Research Article

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Predicting Pharmacokinetics of Drugs Using Physiologically Based Modeling—Application to Food Effects

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Abstract. Our knowledge of the major mechanisms underlying the effect of food on drug absorption allows reliable qualitative prediction based on biopharmaceutical properties, which can be assessed during the pre-clinical phase of drug discovery. Furthermore, several recent examples have shown that physiologically based absorption models incorporating biorelevant drug solubility measurements can provide quite accurate quantitative prediction of food effect. However, many molecules currently in development have distinctly sub-optimal biopharmaceutical properties, making the quantitative prediction of food effect for different formulations from *in vitro* data very challenging. If such drugs reach clinical development and show undesirable variability when dosed with food, improved formulation can help to reduce the food effect and carefully designed *in vivo* studies in dogs can be a useful guide to clinical formulation development. Even so, such *in vivo* studies provide limited throughput for screening, and food effects seen in dog cannot always be directly translated to human. This paper describes how physiologically based absorption modeling can play a role in the prediction of food effect by integrating the data generated during pre-clinical and clinical research and development. Such data include physicochemical and *in vitro* drug properties, biorelevant solubility and dissolution, and *in vivo* pre-clinical and clinical pharmacokinetic data. Some background to current physiological absorption models of human and dog is given, and refinements to models of *in vivo* drug solubility and dissolution are described. These are illustrated with examples using GastroPlus™ to simulate the food effect in dog and human for different formulations of two marketed drugs.

KEY WORDS: food effect; formulation; GastroPlus; pharmacokinetics; physiologically based absorption model.

INTRODUCTION

Prediction of Pharmacokinetics with Physiologically Based Models

A key task for pre-clinical research is the prediction of human pharmacokinetics for compounds entering clinical studies. While empirical methods such as allometric scaling are still widely used, they often result in large prediction errors, and this has led several groups to explore physiologically based modeling approaches. Physiologically based pharmacokinetic models (PBPK) represent the whole body physiology by a series of linked equations with model parameters corresponding to measurable quantities such as blood flow rates and tissue volumes. In addition, these models

can incorporate *in vitro* data relevant to drug absorption, distribution, metabolism, and elimination and scale them to the *in vivo* situation. Recently, several groups have reported that PBPK models deliver more accurate predictions than allometry (1–3).

Physiologically Based Models of Drug Absorption and Prediction of Food Effects

Most drugs are given orally, and so, it is important to have PBPK models of absorption from the gastrointestinal (GI) tract. These models need to account for the impact of food on the GI tract physiology, drug dissolution, and drug permeation. Mechanisms by which food can change drug absorption are well understood. Increased systemic exposure with food is often seen for lipophilic drugs and is attributable to improved solubilization due to higher bile salt and lipid concentrations. Negative food effects are mostly seen for hydrophilic drugs where food impedes permeation (4). Thus, qualitative prediction of food effect is often possible based solely on the Biopharmaceutical Classification System (BCS) class of the drug (5), and Gu was able to categorize 80% of a set of 92 drugs as having negative, positive, or no food effect

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based simply on their dose, solubility, and permeability (4). Quantitative prediction of food effect has been shown with PBPK models. Jones (6) incorporated biorelevant solubility data into the GastroPlus™ absorption model to predict plasma profiles in fed and fasted humans for six diverse molecules, and more recent studies have confirmed the usefulness of this approach (7,8).

Pre-clinical *In Vivo* Models for Food Effect

In vivo studies in animals constitute a useful tool to explore the influence of formulation changes on food effect. The dog is generally considered to be the best species for such studies since solid oral dosage forms designed for human can be administered and canine food composition can be similar to human meals (9). However, physiological differences between dog and human such as those in gastric pH, gastric emptying, and intestinal transit time can result in differences in food effect. Furthermore, if dog studies are to be predictive for human, very careful design of the experimental protocol is essential. This was shown in a study where adjustment in the amount and type of food and lowering of gastric pH with intramuscular doses of pentagastrin was necessary in order to establish a quantitative correlation of dog and human fed/fasted exposure ratios for a set of nine drugs (10).

Strategy for More Reliable Quantitative Prediction of Food Effect and the Influence of Formulation on Food Effect

For many molecules in development, reliable quantitative prediction of the impact of formulation on food effect remains challenging and a recent review paper concluded that no single model is universally applicable (9). In this paper, we describe how physiologically based absorption models can play a role in advancing quantitative food effect predictions. This is achieved by integrating various predictive data, including physicochemical drug properties, *in vitro* data, biorelevant dissolution data, and *in vivo* study results. A strategy is proposed where absorption models of dog and human are developed in parallel, and importantly, verification of model assumptions is performed by comparing simulations to the food effect measured in carefully designed *in vivo* dog studies. A good match of simulated and observed plasma concentrations in the fasted and fed dog should indicate that the model captures the mechanisms responsible for food effect and allow a reliable prediction for human. A mismatch of simulation with *in vivo* data in dog may indicate model misspecification or incomplete input data and require further experimental work or model refinement before reliable prediction in man is possible. This strategy adds value to the use of *in vivo* dog studies alone since PBPK models allow consideration of differences in dog and human physiology as well as in pharmacokinetics, biorelevant solubility, and permeability. The potential of this approach is illustrated with two examples based on literature data where food and formulation effects were reported in dog and human.

