration with pediatricians to collect more data *i.e.* aspirates from intubated infants in order to determine key physiological characteristics of the GIT relevant to oral drug absorption and/or plasma data collected under well-defined conditions is of paramount importance.

Relevant advances will be useful in order to better design food effect studies in adults for the evaluation of pediatric products and will improve *in vitro* methodologies used to evaluate GIT transit and intraluminal dosage form performance in infants. In addition, relevant information will be useful to the development of *in silico* methodologies for evaluating the PK of oral drug products in infants [63,65].

#### 2.2.4. Biorelevant dissolution testing and *in silico* modeling to predict pharmacokinetics in neonates and pediatrics

Although significant progress has been made in recent years to understand and model how oral formulations behave in neonates and pediatrics [40,58,66–71], drug absorption in these populations remains difficult to study and poorly characterized. This limits the ability to (1) confidently extrapolate formulation performance from adult to pediatric/neonate, (2) identify whether changing how a formulation is administered alters performance and (3) meaningfully de-risk BE studies for pediatric formulations.

Of the various pediatric sub-groups, neonates present the biggest challenge, as controlled *in vivo* studies in this age group can be especially difficult (or even impossible) to perform, and because this sub-group is most differentiated from adults.

Attempts have been made to develop *in vitro* dissolution tests for predicting pediatric formulation behavior [40,59,71], including the impact of co-administered food [67,72] to predict *in vivo* performance, but the tools currently available still need more development and validation. The use of compendial *in vitro* methods for pediatric formulations has been advocated and explored, as these have the advantage of relative simplicity, but in practice, it is difficult to build in the appropriate levels of biorelevance required [1].

Flow through methods (United States Pharmacopoeia (USP) IV apparatus) and scaled down versions of compendial methods have been proposed as apparatus more suitable for examining pediatric formulations [40] mainly due to the ability to use smaller volumes, but these methods still cannot mimic other key aspects relevant to pediatric formulation performance, such as gut motility, dynamic fluid movement, the impact of co-administered food digestion, *etc.*

Therefore, a different approach is needed. The use of complex non-compendial *in vitro* tools, capable of mimicking volumes, dynamics, meals and their digestion, in both adults and in different aged children under different dosing conditions could be of high value for pediatric formulation performance prediction scenarios. This would be especially valuable if the output of these models could be combined with PBPK modeling tools, which can in principle take into account other pediatric specific factors, such as relevant levels of metabolizing enzymes.

Limited work has been performed with complex *in vitro* tools. Protocols have been proposed for the TNO (gastro-)intestinal model 1 (TIM-1) [73], but other complex *in vitro* tools may be equally valid for use to simulate pediatric formulation performance [40,67,72]. Ultimately, if proven successful, these approaches could replace some adult relative bioavailability (RBA) formulation comparison studies used in the estimation of pediatric PK. This in turn would generate predictions that can be used with greater confidence when first administering oral medicines to children, or administering a formulation modified for palatability. For higher risk drugs, these approaches could then be used to supplement an adult RBA study to increase the confidence of determining appropriate doses (and appropriate modifications for dosing) for different aged children.

Another scenario where a combination of complex *in vitro* tools and modeling could ultimately be usefully applied is when a pediatric formulation is changed post launch and BE studies would normally be required. BE studies for pediatric formulations in children are challenging to perform, and routes to achieving biowaivers (desirable given the challenges of dosing to children) are poorly defined.

#### 2.2.5. Oral drug absorption in older people and geriatric patients

According to EMA, older people can be defined as individuals older than 65 years of age without any diagnosed chronic disease or impairment, whereas geriatric patients are older people with at least one diagnosed chronic disease or impairment [74]. The elderly (>65 years) represent more than 20% of the global population and this percentage is expected to grow to 35% in 2050. Older age is accompanied by an increase in the number and complexity of illnesses, including that of chronic diseases. Although geriatric patients are the main end-users, they are under-represented in clinical trials due to advanced age, multi-morbidity or polypharmacy [75] and current drug formulations are based on clinical studies of adults aged 18–55 years, meaning that a crucial part of the population in need of medications is not considered during key steps of drug development.

Based on the fact that the majorities of geriatric patients in primary care lives and manage their therapy independently or with the support of relatives or caregivers, the oral route of administration remains the route of choice. Administration of oral drug products can be problematic for geriatric patients with swallowing difficulties (dysphagia). The prevalence of dysphagia in the general population is 16–23% increasing to 27% in people aged over 76 years [76,77]. This number escalates in people suffering from Alzheimer's and Parkinson's diseases, as well as other forms of dementia and neurological conditions [78]. In addition, ageing alters the physiological characteristics of the GIT, thus affecting oral drug absorption. Apart from alterations in gastric pH values compared to healthy adults [79], other luminal characteristics have not been investigated in older people and in geriatric populations. Data on gastric emptying rates in adults vs. older people that have been published for the fed state are conflicting, and/or not relevant for orally administered drug dosage forms [80,81]. The luminal environment in the lower intestine of older people has been investigated for the first time, recently, under both fasted and fed state conditions. Median pH of contents of proximal colon of older people (6.4 in the fasted state and 5.8 in the fed state [82]) were lower than median pH values in young adults (7.8 in the fasted state and 6.0 in the fed state [83]), but the difference reached significance only in the fasted state [82]. Information on age-related differences in bacterial degradation of drugs in the lower intestine is limited as well [84].

Methodologies already applied in adults can be used in order to generate information on the actual dosage from intake conditions for older people and geriatric patients and on physiological characteristics of their GIT lumen relevant to drug absorption. The oesophageal transit of oral dosage forms could be investigated using established clinical techniques such as Magnetic Resonance Imaging [85,86]. The gastric emptying of fluids and solid foods and the transit time of solid oral formulations could be investigated using the salivary tracer technique [87].

Direct sampling procedures could be useful in understanding the physiology of older people, but they have certain limitations. Where direct sampling using intubation is required, multiple sampling can be problematic for older people and geriatric patients as intubation usually cannot last for more than expected for routine medical examination due to ethical reasons. In order to evaluate the potential of paracellular drug absorption after oral drug administration to older people, the leakiness of GIT epithelium of older people and its impact on oral drug absorption can be studied using the triple sugar tests [88]. Bacterial drug degradation in the lower intestine and its clinical relevance can be further investigated using fecal material [89].

Advances in the determination of key physiological characteristics of the GIT relevant to oral drug absorption in older people and geriatric patients will be useful for the optimization of oral dosage forms based on real life dosing conditions. This information will enable the development of *in vitro* methodologies to evaluate swallowability, GIT transit, and intraluminal dosage form performance and *in silico* models for evaluating the PK of oral drug products for older people and geriatric patients.

### 2.3. Disease-specific populations

#### 2.3.1. Cardiovascular, renal and metabolism diseases

Cardiovascular, renal and metabolism (CVRM) diseases are one of the major disease areas by means of prevalence and mortality, and include diseases such as diabetes, obesity, heart failure and infarction, hyperlipidemia, hypertension and chronic kidney disease. Biopharmaceutics aspects of CVRM diseases can have different origins related to physiological influence of the disease itself, common co-morbidities, old age or effects of co-medication. Factors potentially influencing drug absorption that might be affected include GIT transit, GIT fluid volumes and composition, bile secretion and intestinal permeability. A non-comprehensive list of examples is provided for illustration in Table 1. The delay in gastric emptying in diabetic patients even leading to gastroparesis is well known [90]. In addition, diabetes has shown to have impact on mucosa thickness both in the small intestine and the colon [91], which is of potential importance for drug permeability. Furthermore, acute myocardial infarction can also lead to gastroparesis due to the trauma and/or morphine treatment as well influencing bile secretion which can have critical effects on concomitant oral medications [92]. Some data is available in relation to oral drug absorption in obese patients subject to bariatric surgery including *in silico* modeling [93,94]. Overall, systematic investigations are needed, in order to generate the necessary “system characterization” followed by implementation and validation in oral biopharmaceutics tools like *in vitro* dissolution tests and PBPK.

As a first step, there is a need to assemble a literature review based on physiological and clinical science journal papers with the primary purpose of obtaining more comprehensive data regarding factors influencing absorption for a defined list of major CVRM diseases. Second, patient PK data for different relevant diseases should be gathered. A first set of modeling work of these PK data should be performed using existing tools such as GastroPlus® or Simcyp® based on the disease-specific factors obtained from the first stage of the review also including common co-morbidities. These modeling efforts will help to identify gaps by means of understanding PK performance in these patients as well as provide a first validation of initial computer models. In a next stage, novel *in vivo* mechanistic studies in patients are needed to test novel hypotheses and fill knowledge gaps generated through the initial modeling work. This data set will improve the PBPK models for the different disease stages and a final re-validation should be performed. This approach may also be applied to disease areas outside CVRM, if successful. Public-private partnership projects can be especially useful for realization of the outlined future research work.

#### 2.3.2. Inflammatory bowel disease

Oral absorption from the GIT in patients with IBD is of particular interest, as differences in the PK of orally administered drugs between healthy subjects and patients with IBD have been observed, but rarely assessed in clinical trials.

GIT fluids in IBD patients have been shown to be different from healthy adults in terms of their composition (*i.e.* proteins, lecithin, bile salts levels) and their physicochemical properties (*i.e.* osmolality, pH, buffer capacity) [1,29]. Moreover, the IBD population includes a range of sub-populations: the type of disease (*i.e.* Crohn's disease, CD and Ulcerative Colitis, UC) and disease states (*i.e.* remission and relapse) should be considered. The current knowledge with respect to GIT physiology of these populations has recently been reviewed [1,29] and it was shown that altered drug product performance could be attributed to pathophysiological alterations (*i.e.* GIT transit time, fluids composition and permeability).

In order to obtain a better understanding of solubility and dissolution in the GIT of IBD patients, GIT fluids should be collected and characterized. Characterization typically includes pH, osmolality, bile salt composition/concentration, surface tension and buffer capacity. Available data is scarce, but it is being generated: *e.g.* the ascending colonic fluids of UC patients in relapse or remission have been characterized [95]. Such fluids can be used for the characterization of new drugs, specifically intended for IBD patients, or for the optimization of simulated IBD GIT fluids that could be integrated in dissolution tests linked to PBPK models.

## Specific patient populations: CVRM, IBD and COVID-19

### Gaps

Absorption-related pathophysiology of the GIT is insufficiently characterized  
Clinical impact of GIT pathophysiology on drug PK is unclear  
Predictive *In vitro* and *in silico* tools are underdeveloped

### Way forward

Expand the determination of GIT fluid volumes, bile salt composition, permeability, physicochemical properties and variability  
Resolve the link between drug properties, pathophysiology and clinical PK data  
Further improve the *in vitro* & *in silico* tools

Fig. 2. Disease-specific population's summary.

**Table 1**  
Examples of disease related impact on oral drug absorption through biopharmaceutics mechanisms.

Disease	Origin of critical effect	Impact biopharmaceutical factor	Potential impact oral absorption	Ref.
Diabetes	Hyperglycemia effects affecting hormonal control	Reduced gastric emptying rate Thickening intestinal mucosa	Slower rate of absorption Reduced oral bioavailability if non-linear first pass metabolism due to slower rate Delayed onset of action Increased drug degradation if sensitive to acid in stomach Reduced permeability of low permeability drugs	[90] [91]
Acute myocardial infarction	Trauma Morphine treatment	Reduced gastric emptying rate Reduced bile secretion	See above Reduced absorption of low solubility drugs	[92]
Obesity	Gastric bypass surgery	Reducing the gastric volume and bypassing the duodenum and proximal jejunum	Reduced extent of absorption for low permeability and low solubility drugs Increased absorption for drugs with intestinal metabolism	[93,94]

For example, biorelevant media representative of the stomach, intestine and colon of IBD patients were developed and used to assess the risk of altered luminal drug solubility depending on the physicochemical properties (ionization, lipophilicity) of the drug [96,97]. Further developing the concept, biorelevant media for CD patients have been used for drug release studies coupled with mechanistic PBPK modeling, resulting in a successful prediction of drug performance in the patient population [29].

Intestinal permeability in IBD patients should also be considered. Limited information is available on the extent of increased tight junction permeability, or on the impact of inflammation, which decreases the absorption surface area and the permeation rate [1]. These effects are likely to depend on the severity and type of disease. Once this data is obtained, *in vitro* permeation models should be developed that better represent the conditions in IBD patients.

Another critical point when administering oral drugs to IBD patients is the frequent use of formulations based on pH-dependent coatings [98]. In this case, factors such as transit time and pH will impact *in vivo* performance, if altered by disease.

Finally, PK data should be collected comparing the IBD patient populations and healthy volunteers, potentially using formulations with targeted drug release profiles. *In vitro* and PBPK models should be further developed and validated, based on the obtained insights from the clinic. These tools should include all the pathophysiological changes relevant for drug absorption in patients with IBD compared to healthy subjects.

### 2.3.3. COVID-19

Literature data about drug absorption in COVID-19 patients are inconsistent and controversial. The hypothesis that the coronavirus can infect and replicate in the GIT was confirmed by the identification of the SARS-CoV-2 RNA in infected patients stool, and by the high expression of the viral receptor angiotensin converting enzyme 2 (ACE2) in absorptive enterocytes [99–101]. Recent *in vitro* studies on ileal cells exposed to SARS-CoV-2 revealed that the alteration of enterocytes, enteroendocrine and goblet cells differentiation disturb the normal intestinal architecture and consequently impairs the local epithelial barrier integrity. The produced intestinal inflammation and morphological changes may lead in time to more pronounced cellular injuries, including the impairment of oral drug absorption [102–104].

Although we do not have detailed information regarding the mechanisms by which the intestinal inflammation induces the overexpression of ACE2 receptors in the GIT, it appears that modulation of the local intestinal renin-angiotensin system (RAS) with ACE inhibitors or receptor antagonists could attenuate inflammation and COVID-19 progression [105–107]. Multiple datasets of single-cell RNA-seq analysis indicated that SARS-CoV-2 infection of the GIT, by altering the levels of ACE2 at the brush border, may cause microbial dysbiosis and inflammation with important implications for oral drug absorption [108].

High ACE2 expression in cholangiocytes and to a lesser extent in hepatocytes also suggests possible hepatobiliary infection by SARS-CoV-2 [109]. Current data evidenced that the SARS-CoV-2 infection may also be accompanied by mild to severe hepatic damage, resulting in increased activity of liver enzymes, higher serum bilirubin levels, and microvesicular steatosis. Hence, the COVID19-dysregulation of the liver function may add to the impact of the coronavirus disease on drug PK [110,111].

Hence, fundamental research is required to examine the full extent of GIT and liver aspects of COVID-19. In this regard, studies on cultures of intestinal epithelial cells and hepatocytes exposed to SARS-CoV-2 infection may be models to study SARS-CoV-2 infectivity and replication. In order to explore drug administration systems that offer distinct advantages for oral drug administration, it would be of great interest to understand whether and how the epithelium barrier integrity is disrupted, as well as the degree of liver and biliary injury in patients with COVID 19, and their impact on drug absorption.

## 3. Regional differences

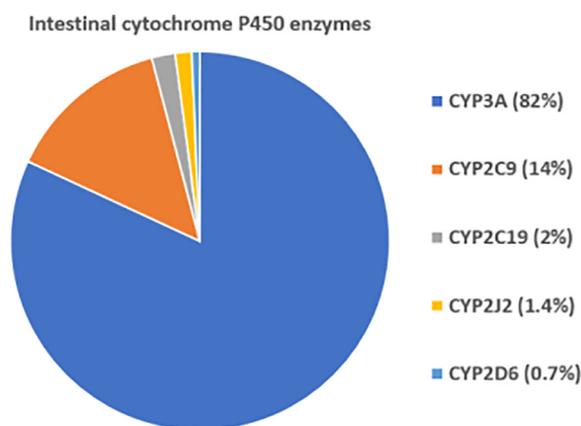
### 3.1. Introduction and scope

Studies on oral drug absorption typically focus on the upper small intestine, as this is the first major absorptive site a drug encounters. However, it is becoming increasingly apparent that the lower parts of the GIT also play an important role in the overall drug absorption process. In particular, there is a renewed interest from pharmaceutical industry in the ileum and colon as potential sites for drug delivery. However, important knowledge gaps remain on the number and function of drug transporters in the ileum and colon, on the composition of colonic fluids, on the drug uptake and permeation process, as well as on the *in silico* modeling of colonic absorption. These knowledge gaps and recent progress in the area will be addressed in the following paragraphs.

Another aspect of oral absorption research, which is frequently overlooked, is the role of intestinal metabolizing enzymes and the interplay with systemic metabolism: the current state-of-the-art and controversies in this area will also be discussed below. Finally, the potential of the lymphatic system as a drug delivery route after oral administration of drugs, including viable approaches to access it, as well as intriguing questions that remain to be answered, is described at the end of the section. The main gaps and the proposed way forward to gain better understanding of the regional differences in oral absorption research are summarized in Fig. 4.

### 3.2. Targeting of oral drugs to the lower intestine and colonic drug absorption

Although oral drug products from which the drug is, at least partly, absorbed from the colon are available for many years, drug targeting



**Fig. 3.** The most important intestinal cytochrome P450 enzymes and their relative expression in comparison with the total cytochrome P450 pool. Data taken from [130].

to the lower intestine (distal ileum and colon) and colonic drug absorption are associated with various challenges relating to the formulation and/or the drug.

Products that target the lower intestine are primarily intended for local action. Performance of relevant products has used physiological cues such as higher pH values in the lumen of lower intestine compared with those in the lumen of upper GIT and/or on the unique metabolism of the bacterial flora of the lower intestine. However, the natural or disease-related variability in pH and microbial levels can result in uncontrolled or variable release. For example, the pH in the proximal colon of healthy adults may not be as high as in the distal ileum and it can be much lower in the cecum [112]. Moreover, a mass movement may empty the caecum, reducing the bacterial content as fresh material enters from the small intestine [113]. Available *in vitro* (or *in silico*) tools for evaluating the performance of drug products in the lower intestine and/or the absorption potential of the drug from this region are validated only for the healthy adult situation [95,114].

On the other hand, the bacterial content in the lower intestine rises gradually from the distal ileum to the caecum, and enzymatic activity is highly variable both between and within subjects. Currently, evaluation of the impact of bacterial drug degradation on drug product or drug performance relies on data collected by using faecal

material (not always collected from humans). The clinical relevance of bacterial drug degradation in the lower intestine and methodological issues related to the human faecal material preparation protocol has been recently addressed [115]. Despite this progress, the procedure for evaluating BE of drug products targeted at the lower intestine is not as straightforward as for conventional drugs that act or are absorbed in the upper GIT. Depending on whether measurements of systemic levels are possible, BE of colon-targeted drugs is evaluated with PK studies and *in vitro* tests or with clinical or pharmacodynamic (PD) equivalence studies [116].

Imaging techniques or direct sampling procedures could be useful in understanding colonic drug disposition, but they are both associated with certain shortcomings. Imaging provides quantitative information only for a limited number of factors, such as the volume of free water [20] and fluids ultrastructure [117]. Direct collection of luminal contents from the colon is more difficult than from the upper GIT [26], because of issues with multiple sampling and alteration of normal physiological conditions. However, colonic content samples and tissue are useful for the assessment of luminal conditions in various populations, bacterial drug degradation and transporter/enzyme expression in the colonic mucosa [26].

### 3.3. Prediction of colonic absorption and physiologically based pharmacokinetic models

For compounds intended to act systemically, extended release (ER) formulations are commonly pursued in clinical development to overcome dosing limitations due to short half-life and thus allow less frequent, ideally once-daily administration, or to blunt the peak-to-trough ratio to mitigate unwanted side-effects associated with  $C_{max}$ . For an ER formulation to fully capitalize on potential, and especially if once-daily administration is targeted, extensive absorption from the colon over a prolonged period is a prerequisite.

