

## Invited Review

# Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions

Jean-Marie Nicolas\*, François Bouzom, Chanteux Hugues, and Anna-Lena Ungell

Non-Clinical Development Department, UCB Biopharma sprl, Braine-l'Alleud, Belgium

**ABSTRACT:** The dissolution, intestinal absorption and presystemic metabolism of a drug depend on its physicochemical characteristics but also on numerous physiological (e.g. gastrointestinal pH, volume, transit time, morphology) and biochemical factors (e.g. luminal enzymes and flora, intestinal wall enzymes and transporters). Over the past decade, evidence has accumulated indicating that these factors may differ in children and adults resulting in age-related changes in drug exposure and drug response. Thus, drug dosage may require adjustment for the pediatric population to ensure the desired therapeutic outcome and to avoid side-effects. Although tremendous progress has been made in understanding the effects of age on intestinal physiology and function, significant knowledge gaps remain. Studying and predicting pharmacokinetics in pediatric patients remains challenging due to ethical concerns associated with clinical trials in this vulnerable population, and because of the paucity of predictive *in vitro* and *in vivo* animal assays. This review details the current knowledge related to developmental changes determining intestinal drug absorption and pre-systemic metabolism. Supporting experimental approaches as well as physiologically based pharmacokinetic modeling are also discussed together with their limitations and challenges. © 2016 UCB Biopharma sprl. *Biopharmaceutics & Drug Disposition* Published by John Wiley & Sons, Ltd.

**Key words:** intestinal absorption; ontogeny; pediatrics; physiologically based pharmacokinetic modeling

## Introduction

The number of reports discussing drug pharmacokinetics in pediatrics has grown substantially. More drugs have been studied in children in the past decade than in the preceding 50 years [1]. This growing interest has roots in the emerging evidence of developmental changes in drug pharmacokinetics and pharmacodynamics, in the increasing regulatory pressure, and in the

overwhelming medical need for safer and more effective pediatric medicines.

Until recently, drugs were developed and approved for use in adults only. Drug labels were silent on pediatric use and generally ignored potential age-dependent effects on drug disposition and drug response [2]. The treatment of children often employed unlicensed or off-label drugs [3], with a dosage formulation not tailored for pediatrics [4]. Drug dosages were based on data obtained in adults, after a simple adjustment for body weight. Such an approach has limited value as many physiological processes do not develop linearly with body size and may show dramatic age-related differences. Occasionally, the

\*Correspondence to: Jean-Marie Nicolas, UCB Biopharma sprl, Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium.  
E-mail: jean-marie.nicolas@ucb.com  
The copyright line for this article was changed on 13 June 2017 after original online publication

lack of pediatric data led to sub-therapeutic outcomes or unanticipated side-effects. The gray baby syndrome in neonates receiving chloramphenicol is an exemplary illustration of such unwanted toxicity due to unadjusted dosing [5].

For years, the paucity of pediatric clinical trials and the very few drugs licensed for pediatric use contrasted with the considerable number of medicines prescribed, averaging 0.8 to 3.2 per child per year [3]. Meanwhile, the World Health Organization has persistently drawn attention to the millions of childhood deaths annually in low income countries, as a result of untreated diseases. Despite their unequivocal value, investigations of pediatric medicines are hampered by numerous barriers. These include the ethical and technical issues limiting the access to pharmacokinetic studies in healthy pediatric subjects, the scarce availability of high quality tissue samples for *in vitro* assays, the poor translatability of animal data and the questionable financial return on investment due to the smaller size of the pediatric patient population [6,7].

In response to the above challenges, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) pressed the pharmaceutical industry to generate pediatric data for new and established medications (2002 Best Pharmaceuticals for Children Act and 2003 Pediatric Research Equity Act in US; 2007, European pediatric Regulation in EU) [7,8]. In the absence of a specific waiver, the sponsor must submit a pediatric development plan at the same time as conducting studies supporting adult use. Also, to incentivize pharmaceutical companies, patent extension and market exclusivity are offered for drugs with approved pediatric use. Finally, private and public funding was assigned for research on the treatment of pediatric patients using off-patent drugs [9]. Eventually, all these concerted efforts from regulatory authorities, the pharmaceutical companies and health care providers has started to show benefit and has generated pediatric data for hundreds of drugs [1].

Most medicines delivered to children are administered orally. Intestinal absorption is influenced by the drug characteristics (e.g. physico-chemical properties) but also by physiological parameters (e.g. gastrointestinal fluid composition and volume, transit time, microbiota, drug

metabolizing enzymes and drug transporters) and environmental factors (e.g. food, drug formulation). It is now recognized that these factors may vary considerably with age and result in age-related changes in drug absorption. The focus of the present review is to summarize the age-related changes in the physiological processes, drug metabolizing enzymes and drug transporters determining intestinal drug absorption and presystemic metabolism. The review is complementary to recent publications in this area [10–14] and intends to focus specifically on case reports, knowledge gaps and discrepancies, and to discuss the available experimental approaches (*ex vivo*, animal studies, clinical studies, physiologically based pharmacokinetics PBPK) together with their limitations.

### Age-mediated changes in physiological processes determining drug intestinal absorption

#### pH

Gastric pH is important for drug stability, drug dissolution and drug ionization, all of which affect the absorption process. At birth, gastric pH is neutral (pH 6–8), presumably as a result of amniotic fluid in the stomach [15]. There is some controversy over the variations of fasting gastric pH during childhood. Review articles often report a decline to pH 2–3 within hours of birth, a rise to neutral pH after 24–72 hours followed by a progressive decrease (over several weeks to several years) to finally reach acidic values similar to those measured in adults [6,13,16–19]. Contrasting with these reviews, a comprehensive examination of original reports shows that gastric pH is continuously maintained around acidic values (< 3) from preterm neonates to adults (Figure 1), a finding already reported elsewhere [14,20].

The supposed transient neutral gastric pH in neonates is regularly discussed for its potential impact on drug disposition, especially for its positive effect on the oral bioavailability of acid-labile drugs (e.g. ampicillin, penicillin or nafcillin) [17]. A higher pH would also favor the ionization, and thus reduce the absorption of weak acids as possibly observed with phenytoin [21] or nalidixic

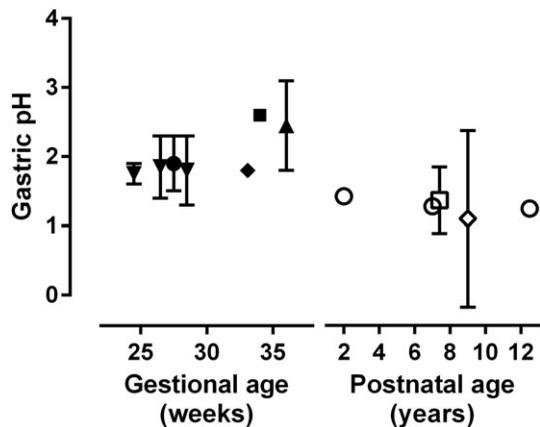


Figure 1. Age-related changes in gastric pH under fasting conditions. Error bars indicate pH range (solid symbols) or standard deviation associated with mean pH (open symbols). References: ▼ [203], ● [204], ◆ [205], ■ [206], ▲ [207], ○ [208], □ [209], ◇ [25] (this latter reference reports pH data for subjects aged 2 months to 18 years)

acid [22]. The higher gastric pH in neonates might also negatively impact on the absorption of weak bases by decreasing their solubility, as observed with ketoconazole [23]. Overall, a clear consensus is still missing concerning the ontogeny of gastric pH and its impact on drug absorption.

*Gastric volume*

In addition to gastric pH, gastric volume is a key determinant of oral drug solubility. According to the biopharmaceutics classification system (BCS), highly soluble drugs in adults are those where the maximum dose unit is soluble in 250 ml aqueous liquid, corresponding to the initial gastric volume [24]. The drug solubility normalized to the dose and to the gastric volume is given by the Dose number  $D_0$ . When normalized per kg body weight, the fasted gastric content volume is broadly similar in children (0.25–0.56 ml/kg, [25–27]) and adults (0.36–0.50 ml/kg, [28,29]) while the drug dosage may differ. As a result, some drugs have a BCS solubility class that changes with age. Chloramphenicol, erythromycin, cefalexin and prednisolone gradually alter from a low solubility classification in 6-month old children ( $D_0$  of 1.3, 2.1, 2.3 and 3.8) to high solubility in adults ( $D_0 \leq 1$ ) [30]. These findings should be taken cautiously as many challenges may confound pediatric BCS consideration,

including the lack of a standardized fluid volume ingested by pediatric patients, the limited literature data across pediatric subpopulations and the knowledge gaps for some pediatric physiological parameters [31].

*Gastric emptying and intestinal transit*

Gastric emptying (GE) and intestinal motility influence the rate and extent of intestinal drug absorption. The GE rate is affected by numerous factors including feeding status, food composition, posture, activity, appetite, psychomotor development, disease states and the method for measuring GE (see [32] for a brief review). Gestational/postnatal age has been also proposed as affecting GE. In adults, the GE of liquid is biphasic with an initial rapid phase (< 10 min) followed by an slower phase [33]. Review articles often report that GE is much slower and linear below the age of 6–8 months, as a result of immature motility neuro-regulation [19,34]. A scintigraphic study in newborns and young infants (aged 1–10 weeks) given milk demonstrated a monophasic GE with a half-life of 45–141 min [35]. An ultrasound study in newborns showed age-dependent changes in the preprandial GE half-time (from 72 min in preterm newborns of 28–32 weeks to 83 min in full-term newborns) [36]. Prolonged GE in the pediatric population is anticipated to decrease the absorption rate and delay the absorption of drugs where GE is rate limiting. This appears to be the case for paracetamol which shows a prolonged absorption half-life in children under 3 months [37] or phenobarbital, sulfonamides and digoxin which all have an absorption rate that increases from 3 weeks to 1 year of age [38]. For similar reasons, cisapride has its plasma peak concentration delayed in neonates when compared with older children [39]. The assertion that GE changes during childhood has been challenged in a recent meta-analysis of 49 published clinical studies (1457 individuals aged from 28 weeks of gestation to adults) [40]. Age was found not to be a significant covariate for GE. However, the authors acknowledged some limitations to the analysis, e.g. the large diversity of the study protocols, and the fact that age and meal type might have been partially confounded. Overall, the effect of age on GE and the consequences

on drug disposition remain poorly understood. As concluded by the authors of the meta-analysis, a well-controlled prospective scintigraphy study measuring GE across a wide range of age is warranted.

The small intestinal and colonic transit time may vary depending of the methodology used. However, within the same method, transit time for the small intestine and colon appears to be minimally affected by age [41,42].

### *Biliary function*

The bile acid pool size and bile flow in newborns are decreased compared with older children and adults [43,44]. In addition, the ileal reabsorption of bile salts remains immature until several months of age [45]. These properties contribute to lower duodenal bile acid concentrations [46]. Bile salts are critical to the intestinal absorption of lipid-soluble xenobiotics by facilitating their solubility and dissolution rate. Although direct clinical evidence is scarce, these compounds are expected to show impaired intestinal absorption in younger age groups. It is tentatively assumed that the above mechanism might account for the 2-fold decrease in the oral bioavailability of the water insoluble antiviral pleconaril in neonates when compared with older children [47].

### *Pancreatic enzymes*

The pancreatic exocrine function is another key determinant of intestinal absorption that changes with age. Pancreatic enzyme levels (e.g. lipase, trypsin) are typically low at birth and develop during the first year of life [48–50]. This might have an impact on the disposition of drugs sensitive to hydrolysis, as exemplified by prodrugs. Decreased intra-duodenal hydrolysis was thought to be at least partially responsible for the incomplete, prolonged and erratic absorption of chloramphenicol palmitate after oral dosing to neonates [51].

