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Prediction of Heptatic and Renal Clearance in Pediatric Populations: A "Bottom-Up" Approach Versus "Top-Down" Recognition of Covariates

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PREDICTION OF HEPTATIC AND RENAL CLEARANCE IN PEDIATRIC POPULATIONS: A "BOTTOM-UP" APPROACH VERSUS "TOP-DOWN" RECOGNITION OF COVARIATES

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ABSTRACT

Medications, including many that are widely used in children, are rarely tested in children. Their safety and effectiveness have not been established in children or have been established only for children of a certain ages. For example, 53 of 140 new medications were labeled for use in children when they were initially approved. Much of pediatric practice, particularly in hospitals and by specialists, has involved "off-label" use of medications. As a consequence, about one-third of the drugs prescribed in office-based pediatric practices and about two-thirds of those prescribed to children in hospital settings are unlicensed or off-label. This proportion reaches 90% in intensive care units. Thus, accurate information about dosage in different age groups is particularly important, otherwise, children might be over or under treated. The safety of a drug in children often cannot be extrapolated from data in adults; medications may be more or less toxic in children and require specific studies. The effectiveness of a drug can be extrapolated from an adult when no relevant differences can be anticipated in disease, disease progression or exposure-effect relationships. Such data are usually supplemented with pharmacokinetic studies. Drug clearance (CL) is a principle PK parameter determining the age-dependent difference. Therefore, predicting pediatric clearance accurately is crucial for appropriate pediatric dosage regimen.

Body weight normalized clearance is widely used because of its simplicity but it does not account the maturation of enzymatic and organ functions. As a result, children is often overdosed because of the over estimation of clearance, especially in neonates and young infants. Recently, anticipation of clearance is built based on more

complex mathematical models, which could take a data or knowledge-driven approach by employing either observed data (top-down) or knowledge of human body (bottom-up). These two approaches depend on different starting information and are likely to be used in conjunction with each other for the purposes of defining pediatric dosing guidelines.

This research first focused on the bottom-up, mechanistic models for predicting age-dependent hepatic and renal elimination. The maturation of specific cytochrome P450 enzymes was modeled based the in vitro ontogeny data from hepatic microsomal studies. The age-related function then was incorporated into a well-stirred liver model with the developmental changes of other physiological factors, achieving the extrapolation from in vitro to in vivo. The model predictions using this physiology-based approach proved to be more accurate and precise than the allometric functions, in neonates and young infants less than 1 year of old for renal and hepatic enzyme eliminated drugs.

In the top-down approach, population analysis is commonly used in pediatric PK studies because of heterogeneity of the study population and sparse sampling. The covariates effects such as age and size was examined in simple exponential relationships. However, these estimates, despite statistical validation in the observed population, may not suffice to predict parameters distribution and drug exposure in a new population. A more complex covariate-parameter relationship was explored to describe the maturation process and changes in physiological functions, based on the clearance values of renal and hepatic eliminated probe drugs. In these cases, the maturation information derived from metabolic probes could be used as system

specific covariate models for other drugs via the same elimination routes in neonates or young infants.

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PREFACE

Manuscript Format is in use for this dissertation.

Chapter 1 (Manuscript-I)

During childhood, as the body weight and its function changes dramatically with age, drug therapy should be arranged according to age-related changes in pharmacokinetics at different age stages. Although the data on drug disposition in infants and children increased considerably over the past 2 decades, the effects of development on pharmacokinetics and pharmacodynamics remain poorly understood. In chapter 1, the impact of developmental pharmacology on drug absorption, distribution, metabolism and elimination in infants and children are reviewed. Absorption may be affected by difference in gastric pH and stomach emptying time. Low plasma protein concentrations and higher body water compositions can change drug distribution. Metabolic processes are often immature at birth and this leads to reduced clearance rates and prolonged half-life for those drugs, which metabolism is a significant mechanism for elimination. Renal excretion is also reduced in neonates due to immature glomerular filtration and tubular secretion. Limited data are available on pharmacodynamics of drugs. Pharmacokinetics processes develop at different rates during the first year of life after birth, thus requiring continual modification of drug dose regimens for their safe and effective use in neonates, infants and children.

Chapter 2 (Manuscript-II)

The maturation of drug metabolizing enzymes is probably the predominant factor accounting for age associated changes. The group of drug-metabolizing enzymes most studied is the cytochrome P450 (CYP) superfamily. In chapter 2, the development of

CYP enzymes involved in xenobiotic metabolism are reviewed from fetal through the life span. These hepatic P450s showed discrepancy in the onset of activities and develops independently. The ontogeny of individual isoform could be described in 4 groups. A first group of CYP3A7 and CYP1A1 expressed at high levels in the fetal liver but silenced in the postnatal period. A second group includes CYP2D6, CYP2E1 and CYP2C19. They surged within hours after birth although proteins could not be detected in fetal samples. A third group of P450s develops later. CYP3A4 and CYP2C9 rose during the first week after parturition and CYP1A2 is the latest isoform to be expressed in the human liver. A fourth group fourth group includes the enzymes with a relatively constant level of expression during hepatic development such as CYP3A5. Individual CYP Enzyme expression/activity data from hepatic microsomes in specific infant age groups are assembled. Tentative general mathematical functions describing the ontogeny of hepatic CYPs are elaborated from these age-specific data. Combined with quantitative changes of other physiological factors during early life stages, these functions have permitted the development of physiological based pharmacokinetic models and the prediction of drug disposition in pediatric population.

Chapter 3 (Manuscript-III)

Drug systemic clearance mechanism matures quickly during the postnatal period and leads to rapid changes in an infant's capacity to eliminate drugs. In chapter 3, a mathematical model describing the maturation of hepatic cytochrome P450 enzyme-mediated clearance and renal clearance due to glomerular filtration was elaborated from developmental physiology and the ontogeny information of specific cytochrome

P450s. The model predicts an age-and elimination pathway-specific clearance for the ontogeny of renal clearance, and metabolic clearance of CYP1A2 and CYP3A4.

The systemic clearance of 6 probe compounds was predicted using the model whose elimination is primarily mediated by a single CYP enzyme or by glomerular filtration. The model performs reasonably well for CYP3A4 substrates with predictions within 2-fold of the observed values in 87% of alfentanil and 74% of midazolam. However, poor predictions were obtained for CYP1A2 substrates caffeine and theophylline with predictions within 2-fold error less than 45%. The overall under predictions for CYP1A2 probes in infants less than 1 year of old suggested the contribution of alternative elimination pathways to the overall clearance of these drugs in neonates. For renal clearance due to glomerular filtration, the model provides reasonable predictions in neonates and young infants.

Clearance scaling is not intended to replace the clinical trials for drugs in development but it will provide a valuable guide on dosing regimens for the first-time doing in children. Furthermore, clearance scaling can greatly benefit the dose adjustment of clinical drugs used in neonates and young infants.

Chapter 4 (Manuscript-IV)

In pediatric population, developmental changes in clearance can be predicted by age and size (body weight or body surface area). Clearance in children is commonly scaled from adults by size using either the per kilogram, body surface area or allometric ¾ power models. But the size model does not account for the maturation process of the elimination organ and therefore may not be appropriate to scale clearance to the very young children. The physiology-based approach accounts for

maturity but requires detailed knowledge regarding developmental changes. In chapter 4, these approaches were compared and appropriate use of these approaches is dependent on the age and clearance pathway.

