

Special Section on Pediatric Drug Disposition and Pharmacokinetics—Minireview

Ontogeny of Hepatic Drug Transporters and Relevance to Drugs Used in Pediatrics

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ABSTRACT

Most of the pharmacokinetic studies conducted to calculate pediatric drug doses are based on scaling from adult data using various allometric parameters related to body size. However, these uniform scaling methods cannot account for all physiologic changes occurring during maturation, which influence various drugs in different ways. The ontogeny of physiologic and biologic functions accompanying the progression from infancy to childhood to adulthood does not proceed in a simple monotonic rate with body size for various elimination pathways. The transporters and their interplay with enzymes have a substantial role in drug metabolism and

disposition. Although much is known about enzymes and their ontogeny, there is a scarcity of information on the ontogenic profile of drug transporters, particularly during the early years of human life. These ontogeny data are required for the enhancement of physiologically based pharmacokinetic models, and consequently for the prediction of pharmacokinetic profiles of new therapeutic compounds in pediatric populations. This review points to the relative ontogeny rate for enzymes and transporters and how these may confound our understanding of the role that transporters may or may not play in childhood compared with adulthood.

Introduction

Commonly Encountered Issues in Pediatric Drug Dosing. One of the most prevalent problems in pediatrics is the high incidence of adverse drug reactions associated with the use of off-label or unlicensed drugs. In 2000, it was reported that about 70% of the drugs prescribed to pediatric patients in five European countries are off-label or unlicensed drugs (Conroy et al., 2000). It is not surprising that at the time of approval of new drugs, they have seldom been tested in children, even when there might be pediatric applications. Of the formulas that have been used to scale adult doses to children, rules such as Clark's body weight and body surface area, which are purely based on allometric scaling (Anderson and Meakin, 2002), are typically used. However, such simple extrapolation methods usually fail to predict pharmacokinetic behavior in younger pediatric patients (Johnson, 2008). This is mainly because of developmental changes in organ function (including the ontogeny of drug disposition pathways) and variations in body composition across the different age ranges of children (Kearns et al., 2003).

Physiologically Based Pharmacokinetic (PBPK) Models in Pediatric Populations. Development of PBPK models in pediatrics has facilitated the evaluation of pediatric population exposure to drugs and xenobiotics. These models take into consideration drug-related data such

as biochemical, demographic, and physiologic data—specifically, the ontogeny of drug disposition and elimination pathways. The models can be used to assist in the extrapolation of *in vitro* data to predict the *in vivo* behavior of drugs in any age group, including children (Barrett et al., 2012).

Robust PBPK models require good data, in particular, quantitative data on pediatric drug metabolizing enzymes (cytochrome P450 or UDP-glucuronosyltransferases and transporters). Furthermore, it is not sufficient to take a single snapshot of the childhood drug metabolizing capability because it changes with age and needs to be described as an age-dependent ontogeny function. Hence, measurements of the levels of these proteins over the whole childhood period are necessary for *in vitro* to *in vivo* extrapolation (Prasad and Unadkat, 2014).

Drug Transporters. According to the direction in which transporters flux their drug substrates through membranes, they can be grouped as efflux or uptake transporters. Efflux transporters drive their substrates out of cells, whereas uptake transporters transfer them into cells. Alternatively, transporters may belong to the ATP-binding cassette, solute carrier transporter, or organic solute transporter families.

Liver Transporters. Liver transporting proteins are crucial factors for the uptake and efflux of various drugs and endogenous substances (Klaassen and Lu, 2008; Klaassen and Aleksunes, 2010). Therefore, they are major determinants of drug efficacy and toxicity; they affect drug concentrations in plasma through their roles in metabolic or biliary clearance (Borst and Elferink, 2002). Figure 1 shows the most

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ABBREVIATIONS: BCRP, breast cancer resistance protein; BSEP, bile salt export pump; MATE, multidrug and toxin extrusion protein; MRP, multidrug resistance-associated protein; NTCP, Na⁺-taurocholate cotransporting polypeptide; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OST, organic solute transporter; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein.

important hepatic uptake and efflux transport proteins and their locations in the hepatocytes.