### *Intestinal villous morphology and surface area*

The villous pattern of the small intestine begins at 8 weeks of gestation [52]. Crypt fission peaks at 6–12 months of age [53]. Young children tend to show short ridge-shaped villi and longer crypts

[54]. The typical adult profile with elongated finger- or leaf-shaped villi appears after 3 years of age [55]. Together with a reduced number of villi, these differences translate into a reduced surface-to-volume ratio in children when compared with adults [56]. A retrospective population pharmacokinetic analysis in children and adolescents (aged 3 months to 18 years) with epilepsy revealed that the absorption rate constant of levetiracetam (LEV) increased over 3-fold within the investigated age range [57]. This finding was found difficult to interpret as LEV is a BCS class 1 drug (high solubility, high permeability) with restricted absorption unexpected. However, considering its physicochemical properties (LogD of  $-0.45$ , MW of 170), LEV might not easily dissolve in the lipid bilayer of the intestinal cell membranes and might be partly absorbed by the paracellular pathway, as demonstrated for other hydrophilic small molecular weight compounds [58,59]. To be fully absorbed, these compounds diffuse further down the length of the villi and require a larger absorptive surface area [60,61]. Thus, it could be tentatively hypothesized that the immature intestinal villi in children, with a lower surface/volume ratio and a reduced absorptive surface, may influence LEV absorption.

### *Intestinal permeability*

The potential age-related changes in the intestinal paracellular and transcellular permeability of drugs have been largely ignored. The polar hydrophilic antidiuretic furosemide was described to be absorbed, at least in part, by the paracellular route [62]. In adults, its absorption is incomplete and its oral bioavailability is variable and rather low (mean value of ca. 56%) [63]. Data about the pharmacokinetics of furosemide in very young children are scarce. Mirochnick *et al.* [64] reported an average oral bioavailability of 84% when furosemide is delivered to children of 39 weeks gestational age. Whether this higher bioavailability originates from higher intestinal paracellular permeability remains to be confirmed as other explanations cannot be excluded [65].

### *Gastrointestinal lumen and microbiota*

At birth, the intestinal tract is generally sterile. Microbial colonization occurs within a few hours

[66] with a microbiota composition that varies and evolves depending upon the mode of birth delivery (vaginal versus cesarean) [67], the first feeding (breast-milk vs infant formula) [68,69], and many other environmental factors (e.g. hospitalization, diet, exposure to antibiotics and environmental toxicants) as listed in recent reviews [70–72]. Both gestational and postnatal age also have significant effects on the intestinal microbiota. Penders *et al.* demonstrated a higher *Clostridium difficile* colonization rate in preterm infants when compared with healthy full term infants [73]. This finding is likely to be related to the use of antibiotics in neonatal intensive care units, rather than to maternal transmission [74]. Koenig *et al.* reported a gradual change in the diversity of the intestinal microbiota with four discrete steps that evolve up to 3 years of age where an adult-like profile is achieved [75]. Each step corresponds to a change in the children’s diet (e.g. breast milk, formula feeding, adult diet), which coincides with a modification of the relative abundance of the bacteria phyla (i.e. firmicutes, bacteroidetes, actino- and proteobacteria). Other authors reported that adult characteristics were reached after the first year of life [66].

The intestinal microbiota has various important functions, some with impacts on drug disposition, e.g. regulation of intestinal barrier development, bile salt metabolism, gastrointestinal motility and drug metabolism. The impact of gut microbiota on drug metabolism is largely overlooked despite being recognized since the 1960s [76] and potentially affecting the disposition of up to one third of drug candidates [77,78]. The effects of gut flora ontogeny on drug metabolism are even less well-documented beyond the often cited digoxin case example. After oral dosing to adults, digoxin is partially eliminated as reduced metabolites containing a saturated lactone ring (e.g. dihydrodigoxin). This reaction is primarily supported by anaerobic bacteria of the gut flora. Lindsay *et al.* [79] investigated the *in vivo* pharmacokinetics of digoxin in over 200 subjects, from 3.5 weeks of age to adults. Reduced metabolites were not detected below 8 months of age and the adult pattern was observed after 16 months (Figure 2).

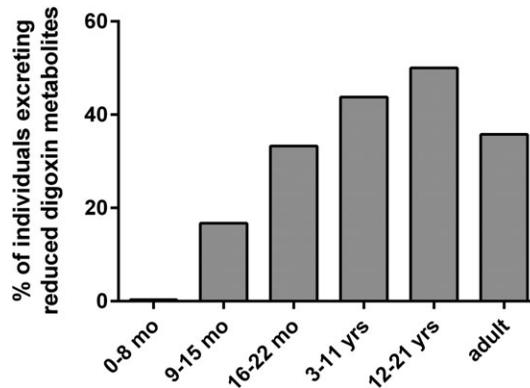


Figure 2. Excretion of digoxin reduced metabolites at different ages. Digitalized children and adults were examined for metabolites recovered in urine samples. Subjects were classified as excretors when digoxin reduced metabolites accounted for at least 5% of the total drug-related material. Adapted from [79]

### Intestinal wall drug metabolizing enzymes

Although less abundant than in the liver, cytochrome P-450 enzymes (CYPs) are also expressed in the intestinal wall and can potentially decrease the oral bioavailability of a wide variety of drugs such as midazolam and cyclosporine [80]. CYP3A4/5 accounts for 80% of the total immuno-quantifiable intestinal CYPs, with its expression decreasing from the duodenum to the ileum [81].

The ontogeny of hepatic drug metabolizing enzymes has been thoroughly documented [82–85]. On the other hand, there are far fewer data on intestinal metabolizing enzyme ontogeny, probably as a result of the scarcity of quality pediatric intestinal tissue samples. Unfortunately, the ontogeny profile of drug metabolizing enzymes varies across organs [86,87] and the developmental changes at the intestinal level cannot be extrapolated from another tissue. The precise mechanisms of this organ-specific developmental regulation remain to be determined. In his pioneering and comprehensive work using biopsies and surgical specimens, Johnson *et al.* [88] demonstrated that duodenal CYP3A4 protein and activity change with age. Both endpoints gradually increased from barely detectable levels in the fetus to maximal levels by the age of approximately 2 years (Figure 3). A subsequent study investigated 59 duodenal biopsies from donors of 1 month to

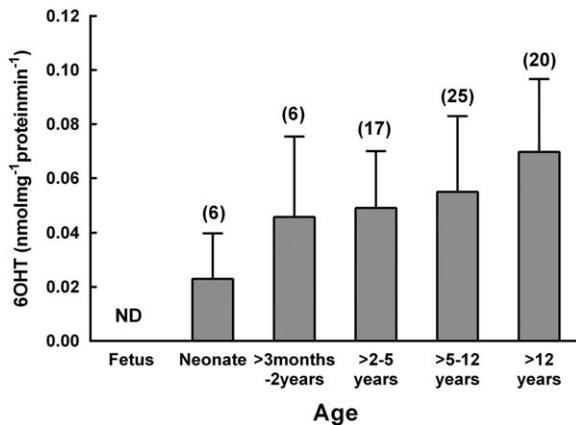


Figure 3. Age-related changes in human intestinal CYP3A4 activity as measured by the rate of testosterone 6- $\beta$  hydroxylation. Sample size given by the numbers in brackets. Reproduced from [88]

17 years of age [89]. As demonstrated by immunohistochemistry, CYP3A4 protein was detected in all enterocytes from 6 months of age. CYP3A4 and CYP3A5 mRNA were elevated during the first year of life and then gradually decreased. Of note, duodenal samples showed low levels of CYP3A7, the fetal liver CYP3A isoform. More recently, Chen *et al.* [86] investigated a total of 112 liver and intestinal samples from human donors (1–198 days of age). While the level of liver CYP3A4 mRNA was positively correlated with age, duodenal CYP3A4 mRNA showed a more complex and almost opposite pattern. Duodenal CYP3A4 mRNA expression tended to increase between day 1 to 96, and then gradually decreased with age. Overall, when compared with the 1–70 days age group, adults showed a 67% decrease in CYP3A4 mRNA. The same adult group showed, however, higher CYP3A4 protein level suggesting that a translational efficiency increases with age. However, these observed changes were not statistically significant and were hampered by a large inter-individual variability. Overall, the above data indicate that intestinal CYP3A4 functional activity increases with postnatal age, a finding that cannot be predicted from mRNA measurement.

Midazolam is extensively transformed by CYP3A4/5. In healthy subjects, about 50% of an oral dose is cleared by intestinal CYP3A4 metabolism, as demonstrated by comparing the kinetics

after oral versus intravenous with and without co-administration with CYP3A4 inhibitors [90,91]. De Wildt *et al.* [92] showed that the midazolam oral bioavailability was substantially higher in preterm infants (49%) when compared with the values reported in adults (24–38%) [93,94]. Similarly, the  $CL/F$  after oral dosing was nearly 10-fold lower in infants than in older age groups [92]. The authors concluded that these findings were due to the ontogeny of both liver and intestinal CYP3A4 activity. Beyond the often-cited midazolam example, many other drugs are extensively transformed by intestinal CYP3A4 [80,95] and could potentially show ontogeny in their disposition. Sirolimus has a low oral bioavailability (14%) mostly linked to its extensive intestinal CYP3A4 metabolism (84% intestinal extraction) [96]. Recent pharmacokinetic data from a multicenter phase II clinical trial in pediatric patients showed a clear effect of age on sirolimus metabolism [97]. The formation rate of the two most abundant circulating metabolites, 16-O-demethylsirolimus and 24-hydroxysirolimus, gradually increased with age. The median metabolite ratios in children (3–14 years) was about 2-fold lower than in adults (25–48 years) which might be explained by immature intestinal CYP3A4. The HIV protease inhibitor saquinavir belongs to those drugs with over 80% of the oral dose cleared by CYP3A4-mediated intestinal clearance [95]. Grub *et al.* reported that the  $CL/F$  of an oral dose of saquinavir was higher in children (3–13 years) than in adolescents or adults. The authors tentatively assumed a higher intestinal clearance and/or lower bioavailability in children [98]. However, this hypothesis is difficult to reconcile with the lower intestinal CYP3A4 activity reported in pediatrics, although it could be argued that the studies explored different age ranges. In adults, the anxiolytic buspirone has an oral bioavailability as low as 4% [99] which parallels its 79% CYP3A4 first-pass intestinal extraction [95]. A pharmacokinetic study in children 2–6 years old receiving a single oral dose of buspirone did not reveal any age-related changes in  $CL/F$  [100], with values indistinguishable from those measured in adults [95].

As illustrated with all the above examples, the impact of intestinal CYP3A4 ontogeny on drug disposition is not yet fully understood and

requires further investigations which should also explore potential confounding factors (e.g. pediatric formulations, feeding regimen, disease states).

Drug metabolism in the intestinal wall is not restricted to CYPs and involves other enzymes that are far less well documented with respect to maturation. Glutathione-S-transferase (GST) is expressed in the liver but also along the length of the small intestine. GST is responsible for the metabolic elimination of busulfan, an alkylating agent used in hematologic malignancies. Hassan first reported an age effect on busulfan pharmacokinetics after oral dosing. The exposure was found to be lower in children under 5 years of age when compared with older children and adults (2 to 3-fold difference), even when the values were normalized for body surface or body weight [101] (Figure 4). Subsequent *in vitro* studies demonstrated that intestinal GSTA1 participates in the first-pass extraction of bisulfan with the activity being up-regulated in young children under 5 years of age when compared with older subjects [102].

Carboxylesterase-2 (CES2) is highly expressed in the intestinal wall of adults, with lower levels in the liver, and the enzyme is known for contributing to the first-pass metabolic hydrolysis of various drug substrates such as the ester prodrugs of the angiotensin receptor blockers candesartan and olmesartan [77]. CES2 mRNA and protein were measured in duodenal samples from human donors of various ages [86]. Both endpoints were found to increase with time to reach adult levels

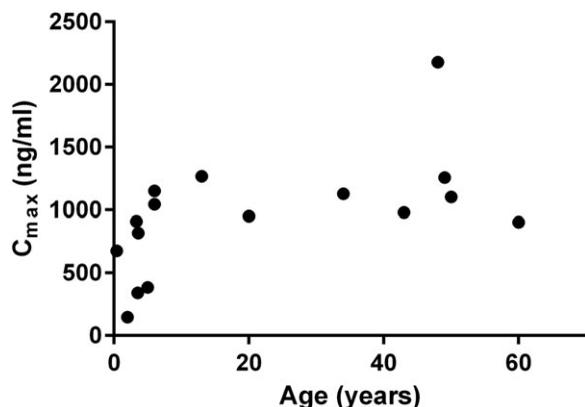


Figure 4. Age-related changes in busulfan plasma exposure of after oral dosing. C<sub>max</sub> values were normalized for the dose and body weight. Adapted from [101]

at 0.5–1 year of age. Despite the reported ontogeny of intestinal CES2, the prodrugs candesartan cilexetil and olmesartan medoxomil show the same pharmacokinetic profile after oral dosing to children when compared with adults [103,104]. Valacyclovir, an ester prodrug of acyclovir, is also claimed to be cleared by intestinal CES2 [105]. Surprisingly, its exposure after oral dosing consistently increases with age (from 2 to 18 years) [106], which contradicts CES2 ontogeny data. It could tentatively be assumed that valacyclovir hydrolysis is preferably supported by an enzyme distinct from CES2 [107], with a different ontogeny pattern.