A child PK database was developed and the dataset of experimentally obtained clearance values was used. Predicted clearance values in children were calculated based on the adult clearance, age and weight data using the four approaches. The ratio of predicted to observed values was graphed separately for probe substrates of three predominant clearance pathways: CYP1A2, CYP3A4 and renal clearance due to glomerular filtration.

Allometric ¾ power model and body surface area approach systemically over estimated clearance in children below 1 year of old for all the compounds. Physiology-based approach accurately predicted clearance at all ages for compounds eliminated via CYP3A4. The clearance of CYP1A2 substrates within the first year were under predicted by the physiology-based approach but over estimated by the other approaches. In the case of renal clearance in children below 1 year of age, the per kilogram and physiology-based model performed similar with a few overestimations.

Physiology-based clearance scaling accurately predicted clearance in children from birth to 18 years. The allometric ¾ power and body surface area model are only accurate when the major clearance pathway is fully matured.

Chapter 5 (Manuscript-V)

Population modeling in pediatric studies often uses size and age as covariates. Covariate-parameter correlations are described in an exponential relationship used by allometric scaling. However, extrapolations based on such parameter estimates have

limited value due to differences in the impact of developmental growth across populations. The quantitative models used to describe the clearance maturation processes across the age range may be required to improve extrapolation and predictive performance.

Previously published pharmacokinetic parameters from probe substrates of hepatic and renal elimination processes are used to develop clearance pathway-specific maturation models. The postconceptional age (PCA) was used as the variable in the modeling practice. Clearance maturation, after standardized to a 70-kg adult, is best fitted with a Hill function. The two renal excreted compounds due to glomerular filtration, gentamicin and vancomycin, presented similar maturation half-life and Hill coefficient, suggesting a system-specific maturation function of glomerular filtration rate (GFR). Similar results were attained with CYP1A2 metabolic probes theophylline and caffeine. Midazolam and alfentanil demonstrated different clearance maturation profiles suggesting a complexity in CYP3A4 metabolism.

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CHAPTER 1

Developmental Pharmacokinetics in Pediatric Populations

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Abstract:

Drug regimens for the pediatric population should be developed in accordance with an understanding of how drug's pharmacokinetics and pharmacodynamics are affected by the dynamic age-related changes in body size and physiology that occur at different stages of development. Although the data on drug disposition in infants and children has increased considerably over the past 2 decades, the effects of development on pharmacokinetics and pharmacodynamics remain poorly understood. In this review, the impact of development on drug absorption, distribution, metabolism and elimination in infants and children is reviewed. Absorption may be affected by the differences in gastric pH and stomach emptying time that have been observed in the pediatric population. Low plasma protein concentrations and higher body water compositions can change drug distribution. Metabolic processes are often immature at birth, which can lead to reduced clearances and prolonged half-lives for those drugs for which metabolism is a significant mechanism for elimination. Renal excretion is also reduced in neonates due to immature glomerular filtration and tubular secretion. Limited data are available on pharmacodynamic behavior of drugs in the pediatric population. Pharmacokinetic processes develop at different rates during the first year of life after birth, thus requiring continual modification of drug dose regimens for their safe and effective use in neonates, infants and children.

Introduction

The pediatric population is very dynamic compared to the adult population. It consists of a collection of highly variable groups that span from new born babies to adolescents of 16 years old. According to the Food and Drug Administration (FDA) guidance, the pediatric population is broken down into the following age-based groups: neonates (birth to 1 month); infants (1 month to 2 years); developing children (2 to12 years); and adolescents (12 to 16 years).[1] In addition to growth in physical size, dramatic changes in body proportions, body compositions, physiology, neurologic biochemistry, and psychological development take place during the transition between infancy and childhood. Growth and development occur particularly rapidly during the first 2 years of life. Body weight typically doubles by 6 months of age and triples by the first year of life. Body surface area doubles during the first year.[2] Proportions of body water, fat and protein continuously change during infancy and childhood. Major organ systems mature in size as well as function during infancy and childhood. Additionally, the pathophysiology of some diseases and pharmacologic receptor functions change during infancy and childhood and differ from adults. For example, most cases of hypertension in children are secondary to renal disease, whereas most cases of hypertension in adults are primary or essential. This has profound effects on the design of antihypertensive drug trials with children. [3] The different sensitivity of the neuromuscular junction to d-tubocurarine (d-TC) among neonates, infants, children and adults has been determined.[4] All of the previously mentioned changes can affect the pharmacokinetics and pharmacodynamics of various drugs and other xenobiotic compounds in the infant and developing child.

Mainly due to the ethical concerns, clinical studies to investigate drug pharmacokinetics and pharmacodynamics in the pediatric population did not begin until 1970's, which is a period also accompanied by the development of sensitive and specific bioanalytical assays. Since that time, numerous studies have reported age-related pharmacokinetic profiles, and to a lesser extent, pharmacodynamic profiles, for many therapeutic agents used in children.[5, 6] Pharmacokinetic parameters including half-life, apparent volume of distribution and total plasma clearance, were shown to have substantial differences among different age groups even when normalized by body weight.[7] These findings were confirmed by population analyses in pediatric patients across broad age ranges, which indicated that in addition to body size, age is an important determinant of pharmacokinetic parameters the pediatric population. [8-12] Age associated developmental changes, such as body compositions, organ functions, ontogeny of drug biotransformation pathways, disease progression, pharmacological receptor functions, have all been shown to affect drug dispositions and response profoundly, especially during the first two years of life.[13] Understanding these age effects can provide a mechanistic way to identify initial doses for the pediatric population.

The purpose of this review is to summarize quantitative and qualitative developmental changes in the neonate, infant and developing child, and discuss how these changes may produce age-associated changes in the drugs' pharmacokinetics (bioavailability, volume of distribution, protein binding, hepatic and renal elimination), and pharmacodynamics. Approaches that can determine age-specific

dosing regimens through a mechanistic understanding of pharmacokinetic and pharmacodynamic are discussed.

Absorption

In contrast to intravenous administration, drugs administrated extravascularly must undergo absorption to reach the systemic circulation. Absorption is frequently assumed to be a single first order process and is often quantified by absorption rate constant (k_a), the time to reach peak concentrations (t_{max}), and the extent of drug absorption by the fraction of the dose absorbed (F) and the value of the peak plasma concentration (C_{max}).

In the gastrointestinal tract, several age-related anatomic and physiological changes have been found that may affect drug absorption (Table 1.1). Gastric pH is neutral at birth but falls to pH 1-3 within 24-48 hours after birth. The pH then gradually returns to neutral again by day 8 and subsequently declines very slowly, reaching adult values only after 2 years of age.[14, 15] The higher pH or relative achlorhydria in neonates and very young infants may partially explain the higher bioavailability reported for acid-labile compounds such as beta-lactam antibiotics because higher gastric pH results in their reduced degradation.[16] Bioavailability of orally administered weak acids, such as phenytoin, acetaminophen and phenobarbital, may be reduced in infants and young children due to increased ionization under achlorhydric conditions.[17, 18]

Gastric emptying and intestinal motility are important determinants for the rate of drug absorption in the small intestine, which is the major site of drug absorption. Gastric emptying time during the neonatal period is prolonged relative to that of the

adult. This may partially account for delayed absorption for orally administered phenobarbital, digoxin and sulfonamides. [19] Other factors such as reduced intestinal absorption surface area and shorter gut transit time may also be responsible for the delayed absorption observed in neonates.