MATERIALS AND METHODS

The ACAT Model

GastroPlus™, based on Yu and Amidon's compartmental absorption and transit (CAT) model (11), was the first

physiologically based absorption model to be produced as a commercial software tool. Simulations Plus (www.simulations-plus.com) extended the CAT model with features such as pH-dependent solubility and permeability and added a user-friendly interface. They renamed the new model the Advanced CAT or ACAT model (12). The theoretical basis and mathematical description of the CAT model is provided in detail in several papers from Yu (13–15), and further details of extensions made in the ACAT model are given in a paper from Simulations Plus (12) and in a book chapter (16). Briefly, the ACAT model of the human gastrointestinal tract comprises nine compartments linked in series, with the first compartment representing the stomach and subsequent compartments corresponding to duodenum, jejunum (two compartments), ileum (three compartments), cecum, and ascending colon. Compartment properties are set according to published data on the pH, volume, and permeability characteristics of the corresponding intestinal region. Transit of drug material between the compartments is modeled as a first order process with the transit time for each compartment based on the physiological value for the corresponding region. For each drug simulated, compound-specific input data for solubility, permeability, $\log P$, pK_a , particle size, and dose are fed into models of dissolution and absorption. For dissolution, a model based on the Noyes–Brunner modification of the Noyes–Whitney equation is implemented. Absorption across the gastrointestinal mucosa is determined by the permeability of the drug in that particular region and by the concentration gradient across the intestinal membrane. The models for estimation of regional permeability and solubility are described below.

Physiologically Based ACAT Models of the Human GI Tract

The default GastroPlus™ human physiological models for fasted and fed states are given in Table I. The pHs, lengths, transit times, and radii for all compartments are based on published values (17,18). Regarding pH, the effect of food is to increase considerably the stomach pH, while that in the upper small intestine is slightly lowered. It should be noted that the values in these standard GastroPlus™ fasted

Table I. ACAT Model Parameterization for Physiological Models of Human (Upper) and Canine (Lower) Gut

Compartment	pH (fasted/fed)	Transit time (h)	Length (cm)
Human			
Stomach	1.3/4.9	0.25/1	28
Duodenum	6.0/5.4	0.3	14
Jejunum (×2)	6.2–6.4/5.4–6.0	1.7	116
Ileum (×3)	6.6–7.4	1.3	174
Cecum	6.4	4.2	13
Asc colon	6.8	12.6	28
Dog			
Stomach	3/5	0.25/1.5	15
Duodenum	6.2	0.3	12
Jejunum (×4)	6.2–6.6	1.5	133
Ileum	6.7/7.05	0.1	4
Cecum	6.75/7.5	1.3	2
Asc colon	6.8	4	4

and fed-state models represent a time average of properties, which actually vary considerably after meal ingestion (19). The volume of fluid in human intestines was set at 40% for the small intestine and 10% for the colon based on measurements made by Schiller (20).

Physiologically Based ACAT Models of the Dog GI Tract

Data published by Yildiz (21) was used to define the regional description and dimensions of the gut physiology for dog (Table 1). As the published dimensions were based on a 28 kg Turkish shepherd dog, these were scaled down proportionally to be appropriate for a 10 kg Beagle dog, which is more commonly used in pre-clinical studies. The resulting model for beagle dog was found to be in good agreement with the limited data available on beagle physiology (22). As the dog has a proportionally longer jejunum and shorter ileum than human, four compartments were assigned to the jejunum in dog compared to two compartments in man, allowing a more equal balance of compartmental transit times. Small intestine transit time in dog is about half that in human (18,23), and the substantially shorter large intestine in this species was set to match the total gastrointestinal transit time of 6–8 h reported for beagle dogs (22). The pH in different regions of the dog GI tract was based on data taken from a number of sources (24,25). In particular, fasting stomach pH of dogs differs from that in humans. Bueno (24) observed that the mean antral pH is 5.8 for half the recording time in fasted dogs due to regurgitation of duodenal contents into the antrum. However, it has also been reported that dosing of placebo capsules causes a decrease in gastric pH in fasted dogs (26). Considering this, a value of 3 is used in the fasted dog model, although it should be noted that pH is extremely variable in dog stomach. In contrast, intestinal pHs in dog were recently shown to be very similar to human values (27) and the dog ACAT model reflects this. No published data on the volume of fluid in dog intestines could be found, and so, these were set equal to the human values.

Prediction of *In Vivo* Solubility

GastroPlus™ calculates regional solubility based on fraction of drug ionized at each compartmental pH according to the Henderson–Hasselbalch relation. This model has been described by Hendriksen (28) who showed that, to simulate absorption reliably for weak bases, it is essential to have at least two measured values of solubility to characterize adequately the solubility *versus* pH curve. Furthermore, Bergstrom (29) measured pH-dependent solubility profiles for 25 amines in phosphate buffer and found that the slope of the pH-dependent solubility profile often differed from that calculated with the Henderson–Hasselbalch equation. Therefore, to achieve the most accurate simulations for drugs with pK_a values in the physiological range, measured data characterizing the solubility changes should be used to calibrate the pH dependence of solubility.