Phosphate prodrugs, designed to improve the absorption of water-insoluble compounds, are based on a prior intestinal dephosphorylation step that is supported by the brush border enzyme alkaline phosphatase. The hydrolysis results in intraluminal supersaturation of the active moiety, which in turn favors the absorptive process. This approach has been used successfully with fosamprenavir, the phosphate prodrug of the water-insoluble amprenavir [108]. Of interest, a population pharmacokinetic analysis of fosamprenavir revealed an effect of age on body weight-adjusted CL/F [109]. The 1.8-fold higher CL/F in the youngest children (2 month) compared with children >4 years possibly reflects a lower F because of immature intestinal alkaline phosphatase activity [110]. The exact mechanisms, however, remain unclear; especially considering that the enzyme has multiple roles in the maintenance of the intestinal function (pH, absorption of lipids, modulation of gut flora) [111].

Glucuronidation enzymes (UGTs) are highly expressed in the intestinal wall and are responsible for the low oral bioavailability of some drugs such as raloxifene [112]. While the ontogeny of hepatic UGTs has been documented [113], there is little published data about the intestinal counterparts. Court *et al.* investigated the mRNA levels of 19 UGT isoforms in various adult and fetal tissues including small intestine and colon [114]. Unfortunately, developmental changes were discussed for liver and nasal tissues, not for intestine. The mRNA of UGT2B7, the enzyme primarily involved in the metabolism of zidovudine [115] and chloramphenicol [116], was found to be highly expressed in adult but not in fetal liver.

Interestingly, the oral bioavailability of zidovudine is significantly higher in infants  $\leq 14$  days (89%) when compared with older children (61%) [117]. The 'gray-baby' syndrome (see Introduction) occurred following chloramphenicol administration to neonates. This serious and life-threatening syndrome is believed to result from reduced metabolic clearance, and hence high exposure, of chloramphenicol associated with immature UGT2B7 [118]. Although immature hepatic UGT2B7 is likely to be involved in zidovudine and chloramphenicol findings, the intestinal form could also contribute.

Other drug metabolizing enzymes demonstrate little change with age, including epoxide hydrolase, glutathione peroxidase [119] and alcohol dehydrogenase [120]. The ontogeny of the other intestinal drug metabolizing enzymes remains to be examined. Developmental patterns based on mRNA expression are not necessarily predictive of the changes occurring at the protein or activity level. This is not unexpected given the numerous regulatory mechanisms occurring after mRNAs are manufactured (see [121] for a review).

### *Intestinal wall transporters*

Transporters are membrane-bound proteins playing a major role in the cellular uptake and efflux transport of endogenous and exogenous compounds. Numerous transporters belonging to the ATP binding (P-gp, BCRP, MRPs) or solute carrier super-families (PEPT1, OATPs) are expressed in the intestine and are important determinants of oral drug pharmacokinetics (see [122] for review).

P-glycoprotein (P-gp) is, by far, the most studied intestinal efflux transporter and is known to markedly affect the oral bioavailability of various drugs, especially those with poor solubility and low passive diffusion permeability. Several studies have demonstrated that intestinal P-gp is undetectable in early fetal life, then increases with age to reach adult expression after 12 weeks of gestation [123,124]. As expected, there are no age-related differences in intestinal P-gp expression between neonates, infants, children and adults [125,126]. It should be pointed out that all these investigations focused on mRNA and therefore need careful interpretation. Indeed, it has been demonstrated that mRNA levels for

intestinal transporters might not correlate with the corresponding proteins or functional activities [121,127]. Where available, the pharmacokinetic behavior of P-gp substrates in the pediatric population *vs* adults is more informative. Cyclosporin has a poor oral bioavailability as a result of high intestinal CYP3A4 first-pass metabolism combined with effective P-gp-mediated efflux. The bioavailability of cyclosporine was found to be constant from 0.4 to 18 years of age, which suggests an absence of changes in intestinal P-gp and/or CYP3A4 activities in this age range [81]. Aliskiren is another P-gp substrate with an oral bioavailability as low as 3% [128] with the possibility of drug interactions when co-administered with P-gp inhibitors [129,130]. A juvenile rat toxicity study revealed a substantial increase in plasma exposure and severe toxicity when aliskiren was delivered to pups compared with adult animals. Immaturity of intestinal P-gp was identified as the causative mechanism [131]. Conversely, the pharmacokinetics and safety profile of aliskiren in pediatric patients (6–17 years old) were found similar to adults [132]. Although incomplete, these data may suggest species differences in the developmental pattern of intestinal P-gp, with less impact in humans.

When compared with P-gp, there are fewer data on the other intestinal transporters. Immunodetectable breast cancer resistant protein (BCRP), another efflux transporter limiting the intestinal absorption of drugs, was demonstrated as early as 6 weeks of gestation [123]. As for BCRP, data related to the multi-drug associated protein 1 (MRP1) efflux transporter are scarce and only one study reported immunodetectable protein levels comparable to adults from 7 weeks of gestation [123]. Mooij *et al.* [125,133] reported stable mRNA expression of MRP2 from neonates to adults. The expression of influx oligopeptide transporter PEPT1 was investigated in intestinal tissue samples across the pediatric age range [133]. PEPT1 mRNA expression of the neonates was only marginally lower (0.8-fold) than the older children. Immunostaining demonstrated specific apical localization of PEPT1 in neonates, comparable to that observed in adults [133,134]. The organic anion transporting polypeptide (OATP) 2B1 uptake transporter appears to have a different ontogeny pattern compared with the

transporters described earlier. OATP2B1 mRNA expression was found to be 3-fold higher in neonates compared with adults [125,133].

Again, mRNA may not be predictive of transporter protein level or activity, and should be interpreted cautiously. Hopefully, absolute transporter protein abundance data are becoming more and more available thanks to the recent developments in mass spectrometry-based targeted proteomics [135,136]. Compared with mRNA, abundant protein level is usually considered to be a more suitable parameter of functional activity. However, some recent reports have emerged showing a disconnection between protein expression and activity for some transporters [137]. One of the proposed hypotheses relates to post-translational modifications (glycosylation, phosphorylation and ubiquitination) as described for P-gp, PEPT2, OATP2B1 and BCRP [138,139]. Because of the limitations of the expression data, more functional and pharmacokinetic studies are needed to understand age-related changes in

intestinal transporter activity. Of note, pharmacokinetic data across age are typically missing for drugs that are substrates of intestinal transporters other than P-gp.

*Experimental approaches to measure developmental changes in intestinal functions (Table 1)*

Many *in silico* (e.g. calculated logP and PSA) and *in vitro* cellular assays (e.g. Caco-2, MDCK cell lines, expressed metabolizing enzymes and transporters) have been successfully applied to characterize and predict the intestinal absorption, metabolism and transport of drugs (see [140–142] for review). These assays cannot directly assess effects of age on drug disposition. Instead, they allow a mechanistic understanding of the drug identifying the properties that might limit its availability (e.g. low solubility, instability in the gastrointestinal tract, efflux, intestinal first-pass). Also, these assays are useful to identify the specific absorption and dissolution mechanisms,

Table 1. Experimental methods to study developmental changes in intestinal drug absorption, metabolism and transport

Model	Applications	Limitations	Examples of use for studying ontogeny of intestinal function
Enzyme and transporter expression in tissue collection and biopsies	mRNA, protein and activity related to metabolizing enzymes and transporters	mRNA might be not predictive of functional activity Difficulties to access healthy tissue samples Limited scope	CYP3A4 protein and activity measured in 74 histologically normal pediatric biopsies [88]
Brush-border membrane vesicles <i>Ex vivo</i> everted sac	Active uptake Permeability, metabolism and transport	Not amenable to human	Intestinal uptake of folate in rats of varying ages [148] Intestinal transport and metabolism of prostaglandin PGF2 in rats of varying ages [210]
<i>Ex vivo</i> Ussing chamber	Permeability, metabolism and transport	Difficulties to access healthy tissue samples	Intestinal permeability of various compounds measured in rats of varying ages [163]
<i>In situ</i> intestinal single pass perfusion	Permeability, metabolism and transport	Complex surgical procedures and instrumentation Hardly amenable to children	Intestinal permeability of various compounds measured in rats of varying ages [156]
<i>In vivo</i> animal pharmacokinetic studies	Absorption, transport, metabolism	Animal data not translatable to human. Ontogeny time frame different than in humans	Ontogeny of intestinal P-gp responsible for the higher bioavailability and higher toxicity of aliskiren in rat pups when compared with adult animals [131]
PBPK	Full integration of all the data. Predicted drug concentration over time in plasma and various tissues	Relative lack of physiological and biochemical data across age groups	Age-dependent change in the intestinal first-pass metabolism of voriconazole. Knowledge gaps discussed [199–201]
Pharmacokinetic trial in children		Restricted to diseased subjects; small sample size; ethical barriers	Pediatric microdose study of [ <sup>14</sup> C] paracetamol using accelerated mass spectrometry detection [180]

enzymes and transporters involved. Once identified, the properties of concern can be scrutinized for potential developmental changes using the relevant *ex vivo*, *in situ* or *in vivo* approaches.

*Ex vivo* and *in situ* methods using intact tissue specimens have been developed to study intestinal drug disposition. Although these methods have some limitations and are not always amenable to humans, they can be instrumental for exploring age effects, as well as species differences, effects of formulation, food, co-administrations and disease states on intestinal functions (see [143] for review). Brush-border membrane vesicles (BBMV) consist of apical membrane preparations isolated from intestinal segments through a number of homogenization and purification steps [144]. The BBMV method was developed to study transport activities across the apical membrane in various species including human [145,146]. When compared with the other techniques listed here below, BBMV are easier to set up and less demanding in terms of tissue quality. Unfortunately, since the apical and basolateral membranes are separated, the use of BBMV is restricted to the study of active uptake into or efflux from enterocytes, not to the prediction of intestinal absorption *in vivo*. BBMV have been used to investigate developmental changes in intestinal brush border functions of mice [147], rats [148,149], minks [150], piglets [151] and calves [152]. To the best of our knowledge, the technology has never been used to study ontogeny of intestinal function in humans.

Everted sac preparations are based on intestinal segments, inverted over a glass rod to expose the serosa to the outside, and tied off at the ends [143,144]. An oxygenated solution is injected into the inner compartment and then the sac is placed in a buffered medium. Transport of drugs can be studied in either direction, mucosa to serosa or serosa to the mucosa. The method allows investigations of intestinal drug absorption, transport and gut wall metabolism. Unfortunately, the method suffers numerous drawbacks, e.g. the limited availability of human tissues, rapid deterioration of intestinal function after tissue removal (within minutes), limited viability of the intestine under *in vitro* conditions, lack of digestive enzymes, and a small serosal compartment that may confound the kinetics. So far, the method has only

been applied to animal species such as mice, rats, guinea-pigs, rabbits, sheep and pigs [153].

The single-pass intestinal perfusion technique is based on the cannulation and perfusion of a segment of the intestine [143,154]. The disappearance of the drug from the perfusate is measured to determine the rate and extent of absorption. The method can also be used to investigate intestinal metabolism and active transport. While *ex vivo* isolated intestinal perfusion is only applicable to laboratory animals, *in situ* perfusion can be used across all species including human and is recommended by the FDA for classifying drugs based on BCS [154]. *In situ* perfusion was used to demonstrate the higher intestinal permeability in suckling rats compared with older rats [155]. In another rat study, the jejunal absorption of metoprolol and antipyrine was found to be constant over 5–30 weeks of age [156]. *In situ* perfusion studies in human adults remain scarce due to the ethical issues and limited availability of healthy volunteers. So far, the method has not been reported in children.