In addition, the ability to solubilize and subsequently absorb some lipophilic drugs can be affected by the age-dependent changes in biliary function and activities of pancreatic enzymes. The infant has decreased pancreatic exocrine function and bile acids secretion, which may influence the absorption of prodrug esters such as erythromycin that require solubilization or intraluminal hydrolysis. [20]

Developmental changes in the activity of intestinal drug-metabolizing enzymes and transporters that could potentially alter the bioavailability of drugs. At this time, these developmental changes have not been completely characterized as few clinical studies have addressed this issue. Midazolam's oral clearance (Cl/F) is markedly decreased in preterm infants because immature intestinal cytochrome P450 3A4 (CYP3A4) metabolism leads to an increased oral bioavailability. [21] One study observed that intestinal biopsy specimens from young children (1- to 3- years old) had a 77% higher busulfan glutathione conjugation rate compared to older children (9- to 17- year old), which may lead to an enhanced first-pass intestinal metabolism and a reduced absorption fraction (F) in young children. [22] Gabapentin is primarily excreted in urine as unchanged drug, and its bioavailability is dose independent because the L-amino acid transporter in the GI membrane is saturated by high drug concentrations. Oral clearance of gabapentin is 33% higher in children younger than 5 years than in older children or adults. Because renal clearance reaches adult levels at 1-2 years of

age and gabapentin is not protein bound, this effect on oral clearance is not likely due to the altered clearance but decreased bioavailability caused by immature L-amino transporter activity. [23]

Developmental changes also can alter the absorption of drugs by other extra vascular routes. Percutaneous absorption of drugs through skin may be high in newborns and infants owing to better hydration of the epidermis, greater perfusion of the subcutaneous layer and the larger ratio of total body surface area to body mass compared to adults. Thus, the relative exposure to steroids applied topically in newborns and infants may exceed that in adults and result in toxic effects in some instances.[24] The absorption of intramuscular administered drugs may be delayed in neonates from reduced blood flow to skeletal muscles, although it is often unpredictable in clinical practice. [25]

Distribution

Independent of the route of administration, once the drug enters the blood stream; it is distributed into the vascular compartments of the body and extra vascular tissues. The volume of distribution (V_d), is mainly determined by the ratio of plasma to tissue binding and the physiological volumes a drug can access. A high volume of distribution indicates extensive drug distribution to the tissues and a low volume suggests that a drug is highly bound to plasma proteins. Other factors, such as lipid and water solubility, may also be important.

The dramatic maturation changes in the relative amount of body water and fat have been well characterized by Friis-Hansen. [26] The total body water content, expressed as percentage of body weight, decreases with age, from approximately 80%

in newborns to 60% by 1 year of age. Conversely, body fat increases with age, from 1-2% in a preterm neonate to 10-15% in a term neonate and 20-25% in a 1-year-old. The impact of these differences on the volume of distribution depends to on the physiochemical characteristics of the drug. Highly water-soluble compounds, such as gentamicin, have larger volumes of distribution in neonates compared to adults. For example, gentamicin's volume of distribution is around 0.5 L/kg in neonates, compared to 0.25-0.3 L/kg in adults. As a result, a larger milligram per kilogram loading dose may need to achieve desired therapeutic concentrations. [27] Lipophilic drugs, such as diazepam, tend to have smaller volumes of distribution in infants than in older children and adults.[18]

Plasma protein binding tends to be reduced in neonates and infants.[28] Factors that influence the drug-protein binding during infancy include: the total amount of plasma proteins such as albumin and alpha1-acid glycoprotein, the binding affinity and the presence of endogenous compounds. Reduced protein binding may result in drugs being distributed more widely and an increased apparent volume of distribution. For example, a clear link between an increased volume of distribution in neonates and the decreased protein binding has been reported for phenobarbital.[18] In addition, the decreased protein binding would alter the ratio of unbound to total plasma concentrations for the highly protein-bound antiepileptic drugs such as phenytoin, which makes the total concentrations of phenytoin difficult to interpret for therapeutic drug monitoring in neonates and young infants. [29] Finally, it is worthwhile to mention that highly bound acid drugs such as sulfonamides can compete for bilirubin-binding sites on albumin and displace bilirubin when plasma albumin level is low. This will

lead to increased blood levels of unconjugated bilirubin and increase risk of kernicterus in the fetus or neonate. [30]

Drug Transport

Drug transporters such P-glycoprotein (P-gp), organic anion transporting polypeptides (OATPs), organic anion transporters (OATs), cation transporters (OCTs), and breast cancer resistance protein (BCRP), may influence drug absorption, distribution and elimination.[31] Limited data in humans suggest that P-gp follows a developmental pattern. P-gp mRNA and protein were detected in human kidney and liver as early as 11 to 14 weeks of gestation but were only detected in brain and intestine in the third trimester of pregnancy. [32] A significant amount of P-gp is expressed in the intestine of neonates and infants with a large interindividual variability.[33] In human intestinal and liver tissues from individuals of different age ranging from neonatal to 85 years, P-gp expression was relatively low in the neonatal group but it increased with development and reached maximum levels in young adults (15 – 38 years of age) and decreased to half the maximal levels in older individuals (67 – 85 years).[34] There is differential expression of P-gp in various tissues. A study in 90 healthy volunteers aged 0 – 86 years showed that P-glycoprotein activity in peripheral blood lymphocytes was highest in cord blood and progressively declined with age. [35] The clinical significance of developmental changes in transporter functions has not been systemically studied in humans.

Hepatic Metabolism

Drug metabolism can be divided into two types of reactions: phase I and phase II metabolism. Phase I metabolism involves structural alterations of drug molecules by

introducing or unmasking a functional group (e.g. oxidation, reduction and hydroxylation). Phase II metabolism involves conjugation of functional groups of molecules (parent drug or phase I metabolites) with hydrophilic endogenous substrates (e.g. glucuronidation, sulfation, acetylation). Although the kidney, intestine, lung and skin are also capable of biotransformations, the liver is quantitatively the most important organ for drug metabolism. [36] Developmental changes in hepatic metabolism have been identified in the past decade and different metabolic pathways mature differently with age.