Currently, in a development setting, *a priori* prediction of colonic absorption is very difficult [118]. Clinical assessment of regional absorption requires the execution of complex regional dosing studies using specialized dosing capsules [119–121] that add to the timeline and cost of drug development; often a solution needs to be dosed thus allowing only for studying the permeation component of the colonic absorption and not the solubility/dissolution. Alternatively, dosing of modified release formulations and retrospective evaluation of the colonic absorption *in silico* (e.g. via deconvolution) is pursued. However,

## Regional differences

### Gaps

- Incomplete understanding of colonic and lymphatic absorption
- Limited availability of *in vitro* and *in silico* biopharmaceutical tools
- Relative contribution of intestinal metabolism to total clearance and genetic variability is poorly understood
- Clinical impact of intestinally mediated absorptive/metabolic pathways is unclear

### Way forward

- Further characterize the colonic and lymphatic absorption pathways using imaging or direct sampling techniques
- Develop improved *in vitro* and *in silico* models to predict colonic and lymphatic absorption
- Explore the impact of intestinal metabolism
- Use industry-academia partnerships to assess the benefits & risks of targeted drug delivery

**Fig. 4.** Regional differences summary.

in that scenario the impact of permeability and dissolution on absorption for BCS class 2 or 4 compounds may be difficult to separate. While preclinical studies can be executed in different species, there are questions on translation of preclinical to clinical data, especially when considering the physiology differences along the GIT.

The increased utilization of PBPK models to facilitate absorption characterization and formulation development [122], raises the question on the readiness of application of these models to ER formulations. While examples of applications have been published, prediction of colonic absorption *via* PBPK models remains challenging and quite often the current “default” models over-predict absorption and model fitting is required to achieve acceptable predictions and establish *in vitro-in vivo* correlations [123–126]. It has been proposed that correction of the available water volume for absorption may at least partially explain this discrepancy [127] for poorly soluble compounds. While preclinical studies can be executed in different species to estimate regional absorption *via* a PBPK model, there are very limited reports of translation of the models from preclinical species to humans [128]. These uncertainties also limit application of these models in a regulatory setting as highlighted during an FDA public workshop where “the lack of *in vivo* data on the dissolution of drug products in the GIT, and local permeability in the GIT” were highlighted as major information gaps [122,129].

Future research should focus on addressing these knowledge gaps with the aim of developing an optimal translational strategy for prediction of colonic absorption and incorporation in PBPK models. This would require the retrospective application of PBPK models to published modified release formulation data across BCS classes utilizing different inputs (e.g. preclinical data, *in vitro* dissolution data, *in vitro* permeability data). Standardization of dissolution methodologies for ER drug products or high dose instant-release products for poorly soluble drugs and standardization of colonic permeability predictions using *in vitro* or *in silico* approaches as proposed by Olivares-Morales and colleagues would help drive a more uniform approach to the models and eventually allow for development of best practices [128]. It is anticipated that improving the robustness of colonic absorption predictions will not only facilitate wider adoption of these models in drug product development but would also provide a path to application of these models for regulatory purposes, addressing the concerns previously raised by regulators.

### 3.4. Intestinal drug-metabolizing enzymes

Human small intestine epithelial cells (enterocytes) and luminal fluids are the first site, where drug metabolism of orally ingested xenobiotics takes place. CYP3A and CYP2C9 are the most abundant cytochrome P450 (CYPs) in the intestinal CYP pool, accounting for approximately 80 and up to 15% of total CYP content, respectively (Fig. 3) [130]. CYP3A4 and CYP3A5, localized on the villous tips, have their highest expression in the duodenum and the proximal jejunum, and the expression decreases along the human GIT [131,132].

Some members of the uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase, UGT) superfamily and some sulfotransferases (SULTs) are among the most expressed phase II enzymes in the intestine (Table 2) [133,134]. The highest levels of UGTs are localized in the proximal gut and decline further to the distal parts of intestine.

It was shown that the small intestine metabolism by CYP3A enzymes contributes to the first-pass metabolism of many drugs eliminated dominantly by CYP3A4 such as cyclosporine, verapamil, felodipine, midazolam, tacrolimus, simvastatin or nifedipine and the effect can be augmented by inducers (such as rifampicin) [135–142], see review by [143]. Confirmation of the intestine as an important site of the first-pass metabolism is mainly based on data from patients undergoing the anhepatic phase of liver transplantation or with porto-caval anastomosis [144–146], from patients with bariatric or gastric bypass surgery,

**Table 2**

Relative expression of human xenobiotic metabolizing enzymes of CYP1, 2 and 3 subfamilies, key phase II enzymes and nuclear receptors/transcription factors in small intestine at the protein or mRNA levels in comparison with the liver.

Intestinal expression			
Enzyme	Relative mRNA expression	Protein expression (pmol/mg of protein)	Liver microsomes (pmol/mg of protein)
Phase I enzymes (liver expression)			
CYP1A1	+	n.d. <sup>a</sup> ; 0.06–2.3 <sup>b</sup>	n.d.
CYP1A2	–	n.d. <sup>a</sup> ; 0.01–0.05 <sup>b</sup>	19 ± 3 <sup>a</sup>
CYP1B1	n.a.	n.d. <sup>a</sup> ; 0.06–0.12 <sup>b</sup>	n.d.
CYP2A6	–	n.a.	n.a.
CYP2B6	++/+	n.d. <sup>a</sup>	10 ± 2 <sup>a</sup>
CYP2C8	+	n.d. <sup>a</sup>	47 ± 3 <sup>a</sup>
CYP2C9	++	4–8.2 <sup>a</sup> ; 1–6 <sup>b</sup>	152 ± 22 <sup>a</sup>
CYP2C19	++	0.7–1.7 <sup>a</sup> ; 0.15–0.84 <sup>b</sup>	5 ± 1 <sup>a</sup>
CYP2D6	++/+	0.3–0.5 <sup>a</sup> ; 0.85–6.1 <sup>b</sup>	12 ± 1.5 <sup>a</sup>
CYP2E1	++/+	n.d. <sup>a</sup>	103 ± 7 <sup>a</sup>
CYP3A4	+++	27–52 <sup>a</sup> ; 8.8–41.6 <sup>b</sup> ; 31 vs 17 <sup>c</sup>	96.6 ± 6 <sup>a</sup>
CYP3A5	+++/+	0.4–0.7 <sup>a</sup> ; 0.24–4.7 <sup>b</sup>	4.7 ± 0.3 <sup>a</sup>
CYP3A7	+/-	n.d. <sup>a</sup> ; 2.3 ± 1.5 <sup>a</sup>	n.a.
CYP4F2	n.a.	2.8–10.7 <sup>a</sup>	20 ± 1 <sup>a</sup>
Phase II enzymes			
UGT1A1	++	UGT1A1 (5.5–18.5) <sup>b</sup>	n.a.
UGT1A3, 1A6, 1A9	+	UGT1A6 (0.62–3.26) <sup>b</sup>	n.a.
UGT1A5, 1A7, 1A8, 1A10	+++		
UGT2B4	–	n.a.	n.a.
UGT2B7	++		
UGT2B15	+		
SULT1B1, 1A3, 1A1, 1E1	+++	n.a.	n.a.
SULT2A1	+	n.a.	n.a.
Nuclear receptors/transcription factors			
PXR (NR1I2)	++	n.a.	n.a.
CAR (NR1I3)	+/-	n.a.	n.a.
AhR (AHR)	+	n.a.	n.a.

+++ = high expression; ++ = moderate expression; + = low expression; – = undetectable expression; +/- = controversial expression or reports. Data in the table were adapted from papers by [133,135,143,152,165,166]. n.a. – not available; n.d. – not detectable.

<sup>a</sup> Commercially available human intestine microsomes or human liver microsomes [167].

<sup>b</sup> Human jejunal tissues excised from morbidly obese subjects during gastric bypass surgery [168].

<sup>c</sup> Expression of CYP3A in duodenal versus jejunal microsomes [131].

gastrointestinal intubation or *in situ* perfusion of human intestine [147,148], see review [149].

Importantly, the clinical significance of the intestinal metabolic barrier as a site of drug-drug interactions (DDI) or food-drug interactions has also emerged [149]. Several juice beverages appeared to alter intestinal metabolism leading to enhanced absorption [150]. Grapefruit juice which contains significant amounts of the furanocoumarins 6',7'-dihydrobergamottin and bergamottin has been demonstrated as clinically relevant inhibitor of intestinal CYP3A4 in metabolism of orally ingested drugs such as midazolam [151].

“Xenobiotic” receptors such as pregnane X (PXR), constitutive androstane receptor (CAR) or by aryl hydrocarbon receptor (AhR) play a prominent regulating role for many drug-metabolizing enzymes [152]. Hepatic and intestinal CYPs gene expression regulated by PXR ligands seems to vary both in animals and humans [136]. Importantly, most clinical or animal data suggest that CYP3A4 orthologues induction in the intestine is absent or significantly lower than that of hepatic CYP3A4 expression in the liver [153–156]. Similarly, other PXR target genes such as CYP2C8 and CYP2C9 have lower inducibility by PXR ligands in human enterocytes compared to the liver [153].

There are controversies with regards to the role of CYP3A4-mediated intestinal metabolism for overall body clearance [157]. For many drugs eliminated by CYP metabolism, it remains unclear whether the gut metabolism could contribute significantly to the whole-body clearance or if it affects drug absorption. Even though the content of CYP3A4 protein and its enzymatic activity in human enterocytes isolated from human duodenal or jejunal mucosa is comparable to that in the liver tissue (Table 2), the total intestinal content of CYP3A4 is only about 1% compared to the total CYP3A4 content in the liver [143,158].

Several Physiologically Based Pharmacokinetic (PBPK) models have been developed in the last years to estimate the intestinal first-pass (or CYP3A-mediated) metabolism or the intestinal availability for many compounds [149,159,160]. In addition, these models allowed to predict the first-pass metabolism in DDI, study induction of intestinal drug-metabolizing enzymes, examine the role of drug metabolism and drug transporters along the gut or model presystemic metabolism in pediatric population, see reviews [149,159,161]. PBPK modeling has more clearly established that intestinal metabolism plays a minor role in systemic but not first pass metabolism. In addition, these models are used in regulatory decisions with predictions of both systemic and first pass metabolism, and intestinal availability nowadays [162,163].

High variability in expression has been reported for CYP3A4 and CYP2C9 (17-fold and 9-fold, respectively) in the human intestine [143]. This may result in significant inter-individual variability in absorption and therapeutic response. Unfortunately, there are no biomarkers or other tools how to predict intestinal metabolism in human personalized pharmacotherapy so far. More knowledge regarding the role of intestinal metabolism in the first-pass metabolism as well as in the total body clearance in humans could give us leads for future pharmacogenetic clinical studies examining polymorphic variants of intestinal drug-metabolizing enzymes in “poor metabolizer” individuals.

And vice versa, little is known about the effect of genetic variability (single nucleotide polymorphisms, SNP) of intestinal drug-metabolizing enzymes on drug absorption. A SNP such as CYP3A5\*3 allele which results in a frameshift and truncation of CYP3A5 enzyme significantly contributes to the total CYP3A activity in the liver and intestinal mucosa [152,164]. Proper understanding of the phenomenon may help us design (i) better formulations to optimize drug intestinal absorption independently of drug-metabolizing enzymes' genetic variability in personalized pharmacotherapy and (ii) avoid drug-drug or food-drug interactions during the intestinal absorption.

### 3.5. The role of lymphatic transport in oral drug delivery

After oral administration and absorption into the enterocytes that line the GIT, the vast majority of drugs (and nutrients) pass into the blood capillaries that drain the intestinal villi and from there are transported *via* the hepatic portal vein through the liver to the systemic circulation. However, an alternate transport pathway exists in parallel – the intestinal lymphatic system. This originates as a single lymphatic capillary or lacteal in the centre of the villi that drains into the mesenteric lymph and *via* the mesenteric lymph nodes directly to the systemic circulation. This route of transport effectively by-passes first pass metabolism in the liver. The intestinal lymph is the pathway by which the majority of dietary lipids are transported to the systemic circulation in the form of lipoproteins and is also the major conduit for the delivery of GIT antigens and antigen presenting cells to the mesenteric lymph node. In this way it provides both an alternate delivery route to the systemic circulation and a specific pathway to the mesenteric lymph nodes [169,170]. The latter plays a significant gatekeeper role in controlling immune or tolerance responses to food components and GIT antigens. Based on data obtained in (mostly) rats, it is clear that some highly lipophilic drugs associate with lipoproteins and gain access to the intestinal lymph. This is typically observed for drugs with very high lipophilicity (typically  $\log D > 5$  and lipid solubility  $> 50$  mg/g) [171,172] and

lymphatic transport is enhanced for more lipophilic drug analogues or prodrugs [173–175]. Since association with lipoproteins is a critical aspect of lymphatic drug transport, co-administration with lipids and food enhances lymphatic drug transport. For a very limited number of drugs, data has been generated in multiple pre-clinical species *e.g.* mouse, rat and dog and in general trends in lymphatic transport are preserved across species and if anything become more prevalent in higher order species [176]. However, there is almost no data for drug transport into the intestinal lymph in humans, since the methods employed to cannulate lymphatic vessels are highly invasive. As such the relevance to human drug absorption and bioavailability remains unknown. The complexity of measuring lymph levels directly and the need for complex surgical models to achieve this has also led to efforts to use surrogate measurements to estimate lymphatic transport. The most common of these is either measuring drug concentrations in lymph nodes or the conduct of bioavailability studies in the presence and absence of compounds such as cycloheximide that block lipoprotein (chylomicron) formation [177]. Both methods are controversial, however, as increased lymph node exposure could occur *via* drug transport to lymph nodes in the blood or after association with lymphocytes in the blood, and most chylomicron blockers are relatively non-specific and likely interrupt other processes in the drug absorption chain. A decrease in oral drug exposure in the presence of cycloheximide, might therefore reflect a reduction in lymph transport, but may also reflect inhibition of other absorption processes. A further controversy is whether or not stimulating lymphatic drug transport actually increases drug absorption. Most workers in the field regard lymphatic transport as a post-absorptive event hence we refer to lymphatic transport rather than lymphatic absorption. Thus, drugs are not absorbed directly into the lymph, but instead are absorbed across the apical membrane of the enterocyte in a similar manner to other drugs, but from there may associate with lipoproteins and enter the lymph. As such the simple answer to the question of drug absorption and lymph transport would be to suggest that “No, increasing lymph transport does not increase drug absorption”. However, it is possible that improved lymphatic absorption may provide an enhanced absorption ‘sink’ for these highly lipophilic compounds and in this way actually increase absorptive flux. To this point there is little clear data to prove this since almost all methods to increase lymph transport (dosing with lipids) also change luminal conditions and may therefore affect absorption. This is especially the case for the highly lipophilic drugs that are likely to be lymphatically transported since absorption is usually solubility and/or dissolution limited and therefore highly susceptible to changes in drug solubilization in the GIT [178].

There are many critical knowledge gaps in this field, ranging from the fundamental to the more applied. For example, we know very little about the process of drug association with lipoproteins. It seems to be favoured by highly lipophilic drugs and so the assumption has been that this is a passive ‘partitioning’ event. However, it is entirely possible that binding proteins and transporters are involved in shuttling drugs to the endoplasmic reticulum where lipoprotein assembly occurs or in moving drugs across membranes. Similarly, the mechanisms by which lipoproteins access lymphatic capillaries in the GIT is also incompletely understood. We do know a good deal about lymphatic capillary structure [179] and it is clear that in general lymphatic capillaries are more permeable than the equivalent blood capillaries. This provides one explanation for the ability of colloidal lipoproteins to enter lymph vessels preferentially when compared to blood vessels. But whether this process is entirely passive and paracellular or whether active transcellular mechanisms [180] are involved has not been shown unequivocally in mesenteric lymphatics. As described above the anatomy of the GIT lymphatics is such that first pass metabolism in the liver is entirely avoided by drugs that pass from the intestine to the systemic circulation *via* the lymph. What is less clear, is whether drug association with lipoproteins in the enterocyte may also avoid enterocyte-based metabolism. Finally, the mesenteric lymph flows firstly to the mesenteric lymph node before

passing into the major central lymph duct (the thoracic duct) and from there emptying into the systemic circulation. But what are the implications of this? The potential for this to provide advantage in targeting immunomodulators to the mesenteric node has been explored [175,181,182], but the amount of data generated so far is limited and does not exist in humans.

There are a number of key directions of research progression that are required to better understand the role of intestinal lymphatic transport on drug absorption. First and foremost there is a critical need to generate more lymphatic drug transport data in humans. This remains challenging, but somewhat less invasive surgical approaches to lymphatic access may provide a route to generating at least small data sets. Similarly, increases in label free visualization approaches may increase our ability to better understand intracellular trafficking. This is currently limited by the need to use fluorophores that typically change drug properties and the appreciation that optical microscopy methods still struggle to generate resolution sufficient to isolate one side of a membrane from the other. A number of studies have sought to progress *in silico* (and other) methods of prediction of lymphatic transport and have met with some success [183–185], however the relatively small data set of high quality lymph transport data and the lack of deep understanding of the mechanisms of lymphatic access have limited both correlative and fundamental approaches to building lymphatic transport models. Finally, with increasing interest in the oral delivery of larger molecules that sit outside traditional rule-of-5 space, there is essentially no data to provide a guide as to whether these types of molecules will access the intestinal lymph in a similar way to relatively lower molecular weight drugs.

## 4. Advanced formulations

### 4.1. Introduction and scope

The new drug candidates emerging from the drug discovery pipelines of the modern pharmaceutical industry frequently display poor biopharmaceutical properties, which limit their oral drug delivery. Whether classical small molecules with very low aqueous solubility, or poorly permeable and fragile biologicals, these drugs require the development of tailored advanced formulations to allow successful

translation to the clinic and the pharmaceutical market. In the following paragraphs, key questions in technologies such as lipid-based drug delivery systems (LbDDS) and amorphous solid dispersions (ASD) are debated in view of their application for the delivery of small pharmaceutical molecules. Afterwards, the current advances and controversies in oral delivery of nucleic acids and peptides are described. The main gaps and the proposed way forward in the research of advanced oral formulations for small molecules and biologicals are summarized in Figs. 5 and 7, respectively.

### 4.2. Poorly water-soluble drugs

#### 4.2.1. Understanding lipid-based drug delivery systems for increasing absorption and reducing food effects

LbDDS are classically used as an enabling technology to increase the oral bioavailability of poorly water-soluble drugs (PWSD) with solvation-limited solubility that restricts their intestinal absorption [5]. Between the different LbDDS, especially self-nanoemulsifying drug delivery systems (SNEDDS) have shown to be efficient. SNEDDS are isotropic mixtures of oils, hydrophilic and lipophilic surfactants, sometimes also containing a co-solvent. SNEDDS spontaneously form nanoemulsion droplets upon dispersion in an aqueous phase, e.g. the GIT fluids. The feasibility of LbDDS technology is well proven as more than 150 such drug products are currently on the market [4].

Despite the fact that the LbDDS technology is widely used in drug products and is widely researched by academics, the exact mechanism by which LbDDS improve drug absorption is still controversial.

The digestibility of LbDDS is well characterized and documented with regard to colloid formation and potential drug precipitation in the case of PWSDs. However, the relation between *in vitro* solubilization and *in vivo* exposure is not straightforward for some PWSD [186]. Existing *in vitro* lipolysis models, which simulate lipid digestion and drug solubilization in the GIT, often show lack of correlation with subsequent animal exposure studies [187]. This can be due to lack of including a gastric step, not simulating the intestinal physiology of the test animal [12], or lack of an absorptive step in the model [188,189].

An important question for the understanding of drug absorption from LbDDS is whether enzymatic hydrolysis of the lipid excipients is needed for drug absorption. Some researchers clearly advocate for

## Advanced formulations for poorly water-soluble drugs

### Gaps

No consensus on lipid-based drug delivery systems formulation in terms of drug solubilization, formulation digestibility and excipients selection for mitigating food effect

Impact of *in situ* generated nanoparticles during the dissolution of amorphous solid dispersions on bioavailability is not clear

Lack of broader validation of tools to bridge between *in vitro*, *in silico* and *in vivo* behavior of advanced formulations and enable links back to primary composition

### Way forward

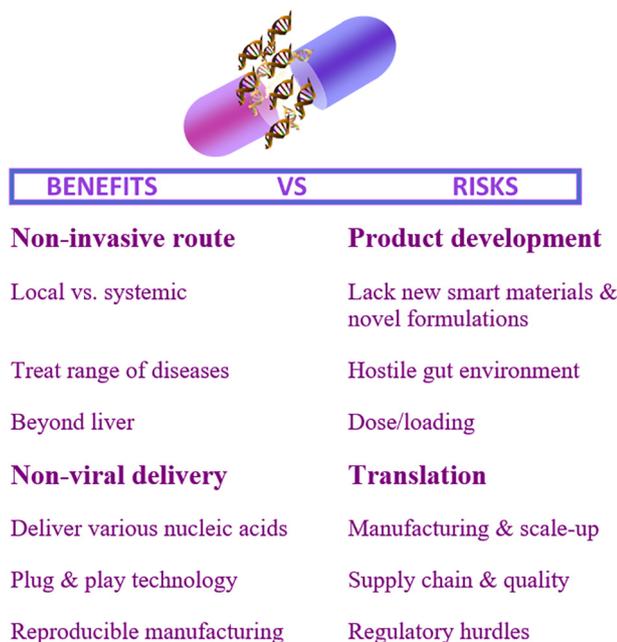
Explore further the role of food and digestion on the mechanisms of drug absorption from lipid-based drug delivery systems

Understand better the role of drug supersaturation in lipid-based drug delivery systems

Use clinical data to develop and validate improved *in vitro* drug release and permeation tools to study drug absorption from amorphous solid dispersions

Characterize further the role of nanoparticles generated during the dissolution of amorphous solid dispersions on drug absorption

Fig. 5. Advanced formulations for oral delivery of poorly water-soluble drugs summary.



