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Lipid-associated Oral Delivery: Mechanisms and Analysis of Oral Absorption Enhancement

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Abstract

The majority of newly discovered oral drugs are poorly water soluble, and co-administration with lipids has proven effective in significantly enhancing bioavailability of some compounds with low aqueous solubility. Yet, lipid-based delivery technologies have not been widely employed in commercial oral products. Lipids can impact drug transport and fate in the gastrointestinal (GI) tract through multiple mechanisms including enhancement of solubility and dissolution kinetics, enhancement of permeation through the intestinal mucosa, and triggering drug precipitation upon lipid emulsion depletion (e.g., by digestion). The effect of lipids on drug absorption is currently not quantitatively predictable, in part due to the multiple complex dynamic processes that can be impacted by lipids. Quantitative mechanistic analysis of the processes significant to lipid system function and overall impact on drug absorption can aid understanding of drug-lipid interactions in the GI tract and exploitation of such interactions to achieve optimal lipid-based drug delivery. In this review, we discuss the impact of co-delivered lipids and lipid digestion on drug dissolution, partitioning, and absorption in the context of the experimental tools and associated kinetic expressions used to study and model these processes. The potential benefit of a systems-based consideration of the concurrent multiple dynamic processes occurring upon co-dosing lipids and drugs to predict the impact of lipids on drug absorption and enable rational design of lipid-based delivery systems is presented.

Keywords

Lipid-based formulation; oral drug delivery; lipid digestion; emulsions; micelles; mechanistic model

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1. Overview

Lipid-based systems have garnered a large amount of excitement based on their ability to markedly increase overall absorption of poorly water soluble drugs, potentially resulting in several-fold increases in bioavailability [1–3]. However, there are few examples of commercial lipid-based formulations (LBFs) [4]. This is due, in part, to lack of mechanistic, quantitative guidance regarding when lipid-based systems will enhance bioavailability and how to best formulate drugs to achieve the desired impact. Lipid-based delivery systems can also potentially result in no enhancement, or even a reduction in bioavailability [5–7]. There have been many excellent reviews on lipid-based delivery systems [8–11]. We focus specifically in this review on the quantitative analysis and modeling of the mechanistic aspects of lipid-based formulation function. The key questions that we seek to answer include: 1. What experimental techniques can be applied to understand mechanisms of LBF function, and what key insights have these techniques revealed? and 2. How can modeling be used to relate mechanistic analyses *in vitro* to *in vivo* function and predict the overall impact on bioavailability? Moreover, much of the noted methodology and associated literature is also applicable to food-based lipids, which can also significantly impact oral bioavailability, and in fact share common mechanisms of function with lipid-based drug carriers.

2. The Importance and Under-utilization of LBFs

As more than 50% of commercially available oral drugs are poorly water soluble (PWS), effective solubilization technologies can make a significant impact on oral drug delivery, potentially reducing the required dose and enabling oral delivery of compounds that may otherwise require injection. While the solubility of some PWS compounds may not be increased by lipids, LBFs have shown efficacy in enhancing the bioavailability of several poorly water-soluble drug compounds as well as nutritive supplements [12–20]. Despite this, commercial LBF-formulated drugs constitute only 2–4% of the pharmaceutical market, according to a survey conducted in the United Kingdom, United States, and Japan [21]. The relatively low number of marketed LBFs, despite the potentially large benefits they offer in oral drug delivery, is due to several factors. For example, manufacturing processes for LBFs can employ complex and costly instruments and procedures [21]. Not all compounds are suitable for LBF formulation due to low solubility or stability issues in the formulation components. Additionally, LBFs can result in drug precipitation upon dilution in gastric fluids, thus failing in their intended function of maintaining drug in solution and consequently resulting in poor bioavailability [22, 23]. Moreover, there is an incomplete mechanistic understanding of and ability to predict LBF function in the gastrointestinal (GI) tract. Predictability of the effect of lipids on overall drug absorption is often elusive due to the multiple concurrent dynamic processes impacted by orally ingested lipids. This last challenge is the focus of this review.

Ingestion of LBFs triggers a cascade of processes that change the physical and chemical nature of the gastrointestinal milieu and directly affect the behavior of oral compounds in the GI tract. The digestion of lipids is significant to LBF function due to impact on both oil emulsion droplets and other colloidal species (most notably bile micelles) into which

digestion products partition. Interaction of lipid digestion products with micelles was first observed by Patton and Carey in 1979 [24]. Size and structure of colloids are important to study as they directly affect oral drug partitioning and capacity to serve as a vehicle for drug transport along the GI tract [2]. The insertion of lipid digestion products, such as fatty acids (FA) and monoglycerides (MG) into bile-originating colloids (e.g., micelles, vesicles) causes them to swell, changing the effective volume, chemical nature, and solubilization capacity of the colloidal phase during digestion. The altered colloidal phase can impact drug release kinetics from LBFs into the colloid-rich aqueous intestinal lumen. The increased drug concentration in the aqueous lumen serves as a driving force for drug absorption, often resulting in enhanced bioavailability. Recent studies have shown that fast depletion of the lipid (triglyceride) phase relative to drug release from formulation can result in precipitation [25], potentially making re-solubilization the rate-limiting step in overall absorption. A mechanistic representation of these concurrent processes that may affect overall drug absorption are shown in Figure 1.

Lipids are not only used in drug delivery, but are also a major component of food consumed daily. Thus, understanding the mechanisms that govern lipid-drug interactions in the GI tract would also aid in predicting the food effect on drug absorption, and could allow rational development of food-drug dosing schemes that enhance drug bioavailability. Several marketed drugs known to have a positive food effect on absorption and bioavailability, including oral chemotherapeutics, are taken under fasting conditions despite potential food benefits of reducing dosing and local GI side effects [26]. One reason patients are instructed to take drugs in the fasted state is concern over varying efficacy and potential safety issues associated with the high variability in dietary intake and its impact on pharmacokinetic (PK) profiles. Telaprevir, a Hepatitis C oral drug, was prescribed in conjunction with a meal containing 20 g of fat [27]. However, production of telaprevir was discontinued due to failing demand in relation to competition from other hepatitis C treatments. Enhanced mechanistic understanding and ability to predict drug-lipid and drug-food interactions could enable more effective oral delivery of drug compounds as well as nutritive supplements.

Similar to LBFs, co-administration with lipid-rich food can allow enhanced solubilization capacity of the aqueous intestinal milieu. The colloidal structures present and evolving during lipolysis directly affect dissolution kinetics, as dissolved drug can partition into them. Figure 2 shows the cascade of processes affecting the absorption of a solid drug in the presence of food-associated lipids. It is important to note that the presence of food also impacts other physiological factors of importance to pharmacokinetics such as gastric emptying, blood flow, and pH [28].

There are several other proposed processes, not explicitly shown in Figures 1 and 2, by which lipids affect oral drug absorption. For example, a study by Yeap et al. indicated that release of lipids from bile micelles at the unstirred water layer adjacent to the intestinal epithelium, and their absorption by enterocytes, can result in drug supersaturation and an associated high driving force for drug absorption [29]. Other studies have indicated that lipid emulsifiers such as bile salts and excipients can inhibit drug efflux transporters, increasing effective drug intestinal permeability and overall drug absorption [3, 30]. Another proposed mechanism by which lipids can mediate drug absorption is via engulfment of the nano-sized

colloids by enterocytes through endocytosis, resulting in an increase in drug absorption relative to dosing of drug alone [31]. These examples attest to the complexity of understanding oral drug behavior in the GI tract upon co-dosing with lipids.

The coexistence and interplay among these mechanisms creates challenges for thorough mechanistic and quantitative understanding of the effect of ingested lipids on oral drug processes and overall intestinal absorption. This review highlights the important processes, mechanisms, and associated parameters to be studied and quantified to model and predict the effect of lipids on pharmacokinetic profiles, and the work that has been conducted so far in understanding such processes and parameters. Analysis techniques to improve understanding of impact of lipid digestion and drug-lipid interactions on drug dissolution, partitioning, precipitation, and absorption processes are presented. Efforts to model the effect of lipids on oral drug bioavailability are reviewed, and future work to further improve such models is considered. In particular, we propose that a quantitative model incorporating these multiple, concurrent processes into a system of mechanism-based equations will facilitate prediction of the impact of lipids on overall absorption and rational design of oral lipid-based delivery systems.

3. Key Processes Governing Lipid Impact on Overall Oral Absorption: Mechanisms and Analysis

To build an effective mechanistic model predicting the impact of lipids on drug absorption, it is necessary to understand the physical and chemical nature of the intestinal milieu post dosing, and how the disposition of a drug is accordingly impacted. In this section we discuss the experimental methods used to analyze key processes impacting the intestinal environment and drug disposition post lipid ingestion. These processes, including drug release (from LBF) or dissolution (from co-dosing with food-associated lipids) (section 3.1), lipid digestion (section 3.2), drug-lipid interactions (section 3.3), and drug and lipid transport across the intestinal mucosa (section 4.4), are described separately in the context of dosing a solubilized drug formulated in LBF or dosing a solid drug with lipids. Additionally, we consider the importance of lipids in mediating uptake by the lymphatic system (section 3.5) and in altering drug transit along the GI tract (section 3.6).