Most phase I drug reactions are mediated by cytochrome P450 (CYP) enzymes, a super family of multiple hemeproteins, among which CYP3A, 2C9/2C19, 2D6, 1A2, 2E1 and 2B6 are involved in the hepatic metabolism of most drugs. [37] Other than CYPs, the flavin-containing monooxygenase (FMO) enzymes are also important for the oxidative metabolism of a wide variety of therapeutics. Figure 1 lists the ontogenesis for primary cytochrome P450 (CYP) enzymes.[38-42] Briefly, CYP3A7 is the primary isoenzyme during the prenatal period and declines rapidly after birth. This enzyme exists in barely measurable quantities in adults. CYP2E1 and CYP2D6 levels begin to rise at the time of birth, and CYP3A4, 2C9 and 2C19 appear within the first weeks of life. CYP1A2 is the last enzyme to develop and is present by 1-3 months of life. The activity of these enzymes increases over time but does not appear to do so in a direct linear manner with age. By 1 -2 years of age, the total CYP activity levels are similar to those of adults. [43] The developmental changes in the enzymatic systems are supported by the age-related changes in the systemic clearance, as well as changes in the metabolic ratios of probe substrates to their metabolites in vivo. For example, the

rise of the amount of CYP2D6 protein was associated with the rise in dextromethorphan *O*-demethylation, as measured in vivo by the urinary ratio of dextromethorphan to dextrorphan. [44, 45] Similarly, the delayed ontogenesis of CYP1A2 protein was consistent with the in vivo data where N3 and N7 demethylation products of caffeine (assigned to CYP1A2) represented 6-8% of the total biotransformation in neonates and increased to about 28% in infants aged 2-10 months. [46] In addition, the developmental sequence of the CYP isoenzymes is also demonstrated with the developmental changes in the relative amount of metabolites produced from the different pathways. For example, *O*-demethylation of diazepam by CYP2D6 is reported to develop sooner than *N*-demethylation by CYP3A4, which is in line with the in vitro observations on the ontogeny of CYP2D6 and CYP3A4. [47]

The ontogeny of hepatic FMO exhibits a similar developmental pattern as the CYP3A family. The expression of FMO1, whose developmental pattern is like that of CYP3A7, is at the highest level at 8 to 15 weeks of gestation and subsequently declines during the fetal development and completely absent within 72 postnatal hours. FMO3, however, analogous to CYP3A4, is negligible in neonatal period and becomes detectable by 1 to 2 years of age. The delayed onset of FMO3 expression results a null FMO phenotype in the neonate. [48]

In contrast to the cytochrome P450 enzymes, isoform-specific quantitative data for the developmental pattern of phase II enzymes is very limited. The timelines of phase II enzymes detected in fetus, neonates and infants are shown in Table 1.2. [49, 50] The uridine 5-diphosphoglucuronic acid glucuronyl transferases (UGT) are the major

contributors to phase II metabolism, and account for nearly 15% of phase II metabolism activity in adult human. [51] The ontogenesis of UGTs has been of greater focus than other phase II enzymes because of the common therapeutic use of several substrates in pediatric patients. These substrates include, acetaminophen (UGT1A6 and, to a less extent, 1A9), morphine (UGT2B7) and zidovudine (UGT1A6). Among the UGT isoforms, UGT 1A1 and 2B7 develop quickly, whereas UGT1A6 and 1A9 develop slowly. [52] The expression of UGT1A1, the major enzyme responsible for bilirubin glucuronidation, is triggered at birth and the activity reaches adult levels by 3 to 6 months postnatal age. UGT2B7 is present in fetus, and increases at birth with adult levels attained by 2 to 6 months of age. UGT1A6 is undetectable in the fetus. It increases slightly in neonates, but does not reach adult levels until 10 years of age. These data are consistent with the pharmacokinetic data of UGT substrates assessed in vivo. For example, the systemic clearance of morphine (substrate of UGT2B7) is low in neonates and reaches adult levels between 2 and 6 months. [53] Similarly, acetaminophen glucuronidation (primarily mediated by UGT1A6 and 1A9 to a less extent), as reflected by urine metabolite data, is low in newborns and young children compared to adolescents and adults. [54]

Although glucuronidation is considerably reduced in newborns, sulfation appears to be well developed at birth. The variation in the function of the two phase II reactions can be seen with the developmental changes of acetaminophen metabolism. In early infancy, acetaminophen is primarily converted into the sulfate conjugates; but with increasing age, glucuronidation becomes the predominant form of metabolism. [13] Acetylation has also been studied and has been found to have reduced activity in the

first month of life, although the effect of age appears to be less dominant than that of polymorphism of N-acetyltransferase (NAT). [50] Esterase activity is also reduced in newborn and this may partly account for the prolong effects of local anesthetics. [55]

In conclusion, both phase I and II metabolic processes may be immature at birth. These deficiencies may result in the increased risk for drug toxicity in infants and young children. An often cited example is "gray-baby" syndrome associated with the administration of chloramphenicol (substrate of UGT2B7). The syndrome consisted of emesis, abdominal distension, abnormal respiration, cyanosis, cardiovascular collapse and death. This response has been primarily assigned to the very high drug plasma concentrations caused by the reduced glucuronidation and clearance of chloramphenicol in the neonate. [56] The ontogeny of drug-metabolizing enzymes in humans will clearly have to be addressed by age-related dosage adjustment for some therapeutic agents in pediatric patients. For example, a positive correlation was demonstrated between age and systemic clearance normalized by body weight for theophylline. [57] Other drugs that undergo extensive metabolism, including caffeine, diazepam, phenytoin and chloramphenicol, are often observed with prolonged apparent half-lives in neonates and young infants. As a result, a decreased daily maintenance dose or increased dosing interval may be needed to avoid drug accumulation. A typical example is the clinical use of theophylline in infants with apnea or chronic lung disease. The elimination half-life of caffeine, a metabolite of theophylline is ~30 hours in neonates, and is decreased to 3 hours by 1 year of age as CYP1A2 enzyme activity increases. Correspondingly, the initial maintenance dose of theophylline is adjusted for different ages to avoid the accumulation of caffeine. [58]

The transporter-mediated uptake of drugs into the hepatocytes and efflux into the bile is often calledPhase III hepatic pathway. Important uptake transporters include the OATPs, OAT2 and OCT1, and clinically important efflux transporter at canalicular membrane include P-glycoprotein (P-gp), breast cancer resistant protein (BCRP), bile salt export protein (BSEP) and multidrug resistance protein 1 (MRP1). P-glycoprotein is also involved in efflux of compounds from the intestines.[59] At this time, there are limited data in humans on the ontogeny expression of liver transporters.[60] A single study suggested that P-gp in the liver follows a developmental pattern of expression in which activity increases during the first few months of life and adult levels reaches by 2 years of age. [61]

In addition to size and ontogeny of enzyme and transporters, other factors such as genetic polymorphism, prenatal or postnatal exposure to inhibiting and inducing agents, might also have an independent impact on the phenotypic metabolic activity observed. [62] Allegaert et al. recently found that CYP2D6 polymorphisms had a significant impact on *O*-demethylation of tramadol in neonates and young infants. The metabolite ratio of tramadol over O-demethyl tramadol decreased progressively with increasing activity score of CYP2D6 (a quantitative classification of CYP2D6 genotypes). [63] There is a large interindividual variability observed in CYP2B6 expression including pediatric populations. This was likely due to the highly inducible and polymorphic nature of this enzyme.[64]