**Fig. 6.** The benefits versus the risks associated with the development of an oral product for delivery of therapeutic nucleic acids.

digestible LbDDS, as the formed colloids can have greater drug solubilization capacity, so the drug does not necessarily need to be dissolved in the LbDDS pre-concentrate [190,191]. As a matter of fact, the digested lipids can sometimes enhance the absorption of drug dosed as suspension in LbDDS [192]. However, absorption from LbDDS suspensions seems to be drug dependent, as other studies have shown reduced absorption from these formulations [12,192,193]. This is most likely related to the interplay between rate of drug solubilization in the digesting LbDDS and the rate of absorption.

Industry would generally prefer digestible formulations to avoid adverse effects and potential accumulation of non-degradable excipients. Two recent PK studies, dosing halofantrine [194] and fenofibrate [193] dissolved in SNEDDS and dosed orally to rats, investigated the effect of incorporating orlistat, a lipase inhibitor, into the SNEDDS on the absorption of the drugs. For none of the drugs, a difference in the drug absorption was seen, but for both a change in the PK plasma curve, towards a later  $T_{max}$  when orlistat was added, was observed.

The general applicability of LbDDS is limited by the low solubility of many drugs in LbDDS, resulting in unacceptably high excipient-to-drug dose ratios. One approach to solve this problem is the formation of lipophilic salts or ionic liquids by pairing weakly acidic or basic drugs with non-traditional lipophilic counterions. This has been shown to significantly enhance lipid solubility and to facilitate the beneficial effects of SNEDDS *in vivo* at high drug dose [195,196]. Another approach to the challenge of low lipid solubility in LbDDS is the concept of generating supersaturated drug concentrations in SNEDDS (superSNEDDS). The degree of supersaturation that can be reached, and the stability that is achieved, is dependent on both the drug properties and the composition of the SNEDDS. It has been shown that halofantrine was stable for at least six months, whereas fenofibrate can only maintain a supersaturated concentration for days [197,198]. The superSNEDDS concept has been tested in PK studies in dogs, pigs and rats [12,193,194,199]. In all cases, the drug absorption from the superSNEDDS was equal or higher compared to conventional SNEDDS, indicating the supersaturated concentrations in the formed emulsions may play a role for absorption.

Recent studies have also suggested that one of the inherent advantages of LbDDS may be the ability to promote *in situ* drug supersaturation in the intestine as the formulation is dispersed and digested

[200,201]. This effect may be further enhanced by the addition of excipients, such as polymers that inhibit drug precipitation [202,203].

Finally, some studies have shown that LbDDS can mitigate food effects, probably depending on the colloidal phases they form upon dispersion. Sandimmune® and Neoral® are good examples of commercial LbDDS, both containing cyclosporin A. Sandimmune® forms a crude emulsion and has a rather large food effect, while the subsequently developed Neoral® is a SNEDDS that forms nanometer size emulsion droplets has a reduced food effect [204]. In accordance, a recent study also found that dosing a PWSD in SNEDDS resulted in a reduced food effect in human volunteers [199,205]. However, very few studies have investigated the mechanism by which the food effect is limited.

There is clearly a need to bridge the knowledge accumulated in material and formulation sciences and biopharmaceutics to discriminate the impact of digestion on drug solubilization/protection/absorption in a dynamic environment. On one hand, the study of colloids and phase transitions *in vitro* is generally performed with pH-stat equipment [206] that can be coupled with various techniques [207], such as small angle X-ray scattering (SAXS) or Taylor Dispersion equipment. Addition of some lipid-based excipients [208,209] can critically modify the type of colloids generated during the lipolysis with potential impact on the drug solubilization.

On the other hand, the impact of digestion on *in vitro* drug solubilization in colloids for absorption (eventually creating supersaturation) is generally performed separately in pH-stat equipment only [188] or more recently coupled to a membrane (cell-based, tissue or synthetic) in order to favor the concurrent absorption of drug during the digestion process [189,210]. This recent improvement of the system could favor a better *in vivo-in vitro* relationship.

However, there are still very few studies looking systematically to the role of colloid formation/transition during digestion on drug solubilization, protection and absorption. Also, comparison between published studies is not possible as very different drugs/formulations/protocols are used.

Based on the above, the identified gaps within LbDDS development are:

- How can *in vitro* lipolysis models be improved to actually be *in vivo* predictive?
- What is the mechanism of absorption from SNEDDS; how important is digestion/lipolysis of the lipid excipients, and what role does the formed colloidal phases play?
- What is the role of supersaturation in SNEDDS and what are the mechanisms of the increased absorption?
- To what extent can SNEDDS eliminate a food effect – and which composition is best?

To close these gaps, which will translate into better LbDDS on the market, it is needed to develop and standardize *in vitro* models that can predict the *in vivo* performance of LbDDS. These models should consider both the media composition, the rate and extent of digestion, the colloidal phases formed and the absorption of the drug. Different *in vitro* models will be needed, depending on the drug in question, the LbDDS to be developed (e.g. digestible vs non-digestible LbDDS, drug solution vs drug suspension) and the *in vivo* model (e.g. fed or fasted, humans or pre-clinical animal models).

#### 4.2.2. Understanding the *in vivo* performance of amorphous solid dispersions

##### 4.2.2.1. Impact of supersaturation and precipitation on drug absorption.

Among advanced formulations, ASDs represent the most commonly used solid formulation approach [211]. To facilitate the development of such formulations, *in vitro* tools that allow accurate predictions of the *in vivo* behavior, as a function of formulation composition, would be highly desirable. This is especially true with the increased interest

in higher drug load ASDs (*i.e.* >40% drug), driven by the need to reduce pill burden, which can become an issue for high dose compounds, or to accommodate development of fixed dose combinations. The primary driver for the bioavailability enhancement of ASDs is the increase in solubility of the amorphous form over the crystalline drug. Thus, *in vitro* tools employed to study ASDs primarily focus on characterization of supersaturation by studying initial dissolution (spring) and precipitation (parachute) effects. Two of the common assays employed include (i) a precipitation inhibition assessment to identify suitable excipients which have the potential to maintain supersaturation and inhibit precipitation of drug candidates [212] and (ii) a two-stage dissolution method mimicking the pH shift between the pH in the stomach and duodenum in a USP2 apparatus using biorelevant dissolution media [213]. If necessary, additional experiments can be conducted (i) using physiologically relevant ratios between the amount of ASD and volumes of dissolution media and (ii) evaluating the effects of crystalline material on supersaturation and precipitation. Information obtained with these models is typically used in PBPK modeling to bridge to the *in vivo* situation.

Although a range of tools are used to develop and evaluate ASDs and these have been successfully implemented at later stages of development, a thorough understanding of the interaction between the drug, polymer and physiology and its effect on dissolution and overall performance *in vivo* is still missing. The composition of ASDs should result in a good spring (*i.e.* fast initial dissolution to the amorphous solubility limit) and parachute (*i.e.* maintained supersaturation and precipitation inhibition) effect [214]. However, these effects are difficult to predict and seem to highly depend on ASD composition (*i.e.* glass transition temperatures of drugs, the type of polymer used and drug load). Moreover, the growing interest in high drug load ASDs can further complicate this composition as these loads can increase the risk of poor wettability and slow dissolution by mechanisms not related directly to the amorphous supersaturation (*e.g.* affecting tablet disintegration/erosion). In an industry setting, screening of different ASD compositions for *in vivo* performance maintains a significant empirical component with *in vivo* (preclinical and ultimately clinical) studies serving as stage-gates for decision making. More focus on linking performance to formulation composition could have a significant impact on improving future formulation screening practices. To understand how to optimize ASD compositions, a broad range of polymer types, polymer molecular weight fractions, drug/polymer ratios and ternary mixtures need to be screened and evaluated in a toolbox of assays. For example, dissolution and precipitation assays that mimic more closely GIT conditions (transfer dissolution systems) and include the solid-state characterization of residual solids in the experiments might be needed. Additional more complex measurements that look at the impact of amorphization/crystallization and supersaturation on membrane flux should be considered. In addition, new developments in the spectroscopic field should be implemented to allow for more systematic solid-state characterization of non-dissolved residues [215,216]. Systematic generation of such datasets and improved *in vitro* - *in vivo* correlation can lead to better positioning of the different screens in the development process. Furthermore, this will provide “validation” of the predictive nature of *in vitro* testing, hence clearing the path to reduce the empirical *in vivo* screening.

**4.2.2.2. Role of nanoparticle formation during ASD dissolution.** While the characterization of supersaturation *via* dissolution/precipitation assays that were discussed in the preceding paragraphs may be sufficient in early development to screen a relatively wide range of formulation compositions, it is now well documented that for some ASDs, generation of nanoparticles during dissolution (often referred to as liquid-liquid phase separation) takes place and the size of these particles has been shown to be composition dependent [217,218]. While these nanoparticles were initially believed to serve as a source of rapidly dissolving drug, the exact impact of nanoparticle formation in increasing

bioavailability remains unclear. More importantly, the available published literature on the impact of the size of these nanoparticles on absorption *in vivo*, is very limited. Most of the previous publications have focused on comparing micronized vs nanosized crystalline drug during the investigation of nanocrystalline formulations. There are very few controlled preclinical or clinical studies with different particle sizes within the submicron range at clinically relevant doses. For example it was recently reported that for an anacetrapib/copovidone/tocopheryl polyethylene glycol succinate (TPGS) ASD prepared *via* hot melt extrusion, a reduction of the size of *in situ* generated nanoparticles from ~200 nm to ~100 nm resulted in approximately two-fold higher bioavailability in dogs in either fasted or fed state [219].

Some authors have proposed that the bioavailability increases with nanoparticles (crystalline or amorphous) can be explained by increases in dissolution rate/solubility [220] while others have suggested impact of nanoparticles through diffusion in mucus resulting in increased apparent permeability [221–223]. If nanoparticle size can indeed affect bioavailability by such mechanisms, this could have significant product quality implications for those formulations where the resulting particle size can be affected by variations that can be seen during routine manufacturing conditions. Under current practices, dissolution and not particle size measurement, is the primary quality control method. Nevertheless, if the mechanism of bioavailability increase is not related to dissolution, dissolution methods would need to be adapted to reflect the impact of nanoparticle size or, perhaps more appropriately, alternative product quality control methods may need to be considered.

A review of available literature raises interesting questions regarding the proposed bioavailability enhancing mechanisms. Most of the manuscripts attempting to explain the bioavailability gains have relied on computational arguments [222–224]. Even so, the identifiability of parameters is a concern with current oral absorption models as estimations of permeability/solubility/dissolution are confounded when it comes to predicting the absorptive flux. Ideally, all input parameters would be supported by experimental data including detailed dissolution rate and diffusion measurements, but this connection is still largely lacking. For example, while diffusion through the unstirred water layer/mucus has been suggested, when the diffusion of polystyrene nanoparticles in mucus was measured it was estimated that particles of 20–100 nm (a size not commonly achieved in formulations) would penetrate ~200 nm of mucus within an hour and the flux of larger particles would be significantly slower [225]. The observed diffusion coefficients are too slow to explain the usually fast absorption observed when employing nanoparticles/solid dispersions. Additionally, while current computational models suggest a favorable effect only for high permeability (BCS class 2) compounds [224], bioavailability advantages have been observed also for BCS class 4 compounds. This opens up the possibility that additional mechanisms, which may not have been characterized or incorporated in computational models to date, may be in play.

To further advance our understanding it is clear that controlled preclinical studies, preferably in larger animals that would allow dosing of clinical formulations, and ideally clinical studies, where particle size is purposefully varied within the submicron range, would be useful. Preparation of the accompanying formulations will need to involve thorough characterization to ensure accurate understanding of what is dosed, including measurements of nanoparticle dissolution rate under discriminating conditions. The increasing utilization of human intestinal aspirate studies to facilitate biopharmaceutics characterization [226] could provide additional insights by comparing the dissolution rate and nanoparticle formation (especially particle size measurements) between simulated intestinal fluids and *in vivo* conditions. On the preclinical map, more detailed measurements of diffusion coefficients of pharmaceutical product relevant nanoparticles in intestinal mucus as well as development of imaging protocols, that would allow visualization and quantitation of mucus associated nanoparticles after oral administration, could provide better insight on the absorption process of nanoparticle formulations. Finally, the incorporation of such input

data in computational models, coupled with appropriate parameter sensitivity analysis (PSA) to understand identifiability issues, would enable to further develop computational models and validate those against controlled datasets.

It is envisioned that such an advancement in our understanding of nanoparticle impacts on absorption could lead to improvements in efficiency during product development. Coupled with further advancements in linking dissolution of ASDs to primary formulation composition, this can allow for more rational design of advanced formulations with an understanding of the limits of bioavailability enhancement, as well as a selection of the most appropriate methodologies for quality control as already discussed earlier in this section. Collaborative research between academic groups with access to characterization tools and industry partners with access to *in vivo* (especially clinical data) is recommended to propel the field forward.

### 4.3. Biologicals

#### 4.3.1. Introduction and scope

There are many examples where the issue of delivery of a drug with a specific advantageous pharmacology is hampered by the lack of an appropriate, tailored delivery system. The current concern with regard to orally administered nucleic acids and peptides is a case in point, where very low and variable absorption has to be acceptable if such drugs are to be used. Peptides such as desmopressin acetate are available as oral tablets, even though the formulation has an oral bioavailability of 0.1% and food delays and decreases absorption [227]. The drug is useful and safe, but bioavailabilities of this order risk huge swings in pharmacological effect if even small increases in bioavailability are achieved by formulation, which represents a high commercial risk [228].

These less permeable drugs require innovative approaches to improve absorption and there are translational difficulties moving from animal to man [229]. Brayden and Alonso have summarized the progress in a recent editorial: synthetically produced peptides can be produced cheaply and companies are developing smaller, less hydrophilic cyclic peptides with lipophilic motifs [230]. They also alert the audience to the advantages of appropriately positioned delivery. If the organ producing the hormone of interest is situated in or near to the gut wall, then oral delivery might be preferred from subcutaneous delivery and that

approach should lead to less bystander side-effects. The formulation approaches of stealth with nanocarriers and conjugates [231,232] or physically broaching the intestinal wall are also being considered. Recently, LbDDS have shown encouraging results for the protection of peptides against metabolic degradation [233] and absorption in preclinical models [234], with the non-digestible excipients/formulations suggested to be key in avoiding peptide degradation [235]. The theoretical risks of intra-oral needle-based systems and approaches using enhancers in order to access the systemic circulation must be considered in the context of both the healthy and the sick gut where appropriate models for safety assessment may be poorly developed.

These and other aspects of the contemporary approaches for oral delivery of nucleic acids and peptides are presented in the following sections.

#### 4.3.2. Oral delivery of therapeutic nucleic acids by non-viral methods

**4.3.2.1. State of the art.** Advances in knowledge regarding the genetic basis of disease has opened up innovative opportunities to develop gene-based therapies where gene silencing or upregulation is now feasible using either siRNA or mRNA, respectively [6,236]. Although this field is still in its infancy, progress has been made mainly using viral delivery methods for parenteral administration. However due to significant disadvantages associated with viral delivery, including immunogenic reactions, poor loading capacity, scale-up and manufacturing issues, research interest in non-viral formulations is growing apace [237,238]. In 2018, the first ever non-viral siRNA product (Patisiran, Onpatro®) [239] using a lipid-based nanoparticle was licensed, followed late in 2019 by licensing of a siRNA-N-acetylgalactosamine (GalNAc) covalent conjugate (givosiran, Givlaari®) [240], both administered parenterally to target liver disease.

The existence of these products shows the technology works and the major challenges remaining are to extend the technology to other sites beyond the liver and to non-invasive routes of administration including oral, the advantages of which are widely known. In addition, many therapeutic targets exist for oral administration of nucleic acids including local treatment of IBD and intestinal cancers, and the potential for systemic absorption to target other disease sites. Several academic groups are already exploring oral delivery [4,241–244] and companies have publicly declared their interest.

## Advanced formulations for biologicals

### Gaps

Advanced planning of target product profiles and commercial feasibility are a challenge for the oral delivery of nucleic acids

Oral peptide formulations that minimise variability and maximise exposure are lacking

Novel biomaterials are urgently needed

Developability, acceptance and uptake of sophisticated technologies remain unclear

The enhanced risk in patients with gastrointestinal dysfunction remains unchecked

### Way forward

Improve the evaluation of the mechanisms of action, PK analysis and toxicity after chronic administration of non-viral oral nucleic acid formulations

Further establish critical quality attributes influencing efficacy and safety of oral biologicals

Explore advanced formulations combined with drug molecular engineering for oral peptide delivery

Design and validate improved scalable manufacturing processes to insure batch to batch reproducibility

Determine how the gut heals after repeated physical and chemical insults (*e.g.* microneedles and novel permeation enhancers)

Fig. 7. Advanced formulations for oral delivery of biologicals summary.

While oral delivery of therapeutic NAs is an attractive clinical and commercial prospect, many unanswered questions still remain, which hamper translation and delay access for patients to these life-altering therapies (Fig. 6). Examples of these knowledge gaps are highlighted below.

**4.3.2.2. Novel chemistries.** The physicochemical properties of nucleic acids including large molecular weights, complex hydrophilic structures and high negative charge result in poor membrane permeability and susceptibility to enzymatic degradation, making oral delivery difficult. However, the chemistry of RNA molecules in particular siRNA and mRNA can be modified to improve stability and enhance efficacy and enzymatic resistance [6,236,245]. The ability of these structural modifications to maintain stability in the gut needs to be addressed.

**4.3.2.3. Site specific delivery systems.** In tandem with synthesizing more stable NA cargos, the other major gap in our knowledge is the design and preparation of delivery systems capable of surviving the harsh environment of the gut. These delivery systems must also possess adequate loading and release properties which will achieve sustained and controlled transfection. The site of action, either locally at the gut wall or systemically following absorption, will influence the formulation design. Local administration will avoid the barriers faced in the circulation and should provide research opportunities likely to yield success in a shorter timeframe.

**4.3.2.4. Will current approaches work in the gut?** To tackle the RNA delivery dilemma, two main approaches based on (1) nanoparticles [245], (2) covalent nucleic acid conjugates have been explored mainly for parenteral administration [246,247]. Nano constructs engineered with a variety of biocompatible materials have been investigated with varying degrees of success [236,248]. Of these materials, pH-dependent ionizable lipids capable of forming lipid nanoparticles (LNPs) incorporating nucleic acid deserve particular mention. LNPs are used to deliver siRNA in the marketed product Patisiran (Onpattro®) and this formulation approach is also used in mRNA Covid-19 vaccines by several companies (NCT04368728, NCT04470427). Both products have shown ≈95% efficacy in Phase 3 trials (Nov 2020) and application for emergency use authorization has been filed to the FDA [249]. Can these approaches be exploited for oral delivery?

**4.3.2.5. What lipids to choose?** The most commonly used LNPs consistently contain a blend of 4 lipids including an ionisable cationic lipid (e.g. heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate, DLin-MC3-DMA), cholesterol, distearoyl phosphatidyl choline and a pegylated-lipid [250,251]. To date while the LNP approach has mainly been successful for targeting the liver, the approach has also been used to target other tissues including the intestine. An antibody targeted LNP encapsulating modified mRNA [252] increased the expression of interleukin 10 by Ly6c+ inflammatory leukocytes in diseased tissues resulting in a therapeutic benefit in an IBD animal model, however intravenous administration was used. The challenge of transfecting the intestine following oral administration of siRNA has been investigated [241]. Following oral gavage, although the LNPs appeared to be taken up by intestinal cells, no gene silencing was detected.