A further complication in estimation of relevant *in vivo* solubility is the presence of bile salts and lipids in the intestinal fluids, which can change the solubility compared to that in aqueous buffers. Several studies have shown the importance of

biorelevant solubility measurement in media such as fasted-state simulating intestinal fluid (FaSSIF) for accurate simulation of absorption of very lipophilic drugs (6,30,31). Recently, further advances have been made to develop media reproducing the changes in upper intestinal tract due to food (32) and to represent the time dependent changes, which occur after meal intake (33). In this paper, we have explored an extension of the GastroPlus™ solubility model based on the hypothesis that, for drugs where biorelevant solubility differs significantly from aqueous solubility, it is appropriate to account for the different bile salt concentrations in different intestinal regions. Previous studies have shown the importance of solubility measurements in biorelevant media for the large intestine (6). However, such detailed measurements are often not made, and so, we developed an approach for scaling of a single jejunal solubility to estimate solubility in other regions of the GI tract. In human, 95% of released bile salts undergo enterohepatic circulation (18) with the majority of the re-absorption occurring in the ileum via the apical sodium dependent bile acid transporter (34). We performed simulations of the human luminal bile acid concentrations at steady state based upon known human fed-state bile flow and bile salt concentrations taken from Zwart (18). Due to lack of quantitative data on the distribution of transporter throughout the small intestine, we assumed that 70% of re-absorption occurs in the two terminal ileum compartments. Although the accuracy of this assumption was uncertain and measurements of human bile salt concentrations in different intestinal regions are limited and variable (35), we found that the profile of regional bile salt concentrations obtained was in good agreement with measured regional concentrations of bile acids in the rat (36), which show a maximum in the upper jejunum and fall off steadily toward the large intestine. As the typical biorelevant media are designed to represent the situation in the upper jejunum (33), we estimated the solubility in other intestinal regions by scaling down the jejunal solubility accounting for the lower concentrations of bile salt. Reasonable prediction of the solubility enhancement of neutral drugs due to bile salts has been demonstrated based on drug lipophilicity (37,38). We applied the relationship published by Glomme: $\text{Log}(\text{SR}) = 0.74 \cdot \text{log}P + 2.01$ ($n=14$; $r^2=0.95$; $\text{SD}=0.26$), where SR is the solubilization capacity of the compound per mol of bile salt divided by the solubilization capacity per mole water, and $\text{log}P$ is the octanol/water partition coefficient.

Prediction of Precipitation *In Vivo*

For poorly soluble weak bases, the increase in pH on moving from the stomach to the small intestine is expected to result in lower solubility, and so, there is a possibility of precipitation. Kostewicz devised a transfer model to measure this precipitation when a solution of drug in simulated gastric fluid was continuously pumped into simulated intestinal fluid (39). It was found that, for three diverse bases, precipitation occurred only under simulated fasted-state conditions, whereas under simulated fed-state conditions, the higher concentrations of bile and the lower pH value in the acceptor medium inhibited precipitation. To model precipitation in GastroPlus™, a mean precipitation time parameter represents the time for particles to precipitate once a supersaturated state is achieved. Based upon the data measured for

transfer into FaSSIF, mean precipitation times of 900 to 2,500 s seem to be realistic for the fasted state.

Prediction of Dissolution Rate *In Vivo*

Dissolution in GastroPlus™ is calculated with a diffusion layer model based on the Noyes–Whitney equation. $\frac{dM_p}{dt} = \frac{D}{\rho h r s} (C_s - C_l)(1 + 2s)M_U$, where M_D and M_U are the dissolved and undissolved amount, respectively, D is the diffusion coefficient of a drug molecule, ρ is the density of the drug particle, r is the current spherical particle radius, h is the diffusion layer thickness, C_s is the solubility, C_l is the drug concentration in the lumen, and s is a particle shape factor (defined as length/diameter; for spherical particles $s=1$). For drugs where the *in vivo* solubility is significantly higher than aqueous solubility, it has been shown that dissolution rate may be over-predicted by this equation (40), possibly because the diffusion of a drug-containing bile salt micelle is slower than that of a free drug molecule. In this case, a modification of the diffusion coefficient to reflect the slower diffusion of bile salt/lecithin aggregates may be appropriate (41). Alternatively, if measured data for dissolution in biorelevant conditions are available, they may be incorporated directly in GastroPlus™ as tabulated release *versus* time profiles.