Renal Elimination

Excretion of drugs by the kidneys is dependent on three processes. First, glomerular filtration, a passive process whereby a drug not bound to plasma proteins is

filtered into the renal tubule. Clearance by glomerular filtration is principally determined by the glomerular filtration rate (GFR) and the extent of plasma protein binding. Second, drug excretion may be augmented by the action of uptake and efflux transporters in the renal tubule epithelium of the proximal convoluted tubule. Finally, drugs may be re-absorbed from the tubule back into the blood by passive diffusion, which is determined by the physiochemical characteristics of the drug and urinary pH. The renal clearance (CL_r) of drugs is the sum of three processes (Eq.1). Each of these processes exhibit independent rate and pattern of development.

$$CL_r = CL_{glomerular\ filtration} + CL_{tubular\ secretion} - CL_{reabsorption} \quad Equation\ 1$$

Glomerular filtration is the major renal elimination pathway for many drugs. As shown in Figure 2,[65, 66] the glomerular filtration rate (GFR), measured by the renal mannitol clearance, is around 10-20 mL/min/m^2 at birth for a full-term newborn. This increases rapidly to 20-30 mL/min/m^2 during the first weeks of life and typically reaches adult values (70 mL/min/m^2) by 3-5 months. Furthermore, the increase in GFR is highly dependent on postnatal age (PNA, the chronological age since birth). Hayton et al. described the maturation of GFR with PNA by a nonlinear function. [65] A more practical equation (Eq.2) for estimating age-specific renal glomerular filtration rate (CL_{GFR}) was proposed by Schwartz and coworkers. [67]

$$CL_{GFR} = CL_{r,Cr} = K \times \frac{Ht}{SCr} \quad Equation\ 2$$

Where $CL_{r,\ Cr}$ is renal creatinine clearance (ml/min/1.73m^2), Ht is height (cm) and SCr is serum creatinine concentration (mg/dl). K is a constant of proportionality and

different for children in different age bands. K is 0.33, 0.45, 0.55, 0.55 and 0.7 for infants with low birth weight (0-12 months), full term infants (0-12 months), children (2-12 years), female adolescents (13-21 years) and male adolescents (13-21 years), separately. For drugs that are mainly excreted by glomerular filtration (e.g. aminoglycosides), the initial dose adjustment can be made based on the knowledge of serum creatinine levels.

In contrast to glomerular filtration, tubular secretary and re-absorptive capacity appear to mature at a much slower rate. The tubular secretion rate, measured by the renal clearance of p-aminohippurate (a substrate of kidney the uptake transporter, organic anion *transporter*), is reduced at birth to approximately 20-30% of adult capacity but matures by 15 months of age.[66] The development of other renal uptake transporters such as organic cation transporters (OCT) and organic anion transporting polypeptides (OATPs) is unknown. Tubular reabsorption is the last renal function to mature and does not reach adult levels until 2 years of age. This delay in the development of tubular functions may have variable effect on some drugs' clearance for which tubular secretion or reabsorption is important in adults. For example, digoxin is both filtered by the glomerulus and secreted by the tubules, and average renal clearance of 1.97, 5.33 and 8.67 L/h/1.73m2 have been reported in full term infants less than 1 week of age, 3-month-old infants and children of 1.5 years of old, respectively.[68] At this time there is little information in the literature about the ontogeny of renal drug transport systems and their impact on renal elimination in infants and children.

Generally, for drugs principally eliminated by kidney, immature renal clearance processes result in the inefficient elimination of drugs and prolongation of their residence time in the body (e.g. the prolonged apparent half-life in the newborns for digoxin and penicillins [69]).

Pharmacodynamics

Unlike the rapidly accumulating knowledge of the pharmacokinetic changes associated with development, little is known about receptor development and sensitivity and how maturation affects the response to the drug-receptor interaction. Most often, the apparent developmental differences in pharmacodynamics (e.g. higher acid inhibition effect of lansoprazole in young infants) or the incidence of adverse effects (e.g., increased hepatoxicity of valproic acid in young infants) are linked with pharmacokinetic differences. [29, 70] The existence of true age-dependent differences in receptor sensitivity appears to be supported by data on certain drugs (e.g., warfarin and tubocurarine). For example, Takahashi et al. reported that the mean international normalized ratio obtained from the prepubertal patients was significantly greater than that obtained from the adult patients, despite no differences in unbound fraction of (S)-warfarin observed between pediatric and adult patients.[71] This finding suggested that children might possess a greater sensitivity to warfarin than adults due to pharmacodynamic rather than pharmacokinetic differences. Marshall and Kearns reported the in vitro developmental pharmacodynamics for cyclosporine.[72] The peripheral blood monocytes of the infants showed a twofold lower mean IC_{50} (peripheral blood monocyte proliferation) and sevenfold lower mean IC_{90} (interleukin-2 expression) than peripheral blood monocytes from older subjects. The study

provided relevant information on developmental changes in receptor binding characteristics in vitro, but this may not be reflective of the pharmacodynamic response in vivo. Future clinical research in age-related changes in the pharmacodynamic response chain is important to fully understand drug response in the pediatric population and to identify optimum therapeutic plasma concentrations in this group.

Age-related Dosing Regimen

Simple dosage formulas (normalized by body weight or body surface area) and allometric scaling may be clinically applicable in children older than 2 years of age.[73] In neonates and young infants, where age related developmental changes in drug disposition are underway, age-specific dosing regimens are needed based on observed age-related changes in bioavailability (F), volume of distribution (Vd) and overall clearance (CL).

In clinical practice, when pharmacokinetic data in children is available standard pharmacokinetic equations can be used to estimate doses. Without established dosing guidelines or complete pharmacokinetic data in children, methods to approximate the initial dose or maintenance dose for an infant are proposed as "bottom-up" approaches. To date, there are several "bottom-up" approaches for pediatric dose selection without PK data in children. Bartelink et al. proposed a refined dosing guideline based on the route of administration, the characteristics of the drug, the age of the child. For example, the dose of drugs that are excreted by glomerular filtration is indexed to body surface area in children over 2 years of age, but uses GFR as descriptor in children < 2 years of age.[74] This guideline is an integration of four

important pharmacokinetic processes: absorption, distribution, metabolism and renal excretion and its decision tree designation is simple to use in clinical practice. However, the approach ignored the complexity of PK characteristics of individual drugs and the large interindividual variability across the pediatric population. Another possibly more accurate approach is pediatric physiologically based pharmacokinetic (PBPK) modeling. Many pediatric PBPK models have been developed to predict pharmacokinetics in children, one of which is the model presented by Edginton et al. using PK-Sim for five compounds: acetaminophen, alfentanil, morphine, theophylline and levofloxacin.[75] In general, an existing adult PBPK model is extended to reflect age-related physiological changes in children from birth to age eighteen. The age-modified model was then used together with a previously developed age-specific clearance model to predict pediatric plasma concentrations.[76-78] PBPK models combine the developmental physiological processes of the child with adult PK data, and require the compound-related information and compound-independent information on the ontogeny of anatomical, physiological and biochemical variables, which are often from multiple resources in the literature.[79] However in many cases it seems unlikely that accurate data from humans of all ages will ever be available. Moreover, there is no consensual input for physiological parameters. Regression equations fitted from the literature sources are validated internally by each author or modeling team. Many of the values are often estimates or educated guesses. For example, the data on the tissue composition (proportion of lipids, protein and water) are limited and sometimes unavailable for children. Because this information is important to predict the tissue blood partition coefficient,[80] assumptions are made in