The Ussing chamber is a powerful and well validated *in vitro* method involving a small section of intestinal mucosa mounted between two chambers (i.e. donor and receiver compartments). Samples are collected from the receiver side at intervals and analysed for drug appearance [143]. The Ussing chamber technique has been successfully applied to animal and human tissues to study drug permeability, metabolism and transport for many compounds. Recently, using a large set of compounds, the *in vitro* permeability as measured in pig intestinal tissues was found to correlate well with the values measured in human samples [157]. As for the other *in vitro* methods, the Ussing chamber technique requires fresh surgical samples (to be used within 1 hour following surgical excision) which hampers studies with human material [158]. Endoscopic biopsies have been introduced as an alternative amenable to healthy adult volunteers [159] or diseased children [160–162]. Using the Ussing chamber technique, rat intestinal segments and various marker drugs, Annaert *et al.* [163] showed a decrease in the paracellular pathway in aging rats. This finding was paralleled with an increase in the transcellular flux and P-gp mediated-efflux. There has been no reported attempt to use human

excised intestinal segments to study developmental changes in intestinal drug absorption, metabolism or transport.

The access to human surgical samples is restricted and limits the use of the above mentioned *in vitro* methods. In most cases, specimens originate from patients undergoing gastrointestinal surgery [159] or from organ donors [164]. The conditions of the human tissue samples are variable due to differences in diet, medication, environmental conditions, surgical procedure, separation from adjacent diseased tissue, which all may affect the tissue morphology, the functional activity and the data collected *in vitro* [157]. Finally, irrespective of the technique, the tissue has a limited viability *in vitro* and only incubations of less than 3 hours can be envisaged [158]. Biopsy samples are easier to obtain but provide a small amount of tissue (2–21 mm<sup>2</sup>) and so may not be compatible with all *ex vivo* methodologies or expression analysis (e.g. mRNA and protein). Healthy intestinal biopsies can be obtained in adult volunteers, but rarely from healthy children, which limits the investigation on age-dependent changes in the control population. Very recently, precision-cut intestinal slices have re-emerged as a tool for *in vitro* metabolism, transport, drug interaction and toxicity studies both in preclinical animal species and in humans [165]. It allows the study of numerous compounds in parallel using tissues from the same individual. Although potentially powerful, this latter technique is likely to have the same shortcomings as other methods using excised tissues (i.e. limited access to healthy specimens).

*In vivo* studies using preclinical animals are routinely used to explore the pharmacokinetic profile of drug candidates and predict the clinical outcome. Unfortunately, species differences in drug disposition usually make animal studies poorly translatable to humans. The most commonly used species are mice, rats, dogs and monkeys, but also recently minipigs [166]. There are reports describing a good correlation between oral drug absorption in rodents and humans [167], however, using different sets of test compounds, other studies concluded poor predictivity [168–170]. Similarly, absorption in monkey appears to be predictive of humans [171], except for highly transformed compounds [172]. Drug absorption in dogs was first described as poorly predictive

of the human outcome [173]. Subsequent studies indicated that the paracellular transport route for polar compounds is overestimated in dogs. Consequently, when polar compounds are excluded from the dataset, dog might be a more relevant to humans [168]. Overall, the translation of drug absorption and intestinal permeation from animals to humans remains challenging. This is not unexpected given the numerous processes involved and their propensity for species differences [174]. The use of laboratory animals to predict age-related changes in human drug absorption is likely to be even more hazardous. Although never fully scrutinized, the ontogeny of the various gastrointestinal functions is likely to have a time course that varies across species. This deserves more attention as it may improve the design and interpretation of toxicity studies in juvenile animals [87].

### Pediatric clinical pharmacokinetic studies

Pending more predictive preclinical models and a better understanding of the ontogeny of intestinal processes, there is an overwhelming need for more pharmacokinetic trials in the pediatric population, especially those that measure changes in absorption parameters with age. Clinical data are necessary to provide a rational basis for dosage adjustment in this vulnerable population, to generate scientific evidence for the developmental changes in intestinal processes (e.g. population pharmacokinetic analysis), and to gradually fill the knowledge gaps in the field. Often, because of the paucity of clinical data, the pharmacokinetic profile in the pediatric population is predicted from adult data assuming that the absorption rate constant and the fraction absorbed are conserved across the age spectrum. A recent review of 1081 registered trials in children revealed that only 24% of the trials incorporated pharmacokinetic measurements [175]. Also, 74% of the eligible trials were conducted in children aged over 2 years, while changes in drug disposition and drug response tend to predominate in younger children. The limited number of pediatric studies, especially in very young children, illustrates the numerous obstacles associated with those studies,

from practical and methodological difficulties to regulatory requirements and ethical concerns.

Pediatric trials typically involve fewer study participants compared with trials in adults [176]. Innovative study designs (e.g. adaptive design, Bayesian approach, randomized withdrawal design) are required to accommodate the small group sizes, and possibly facilitate ethical acceptance.

Blood sample collection in children is more challenging than in adults. The blood volume that can be safely collected at a time in newborns is much smaller than in adults (1 ml versus up to 10 ml, respectively) [16]. Venipuncture is also technically more challenging in children, a needle prompting an emotional response in the child and the parents, with the practitioner being reluctant to have more than one or two attempts at venipuncture before sampling [177]. Microsampling from finger and heel prick can be used as an alternative to venipuncture. A novel dried blood sampler (termed volumetric absorptive micro-sampler) was recently introduced to overcome the technical issues observed when taking a sub-punch of dried blood spot samples [178]. Hopefully, the newer bioanalytical techniques are sensitive enough to accommodate those smaller volume samples.

From a regulatory perspective, drugs investigated in pediatric trials should either provide a direct benefit to the subjects (precluding studies in healthy volunteers) or be used at a dose where risks are minimal. The latter includes the use of microdosing studies in children who have a disorder or condition which is the object of the research [179]. A proof of concept pediatric microdose study investigating [ $^{14}\text{C}$ ]-paracetamol in diseased children and using accelerated mass spectrometry was recently reported [180]. Microdosing in control healthy children is not authorized. In addition, as for adults, microdosing has some limitations with respect to saturable processes such as intestinal transport and metabolism [181–183].

The absence of age-appropriate formulations has been identified as another factor limiting pharmacokinetic studies in the pediatric population [16]. Pediatric trials with oral drugs often require dedicated formulations differing from those used in adults. Pediatric formulations

typically include liquids, chewable tablets, rapidly dissolving tablets, and more palatable flavors.

### Physiologically based pharmacokinetics (PBPK)

Traditionally, the drug dosage to be administered to children has been estimated from adult data using allometric scaling related to body weight or body surface area [184]. Recently, a move towards more mechanistic approaches has been made, integrating developmental changes in key anatomical, physiological and biochemical processes relevant for drug disposition [185]. Nowadays, physiologically based pharmacokinetic (PBPK) modeling is routinely used for this mechanistic purpose, and is available as various ready-to-use 'designed' software (e.g. Simcyp<sup>®</sup>, PKSim<sup>®</sup>, Gastroplus<sup>®</sup>) [186].

Initially, PBPK software primarily focused on adult populations. Subsequently, pediatric modules have been added to integrate the physiological differences between adults and children. These modules, derived from the most recent literature, describe in mathematical terms the relationships between age and various physiological and biochemical variables such as body weight, body height, organ volume, cardiac output, renal function, plasma protein binding and some key drug metabolizing enzymes (e.g. CYPs) [187–189]. The gastrointestinal function is covered as well including e.g. age-related changes in organ volume, absorptive surface area, permeability, enzymes activity, pH, intestinal fluid content and transit time [190,191].

There are several recent examples illustrating the value of PBPK for predicting drug disposition in the pediatric population, and for supporting pediatric approval [192–195]. Often, the strategy used to predict disposition in pediatrics involves an initial step where a PBPK model is built to describe the drug pharmacokinetics in adults [195–197]. It is an iterative process where predicted and observed data are compared with further improve the model. Then, once the model has been qualified in adults, its physiological variables are scaled down to the pediatric population, whereas the drug-related parameters remain unchanged.

Such a workflow allows the sponsor and the regulatory authorities to gain confidence in the understanding of the drug pharmacokinetics before performing studies in the vulnerable pediatric population.

Although successful predictions for pediatrics have been reported, one should recognize the knowledge gaps that still limit the predictive value of PBPK. Indeed, the predictability of PBPK models is strongly dependent on the understanding of the drug properties and of the key physiological processes involved in its disposition. Unfortunately, as highlighted earlier, most of the developmental changes affecting drug absorption are still poorly understood. A recent study from a PhRmA initiative [198] showed that the current methods to predict the pharmacokinetics in adults were about three times less predictive for oral when compared with intravenous drugs. The plasma time course profiles after oral dosing were accurately predicted in only 23% of the cases, illustrating the need for a better understanding of the gastrointestinal physiology, its sensitive factors as well as the interplay between all the underlying processes driving drug absorption. Modeling for pediatrics is even more challenging considering the additional hurdle of our limited understanding of the developmental changes in gastrointestinal processes. This is nicely illustrated with voriconazole which shows an oral bioavailability of 45–66% in pediatrics to be compared with 96% in adults, a difference that cannot be explained by age-dependent changes in hepatic clearance. The data were recapitulated in a retrospective PBPK analysis incorporating an extensive intestinal first-pass in pediatric, but not in adult, subjects [199]. This would imply that the enzymes involved in voriconazole extraction are overexpressed in the pediatric intestine, which either has not been unambiguously established (CYP3A4) or has not been studied (CYP2C19 and FMO3) [200,201]. This case study illustrates how PBPK modeling is undoubtedly useful for mechanistic investigations but still suffers limitations for prospective predictions of complex drugs.

In order to fill these needs, the Innovative Medicines Initiative (IMI) launched a dedicated initiative in 2012, the Oral Biopharmaceutical Tools (OrBiTo) project [202]. The project is intended to refine existing tools and to explore newer assays

in order to better understand and predict drug absorption. Ultimately, the project plans to include the full complexity of drug absorption integrating drug release and dissolution (as a function of both pH and intestinal content), potential precipitation all along the gastrointestinal tract, potential impact of gut flora, intestinal enzymes and transporters. Because the IMI is Europe's largest public-private initiative in life sciences combining academia, pharmaceutical companies and software companies, the OrBiTo project will be an important and useful step to better understand and predict the pharmacokinetic profile of oral drugs, including in pediatrics.

## Conclusion

A recent FDA survey showed that 42% of recently completed pediatric trials have failed to demonstrate either safety or efficacy, and pediatric approval could not be obtained [175]. One quarter of the efficacy failures was attributed to suboptimal exposure, probably from a misapprehension of age-related changes in drug disposition.

Thanks to coordinated efforts from the pharmaceutical industry, academics and regulatory agencies, enormous progress has been made in our understanding of the age-related changes in drug disposition, particularly those relating to liver drug oxidation and transport. So far, ontogeny of intestinal drug absorption and pre-systemic metabolism has been overlooked with gaps in our knowledge remaining.

Specific research efforts are needed on the age-dependent expression of intestinal metabolizing enzymes, beyond CYP3A4, and drug transporters. Although mRNA data can be easily generated, it may not be predictive. Thus, protein quantitation using LC-MS-MS and *ex vivo* functional assays should be given a high priority. Expression data are often reported for fetal and neonatal intestinal samples while investigations should cover the whole age range, with more information in infants and children. The relative lack of physiological data across age groups restricts the use of PBPK modeling to predict drug disposition in the pediatric population. Although encouraged by regulatory agencies, clinical trials in the pediatric population are still limited. More clinical data in

the pediatric population will help validating PBPK models and building more evidence based information on the effects of age on intestinal absorption. All these information gaps need to be filled to ensure the development of safer and more efficacious drugs for children.

## Acknowledgments

We thank Drs C. Peters and J. Tibbitts for their careful review of the manuscript and their many insightful comments and suggestions.

## Conflict of Interest

The authors have no conflict of interest.