These studies identified pepsin in the stomach and bile salts as the main barriers limiting the transfection efficiency of the LNPs particularly under fed state conditions. The influence of the bile salts while unclear maybe related to solubilisation of the NP lipids causing deconstruction of the LNP. Although extensive research is ongoing into the design and modification of lipids [253] a detailed characterisation of the orientation and packing of these 'newer' lipids within NPs is currently lacking, such studies may help identify more stable LNPs with potentially enhanced resistance to the influence of bile salts.

**4.3.2.6. Alternative excipients combinations.** Alternatively, lipids such as those found in exosomes may help to improve the design and efficacy

of lipid-based formulations. Exosomes are a type of extracellular vesicle of which lipids are a major component [254]. Exosomes derived from milk have been investigated as potential oral delivery systems for macromolecules including siRNA [255] and microRNA. Milk exosomes have been shown to be resistant to digestion in the gut and have wide biodistribution including to the brain following oral delivery [256]. However, in order to fully exploit the drug delivery potential, more needs to be discovered regarding the biological function of these 'natural' lipids, in addition, challenges regarding large scale production and consistent quality also need to be overcome.

An interesting alternative approach for future investigation in the oral space may be the combination of the lipids described above with newer materials including modified cyclodextrins and biocompatible polymers [257,258]. Such combinations may enable higher loading and increased stability in the harsh environment of the gut.

**4.3.2.7. Conjugates - beyond the liver.** In contrast to NPs, covalent conjugates of siRNA may also provide a solution to oral delivery in particular for systemic versus local availability. The siRNA.GalNac conjugate, in combination with a permeation enhancer was reported to produce a dose dependent reduction in a serum biomarker post oral gavage of siRNA conjugate [259]. While this conjugate is again targeted for liver disease the potential exists to explore this approach for other therapeutic applications beyond the liver. To enhance the stability and absorption capacity, the concept of more lipophilic conjugates capable of self-assembly into nano-constructs, similar to that described recently, may be an exciting future possibility [260].

**4.3.2.8. Regulatory constraints.** Regulatory hurdles for novel materials used in innovative drug delivery systems, including oral products, are a major concern for the industry. While of course safety is paramount, translational progress is and will be hampered if drug delivery scientists are limited to a set list of traditionally used GRAS (generally recognized as safe) excipients. This is a particular issue for delivery of macromolecules like proteins and nucleic acids where excipients are often integral to the mechanism of action and efficacy. To help break this deadlock, more research is urgently needed to identify the issues, explore potential solutions and develop regulatory guidelines for the use of novel excipients based on a risk-informed decision-making process.

One potential solution may be to consider a streamlined approach and to assess and license the product as a whole (NA plus excipients) on a case-by-case basis, in this way innovative delivery systems for rare diseases with unmet clinical needs could reach the patient in a timely fashion. In tandem such studies would progressively build a dossier of information on the individual novel excipient while mitigating risk by assessing its role within the product.

### 4.3.3. Oral peptide delivery

Peptide drugs are substantially larger than traditional drugs and can therefore interact with promising targets that are "difficult-to-drug" using traditional membrane permeable molecules. While peptides offer the opportunity to expand the number of "druggable" targets, they have well known drawbacks, including poor membrane permeability (as the nucleic acids, described in the previous section) and negligible oral bioavailability [261]. In the following paragraphs, the state-of-the-art, current challenges and knowledge gaps in the various oral peptide delivery approaches will be outlined.

**4.3.3.1. Transient permeation enhancers.** To overcome the formidable physiological barriers and enable oral delivery of peptides, scientists and formulators have investigated the use of transient permeation enhancers (TPEs) with/without enzyme inhibitors. TPEs are compounds that can temporarily and reversibly modulate the permeability of the intestinal epithelium. Examples include sodium caprate, medium chain glycerides and derivatives, acylated amino acids such as N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC), acyl carnitines, bile salts

**Table 3**  
Examples of peptide/TPE formulations that have advanced to clinical testing or registration.

Peptide	TPE/delivery technology	Oral bioavailability in humans (%)	Variability (CV%)	Subjects No.	Clinical stage	Ref
Basal Insulin 338 (a long-acting, basal insulin analogue)	Sodium caprate (GIPET I®, Merrion)	Not evaluated	82–132	44	Phase II	[262]
Desmopressin	Mono-, diglycerides of C8 & C10 (GIPET II®, Merrion)	2.4	124	18	Phase I	[263]
Salmon calcitonin	Acylated amino acid (Eligen®, Emisphere)	0.41–1.44 (dose dependent)	59–99 (dose dependent)	8	NDA accepted for review 2015	[264]
Parathyroid hormone (PTH1-34)	Acyl carnitine/citric acid (Peptelligence®, Enteris)	0.58 (week 0)	99 (week 0)	32	Phase II	[265]
Octreotide	Oily suspension containing sodium caprylate, glyceryl mono- and tricaprylate (TPE®, Chiasma)	0.8	62	71	Approved, 2020	[266]
Semaglutide	Acylated amino acid, SNAC (Eligen®, Emisphere)	0.4–1	65–84	11–32 (for variability)	Approved, 2019	[267,268]

and various polymers, amongst others. The use of TPEs (+/– enzyme inhibitors) has resulted in some degree of success, with a number of peptide/TPE combinations entering clinical studies and some advancing to late stage clinical testing and regulatory “new drug” applications and approvals. Table 3 summarizes some of the more advanced clinical studies that have been reported in the literature using TPEs to enable oral delivery of peptides.

Despite the progress made using TPEs to enable oral delivery of peptides and the success (albeit still limited) in advancing these to clinical studies, oral bioavailabilities are still very low (low single digit at best) and variability is still very high (coefficients of variation typically >50% together with dosing periods when negligible drug is absorbed). These issues hinder the commercial “developability” of oral peptides (Table 3).

Hence, considerable efforts are on-going aimed at identifying better TPEs or delivery systems to enable higher oral bioavailabilities. Notable recent examples of TPEs include pelargonidin (a strawberry extract component), which reportedly had impressive effects on oral bioavailability of insulin yielding levels resembling or superior to those achieved following subcutaneous administration [269] and the ionic liquids based on choline and geranate reportedly yielding an oral bioavailability of insulin of approximately 50% following intrajeunal administration [270]. Building on the ionic liquids concept, lipid formulations incorporating peptides hydrophobized via non-covalent interactions (e.g. ion pairing, hydrogen bond pairing), are receiving increasing attention in this area and will be described in more detail in the following subsection.

In addition, an emerging trend is to chemically engineer peptides to render them more suitable for oral administration. This includes modifications to make the peptide more resistant to enzymatic degradation in the GIT as well as increasing the systemic half-life of the peptide to better accommodate variability. The latter, viz. half-life extension strategies such as fatty acid-acylation of the peptide, has been a fortunate gain from efforts being made in the parenteral space aimed at reducing dosing frequency from daily to weekly (or longer). Having a long half-life (>100 h), allows the high variability associated with oral delivery of peptides to be somewhat accommodated because the resulting drug accumulation can “keep drug onboard” even over days where no or minimal drug is absorbed. This strategy has been adopted for the successful development of the oral glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide product, Rybelsus®, approved by the FDA in September 2019 and represents the first successful commercial oral peptide product delivered using a TPE.

However, the success of Rybelsus® raised several questions. Can peptide engineering be used to make peptides more amenable to formulating with TPEs? Is promoting the interactions between the peptide and TPE (as exemplified by SNAC that reportedly interacts with Semaglutide to promote monomerization of the peptide and enabling stomach absorption [271]) beneficial for all types of peptides or what kind of interaction is optimal?

If the aspect of peptide/TPE interaction can be better understood, it could open the way for the use of *in vitro* methods that can characterize such interactions (e.g. isothermal calorimetry, nuclear magnetic resonance (NMR), dynamic light scattering etc.) to be used in the selection of the preferred TPE to combine with a particular peptide (e.g. [272]). With an improved understanding of the preferred types of interactions, ultimately *in silico* techniques such as molecular dynamic simulations (e.g. [273,274]) could be employed to identify preferred combinations thereby overcoming the need, or at least reducing the need for multiple *in vivo* studies to screen and select peptide/TPE combinations.

Methodology issues in TPE screening should also be addressed. Typically, TPEs are screened for their suitability to promote systemic absorption of peptides by *in vitro* techniques such as the parallel artificial membrane permeability assay (PAMPA), Caco2 (or similar) or Ussing permeability assays before progressing to *in vivo* rodent and subsequently larger pre-clinical animal models. These *in vitro* systems lack many of the functionalities of a “live” intestine such as the ability to regenerate and re-model following exposure to TPEs, as well as lacking the existence of a native 3D mucus structure; and many of the *in vivo* preclinical models give overly optimistic predictions of bioavailabilities. To advance our understanding and use of TPEs for promoting oral delivery of peptides, we need to improve and expand our “Biopharm” toolbox such that peptide/TPE combinations can ultimately be developed as efficiently as currently being done for small molecules. As an example, can 3D organoids (intestine-on-a-chip) or similar be developed to enable such screening, and simplified such that high throughput screening can be conducted on a scale similar to that done in the “developability” assessment of small molecules [275]. Similarly, the existing “Biopharm” toolbox would also need to be expanded to better evaluate oral peptide technologies reliant on transporters as well as nanoparticulate delivery systems.

The chronic use of TPEs also requires careful safety profile considerations. As mentioned, TPEs increase intestinal permeability by opening tight junctions or modifying membrane structure to allow large hydrophilic molecules such as peptides to be absorbed. By inference, if peptides can be absorbed then it is possible that other molecules usually excluded by the intestinal epithelium can also be absorbed. Chronic use of TPEs, as could be expected for many indications, would also mean repeat “permeabilization” of the intestinal membrane (although unlikely to occur to the same place in the intestine). Despite the safety of SNAC (300 mg) being evaluated in studies with a mean duration of ~60 weeks of daily dosing to support the approval of Rybelsus® [267], the long term safety following chronic use of other TPEs and at various dose levels needs to be further investigated and understood.

**4.3.3.2. Hydrophobic pairing as a strategy to enhance permeation.** It is generally accepted that both ionic and non-ionic surfactants exhibit a permeation enhancing effect on the intestinal mucosa as they are interacting with epithelial cells via different mechanisms [276].

According to recent results, however, we assume that the interaction of these permeation enhancers with the drug itself is at least as important for an improved oral bioavailability as their interaction with the membrane [277,278]. Due to the formation of hydrophobic ion pairs (HIPs) and hydrophobic hydrogen bond pairs (HHPs) H-bond donor and H-bond acceptor substructures on poorly permeable drugs can be masked resulting in improved membrane permeability. According to Lipinski's rule of 5, the number of H-bond donors has to decrease below 5 and that of H-bond acceptors below 10 using HIPs and HHPs for such a masking. For drugs > 500 Da a log D of 3 seems advantageous to provide high membrane permeability of such complexes [279].

Although the concept of hydrophobic ion pairing has been known for many decades and there are numerous publications providing evidence for its potential in form of *in vitro* permeation studies [280,281], to the present date this potential could not be translated into an improved oral bioavailability. The reason for this failure in most *in vivo* studies is the poor stability of HIPs and HHPs under the harsh intestinal conditions, as there are simply too many competitive counter ions as well as H-bond donors and acceptors available destroying these complexes on their way to the absorption membrane [277,282]. Nazir and colleagues demonstrated that the dissociation of HIPs increases with increasing salt concentrations over time. At salt concentrations equivalent to that of GIT-fluids, half of the HIPs dissociated within 4 h [282]. In case of HHPs, in particular mucus glycoproteins bearing countless competitive H-bond donor and acceptor substructures, represent an immense threat to the stability of these complexes. Accordingly, the likelihood that intact HIPs or HHPs reach the absorption membrane, as in the case in well-designed *in vitro* permeation studies, is low.

Due to the incorporation of HIPs and HHPs in lipid-based nanocarriers such as solid lipid nanoparticles (SLN), nanostructure lipid carriers (NLC), o/w nanoemulsions or SNEDDS, a sufficient high stability of these complexes can be guaranteed. As most competitive counter ions as well as H-bond donors and acceptors are simply too hydrophilic, they cannot penetrate into lipid-based nanocarriers in order to destroy the incorporated HIPs and HHPs. Moreover, as the dielectric constant of lipophilic excipients is up to 80-fold lower than that of GIT-fluids, HIPs are even further stabilized within lipid-based nanocarriers. In case of HHPs, competitive H-bond donor and acceptor interactions can be avoided to a high extent by lipid-based nanocarriers comprising aprotic and apolar excipients such as paraffins or squalene. Furthermore, by the exclusion of lipophilic excipients exhibiting ester substructures, the degradation of lipid-based nanocarriers by lipases can be ruled out [235]. Once these nanocarriers have permeated the mucus gel layer and reached the absorption membrane, they are supposed to fuse with the outer cell membrane releasing intact HIPs and HHPs.

Although our knowledge about the membrane transport of HIP/HPP is still very limited, there are at least some studies providing evidence [283]. Motion and colleagues demonstrated that the delivery mechanism can be further improved by engineering phosphatase-triggerable lipid-based particles displaying phospho-peptides on their surface. After removal of the phosphate groups by membrane-bound phosphatases, the specific peptide can then activate membrane fusion and provide cytosolic delivery of encapsulated compounds in a highly efficient manner [284]. An improved knowledge about this fusion process and other likely involved mechanisms such as endocytosis will certainly be the key to success for this emerging technology. In addition, the design of lipid-based nanocarriers releasing their payload in a targeted manner directly at the absorption membrane might be another promising approach. Lipid-based nanocarriers such as o/w nanoemulsions or SNEDDS being emulsified by a phosphorylated surfactant, for instance, might be designed in a way that they release their payload directly at the absorption membrane. As the phosphate group of such surfactants is rapidly cleaved off from its lipophilic tail by the absorption membrane-bound alkaline phosphatase [285], the surfactant loses its emulsifying property and the oily droplets will disintegrate and liberate HIPs and HHPs in a targeted manner. Addressing the interaction of

lipid-based nanocarriers with the absorption membrane by a two-track strategy focusing on one hand on an improved knowledge about their fate on the membrane and parameters controlling it and on the other hand on the improvement of already established lipid-based nanocarriers for HIPs and HHPs delivery is likely most straight forward. It will open the door for more efficient oral delivery systems especially for BCS class 3 and 4 drugs.

**4.3.3.3. Epithelial transport systems and cell penetrating peptides.** To avoid having to modulate the permeability of the intestinal membrane, which may come with associated safety concerns, or to use complex hydrophobic pairing strategies, researchers have also investigated the exploitation of inherent transport systems present in the intestinal epithelium.

For example, the proton-assisted small amino acid transporter (PAT-1/SLC36A1) was recently evaluated for the oral delivery of insulin wherein insulin was encapsulated in zwitterionic betaine (a PAT-1 substrate) polymer micelles stabilized by an unusually low critical micelle concentration. Limited proof for the uptake mechanism was presented, but nevertheless, cellular accumulation and systemic delivery of insulin followed by a glucose lowering effect in diabetic rats was observed [286]. In another example, the domain 1 of the *V. cholerae* toxin (cholix) was investigated as a strategy to promote peptide absorption across the intestinal epithelium, taking advantage of the inherent vesicular transport mechanism that enables the delivery of the toxin across the epithelial barrier. In this case, significant systemic levels of human growth hormone were reported when the peptide-cholix conjugate was orally administered, but bioavailability was not determined [287].

Conjugation with cell penetrating peptides (CPPs) is another approach that has been investigated for promoting oral drug absorption but mechanistic information for transcellular transport has not yet been presented at the level of e.g. cholix-mediated transcytosis [288]. Since the main uptake mechanism for membrane penetration is binding to the cell surface followed by endocytosis, the most promising approach has been to use CPP-peptide conjugates to enhance the intracellular delivery of peptides by an endosomal escape mechanism. A particularly promising CPP that provides efficient endosomal escape and thereby promotes intracellular target access is CPP9 [289]. For instance, conjugation with this CPP enhanced the intracellular efficacy of a variety of stapled peptides between 10 and 100-fold [290].

**4.3.3.4. Nanoparticle formulations.** The multiple reasons behind the unfulfilled promise of nanoparticles for oral delivery of peptides were unraveled in the pan-European project "TransInt" (2012–2017) and pointed at the complexity of developing such systems to translatable oral delivery systems [230]. When challenged by preset specifications, a lack of connection between elegant nanotechnology and realistic formulation requirements, such as required peptide payload, loading efficiency and release kinetics was revealed. Insufficient consideration of biochemical and physiological barriers in the GIT was also a problem. Examples of biochemical shortcomings include enzymatic degradation of the peptide or little knowledge of how the entrapment or encapsulation influence the native peptide conformation. Examples of physiological shortcomings include failure to overcome the various physiological barriers in the GIT and lack of scalability to larger species. None of the nanoparticle candidates were able to deliver sufficient amounts of the therapeutic peptides to be of interest for further development. An informative summary of the problems encountered is given in a recent review [291].

Strategies such as those developed within the TransInt consortium however could be applied more rigorously in order to reduce efforts on those nanoparticle formulations which have little chance of success. Factors such as robustness, stability and batch-to-batch reproducibility (many formulations in the TransInt consortium did not survive transfer between laboratories and others did not give reproducible results between batches) and compatibility with the peptide of interest at amounts predicted to give the desired systemic effect (e.g. loading capacity, maintenance of native peptide structure, protection of the

peptide against chemical and enzymatic degradation, release of the peptide at the desired rate) can be investigated in most laboratories at relatively low costs. Early consideration of such *in vitro* liabilities could save resources by reducing the number of unnecessary studies in intestinal barrier models of various complexity as well as *in vivo* studies.

**4.3.3.5. Ingestible injectable devices.** To further exemplify the interest in oral delivery of peptides in an attempt to offer patients an alternative to injectable parenteral administration, researchers and companies are investigating and developing “ingestible injectable” devices, hence moving the invasive administration site from the subcutis, which has a high density of sensory neurons, to the GIT, which is devoid of such receptors. Examples include the self-orienting millimeter-scale applicator (SOMA) and luminal unfolding microneedle injector (LUMI) designed to inject the peptide directly into the gastric or intestinal epithelium respectively and reporting double digit bioavailabilities [292,293]. The most established of these ingestible injectable technologies is the “robotic” RaniPill™. Recently released details on its first Phase I trial reported an oral bioavailability of octreotide greater than 70%, with an acceptable tolerability profile [294]. Although the Phase I study reported the administration protocol to be safe, “Real World” evidence of the use of these kinds of devices needs to be generated as issues related to obstruction rates and injury following multiple, long term dosing needs to be generated. It also needs to be evaluated whether the additional cost-of-goods and the manufacturability of these “ingestible injectable” devices can be accommodated to enable the successful clinical and commercial translation of these devices.

**4.3.3.6. Future outlook.** Despite extensive efforts having been made over the past 30+ years to enable oral administration of peptides, there has been little success since the approvals of cyclosporin and desmopressin (DDAVP, a synthetic vasopressin analogue) in the 1990s beyond a number of candidates entering late stage clinical testing (see Table 3 for examples). These candidates have almost exclusively used TPE's as the strategy to enable systemic absorption of peptides following oral administration, with a couple of exceptions being silica particles and the hepatic-directed vesicle (HDV), and a lipid-nanoparticle carrier (both in Phase II as of mid-2020). Despite this hiatus, in the last year we have seen approval of two oral peptide products, Rybelsus® (semaglutide; September 2019) and Mycapssa® (Octreotide; June 2020). The former, semaglutide, is an engineered peptide designed to have a long half-life, thereby able to accommodate the high variability associated with oral peptide delivery. It remains however to be seen whether peptide engineering will finally enable TPEs (or indeed other strategies described above) to be used as a routine strategy for oral peptide delivery. Even if so, bioavailabilities are still likely to be very low (low single digit) and cost of goods hence a challenge.