Ontogeny of Liver Transporters. Although there are some metabolizing enzymes known to be overexpressed in the early days of infant life compared with adulthood (for example, CYP3A7), drug transporter expression is expected to be fairly low at birth because of the observation that infants' disposition machineries are unable to handle toxic xenobiotics. The liver matures rapidly after birth from simply an organ for formation of blood cells to an organ holding the major metabolism and elimination machinery for drugs and xenobiotics. Therefore, liver transporters maturation is important for the proper flux of xenobiotics across the cells (Cui et al., 2012). Despite improved knowledge of drug transporters in humans, knowledge of the developmental patterns of individual drug transporters remain incomplete, particularly in relation to transporter developmental and ontogenic expression in the pediatric population. This is largely because of the scarcity of pediatric clinical studies in this area (Wei et al., 2014).

In order to determine the ontogenic profile of liver transporters, a literature search was conducted to identify studies dealing with major drug transporters in the liver [i.e., breast cancer resistance protein (BCRP), bile salt export pump (BSEP), multidrug and toxin extrusion protein (MATE), multidrug resistance-associated protein (MRP), Na⁺-taurocholate cotransporting polypeptide (NTCP), organic anion transporter (OAT), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), organic solute transporter (OST), and P-glycoprotein (P-gp)]. A search of relevant publications on the abundance data of the efflux, uptake, and bidirectional transporters was done through PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) using the following keyword combinations: "hepatic/liver" plus "MATE1, BSEP, BCRP, MRP2, MRP3, MRP4, MRP6, NTCP, P-gp, OATP1B1, OATP1B3, OATP2B1, OAT2, OCT1, OAT7, OST α/β " plus "uptake or influx or efflux or flux or transport" plus "abundance, ontogeny, correlation of expression, quantification." Searches were limited to humans (children: birth to 18 years). Titles and abstracts were reviewed to keep the search centered on the levels of transporter expression in pediatric subjects. The literature was reviewed to assess the development of drug transporter expression with age, and the following data were obtained regarding each of the relevant liver transporter ontogeny.

Hepatic Uptake Transport Proteins.

NTCP. NTCP is a basolateral transporter entirely expressed in the liver and constituting the main hepatic pathway for conjugated bile

acids uptake. NTCP was detectable in the liver samples of fetuses of 14–20 weeks of gestation (Chen et al., 2005). NTCP protein expression was not significantly different between adults and neonates (Yanni et al., 2011).

OATPs. Out of the 11 OATPs in humans, there are only four transporters with an extensive role in substrate uptake at the hepatic sinusoidal membrane; these are OATP1A2, OATP1B1, OATP1B3, and OATP2B1 (Kalliokoski and Niemi, 2009). Fetal livers showed mRNA expression for OATP1B1, OATP1B3, and OATP2B1. Some studies suggested that there were no significant differences between neonatal and adult livers in the expression of OATP1B1 and OATP1B3 (Yanni et al., 2011). However, one study on 45 liver samples suggested that the mRNA expression for both transporters was age dependent until the seventh year of life, at which point the levels stabilized (Mooij et al., 2014). The only available study on protein expression for OATPs showed no correlation between the OATP1B1, OATP1B3, and OATP2B1 expression and age (Prasad et al., 2014).

OATs. Although most of the available data about organic anion transporters are related to kidney drug transport, they are highly expressed in sinusoidal hepatocyte membrane. OAT2 was detected in fetal liver and showed an increased expression with age in livers from neonatal to older children and adults (Klaassen and Aleksunes, 2010).

OCTs. There are two expressed isoforms of human OCTs in the human liver, OCT1 and OCT3. OCT1 is the major transporter in humans in terms of its expression and is believed to be confined to the liver sinusoidal membrane (Zhang et al., 1997; Hilgendorf et al., 2007). It has 13 times the expression level of OCT3, which has broader tissue distribution (Nies et al., 2009). While there were no studies about OCT3 ontogeny, the very limited number of studies available assessing the age-related maturation of OCT1 did not report any significant difference in mRNA expression between adults and pediatric populations (Kim et al., 2012).

Hepatic Efflux Transport Proteins. These may be classified into either canalicular or basolateral efflux transport proteins.

Canalicular Transport Proteins. The role of canalicular transport proteins is to excrete drugs and their metabolites through the hepatic apical membrane to the bile (canalicular membrane).