## References

- Christensen ML. Best pharmaceuticals for children act and pediatric research equity act: time for permanent status. *J Pediatr Pharmacol Ther* 2012; **17**: 140–141. doi:10.5863/1551-6776-17.2.140.
- Shirkey H. Therapeutic orphans. *J Pediatr* 1968; **72**: 119–120.
- Kimland E, Odlind V. Off-label drug use in pediatric patients. *Clin Pharmacol Ther* 2012; **91**: 796–801 .DOI: clpt201226 [pii];10.1038/clpt.2012.26 [doi]
- Standing JF, Tuleu C. Paediatric formulations – getting to the heart of the problem. *Int J Pharm* 2005; **300**: 56–66 .DOI: S0378–5173(05)00313–3 [pii];10.1016/j.ijpharm.2005.05.006 [doi]
- Sutherland JM. Fatal cardiovascular collapse of infants receiving large amounts of chloramphenicol. *AMA J Dis Child* 1959; **97**: 761–767.
- Sage DP, Kulczar C, Roth W, Liu W, Knipp GT. Persistent pharmacokinetic challenges to pediatric drug development. *Front Genet* 2014; **5**: 281 . DOI: 10.3389/fgene.2014.00281 [doi]
- Kalra A, Goindi S. Issues impacting therapeutic outcomes in pediatric patients: an overview. *Curr Pediatr Rev* 2014; **10**: 184–193 .DOI: CPR-57936 [pii]
- Corny J, Lebel D, Bailey B, Bussieres JF. Unlicensed and off-label drug use in children before and after pediatric governmental initiatives. *J Pediatr Pharmacol Ther* 2015; **20**: 316–328. doi:10.5863/1551-6776-20.4.316.
- Ruggieri L, Giannuzzi V, Baiardi P, et al. and Consortium GR. Successful private-public funding of paediatric medicines research: lessons from the EU programme to fund research into off-patent medicines. *Eur J Pediatr* 2015; **174**: 481–491. doi:10.1007/s00431-014-2398-z.
- Merchant HA, Liu F, Orlu GM, Basit AW. Age-mediated changes in the gastrointestinal tract. *Int J Pharm* 2016 .DOI: S0378–5173(16)30308–8 [pii];10.1016/j.ijpharm.2016.04.024 [doi]
- Batchelor HK, Fotaki N, Klein S. Paediatric oral biopharmaceutics: key considerations and current challenges. *Adv Drug Deliv Rev* 2014; **73**: 102–126 . DOI: S0169-409X(13)00248–2 [pii];10.1016/j.addr.2013.10.006 [doi]
- Kaye JL. Review of paediatric gastrointestinal physiology data relevant to oral drug delivery. *Int J Clin Pharm* 2011; **33**: 20–24. DOI: 10.1007/s11096-010-9455-0
- Bowles A, Keane J, Ernest T, Clapham D, Tuleu C. Specific aspects of gastro-intestinal transit in children for drug delivery design. *Int J Pharm* 2010; **395**: 37–43 .DOI: S0378–5173(10)00345–5 [pii];10.1016/j.ijpharm.2010.04.048 [doi]
- Mooij MG, de Koning BA, Huijsman ML, de Wildt SN. Ontogeny of oral drug absorption processes in children. *Expert Opin Drug Metab Toxicol* 2012; **8**: 1293–1303. doi:10.1517/17425255.2012.698261.
- Avery GB, Randolph JG, Weaver T. Gastric acidity in the first day of life. *Pediatrics* 1966; **37**: 1005–1007.
- Batchelor HK, Marriott JF. Paediatric pharmacokinetics: key considerations. *Br J Clin Pharmacol* 2015; **79**: 395–404.
- Milsap RL, Jusko WJ. Pharmacokinetics in the infant. *Environ Health Perspect* 1994; **102**(Suppl 11): 107–110.
- Bartelink IH, Rademaker CM, Schobben AF, van den Anker JN. Guidelines on paediatric dosing on the basis of developmental physiology and pharmacokinetic considerations. *Clin Pharmacokinet* 2006; **45**: 1077–1097. doi:10.2165/00003088-200645110-00003.
- Koren G. Therapeutic drug monitoring principles in the neonate. National Academy of Clinical Biochemistry. *Clin Chem* 1997; **43**: 222–227.
- Yu G, Zheng QS, Li GF. Similarities and differences in gastrointestinal physiology between neonates and adults: a physiologically based pharmacokinetic modeling perspective. *AAPS J* 2014; **16**: 1162–1166. doi:10.1208/s12248-014-9652-1.
- Al ZM, Lanner A, Xiaonian X, Donovan T, Charles B. Application of routine monitoring data for determination of the population pharmacokinetics and enteral bioavailability of phenytoin in neonates and infants with seizures. *Ther Drug Monit* 2006; **28**: 793–799 .DOI: 10.1097/01.ftd.0000249948.95749.f3 [doi];00007691–200612000-00011 [pii]
- Rohwedder HJ, Simon C, Kubler W, Hohenauer M. Studies on the pharmacokinetics of nalidixic

- acid in children of different ages. *Z Kinderheilkd* 1970; **109**: 124–134.
23. van den Anker JN, van Lingen RA, Koster M, Heykants J, Sauer PJ. Insufficient ketoconazole concentrations in preterm infants with fungal infections. *Eur J Pediatr* 1993; **152**: 538.
  24. Batchelor H. Paediatric biopharmaceutics classification system: current status and future decisions. *Int J Pharm* 2014; **469**: 251–253 .DOI: S0378-5173(14)00139-2 [pii];10.1016/j.ijpharm.2014.02.046 [doi]
  25. Schwartz DA, Connelly NR, Theroux CA, *et al.* Gastric contents in children presenting for upper endoscopy. *Anesth Analg* 1998; **87**: 757–760.
  26. Crawford M, Lerman J, Christensen S, Farrow-Gillespie A. Effects of duration of fasting on gastric fluid pH and volume in healthy children. *Anesth Analg* 1990; **71**: 400–403.
  27. Meakin G, Dingwall AE, Addison GM. Effects of fasting and oral premedication on the pH and volume of gastric aspirate in children. *Br J Anaesth* 1987; **59**: 678–682.
  28. Goetze O, Treier R, Fox M, *et al.* The effect of gastric secretion on gastric physiology and emptying in the fasted and fed state assessed by magnetic resonance imaging. *Neurogastroenterol Motil* 2009; **21**: 725–e42. doi:10.1111/j.1365-2982.2009.01293.x.
  29. Mudie DM, Murray K, Hoad CL, *et al.* Quantification of gastrointestinal liquid volumes and distribution following a 240 ml dose of water in the fasted state. *Mol Pharm* 2014; **11**: 3039–3047. doi:10.1021/mp500210c.
  30. Shawahna R. Pediatric biopharmaceutical classification system: using age-appropriate initial gastric volume. *AAPS J* 2016; **18**: 728–736 .DOI: 10.1208/s12248-016-9885-2 [doi];10.1208/s12248-016-9885-2 [pii]
  31. Gandhi SV, Rodriguez W, Khan M, Polli JE. Considerations for a Pediatric Biopharmaceutics Classification System (BCS): application to five drugs. *AAPS PharmSciTech* 2014; **15**: 601–611. doi:10.1208/s12249-014-0084-0.
  32. Gomes H. Gastric emptying in children. *J Pediatr Gastroenterol Nutr* 2004; **39**: 236–238.
  33. Ziessman HA, Fahey FH, Collen MJ. Biphasic solid and liquid gastric emptying in normal controls and diabetics using continuous acquisition in LAO view. *Dig Dis Sci* 1992; **37**: 744–750.
  34. Tayman C, Rayyan M, Allegaert K. Neonatal pharmacology: extensive interindividual variability despite limited size. *J Pediatr Pharmacol Ther* 2011; **16**: 170–184. doi:10.5863/1551-6776-16.3.170.
  35. Signer E. Gastric emptying in newborns and young infants. Measurement of the rate of emptying using indium-113 m-microcolloid. *Acta Paediatr Scand* 1975; **64**: 525–530.
  36. Riezzo G, Indrio F, Montagna O, *et al.* Gastric electrical activity and gastric emptying in term and preterm newborns. *Neurogastroenterol Motil* 2000; **12**: 223–229.
  37. Anderson BJ, Woollard GA, Holford NH. A model for size and age changes in the pharmacokinetics of paracetamol in neonates, infants and children. *Br J Clin Pharmacol* 2000; **50**: 125–134 . DOI: bcp231 [pii]
  38. Heimann G. Enteral absorption and bioavailability in children in relation to age. *Eur J Clin Pharmacol* 1980; **18**: 43–50.
  39. Kearns GL, Robinson PK, Wilson JT, *et al.* Cisapride disposition in neonates and infants: *in vivo* reflection of cytochrome P450 3A4 ontogeny. *Clin Pharmacol Ther* 2003; **74**: 312–325 .DOI: 10.1016/S0009-9236(03)00225-X [doi]; S000992360300225X [pii]
  40. Bonner JJ, Vajjah P, Abduljalil K, *et al.* Does age affect gastric emptying time? A model-based meta-analysis of data from premature neonates through to adults. *Biopharm Drug Dispos* 2015; **36**: 245–257. doi:10.1002/bdd.1937.
  41. Maharaj AR, Edginton AN. Examining small intestinal transit time as a function of age – is there evidence to support age-dependent differences among children? *Drug Metab Dispos* 2016 .DOI: dmd.115.068700 [pii];10.1124/dmd.115.068700 [doi]
  42. Wagener S, Shankar KR, Turnock RR, Lamont GL, Baillie CT. Colonic transit time – what is normal? *J Pediatr Surg* 2004; **39**: 166–169 .discussion 166–169
  43. Watkins JB, Ingall D, Szczepanik P, Klein PD, Lester R. Bile-salt metabolism in the newborn. Measurement of pool size and synthesis by stable isotope technic. *N Engl J Med* 1973; **288**: 431–434. doi:10.1056/NEJM197303012880902.
  44. Bongiovanni AM. Bile acid content of gallbladder of infants, children and adults. *J Clin Endocrinol Metab* 1965; **25**: 678–685. doi:10.1210/jcem-25-5-678.
  45. de Belle RC, Vaupshas V, Vitullo BB, *et al.* Intestinal absorption of bile salts: immature development in the neonate. *J Pediatr* 1979; **94**: 472–476.
  46. Challacombe DN, Edkins S, Brown GA. Duodenal bile acids in infancy. *Arch Dis Child* 1975; **50**: 837–843.
  47. Kearns GL, Bradley JS, Jacobs RF, *et al.* Single dose pharmacokinetics of pleconaril in neonates. *Pediatric Pharmacology Research Unit Network Pediatr Infect Dis J* 2000; **19**: 833–839.
  48. Clegghorn G, Durie P, Benjamin L, Dati F. The ontogeny of serum immunoreactive pancreatic lipase and cationic trypsinogen in the premature human infant. *Biol Neonate* 1988; **53**: 10–16.
  49. Lebenthal E, Lee PC. Development of functional responses in human exocrine pancreas. *Pediatrics* 1980; **66**: 556–560.
  50. McClean P, Weaver LT. Ontogeny of human pancreatic exocrine function. *Arch Dis Child* 1993; **68**: 62–65.