It is noted that researchers have investigated ingested lipids over several decades, from understanding and quantifying processes *in vivo* to simulating them *in vitro*. We describe here a few key organizations formed to promote study, understanding, and use of lipid-based drug delivery systems and foods, as they have played integral roles in shaping and standardizing the experimental methods for analyzing lipid-based systems: the Lipid Formulation Classification System (LFCS) consortium, the COST Infogest scientific community, and the Innovative Medicines Initiative (IMI). The LFCS consortium is a non-profit organization that sponsors and conducts research on lipid-based drug delivery systems (LBDDS) for the oral administration of poorly water-soluble drugs [32]. The COST Infogest community was formed to improve scientific knowledge on how foods are disintegrated during digestion, with the hope of designing new foods with improved nutritional and functional properties [33]. The IMI community focuses in the Oral Biopharmaceutics Tools

(OrBiTo) project [34] whose goal is to improve the ability to predict *in vivo* performance of oral drug products in the absence and presence of other co-dosed compounds, including lipids, by developing a fundamental knowledge of the GI environment and predictive tools. While these three groups are interested in mechanistically understanding and describing processes in the GI tract, their overall goals are slightly different, as are the experimental methods and approaches adopted and recommended, as noted below.

3.1. Drug Release/Dissolution in the Presence of Lipids

The most frequently considered and analyzed facet of oral drug delivery in the presence of lipids is the impact of lipids on solubility and dissolution kinetics. Co-dosing of oral compounds with lipids, either as part of a lipid formulation or in food, often alters release/dissolution rate relative to dosing as a solid in the fasted state. While the mixing and flow hydrodynamics of the GI environment is highly complex and poorly understood, simple rather than complex *in vitro* experimental setups are necessary. For example, drug dissolution studies are conducted in well-mixed vessels (e.g., USP dissolution apparatuses) to reproducibly and reliably analyze solubility and release/dissolution kinetics. Results from such studies are used in conjunction with empirical relationships between *in vitro* and *in vivo* dissolution [35, 36]. The development of standardized dissolution media has been a major topic of interest for the pharmaceutical scientific community due to the direct impact of media on release/dissolution kinetics and the need for standardized protocols to enable comparison of studies. Fluid pH and composition vary with location within the GI tract and also with ingestion of food or lipid-based delivery systems [37–39]. Early media used to mimic GI contents, or biorelevant media, included aqueous solutions of salts (KH_2PO_4) that were pH adjusted with NaOH or HCl based on the location in the GI tract being modeled, for example, 6.5–7.5 for the small intestine [40, 41], and 1.2 for the stomach [42]. In 1998, Galia et al. introduced simulated fasted and fed state intestinal fluids containing bile salts and phospholipids, components of bile secretions in humans [43], typically at a ratio of 4:1 to represent relative physiological concentrations [1, 44]. Galia et al. also proposed a 5× increase in concentration of bile salt (sodium taurocholate) and phospholipid (lecithin) to mimic fed state as compared with the fasted state. A postprandial response, or “fed state”, is observed *in vivo* after dosing of at least 7 g of lipid [45] and thus LBFs that contain less than 7g of lipid are tested in a medium mimicking the fasted state whereas food-associated lipids are tested in a fed state medium.

3.1.1. *In Vitro* Simulation of the Fasted State for Analyzing LBFs—Simulated fasted state gastric fluids (FaSSGF) used for LBF study consist of a 0.1M HCl solution at pH 1.2 [42]. FaSSGF are rarely used to conduct drug release testing from LBF due to the negligible release rates observed in these fluids. They are, however, used to conduct stability testing upon LBF dilution in gastric media. To mimic intestinal fluid content, various salts are used to introduce electrolytes that are found in the body and reduce surface tension [46]. While different mixtures of salts have been considered, building on the preparation from Galia et al. [43] the LFCS consortium recommends the use of a 2mM maleate buffer at pH 6.5 and bile components consisting of 3 mM sodium taurodeoxycholate (NaTDC), and 0.75 mM phosphatidylcholine (PC), at a 4:1 ratio [47]. This is within the range (5:1 to 3:1) of bile salt:phospholipid ratios found in normal human bile [48, 49]. Commercially available

simulated intestinal fluid powders that also have a 4:1 ratio of NaTDC to PC can be used to ensure comparable work between groups (biorelevant.com). Table 1 summarizes some of the most widely used fasted state preparations, but there are others that have been proposed [50, 51]. Analytical methods for quantifying drug release kinetics from LBFs are described in section 3.3.

3.1.2. *In Vitro* Simulation of the Fed State—*In vitro* dissolution studies with solid dosage forms simulating fed conditions are generally performed using fed state simulated gastric and intestinal fluids (FeSSGF and FeSSIF) as recommended by the LFCS [51]. FeSSGF consists of an acetate buffer at pH 5.0. The Infogest scientific community, which focuses on understanding the disintegration of a variety of food systems during digestion, uses a relatively more complex solution consisting of a carbonate/phosphate buffer and salts at pH 3.0 [52]. As phospholipids have been found to be present in gastric juices [53], the Infogest community recommends to include small quantities (0.17mM) of egg yolk lecithin when no phospholipids are present in the tested food [52]. Gastric fluid pH is an important parameter as the activity of digestive enzymes is pH dependent. Thus, pH can impact the rate and extent of digestion, and may change the size and composition, and thus the solubilization capacity, of the colloidal structures (micelles and vesicles). Dissolution of drugs that are weak bases may also be affected by pH. Gastric pH has a median pH of 5.0, but does not remain constant due to gastric acid secretions by the chief cells. A pH of 3.0, is suggested by the Infogest community due to maximum activity of the gastric protease, pepsin, at this pH. Thus, the choice of model gastric fluid must be carefully considered depending on one's study purposes.

FeSSIF also consists of an acetate buffer at pH 5.0, and elevated concentrations of bile components (15mM sodium taurocholate and 3.75mM lecithin) compared to fasted state. The presence and high concentration of bile components has been shown to significantly increase the rate and extent of PWS drug dissolution, which increases further in the presence of lipids and lipid digestion products [3, 54]. The Infogest community suggests use of a mixture of bile extract from bovine or porcine bile ducts. Both bovine and porcine bile contain considerably less phospholipid (at a ratio of 10:1 with bile salt [55]), which may hinder comparisons with studies using a 4:1 ratio of bile salt and PC. In addition, while the complex mixture present in native bile is more representative of the intestinal environment *in vivo*, Williams et al. have shown that a more complex simulation of bile salts *in vitro* has little effect on the digestion kinetics of lipids, supporting use of simpler, more cost effective media [47]. Similarly to fasted state preparations, the fed state preparations discussed here are only a subset of the various simulated intestinal fluids used in the literature. They are meant to represent the most commonly used preparations by the scientific community. Experimental methods for quantifying drug dissolution kinetics in the presence of lipids are described in section 3.3.

3.2. Lipid Digestion

Several *in vitro* methods have been developed to mimic GI digestion of food, lipids, proteins, and formulations [56–59]. To facilitate cross-laboratory comparisons, the LFCS consortium has suggested standardized *in vitro* digestion models for LBFs as well as food

substances [47, 52, 60, 61]. Digestion starts with the addition of an enzymatic cocktail prepared from pancreatin extract, and continues while maintaining a constant pH by NaOH titration. The NaOH titration step is automated using a pH-stat instrument, and is necessary to maintain a constant pH due to the release of FA, from the hydrolysis of TG and DG. Therefore, the amount of NaOH added during lipolysis indirectly provides information on the kinetics of FA release under the assumption that equimolar amounts of NaOH are used for the neutralization of equivalent amounts of ionized acidic species (FA^-). Depending on the pKa of the FA and the pH of the medium, often only some of the FA in solution is ionized. As a result, only some of the FA is accounted for by base titration and hence, the amount of FA produced is underestimated. The accuracy of the titration method has been assessed under various conditions. For example, Sek et al. observed that the titration method accurately assessed extent of MCT and LCT digestion in fed conditions, but indicated only 80% of actual FA production for LCT digestion in fasted conditions when compared against more rigorous FA quantification techniques [62]. To allow quantification of total FA content as opposed to ionized FA content, all FA released during lipolysis can be titrated by shifting the end-point pH to 9.0. This technique is generally employed at the end of digestion experiments to quantify the extent of lipolysis, as enzyme function is pH-dependent [60]. It is noted that the presence of other formulation components, such as certain emulsifiers ionizable at intestinal pH (e.g., phosphatidylcholine, whey protein) can also affect the accuracy of the pH-stat titration technique [63]. Despite its limitations [64], pH-stat titration is widely employed in the quantification of FA concentrations due to ease of measurements and calculations.