P-gp. Having different functions in various tissues, P-gp is the transport protein with the highest number of studies. The mRNA levels of P-gp in a group of 61 post-mortem liver samples from fetuses and neonates were about 20- to 30-fold lower when compared with the

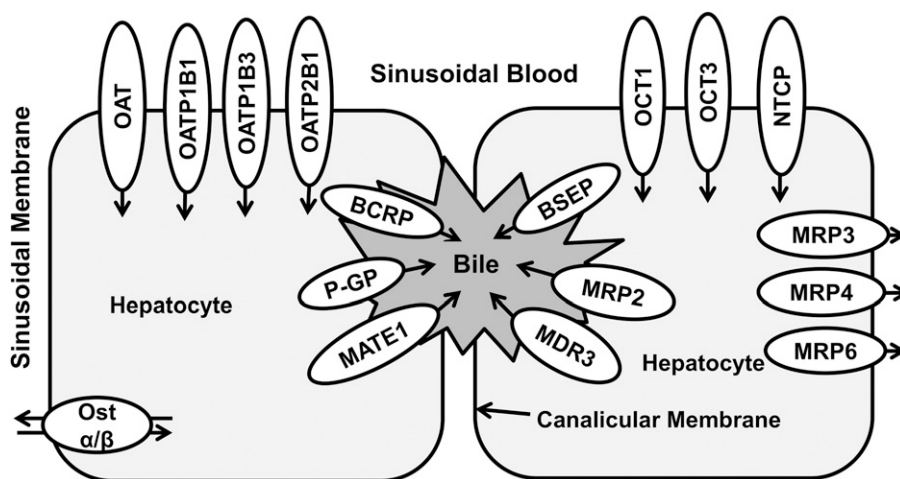


Fig. 1. The most important hepatic uptake and efflux transporters and their locations in the hepatocytes: BCRP, BSEP, MATE1, P-gp, MRP, NTCP, OAT, OATP, OCT, and OST α/β .

adult group. P-gp mRNA expression of first-year infants was found to be higher than the neonatal levels, and was estimated to be about 5-fold lower than adults, whereas there were similar expression levels in older children, adolescents, and adults (Mooij et al., 2014). This suggests that the P-gp mRNA increases throughout the first year of human life, and these findings were consistent with previous results (van Kalken et al., 1992; Miki et al., 2005; Fakhoury et al., 2009). P-gp protein was expressed in neonates including premature neonates, but its expression was independent of age in a pediatric group from 4 months to 12 years of age, where P-gp expression levels were not statistically or significantly different in 65 different liver samples (Tang et al., 2007). This result was somewhat supported by a study for P-gp quantification through the age interval from 7 to 70 years, which revealed that age and P-gp protein expression were not correlated (Prasad et al., 2014).

MDR3. Available literature data proves a similar MDR3 substrate specificity to P-gp. However, there are not enough data available about its ontogenic profile.

BSEP. The mRNA expression of BSEP was found to be very low in fetuses with a 10- to 20-fold increase in expression in neonates; it continues to rise in adulthood (Chen et al., 2005).

MRP2. MRP2 was found to be expressed in the second trimester of gestation. The expression level was higher in 19-week-old fetuses than those at 14 weeks of age (Cízková et al., 2005). The expression continues to increase from the fetal period to neonatal and infantile periods (Klaassen and Aleksunes, 2010; Mooij et al., 2014). The MRP expression was stable over the age range from 7 to 70 years (Deo et al., 2012).

BCRP. Although BCRP was found in samples from fetuses aged only 6 weeks (Konieczna et al., 2011), BCRP protein expression did not differ significantly between neonates and adults (Yanni et al., 2011). Analysis by liquid chromatography–tandem mass spectrometry revealed no association between BCRP levels and age in 65 liver samples from 7 to 70 years of age (Prasad et al., 2013).

MATE1. Analysis of early fetuses' livers detected the expression of mRNA of MATE1 with an increase in expression with age until adulthood (Klaassen and Aleksunes, 2010).

Basolateral Efflux Transport Proteins. These transporters are a major class of export proteins that mediate xenobiotic excretion from the liver into the sinusoidal blood across the basolateral membrane.

MRP3. There was a similarity in the ontogeny of MRP3 to that of MATE1 and OAT2 in terms of its expression in fetal liver and its increase with development from early neonatal life to adulthood (Klaassen and Aleksunes, 2010).