51. Shankaran S, Kauffman RE. Use of chloramphenicol palmitate in neonates. *J Pediatr* 1984; **105**: 113–116.
52. Lacroix B, Kedinger M, Simon-Assmann P, Haffen K. Early organogenesis of human small intestine: scanning electron microscopy and brush border enzymology. *Gut* 1984; **25**: 925–930.
53. Cummins AG, Catto-Smith AG, Cameron DJ, et al. Crypt fission peaks early during infancy and crypt hyperplasia broadly peaks during infancy and childhood in the small intestine of humans. *J Pediatr Gastroenterol Nutr* 2008; **47**: 153–157. doi:10.1097/MPG.0b013e3181604d27.
54. Penna FJ, Hill ID, Kingston D, Robertson K, Slavina G, Shiner M. Jejunal mucosal morphometry in children with and without gut symptoms and in normal adults. *J Clin Pathol* 1981; **34**: 386–392.
55. Stenling R, Fredrikzon B, Nyhlin H, Helander HF, Falkmer S. Surface ultrastructure of the small intestine mucosa in healthy children and adults: a scanning electron microscopic study with some methodological aspects. *Ultrastruct Pathol* 1984; **6**: 131–140.
56. Risdon RA, Keeling JW. Quantitation of the histological changes found in small intestinal biopsy specimens from children with suspected coeliac disease. *Gut* 1974; **15**: 9–18.
57. Toubanc N, Sargentini-Maier ML, Lacroix B, Jacqmin P, Stockis A. Retrospective population pharmacokinetic analysis of levetiracetam in children and adolescents with epilepsy: dosing recommendations. *Clin Pharmacokinet* 2008; **47**: 333–341. doi:10.2165/00003088-200847050-00004.
58. Artursson P, Ungell AL, Lofroth JE. Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm Res* 1993; **10**: 1123–1129.
59. Ungell AL, Nylander S, Bergstrand S, Sjoberg A, Lennernas H. Membrane transport of drugs in different regions of the intestinal tract of the rat. *J Pharm Sci* 1998; **87**: 360–366. doi:10.1021/js970218s.
60. Artursson P, Palm K, Luthman K. Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* 2001; **46**: 27–43.
61. Strocchi A, Levitt MD. Role of villous surface area in absorption. Science versus religion. *Dig Dis Sci* 1993; **38**: 385–387.
62. Flanagan SD, Takahashi LH, Liu X, Benet LZ. Contributions of saturable active secretion, passive transcellular, and paracellular diffusion to the overall transport of furosemide across adenocarcinoma (Caco-2) cells. *J Pharm Sci* 2002; **91**: 1169–1177.
63. Grahnen A, Hammarlund M, Lundqvist T. Implications of intraindividual variability in bioavailability studies of furosemide. *Eur J Clin Pharmacol* 1984; **27**: 595–602.
64. Mirochnick MH, Miceli JJ, Kramer PA, Chapron DJ, Raye JR. Furosemide pharmacokinetics in very low birth weight infants. *J Pediatr* 1988; **112**: 653–657.
65. Pacifici GM. Clinical pharmacology of furosemide in neonates: a review. *Pharmaceuticals (Basel)* 2013; **6**: 1094–1129. doi:10.3390/ph6091094.
66. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**. e177DOI: 07-PLBI-RA-0129 [pii];10.1371/journal.pbio.0050177 [doi]
67. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010; **107**: 11971–11975. doi:10.1073/pnas.10026011107.
68. Fallani M, Young D, Scott J, et al. and Other Members of the IT. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J Pediatr Gastroenterol Nutr* 2010; **51**: 77–84. doi:10.1097/MPG.0b013e3181d1b11e.
69. Bezirtzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence *in situ* hybridization (FISH). *Anaerobe* 2011; **17**: 478–482. doi:10.1016/j.anaerobe.2011.03.009.
70. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. *Pediatrics* 2012; **129**: 950–960 .DOI: peds.2011–2736 [pii];10.1542/peds.2011–2736 [doi]
71. Madan JC, Farzan SF, Hibberd PL, Karagas MR. Normal neonatal microbiome variation in relation to environmental factors, infection and allergy. *Curr Opin Pediatr* 2012; **24**: 753–759 .DOI: 10.1097/MOP.0b013e32835a1ac8 [doi]
72. Di MA, Neu J, Riezzo G, et al. Gastrointestinal function development and microbiota. *Ital J Pediatr* 2013; **39**: 15 .DOI: 1824–7288–39-15 [pii];10.1186/1824–7288–39-15 [doi]
73. Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006; **118**: 511–521. doi:10.1542/peds.2005-2824.
74. Al-Jumaili IJ, Shibley M, Lishman AH, Record CO. Incidence and origin of *Clostridium difficile* in neonates. *J Clin Microbiol* 1984; **19**: 77–78.
75. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* 2011; **108**(Suppl 1): 4578–4585 .DOI: 1000081107 [pii];10.1073/pnas.1000081107 [doi]
76. Scheline RR. Drug metabolism by intestinal microorganisms. *J Pharm Sci* 1968; **57**: 2021–2037 . DOI: S0022-3549(15)36680-6 [pii]

77. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int J Pharm* 2008; **363**: 1–25 .DOI: S0378-5173(08)00498-5 [pii];10.1016/j.ijpharm.2008.07.009 [doi]
78. Tralau T, Sowada J, Luch A. Insights on the human microbiome and its xenobiotic metabolism: what is known about its effects on human physiology? *Expert Opin Drug Metab Toxicol* 2015; **11**: 411–425. doi:10.1517/17425255.2015.990437.
79. Linday L, Dobkin JF, Wang TC, Butler VP Jr, Saha JR, Lindenbaum J. Digoxin inactivation by the gut flora in infancy and childhood. *Pediatrics* 1987; **79**: 544–548.
80. Gavhane YN, Yadav AV. Loss of orally administered drugs in GI tract. *Saudi Pharm J* 2012; **20**: 331–344 .DOI: 10.1016/j.jsps.2012.03.005 [doi]; SPJ145 [pii]
81. Johnson TN, Thomson M. Intestinal metabolism and transport of drugs in children: the effects of age and disease. *J Pediatr Gastroenterol Nutr* 2008; **47**: 3–10 .DOI: 10.1097/MPG.0b013e31816a8cca [doi];00005176-200807000-00002 [pii]
82. Sadler NC, Nandhikonda P, Webb-Robertson BJ, et al. Hepatic cytochrome P450 activity, abundance, and expression throughout human development. *Drug Metab Dispos* 2016 .DOI: dmd.115.068593 [pii];10.1124/dmd.115.068593 [doi]
83. Hines RN. Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* 2007; **21**: 169–175.
84. Ince I, Knibbe CA, Danhof M, de Wildt SN. Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from *in vitro* and *in vivo* investigations. *Clin Pharmacokinet* 2013; **52**: 333–345. doi:10.1007/s40262-013-0041-1.
85. Neims AH, Warner M, Loughnan PM, Aranda JV. Developmental aspects of the hepatic cytochrome P450 monooxygenase system. *Annu Rev Pharmacol Toxicol* 1976; **16**: 427–445. doi:10.1146/annurev.pa.16.040176.002235.
86. Chen YT, Trzoss L, Yang D, Yan B. Ontogenic expression of human carboxylesterase-2 and cytochrome P450 3A4 in liver and duodenum: postnatal surge and organ-dependent regulation. *Toxicology* 2015; **330**: 55–61 .DOI: S0300-483X(15)00043-8 [pii];10.1016/j.tox.2015.02.007 [doi]
87. de Zwart L, Scholten M, Monbaliu JG, et al. The ontogeny of drug metabolizing enzymes and transporters in the rat. *Reprod Toxicol* 2008; **26**: 220–230. doi:10.1016/j.reprotox.2008.09.010.
88. Johnson TN, Tanner MS, Taylor CJ, Tucker GT. Enterocytic CYP3A4 in a paediatric population: developmental changes and the effect of coeliac disease and cystic fibrosis. *Br J Clin Pharmacol* 2001; **51**: 451–460 .DOI: bcp1370 [pii]
89. Fakhoury M, Litalien C, Medard Y, et al. Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age. *Drug Metab Dispos* 2005; **33**: 1603–1607 .DOI: dmd.105.005611 [pii];10.1124/dmd.105.005611 [doi]
90. Andersen V, Pedersen N, Larsen NE, Sonne J, Larsen S. Intestinal first pass metabolism of midazolam in liver cirrhosis – effect of grapefruit juice. *Br J Clin Pharmacol* 2002; **54**: 120–124 .DOI: 1615 [pii]
91. Tsunoda SM, Velez RL, von Moltke LL, Greenblatt DJ. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an *in vivo* probe: effect of ketoconazole. *Clin Pharmacol Ther* 1999; **66**: 461–471. DOI: S0009923699001563 [pii];10.1016/S0009-9236(99)70009-3 [doi]
92. de Wildt SN, Kearns GL, Hop WC, Murry DJ, Abdel-Rahman SM, van den Anker JN. Pharmacokinetics and metabolism of oral midazolam in preterm infants. *Br J Clin Pharmacol* 2002; **53**: 390–392 .DOI: 1223 [pii]
93. Paine MF, Shen DD, Kunze KL, et al. First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* 1996; **60**: 14–24. doi:10.1016/S0009-9236(96)90162-9.
94. Mandema JW, Tuk B, van Steveninck AL, Breimer DD, Cohen AF, Danhof M. Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite alpha-hydroxymidazolam in healthy volunteers. *Clin Pharmacol Ther* 1992; **51**: 715–728.
95. Gertz M, Houston JB, Galetin A. Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab Dispos* 2011; **39**: 1633–1642 .DOI: dmd.111.039248 [pii];10.1124/dmd.111.039248 [doi]
96. Emoto C, Fukuda T, Cox S, Christians U, Vinks AA. Development of a physiologically-based pharmacokinetic model for sirolimus: predicting bioavailability based on intestinal CYP3A content. *CPT Pharmacometrics Syst Pharmacol* 2013; **2**: e59 .DOI: psp201333 [pii];10.1038/psp.2013.33 [doi]
97. Emoto C, Fukuda T, Mizuno T, et al. Age-dependent changes in sirolimus metabolite formation in patients with neurofibromatosis type 1. *Ther Drug Monit* 2015; **37**: 395–399 .DOI: 10.1097/FTD.0000000000000130 [doi]
98. Grub S, Delora P, Ludin E, et al. Pharmacokinetics and pharmacodynamics of saquinavir in pediatric patients with human immunodeficiency virus infection. *Clin Pharmacol Ther* 2002; **71**: 122–130 .DOI: S000992360273292X [pii];10.1067/mcp.2002.121423 [doi]
99. Mahmood I, Sahajwalla C. Clinical pharmacokinetics and pharmacodynamics of buspirone, an