PBPK models to consider the coefficient in children as equal to that in adults. The ontogeny of hepatic cytochrome P450 enzymes are still missing for some age ranges.[81]

Although these bottom-up approaches provide a valuable aid for the first-time dosing in children and for the study design, none of them could be a substitute for a clinical study on the effect of age on the pharmacokinetics of a specific drug.[82] A population pharmacokinetic approach is commonly used to obtain these age-associated PK parameters. The selected population model usually includes covariates such as body weight and age. Weight is often included in the model using allometric scaling according to the formula (Eq. 3):

$$\theta_i = \theta_{TV} \times (\frac{BW_i}{median\ BW})^{EXP} \qquad \text{(Equation 3)}$$

where θ_i represents the individual parameter of interest, θ_{TV} the typical value for the parameter, BW_i the individual body weight, median BW is the median body weight of the population and exponent (EXP) is the power factor. The allometric exponent (EXP) can be either fixed or estimated during the model building procedure.[12] In the example of zidovudine PK in HIV-infected infants and children, an allometric size model with fixed coefficients of 0.75 for clearance and 1 for volume of distribution were incorporated in the base model and then other potential covariates such as age, liver enzyme measurements were evaluated.[9] The population approach is ideal for the pediatric population since a large heterogeneous population can be studied by taking only a few samples per patients at flexible sampling times. [83] Moreover, if clinical response measurements are available, it may be possible to create integrated pharmacokinetic-pharmacodynamic models, which would allow better

optimization of pediatric dosing. For example, a population PK-PD model was used to refine postoperative sedative effect and optimize the dose of midazolam in nonventilated infants aged 3 months to 2 year old.[84]

Conclusion

An advance in developmental pharmacology during the past decades have improved our understanding of the influence of growth and maturation on the disposition (and actions) of drugs. Pediatric clinical studies encouraged by regulatory agencies have facilitated improvements in drug therapy for this population. [85] Based on the current knowledge, it should be obvious, that the dosing regimen for adults cannot be simply or linearly extrapolated to children, particularly in neonates and infants. The application of population pharmacokinetic-pharmacodynamic methods has been widely advocated and is described in the FDA's guidance document. [1, 86] The use of physiologically based pharmacokinetic models has been recommended to help in the first time dosing in children as well as the study design. [87, 88] However there is a strong need for more research on developmental pharmacology such as the ontogeny of drug metabolizing enzymes, transporters, receptor system, and disease progress. As the gaps in knowledge are gradually filled in, the development of therapeutic pediatric dosing regimen will be enhanced, and drugs will eventually be provided to children with greater precision and safety.

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Table 1 Developmental factors affecting drug pharmacokinetics in neonates and infants.

Physiologic Factors	Difference compared to adults	PK implications	Example Drug
Oral absorption			
Gastric PH	↑	↓ bioavailability (weak acids)	phenytoin, phenobarbital, ganciclovir
		↑ bioavailability (weak bases)	penicillin G, ampicillin, nafcillin
Gastric emptying time	↑	delayed absorption	phenobarbital, digoxin and sulfonamides
Intestinal CYP3A4	↓	↑ bioavailability	midazolam
Intestinal GST	↑	↓ bioavailability	busulfan
Intestinal drug transporters	↓	↓ bioavailability	gabapentin
Percutaneous absorption			
hydration of epidermis	↑	↑ bioavailability	steroids
Intramuscular absorption			
skeletal muscle blood flow	variable	unknown	n.a.
Distribution			
Body water : body fat ratio	↑	↑ volume of distribution (hydrophilic drugs)	gentamicin, linezolid, phenobarbital, propofol
		↓ volume of distribution (lipophilic drugs)	diazepam, lorazepam
Protein binding	↓	↑ free fraction of drugs	sulfonamides
Hepatic metabolism			
Phase I enzyme activity	↓	↓ hepatic clearance	theophylline, caffeine, midazolam
Phase II UGT enzyme activity	↓	↓ hepatic clearance	morphine
Renal excretion			
Glomerular filtration rate	↓	↓ renal clearance	aminoglycosides
Renal tubular absorption and secretion	↓	↓ renal clearance	digoxin

↑ = changes increased in values; ↓ = changes decreased in values; n.a. = not available

CYP: cytochrome P450; GST: glutathione S-transferases; UGT: UDP-glucuronosyltransferase

Table 2 In vitro ontogeny of human hepatic phase II enzymes (adapted from Ref. [36-37]).

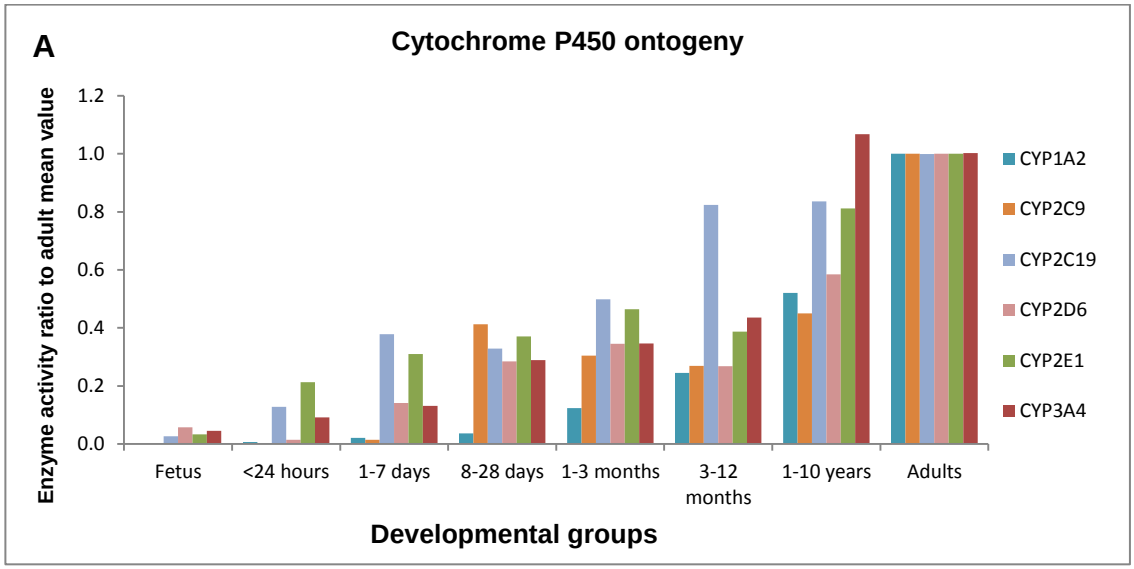
Isoenzyme	Fetus	Neonate (0-1 month)	1 month to 1 year	Adult	Ontogeny Facts
UDP glucuronosyltransferase (UGT)					
UGT1A1	-	+	+	+	adult levels attained by 3-6 month
UGT1A6	-	+	+	+	maturation complete until puberty
UGT2B7	+	+	+	+	adult levels attained by 2-3 month
Sulfotransferases (SULT)					
SULT1A3	++	+	+	-	substantial decrease in perinatal period
Glutathione S-transferase (GST)					
GSTA1/2	+	++	++	++	increase dramatically to adult levels shortly after birth
GSTM	+	++	++	++	increase dramatically to adult levels shortly after birth
GSTP	++	+	+	-	substantial decrease in perinatal period
Epoxide hydrolase (EPH)					
EPHX1	+	+	+	+	no correlation between EPHX1/EPHX2 activity and gestational or postnatal age
EPHX2	+	+	+	+	
N-acetyltransferase (NAT)					
NAT2	+	+	+	+	Enzyme polymorphisms affect isoniazid metabolism more importantly than ontogeny