More rigorous methods for the measurement of FA concentrations have been developed which can also quantify concentrations of other products of lipid digestion such as MG, DG, and TG. Such methods include thin layer chromatography and gas chromatography coupled with a flame ionization detector (FID) [65, 66]. The ability to quantify other digestion products enhances understanding of the mechanism of intestinal lipolysis as well as the evolution of colloidal structures during digestion. More recently, HPLC methods using evaporative light scattering detection (ELSD) [67–69], and charged aerosol detection (CAD) [69–71] have increased the sensitivity and accuracy of quantification of the concentration of TG, DG, MG, and phospholipids in addition to the concentration of FA. Knowledge of the quantities of all products of digestion is clearly required for complete characterization of the chemical composition of the GI environment during digestion. These digestion products alter the stabilization of emulsion droplets and physicochemical nature of the micelles they partition into. Thus this knowledge could ultimately contribute to detailed mechanistic understanding of the impact of the digestion process and associated changes in the intestinal milieu on drug disposition and overall absorption. However, for the purposes of modeling, while some detailed mechanisms of lipid digestion have been proposed and relative kinetic expressions have been developed (as discussed in section 4.3.3 below) the complexity of the kinetics of GI lipolysis and the factors affecting it creates experimental and computational challenges that have resulted in simplistic kinetic expressions generally based on fatty acid production, at best, being employed.

3.3. Interaction between Drugs and Colloidal Structures

While the total amount of solubilized drug in the GI environment includes both free drug and drug that is partitioned into colloidal species (emulsion droplets, micelles, vesicles) at a given point in time, it is generally accepted that only the free drug fraction is absorbed [2]. It is thus important to understand kinetics and extent of partitioning between colloidal species and the aqueous environment of the GI tract to accurately determine the amount of drug available for absorption. Several techniques have been used to measure drug partitioning and associated relative solubility in different phases. Ultracentrifugation (above $100,000 \times g$) is the most widely used technique to separate the lipid and micelle-rich aqueous phase [60, 72, 73], enabling measurement of compound concentration in each phase generally by HPLC. While a simple technique, ultracentrifugation requires long sample processing times during which drug distribution between phases (e.g., lipid droplet, micelles, water) could be altered.

Nuclear magnetic resonance (NMR) has also been employed for measuring drug partitioning in emulsions [74] and offers the advantage of *in situ* analysis without sample processing. Different environments (e.g., water, micelles, oil droplets) result in chemical shifts of the drug NMR peak. Solutions containing a mixture of two or more phases may generate two or more peaks, each corresponding to a phase (Figure 3). This peak separation provides qualitative and quantitative means to analyze drug partitioning [75, 76]. Sometimes, two peaks corresponding to drug partitioned into two phases may not be fully separated. In this case line broadening is observed, suggesting fast drug exchange between phases, i.e. a quasi-instantaneous rate of drug partitioning [77]. There are some drawbacks associated with NMR, including low sensitivity (drug can only be detected in the mM concentration range), limiting application to low solubility compounds. To increase sensitivity, measurement times can be increased up to several hours, possibly compromising sample and colloidal structure stability, and precluding the measurement of partitioning kinetics.

Electron paramagnetic resonance (EPR) has recently been employed to measure compound partitioning in lipid-based systems [78–81]. EPR spectra reflect the nature of the microenvironment of a molecule of interest. While EPR is based on principles similar to those of NMR, it offers the advantage of being highly sensitive (drug can be detected in the μM concentration range) and short sample measurement time (a few minutes), making it a good technique for analyzing kinetics of partitioning. Additionally, the method is nondestructive to the sample. A major drawback of this method is that only compounds containing free radicals can be “seen” by EPR. The vast majority of drugs are thus not detectable by this method. Commercially available stable radicals (spin probes) can be used as “model drugs,” to study partitioning behavior in the presence of lipids and simulated intestinal fluids and also to understand changes in the physicochemical nature of intestinal fluids during lipid digestion [81]. Spin probes can also be covalently linked to drugs to allow their detection by EPR [82]. However, effective physicochemical properties of the compound of interest are altered by this procedure, potentially skewing partitioning analysis of the original drug. EPR spectra can be modeled, and a given model fit for a multicomponent system can be expressed as weighted contributions of the system components, providing quantitative partitioning information in two- and three- phase (e.g., water, micelles, oil) systems (Figure 4). This information is important, for example, for

understanding the amount of free drug available for absorption in a given system, and for calculating the associated partition coefficient.

3.4. Drug and Lipid Absorption into Enterocytes

The relative rates of lipid digestion (i.e. production of MG and FA) and absorption (i.e. removal of MG and FA) determine the lipid content in the intestinal lumen. Understanding of mechanisms and kinetics of lipid absorption is thus important to overall understanding of the impact of lipids on drug absorption. In this section, we describe methods used to understand mechanisms and kinetics of drug and lipid absorption.

Several methods (*in vitro*, *ex vivo*, and *in situ*) have been used to understand the mechanisms and describe the kinetics of absorption of oral compounds. Due to relative complexity of measuring compound absorption kinetics *in vivo*, *in vitro* studies, most notably using Caco-2 monolayers on porous cell culture insert membranes, have commonly been used. Differentiated Caco-2 cell lines have key characteristics of intestinal epithelium, including microvilli and intercellular tight junctions [84, 85], which make them a useful tool for mimicking passive intestinal diffusion. Active transport, however, is only partially represented by this model due to much lower levels of transport protein expression in Caco-2 than in human and animal enterocytes [84, 86]. In an effort to overcome this limitation, researchers have overexpressed specific transporters in Caco-2 [87–89]. While these *in vitro* cell culture systems serve as useful tools for understanding compound absorption mechanisms, translation of *in vitro* results to rate or extent of compound absorption in humans is often not clear.

An additional experimental limitation of using Caco-2 monolayers for studying impact of lipid-based systems on absorption is limited tolerance for bile salts, phospholipids, and fatty acids. As such, scientists resort to very short (1 minute long) experimental timeframes [84], or dilution of these species several fold below physiologic concentrations and consequently, below model bile component critical micellar concentration [90]. To better mimic the mucosal barrier and in particular the mucus layer at the intestinal wall, co-culture systems have been adopted using mucus-producing cells (e.g., HT29-MTX) [91]. Mucus is known to have an impact on diffusive transport of particulate and some molecular species, with the impact being dependent on the properties of the diffusing entity [92–95]. Interestingly, mucus has been shown to be significant to the absorption of certain nutrients, and lipids and lipid digestion products significantly modulate the mucus barrier properties [96]. These examples highlight the importance of consideration of mucus when analyzing compound transport across the intestinal mucosal barrier.

Single-pass intestinal perfusion (SPIP) is an *in situ* technique used for quantifying compound absorption [97]. In this technique, a section of an anesthetized animal's small intestine is cannulated at both ends. The test compound is pumped over time at one end, while samples are collected at the other end to determine the amount of drug absorbed over time. Drawbacks of the SPIP technique include complexity, cost, and limited utility in studies of lipids, as digestion almost completely stops when the animal is under anesthesia. In addition, as they utilize animals, SPIP studies are inherently associated with additional drawbacks including questionable translation to human results and ethical issues.

An alternative *ex vivo* approach to studying compound transport is to use excised intestinal tissue mounted on Ussing diffusion chambers [98–100]. This technique offers the advantage of facile study of absorption on samples from different regions of the small and large intestine. Tissues from sacrificed animals (e.g., rats, pigs, dogs, horses) and from patients undergoing GI surgery, commonly for tumor removal or obesity treatment, have commonly been used for these studies [97]. Absorption kinetics is highly correlated between *ex vivo* and *in situ* studies when transport is passive, but often under- or over-predicted when active transport is significant if animal tissue is utilized. This is, in part, due to differences in transporter types and/or transporter amounts if animal tissue is utilized. In addition, interspecies intestinal pH differences become very important for pH-dependent transport processes [97].

While the absorption kinetics of oral drugs have been extensively studied using *in situ* and *ex vivo* techniques [99–101] there has been less focus on quantification of absorption kinetics of ingested lipids. Knowledge of the absorption kinetics of lipids along the intestine is important in understanding and predicting lipid concentrations in the GI tract, a critical parameter that impacts compound partitioning among the different phases in the lumen and ultimately regulates drug absorption. The mechanism of lipid uptake has been studied primarily *in vitro* using cell culture models [84, 102–107]. The kinetics of uptake, however, has been harder to quantify due to the toxicity of prolonged lipid exposure to such cell culture systems. A few *in vivo* studies in humans have been reported that quantify the kinetics of absorption of FA. A study by Park et al. used single-chain radiolabeled triolein (C18:1) emulsions to monitor the kinetics of oleic acid (product of triolein digestion) absorption, by measuring the rate of chylomicron release into plasma following ingestion of a diet containing almost 40% lipids [108]. A similar study was conducted by Labbe et al., in which human volunteers were fed labeled ^3H -FA and ^{14}C -TG (heavy atom in one of the TG chains only). Equal rates of appearance of the FA label and the TG label in plasma were observed [109]. As TG needs to be digested into FA before it can be absorbed, these results indicated that the digestion of TG in the intestinal lumen was not a rate-limiting step for FA absorption, and FA absorption occurred significantly more slowly than TG digestion. Another study compared the rates of lipid digestion and absorption when patients were dosed coarse vs. fine oil-in-water emulsions [104]. While significant differences in rates of lipid digestion were observed, the amount of triglycerides and chylomicrons in plasma were similar in both cases, again supporting a rate of lipid absorption significantly slower than the rate of lipid digestion.