Prediction of Intestinal Permeability

Human jejunal permeability values measured with *in vivo* perfusion are available for relatively few drugs (42). Approaches to predict the human jejunal permeability of novel molecules include computer models based on *in silico* properties derived from chemical structure (43) and correlation methods where *in vitro* values for selected reference drugs are used to build a relationship to the *in vivo* values for those drugs. This relationship is then used to transform permeability measured *in vitro* for novel molecules (7). The modeling of passive absorption from each compartment is further complicated by regional permeability changes. These are due to the changing mixture of trans- and paracellular transport, changing surface area with different dimensions and density of villi and microvilli, and varying drug ionization with different regional pH. GastroPlus™ provides a log D model, which scales regional permeability so that effective permeability decreases as the ionized fraction of a compound increases. This model was optimized by Simulations Plus to reproduce fraction of dose absorbed for a set of drugs where measured human or rat permeability values are available. The log D model was used in the current study for simulations of both dogs and humans. Transporter effects on regional permeability are not considered here. Dog is believed to have a higher permeability compared to human, and GastroPlus™ accounts for

this by adjusting the permeability to be roughly threefold higher than in man (personal communication—Dr. G. Amidon).

RESULTS AND DISCUSSION

Properties of the Studied Drugs

The modeling described here applies the human and dog ACAT models described in Table I based on the physicochemical inputs listed in Table II. Any changes made to models or inputs in order to simulate specific *in vivo* data are mentioned.

PBPK Absorption Modeling of the Food Effect for Theophylline

Theophylline is a methylxanthine drug, which has been used in the treatment of asthma and chronic obstructive pulmonary disease for over 60 years. It has a narrow therapeutic range, and since immediate release dosage forms produced large fluctuations in plasma concentrations, controlled release formulations were developed to maintain steady-state concentrations within a narrower range. Theophylline is hydrophilic and weakly acidic and exhibits good solubility and permeability, resulting in complete absorption of an orally administered solution (44).

To model the disposition pharmacokinetics in the dog, the mean of plasma concentration *versus* time profiles obtained after intravenous dosing at 10 mg/kg to 12 dogs (45) was used. A one compartment model with a volume of 0.73 L/kg and a clearance of 0.91 L/h gave a good fit to this data, and as this clearance is very low compared to hepatic blood flow in dog, the first pass effect was assumed to be negligible. Aqueous solubility and permeability inputs for the absorption model were taken from Roche databases. The permeability value, measured in Caco-2 cells, was scaled to human effective jejunal permeability using an in-house correlation based upon reference drugs (46). GastroPlus™ was used to simulate plasma concentrations in dogs dosed orally with immediate and controlled release formulations as obtained from a study of Cook (47). Cooks' data were measured in six healthy female beagle dogs. Each dog received 200 mg of theophylline under fasting and fed conditions in a two-way randomized crossover study. In the non-fasting state, a homogenized, standardized human high-fat meal was offered to the dogs 15 min prior to dosing. *In vitro* dissolution data for the controlled release formulation was measured in 0.05 M phosphate buffer at pH 6.6 using USP apparatus I at 37°C with a stirring rate of 100 rpm.

Table II. Physicochemical Properties and Pharmacokinetic Properties Used in the GastroPlus™ Modeling

	Log P	p K_a	Solubility (μ g/mL)	Human jejunal permeability (cm/s)	References
Theophylline	−0.02	8.8 acid	Aqueous: 14,300 at pH 6.5	$4.4 \times 10E-4$ (scaled from Caco-2 value)	Log P , p K_a (55) Solubility and permeability (56)
Aprepitant	4.8	9.7 base	Aqueous buffer: 0.8 FaSSIF (human) 6 FeSSIF (human) 119 FaSSIF (dog) 21	$3 \times 10E-4$ (predicted)	Log P and p K_a (55) Solubility (8) and (31) Permeability (51)

Fig. 1. Simulated and observed food effect for theophylline. *Top* An immediate release formulation in dogs. *Middle* A controlled release formulation in dogs. *Bottom* A controlled release formulation in human

Dissolution data were incorporated into GastroPlus™ as a tabulated release *versus* time entry.

Simulation of the immediate release anhydrous formulation showed that the reduced C_{max} and later T_{max} in the fed state were due to delayed gastric emptying. The best match of simulation to observations was achieved when the fed-state gastric emptying time was extended from the default 1.5 to 3.5 h (Fig. 1), a change which is justified by the large and high fat content of the canine meal in this study. Simulation of a controlled release formulation was performed by changing to the *controlled release dispersed* dosage form in GastroPlus™ and incorporating the *in vitro* dissolution data, which showed a slow release of drug over 18 h. Interestingly, the observed reversal of the immediate release food effect was matched by the simulation. With controlled release, C_{max} was higher with food (Fig. 1). Investigation of the cause of this effect with the model showed that for the immediate release formulation, all drug is dissolved in the stomach and absorption occurs very rapidly in the duodenum and jejunum on stomach

emptying. Thus, C_{max} is higher in the fasted state since faster gastric emptying provides more drug for rapid absorption. In contrast, with the controlled release formulation, slowed dissolution means that only a small fraction of drug can dissolve during the short time of fasted-state gastric residence. However, in the fed state, longer gastric residence allows more time for drug to dissolve, and the C_{max} is higher even though T_{max} is later due to the slower emptying rate. The food effect seen in man for the controlled release formulation (47) was similar to that seen in dog, and this was also reasonably matched by simulations with the default GastroPlus™ models for human fed and fasted physiology (Fig. 1). (Human disposition pharmacokinetics was modeled based on data taken from (48).)