MRP4 and MRP5. While there was a scarcity of ontogeny data related to MRP5, the mRNA expression of MRP4 was found to be unrelated to age (Sharma et al., 2013).

MRP6. In line with the ontogeny data of MRP3, MATE1, and OAT2 transporters, MRP6 was also detected in fetal livers and its expression increased with age from neonatal to older children and adult livers (Klaassen and Aleksunes, 2010).

OST α -OST β . OST α -OST β are basolateral hepatic bidirectional liver transporters mediating bile acid flux. The mRNA for both transporters was detected at low levels in pediatric livers (Chen et al., 2008), but there is little reported data on their ontogeny.

Drugs with Known Pediatric Applications that Are Substrates for Transporters. To appreciate the importance (or lack of relevance) of transporter ontogeny for pediatric drug treatment, it seems natural to assess the overlap between the sets of drugs that are used in pediatric drug treatment and the drugs acting as substrates or modulators of drug transporting proteins. However, as will be described subsequently, this approach might be misleading.

Management of preterm infants and children of all ages often involves using a variety of drugs that are frequently transported by one or more transporter proteins. In order to identify drugs with important uses in pediatric populations that are substrates for liver transporters, a review of the literature was carried out in PubMed using the keywords combination (hepatic or liver) plus (NTCP or OATP or OAT or OCT or P-gp or P-glycoprotein or MDR or BSEP or MRP or BCRP or MATE1 or OST) plus (substrate); only human studies were taken into account and titles/abstracts were looked into for relevant information. References in each report were scrutinized for further sources of published data on drug transporter substrates. The data collected are shown in Table 1. Drugs known to be substrates of liver transporters were then compared with drugs used in pediatrics from the British National Formulary for Children 2014/2015, and the matching drugs are indicated in bold in Table 1.

According to Table 1, there are about 175 drug substrates for liver transporters, 104 of which are of pediatric application. This suggests that about 60% of the drugs prescribed to children may be affected by the function of one or more liver transporters (see Fig. 2). The accuracy of this calculated proportion may, of course, be compromised by the fact that there are many off-label or unlicensed drugs that are used. However, it seems likely that the proportion of off-label drugs that are substrates for transporters is similar to the value obtained for the British National Formulary for Children drugs, and that the calculated value might be taken as a rough estimate for the scale of the relevance of transporters in pediatric drug treatment. Obviously, the involvement of transporters does not necessarily translate to a crucial impact for them in the drug disposition; assessing the significance of transporter involvement is an area that has only started to mature (see the subsequent sections).

Based on the increasing number of drugs used in pediatric populations that are substrates of or modulators for liver transporters, it is therefore apparent that studies about developmental changes of these transport proteins should be improved. Most of the available studies on liver transporters have concentrated only on snapshots of gene or protein expression and few have focused on the determination of age-dependent transporter activities (Fattah et al., 2015).

In the next section, the theoretical aspects related to the relative importance of transporters, as opposed to enzymes, with age are discussed. It cannot be assumed that the transporter effect in a certain drug's disposition (as a prominent determinant, regardless of absolute value) does not vary with age.

Problems Associated with Estimating Drug Transporter Relevance to Pediatric Drugs. There is a general deficiency of data on the developmental changes in transporters in humans. However, from the available studies it is clear that there is great variability in the developmental scenarios between individual transporters. This is also known for enzymes (Salem et al., 2013). Although several efflux and uptake transporters were found to be expressed in the fetal liver, some transporters show some developmental maturation in expression from fetal to neonatal and adulthood periods such as BSEP, MRP3, MRP6, MATE1, and OAT2, while the expression of other transporters such as MRP2, MDR1, OAT1B1, and OAT1B3 increases from the neonatal period to some point in childhood and then stabilizes at adult levels. On the other hand, the maturation of some transporters is not related to age; these include BCRP, OCT1, and NTCP. These results are broadly in line with a recently published review that assessed the ontogeny of human transporters in intestine, liver, and kidney (Brouwer et al., 2015).