3.5. Routes of Systemic Transport

After passing through the enterocyte, absorbed compounds can either enter into the circulation via the hepatic portal vein or into the lymphatic system. The majority of water soluble drugs enter into the hepatic portal vein, where they are delivered to the liver and undergo first pass metabolism. However, highly lipophilic compounds have the potential for entering the lymphatic system with ingested lipids. This route avoids first pass metabolism by bypassing the liver, increasing the effective drug concentration in plasma.

Multiple studies indicate that lymphatic transport can be significant for lipophilic drugs and lipid delivery systems [110–118]. There are several review articles that focus on the lymphatic system physiology and function [119, 120], lipid and particulate uptake [121], animal models used to study lymphatic transport [122], and mechanism and extent of drug delivery to the lymphatics [118, 123–126]. It is known that the lymphatic system is the major transport system for dietary lipids in the form of chylomicrons [127], and that lymph flow increases in the presence of lipids [128]. There are still many unknowns, however, such as how lymphatic lipid load varies *in vivo* after a meal [120] or what percent of a co-dosed drug generally enters the body through the lymphatic system.

It has been shown that the type of lipid with which a drug molecule is dosed affects the extent of lymphatic transport. In a study by Caliph et al., halofantrine, a BCS Class II drug, was dosed in lipid-based vehicles consisting of LCT, MCT, or SCT. When administered with LCT, the authors observed that 15.8% of the drug dosed was present in the lymphatic system. When delivered with MCT and SCT they observed that 5.5% and 2.2% of the original dose was present in the lymph, respectively [129]. This study suggested that co-administration with LCT promotes transport via the lymphatic pathway relative to co-administration with MCT or SCT. Other researchers have also observed increased bioavailability of PWS drugs using LCT due to enhanced lymphatic transport [115]. The solubility of the drug in lipid is also a factor in lymphatic transport. Charman et al. proposed that drugs with a logD greater than 5 and a solubility of greater than 50 mg/g in LCT are preferentially transported via the lymph system [130].

Lymph flow transports molecules through the lymphatic system and into blood circulation. Swartz noted that the lymph flow rate is 100–500 times lower than that of blood [119]. The transport of lipids from the intestine into the lymphatic circulation significantly increases the lymph hydrostatic pressure, enhancing the lymph flow rate per Starling's Law [119], and resulting in faster delivery of lymph-associated drug into the bloodstream and altered pharmacokinetics.

Several experimental techniques have been used to study the lymphatic system and its role in transporting compounds and lipids into the bloodstream. Dixon et al. created an *in vitro* system using a Caco-2 and lymphatic epithelial cell monolayer co-culture to study lymphatic permeability in the presence of various lipids [131]. This model was reported to accurately replicate certain aspects of *in vivo* morphology, and may be a useful tool in studying lymphatic permeability of both lipids and drugs. However, it does not give insight into the lymph flow rate or the relative amounts of drug or lipid that enter the lymph system compared to the hepatic portal vein.

Ex vivo methods have recently been developed using excised sections of the small bowel which are connected to a fluidic device that perfuses luminal fluid inside the intestinal segment and vascular fluid on the outside of the segment, while enabling monitoring and collection of lymphatic fluid effluent [132]. This system could enable quantification of the amount of compound that enters lymphatic circulation, as well as enabling analysis of the transport drug and lipids. The downside of this technique is that it requires very specialized equipment, including an intricate system of weights, balances, and pressure transducers.

Different animal models have been used to study lymphatic transport *in vivo*. Rodents are commonly used as animal models for lymphatic studies due to low cost and accessibility. However, rats have different GI physiology, such as continuous bile secretions, which does not reflect human digestive conditions [133]. There are several reviews that discuss different larger animal models, such as pig, sheep, and dog, which have been used to study lymphatic transport [122, 134]. Sheep are not ideal models as they are ruminants, and therefore have very different GI physiology compared to humans. Both dog and pig models have been successfully used to measure lymphatic transport through cannulation of the thoracic lymph duct. Sampling at the thoracic lymph duct can be conducted in a conscious animal and thus in long-term studies, but in such studies the thoracic lymph fluid is returned to circulation, which only allows for lymph sampling and not total lymph collection. Therefore, the absolute mass of the drug being transported by the lymph cannot be calculated. In addition, after absorption at the enterocyte, drug can gain access to lymph fluid both directly and indirectly from systemic blood which may cause overestimation of lymphatic transport sampled from the thoracic lymph duct.

In situ dual optical imaging has been used in rats as a new technique to measure lymphatic transport of lipids through the mesenteric duct [135]. After orally dosing with oil mixed with BODIPY FL dye (a model LCT molecule), a rat is placed under anesthesia, and an incision is made in its abdomen to expose the mesentery (specifically a lymphatic vessel) over an imaging window. Using a fluorescence and bright field microscope, the concentration of BODIPY is assessed over time. Local flow velocity can be determined through lymphocyte tracking. Compounds must exhibit fluorescence to be tracked via this technique. However, the use of anesthesia generally decreases lymph flow rate which may skew results [136, 137]. Arnold et al. were able to sample lymph fluid from awake, unrestrained rats via a surgical implantation [138]. However, this procedure requires advanced surgical techniques and does not allow measurement of intestinal lymph flow rate.

In summary, while a significant amount of work has been done to quantify and study the lymphatic transport of drugs and lipids, the currently available experimental methods are either relatively complex and/or lack a clear translation to relevant *in vivo* phenomena. Further advances are required to enable accessible experimental systems for understanding and quantifying the relative contribution of lymphatic transport to overall absorption of drug co-dosed with lipids.

3.6 Effect of Lipids on GI Transit

The kinetics of processes central to drug-lipid system function (e.g., dissolution, digestion) depend on the compartment (e.g., stomach, intestine) in which the drug-lipid bolus resides as it travels along the GI tract. Every compartment is characterized by specific physiological properties that affect lipid digestion, compound dissolution, and absorption, such as pH, enzymatic content, aqueous secretions, gut epithelial absorption area, etc. As a result, it is important to know the time it takes for compounds to travel through each compartment in order to understand and accurately model the rates and extents of such processes. Transit time is highly impacted by the type and amount of compounds (e.g., water, food, lipids) ingested. Determination of the transit time of compounds in the GI tract has been

experimentally challenging, involving endoscopic intubation and use of measuring probes *in vivo*.

The simplest and oldest method used to determine gastric emptying (GE) and transit parameters in humans is the method of George [139], which employs a non-absorbable marker of a known starting concentration. Upon GI content sampling, the diluted marker concentration can provide gastric volume and transit time information. As the method of George required continuous intubation for extended periods of time, less invasive techniques were desired. Gamma scintigraphy was developed as a noninvasive method that uses a gamma camera to collect images of the abdominal radioactivity distribution. The frames collected over time are analyzed to construct a profile for GE and GI transit [140]. Scintigraphy, however, employs expensive equipment and exposes patients to significant radiation. The ^{13}C breath test uses radiolabelled octanoic acid, which is believed to be readily absorbed by the intestine and metabolized by the liver [141]. The heavy atom is exhaled by the lungs in its oxidized form, $^{13}\text{CO}_2$. The presence/amount of heavy atom exhaled indicates octanoic acid has been emptied into and absorbed by the intestine. While good correlations with scintigraphy have been demonstrated [142], the method operates under the assumption that absorption, metabolism, and release in the lung are instantaneous. The paracetamol absorption test involves determining GE times by collecting and quantifying the amount of absorbed paracetamol present in plasma over time [143], and suffers from a similar limitation as the ^{13}C breath test. As paracetamol is a lipid soluble weak acid with $\text{pK}_a \sim 9.5$, it is neutral at intestinal pH and is rapidly absorbed from the small intestine. As a result, the overall absorption rate of paracetamol is likely dependent on the rate of GE. A paracetamol T_{max} vs. gastric emptying time correlation is used to determine GE.

In the last two decades, more sophisticated techniques have been developed to study the effect of lipids (and other ingested material) on GI transit. For example, telemetric capsules can be ingested and used to collect and wirelessly transmit intestinal pH and temperature recordings. The capsule location can be deduced from pH. For example, a drastic increase in pH can correspond to GE. Since their approval by the Food and Drug Administration in 2001, several capsules of this type have become commercially available, some of which have video capturing capabilities [144]. MRI has also been used to measure fluid volumes and distribution in the GI tract, which have been interpreted to understand transit times as well as distribution of lipids/water in the human stomach [145]. In fasting conditions, such as during LBF dosing with water, recent findings indicate that gastric transit is completed within 15–30 min [146]. In the case of enteric coated formulations transit time may take longer depending on the timing of the peristaltic contractions by the inter-digestive migration motor complex (IMMC). MRI studies have also shown that the wall of the small intestine is generally collapsed, and non-absorbed fluid is located in several fluid pockets along the intestine [28]. Water ingested in fasted state is rapidly absorbed in the intestine, and the amount of fluid in the small intestine remains relatively constant at all times.