PBPK Absorption Modeling of the Food Effect for Aprepitant

Aprepitant is a poorly soluble, lipophilic drug, approved for prevention of chemotherapy-induced nausea and vomiting. An early clinical tablet formulation containing 100 mg of micronized drug showed a threefold higher area under the plasma curve (AUC) when given with a high-fat breakfast, and this food effect was even more pronounced at a dose of 300 mg (49). Subsequent studies in the dog showed a similar quantitative food effect when the same formulation was dosed at 2 mg/kg. Mechanistic and screening studies in the dog were helpful in guiding development of a nanoparticle formulation, which successfully reduced the food effect and subsequent clinical studies with final market capsules based on nanoparticles showed only a small food effect (1.2-fold) (50). Kesiosoglou (8) has described GastroPlus™ modeling of Aprepitant in dog and human based on measured solubility in human biorelevant media for fasted and fed state and on permeability measured in Caco-2 cells. In that study, a positive food effect was predicted in both species, although the C_{max} in the fed state was over-predicted, which was presumed to be due to an over-estimation of dissolution rate. A dissolution rate slower than predicted by the default Noyes–Whitney model in GastroPlus™ was confirmed by a study of Takano (31). Takano measured dissolution for particles of 5-μm diameter in biorelevant FaSSIFdog medium (containing 5 mM bile salts) and fit a Noyes–Whitney model to the *in vitro* dissolution *versus* time data to estimate the composite parameter z ($z=3D/phr$, see Methods for the

Fig. 2. Estimated regional solubility values used to simulate Aprepitant absorption in fasted and fed dogs

Noyes–Whitney model in GastroPlus™). We compared this z value with the value computed in GastroPlus™ based on *in silico* estimates of diffusion coefficient, D (0.65×10^{-5} cm/s), drug density, ρ (1.2 g/mL), particle radius r , and diffusion layer thickness h (set equal to r). The default GastroPlus™ model was leading to a 21-fold over-estimation of initial dissolution rate, and so the diffusion coefficient in our model was reduced so that z corresponded to the measured value.

Takano also measured Aprepitant equilibrium solubility in phosphate buffer (0.8 μ g/mL) and in FaSSIFdog medium (21 μ g/mL). This big increase in *in vivo* solubility indicates a strong dependence of solubility on bile salt level, and so, we generated a solubility profile based on regional bile salt concentration ratios (see “Materials and methods” section). For fasted state, the FaSSIFdog value was used in the upper jejunum, while in the fed state, the measured value for fed-state simulating human intestinal fluid (FeSSIF) of 119 μ g/mL was used. No measured values for biorelevant stomach solubility were found, and so, 25 μ g/mL was used in both fasted and fed states (Fig. 2).

A permeability value of 7.85×10^{-6} cm/s was measured in Caco-2 cells by Wu (49). Takano (31) obtained a similar Caco-2 value of 2.1×10^{-5} cm/s but argued that this is not representative of the *in vivo* situation due to differences in unstirred water layer *in vitro* and *in vivo* and used a higher calculated value of 11×10^{-4} cm/s for simulations (40). For our model, we applied the *in silico* model for human permeability in ADMET Predictor (51), which estimated human permeability as 3×10^{-4} cm/s.

Plasma concentration measurements obtained after an intravenous dose are needed to characterize accurately the disposition in the dog. However, such data could not be found, and so, an estimate was made by fitting a two-

Fig. 3. The effect of adjusting regional solubility values used to simulate Aprepitant absorption of a suspension of 5.5 μ m particles in fasted dogs

Fig. 4. Simulated and observed food effect for a micronized and nanoparticle formulation of Aprepitant in dog

compartment disposition model to the plasma profile measured after oral dosing of the nanoparticle formulation assuming complete absorption and negligible liver first pass (which is supported by the high absolute bioavailability of a solution dose (31)). The estimated clearance was low at 1 L/h and volume of distribution was 1.5 L/kg. The uncertainty of an estimation of disposition pharmacokinetics in this manner is acknowledged and should normally be addressed by measuring concentrations after an intravenous bolus dose ideally in the same dogs used for the oral studies.

Wu measured plasma concentrations after oral administration of a suspension of 5.5 μ m particles (in 0.5% methylcellulose in water with 0.02% sodium dodecyl sulfate) to five fasted, male beagle dogs at a dose of 2 mg/kg. Our

Fig. 5. Simulated and observed food effect for a nanoparticle formulation of Aprepitant in humans

simulations for the suspension matched the observed data only when using the regional solubility profile. The adjustment to account for reducing levels of bile salts proved to be important for realistic simulations since simulated absorption from distal jejunum, ileum, and large intestine was much higher when solubility was left unchanged and resulted in a significant over-prediction of plasma concentrations (Fig. 3).

Wu measured the food effect for both the micronized suspension and the nanoparticle formulations at 2 mg/kg. The fasted dogs were without food or water overnight prior to each study day, while fed dogs ate 374 g of dog food containing 55% of total calories from fat, approximately 30 min prior to dosing. To model the fed-state absorption for such a large and fatty meal, an extension of the gastric emptying time to 3.5 h was made. The simulations matched well the food effect with a 2.7-fold increase in AUC predicted with food for the micronized formulation (3.2 observed) and no change in AUC for the nanoparticle formulation (Fig. 4).