To overcome these limitations, recent focus has turned to more “disruptive technologies” with the aim of achieving systemic bioavailabilities similar to sub-cutaneous delivery but with the convenience of oral administration. Notable developments in this area have included the “ingestible injectable” devices recently appearing in high impact journals and reporting double digit bioavailabilities [292,293] and the report that the RaniPill™ was able to achieve 70% oral bioavailability of octreotide whilst being well tolerated in a Phase I study. It will be interesting to see the adoption of such “disruptive” technologies by both industry and patients, and whether these innovations will ultimately revolutionize peptide delivery.

## 5. *In vitro* and *in silico* tools for exploring advanced drug formulations

### 5.1. Introduction and scope

Although advanced formulations are used to solve various hurdles encountered in drug development, these benefits come at the expense

of stability issues and complex behavior in the dynamic conditions of the GIT. Events such as supersaturation, precipitation and formulation digestion often render classical *in vitro* and *in silico* tools inadequate for the study or description of the fate of the drug formulation after ingestion.

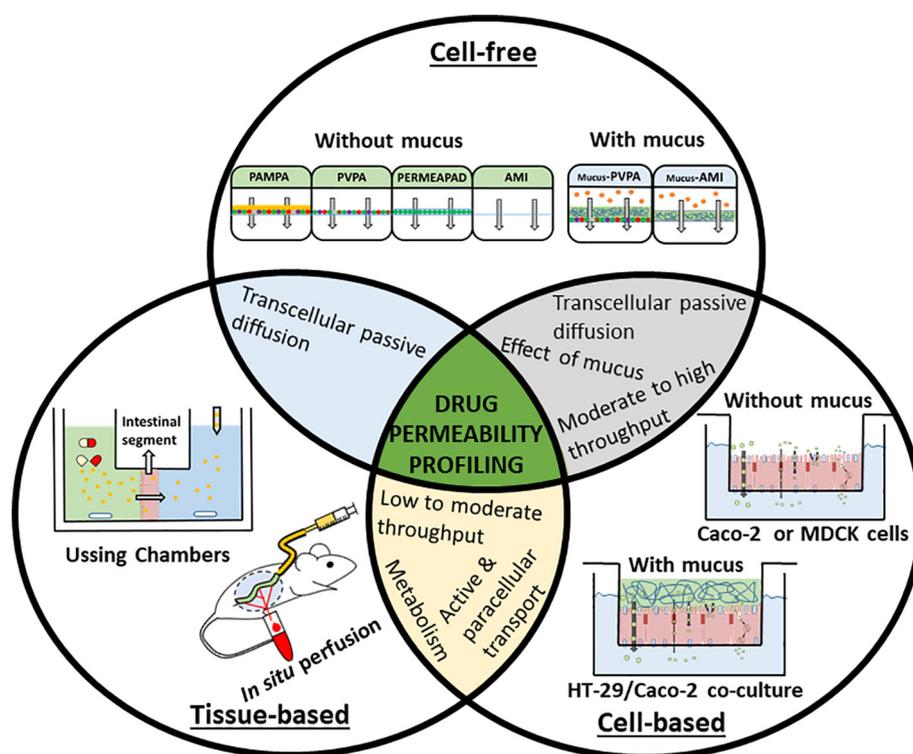
Therefore, biorelevant tools with improved capacity to predict the *in vivo* behavior of such formulations are required. In the following paragraphs, controversies in biorelevant dissolution, permeation and combined dissolution/permeation models are addressed, followed by an update on cutting-edge analytical methods used in this context. Afterwards, a vision for the combination of *in vitro*, *in silico* and *in vivo* data for the prediction of the performance of oral drugs is presented. Finally, the current limitations and future opportunities in PBPK modeling are discussed. The specific applications of PBPK modeling in the context of special populations, regional differences, advanced formulations and food-drug interactions is situated in the corresponding sections of the paper. The main gaps and the proposed way forward in the area of biopharmaceutical tools are summarized in Fig. 9.

### 5.2. Biorelevant dissolution testing

The development of biopredictive dissolution tests aims at realistic simulation of mechanical and chemical conditions of the GIT. The development follows one of two main strategies: complex devices mimicking the GIT anatomy such as the dynamic gastric model (DGM) [295], TNO-TIM-1 [296] and tiny-TIM [297], or simple abstract models. The main advantage of complex devices is the representation of the GIT conditions by simultaneous simulation of multiple factors. Unfortunately, these experiments are time-consuming, costly and have low throughput. Here is where the simpler models come in: although they do not reflect the anatomy of the GIT, they can successfully simulate individual physiological factors *via* working principles and parametrization [298,299]. Some examples are the rotating beaker apparatus [300], BioGIT [301], bio-relevant dissolution stress tester [302], or its later modifications such as Gastro Duo [303] and the Dynamic Open Flow-Through Apparatus [304,305]. The devices were successfully evaluated on numerous examples of clinical relevance [302,306–310]. Use of the bio-predictive methods enabled effective support of the development of dosage forms, identification of undesired drug delivery performance of formulations, such as dose dumping or decreased availability of the drug and checking the pharmaceutical equivalence of various products [298,302,306,308,309,311–315].

The key issue in biopredictive evaluation is the realistic simulation of the conditions along the GIT, especially considering the variation of the physiologically relevant factors. Therefore, the appropriate test scenario is as important as the device itself. The pragmatic question is what are the truly “biorelevant” conditions to simulate? Temperature, pH gradients and pressure values along the GIT have been studied with the use of telemetric capsules [316–318]. However, the results should be treated with caution as the reliability of measurements might be compromised due to technical reasons (*i.e.* construction, measurement range and size). Additionally, the results confirm high intra- and inter-subject variability. Consequently, there cannot be a universal test protocol covering the wide range of pH values, intensity of mechanical agitation and passage times in one experiment. Applying average conditions is not justified either as they do not reflect any of the physiological situations. The biggest challenge to overcome is finding a way to implement the variability range of the conditions of the GIT passage into a set of biopredictive experiments.

Another problem to solve is the simulation of GIT fluids. Commercially available solutions simulating both fasted and fed state are easy to use and provide reproducible results. However, they neither reflect pH variability among GIT nor enable performing analysis in high-resolution pH gradients [307,319]. It should be also noted that most dissolution tests are performed in unrealistic high volumes to provide sink conditions [20]. This does not reflect the physiological conditions and



**Fig. 8.** Overview of the currently available permeation tools used for intestinal permeability profiling. Permeation tools are subdivided into three classes including tissue-based, cell-based and cell-free permeation tools supplemented with their common characteristics.

can lead to the omission of phenomena such as supersaturation or precipitation, compromising the predictability of the test [20,320,321].

As the impact of the compositional variability of GIT fluids on drug solubility is well established [26,322], sets of multiple media defined by a design of experiment approach have been developed to tackle the issue [23,323].

An additional important issue is the impact of dynamic digestion and its products on drug delivery performance, especially in case of

lipid-based formulations [27,324]. Simple dissolution tests performed with a single biorelevant media with a constant composition usually do not reflect the digestion and might significantly underestimate the delivery rate.

To our understanding the point by point simulation of every individual GIT passage via extremely complex models is unreasonable. It is crucial to understand the interplay between the working principles of the formulation and GIT conditions to determine rate-limiting conditions.

## The biopharmaceutical toolbox

### Gaps

GIT digestion dynamics and variability are only integrated in complex, low throughput models

*In vitro* permeation models do not capture the true permeation rate and complexity of the intestinal barrier *in vivo*

Combined dissolution/permeation models are not sufficiently validated

Real-time analytical methods to study supersaturation are available, but rarely used

The scientific integrity of the middle-out approach in oral PBPK modeling is questioned

### Way forward

Novel approaches (e.g. organoids) should be applied to develop and validate predictive permeation and dissolution/permeation tools

Further development and validation of simple *in vitro* dissolution models that combine clinically relevant aspects of GIT dynamics and variability

The benefits of real-time analytics to reveal the release mechanisms of advanced formulations should be convincingly demonstrated

Best practices and transparent policy should be adopted to increase confidence in PBPK modeling

**Fig. 9.** Biopharmaceutical tools summary.

One approach to test formulations would be by using simple models to evaluate the influence of individual physiological factors, simulated as a set of consecutive experiments. Careful selection of proper dissolution setup from a pool of various tests should be based on their usability range and limitations. The problem of high variability of the GIT transit can be overcome by covering the boundary conditions (e.g. the 25th and 75th percentile) of pH gradients, intensity of mechanical stress agitation, time of passage, etc. It would lead to determination of the variability range of the drug delivery performance that might be expected *in vivo*.

Finally, the challenging goal of truly bio-predictive dissolution testing can be achieved only by integration of advanced techniques and pragmatic interpretation of the outcomes.

### 5.3. Permeation models

In combination with solubility, permeability is a key parameter dictating intestinal drug absorption following oral administration, and as a result should be carefully explored during early phases of drug discovery and development. As illustrated in Fig. 8, a diversity of well-established *in vitro* permeation tools is available to obtain a first estimation of intestinal permeability, including cell-based, tissue-based and cell-free models. Comprehensive information on the advantages and limitations of the different approaches is available in dedicated reviews [325–327].

Most of the developed permeation tools result in a good estimation of the rank order of human intestinal permeability, however, the outcome is highly affected by the experimental test design [328]. Extensive efforts have been made to demonstrate the importance of selecting the appropriate media in the donor and acceptor compartment in order to maximize drug recovery and consequently, offering improved reliability of the apparent permeability results [329,330]. For instance, pharmaceutical companies typically use apparent permeability coefficients obtained in cell-based (Caco-2, MDCK, LLC-PK etc.) assays to support their drug development programs. However, these cell-based permeation tools are not advised to be implemented as a permeation compartment in *in vitro* dissolution models for the evaluation of absorption-enabling formulation strategies given their (i) moderate throughput, (ii) lengthy and cost-consuming preparation steps and (iii) limited compatibility towards solubilizing pharmaceutical excipients and food components. Cell-free permeation barriers are considered as attractive alternatives to be used in combined dissolution/permeation tools for assessing formulation performance, as these artificial membranes are compatible to biorelevant media, easily resist pharmaceutical excipients, food components and a wide range of pHs [14]. Unfortunately, only a limited number of case examples are described in literature implying that more efforts are needed before the full potential of these combined dissolution/permeation systems could be achieved. Particularly for the evaluation of lipid-based drug delivery systems (LbDDS), as already mentioned in the “Advanced formulations” section of the paper, *in vitro* tools combining dissolution-digestion-permeation evaluation are scarce due to the complexity of properly capturing the continuously changing intraluminal environment during lipid digestion. In addition to a cell-based lipolysis-permeability system using Caco-2 cell line as the permeation step [331], several cell-free systems, i.e. Permeapad [332], PVDF filters treated with lecithin in *n*-dodecane solution [333] and mucus-PVPA (phospholipid vesicle-based permeation assay [334]), have been utilized in these combined model systems. Further development of combined lipolysis-permeation models is however required to obtain better predictions of the *in vivo* performance of LbDDS [188].

For lipophilic compounds, intestinal mucus is considered as an additional physical barrier that these compounds must cross before permeating the intestinal epithelium layer. Several approaches were introduced to investigate the impact of mucus on intestinal drug permeation including mucus-producing cell lines, collection of mucus from

animals or humans and the artificial preparation of mucus simulating the *in vivo* mucus layer [335,336]. However, it is widely reported that the composition of the mucus layer is changing according to the region within the intestinal tract and between the luminal mucus layer and adherent mucus layer which has an impact on its rheological properties and barrier functions [337,338]. Further, several diseases are also known to influence the mucus properties. Therefore, it becomes challenging to produce a model able to mimic physiological mucus, and the differences between native and reconstituted mucus can lead to variations in the obtained drug permeability outcome. A standardized approach mimicking different types of mucus would thus allow to improve our mechanistic understanding of the impact of mucus characteristics on intestinal drug transport.

In addition, it should be noted that the currently available permeation models cannot mimic the true permeation rate observed *in vivo*, due to a surface-to-volume ratio that does not correspond to *in vivo* conditions: an aspect that is particularly important for the combined dissolution-permeation models and is discussed in more details in the next section.

These comments clearly indicate that the multitude of factors affecting oral drug absorption will always complicate the use of predictive *in vitro* experiments. Indeed, the dynamic aspect of the GIT environment is not well captured by most *in vitro* tools, usually considering the GIT as a static environment with constant hydrodynamics, constant pH, high GIT volumes and constant concentrations of surface-active substances (e.g. bile salts, phospholipids, lipid digestion products) contributing to colloidal structures. A single comprehensive tool mapping the full dynamic and complex nature of the human body, or even the intestinal absorption process will probably never exist. However, recent advancements in the development of intestinal organoids are aiming to bridge the gap between two-dimensional cell-based models and *in vivo* models, thereby drastically improving physiological relevance and *in vivo* similarity resulting in improved drug permeability evaluation. Despite their complexity, intestinal organoids might be useful to increase our mechanistic understanding for various specific biopharmaceutical challenges and improving *in vivo* predictions [339]. Furthermore, *in vitro* experiments are typically designed using default conditions representing averaged data from healthy volunteers for predicting performance of drug products intended for a specific disease. It is not surprising that intestinal permeability might be altered in specific diseases compared to healthy volunteers [31]. This could result in a misprediction of drug product performance in the targeted patient population and may potentially be a cause for discontinuing the project due to insufficient understanding of the problem. Consequently, it would be relevant to focus on the development of disease-specific *in vitro* (permeation) tools considering the GIT physiological modifications by the disease of interest which would finally result in better performing drugs for the targeted disease.

### 5.4. Combined dissolution-permeation models

The assessment of drug permeation together with dissolution testing could provide valuable mechanistic knowledge that could be used for better *in vitro-in vivo* correlations, especially when considering food effects or advanced oral formulations with complex behavior in the GIT.

In recent years, a range of dissolution-permeation assays has been developed (Table 4) [14,340–346]. These typically consist of a donor and receiver compartment separated by a membrane (i.e. cell monolayers [342,347], lipid (bi) layers [340,342,344–346] or dialysis membranes [14,341]), mimicking the intestinal epithelium. In some of these devices, intact dosage forms can be evaluated, whereas other devices require downscaling of the dosage form (e.g. to powders) or processing (e.g. pre-dispersion). Several dissolution/permeation setups can be used in existing tools including the USP 2 [340] or the  $\mu$ DISS

**Table 4**  
Dissolution/permeation setups.

	Barrier	A (cm <sup>2</sup> )	V (mL)	A/V (cm <sup>-1</sup> )	Formulation	Setup	IVIVR	Time (min)
IDAS2 [343]	Caco-2	2.26	500	0.005	Intact	Continuous	Yes	120
MacroFLUX™ [340]	lipid layer	3.8	1062	0.004	Intact	Continuous	Yes	240
MicroFLUX™ [346]	lipid layer	1.54	20	0.08	Down-scaled/processed	Continuous	Yes	300
Vertical membrane flux [345]	lipid layer	4.9	5	0.98	drug	Continuous	Yes	120
Hollow fiber module [341]	dialysis membrane	100	50	2	Intact	Continuous	ND	300
AMI-system [14]	dialysis membrane	4.91	0.7	7.38	Intact	Discontinuous	Yes	60–120
Permealop® [344,350]	Permealop®	27.6	20	1.38	Down-scaled/processed	Continuous	ND	360
Digestion-permeation setup [342]	lipid layer /Caco-2	27.28	20–60	0.45–1.35	Down-scaled/processed	Continuous	Yes	30–90

A: absorption surface, V: donor volume, A/V: absorption-surface-to-donor-volume-ratio, IVIVR *in vitro in vivo* ranking determined, ND not determined. Partly adopted from: 'Dynamic dissolution-/permeation-testing Martin Brandl UNGAP/NordicPOP training school *in vitro* tools for evaluating the intraluminal and absorption behavior of advance drug formulations Odense, June 17<sup>th</sup> 2019'.

profiler™ [345,346], although, the routine implementation of such methods in the pharmaceutical industry is limited.

Some of the reasons for the limited implementation of these novel tools by the industry are poor validation, laborious preparation and low throughput. For example, validation has often been performed for one or a few compounds and formulation strategies and exploration of food effect has only been done sporadically [340,348]. Resource optimization (materials and time) is also critical: cell- and tissue-based setups are usually associated with lower throughput and feasibility issues, compared to the more simple, membrane-based assays [14,341,346]. Hence, the adoption and routine use of dissolution-permeation tools to support formulation development has yet to reach its full potential.

The permeation rates achieved in the dissolution-permeation models are another important issue. In order to achieve a sufficiently high mass transfer, setups should be optimized with regards to geometry and hydrodynamics to avoid aqueous boundary layers that limit drug permeation, especially for lipophilic compounds. In addition, optimal absorption-surface-to-donor-volume-ratios (A/V) should be applied: commercially available setups provide A/V values between 0.004 and 0.08 cm<sup>-1</sup> whereas academically developed tools, reported in the literature, reach values of 0.45–7.4 cm<sup>-1</sup> (Table 4) which more closely resembles the A/V in the human small intestine. Amidon et al. have estimated that the A/V in the human small intestine ranges between 1 and 14 cm<sup>-1</sup>, depending on the degree of compression of the cylinder-approximated intestinal geometry [349]. Evidently, sink conditions, affected by the composition of acceptor media and the donor-volume-to-acceptor-volume-ratio, are also crucial to maintain the flux [329,330].

To facilitate implementation, existing approaches need to be optimized with respect to their predictive value. This comprises the use of *in vitro* data to predict *in vivo* PK, potentially through PBPK modeling. For certain formulations, *in vitro* data needs to be generated with the intact dosage form, as scaling down or processing can alter release and/or dissolution profiles.

Moreover, it would be of interest to downscale and automate dissolution-permeation assays. This has been successful in the past for other biopharmaceutical tools (*i.e.* solubility testing, solid dispersion selection and precipitation inhibition). High throughput screening capabilities could be explored to satisfy the needs of biopharmaceutical assessment although exploration of intact dosage forms might prove to be challenging.

Finally, it should be mentioned that to really simulate the *in vivo* process of drug absorption, both the dissolution and permeation rates achieved in the *in vitro* setup must mimic to an adequate extent (*e.g.* at least in the order of magnitude) the corresponding processes in the human GIT. A potential mismatch in the dissolution and permeation kinetics could lead to results that are not biorelevant and would require careful analysis before any conclusions regarding the *in vivo* mechanisms of drug absorption can be drawn based on the *in vitro* data.

### 5.5. Novel real-time analytics for supersaturating formulations

Supersaturating drug formulations are currently viewed as the most promising to enhance the oral absorption of poorly soluble drugs. These approaches include lipid-based systems, solid dispersions, as well as other formulation techniques [351]. Such systems typically produce complex colloidal dispersions in water that are important to understand along with the release kinetics of the drug [352]. While much progress has been made to study overall formulation performance *in vitro*, there is still limited understanding of such colloids formed in the GIT fluids and how they interact with the drug, endogenous bile salts, and phospholipids.

Different on-line, in-line and (to some extent) at-line analytical tools can facilitate real-time analysis of supersaturating formulations. The spectroscopic or other analytical techniques can be implemented in classical compendial as well as non-compendial *in vitro* tests. It is possible to use for example immersion probes or flow-through cells for real-time analysis of drug release and different methods were reviewed in the literature [353,354]. While ultraviolet (UV) fiber optical probes are these days widely used, other approaches such as UV imaging, Raman or Fourier transform infrared (FTIR) spectroscopy or laser scanning techniques have been used only occasionally for drug release/precipitation analysis [353,354]. It is important to clarify what these analytical methods can offer to biopharmaceutical testing as well as to learn about these emerging new tools.

The different analytical probes have their particular advantages, but also conceptual limitations. UV probes, for example, struggle with analysis of optically dense samples despite some improvement through the preprocessing of raw data and multivariate data analysis [355]. For in-line dispersive Raman spectroscopy, such data treatment allows measurements in complex media, such as digested lipid-based systems, but extensive calibration work is required when drug release is monitored in solution parallel to the kinetics of optional precipitation [356]. While Raman spectroscopy is not particularly disturbed by the presence of water, this is different in case of FTIR for which sophisticated sample cells were used to study how dosage forms interact with aqueous media and how the drug is then released [357]. An even higher level of sophistication is generated with synchrotron-based analysis of colloids formed during dosage form disintegration and drug release [207]. This is certainly very attractive from a research perspective, but is hardly accessible for routine laboratory practice.