GI transit in fed conditions is highly variable as it is affected by the type (e.g., solid vs. liquid), volume, and caloric content of the meal [147]. Solid meals can delay GE relative to liquid meals, and fat content delays GE of liquid meals [148]. Liquid meals containing lipids

may experience different transit times depending on the lipid emulsion stability in GI fluids, despite having the same volume and caloric content [145, 149–152]. It was observed that emulsions that are stable and well-dispersed in the acidic gastric environment displayed longer GE times compared to oil emulsions that were unstable in the gastric phase. Well-dispersed emulsions release into the intestine uniformly [145] while unstable emulsions result in layering of the lipid phase above the aqueous phase in the gastric compartment and biphasic kinetics of fluid release into the intestine (Figure 5). Kunz et al. observed significant impact of order of dosing lipid-free and lipid-rich material on GE time [153]. When ingested lipid emulsions are stable and well-dispersed in gastric fluids, the size of the emulsion droplet has been shown to also affect the rate of GE. Hussein et al. observed that fine emulsions (0.4 μm diameter) containing 60g lipid/bolus (mostly LCT) reside in the stomach longer than coarse emulsions (6 μm diameter) [151]. Similarly, Steingoetter et al. observed that 0.33 μm diameter emulsions containing 80g lipid/bolus (mainly LCT) have a longer residence time than 52 μm diameter emulsions [152]. Additionally, the length (MCT vs. LCT) and saturation level (saturated vs. mono- vs. polyunsaturated) of triglycerides may affect GE rates [154]. In summary, results from MRI studies have highlighted the importance of considering multiple parameters, including fluid volume, lipid concentration, emulsion stability and size, and type of lipid, in understanding the impact of lipids and other ingested materials on GE and intestinal transit.

4. Value of Mathematical Modeling and Existing Modeling Approaches

Mathematical expressions can be used to describe and aid in quantitative characterization of the processes presented above. For example, analysis of digestion kinetics can be used to determine digestion kinetic parameters for comparison between different lipid systems. However, while the experimental techniques and mechanisms discussed above provide useful insight into certain aspects of function of orally dosed lipids, the translation of experimental measurements to the overall impact of lipids on oral absorption is not always immediately clear or obvious. In this section, we review existing frameworks used to model the impact of drug delivery parameters on overall absorption, approaches to account for the impact of lipids or food within these models, and the predictability of these models. We propose that explicit consideration of the impact of lipids on the processes relevant to overall absorption (as outlined above), and consideration of these simultaneous dynamic processes in a systems-based model may enable quantitative prediction of the impact of lipids on drug absorption and bioavailability. We thus review model expressions proposed for these individual key processes and the impact of lipids on the expressions. Using the *in vitro* and *in vivo* experimental techniques described above, parameters for these processes that reflect the physicochemical properties of the drug, lipid or LBF, and the GI environment can be determined and incorporated into these expressions. These mathematical expressions can be assembled and solved simultaneously to understand their concurrent dynamic impact on absorption.

4.1. Frameworks for Modeling Overall Oral Absorption

Dynamic models of absorption of orally delivered drugs taking into account concurrent drug dissolution and permeation across the intestinal wall were first described as a class in 1996

by Yu and Amidon [155]. There are several reviews describing in detail the different dynamic models and software packages in which they are employed [36, 156].

One of the first dynamic models predicting drug plasma concentrations for orally dosed drugs was the Dispersion Model. This model considers the small intestine to be a uniform tube through which contents travel with constant axial velocity and constant concentration profile across the tube diameter. Following this approach, drug concentration in the intestine can be represented using a convection-dispersion non-steady state model [156, 157].

$$\frac{\partial C}{\partial t} = \alpha \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z} - K_a C \quad (1)$$

where C is the concentration of the solubilized drug, z is the axial distance of the drug bolus from the pyloric sphincter marking the start of the intestine, α is the coefficient that accounts for mixing caused by both molecular diffusion and physiological effects (such as peristaltic activity), v is the fluid velocity in the axial direction, and K_a is the absorption rate constant. There were multiple drawbacks to this model. The computational complexity of the model required several assumptions and simplifications (e.g., instantaneous dissolution) to obtain a solution. As discussed in section 3.6, recent MRI studies indicate that fluid flow in the small intestine does not resemble flow in a water-filled tube, but rather transfer of fluid among pockets of different volumes along the intestine [28]. Additionally, the model does not inherently allow for the consideration of the effect of ingested food on drug absorption. Model frameworks developed later separated the GI tract into compartments, eliminating the explicit consideration of changes in drug concentration with distance within a given compartment, and thus the requirement for partial differential equations.

The Compartmental Absorption Transit (CAT) model [155] breaks the human GI tract into a series of well-mixed compartments analogous to continuously stirred reactors (CSTRs) from classic chemical process analysis [156]. Some of the assumptions of this model included minor/no absorption occurring from the stomach and the colon, passive absorption, and instantaneous drug dissolution [155]. In addition, the model assumed that residence times and transit rate constants describing the kinetics of mass transfer from one compartment to the next were the same for all intestinal compartments.

The Advanced CAT model, or ACAT model addressed some of these limitations. It included more compartments (total of 9 including one for the stomach and one for the colon) and enhanced attention to reflection of physiological conditions (e.g., GE, intestinal transit rate). Additionally, 6 drug states (unreleased, undissolved, dissolved, degraded, metabolized, and absorbed), dissolution kinetic expressions, and nonlinear absorption and metabolism kinetics were incorporated. The ACAT model also took into account drug physicochemical parameters such as pKa, solubility, particle size, and density, which were incorporated as parameters in the kinetic expressions. The Advanced Dissolution, Absorption and Metabolism (ADAM) model is another mathematical framework that was developed based on the CAT model, but differs in the type of expressions used to predict dissolution kinetics

and also considers the heterogeneous distribution of blood flow and enzymes in the gut wall [158].

Both the ACAT and ADAM frameworks are termed physiologically-based pharmacokinetic (PBPK) models. These frameworks have been used in conjunction with models predicting drug distribution and clearance after absorption from the intestinal lumen to predict oral PK profiles, including those reflecting interactions with formulation excipients and food. A PBPK model derives its structure from the anatomy and physiology of the organism studied (humans or animals) [159]. PBPK models consist of mathematical equations that represent the kinetics of processes determining the drug fate in the body (e.g., dissolution, absorption, metabolism, transport in plasma and between different tissues) [160]. Therefore, model inputs include not only drug-specific properties such as solubility, intestinal permeability, plasma-bound drug fraction etc., but also parameters characterizing the physical properties of organ and tissue systems under consideration. For example, in modeling the GI tract, volumes, pH values, and transit times reflective of specific GI compartments are utilized [159]. Some of the kinetic expressions relevant to processes occurring in the GI tract (i.e. dissolution, absorption) used in these models will be discussed below.

4.2. The Use of PBPK Models to Predict Lipid Effects

Bioavailability is commonly quantified as the area under the plasma drug concentration vs. time curve (AUC) relative to that obtained upon intravenous injection. Commercially available PBPK software can be used to predict the impact of lipids on drug PK profiles *in silico* [161–164]. These software packages can account for the enhanced solubilization capacity due to the presence of lipids in solution, which affects drug release/dissolution and absorption kinetics. These models provide the option to incorporate precipitation and re-dissolution expressions, which may be relevant for LBF formulated drugs [165]. There are limited examples of comparisons of PBPK model predictions of LBF impact on PK to actual data, making it difficult to assess the robustness and reliability of these models for such applications. In contrast, prediction of the effect of food on drug PK has been explored more extensively.

Several studies have predicted the pharmacokinetic profiles of several drug compounds in fasted state and in combination with a meal using PBPK software packages such as GastroPlus™ [163, 166] and SimCyp® [167]. Food effect predictions were obtained by changing the physiological parameters of the GI tract (e.g., gastric pH, GI transit times) and also the effective solubilities and diffusion coefficients of the drugs, based on *in vitro* measurements in simulated intestinal fluids and empirical expressions related to the concentration of bile components in the lumen [168, 169]. The software were able to predict overall trends in food effect. However, adjustment of certain model parameters, enabled by the availability of *in vivo* animal and human data, was required for accurate prediction of plasma profiles. Additional software including IDEA™, PK-Sim®, and STELLA employ similar frameworks (compartment-based) and have been used to predict the effect of ingested food on drug absorption [167, 170, 171]. Overall, literature reports support the promise of employing PBPK models in predicting food effect, but indicate that models currently widely employed require *in vivo* data to optimize model parameters for confidence

in PK predictions. To the best of our knowledge, the type of food, specifically the amount and composition of food content, is not explicitly accounted for in current PBPK models. In addition, these models do not account for changes in ingested lipids in food as digestion and absorption occur. This is due in part to limited knowledge of the fate of food components (e.g., lipids, proteins, carbohydrates) upon ingestion and how they affect drug behavior (e.g., solubility, distribution, supersaturation, absorption). Explicit incorporation of the interaction between drug molecules and colloidal species present during lipid/food ingestion, digestion, and absorption, as described extensively above, may improve predictions of the food effect. OrBiTo, along with other groups, have emphasized the need for a better mechanistic understanding of food (particularly lipid)-drug interactions in the GI tract [36, 172].