- anxiolytic drug. *Clin Pharmacokinet* 1999; **36**: 277–287. doi:10.2165/00003088-199936040-00003.
100. Edwards DJ, Chugani DC, Chugani HT, Chehab J, Malian M, Aranda JV. Pharmacokinetics of buspirone in autistic children. *J Clin Pharmacol* 2006; **46**: 508–514 .DOI: 46/5/508 [pii];10.1177/0091270006286903 [doi]
  101. Hassan M, Ljungman P, Bolme P, et al. Busulfan bioavailability. *Blood* 1994; **84**: 2144–2150.
  102. Gibbs JP, Liacouras CA, Baldassano RN, Slattery JT. Up-regulation of glutathione S-transferase activity in enterocytes of young children. *Drug Metab Dispos* 1999; **27**: 1466–1469.
  103. Wells TG, Blowey DL, Sullivan JE, et al. Pharmacokinetics of olmesartan medoxomil in pediatric patients with hypertension. *Paediatr Drugs* 2012; **14**: 401–409. doi:10.2165/11631450-000000000-00000.
  104. Schaefer F, van de Walle J, Zurowska A, et al. Efficacy, safety and pharmacokinetics of candesartan cilexetil in hypertensive children from 1 to less than 6 years of age. *J Hypertens* 2010; **28**: 1083–1090. doi:10.1097/HJH.0b013e328336b86b.
  105. Laizure SC, Herring V, Hu Z, Witbrodt K, Parker RB. The role of human carboxylesterases in drug metabolism: have we overlooked their importance? *Pharmacotherapy* 2013; **33**: 210–222. doi:10.1002/phar.1194.
  106. Bomgaars L, Thompson P, Berg S, Serabe B, Aleksic A, Blaney S. Valacyclovir and acyclovir pharmacokinetics in immunocompromised children. *Pediatr Blood Cancer* 2008; **51**: 504–508. doi:10.1002/pbc.21638.
  107. Kim I, Chu XY, Kim S, Provoda CJ, Lee KD, Amidon GL. Identification of a human valacyclovirase: biphenyl hydrolase-like protein as valacyclovir hydrolase. *J Biol Chem* 2003; **278**: 25348–25356. DOI: 10.1074/jbc.M302055200 [doi];M302055200 [pii]
  108. Brouwers J, Tack J, Augustijns P. *In vitro* behavior of a phosphate ester prodrug of amprenavir in human intestinal fluids and in the Caco-2 system: illustration of intraluminal supersaturation. *Int J Pharm* 2007; **336**: 302–309 .DOI: S0378-5173(06)01069-6 [pii];10.1016/j.ijpharm.2006.12.011 [doi]
  109. Barbour AM, Gibiansky L, Wire MB. Population pharmacokinetic modeling and simulation of amprenavir following fosamprenavir/ritonavir administration for dose optimization in HIV infected pediatric patients. *J Clin Pharmacol* 2014; **54**: 206–214. doi:10.1002/jcph.205.
  110. Mulivor RA, Hannig VL, Harris H. Developmental change in human intestinal alkaline phosphatase. *Proc Natl Acad Sci U S A* 1978; **75**: 3909–3912.
  111. Lalles JP. Intestinal alkaline phosphatase: novel functions and protective effects. *Nutr Rev* 2014; **72**: 82–94. doi:10.1111/nure.12082.
  112. Furukawa T, Yamano K, Naritomi Y, Tanaka K, Terashita S, Teramura T. Method for predicting human intestinal first-pass metabolism of UGT substrate compounds. *Xenobiotica* 2012; **42**: 980–988. doi:10.3109/00498254.2012.680620.
  113. Krekels EH, Danhof M, Tibboel D, Knibbe CA. Ontogeny of hepatic glucuronidation; methods and results. *Curr Drug Metab* 2012; **13**: 728–743.
  114. Court MH, Zhang X, Ding X, Yee KK, Hesse LM, Finel M. Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica* 2012; **42**: 266–277. doi:10.3109/00498254.2011.618954.
  115. Barbier O, Turgeon D, Girard C, et al. 3'-Azido-3'-deoxythymidine (AZT) is glucuronidated by human UDP-glucuronosyltransferase 2B7 (UGT2B7). *Drug Metab Dispos* 2000; **28**: 497–502.
  116. Chen M, LeDuc B, Kerr S, Howe D, Williams DA. Identification of human UGT2B7 as the major isoform involved in the O-glucuronidation of chloramphenicol. *Drug Metab Dispos* 2010; **38**: 368–375. doi:10.1124/dmd.109.029900.
  117. Boucher FD, Modlin JF, Weller S, et al. Phase I evaluation of zidovudine administered to infants exposed at birth to the human immunodeficiency virus. *J Pediatr* 1993; **122**: 137–144.
  118. Lu H, Rosenbaum S. Developmental pharmacokinetics in pediatric populations. *J Pediatr Pharmacol Ther* 2014; **19**: 262–276. doi:10.5863/1551-6776-19.4.262.
  119. Stahlberg MR, Hietanen E, Maki M. Mucosal biotransformation rates in the small intestine of children. *Gut* 1988; **29**: 1058–1063.
  120. Smith M, Hopkinson DA, Harris H. Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann Hum Genet* 1971; **34**: 251–271.
  121. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* 2012; **13**: 227–232. doi:10.1038/nrg3185.
  122. Estudante M, Morais JG, Soveral G, Benet LZ. Intestinal drug transporters: an overview. *Adv Drug Deliv Rev* 2013; **65**: 1340–1356. doi:10.1016/j.addr.2012.09.042.
  123. Konieczna A, Erdosova B, Lichnovska R, Jandl M, Cizkova K, Ehrmann J. Differential expression of ABC transporters (MDR1, MRP1, BCRP) in developing human embryos. *J Mol Histol* 2011; **42**: 567–574. doi:10.1007/s10735-011-9363-1.
  124. van Kalken CK, Giaccone G, van der Valk P, et al. Multidrug resistance gene (P-glycoprotein) expression in the human fetus. *Am J Pathol* 1992; **141**: 1063–1072.
  125. Mooij MG, Schwarz UI, de Koning BA, et al. Ontogeny of human hepatic and intestinal transporter gene expression during childhood: age matters. *Drug Metab Dispos* 2014; **42**: 1268–1274. doi:10.1124/dmd.114.056929.
  126. Mizuno T, Fukuda T, Masuda S, et al. Developmental trajectory of intestinal MDR1/ABCB1

- mRNA expression in children. *Br J Clin Pharmacol* 2014; **77**: 910–912. doi:10.1111/bcp.12211.
127. Berggren S, Gall C, Wollnitz N, *et al.* Gene and protein expression of P-glycoprotein, MRP1, MRP2, and CYP3A4 in the small and large human intestine. *Mol Pharm* 2007; **4**: 252–257. doi:10.1021/mp0600687.
  128. Vaidyanathan S, Jarugula V, Dieterich HA, Howard D, Dole WP. Clinical pharmacokinetics and pharmacodynamics of aliskiren. *Clin Pharmacokinet* 2008; **47**: 515–531. doi:10.2165/00003088-200847080-00002.
  129. Tapaninen T, Backman JT, Kurkinen KJ, Neuvonen PJ, Niemi M. Itraconazole, a P-glycoprotein and CYP3A4 inhibitor, markedly raises the plasma concentrations and enhances the renin-inhibiting effect of aliskiren. *J Clin Pharmacol* 2011; **51**: 359–367. doi:10.1177/0091270010365885.
  130. Tsukimoto M, Ohashi R, Torimoto N, *et al.* Effects of the inhibition of intestinal P-glycoprotein on aliskiren pharmacokinetics in cynomolgus monkeys. *Biopharm Drug Dispos* 2015; **36**: 15–33. doi:10.1002/bdd.1920.
  131. Hoffmann P, Beckman D, McLean LA, Yan JH. Aliskiren toxicity in juvenile rats is determined by ontogenic regulation of intestinal P-glycoprotein expression. *Toxicol Appl Pharmacol* 2014; **275**: 36–43. doi:10.1016/j.taap.2013.12.019.
  132. Sullivan JE, Keefe D, Zhou Y, Satlin L, Fang H, Yan JH. Pharmacokinetics, safety profile, and efficacy of aliskiren in pediatric patients with hypertension. *Clin Pediatr (Phila)* 2013; **52**: 599–607. doi:10.1177/0009922813483875.
  133. Mooij MG, de Koning BE, Lindenbergh-Kortleve DJ, *et al.* Human intestinal PEPT1 transporter expression and localization in preterm and term infants. *Drug Metab Dispos* 2016; **44**: 1014–1019. doi:10.1124/dmd.115.068809.
  134. Groneberg DA, Doring F, Eynott PR, Fischer A, Daniel H. Intestinal peptide transport: ex vivo uptake studies and localization of peptide carrier PEPT1. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G697–G704.
  135. Prasad B, Gaedigk A, Vrana M, *et al.* Ontogeny of hepatic drug transporters as quantified by LC–MS/MS proteomics. *Clin Pharmacol Ther* 2016; **100**: 362–370. doi:10.1002/cpt.409.
  136. Harwood MD, Achour B, Russell MR, Carlson GL, Warhurst G, Rostami-Hodjegan A. Application of an LC–MS/MS method for the simultaneous quantification of human intestinal transporter proteins absolute abundance using a QconCAT technique. *J Pharm Biomed Anal* 2015; **110**: 27–33. doi:10.1016/j.jpba.2015.02.043.
  137. Fattah S, Augustijns P, Annaert P. Age-dependent activity of the uptake transporters Ntcp and Oatp1b2 in male rat hepatocytes: from birth till adulthood. *Drug Metab Dispos* 2015; **43**: 1–8. doi:10.1124/dmd.114.059212.
  138. Xu D, You G. Loops and layers of post-translational modifications of drug transporters. *Adv Drug Deliv Rev* 2016. doi:10.1016/j.addr.2016.05.003.
  139. Hong M. Biochemical studies on the structure–function relationship of major drug transporters in the ATP-binding cassette family and solute carrier family. *Adv Drug Deliv Rev* 2016. doi:10.1016/j.addr.2016.06.003.
  140. Alqahtani S, Mohamed LA, Kaddoumi A. Experimental models for predicting drug absorption and metabolism. *Expert Opin Drug Metab Toxicol* 2013; **9**: 1241–1254. doi:10.1517/17425255.2013.802772.
  141. Bergstrom CA, Holm R, Jorgensen SA, *et al.* Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. *Eur J Pharm Sci* 2014; **57**: 173–199. doi:10.1016/j.ejps.2013.10.015.
  142. Balimane PV, Chong S, Morrison RA. Current methodologies used for evaluation of intestinal permeability and absorption. *J Pharmacol Toxicol Methods* 2000; **44**: 301–312.
  143. Luo Z, Liu Y, Zhao B, *et al.* Ex vivo and in situ approaches used to study intestinal absorption. *J Pharmacol Toxicol Methods* 2013; **68**: 208–216. doi:10.1016/j.jvascn.2013.06.001.
  144. Kararli TT. Gastrointestinal absorption of drugs. *Crit Rev Ther Drug Carrier Syst* 1989; **6**: 39–86.
  145. Osiecka I, Porter PA, Borchardt RT, Fix JA, Gardner CR. In vitro drug absorption models. I. Brush border membrane vesicles, isolated mucosal cells and everted intestinal rings: characterization and salicylate accumulation. *Pharm Res* 1985; **2**: 284–293. doi:10.1023/A:1016341601273.
  146. Hillgren KM, Kato A, Borchardt RT. In vitro systems for studying intestinal drug absorption. *Med Res Rev* 1995; **15**: 83–109.
  147. Lopez V, Suzuki YA, Lonnerdal B. Ontogenic changes in lactoferrin receptor and DMT1 in mouse small intestine: implications for iron absorption during early life. *Biochem Cell Biol* 2006; **84**: 337–344. doi:10.1139/o06-059.
  148. Balamurugan K, Said HM. Ontogenic regulation of folate transport across rat jejunal brush-border membrane. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G1068–G1073. doi:10.1152/ajpgi.00188.2003.
  149. Nabokina SM, Subramanian VS, Said HM. Comparative analysis of ontogenic changes in renal and intestinal biotin transport in the rat. *Am J Physiol Renal Physiol* 2003; **284**: F737–F742. doi:10.1152/ajprenal.00364.2002.
  150. Buddington RK, Malo C, Sangild PT, Elnif J. Intestinal transport of monosaccharides and amino acids during postnatal development of mink. *Am J Physiol Regul Integr Comp Physiol* 2000; **279**: R2287–R2296.
  151. Liao Y, Lopez V, Shafizadeh TB, Halsted CH, Lonnerdal B. Cloning of a pig homologue of the