-, activity or protein not detectable; +, activity or protein detectable; ++, high level of activity or protein expression

Figure 1 Developmental profiles of major hepatic cytochrome P450s (A) and CYP3A7 (B). The postnatal evolution of P450 isoforms was explored in a liver bank comprising samples from fetus, neonates, infants and adults. Isoform enzyme activity was characterized by the following measurements: methoxyresorufin demethylation (MEROD) for CYP1A2, tolbutamide hydroxylation and for Diazapam N-demethylation for CYP2C9 and 2C19, dextromethorphan O-demethylation for CYP2D6, chlorzoxazone hydroxylation for CYP2E1, testosterone 6beta-hydroxylation for CYP3A4, DHEA 16alpha-hydroxylation for CYP3A7. (Data are adapted from ref. [38-42]).

Figure 2 Developmental changes of renal glomerular filtration rate (GFR) measured by mannitol clearance. (Data adapted from Ref. [65-66]).

Figure 1


A
Cytochrome P450 ontogeny
Enzyme activity ratio to adult mean value
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Fetus
<24 hours
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
Adults
Developmental groups
CYP1A2
CYP2C9
CYP2C19
CYP2D6
CYP2E1
CYP3A4

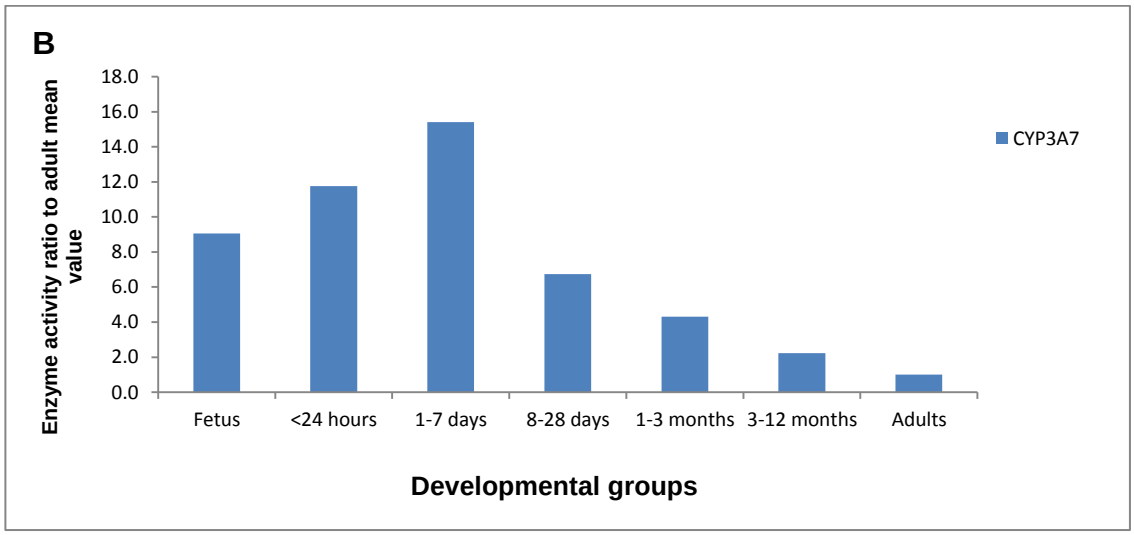
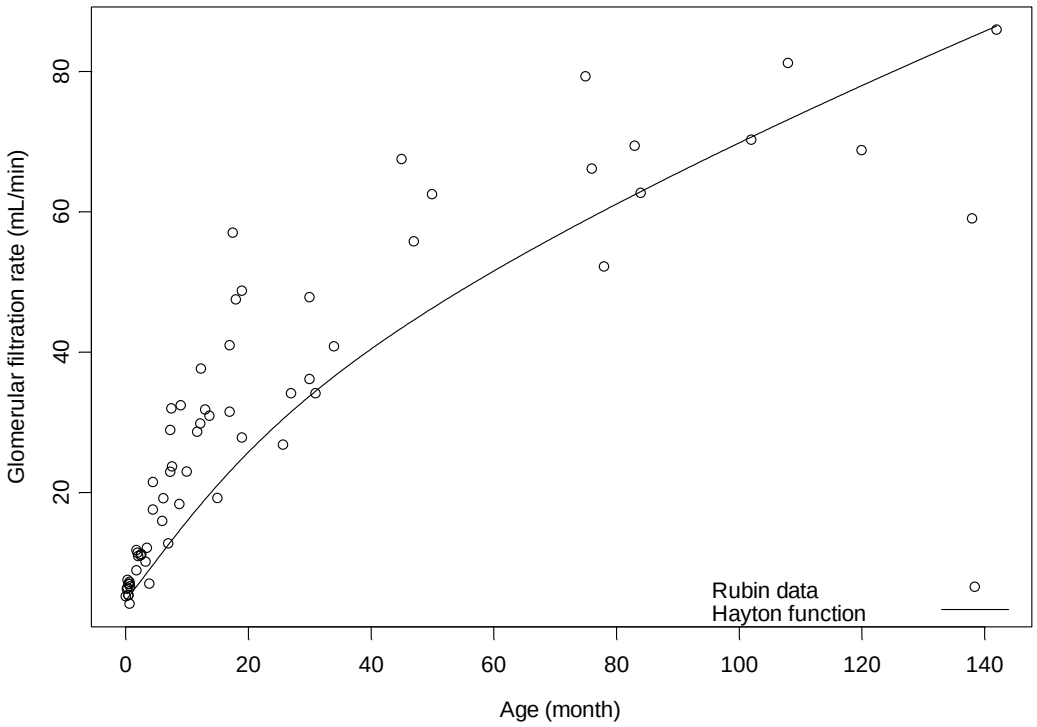

B
Enzyme activity ratio to adult mean value
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Fetus
<24 hours
1-7 days
8-28 days
1-3 months
3-12 months
Adults
Developmental groups
CYP3A7

Figure 2


Glomerular filtration rate (mL/min)
20
40
60
80
0
20
40
60
80
100
120
140
Age (month)
Rubin data
Hayton function

CHAPTER 2

Ontogeny modeling of Human Hepatic Cytochrome P450s

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Abstract

Objectives: The maturation of drug metabolizing enzymes is the predominant factor that accounts for age associated changes in pediatric pharmacokinetics. Each enzyme demonstrates an independent rate and pattern of development. In this study, individual CYP Enzyme expression/activity data from hepatic microsomes in specific infant age groups were assembled from the literature published from 1970-2012 in PubMed, and the characteristic maturation patterns of the most important hepatic CYP enzymes including CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4, were quantified as a continuous function of age from birth and the age of reaching the adult level.