Of the available transporter ontogeny data, some are in the form of mRNA and others are in the form of protein data. Furthermore, most of these data still need to be correlated with activity, and the relative significance of the transporters in the distribution of drugs and the ways

TABLE 1

Drugs known to be substrates for liver transporters

Drugs that are given in boldface have pediatric applications. Drugs that are given in italics have high f_T values. Drugs that are given in both boldface and italics have pediatric applications and high f_T values. SN-38 is the active metabolite of irinotecan.

Transporter	Substrate
NTCP	Atorvastatin, fluvastatin, pitavastatin, rosuvastatin , and taurocholate covalently bound drugs
OATP1A2	Acebutolol , atenolol , atrasentan, celiprolol , D-penicillamine , deltorphin-II, erythromycin, <i>flexofenadine</i> , imatinib, levofloxacin , lopinavir , methotrexate , microcystin-LR, ouabain, pitavastatin, rosuvastatin , rocuronium , <i>N-methyl quinine</i> , <i>saquinavir</i> , sotalol , thyroxine , talinolol, tebipenem pivoxil, and unaprostone
OATP1B1	Atrasentan, atorvastatin , benzylpenicillin , bosentan , caspofungin , cerivastatin, D-penicillamine, enalapril, ezetimibe glucuronide, <i>flexofenadine</i> , fluvastatin , methotrexate , microcystin-LR, olmesartan , ouabain, pitavastatin, phalloidin, pravastatin , repaglinide , rifampicin , rosuvastatin , simvastatin , SN-38, troglitazone-sulfate, temocapril, and valsartan
OATP1B3	Atrasentan, amanitin, bosentan , cyclosporin , digoxin , docetaxel , deltorphin-II, D-penicillamine, enalapril , erythromycin, <i>flexofenadine</i> , fluvastatin , imatinib, methotrexate , microcystin, olmesartan , ouabain, paclitaxel , pitavastatin, phalloidin, pravastatin, rifampicin , rosuvastatin , SN-38, telmisartan , and valsartan
OATP2B1	Atorvastatin , benzylpenicillin , bosentan , fluvastatin , <i>flexofenadine</i> , glibenclamide, pravastatin , pitavastatin, rosuvastatin , and unaprostone
OAT2	Allopurinol , L-ascorbic acid, bumetanide , erythromycin , 5-fluorouracil , methotrexate , paclitaxel, ranitidine , salicylate , tetracycline , taxol, theophylline , and zidovudine
OCT1	Acyclovir , azidoprocainamide, berberine, citalopram , cimetidine , cisplatin , famotidine , furamide, ganciclovir , imatinib , irinotecan, lamivudine, metformin , methiodide, <i>morphine</i> , oxaliplatin , ondansetron , procainamide , pentamidine , picoplatin, paclitaxel, quinidine, ranitidine , tropisetron, tramadol , and verapamil
OCT3	Adefovir , atropine , amantadine , d-amphetamine , cimetidine , clonidine, <i>citalopram</i> , desipramine, diphenhydramine, dizoclopine, etilefrine, granisetron , imipramine , ketamine , lidocaine , Lamivudine, Metformin, Mitoxantrone, Memantine , O-methylisoprenaline, nicotine , phenoxybenzamine , phencyclidine , prazosin , procainamide , quinidine , ranitidine , tropisetron, and verapamil
P-gp	<i>Amprnavir</i> , atorvastatin , aldosterone , berberine, corticosterone; cimetidine , cyclosporin A , dexamethasone , digoxin , daunorubicin , doxorubicin , debrisoquine, diltiazem , erythromycin , etoposide , <i>flexofenadine</i> , grapafloxacin , hydrocortisone , <i>indinavir</i> , imatinib , irinotecan, lovastatin, losartan , levofloxacin , loperamide , mitoxantrone , morphine , norverapamil, <i>nelfinavir</i> , paclitaxel , pitavastatin, phenytoin , quinidine, rosuvastatin , ritonavir , rhodamine 123, <i>saquinavir</i> , simvastatin , tacrolimus , taxanes , talinolol, terfenadine , verapamil , vinblastine , and vincristine
BSEP	<i>Flexofenadine</i> , pravastatin, vinblastine
MRP2	Acetaminophen glucuronide, carboxydichlorofluorescein, camptothecin, cerivastatin, cisplatin , doxorubicin , etoposide , <i>flexofenadine</i> , glibenclamide , indomethacin , MTX , mitoxantrone, olmesartan, pitavastatin, pravastatin , rifampin conjugates, rosuvastatin , spiramycin , SN-38 glucuronide, vincristine , and valsartan
BCRP	Albendazole sulfoxide, cerivastatin, ciprofloxacin , daunorubicin , doxorubicin , dirithromycin, erythromycin , epirubicin , genistein, irinotecan , imatinib , lamivudine , methotrexate , MX , norfloxacin , nitrofurantoin , oxfendazole, ofloxacin , pitavastatin, prazosin , pantoprazole, rifampicin , rhodamine 123, rosuvastatin , sulfasalazine , SN-38, testosterone , tamoxifen , topotecan, and zidovudine
MATE1	Acyclovir, cephalixin, <i>flexofenadine</i> , gancyclovir, metformin, oxaliplatin
MRP1	Adfovir, apicidin, berberine, camptothecins, ciprofloxacin, <i>citalopram</i> , daunorubicin , doxorubicin , difloxacin, etoposide , edatrexate, epirubicin , flutamide , filfoxacin, grapafloxacin , irinotecan , <i>indinavir</i> , idarubicin , methotrexate , pirarubicin , paclitaxel, ritonavir , romidepsin, raltitrexed , SN-38, <i>saquinavir</i> , tomudex, vincristine , and vinblastine
MRP3	Acetaminophen glucuronide, etoposide , <i>flexofenadine</i> , MTX , teniposide, and vincristine
MRP4	Azidothymidine , lamivudine , methotrexate , PMEA , and stavudine zidovudine
MRP5	Adefovir , atorvastatin, 5-fluorouracil, methotrexate , PMEA , and rosuvastatin
MRP6	Endothelin receptor antagonist BQ-123
OST α /OST β	Digoxin