4.3. Mathematical Representation of Key Processes Occurring in the Intestinal Lumen upon Dosing of Drugs and Lipids

In this section we focus on mathematical expressions for modeling key processes determining drug fate in the intestinal lumen, and how these expressions could be altered to explicitly consider drug-lipid interactions, including drug dissolution, drug absorption, lipid digestion, and drug partitioning into lipid-based colloidal species.

4.3.1 Drug Release Kinetic Models—The release of drug by diffusion from a drug reservoir, such as a lipid formulation, into the aqueous gastrointestinal environment can be modeled using a mass transfer expression [173, 174], as shown below:

$$\frac{dC_{aq}}{dt} = -\frac{A_{em}P_{rel}}{V}(C_{aq} - C'_{aq}) \quad (2)$$

where A_{em} is the total area of the lipid emulsions, P_{rel} is the relative drug permeability at the oil-water interface, V is the aqueous volume, C_{aq} is the drug concentration in the aqueous phase, and C'_{aq} is a hypothetical aqueous drug concentration in equilibrium with the drug concentration inside the oil droplets (described more in detail in section 4.3.5). This expression can be modified to account for the impact of LBF digestion on drug release, with the fraction of drug released due to digestion being proportional to the fraction of the emulsion droplet digested [81]:

$$\frac{dC_{aq}}{dt} = -\frac{A_{em}P_{rel}}{V}(C_{aq} - C'_{aq}) + k_{dig}\frac{A_{em}}{V}\frac{V_{em}}{n_{FA}}C_{em} \quad (3)$$

where V_{em} is the total emulsion volume, n_{FA} is the number of moles of FA produced due to lipolysis, k_{dig} is the lipolysis rate constant (see eqn 9 below), and C_{em} is the drug concentration inside the lipid emulsions.

4.3.2. Dissolution Kinetic Models—Drug dissolution, the release of dissolved drug from a solid form, has classically been described using a Nernst-Brunner modification of the Noyes-Whitney Equation [175, 176]. This equation is essentially a solution to a conservation of species equation in Cartesian coordinates, and describes a linear dependence of

dissolution rate on concentration difference between the dissolving particle surface and the bulk concentration:

$$\frac{dC_{aq}}{dt} = \frac{D_{eff}S}{Vh_{eff}}(C_{aq}^{eq} - C_{aq}) \quad (4)$$

where S is the surface area of the solid drug particle, D_{eff} is the effective diffusivity of the dissolved drug into the aqueous environment, h_{eff} is the effective thickness of the boundary layer surrounding the solid particle (Figure 6), V is the volume of the dissolution compartment, and C_{aq}^{eq} and C_{aq} are the drug solubility and drug concentration in the aqueous bulk lumen, respectively. This equation is derived based on the assumption that the particle radius is significantly larger than its boundary layer thickness. However, when the boundary layer thickness is in the same range as the radius of the particle ($r \approx h_{eff}$; generally when the particle radius is less than 30 μm [176]), the conservation of species equation and Fick's law of diffusion in spherical coordinates can be used to derive a dissolution expression [177, 178]:

$$\frac{dC_{aq}}{dt} = - \frac{4\pi r^2 N D_{eff}}{V} \left(\frac{1}{r} + \frac{1}{h_{eff}} \right) (C_{aq}^{eq} - C_{aq}) \quad (5)$$

where N is the number of undissolved particles, and r is the particle radius (Figure 7). To account for non-sphericity of the dissolving particle, a shape factor S is incorporated as a multiplier of the dissolution rate equations [166, 179]. The effective boundary layer thickness, h_{eff} , is dependent on the undissolved particle size as well as the viscosity and relative velocity of the bulk medium along the intestine. The relationship between h_{eff} and these parameters has been empirically determined [176].

The impact of lipids on drug dissolution is important to consider when a solid drug dissolves in the presence of lipids, for example if they are co-dosed (Figure 2). Dissolution can also be important to consider for LBF if precipitation occurs, followed by solid re-dissolution (Figure 1). Some drugs precipitate from LBFs in an amorphous rather than crystalline form typical of solid drug formulations [25]. While the type of solid form (i.e. amorphous, crystalline) can significantly impact dissolution, with amorphous forms experiencing higher dissolution rates than crystalline forms, in both instances this process is modeled using eqns 4 or 5, taking into account the varying solubility of amorphous vs. crystalline forms [180].

Lipids can affect drug dissolution by changing the hydrodynamics of intestinal fluids (e.g., viscosity, agitation), for example by inducing bile secretions and increasing GI transit times. The impact of lipids in the intestine on drug dissolution is typically accounted for using existing commercially available PBPK models by modifying parameters, for example effective diffusion coefficient (D_{eff}), which has been incorporated into a "z" factor determined by measured dissolution rates [181], and drug solubility, in the dissolution expression (e.g., eqns 3 and 4 above). ACAT and ADAM based models employ different correlations to quantify these parameters [166, 167].

An alternative approach to accounting for the impact of lipid-based colloidal species on drug dissolution is to explicitly consider drug-colloid interactions. This mechanistic approach has been shown to enable accurate prediction of the impact of lipid-based colloidal species in simulated intestinal fluids on dissolution of pharmaceutical compounds ranging in physicochemical properties [182]. The presence of bile micelles and other colloidal structures fundamentally affects the dissolution kinetics of solid particles due to partitioning of dissolved drug into colloidal structures at the aqueous boundary layer [183]. The diffusion of micelle-bound drug through the boundary layer is typically slower than that of the free solubilized drug given the larger size of micelles relative to free drug, and can be accounted for with the addition of a second term to the dissolution equation [81, 183].

$$\frac{dC_{aq}}{dt} = \frac{S}{Vh_{eff}} [D_s (C_{buffer}^{eq} - C_{buffer}) + D_m (C_{micelles}^{eq} - C_{micelles})] \quad (6)$$

C_{aq} is the total drug concentration in the micelle-rich aqueous bulk medium, C_{buffer}^{eq} and C_{buffer} are the drug solubility and bulk concentration in the buffer phase, respectively, $C_{micelles}^{eq}$ and $C_{micelles}$ are the drug solubility and bulk concentration in the micellar phase, respectively, D_s is the drug diffusion coefficient in the boundary layer, and D_m is the micelle diffusion coefficient in the boundary layer. To employ this equation, specific knowledge on drug partitioning in micelles is necessary, and can be addressed with measurement and modeling of partitioning (sections 3.3 and 4.3.5).

4.3.3. Absorption Kinetic Models—Transport of oral drugs across the intestinal epithelial layer is modeled, generally, as a combined contribution of passive (diffusive) and active processes. The diffusive process follows a first order dependence on drug concentration in the intestinal lumen (for paracellular and transcellular transport) and carrier-mediated transport is reflected with Michaelis-Menten kinetics:

$$\frac{dC}{dt} = -\frac{AP_{eff}C}{V} - \frac{V_{max}C}{K_m + C} \quad (7)$$

where C is the solubilized drug concentration in the buffer phase, P_{eff} is the effective intestinal permeability due to passive diffusion, A is the intestinal surface area available for absorption, V is the luminal volume, V_{max} and K_m are the Michaelis-Menten coefficients, and C is the aqueous drug concentration in the lumen. The effective intestinal permeability, P_{eff} , reflects both the permeability of the epithelial barrier and the effect of the unstirred water layer [184]. Balakrishnan et al. altered the kinetic model to explicitly incorporate the effect of the aqueous boundary layer as an added resistance to passive and active transport [185]:

$$\frac{dC}{dt} = -\frac{A}{V} \frac{P_{ABL} \left(\frac{V_{max}}{K_m + C} + P_p \right) C}{P_{ABL} + \frac{V_{max}}{K_m + C} P_p} \quad (8)$$

where P_{ABL} is the permeability of the aqueous boundary layer, P_p is the passive intestinal permeability (e.g., obtained from *in vitro* cell culture transport studies), and V_{max} and K_m are the Michaelis-Menten kinetic parameters for carrier-mediated transport. The incorporation of the effect of the aqueous boundary layer at the intestinal wall has been shown to be important in improving *in silico* absorption simulations for some nano-scale LBFs [184]. Yet, it has not been widely explicitly incorporated in commercially available PBPK models due, in part, to lack of quantitative knowledge of the contribution of this barrier to overall resistance to transport across the intestinal mucosa. Indeed, the “unstirred water layer” in the intestine coincides with the intestinal mucus barrier, and knowledge of the impact of this hydrogel layer on drug transport is limited [92, 184].