Majumdar reported on a clinical food effect study with a final market formulation of Aprepitant. Capsules (125 mg) were dosed in the fasted state and after a standard breakfast. Disposition pharmacokinetics in man was modeled with a two-compartment model fit to plasma levels measured after an intravenous dose. The clearance was low at 3.7 L/h and volume of distribution was 1 L/kg. Simulations of the fed and fasted-state profiles in man used the same model adjustments as had been applied in modeling the dog data. The regional solubility profiles were adjusted to account for reduced bile salt levels, but in the fasted state, lower solubilities were used since the measured solubility in FaSSIF for human (8) was lower (6 $\mu\text{g/mL}$) than had been reported for dog. As for dog, the solution dosage form was used to model the nanoparticle formulation (Fig. 5).

CONCLUSION

Predictions of food effect can be useful throughout the drug discovery and development process. The required accuracy depends on how far a molecule has advanced. During early phases, molecules likely to show significant positive or negative changes in pharmacokinetics with food can be recognized simply based on biopharmaceutical properties. For such molecules, once biorelevant solubility measurements are made, a more quantitative estimation of the magnitude of changes with food is possible via incorporation of these data into PBPK models (6,52). However, many molecules reaching clinical trials have distinctly sub-optimal biopharmaceutical properties and show quite large food effects. If this is a hindrance to development, advanced formulation efforts are necessary to reduce the food effect, and *in vivo* studies in the dog are the most commonly used tool for such formulation optimization. *In vivo* screening in the dog can result in successful clinical formulations (49), although physiological and biochemical species differences between dog and man can result in misleading predictions of clinical food effect (53).

Physiologically based absorption modeling can play a role in the prediction of food effect for drug formulations by integrating *in vitro* biorelevant dissolution data as has been illustrated here for two drugs showing very different biopharmaceutical properties. Theophylline is a BCS class 1

drug, and the food effect for immediate and controlled release formulations was well simulated by the GastroPlus™ models of dog and human. On the other hand, simulations for Aprepitant, a BCS II drug, were more challenging. Like many molecules currently reaching development (54), Aprepitant is highly lipophilic and has very low aqueous solubility. Accurate simulation for different formulations of this molecule required several changes to the default GastroPlus™ models. Aqueous solubility did not adequately represent *in vivo* solubility as shown by the measurements in biorelevant media, and furthermore, the measured dissolution rate was much slower than predicted by the Noyes–Whitney model based on default parameter estimates. Together with uncertainty over the relevant permeability value, there was also uncertainty over the changes in permeability and solubility in different regions of the GI tract, factors which were shown to have a considerable impact of the simulation of food effect. Considering these uncertainties, it is clear that direct prediction of the clinical food effect for Aprepitant without prior verification against pre-clinical *in vivo* data would not be advisable. Rather, PBPK modeling based on *in vitro* data should proceed in conjunction with *in vivo* dog studies. In this way, translation of *in vitro* results to the *in vivo* situation can be verified, and the known physiological differences between dog and human can be accounted for. Such verification and refinement of the models leads to better understanding of formulation effects and thus more reliable clinical predictions.

In the near future, as more advanced *in vitro* tests and PBPK models are developed and verified with more molecules, the level of confidence in *a priori* predictions will increase and models will play an increasing role in the design of formulations to reduce clinical food effect. A model-based approach integrating predictive data from various sources will lead to more reliable predictions, better designed experiments, and better decisions.

REFERENCES

1. S. S. De Buck, V. K. Sinha, L. A. Fenu, M. J. Nijssen, C. E. Mackie, and R. A. H. J. Gilissen. Prediction of human pharmacokinetics using physiologically based modeling: a retrospective analysis of 26 clinically tested drugs. *Drug Metab. Dispos.* **35**(10) (2007).
2. H. Jones, N. Parrott, K. Jorga, and T. Lavé. A novel strategy for physiologically based predictions of human pharmacokinetics. *Clin. Pharmacokinet.* **45**(5):511–542 (2006).
3. M. Shiran, N. Proctor, E. Howgate, K. Rowland-Yeo, G. Tucker, and A. Rostami-Hodjegan. Prediction of xenobiotic clearance in humans: *in vitro*—*in vivo* extrapolation vs allometric scaling. *Xenobiotica*. **36**(7):567–580 (2006).
4. C.-H. Gu, H. Li, J. Levons, K. Lentz, R.B. Gandhi, K. Raghavan, and R.L. Smith. Predicting effect of food on extent of drug absorption based on physicochemical properties. *Pharm. Res.* **24**(6):1118–1130 (2007).
5. D. Fleisher, C. Li, Y. Zhou, L.-H. Pao, and A. Karim. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. *Clin. Pharmacokinet.* **36**(3):233–254 (1999).
6. H. Jones, N. Parrott, G. Ohlenbusch, and T. Lavé. Predicting pharmacokinetic food effects using biorelevant solubility media and physiologically based modelling. *Clin. Pharmacokinet.* **45**(12):1213–1226 (2006).
7. N. Parrott, and T. Lave. Applications of physiologically based absorption models in drug discovery and development. *Mol. Pharmaceutics*. **5**(5):760–775 (2008).