MTX, methotrexate.

in which this significance changes with age requires further investigation. For a transporter to be of relevance to the disposition of a certain drug, the following range of factors should be considered:

- First, consider the availability of this transporter and its abundant expression in the tissue of interest;
- Second, consider the relative contribution from this transporter in drug distribution compared with the contribution from other transporters or from passive diffusion (i.e., the fraction of drug transported, f_T , for each transporter);
- Third, consider the degree of the drug affinity to the transporter and the proportion of the drug transported by this specific transporter; and
- Finally, consider the modulation of this transporter by induction or inhibition through endogenous or exogenous substances.

Drugs may be affected by transporters to varying degrees according to the fraction of drug dose being absorbed, distributed between body tissues, or cleared out of the body. Therefore, the concept of fraction transported is of great importance in the determination of the transporter effect on the concentration of drugs in any organ by estimating the transporter's role in the absorption, distribution, metabolism, and excretion profile of drugs. When this fraction of drug transported is

high, then the transporters influencing this drug and their modulation by drug-drug interactions is very important to the drug absorption, distribution, metabolism, and excretion.

Prasad and Unadkat (2015) summarized the central nervous system affecting drugs and the antiretroviral agents that are at the same time substrates for P-gp, BCRP, or MRP in the blood-brain barrier, together with their f_T values. Drugs with high f_T values (0.67–0.98) are set in italics in Table 1. However, this picture becomes more complex when

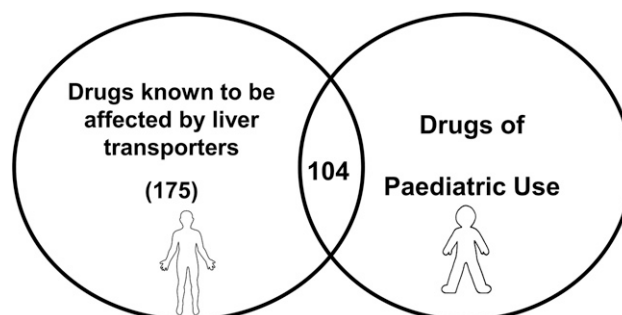


Fig. 2. The number of drugs of pediatric application that are substrates for liver transporters.

the dimension of age is added to it, where the fraction of drug transported may not be constant across various age groups.