In general, mechanism-based or empirical correlations incorporating the potential impact of lipids on drug absorption kinetics have not been widely employed, due in part to the limited knowledge of, and challenges of experimentally quantifying their effect on absorption kinetics. Lipid absorption kinetics are also a composite of passive and active effects, as described in section 3.4, but are also not explicitly taken into account in current PBPK models, but rather are reflected in model parameters, most notably altered solubility and dissolution parameters reflecting changes in bile concentrations due to absorption of bile salts [166].

4.3.4. Lipid Digestion Modeling—Quantitative analysis of products of lipolysis has allowed scientists to propose mechanisms for the digestion of lipids and to develop associated kinetic expressions. Verger et al. proposed a digestion mechanism and incorporated Michaelis-Menten kinetics to describe the enzymatic reactions and associated adsorption/desorption steps at the oil-water interface [186] (Figure 8).

This proposed mechanism was extended to incorporate two- or three-step hydrolysis reactions, concurrently with enzyme inhibition, due to the available surface area for enzyme adsorption being coated by products of lipolysis [187]. The impact of ionic interactions between the enzyme and products of lipolysis on enzyme adsorption to the substrate has also been considered [188]. Hermansyah et al. proposed a digestion mechanism for triolein, a long chain triglyceride (C18:1), based on experimental studies using a fungal lipase [187]. In modeling their experimental results, they included the hydrolysis of TG, DG, and MG, (i.e. $TG \rightarrow DG + FA \rightarrow MG + 2FA \rightarrow 3FA$), which added considerable complexity to the reaction mechanism and associated kinetic model proposed by Verger et al. [186]. Although this model explicitly incorporates the multiple hydrolysis reactions important in overall lipolysis kinetics, it is intrinsically complex, with four differential equations and 13 kinetic coefficients. The large number of coefficients increases solution uncertainty and chances of overfitting.

The complexity associated with fully mechanistic kinetic TG lipolysis models has motivated use of simplified digestion models. Li et al. proposed simplified lipolysis kinetic expressions based on a one-step reaction mechanism ($TG \rightarrow MG + 2FA$) and the assumption that the rate of consumption of the TG is directly proportional to the droplet surface area [189]:

$$\frac{dC_{FA}}{dt} = \phi_{\max} \frac{k_{\text{dig}} A_{\text{em}}}{V} \quad (9)$$

where C_{FA} is the concentration of FA produced during lipolysis that is released out in the aqueous environment containing micelles, k_{dig} is the digestion rate constant, A_{em} is the total oil droplet surface area, and V is the total solution volume. As the authors observed incomplete digestion during their *in vitro* experiments, likely due to the inhibitory effect of FA accumulation at the surface of the oil droplets, they also incorporated a fitting parameter reflecting a maximum extent of digestion ($\phi_{\max}$). Buyukozturk et al. modified this expression to explicitly include the inhibition of digestion associated with the coating of the oil droplet surface by lipolysis products:

$$\frac{dC_{FA}}{dt} = \frac{k_{\text{dig}} A_{\text{em}}}{V} - k_{\text{inh}} C_{FA} \quad (10)$$

where k_{inh} is the inhibition rate constant. This model was able to accurately predict digestion kinetics and extent of digestion of soybean oil. This model was also employed to predict the impact of simultaneous drug dissolution and emulsion digestion, as well as simultaneous drug release from a LBF and emulsion digestion, on drug release into the aqueous environment in simulated intestinal fluids [81]. It is important to note that the kinetic coefficients k_{dig} and k_{inh} are not dependent on the activity of the pancreatic enzymes. The coefficients are determined by fitting [FA] production kinetic data at the enzyme activities matching those in humans present in fasted and fed conditions. Hence, fasted state enzyme concentrations would result in different kinetic coefficients (i.e. k_{dig} and k_{inh}) compared to fed state enzyme concentrations.

Giang et al. also described a modified version of the model proposed by Li et al., where they accounted for coalescence effects of lipid particles during digestion limiting the available surface area and slowing lipolysis [190, 191]. Droplet coalescence was determined experimentally using a particle sizer. They demonstrated the proposed model described the experimentally measured lipolysis kinetics of a variety of MCT and LCT. The *in vitro* digestion method used in this study, however, did not control pH due to experiments being conducted in hermetically sealed headspace vials. Most *in vitro* digestion experiments in the literature maintain a constant pH to reflect the relatively constant pH values in the intestinal lumen, and hence this kinetic model may not be able to accurately fit such digestion data.

In conclusion, the kinetics of lipid digestion is complex due to multiple concurrent reactions (Figure 8) and requires considerable experimental and computational efforts for accurate modeling of all relevant steps. This degree of model complexity may not be necessary, however, for capturing the overall rate at which digestion products are entering the aqueous intestinal environment and therefore increasing the capacity of micelles for solubilizing drug compounds.

4.3.5. Drug-Lipid Partitioning Modeling—Drug (and other compounds') partitioning into lipid-based colloidal structures can be modeled as instantaneous, and thus described at any point in time by a partition coefficient relating the concentrations within and outside of the colloidal structures at equilibrium, or as occurring with defined kinetics. Modeling partitioning as instantaneous is appropriate when the kinetics of exchange between phases is very fast relative to other relevant processes occurring in a given system (e.g., dissolution, absorption). This “pseudo-equilibrium” assumption is often employed to account for partitioning between bile micelles and the aqueous phase in the intestinal lumen [81]:

$$K_{m/b} = \frac{C_{micelles}}{C_{buffer}} \quad (11)$$

where $C_{micelles}$ is the drug concentration in micelles defined as the amount of drug in micelles in a unit volume, and C_{buffer} is the drug concentration in the buffer phase defined as the amount of drug in buffer per unit buffer volume.

Modeling a partitioning process as one occurring with defined kinetics is appropriate for processes occurring on a time scale comparable to other relevant processes (e.g., minutes to hours for a drug dissolving and being absorbed in the intestinal lumen). Similarly to LBF release (eqns 1 and 2), partitioning across the oil/water interface in emulsions (such as are present in the GI tract after ingestion of lipids) has been modeled as an interfacial transport process [81, 174, 192, 193]:

$$\frac{dC_{em}}{dt} = -\frac{A_{em}P_{rel}}{V_{em}}(C_{aq} - C'_{aq}) \quad (12)$$

where A_{em} is the total surface area of the oil droplets, P_{rel} is the relative drug permeability at the oil-water interface, C_{aq} is the drug concentration in the aqueous medium containing micelles, and C'_{aq} is the hypothetical aqueous drug concentration in equilibrium with the drug concentration inside the oil droplets, defined as:

$$K_{em/aq} = C_{em}/C'_{aq} \quad (13)$$

where $K_{em/aq}$ is the drug partition coefficient between the oil droplets and the micelle-rich aqueous environment. The relative permeability, P_{rel} , is dependent on the physical and chemical nature of the boundary layer at the oil-water interface [194]. A pure oil-water interface is associated with large surface tension, leading to high partitioning resistance, low interfacial permeability, and ultimately slow diffusion kinetics from one phase to the other. The presence of surfactants (e.g., emulsifiers, surface proteins, FA, MG) lowers the surface tension and can help increase interfacial diffusion kinetics.

4.3.6. Precipitation Modeling—Precipitation can occur with LBF delivery [22], for example upon dilution of an LBF in GI fluids or digestion by GI lipases if there is

insufficient solubilization capacity of the milieu to maintain the formulated drug in solution. Mechanistically, precipitation is characterized by a nucleation phase, where dissolved molecules spontaneously aggregate, and a particle growth phase, where dissolved molecules aggregate into existing precipitated (aggregated) particles. The intrinsic complexity of nucleation and particle growth make precipitation a complicated process to model [195]. Precipitated drug can form either an amorphous solid structure or a crystalline structure (as in typical solid dosage forms) [25]. Depending on the type of solid structure present, different precipitation kinetics may occur.

One common approach used to model precipitation incorporates classical nucleation theory to describe nucleation rate and a power law to describe particle growth [187]. The nucleation rate is a function of supersaturation (defined as the ratio of actual drug concentration to the drug solubility in the medium of interest) [196]:

$$\text{Nucleation: } \frac{dC_{pr}}{dt} k_{nuc} \frac{C}{C_{eq}} e^{\frac{B}{\left(\ln \frac{C}{C_{eq}}\right)^2}} \quad (14)$$

$$\text{Growth: } G = k_{growth} (C - C_{eq})^\beta \quad (15)$$

where C_{pr} is the number of nuclei formed per unit volume (m^{-3}), G is the rate of particle growth (m/s), C is the concentration of drug dissolved, C_{eq} is the drug solubility, k_{nuc} and k_{growth} are the nucleation and growth rate constants, B is a thermodynamic constant dependent on the molecular volume of the solid phase, interfacial energy of the solid, and the solution temperature, and β is the order of particle growth. The impact of lipids on precipitation kinetics as described by this model has been taken into account by incorporating the change in effective solubility due to the presence of lipids [196]. The approach was used to model concurrent precipitation and LBF digestion.