It would be desirable, especially for supersaturating formulations, to measure the free drug fraction as this drives the absorptive flux across the intestinal membrane. Ion-selective electrodes have been used for this purpose and enabled measurement in turbid media [358]. However, an obvious disadvantage is the limitation to the ionized drug fraction. In summary, the different real-time analytical methods provide to some extent congruent, as well as complementary, information. All techniques have their individual strengths and limitations for

laboratory implementation. Therefore, combinations of probes are of interest and evaluation of new sensor technologies is encouraged.

An emerging analytics procedure for *in vitro* testing is fluorescence analysis. There are many techniques of fluorescence spectroscopy and interestingly, they have hardly been explored to study supersaturating formulations. Lynne Taylor's group reported on such an analytical fluorescence approach to study supersaturated solutions from solid dispersions [359,360]. The researchers employed fluorescence lifetime and steady-state fluorescence spectroscopy to study felodipine as it is autofluorescent. The lifetime of free felodipine was shorter in simple media compared to rich aggregates or in crystals. Subsequently, a reporter molecule (PRODAN) was further used to study its fluorescent lifetime in presence of liquid-liquid phase separation of the model compounds clotrimazole and efavirenz [359].

Another research group used fluorescence quenching of celecoxib and in particular the introduced (modified) Stern Volmer analysis appeared to be highly interesting to study colloids emerging from solid dispersions [361]. The accessibility of celecoxib by the quencher was studied in physical mixtures as well as in solid dispersions and results were highly interesting to achieve an improved understanding of drug-polymer interactions of the dispersed amorphous systems [361].

Interesting is also a recent application of diffusion-ordered  $^1\text{H}$  NMR to characterize the colloid species in biorelevant media and to study surfactant-bile salts-drug interactions, which is promising to achieve a better understanding of drug precipitation from supersaturating formulations even in the absence of real-time measurement [362].

A further emerging technique is diffusing wave spectroscopy (DWS) that was earlier used as a process analytical technology in pharmaceuticals [363]. More recent research applied DWS for the first time to *in vitro* testing of solid dispersions and this technique bears much promise from a biopharmaceutical perspective [364]. The obtained microrheological information by DWS was found to be complementary to drug concentration kinetics studied by UV-fiber optical analysis, which is a good example of how different analytical tools can be combined [364].

There are certainly other analytical techniques that bear potential for biopharmaceutical *in vitro* testing and more research is needed to develop viable and complementary real-time analytics that can also be industrially implemented in drug release/precipitation testing.

### 5.6. Combining *in vitro*, *in silico* and *in vivo* data: ambitions for prediction of oral product performance

Oral drug product design requires a thorough understanding of the attributes which are critical for consistent performance in the GIT environment. Traditionally, formulation scientists have relied on *in vitro* dissolution to guide product development with confirmation of product performance coming from *in vivo* preclinical or clinical studies. More recently, the limitations of compendial dissolution methods to adequately predict *in vivo* performance has been widely recognized and a number of alternative systems have been designed, as described in more details in Section 5.2. Whilst undoubtedly useful for profiling formulation performance and informing decisions on formulation design aspects, the interplay between different variables can be difficult to deconvolute cleanly as is necessary to establish a primary scientific relationship to critical material and quality attributes.

The quest to improve predictive tools for oral absorption will need to consider different paths towards delivering a step-change in our ability to simulate the GIT and absorption processes. Significant, incremental advances have been made with the adoption of new biorelevant media [13,365], combined dissolution-permeation systems [343,366–368] and models of the GIT, which integrate physiological variables such as motility, intraluminal pressure and dynamic digestion [304,369–371]. Innovative engineering solutions continue to be developed to accommodate physiological factors within *in vitro* GIT simulators [372] and biologically based solutions such as intestine on a chip

models [373] or intestinal organoids [374] offer orthogonal approaches for assessing oral absorption potential.

However, can one realistically anticipate the development of a truly holistic and readily accessible model absorption system which simulates the totality of the physiological environment and integrates physiologically-relevant hydrodynamics, transit and motility, dissolution, digestion and absorption? The challenges facing such a task are amplified when we begin to consider how we would adequately accommodate true population patient variability incorporating physiological and disease state factors. The use of computational approaches to predict oral absorption offers an alternative path forward to assess the myriad of variables that need to be considered in tackling this challenge [15,375–377]. However, the typical inputs for such software packages capture the physicochemical properties of the drug and do not routinely consider the impact of formulation or how excipients can modify the biopharmaceutical performance of the active substance [378]. Combining dissolution results, ideally derived from a biorelevant method which incorporates appropriate physiological variables, with a computational approach is an extended solution to address this limitation [379–386] but is limited in the sense that it delivers a bespoke solution for the compound of interest. To extend this concept of combined *in vitro* and *in silico* analysis to a broader compound set would require a multi-component first principles approach to be applied to all those properties which determine bioperformance for both the drug and the formulation. Such a functional biopharmaceutics profile “2.0” dataset would need to move beyond static single point measurements for solubility and intrinsic dissolution to a range of measurements which capture the true range of clinical inter- and intra-subject variability. The same concepts apply to dissolution measurements and in some cases (possibly where active mechanisms predominate) to permeability values. For ionized molecules, more nuanced datasets are required to fully evaluate the potential impact of precipitation processes on dissolution. Studies of supersaturation and precipitation phenomena are widely reported in the literature [214,387,388], and several reports describe successful predictions of *in vivo* impact using *in vitro* data to guide *in silico* models [389–393]. However, the ability to accurately predict solid form, particle size and re-dissolution for precipitated weak bases after gastric transfer remains beyond the current capabilities of the typical biopharmaceutics toolkit. Another factor which requires consideration is that much of our focus remains on physicochemically profiling the drug, yet the conversion to drug product brings with it a number of interactions and potential transformations which are very relevant from a biopharmaceutics performance perspective. This is clearly exemplified when we consider just two key properties for dissolution – drug particle size and surface properties. Formulation processing can significantly impact the final particle size in drug product through attrition or agglomeration events. In some cases, it may be possible to utilize *in vitro* dissolution to determine the effective particle size in drug product and use this information as an improved input for absorption modeling [394,395]. Surface properties of the drug are also altered by formulation composition and processing. Lubricants have long been known to have the potential to adversely affect the dissolution properties of a drug and have come under the spotlight again in recent times with the trend to move from batch to continuous processing sometimes resulting in extended lubrication levels due to the mixing technologies deployed with some continuous processing platforms [396]. Empirically determining such effects may be necessary to better inform predictive models of product performance. Excipients can also positively impact the surface wettability of drug and thereby improve product bioperformance [397]. Other opportunities exist to produce a functional biopharmaceutics profile with a richer dataset than that of a traditional general pharmaceuticals profile and which critically inform the formulation scientist of the properties which are essential for consistent bioperformance. Placing a focus on the functional biopharmaceutics aspects of the drug to drug product development transition will enable more sophisticated risk assessments to be performed than currently

achievable with a conventional approach. Returning to the question posed on the future prospects for the development of a truly holistic model of oral absorption, it is proposed that a more feasible near-term target is to consider combining an end-to-end mindset and enhanced technology toolkit to create a functional biopharmaceutics profile, assembled from a better informed composite array of *in vitro*, *in silico* and *in vivo* data, which reflect the pharmaceutical complexity of the drug product and the physiological variability of the patient.

### 5.7. Biopharmaceutics modeling and simulation: limitations and strengths

PBPK modeling is an evolving toolbox with multiple applications in drug research. In the following paragraphs, we report two emerging points of view that can advance the field by (1) scrutinizing the methodological approaches used or (2) focusing on the application in industrial settings and translation to regulatory decision-making. The ensemble of both views presents a holistic picture of the field that spans from challenges in technical implementation to successes in real-world drug development applications.

#### 5.7.1. Limitations and threats in current PBPK modeling approaches

The limits and threats in PBPK modeling are mainly attributed to two factors: drug-by-drug parameter optimization (fitting) and publication bias. These will be considered in the next paragraphs. Then, a discussion on other methodological and conceptual issues will be presented.

The parameter optimization of a PBPK model using clinical  $C_p$ -time data is generally referred as “middle-out” approach that is different from the “bottom-up” approach based on *in vitro* data and “top-down” PK parameter calculation. There are two kinds of middle-out approaches, “global” and “local (drug-by-drug)”. According to the principles of mathematics, the number of coefficients identifiable from observed data is limited by the degree of freedom and the model structure (parameter identifiability) [398,399]. In the case of the global middle-out approach, parameter identifiability is usually well taken care of (usually more than 4 drugs per parameter). Li et al. provide a good example [400].

However, the local middle-out approach is controversial. The issues of the local middle-out approach have been pointed out by FDA [162]. An oral PBPK model consists of a lot of parameters and equations, whereas an oral  $C_p$ -time data of a drug offers only limited information (the model is said to be “overparameterized”). Therefore, an *i.v.* PK data arm is required to reliably calculate clearance, volume of distribution, and bioavailability of a drug (this is the invariable principle of PK). However, the local middle-out approach is often performed without *i.v.* data [401–405].

The local middle-out approach is usually used when the initial bottom up prediction failed, because of inappropriate drug input data, default physiological parameters, incorrect model equations *etc.* In a significant number of case studies of oral PBPK modeling reported in the literature, parameter optimization has been used [401–409]. When the initial bottom-up prediction fails, a user of commercial software may first suspect the drug input data. However, the physiological parameters and/or model equations are not necessarily correct. Several kinds of parameters can be altered in a drug-by-drug manner, until the simulated  $C_p$ -time curve matches the clinical data. In the past,  $P_{eff}$  and the precipitation rate tended to be the target of parameter optimization as these are not reliably predicted from *in vitro* data [401,406,407]. Recently, scaling factors have been employed for parameter optimization (c.f. scaling factors should be constant among drugs [400]). In some cases, even physiological parameters were optimized in a drug-by-drug manner [403]. In almost all cases, the failed prediction by default setting is not shown, and the optimized data is published and being referred as “predicted”.

The use of parameter optimization or an empirical scaling factor brings empirical statistic modeling into PBPK modeling (semi-empirical PBPK model). Therefore, we should follow the practice of empirical

statistic modeling. The parameter optimization function is provided in commercial software and recommended in the literature. However, before performing parameter optimization, the identifiability of the parameters must be carefully examined. The degree of freedom must be enough to avoid overfitting. The prediction skill should be evaluated by cross-validation. The initial input parameters and simulation curve before parameter optimization must be reported. A semi-empirical PBPK model that is specifically optimized for a drug and a clinical situation should be used as interpolation within a limited parameter space defined by the clinical data used for parameter optimization. Optimizing one parameter hides the errors of the other parameters (the errors of the other parameters are carried over to the error of the back-calculated parameter).

With respect to the publication bias, the issue lies in the fact that usually only the successful results from drug-by-drug studies are published and used for regulatory submission. It is well known that a case study is not regarded as reliable evidence in the medical sciences. Systematic studies are required to populate a reliable knowledge base (like randomized controlled trials). Systematic validation studies have been performed for the bottom-up prediction [17,410]. Similarly, systematic validation studies are required for the local middle-out approach using a standardized procedure.

A number of critical gaps in knowledge and conceptual methodological issues can be identified in PBPK modeling. For example, the “local” middle-out approach has not been systematically validated. It is not well known when and under which condition, a parameter in a complex PBPK model can be reliably identified from  $C_p$ -time data.

In addition, the interpretability of a model is often ignored. A “black box” is often used. A “glass box” can be indecipherable when it is too much complicated (“messy glass box”). In mathematical modeling, PSA has been utilized to interpret black-box models, such as deep learning. PSA could also be a useful tool for PBPK modeling. However, relying on PSA alone may inhibit a deeper understanding or an insight into the system. PSA can be used as a support to diagnose the structural identifiability. However, being a sensitive parameter is a necessary but not sufficient condition to be structurally identifiable. Being an insensitive parameter means that the clinical data offers no information about the validity of the parameter. The knowledge about the rate-limiting step is more important for formulation development. Once we know the rate-limiting step of oral absorption of a drug, sensitivity analysis is not required, because it is trivial that the parameters related to the rate limiting step become a sensitive parameter. The sensitivity analysis is necessary for a “black box” or a “messy glass box” model.

A PBPK model consists of a lot of parameters and equations (several dozens to hundreds, or even thousands). Therefore, its descriptive power is tremendous. It can perfectly reproduce every observed  $C_p$ -time data by retrospective parameter optimization. However, a good descriptive power does not mean a good predictive power. Even though it is counter intuitive, as the number of model parameters increases, the predictability often decreases because the errors of parameters and model equations exponentially accumulate as the model becomes more complex. Therefore, practically, a simpler model often shows a better predictive skill [410]. In science, this paradox has been solved by applying the Occam’s razor principle. This principle has been applied to PK, population PK, and PK/PD area [411]. Therefore, the same principle should also be applied to oral PBPK modeling. A simple model should be used unless a more complex model shows statistically significant improvement by systematic studies for prospective prediction.

A local middle-out approach is usually used to simulate RBA such as the food effect and the gastric pH effect. In these cases, one or two compartment models can be used to simply describe the post absorptive processes.

It is critically important to improve the literacy of modeling and simulation. A publication guideline is required for PBPK modeling. The scientific validity of a research article is the author’s responsibility, even when using commercial software. All model equations and

physiological parameters should be provided by the authors to properly perform peer-review by the referees and the readers who do not have access to commercial software. The model must be a “glass box”.

Current commercial software products show markedly different prediction characteristics, even when using the same input drug data [410]. When two commercial software products show different results, which one should be used for regulatory submission? A potential solution is to use harmonized model equations and physiological data sets. The model equations and physiological parameters should be open and freely accessible to everyone to enable public check. At the same time, commercial software vendors can provide a useful graphic user interface and various utility tools. This business model has been successful in chemistry and the fields, maintaining the scientific integrity while promoting the use of the model for the benefit of society [412–414]. A systematic validation study of the local middle-out approach should be performed before it is used for regulatory submission.

### 5.7.2. Progress in PBPK modeling for regulatory questions

The concept of the PBPK model was first published in 1937 by Torsten Teorell [415]. However, the approach remained unexplored for 40 years until, towards the end of the 1970s, a few academic papers appeared. In the 1990s scientists working on environmental chemicals began to apply and publish on PBPK and eventually in the early 2000s use within the pharmaceutical industry started [416]. Shortly after 2010, use of PBPK picked up and a rapid rise was seen in pharmaceutical related publications until 2017, when both the FDA [417] and the European Medicines Agency (EMA) [418] released draft guidance documents. Initially, regulatory use of PBPK at the FDA was limited to the evaluation of metabolism-related drug–drug interaction potential, specifically to substrates of cytochrome P450 (CYP) 3A and 2D6 enzymes [419]. This was supported by confidence in this area of application established based on systematic analyses for multiple compounds. For example submissions from 9 sponsors for 15 different substrate PBPK models were reanalyzed at the FDA to support the reliability of application for metabolic related DDI [420] and subsequent analyses have provided further support [421]. Confidence in other areas of application was limited due to the lack of sufficient experience of the regulators in these areas due to a more limited number of submissions received [419]. In contrast, workers in industry had higher confidence in broader applications, including those related to drug absorption where PBPK was predominantly used to answer questions related to company internal decision taking [422].

Expansion of the areas of application of PBPK for regulatory questions has continued steadily, building upon the collection of further examples and the sharing of combined analyses for different types of application. Thus, use for prediction of changed PK due to CYP3A enzyme induction is supported by an analysis of 11 substrate PBPK models, developed by six sponsors [423] while multiple published analyses performed in the past 10 years have supported the application of PBPK for scaling of PK to children [424–427]. While oral absorption modeling was initially of lesser impact for regulatory questions, this situation is changing. Recent publications have presented examples where consistent methods have been applied across a range of compounds to simulate food effects [409,428,429] and gastric pH-dependent DDI [430,431] and the potential for wider application is well recognized [432,433].

Regarding the steps needed to build confidence in PBPK modeling for specific applications, it is notable that the PBPK models used to waive clinical studies for metabolic DDI are rarely purely bottom up models which rely solely on *in vitro* data. For example, prior to use of a PBPK model to waive a clinical study with a moderate or weak CYP3A4 inhibitor, it is recognized that the model must first be verified (and potentially optimized) using data obtained in a clinical study with a strong inhibitor. This type of middle-out fitting to clinical data requires that best practices are developed and aligned within the PBPK modeling community. Best practices cover the need for PBPK model

input data of sufficient quality, appropriate level of verification of models with clinical data, the degree of parameter optimization which is acceptable and confirmation of the plausibility of the final model used for predictions [434]. This process of definition and alignment of best practices is key to the advancement of PBPK ensuring that decisions taken based on modeling are made with sufficient confidence. Therefore, open discussion at scientific conferences and workshops has been essential [435,436] and continues to be key as the application to biopharmaceutics attracts more interest [433,437]. A recent publication of and requests for comments on a draft guidance covering “The Use of Physiologically Based Pharmacokinetic Analyses-Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls” [438] is a further step towards wider application of PBPK for regulatory questions.

## 6. Food-drug interactions

### 6.1. Introduction and scope

The administration of drugs at mealtime is a decades-old problem in drug development, due to the frequent and sometimes extreme effects of food (positive or negative) on drug PK. As the food–drug interactions can occur on many levels and stages of drug action in the body, they still remain very difficult to predict both *in vitro* and *in silico*. Hence, a number of aspects of the current knowledge on food–drug interactions are critically examined and questioned in the following paragraphs. The uncharted area of gut microbiota interactions with pharmaceutical compounds and the possible significant impact on drug PK are also explored in detail, providing valuable insight on an emerging topic in the domain of oral drug absorption. The impact of dietary fiber–colon interactions on the general well-being and drug absorption is also discussed. Finally, the opinion of two COST actions focused on food and nutritional supplement-related topics that can interact with intestinal drug absorption provides a wider, interdisciplinary view on the topic and demonstrates its significance to the general audience. The main gaps and the proposed way forward in the area of food–drug interactions are summarized in Fig. 12.

### 6.2. Administration of drugs with food as a strategy to increase oral bioavailability

The observation of strongly positive food effects in the case of various oral anticancer drugs was the starting point of an intense debate about the intake recommendations for these drugs [439–441]. For drugs such as abiraterone acetate or sonidegib, the oral bioavailability after a high-fat meal can be up to 10 times higher than in the fasted state [442,443]. However, since this food effect may strongly depend on the type of food consumed and is therefore difficult to control in real life, many oral anticancer drugs with positive food effects are recommended to be taken on an empty stomach [444]. In this way, the risk of undesired overdosing shall be minimized. Various scientists argue, however, that for drugs such as lapatinib the co-administration together with food can reduce the pill burden and thus, drug therapy costs [445]. In addition, an enhanced drug absorption may also reduce the risk of local side effects in the GIT. The question is therefore: how can we use the occurrence of a positive food effect for the benefit of the patient?