8. F. Kesiosoglou, Y. Wu, and A. Chin. Prediction of micronized aprepitant food effect through absorption modeling. AAPS Annual Meeting and Exposition 2007, (2007).
9. K. A. Lentz. Current methods for predicting human food effect. *The AAPS Journal*. **10**(2):282–288 (2008). doi:10.1208/s12248-008-9025-8.
10. K. A. Lentz, D. G. M. Megan Quitko, J. E. Grace Jr., C. Gleason, and P. H. Marathe. Development and validation of a preclinical food effect model. *J. Pharm. Sci.* **96**:459–472 (2007).
11. L. X. Yu, E. Lipka, J. R. Crison, and G. L. Amidon. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Adv. Drug Deliv. Rev.* **19**:359–376 (1996).
12. B. Agoram, W. S. Wolosz, and M. B. Bolger. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Adv. Drug Deliv. Rev.* **50**(Supplement 1):S41–S67 (2001).
13. L. X. Yu, J. R. Crison, and G. L. Amidon. Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. *Int. J. Pharm.* **140**:111–118 (1996).
14. L. X. Yu, and G. L. Amidon. A compartmental absorption and transit model for estimating oral drug absorption. *Int. J. Pharm.* **186**:119–125 (1999).
15. L. X. Yu. An integrated model for determining causes of poor oral drug absorption. *Pharm. Res.* **16**(12):1883–1887 (1999).
16. M. B. Bolger, B. Agoram, R. Fraczekiewicz, and B. Steer. Simulation of absorption, metabolism, and bioavailability. In H. v.d. Waterbeemd, H. Lennernäs, and P. Artursson (eds.), *Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability*, Wiley, Weinheim (2003).
17. J. B. Dressman, G. L. Amidon, C. Reppas, and V. P. Shah. Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms. *Pharm. Res.* **15**(1):11–22 (1998).
18. L. L. d. Zwart, C. J. M. Rempelberg, A. J. A. M. Sips, J. Welink, and J. G. M. v. Engelen. Anatomical and physiological differences between various species used in studies on the pharmacokinetics and toxicology of xenobiotics. A review of literature. Rijksinstituut voor Volksgezondheid en Milieu. RIVM report 623860 010, (1999).
19. L. Kalantzi, K. Goumas, V. Kalioras, B. Abrahamsson, J. Dressman, and C. Reppas. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm. Res.* **23**(1):165–176 (2006).
20. C. Schiller, C.-P. Froehlich, T. Giessmann, W. Siegmund, H. M. Nikes, N. Hosten, and W. Weitschies. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment. Pharmacol. Ther.* **22**:971–979 (2005).
21. H. Yildiz, K. Arslan, I. Copkun, and B. Yildiz. A geometric modeling of dog intestine. *Turk. J. Vet. Anim. Sci.* **30**:483–488 (2006).
22. T. T. Kararli. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* **16**(5):351–80 (1995).
23. J. B. Dressman. Comparison of canine and human gastrointestinal physiology. *Pharm. Res.* **3**(3):123–131 (1986).
24. L. Bueno, J. Fioramonti, and Y. Ruckebusch. Gastric pH changes associated with duodenal motility in fasted dogs. *J. Physiol.* **316**:319–325 (1981).
25. H. W. Smith. Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J. Path. Bact.* **89**:95–122 (1965).
26. M. Akimoto, N. Nagahata, A. Furuya, K. Fukushima, S. Higuchi, and T. Suwa. Gastric pH profiles of beagle dogs and their use as an alternative to human testing. *Eur. J. Pharm. Biopharm.* **49**(2):99–102 (2000).
27. L. Kalantzi, E. Persson, B. Polentarutti, B. Abrahamsson, K. Goumas, J. B. Dressman, and C. Reppas. Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. *Pharm. Res.* **23**(6):1373–1381 (2006).
28. B. Hendriksen, M. S. Felix, and M. Bolger. The composite solubility *versus* pH profile and its role in intestinal absorption prediction. *AAPS PharmSci.* **5**(1):1–15 (2003).
29. C. A. S. Bergström, K. Luthman, and P. Artursson. Accuracy of calculated pH-dependent aqueous drug solubility. *Eur. J. Pharm. Sci.* **22**:387–398 (2004).
30. E. Nicolaides, M. Symillides, J. B. Dressman, and C. Reppas. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. *Pharm. Res.* **18**(3):380–388 (2001).
31. R. Takano, K. Furumoto, K. Shiraki, N. Takata, Y. Hayashi, Y. Aso, and S. Yamashita. Rate-limiting steps of oral absorption for poorly water-soluble drugs in dogs; prediction from a miniscale dissolution test and a physiologically-based computer simulation. *Pharm. Res.* **25**(10):2334 (2008).
32. J. B. Dressman, M. Vertzoni, K. Goumas, and C. Reppas. Estimating drug solubility in the gastrointestinal tract. *Adv. Drug Deliv. Rev.* **59**(7):591–602 (2007).
33. E. Jantravid, N. Janssen, C. Reppas, and J. B. Dressman. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm. Res.* **25**(7):1663 (2008).
34. A. Balakrishnan, and J. E. Polli. Apical sodium dependent bile acid transporter (ASBT, SLC10A2): a potential prodrug target. *Mol. Pharmaceutics.* **3**(3):223–230 (2006).
35. C. J. H. Porter, N. L. Trevaskis, and W. N. Charman. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.* **6**:231 (2007).
36. J. M. Dietschy. Mechanisms for the intestinal absorption of bile acids. *J. Lipid Res.* **9**(3):297–309 (1968).
37. S. D. Mithani, V. Bakatselou, C. N. TenHoor, and J. B. Dressman. Estimation of the increase in solubility of drugs as a function of bile salt concentration. *Pharm. Res.* **13**(1):163–167 (1996).
38. A. Glomme, J. März, and J. B. Dressman. Predicting the intestinal solubility of poorly soluble drugs. In D. S. D. K. Bernard Testa, H. Wunderli-Allenspach, G. Folkers (eds.), *Pharmacokinetic Profiling in Drug Research*, Wiley, Zürich, 2007, pp. 259–280.
39. E. S. Kostewicz, M. Wunderlich, U. Brauns, R. Becker, T. Bock, and J. B. Dressman. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. *JPP.* **56**:43–51 (2004).
40. R. Takano, K. Sugano, A. Higashida, Y. Hayashi, M. Machida, Y. Aso, and S. Yamashita. Oral Absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. *Pharm. Res.* **23**(6):1144–1156 (2006).
41. A. Okazaki, T. Mano, and K. Sugano. Theoretical dissolution model of poly-disperse drug particles in biorelevant media. *J. Pharm. Sci.* **97**(5):1843–1852 (2008).
42. J.-S. Kim, S. Mitchell, P. Kijek, Y. Tsume, J. Hilfinger, and G. L. Amidon. The suitability of an *in situ* perfusion model for permeability determinations: utility for BCS class I biowaiver requests. *Mol. Pharmaceutics.* **3**(6):686–694 (2006).
43. C. A. S. Bergström. In silico predictions of drug solubility and permeability: two rate-limiting barriers to oral drug absorption. *Basic Clin. Pharmac. Toxicol.* **96**:156–161 (2005).
44. R. A. Upton, L. Sansom, T. W. Guentert, J. R. Powell, J.-F. Thiercelin, V. P. Shah, P. E. Coates, and S. Riegelman. Evaluation of the absorption from 15 commercial theophylline products indicating deficiencies in currently applied bioavailability criteria. *Journal of Pharmacokinetics and Pharmacodynamics.* **8**(3):229–242 (1980).
45. H. Kawai, S. Kokubun, T. Matsumoto, J. Kojima, and K. Onodera. Pharmacokinetic study of theophylline in dogs after intravenous administration with and without ethylenediamine. *Methods Find.* **22**(3):179–184 (2000).
46. J. Alsenz, and E. Haenel. Development of a 7-day, 96-well Caco-2 permeability assay with high-throughput direct UV compound analysis. *Pharm. Res.* **20**(12):1961–1969 (2003).
47. C. S. Cook, C. L. Hauswald, A. Y. Grah, K. Kowalski, A. Karim, R. Koch, G. L. Schoenhard, and J. A. Oppermann. Suitability of the dog as an animal model for evaluating theophylline absorption and food effects from different formulations. *Int. J. Pharm.* **60**(2):125–132 (1990).