More recently, Jakubiak et al. proposed the growth rate to be directly proportional to both the amount of solid material (m_s) and the concentration of dissolved material (C), and the nucleation rate to be the result of the encounter of two or more (α) drug molecules into an aggregated form [197]. Processes of growth and nucleation were combined in a single precipitation rate expression:

$$\frac{dm_s}{dt} = k_{growth} m_s C + V k_{nuc} C^\alpha \quad (16)$$

where m_s is the amount of undissolved drug, α is the molecularity index representing the average number of molecules involved in the formation of a nucleating particle, V is the volume of fluid where the reaction takes place, and k_{growth} and k_{nuc} are the growth and nucleation rate constants, respectively. This approach was used to describe concurrent precipitation and dissolution [197]. It was reported that this precipitation model, when incorporated into a PK model, predicted compound PK profiles for two test drugs (erlotinib

and dipyridamole). It is noted that precipitation in this study was caused by changes in solubility of weakly basic drugs upon pH changes associated with transit from the stomach into the intestine, rather than LBF digestion by intestinal lipases. However, the mechanism and kinetic expressions for precipitation are translatable.

4.4 Benefits of Systems-based Modeling Approach

As noted above, the impact of lipids on drug behavior in current models is considered by adjusting parameters in dissolution equations known to be affected by lipids (e.g., solubility, diffusion coefficients). This approach has often resulted in under- or overpredictions of plasma concentrations in PK profiles for some drugs are co-administered with food-associated lipids, as reported by some studies [166, 167]. These results motivate model modifications, such as explicit incorporation of additional processes central to lipid function in the GI tract. For example, explicit incorporation of lipid digestion and associated changes in intestinal contents (e.g., increased concentration of micelle-forming species and associated solubility changes); impact of mixed bile micelles on dissolution kinetics (eqn 6); partitioning between micelles, lipid droplets, and the aqueous environment; and lipid absorption may improve model predictions. While it is recognized that much information pertaining to processes central to lipid function in the GI tract is still lacking (e.g., impact of lipids on drug permeation across the intestinal wall), there are tremendous efforts in the scientific community (e.g., OrBiTo) to increase understanding of the mechanisms of and model lipid effects.

Support for the significance of explicit consideration of the processes important in lipid function is found in studies that have modeled isolated processes as well as combined processes and compared kinetic model predictions to *in vitro* results. Stillhart et al. modeled simultaneous lipid formulation digestion and drug precipitation using a separate kinetic expression for each process, solved simultaneously. The solution to this system of equations was able to predict the solubilized drug concentration profile in *in vitro* studies [196]. In another study, *in vitro* studies of lipolysis and simultaneous drug release (for drug formulated in an LBF) or dissolution (for drug dosed as a solid together with lipids) were modeled. Separate kinetic expressions describing lipid digestion and concurrent compound release (from an LBF), drug dissolution (from a solid form), and drug partitioning into colloids were solved simultaneously, and the solution to the system of equations was shown to predict *in vitro* profiles of drug in solution [81], suggesting that the mechanisms proposed were representative of the systems and conditions studied. A later study by Stillhart et al. proposed an explicit consideration of the impact of lipids and lipolysis kinetics on drug solubility and distribution in the GI environment (i.e. in the lipid emulsions or in the micelle-rich aqueous environment) by considering a linear contribution of each of the lipids and lipid digestion products on drug concentration during lipolysis [198]. The authors reported excellent agreement between experimental and modeled drug solubility profiles for three different types of fenofibrate-loaded LBFs, attesting to the robustness of the mechanism and kinetic model. Additionally, the study aimed to predict oral PK profiles by incorporating first order drug absorption, distribution, and elimination kinetic expressions. The kinetic parameters for these expressions were obtained from *in vitro* (e.g., absorption parameter determined in part by Caco-2 transport studies) and *in vivo* studies (e.g., distribution and

elimination parameters determined by IV bolus injection). Comparisons between model predictions and oral PK profiles in pigs revealed a notable loss of absorbable drug *in vitro*, well below the fraction of dose that was absorbed *in vivo*, suggesting significant drug precipitation occurring during *in vitro* LBF digestion experiments. However, when drug absorption is incorporated as a process, as is the case in *in vivo* studies, the extent of drug precipitation is reduced. To achieve desirable oral PK profiles it was assumed that the entire oral dose was available for absorption and no precipitation occurred in the GI lumen.

As noted by these examples, mechanism-based systems offer several benefits. For example, solutions to these systems can provide insight into system function, such as relative kinetics of different processes and concentrations of different species in solution (e.g., micelle-associated vs. free drug). In addition, they offer the ability to adjust input parameters to represent characteristics of a particular system (e.g., dose amount, solid particle size, lipid droplet size, lipid amount). In combination with explicit consideration of lipid function on drug absorption kinetics, such models may allow more rigorous predictions of the impact of lipids on overall drug bioavailability.

5. Conclusions

LBFs have demonstrated great potential for enabling effective oral drug delivery, particularly for PWS drugs. However, they have not been widely used in commercial formulations, partially due to lack of a complete mechanistic understanding of their role in impacting drug absorption, and lack of ability to quantitatively predict their effects *in vivo*. Similarly, opportunities may be missed for exploiting the impact of food-based lipids on drug absorption to enable effective oral delivery due to lack of ability to predict this effect. PBPK models have been developed and employed to aid quantitative predictions of food effect on drug absorption and to dictate oral dosing decisions in clinical studies. Current models require *in vivo* data for proper fitting and prediction of food effect. A systems-based approach explicitly considering the impact of lipid-drug interactions on the simultaneous dynamic processes impacting overall drug absorption in the GI tract may enable improved quantitative predictions and facilitate rational design of effective lipid-based oral drug delivery strategies.

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Figure 1.

Processes affecting oral drug absorption when a drug is dosed formulated in a LBF in the GI tract. Kinetic parameters utilized to model each process are also indicated in black and described in section 4.

Figure 2.

Processes affecting oral drug absorption when a drug is dosed in its solid dosage form in the presence of food-associated lipids in the GI tract. Kinetic parameters utilized to model each process as described in section 4 are also indicated in black.

Figure 3.

Fluorine-NMR (F-NMR) spectra of flurbiprofen in oil emulsions stabilized with model bile micelles. F-NMR was chosen due to the presence of a fluorine atom in the drug chemical structure and absence of fluorinated compounds in the solvent. Emulsion concentrations are on a volume/volume basis.

Figure 4.

EPR spectra of spin probe 4-hydroxy TEMPO benzoate (chemical structure shown) in water (top graph), oil (bottom graph), and in a 30% (v/v) oil-in-water emulsion (middle graph). The spectrum from the emulsion (middle graph) was fit using an open-source software, MultiComponent [83], as a weighted average of the water (top graph) and oil spectra (bottom graph), and the fit reflects a 30% intensity contribution from the oil phase and a 70% intensity contribution from the water phase.

Figure 5.
Profile of gastric emptying for a stable (A) vs. unstable (B) emulsion.

Figure 6. Drug concentration profile as a function of distance from the solid-water interface for $r \gg h_{eff}$ [176].

Figure 7.
Drug concentration profile as a function of distance from the solid-water interface for $r \approx h_{eff}$.

Figure 8.
Lipolysis mechanism as proposed by Verger et al. [186].

Table 1

Fasted State Simulated Intestinal Fluid (FaSSIF) compositions

	Galia et al. Preparation [43]	LFCS Consortium Preparation [47]	Commercially Available
Buffer Type	Phosphate (29 mM)	Maleate (2 mM)	Phosphate (28 mM)
NaCl	N/A	150 mM	106 mM
KCl	103 mM	N/A	N/A
CaCl₂	N/A	1.4 mM	N/A
NaTC/NaTDC	3 mM (NaTC)	3 mM (NaTDC)	3 mM (NaTC)
Lecithin	0.75 mM	0.75 mM	0.75 mM
pH	6.5	6.5	6.5

Table 2

Fed State Simulated Intestinal Fluid (FeSSIF) compositions

	Galia et al. Preparation [43]	Infogest Community Preparation [52]	Commercially Available
Buffer Type	Acetate (144 mM)	Carbonate/Phosphate (85 mM/0.8 mM)	Acetate (144 mM)
NaCl	N/A	38.4 mM	N/A
KCl	203.9 mM	6.8 mM	N/A
CaCl₂	N/A	0.3 mM	N/A
MgCl₂	N/A	0.3 mM	N/A
NaTC/NaTDC	15 mM (NaTC)	10 mM *	15 mM (NaTC)
Lecithin	3.75 mM		3.75 mM
pH	5.0	7.0	5.0

* Infogest uses bile extract from bovine or porcine bile ducts which contains a mix of bile salts and lecithin.