The occurrence of positive food effects can be triggered by various physiological effects that are caused by the intake of food and/or caloric liquids [3]. These include increased concentration of bile salts in the small intestine, delay of gastric transit or the presence of dietary lipids. For many drug products, however, the key mechanism driving the food effect is often unknown. Thus, the food effect is hardly controllable since concrete, actionable recommendations cannot be derived for the patient. Against the background of very different dietary habits and disease-related changes in the GIT, the administration of drugs with a

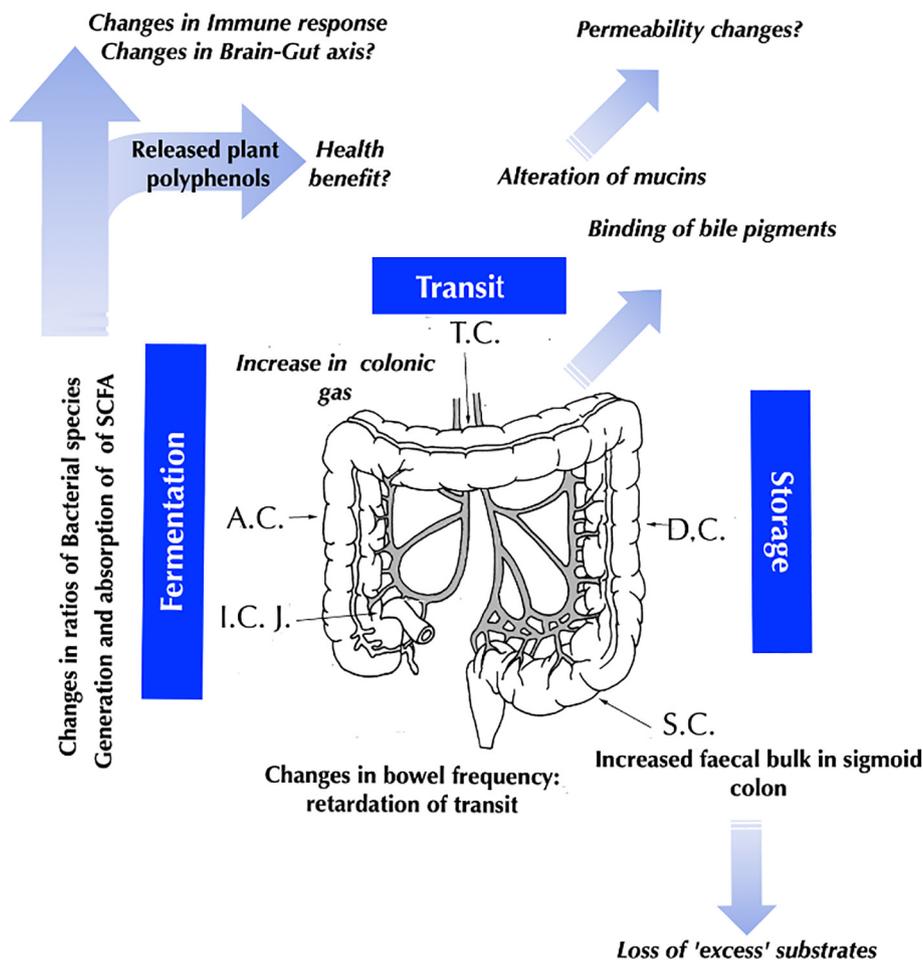


Fig. 10. Physiological factors associated with the intake of fibre.

narrow therapeutic index in the fed state can pose an incalculable risk in real life as strong intra- and inter-individual fluctuations in drug  $C_p$  may occur. In particular, the recommendation to take a drug with a well-defined meal that shall lead to a certain food effect, should remain an exception. Such a procedure is hardly practicable in the long run (e.g. due to polypharmacy) and also has negative effects on the patient's compliance.

It is therefore necessary that we closely examine the mechanisms leading to the occurrence of food effects. Thanks to various initiatives such as the EU project OrBiTo (Oral Biopharmaceutics Tools) or the IQ consortium, a whole range of powerful *in vitro* and *in silico* models are already available to study and predict food effects [27,409,446]. Thereby, the selection and application of a suitable biorelevant *in vitro* or PBPK model should be based on the properties of the drug substance

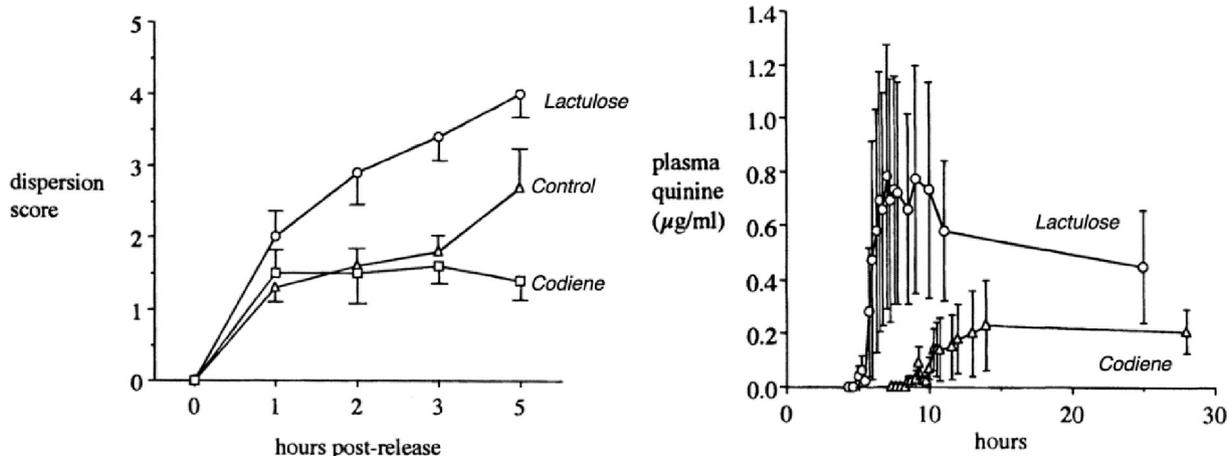


Fig. 11. Effect of Lactulose and codeine on dispersion of  $[^{111}\text{In}]$  labelled Amberlite resin in the colon and systemic concentrations of quinine delivered by a colon targeted device. Reproduced from Hebden et al. with permission [489].

## Food-drug interactions

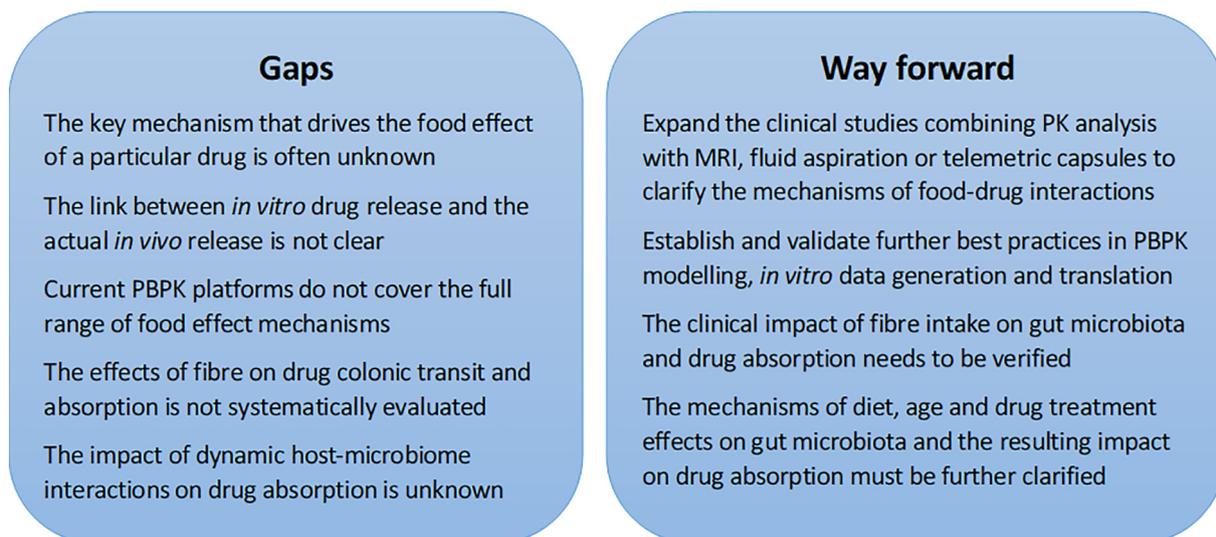


Fig. 12. Food-drug interactions summary.

and the formulation as well as on the potential cause of the food effect. Unfortunately, a one-size-fits-all approach is currently not available. To further improve and optimize these tools, a deeper understanding of the various processes occurring after oral drug administration in the human GIT is needed. Amongst others, this also includes the transit and release behavior of orally administered drug products in both fasted and fed state. In this respect, combined studies in which classical PK analysis is combined with a specific imaging or diagnostic method (e.g. MRI, aspiration, telemetric capsules) can provide further insights into the mechanisms leading to the occurrence of food effects [447]. However, in many cases the link between the *in vitro* release behavior of an oral drug product and the actual *in vivo* release is still unknown. To improve the physiological relevance of the *in vitro* data, the different methods used to assess drug release and absorption need further optimization and validation based on *in vivo* data. In addition, the PBPK models must be optimized as well and should be increasingly used to generate and verify hypotheses.

If the mechanism of a food effect can be identified with the help of these tools, an attempt should be made to minimize the effect by changing the formulation. In the sense of an effective use of the administered dose, however, the formulation strategy should aim at maximizing the bioavailability in the fasted state and not at minimizing the bioavailability in the fed state. This can be done, for example, by advanced or 'enabling' formulations. In the past, it has already been successfully shown that a food effect can be minimized by certain formulation concepts [448].

In conclusion, the recommendation to take the drug together with a meal represents a barely controllable risk, especially in the case of drugs with a narrow therapeutic window. Instead, a better understanding of the mechanisms leading to food effects on oral drug bioavailability is needed to minimize food effects in the drug development process. For this purpose, we need a deeper comprehension of human GIT physiology along with improved *in vitro* and *in silico* tools able to describe and predict food effects.

### 6.3. Application of physiologically based models to predict food effects: current status, strengths and limitations, next steps

PBPK modeling is often applied for the prediction of food effects using the advanced absorption models in commercial software

platforms such as GastroPlus™ and Simcyp®. However, despite multiple published examples for individual drugs [449–451], the health authorities still lack confidence in predictions and neither the recently updated FDA guidance on the conduct of food effect studies [452] nor guidance from other agencies have mentioned the utility of mechanistic studies of food effect using *in vitro* and *in silico* models. This mismatch in confidence assigned to PBPK applied for food effects currently hampers regulatory impact.

Current PBPK model platforms cover only a subset of the full range of mechanisms which can lead to food effect and so predictions may be reliable when the predominant food effect mechanisms are captured but will be poor if key mechanisms are missing. To assess confidence in the food-effect prediction for a particular drug and formulation it is important to identify the major mechanisms a priori based on, for example, molecular properties and *in vitro* measurements and also to establish a consensus on the mechanisms where PBPK predictions are reliable.

The success of simulation approaches is also critically dependent on the quality of input data, which should be generated using biopredictive methods. Definition of best practices for assay selection and measurement methods for generation of input to models for prediction of food effect is needed. Furthermore, appropriate translation of parameters from *in vitro* to *in vivo* needs to be applied, which may involve model-based analysis of data generated with biopredictive methods [16]. The mismatch in confidence levels between industry and regulators is also, in part, due to a lack of agreed best practices for model development. Therefore, a broad effort to define a consistent modeling strategy for food effect prediction and to agree on the definition of adequate verification data is required.

To this end, a recent cross-company effort proposed a decision tree for model verification and optimization and supported this strategy with five case studies [409]. The strategy did not recommend complete elimination of clinical food effect studies via PBPK modeling but rather proposed a streamlined approach where verified food effect models could be leveraged to project effects into different dosing situations e.g. new formulations or different dose levels. Expanding on this work, food effects for 30 diverse drugs were analyzed in a larger cross-industry consortium study [428]. This study performed a well-controlled assessment of PBPK food effect modeling, minimizing confounding factors, such as inconsistent data generation, subjective

model verification/optimization and variable modeler experience. The compound types and mechanisms, which could be modelled with confidence, were found to be those related to changes in GIT motility, pH and luminal fluids (e.g. changes in bile salts).

A recent UNGAP review on the “Food-Drug Interface”, summarized the many different mechanisms that can lead to PK food-drug interactions considering also formulation performance and how specific food types can lead to food-drug interactions [3]. PBPK models already capture some, but not all, of these mechanisms. However, work is ongoing to verify predictions for the mechanisms which are captured and highlight areas where current *in silico* and *in vitro* approaches are inadequate. Verification needs to continue, and the gaps should be prioritized accounting for the molecule types common in current pharmaceutical development pipelines. Having done this, research efforts should be proposed to fill the gaps and extend the range of confidence in predictions.

#### 6.4. Opinions from other COST actions

##### 6.4.1. Food-drug interactions: a perspective from the INFOGEST network

INFOGEST is an international network on food digestion, gathering scientists from more than 150 institutions in 45 countries. It has published consensus protocols for performing *in vitro* digestion in static [453] and semi-dynamic conditions [454] and has recently underlined the advantages and limits of static and dynamic *in vitro* digestion models [455,456].

The potential for food to affect drug absorption has been known for a long time [457] and multiple examples and aspects have already been covered in the previous section. Although we now have increasing knowledge of how food can affect drug metabolism and absorption, the opposite effect *i.e.* how a drug will affect food digestion has scarcely been studied. This is still a significant knowledge gap that needs to be filled. For instance, it is unclear how salivary stimulants or sialagogues affect bolus formation in the mouth and how subsequent dilution in the GIT affects the kinetics of nutrient release. Also, in the stomach, proton pump inhibitors limit gastric acid secretions and increase the gastric pH. An increase in gastric pH would decrease proteolysis since the optimal pH of pepsin is around 2 and is inactive at pH 3 and would therefore increase the relative activity of  $\alpha$ -amylase that has an optimum above pH 5. However, to the best of our knowledge, this has never been studied in detail. Similarly, laxative agents that evacuate soft-formed stools without griping and loss of water will affect transit time and thus the kinetics of nutrient absorption.

There is therefore a strong need for new research to clarify the effect of food on drug absorption, but also the effect of drugs on food digestion and absorption. *In vitro* models of digestion are crucial tools to unravel these interactions. Among them, static *in vitro* digestion models can be considered as screening tools to quickly see, in simplified conditions, if a drug will interact with a food or vice-versa. Dynamic models are more complex and more physiologically relevant since they take into account factors such as food transit, the evolution of pH and the secretion of enzymes and bile in real time. They are promising tools for diving more profoundly into the mechanisms of interaction between drugs and food. By joining their efforts, UNGAP and INFOGEST will have all the skills needed to fill remaining gaps in knowledge about food-drug interactions and their long-term impact on health.

##### 6.4.2. Absorption of carotenoids: a perspective from the Eurocaroten consortium

6.4.2.1. Carotenoids: versatile bioactive compounds of interest for the food, pharmaceutical and (nutri)cosmetic industries. Carotenoids are dietary components of great importance as natural colorants and, some of them, as precursors of vitamin A. More recently, evidence has accumulated that carotenoids can be involved in health-promoting actions contributing to a reduced risk of developing cancers, cardiovascular disease,

skin-, bone- or eye conditions, and even metabolic disorders [458,459]. Hence there is an increased interest in the context of the development of functional foods and related products, such as supplements used as nutraceuticals. Being one of the main contributors of skin colour (along with melanin), carotenoid supplements are also used as nutricosmetics. Furthermore, some carotenoids (canthaxanthin,  $\beta$ -carotene) have been used to treat skin disorders such as erythropoietic protoporphyria [459–461]. Thus, carotenoids are very versatile compounds that, beyond the food industry, are of interest for the pharmaceutical and (nutri)cosmetic industry.

6.4.2.2. Assessment of the main phenomena related to carotenoid absorption. Beyond matrix differences, the bioavailability of carotenoids depends on factors including interindividual dietary, genetic and physiological differences. The use of *in vitro* digestion models with controlled conditions has become very popular to assess the potential for drug absorption. Nowadays, the methodologies used are often based on the consensus static protocol of the INFOGEST COST Action [453]. This approach is helpful to assess bioaccessibility, which usually refers to the fraction of carotenoid ingested that is potentially absorbable. The term “bioaccessibility” is not commonly used in intestinal drug absorption research; however, it is also being used as an output parameter of TIM experiments (see Section 5.2). Normally, the carotenoid fraction incorporated into micelles is considered as bioaccessible [462]. Bioaccessibility of carotenoids has been shown to be well-correlated to their bioavailability [463–465]. In a typical bioavailability study, a carotenoid-rich matrix is provided to human volunteers and changes in the carotenoid plasma levels are evaluated, either after a single meal [466,467] or over several days. [468,469].

Key phenomena involved in the absorption of carotenoids have been explored in detail in various recent studies. For instance, in order to gain insight into differences in uptake among several dietary carotenoids, Mapelli et al. [470] assessed their incorporation into artificial mixed micelles, as well as their uptake and efflux by Caco-2 cells. The possible involvement of proteins, such as cluster determinant 36 (CD36), scavenger receptor class B type I (SR-BI) and NPC1 like intracellular cholesterol transporter 1 (NPC1L1) in the absorption was assessed in Caco-2 cells. The results were compared to those obtained in GripTite cells (genetically engineered Human Embryonic Kidney (HEK 293-T) cells) [470]. On the other hand, differences in site-dependent intestinal absorption of dietary carotenoids (phytoene, phytofluene, lycopene and  $\beta$ -carotene) have been studied in wild-type C57BL/6 Rj mice. It was demonstrated (1) that phytofluene presented a significantly higher bioavailability compared to lycopene and  $\beta$ -carotene, (2) that  $\beta$ -Carotene was mostly converted to vitamin A in the proximal and median intestine, and that the accumulation of phytoene and phytofluene tended to be more important in the distal intestine [471].

6.4.2.3. Research needs. Being lipophilic dietary components of great interest for well-being promotion, the demand of carotenoid supplements has experienced an important increase in recent years. Since they can adopt pharmaceutical formats, interdisciplinary research by scientists with a background in food science and technology, nutrition and pharmaceutical sciences could contribute to carotenoid-rich supplements with optimized bioavailability. For instance, reduced particle size or formulation with oil could result in a markedly higher bioavailability as compared to that of food sources [462]. However, further studies on the absorption of carotenoids formulated as supplements are needed, including studies on the fate of compounds not absorbed in the small intestine, absorption in various populations (age, ethnic groups, individual with particular diseases/digestive conditions), the influence of habits (alcohol, smoking, sports), the role of the mucus layer which covers the intestinal lining, or the interaction with other compounds (formulation ingredients, dietary compounds, drugs) [465,472]. Within this context, adoption of advanced *in vivo*, *ex vivo*, *in vitro* and *in silico* tools and technologies, as described in previous sections, by the food

science/nutrition fields would boost research and innovation in the promotion of health through the consumption of carotenoid-rich products.

### 6.5. Exploring the impact of colon-fibre interactions on health and drug absorption: historical perspective and recent results

“Dis-moi ce que tu manges, je te dirai ce que tu es”

[Anthelme Brillant Savarin]

Humans in pre-civilisation times moved from living in woodland and patchy forest, encountering harder, mechanically-challenging nutrition including legumes, grain and large nuts which could be stored when food availability became scarce [473]. The modal life span of these paleolithic humans was 68–78 years and death occurred by starvation, traumatic injury or infection. Researching extant tribes, Gurvan & Kaplan identified three established behaviours for sourcing food: hunter-gatherers, forager-horticulturalists and third group including acculturated foragers [474]. In the paleolithic era (the old Stone age), selective genetic pressure assisted the survival and reproductive success of *Homo sapiens*, spilling the species out of Africa into the modern world over an era that spanned from 2.5 million to 11,000 years ago [475]. The agricultural revolution started in the Middle East in the Neolithic era (10000–4500 B.C.) and spread the population globally. Carrera-Bastos and colleagues point out that the period from the end of the Paleolithic era spans only 366 generations, that from the Industrial Revolution seven generations and modern times encompassed within four generations! [476] This means that to understand the ability of the gut to process food, we should always look back in time to our earlier diets because the human genome has not evolved fast enough to cope with starkly changed meals of today.

The function of the colon in nutrition is well versed. Although human beings can survive without a colon, surgical intervention to divert a section of small bowel or stoma surgery and changes to eating pattern are needed to survive. Solubilisation and extraction of nutrients by digestion requires physical processing of the food matrix and enzymatic action. Ease of access of nutrients to the systemic circulation is therefore conferred by homogenization to present a high surface area as in processed foods, snacks and treats. Although this generally increases the energy density of the matrix, processing is increasingly used to generate putative health benefits centred around the consumption of extra fibre.

In a varied healthy diet, there will be some components, principally carbohydrates (soluble fibre) which will resist degradation in the small intestine and be presented to the colon. This will be in addition to insoluble fibre components, collagenous matter, cells and mucus shed by surfaces. The papers by Cummings and others underpin our knowledge of the interaction of fibre with the GIT [477]. The gastroenterologists fed volunteers wheat fibre and examined faecal output. As the fibre intake was increased there was an elevation in faecal fat, calcium and nitrogen output and more mixing occurred in the gut resulting in a spread of co-ingested radio-opaque markers. The faecal weight increased three fold, primarily by water retention in the fibre and the excretion of volatile free fatty acids increased. The identity of the short chain fatty acids – acetate, butyrate and propionate generated by colonic anaerobic bacteria and the redox potential generated after feeding complex carbohydrate diets can be sufficient to produce hydrogen and methane [478]. This is detectable in breath.