To indicate the varying relative contribution of different transporters with age, two hypothetical transporters (T1 and T2) were assumed to have relative importance in adults, f_{T1} and f_{T2} of 0.1 and 0.9, respectively. In case A, both transporters were assumed to be expressed at birth with relative values of 0.6 and 0.5 for T1 and T2, respectively, compared with the adults. In case B, T2 was assumed not to be expressed at birth, while the T1 relative value was the same as in case A. The relative abundance values for each transporter were assumed to follow a different trajectory (Fig. 3B), unlike the first scenario when the ontogeny was similar for both transporters (Fig. 3A). Under these circumstances it can be shown that the relative importance of transporters (f_T) can be age dependent for the scenario described for case B (Fig. 3D) while the relative importance may remain the same in adults despite the ontogeny of transporters in case A (Fig. 3C).

This concept is a general case and can be considered not just between two transporters but also in terms of the relative importance of a nontransporter route versus a transporter-related route. The parallels between the aforementioned case with the results of another recent investigation by Salem et al. (submitted manuscript) on the selection of covariates and their age dependence are obvious. This study by Salem et al. (F. Salem, K. Abduljalil, Y. Kamiyama, and A. Rostami-Hodjegan, submitted manuscript) discussed the validity of the commonly known fact that the hepatic extraction ratio, E_H , is a characteristic property of the drug. Salem et al. (F. Salem, K. Abduljalil, Y. Kamiyama, and A. Rostami-Hodjegan, submitted manuscript) concluded that caution should be taken before assuming that the extraction ratio in pediatric populations is the same as in adults because classification of a drug as a high or low extraction ratio does not take into account the variation of the E_H calculation parameters with age. In conclusion, it is clear that the relative ontogeny of transporters and enzymes may not follow the same

trajectory and they can differ in their relative importance to a specific drug and its disposition.

Progress with Experimental Methods for the Study of Transporter Expression. The majority of data about the abundance of drug transporters originates from in vitro methods based on, for example, primary hepatocytes or cell lines with transfected human transporting protein (Hirano et al., 2004; Kitamura et al., 2008). Nevertheless, these in vitro models are not available for the pediatric population. Preclinical animal studies are of value in assessing the ontogenic profile of transporters, and much of that information seems to be in agreement with clinical findings in adults. Nonetheless, most of these data are in the form of transcriptional information limited to the gene expression level and most of the abundance data are obtained through western blot analysis and immunohistochemistry, which suffer from poor reproducibility (Al Feteisi et al., 2015).

Transcript levels (as measured by conventional mRNA methods) have been shown to have only a weak association with the expression levels of proteins. However, liquid chromatography–tandem mass spectrometry experiments can be designed to give sensitive characterization and quantification of proteins. Unlike relative quantification methods that rely on comparing the protein concentrations in two samples relative to each other, absolute quantification is highly recommended to aid in the measurement of the absolute values of proteins in samples, and consequently it facilitates interlaboratory comparisons.

Mass Spectrometry-Based Absolute Quantification. Because of the high sensitivity and selectivity of mass spectrometry, it is of crucial importance in quantitative analysis of pediatric samples, especially because of the minute size and the limited availability of these samples. Mass spectrometry-based absolute quantification is mainly based on isotope dilution, where a predetermined amount of a heavy and isotope-labeled internal standard is mixed with the analyte protein as a reference. Comparison of the signal intensity of labeled and unlabeled peptides leads to the concentration of the protein of interest.