- human lactoferrin receptor: expression and localization during intestinal maturation in piglets. *Comp Biochem Physiol A Mol Integr Physiol* 2007; **148**: 584–590. doi:10.1016/j.cbpa.2007.08.001.
152. Steinhoff-Wagner J, Schonhuse U, Zitnan R, Hudakova M, Pfannkuche H, Hammon HM. Ontogenic changes of villus growth, lactase activity, and intestinal glucose transporters in preterm and term born calves with or without prolonged colostrum feeding. *PLoS One* 2015; **10**. doi:10.1371/journal.pone.0128154.e0128154
  153. Alam MA, Al-Jenoobi FI, Al-Mohizea AM. Everted gut sac model as a tool in pharmaceutical research: limitations and applications. *J Pharm Pharmacol* 2012; **64**: 326–336. doi:10.1111/j.2042-7158.2011.01391.x.
  154. Dezani TM, Dezani AB, Junior JB, Serra CH. Single-Pass Intestinal Perfusion (SPIP) and prediction of fraction absorbed and permeability in humans: a study with antiretroviral drugs. *Eur J Pharm Biopharm* 2016; **104**: 131–139. doi:10.1016/j.ejpb.2016.04.020.
  155. Moran JR, Ghishan FK. Postnatal intestinal maturation in rats: alpha-aminoisobutyric acid loss during *in vivo*, perfusion by hypertonic solutions. *J Dev Physiol* 1983; **5**: 259–266.
  156. Lindahl A, Krondahl E, Gruden AC, Ungell AL, Lennernas H. Is the jejunal permeability in rats age-dependent? *Pharm Res* 1997; **14**: 1278–1281.
  157. Westerhout J, van de Steeg E, Grossouw D, *et al.* A new approach to predict human intestinal absorption using porcine intestinal tissue and biorelevant matrices. *Eur J Pharm Sci* 2014; **63**: 167–177. doi:10.1016/j.ejps.2014.07.003.
  158. Sjoberg A, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell AL. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. *Eur J Pharm Sci* 2013; **48**: 166–180. doi:10.1016/j.ejps.2012.10.007.
  159. Wallon C, Braaf Y, Wolving M, Olaison G, Soderholm JD. Endoscopic biopsies in Ussing chambers evaluated for studies of macromolecular permeability in the human colon. *Scand J Gastroenterol* 2005; **40**: 586–595. doi:10.1080/00365520510012235.
  160. Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996; **97**: 985–990.
  161. Reims A, Redfors S, Ascher H, Strandvik B. Electrotonic ion transport in duodenal biopsies from children with coeliac disease. *Scand J Gastroenterol* 2002; **37**: 43–50.
  162. Reims A, Strandvik B, Sjovall H. Epithelial electrical resistance as a measure of permeability changes in pediatric duodenal biopsies. *J Pediatr Gastroenterol Nutr* 2006; **43**: 619–623.
  163. Annaert P, Brouwers J, Bijmens A, Lammert F, Tack J, Augustijns P. *Ex vivo* permeability experiments in excised rat intestinal tissue and *in vitro* solubility measurements in aspirated human intestinal fluids support age-dependent oral drug absorption. *Eur J Pharm Sci* 2010; **39**: 15–22. doi:10.1016/j.ejps.2009.10.005.
  164. Glaeser H, Drescher S, van der Kuip H, *et al.* Shed human enterocytes as a tool for the study of expression and function of intestinal drug-metabolizing enzymes and transporters. *Clin Pharmacol Ther* 2002; **71**: 131–140. doi:10.1067/mcp.2002.121370.
  165. Li M, de Graaf IA, Groothuis GM. Precision-cut intestinal slices: alternative model for drug transport, metabolism, and toxicology research. *Expert Opin Drug Metab Toxicol* 2016; **12**: 175–190. doi:10.1517/17425255.2016.1125882.
  166. Yoshimatsu H, Konno Y, Ishii K, Satsukawa M, Yamashita S. Usefulness of minipigs for predicting human pharmacokinetics: prediction of distribution volume and plasma clearance. *Drug Metab Pharmacokinet* 2016; **31**: 73–81. doi:10.1016/j.dmpk.2015.11.001.
  167. Chiou WL, Barve A. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharm Res* 1998; **15**: 1792–1795.
  168. McGinnity DF, Collington J, Austin RP, Riley RJ. Evaluation of human pharmacokinetics, therapeutic dose and exposure predictions using marketed oral drugs. *Curr Drug Metab* 2007; **8**: 463–479.
  169. Bueters T, Juric S, Sohlenius-Sternbeck AK, Hu Y, Bylund J. Rat poorly predicts the combined non-absorbed and presystemically metabolized fractions in the human. *Xenobiotica* 2013; **43**: 607–616. doi:10.3109/00498254.2012.752117.
  170. Cao X, Gibbs ST, Fang L, *et al.* Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. *Pharm Res* 2006; **23**: 1675–1686. doi:10.1007/s11095-006-9041-2.
  171. Chiou WL, Buehler PW. Comparison of oral absorption and bioavailability of drugs between monkey and human. *Pharm Res* 2002; **19**: 868–874.
  172. Akabane T, Tabata K, Kadono K, Sakuda S, Terashita S, Teramura T. A comparison of pharmacokinetics between humans and monkeys. *Drug Metab Dispos* 2010; **38**: 308–316. doi:10.1124/dmd.109.028829.
  173. Chiou WL, Jeong HY, Chung SM, Wu TC. Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. *Pharm Res* 2000; **17**: 135–140.
  174. Jones CR, Hatley OJ, Ungell AL, Hilgendorf C, Peters SA, Rostami-Hodjegan A. Gut wall metabolism. Application of pre-clinical models for the prediction of human drug absorption and first-pass elimination. *AAPS J* 2016; **18**: 589–604. doi:10.1208/s12248-016-9889-y.
  175. Viergever RF, Rademaker CM, Ghersi D. Pharmacokinetic research in children: an analysis of

- registered records of clinical trials. *BMJ Open* 2011; **1**. doi:10.1136/bmjopen-2011-000221.e000221
176. Ethical conduct of clinical research involving children. *Bull Med Ethics* 2004; 8–11.
177. Mulberg AE, Murphy MD, Dunne J, Mathis LL. Pediatric Drug Development: Concepts and Applications. John Wiley & Sons Inc.: City, 2013.
178. Spooner N, Denniff P, Michielsen L, *et al.* A device for dried blood microsampling in quantitative bioanalysis: overcoming the issues associated blood hematocrit. *Bioanalysis* 2015; **7**: 653–659. doi:10.4155/bio.14.310.
179. Roth-Cline M, Nelson RM. Microdosing studies in children: a US Regulatory Perspective. *Clin Pharmacol Ther* 2015; **98**: 232–233. doi:10.1002/cpt.165.
180. Mooij MG, van Duijn E, Knibbe CA, *et al.* Pediatric microdose study of [(14)C]paracetamol to study drug metabolism using accelerated mass spectrometry: proof of concept. *Clin Pharmacokinet* 2014; **53**: 1045–1051. doi:10.1007/s40262-014-0176-8.
181. Bosgra S, Vlaming ML, Vaes WH. To apply microdosing or not? recommendations to single out compounds with non-linear pharmacokinetics. *Clin Pharmacokinet* 2016; **55**: 1–15. doi:10.1007/s40262-015-0308-9.
182. Tachibana T, Kato M, Sugiyama Y. Prediction of nonlinear intestinal absorption of CYP3A4 and P-glycoprotein substrates from their *in vitro*  $K_m$  values. *Pharm Res* 2012; **29**: 651–668. doi:10.1007/s11095-011-0579-2.
183. Abuasal BS, Bolger MB, Walker DK, Kaddoumi A. *In silico* modeling for the nonlinear absorption kinetics of UK-343,664: a P-gp and CYP3A4 substrate. *Mol Pharm* 2012; **9**: 492–504. doi:10.1021/mp200275j.
184. Baber N, Pritchard D. Dose estimation for children. *Br J Clin Pharmacol* 2003; **56**: 489–493.
185. Samant TS, Mangal N, Lukacova V, Schmidt S. Quantitative clinical pharmacology for size and age scaling in pediatric drug development: a systematic review. *J Clin Pharmacol* 2015; **55**: 1207–1217. doi:10.1002/jcph.555.
186. Bouzom F, Ball K, Perdaems N, Walther B. Physiologically based pharmacokinetic (PBPK) modeling tools: how to fit with our needs? *Biopharm Drug Dispos* 2012; **33**: 55–71. doi:10.1002/bdd.1767.
187. Johnson TN, Rostami-Hodjegan A, Tucker GT. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. *Clin Pharmacokinet* 2006; **45**: 931–956. doi:10.2165/00003088-200645090-00005.
188. Parrott N, Davies B, Hoffmann G, *et al.* Development of a physiologically based model for oseltamivir and simulation of pharmacokinetics in neonates and infants. *Clin Pharmacokinet* 2011; **50**: 613–623. doi:10.2165/11592640-000000000-00000.
189. Edginton AN. Knowledge-driven approaches for the guidance of first-in-children dosing. *Paediatr Anaesth* 2011; **21**: 206–213. doi:10.1111/j.1460-9592.2010.03473.x.
190. Dressman JB, Reppas C. Oral Drug Absorption: Prediction and Assessment. Informa Healthcare: City, 2010.
191. International Commission on Radiological Protection. Task Group on Reference Man. In Report of the Task Group on Reference Man: A Report. Pergamon Press: City, 1975.
192. Mehrotra N, Bhattaram A, Earp JC, *et al.* Role of quantitative clinical pharmacology in pediatric approval and labeling. *Drug Metab Dispos* 2016; **44**: 924–933. doi:10.1124/dmd.116.069559.
193. Rioux N, Waters NJ. Physiologically based pharmacokinetic modeling in pediatric oncology drug development. *Drug Metab Dispos* 2016; **44**: 934–943. doi:10.1124/dmd.115.068031.
194. T'Jollyn H, Snoeys J, Vermeulen A, *et al.* Physiologically based pharmacokinetic predictions of tramadol exposure throughout pediatric life: an analysis of the different clearance contributors with emphasis on CYP2D6 maturation. *AAPS J* 2015; **17**: 1376–1387. doi:10.1208/s12248-015-9803-z.
195. Johnson TN, Zhou D, Bui KH. Development of physiologically based pharmacokinetic model to evaluate the relative systemic exposure to quetiapine after administration of IR and XR formulations to adults, children and adolescents. *Biopharm Drug Dispos* 2014; **35**: 341–352. doi:10.1002/bdd.1899.
196. Maharaj AR, Edginton AN. Physiologically based pharmacokinetic modeling and simulation in pediatric drug development. *CPT Pharmacometrics Syst Pharmacol* 2014; **3**. doi:10.1038/psp.2014.45.e150
197. Villiger A, Stillhart C, Parrott N, Kuentz M. Using physiologically based pharmacokinetic (PBPK) modelling to gain insights into the effect of physiological factors on oral absorption in paediatric populations. *AAPS J* 2016; **18**: 933–947. doi:10.1208/s12248-016-9896-z.
198. Poulin P, Jones RD, Jones HM, *et al.* PHRMA CPCDC initiative on predictive models of human pharmacokinetics, part 5: prediction of plasma concentration–time profiles in human by using the physiologically-based pharmacokinetic modeling approach. *J Pharm Sci* 2011; **100**: 4127–4157. doi:10.1002/jps.22550.
199. Zane NR, Thakker DR. A physiologically based pharmacokinetic model for voriconazole disposition predicts intestinal first-pass metabolism in children. *Clin Pharmacokinet* 2014; **53**: 1171–1182. doi:10.1007/s40262-014-0181-y.
200. Zane NR, Thakker DR. Response to: 'Prediction of voriconazole non-linear pharmacokinetics using a paediatric physiologically based

- pharmacokinetic modelling approach'. *Clin Pharmacokinet* 2015; **54**: 569–572. doi:10.1007/s40262-015-0254-6.
201. Johnson TN, Salem F, Jamei M, Rostami-Hodjegan A. Prediction of voriconazole non-linear pharmacokinetics using a paediatric physiologically based pharmacokinetic modelling approach. *Clin Pharmacokinet* 2015; **54**: 567–568. doi:10.1007/s40262-015-0247-5.
202. Lennernas H, Aarons L, Augustijns P, et al. Oral biopharmaceutics tools – time for a new initiative – an introduction to the IMI project OrBiTo. *Eur J Pharm Sci* 2014; **57**: 292–299. doi:10.1016/j.ejps.2013.10.012.
203. Kelly EJ, Newell SJ, Brownlee KG, Primrose JN, Dear PR. Gastric acid secretion in preterm infants. *Early Hum Dev* 1993; **35**: 215–220.
204. Kelly EJ, Chatfield SL, Brownlee KG, et al. The effect of intravenous ranitidine on the intragastric pH of preterm infants receiving dexamethasone. *Arch Dis Child* 1993; **69**: 37–39.
205. Smith LJ, Kaminsky S, D'Souza SW. Neonatal fat digestion and lingual lipase. *Acta Paediatr Scand* 1986; **75**: 913–918.
206. Sondheimer JM, Clark DA, Gervaise EP. Continuous gastric pH measurement in young and older healthy preterm infants receiving formula and clear liquid feedings. *J Pediatr Gastroenterol Nutr* 1985; **4**: 352–355.
207. Omari TI, Davidson GP. Multipoint measurement of intragastric pH in healthy preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2003; **88**: F517–F520.
208. Seo JH, Park HK, Park JS, et al. Association between gastric pH and *Helicobacter pylori* infection in children. *Pediatr Gastroenterol Hepatol Nutr* 2015; **18**: 246–252. doi:10.5223/pghn.2015.18.4.246.
209. Splinter WM, Stewart JA, Muir JG. The effect of preoperative apple juice on gastric contents, thirst, and hunger in children. *Can J Anaesth* 1989; **36**: 55–58. doi:10.1007/BF03010888.
210. Bedrick AD, Johnson SM, Koldovsky O. *In vitro* intestinal processing of prostaglandin F2 alpha in rats: developmental aspects. *J Pediatr Gastroenterol Nutr* 1988; **7**: 755–761.