The principal sugars unabsorbed in the small intestine were noted as lactose, raffinose and stachyose [479]. These sugars and the sugar alcohols, sorbitol and xylitol are broken down by the bacteria. The total fermentable carbohydrate as sugars, starches and other compounds presented to the colon was estimated to be 5 g per day. Bacterial digestion of fruit fibre releases polyphenols, which *in vitro* screens suggested to have putative anti-oxidant, anti-inflammatory and even anti-cancer

properties [480]; however, the agents such as anthocyanins are released as glycosides and the individual bioavailability may vary according to the host microbiota [481]. It has been speculated that the polyphenols might influence the digestion of the fibres as both anti-bacterial and pro-biotic effects have been recorded [482]. Pre-biotics, fibre-based matrices which increase the growth or control the diversity of microbiota have received much interest and were recently reviewed [483]. The early origin of their health benefit was probably the research showing oligosaccharides in breastmilk are important for the establishment of the infant microbiome. They generally consist of inulin or galactan structures and the benefits are vague since the compounds are weak pharmacophores. The principal finding after longer term exposures is an increase in bifidobacter sp. and perhaps a reduction in harmful bacteria although the latter has not been accepted by the European Commission [484].

The physiological factors associated with intake of fibre, assimilated from a number of authors, are presented in Fig. 10.

Fibre contributes to the viscosity of GIT contents, which may impact on availability of drugs or nutrient. The overall effects are complex because stomach and colon motility may be affected differentially. Lactulose administration (20 mL t.d.s.) has been used to produce a model of diarrhoea [485,486] to study GIT transit of dosage forms, the absorption of drugs and strategies to control diarrhoea. Psyllium is obtained from plantago seed husk and forms a gel that resists digestion and increases stool water. It delays gastric emptying through viscosity effects and reduces the accelerating effects of lactulose by decreasing fermentation rates in the caecum and ascending colon [487]. The colon timed release system ‘Pulsincap’ was used to isolate the colonic drug delivery component [488]. Using the Pulsincap loaded with [<sup>111</sup>In] labelled Amberlite resin, [<sup>51</sup>Cr]-EDTA and quinine dihydrochloride (10 mg), Hebden and colleagues produced models of constipation and diarrhea by treatment for 5 days extending into the study day with codeine (30 mg qds) or lactulose 20 mL tds [489]. The control arm and all treatment arms had controlled fibre intake prior to the study day. Dispersion, drug absorption and urinary recovery were measured in sixteen subjects in a 3-way randomized crossover design using Pulsincaps with 5, 6 or 8 h delay plugs. Lactulose pretreatment resulted in an increase in colon water leading to greater dispersion of the particles and a faster rise to C<sub>max</sub> for quinine as shown in Fig. 11 whereas codeine treatment produced lowest dispersion scores and a decrease in urinary [<sup>51</sup>Cr]-EDTA excretion.

In summary, the food interacting with the colon has passed by the main sites of absorption, but fermentation of complex carbohydrates control glucose utilization, release micronutrients that can be absorbed and alter the microbial population. The intake of poorly fermentable fibres supplanting soluble, fermentable fibres stimulates peristalsis by removing the disruptive effects of gas on the efficacy of weak peristaltic waves in propelling colonic contents towards the descending colon. The individual differences produced in a sustained dietary habit change the response to fibre and diverse exposure broadens the genera of microbes that will occupy the microbiome. The physiological roles of fibre in the gut are diverse and probably affect immunity and many aspects of health status [490].

To revisit the famous Brillant-Savarin aphorism we might consider this conversation between a scientist now and a person from the Old Stone Age:

“Tell me what you eat and I will help you understand how your colon works!”

### 6.6. The role of the intestinal microbiome

The fate and activity of drugs are frequently dictated not only by the host *per se* but also by the microorganisms present in the GIT. While the ever-increasing scientific breakthroughs have highlighted the influence of the gut microbiota on host health, mechanistic insights on the role of

the microbiota on the absorption of orally administered drugs remain relatively understudied. Elucidating the possible mechanisms by which the gut microbiota might influence or contribute to altered drug absorption *in vivo* is thus warranted.

It is being increasingly recognized that the gut microbiota is not static, but subject to dynamic transformations as a consequence of pharmacological interventions (e.g. antibiotic therapy), pathology (gastroenteric and systemic infection), nutritional status, circadian rhythm and environmental influences [491,492]. In addition, the gut microbiome is now known to play a critical role in health homeostasis, and directly influences host metabolic processes and immunological functionality. Inter-individual temporal microbiota diversity, or dysbiosis, is thus of potential clinical significance, leading to changes that may directly impact drug metabolism such as bacterial enzymatic activity (e.g. fecalase) but also indirect changes in host metabolic activity (e.g. change expression of host genes implicated in drug metabolism and/or transport). However, it remains unclear how these changes in gut microbiota will impact drug absorption and bioavailability after oral administration.

While gut microbiota can both directly metabolise drugs and indirectly influence host-metabolising capacity of drugs, as recently reviewed [493,494], the molecular understanding of microbiota-mediated effects on drug bioavailability is still rudimentary. A limited number of reports in the literature have explored the impact of microbiota mediated changes on oral bioavailability *in vivo*. Microbiota mediated changes, *via* administration of the probiotic *Escherichia coli* strain 1917 (EcN) for seven days to rats, significantly increased amiodarone bioavailability by 43% in comparison to saline-treated control rats [495]. The effect was not evident when using a reference non-probiotic *E. coli* strain (non-commensal), which suggests that the microbiota-mediated changes in amiodarone bioavailability may be strain-specific. In the case of gliclazide, the oral bioavailability was significantly reduced 3-fold in rats following three days administration of a probiotic cocktail [496]. Interestingly, probiotic treatment did not influence systemic clearance of gliclazide after *i.v.* administration, which indicated that the microbially-mediated alterations influence the intestinal absorption of this drug. While neither of these studies demonstrated a confirmatory mechanism for altered absorption of these two drugs, it was speculated that altered intestinal transporter/enzyme expression may be involved. In the case of paracetamol, probiotic treatment (*Lactobacillus reuteri*) significantly reduced oral bioavailability by ~30% in mice, whereas drug levels after *i.v.* administration remained unaffected [497]. In this study, a mechanistic basis for the reduced oral absorption was provided: *L. reuteri* was shown to be capable of directly metabolizing paracetamol; this probiotic was also shown to alter arylsulfate transferase and  $\beta$ -glucuronidase activity, which are bacterial enzymes that are specifically involved in paracetamol metabolism.

It is also increasingly being recognized that the gut microbiota plays a role in intestinal homeostasis, such as metabolism, and that the expression of hepatic genes implicated in drug metabolism displays sensitivity to the alteration in the microbiome effects. For example, the production of two short chain fatty acids, butyrate and propionate, by intestinal bacteria has been shown to alter liver gene expression, leading to changes in CYP450 activity in mice [498]. In addition, the intestinal microbiome plays a role in homeostasis of bile acid composition, which can influence bile acid signaling in the intestine, but also affect the solubilization capacity of bile micelles for poorly water soluble drug [499].

Another potential factor influencing inter-individual variation in microbiota-drug associations relates to the impact of drug induced changes of the gut microbiome. Accordingly, coadministration of drugs affecting the metabolic activities of gut microbes may lead to DDI. The interaction between broad-spectrum antibiotics and oral contraceptives represent a classical clinically-relevant example of the potential impact of depletion of gut mediated deconjugation on reducing overall drug levels of estrogens in patients.

However the impact of antibiotic-induced suppression of gut microbiome may also impact other drugs, such as the increased bioavailability of aspirin observed in rats [497]. The potential effects are not limited to antibiotic-induced microbiota depletion, given that any drug that directly alters the GIT environment (e.g. pH and transit time), mucosa integrity, host and bacterial metabolic activity may consequently affect microbiome composition with the potential to lead to DDI [494]. This is particularly relevant given recent reports of the extensive impact of non-antibiotic drugs on the gut microbiota [491]. The study of Maier et al. screened >1000 marketed drugs against 40 representative gut bacterial strains, and found that 24% of the drugs with human targets (i.e. non-antibiotics), inhibited the growth of at least one bacterial strain with 40 drugs inhibiting the growth of up to 10 different bacteria [491]. While the increasing *in vitro* and preclinical studies re-affirms the importance of accounting for drug-induced changes in the gut microbiota as a potential confounder in drug PK there is presently a paucity of studies in humans to support the translation of these findings to the clinical setting. A clinically relevant example is that of levodopa treatment of patients with Parkinsonism, where the presence of the bacterium *H. pylori* decreases the absorption of levodopa. Hence, antibiotic mediated eradication of *H. pylori* was shown to significantly improve clinical symptoms of patients with Parkinson's disease [500]. However, these findings merely explained the potential source in inter-individual variability in Parkinson's disease patients which have a high abundance of *H. pylori*. More recently, the ability of enterococci bacteria harboring tyrosine decarboxylases (TDC) to reduce absorption of levodopa from the small intestine has been reported. Studies in Parkinson's disease patients revealed that the relative abundance of bacterial-derived TDC enzymes in faecal samples was positively correlated with a higher daily dose of levodopa [501]. This study may therefore potentially identify a 'Pharmacomicrobiomics' approach for individualising levodopa dosing in Parkinson's disease patients where gut bacteria, or their encoded TDC gene, may be used as a predictive biomarker to adjust levodopa dosage levels in Parkinson's disease patients. There is clearly a need for more clinical studies to explore further examples of drug-microbiota interactions, as a potential cause of significant inter-patient variability.

The role of the gut microbiota on influencing oral absorption of polyphenol-based nutraceuticals has also been extensively reported. The bioavailability of polyphenols are known to display high inter-individual variation depending on factors such as age, gender, genotype, and more recently gut microbiome composition [502–507]. For example, rutin (quercetin-3-O-rutinoside), is a dietary flavonoid that is converted to isoquercitrin and further to quercetin by the colonic microflora, and therefore overall bioavailability of quercetin is dependent on their cleavage by intestinal microbiota [508]. Variability among individuals in rhamnosidase-producing bacterial strains may therefore lead to inter-individual variability in the bioavailability of quercetin [509]. However, as for other polyphenols, clinical data supporting the link between altered gut microbiota and reduced bioavailability of such polyphenol-based nutraceuticals is still lacking.

In summary, there is an increasing understanding of the dynamic host-microbiome interplay, shedding new insights into disease progression and affording novel opportunities to develop microbiome-based therapeutics. Within this emerging field, there is a need to address gaps in our knowledge on how these dynamic host-microbiome interactions influence drug absorption. Further studies are therefore required to provide more mechanistic insights on how changes in the microbiota evolve, as a result of diet, age, health status and drug treatment, that can lead to clinically relevant changes in drug absorption [498]. On a broader level, the functionally modifiable nature of the microbiome offers the unparalleled opportunity to apply microbiota-targeted interventions and pharmacomicrobiomics tools to address related variability in drug absorption. Further studies on microbiota-targeted interventions, such as probiotics, to reduce interpatient variability are required, including

## COLOTAN: Boosting advanced doctoral training in innovative colon-targeting drugs

### Scientific projects offered to early stage researchers

Chemically modified polysaccharide (CMP) coatings for site-specific drug delivery to the colon

The influence of altered gut microbiota on the metabolism of and drug release from bacterially-triggered film coated colonic drug delivery for patients with IBD: *in vitro* and *in vivo* evaluation

Evaluate the usefulness of innovative orally administered modified release dosage forms for removing potentially harmful agent(s) from the lower intestine

Delivery of peptides, proteins, antibodies and nucleic acids to the colon

Transporter/enzyme assessment in colonic tissue from healthy volunteers and colorectal cancer patients

Influence of intra and extracellular free drug concentrations in the human colon on local and systemic drug exposure

Importance of diffusion, dissolution and release mechanisms for dosage forms delivering drugs to the colonic environment

Metabolomics investigation of the colonic intraluminal environment

Investigating the effect of age, sex, prebiotics, and drug treatment on colonic microbiota and drug absorption

Mechanistic *in silico* modeling of colonic drug absorption

Evaluating the suitability of novel colon-specific *in vitro* models to investigate drug disposition, metabolism and safety in medium to high-throughput setting

Optimisation of *in vitro* and *in silico* tools to facilitate development of colonic delivery systems

Enteroids and cytokines: an *in vitro* model of drug absorption in the inflamed gut

Fig. 13. Scientific projects offered to early stage researchers by the COLOTAN ETN.

precise determination of the optimal concentration, dosage frequency and potential for adverse reactions of these microbiome-based interventions, before they can be validated as effective treatment options.

### 7. Future outlook

The mission to advance oral absorption research by training a new generation of scientists will be sustained by the UNGAP network while exploring several areas of interest in the field: (1) colonic absorption and drug delivery, (2) GIT physiology and drug delivery in the ageing

population and (3) computational and *in vitro* tools for the eradication of animal experiments in drug development. Building on the success of previous public-private research partnership, such as the OrBiTo project (funded under the Innovative Medicine Initiative), which was focused on advanced biopharmaceutics tools and drug developability classifications, these endeavors are integrated in 3 European training network (MSCA-ETN) projects that have been approved for funding in 2020 and are presented briefly below.

COLOTAN is a network for “Boosting advanced doctoral training in innovative colon targeting drugs”. Its overarching goal is to provide

## AGePOP: Drug Absorption in Geriatric Patients and Older People - a training network innovating drug development for the advanced age population

### Scientific projects offered to early stage researchers

The oral formulations swallowability and esophageal transit in older populations

Gastric emptying in older people assessed by salivary caffeine concentrations

The protein digestion and related micronutrient absorption profile in geriatric patients undergone bariatric surgery

Physicochemical characteristics of upper gastrointestinal contents of older people and their impact on oral drug absorption

Physicochemical characteristics of upper gastrointestinal contents of geriatric patients and their impact on oral drug absorption

The real-life dosing conditions of geriatric patients

The effect of malnutrition and polypharmacy in aging on gastrointestinal (GIT) transit time, gastric fluid emptying and pH

Intestinal drug transporters and metabolizing enzymes in older people and in geriatric patients with Alzheimer’s disease

The leakiness of gastrointestinal epithelium of older people and its impact on oral drug absorption

Application of PBB/PBPK software tools to predict oral drug absorption in older people and geriatric patients and development of an absorption risk calculator to assess the probability of adverse effects in older people due to altered pharmacokinetics

Development of open access PBB/PBPK models to predict oral drug absorption in Geriatric Patients and Older People

The oral formulations swallowability and esophageal transit in older populations

Gastric emptying in older people assessed by salivary caffeine concentrations

Fig. 14. Scientific projects offered to early stage researchers by the AGePOP ETN.

## InPharma: Developing an integrated, model-informed, animal-free approach to oral drug product development

### Scientific projects offered to early stage researchers

Optimizing drug co-crystal formulation using *in vitro* dissolution/permeation models

Modeling drug-excipient solubilization interactions to predict excipient selection

Computational tools to predict co-grinding approaches to overcome dissolution rate limited absorption

Molecular dynamics simulations to predict drug supersaturation (Dosup) in microemulsion formulations

Novel therapeutic deep eutectic systems (THEDES)

Machine learning using supersaturating drug delivery systems

Comparison of rDCS to the pre-clinical suspension/solution approach for formulation selection

Predicting the dissolution gains and precipitation risks of salt and co-crystal forms of drugs

Development of an *in vitro* method for evaluating the impact of gastrointestinal (GIT) transfer on performance of enabling formulations under fed state conditions

*In silico* methods for selecting oral formulations and dosing conditions in early clinical studies.

Integrating *in vitro* dissolution-permeation data with biopharmaceutical models to predict the *in vivo* performance of drug formulations

Application of an end-to-end, animal-free modeling approach to the development of enabling drug products: focus on amorphous solid dispersions (ASD)

Developing a novel two-stage *in vitro* lipolysis model to predict impact of digestion on drug absorption from supersaturating formulations

Fig. 15. Scientific projects offered to early stage researchers by the InPharma ETN.

high-level training in drug delivery, drug disposition and GIT (patho) physiology to improve targeting of drugs to the colon. This program focuses on innovative technological and scientific developments across a range of interdisciplinary fields such as (physical, analytical and organic) chemistry, drug delivery, drug disposition, cell biology, gastroenterology, microbiology and modeling and simulation. The scientific projects offered to early stage researchers (ESRs) are summarized in Fig. 13. The COLOTAN consortium consists of leading research groups from universities and from innovative pharmaceutical companies.

AGePOP is a multidisciplinary training and research programme for elucidating “Drug Absorption in Geriatric Patients and Older People”. AGePOP will generate information on the actual dosage form intake conditions for older people and geriatric patients and on physiological characteristics of their GIT relevant to oral drug absorption. Based on relevant data, AGePOP will then deliver novel *in vitro* and *in silico* tools for the evaluation of oral drug products for older people and geriatric patients. The consortium brings together expertise from academia, industry and clinics and will train 11 ESRs to become the first highly-qualified scientists experienced in the development of safe and effective oral drug products for the advanced age population, see Fig. 14 for the scientific projects offered.

Another recent example of a pan-European research consortium that will advance research in biopharmaceutics is the newly announced InPharma European Industrial Doctorate network (funded under the H2020-MSCA-ITN-2020), see Fig. 15. Based on the success of the PEARL project for streamlining formulation development and regulatory tools (funded as an MSCA ITN from 2016 to 2020), the InPharma consortium is working towards developing a fully integrated, end-to-end modeling approach to the development of oral drug products. The research objective is to link data emerging from drug discovery into computational models that can predict the optimal formulation design (in Work Package 1), and subsequently integrate biorelevant *in vitro* input for formulation prototypes into *in silico* PBPK models to predict formulation performance in simulated human clinical trials (Work Package 2), thus side-stepping the use of animals in oral drug development. InPharma will therefore advance the use of computational tools in drug developability decisions, and at the same time support the

collective societal goal of reducing pre-clinical testing of prototype formulations in animals. The InPharma network, will bring together the complementary expertise of six multi-national pharmaceutical companies, five academic research institutions and eight partner organizations to collaborate on achieving the overall project goals.

### 8. Concluding remarks

The challenges in oral delivery of medicines, with appropriate consideration of safety, cost and effectiveness, generates opportunities for enterprise and collaboration. In order to accommodate the need to significantly strengthen and advance drug innovation in support of public health [510,511], adjusted models and the improved organisation of pharmaceuticals research will be needed to generate innovative and appropriate methodology, address niche areas and broadcast knowledge to the public. The UNGAP programme is an example of a vehicle in which information relevant to oral drug absorption and nutrition is freely exchanged, scientists find ways to advance and disseminate knowledge and training for the next generation and discoveries are made.

The research efforts within UNGAP build on a multi-disciplinary approach covering the journey from discovery of a new molecule to the development of the final drug product, applying a broad framework of therapy-driven drug delivery scenarios [408].

Focused on the needs of specific age and patient populations by initiating broad academic/industry collaboration in the development of relevant *in vitro* and *in silico* techniques linked to *in vivo* ontogeny and disease states, important steps were taken in a move towards a more patient-centric drug delivery paradigm. In order to finetune the formulation development approach, the specificities of the different parts in the GIT have been thoroughly characterized and translated into appropriate *in vitro* and predictive *in silico* characterization techniques. The aim was to leverage available and emerging technologies in the understanding of the gastrointestinal absorption of a broad spectrum of compounds including small pharmaceutical molecules, nucleic acids and peptides. The same principles have been applied to study the interaction between medication and food, which resulted in engagement with

other science networks, leading to active implementation of knowledge arising from the intestinal microbiome exploration and novel computational *in silico* simulations.

In conclusion, this UNGAP position paper used the wide membership across European pharmaceutical scientists, nutritionists, gastroenterologists, physical scientists and biochemists to explore five areas, which require interdisciplinary input. These scope the pharmaceuticals-based medicines research from the dose to the patient at a dinner table and allow a better understanding of the drug absorption process. A fundamental problem has been that the gut is a black box, not easily accessible and the absorption process is a conundrum, with rate-limiting processes occurring at various dynamic boundaries which might not be the same in each individual. Once we attempt to increase patient convenience by moving to sustained release products or address the needs of specific patient groups, a new set of problems emerges. Advanced medicines for specific patient populations, with increased specificity in terms of regional targeting with differences observed in the fed or fasted state and sensible *in silico* or *in vitro* tools summarizes in one sentence the global picture we have tackled. In the future, if the models of medicine need to change to further advance public health, an interdisciplinary effort with similar (or even wider) breadth and scope would be required.

### Declaration of Competing Interest

None.

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