48. A. Onishi. Differential pharmacokinetics of theophylline in elderly patients. *Drugs Aging*. **20**(1):71–84 (2003).
49. Y. Wu, A. Loper, E. Landis, L. Hettrick, L. Novak, K. Lynn, C. Chen, K. Thompson, R. Higgins, U. Batra, S. Shelukar, G. Kwei, and D. Storey. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. *Int. J. Pharm.* **285**(1–2):135–146 (2004).
50. A. K. Majumdar, L. Howard, M. R. Goldberg, L. Hickey, M. Constanzer, and *et. al.* Pharmacokinetics of aprepitant after single and multiple oral doses in healthy volunteers. *J. Clin. Pharmacol.* **46**:291–300 (2006).
51. Simulations Plus Inc., W.A.J., Lancaster, California 93534–2902.
52. N. Parrott, and T. Lave. Applications of physiologically based absorption models in drug discovery and development. *Mol. Pharmaceutics*. **5**(5):760–775 (2008).
53. S. K. Paulson, M. B. Vaughn, S. M. Jessen, Y. Lawal, C. J. Gresk, B. Yan, T. J. Maziasz, C. S. Cook, and A. Karim. Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. *J. Pharmacol. Exp. Ther.* **297**(2):638–645 (2001).
54. P. D. Leeson, and B. Springthorpe. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* **6**(11):881–890 (2007).
55. (Roche data on file). This is Roche internal measured data which has not been published.
56. A. Leo, C. Hansch, and D. Elkins. Partition coefficients and their uses. *Chem. Rev.* **71**(6):525 (1971).