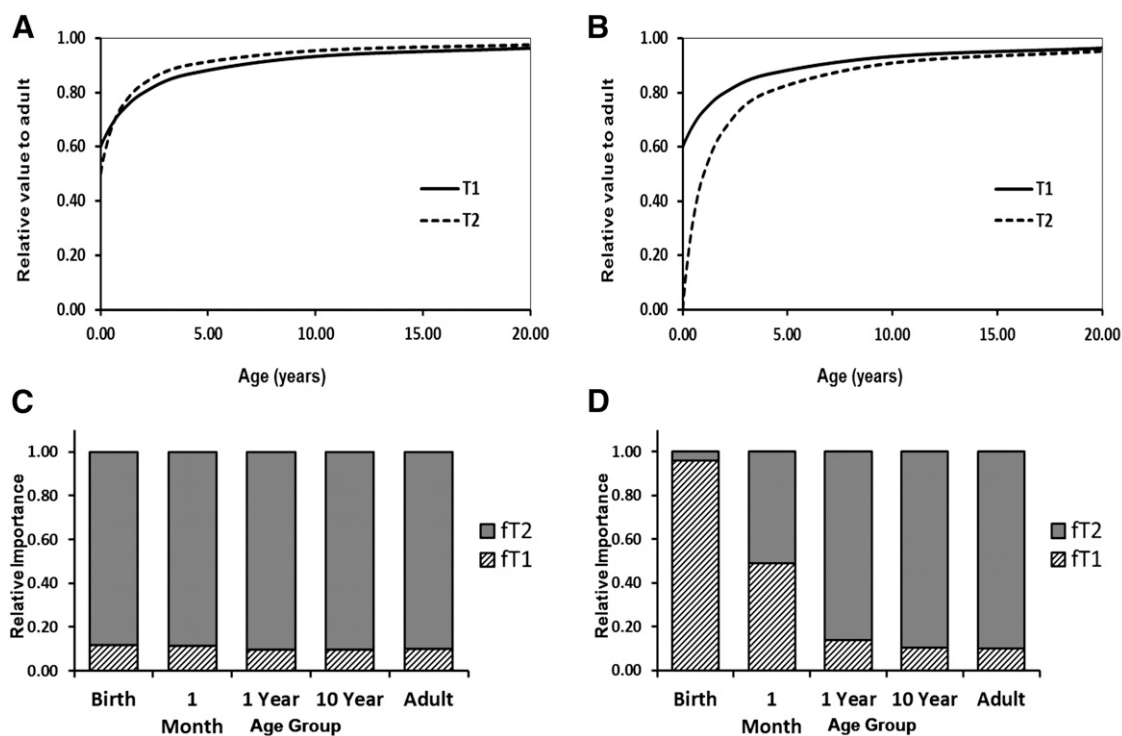


Fig. 3. The relative abundance values (A) and (B), and the age-related changes in the relative importance of two transporters T1 (C) and T2 (D) in different age groups. (A) The relative values of T1 and T2 are 0.6 and 0.5, respectively, at birth compared with the adult value. (B) T1 relative value at birth is 0.6 compared with the adult value, while T2 is not expressed at birth. (C) Both T1 and T2 have a constant relative importance across the different age groups. (D) T1 has a higher relative importance than T2 in neonates compared with adults.

Internal standards may be isolated peptides labeled with stable isotopes (sometimes known as absolute quantification peptides) (Brun et al., 2007); this technique has been successfully used in the hepatic CYP2D6 quantification in humans (Langenfeld et al., 2009). Alternatively, marker quantotypic peptides of various proteins of interest may be expressed (using an artificial gene) concatenated in an artificial protein and released on proteolytic digestion. This approach (known as the quantification concatemer method) allows the quantification of up to 50 proteins using a single standard (Beynon et al., 2005; Pratt et al., 2006). The robustness of the quantification concatemer approach for simultaneous quantification of a few tens of proteins has been demonstrated by Al-Majdoub et al. (2014), and the approach has been used in the quantification of enzymes and transporters (Russell et al., 2013).

Label-free quantification of transporters and enzymes by liquid chromatography–tandem mass spectrometry is also possible and is especially useful for establishing an initial overview (Kito and Ito, 2008). Labeled standards allow for more direct measurements. They are dependent upon fewer assumptions about the relationship of the peptide signal to protein abundance and are normally preferred for repeated precise measurements.

Conclusions

The quest to understand and manage the pediatric drug dose requires knowledge of changes that occur to various body functions with age. The biology of enzyme metabolizing drugs and the ontogeny has been ahead of the efforts on transporters. Some of the concepts regarding the importance (or lack of importance) of transporters are challenged when data on adults are used without consideration of the age-dependent impact of these transporting proteins in the disposition of any particular drug. The invention of methodologies that enable quantitative measurement of transporter proteins using small biologic samples will help to gain insight into ontogeny trajectories of various transporters. These, in turn, will assist with building more robust PBPK models making use of the in vitro data on drugs and their affinities to various transporters and enzymes to aid in the prediction of drug behavior in pediatrics.

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Authorship Contributions

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Performed data analysis: Elmorsi, Rostami-Hodjegan.

Wrote or contributed to the writing of the manuscript: Elmorsi, Barber, Rostami-Hodjegan.

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