Methods: Individual CYP isozyme expression/activity data from hepatic microsomes in specific infant age groups were assembled from the literature published from 1970-2012 in PubMed. The mean reported values stratified by age groups were compiled in the database. For those data reported in graphs instead of tables, the open digitizing software (Engauge Digitizer 5.1, http://digitizer.sourceforge.net/) was used to extract data. For each study reviewed, the in vitro enzyme expression and/or activity data were expressed as a fraction of the mean adult values presented in the same study. For each individual enzyme isoform, the mean values for each age group from different studies were pooled naively and fitted with several plausible non-linear regression functions using NONMEM version VI (Globomax LLC, Hanover, MD, USA). A proportional residual error model was used. Structural models were compared based on the visual inspection of fits and the Akaike information criteria (AIC).

Results: The development of CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 was best described by the hyperbolic function (Eq. 1) in the age range of observations. The parameter MF_0 is the enzyme activity at birth (at postnatal age of 0 day) expressed as a fraction of the adult activity. MF_{max} is the maximal mean activity observed in the investigated samples (expressed as a fraction of the adult levels). TM_{50} is the postnatal age (day) to reach 50% of the maximal enzyme activity (MF_{max}) observed in the investigated age range. This parameter is indicative of the development rate of each individual isoenzyme.

$$MF = MF_0 + \frac{(MF_{max} - MF_0) \times AGE(day)}{TM_{50} + AGE(day)} \qquad \text{Equation 1}$$

Conclusions: The high MF_0 values for CYP2E1 suggested its fetal expression or prenatal onset, while the extremely low values of MF_0 for CYP2C9 and 1A2 suggested the two isoenzymes expression were triggered by birth effect. CYP2C9, 2C19 and 2D6 appeared to mature faster than CYP2E1, 3A4 and CYP1A2, as shown by the short half-life values within days compared to those in months. Because the maximal ratios of CYP1A2, 2C9, 2C19 and 2D6 are way below 1 due to insufficient data, the models could not describe the ontogeny profiles of these four enzymes beyond their observed age range.

Keywords: ontogeny, cytochrome P450, drug metabolism, maturation

Introduction

The human cytochrome P450 enzymes are a haem-containing superfamily that consists of a total 270 CYP gene families divided into 18 families and 42 subfamilies of enzymes based on converged amino acid sequences [21]. Despite the large number of CYP genes and enzymes, only the *CYP1*, *CYP2* and *CYP3* families play a major role in drug and toxicant metabolism. The remaining CYP families are more specialized enzymes involved in the synthesis and degradation of important endogenous molecules. CYP3A, CYP1A2 , CYP2C and CYP2E1 are the most abundant CYP enzymes comprising 29%, 13%, 18% and 7% of the total CYP protein content in the adult human liver, respectively [25]. When the number of known drug substrates is considered, CYP3A is the most important isozyme, metabolizing around 52% of therapeutic drugs currently on the market, followed by followed by 2D6 (31%), 2C9 (10%) and CYP1A2 (3%) [10].

In adults, a wide inter-individual variation in hepatic P450 expression and associated metabolic activity has been reported for each isoform. These variations may be the result of genetic polymorphisms and/or the induction or inhibition of enzyme activity by concomitant medications or exposure to environmental xenobiotics [20,24]. In the pediatric populations, ontogenesis, or the change of enzyme expression/activity as a function of age, is superimposed upon the genetic and or environmental factors, and adds further to variability in enzyme activity and unpredictability. The current knowledge of the ontogeny of hepatic CYP enzymes during the fetal and infant development has been reviewed in-depth [1,3,8,13,22]. It is well recognized that each individual CYP enzyme demonstrates an independent rate and pattern of development.

However the characteristic maturation patterns of each CYP enzyme have rarely been quantified as a continuous function of age from birth and the age of reaching adult level. Such enzyme maturation functions, in conjunction with other age-related physiological changes in neonates and infants, constitute the non-drug specific inputs to physiologically based pharmacokinetic (PBPK) models in pediatric studies. Therefore, the aim of this study is to fill in a piece of knowledge gap in the developmental pharmacology. In this study, published in vitro enzyme ontogeny data on the most important hepatic CYP enzymes: CYP1A, 2B6, 2C, 2D6, 2E1 and 3A, were assembled, and the age-related changes of each CYP isoenzyme were fitted with mathematical functions.

METHODS

Compilation of literature data

Computerized literature search of Medline database were conducted to find references to publications describing ontogenesis of hepatic cytochrome P450 enzymes, using search words such as CYP, human, liver or microsome or hepatocyte, fetal or infant or children or age. Additionally, bibliographies from review articles and pediatric clearance scaling papers were examined to identify relevant information.

Several methods are used to investigate the ontogeny of P450 expression in the liver. These include: including analyzing the immunoquantifiable enzyme protein levels using specific antibodies; measuring mRNA levels using nucleic acid probes; and measuring enzyme catalytic activities using probe substrates. Significant discrepancies exist between developmental profiles obtained from mRNA compared to

protein expression or activity levels. As a result, the enzyme-specific ontogeny is based on in vitro literature values of age-dependent enzyme expression and activity from hepatic microsome or hepatocyte studies.

In most studies individual data were not reported. The mean values and variability were reported and stratified by age groups. The small amount of available individual data from the studies was grouped into different age bands, with variability, to reduce residual errors generated from such data. For those data reported in graphs instead of tables, an open digitizing software (Engauge Digitizer 5.1, http://digitizer.sourceforge.net/) was used to extract data by converting image files into numbers. Where developmental changes were reported for age bands, the median age was used for modeling purpose.

The age groups used in this study were: fetus I (<30 weeks of gestation); fetus II (>30weeks of gestation), newborn (<1 day since birth), neonate (1-30 days), infant I ((1-3 month), infant II (3-12 month), prepuberty children (1 - 10 years old), adolescent (10-16 years old) and adults (> 16 years old), which was similar to that used by McNamara and Alcorn [1]. All attempts were made to divide in vitro data into relatively narrow infant age groups.

For each study reviewed, the in vitro enzyme expression and activity data were expressed as a ratio of adult values presented in the same study so that the absolute values/units were not an issue when comparing studies. The original enzyme protein expression and activity data compiled from published studies is summarized in Table 1.

Ontogeny modeling approach

The age-dependency for one individual enzyme isoform can be described by either protein or a probe substrate reaction. A naïve data pool approach was used to develop ontogeny models for individual isoenzymes: CYP1A2, 2C9, 2C19, 2D6, 2E1 and CYP3A4. Changes with age were evaluated by applying four different models, including hyperbolic, sigmoid (hill-type), logarithmic and mono-exponential functions [2], with minimization of residual errors (proportional error model) using nonlinear regression with NONMEM (NONMEM VI; Globomax LLC, Hanover, MD, USA). The most parsimonious models were identified by visual inspection of fits and using the Akaike information criteria (AIC). AIC values were calculated from the following equation: AIC = NONMEM objective function value + 2 × number of parameters in each structural model.

Development of hepatic CYP enzymes

Total hepatic cytochrome P450s

Not sure this is relevant. It is standard and you didn't personally do it. Among the studies indentified, the P450 content in adult liver varied from 0.25 to 0.5 nmol per mg microsomal protein with a mean of 0.3 nmol per mg microsomal protein. Fetal liver was reported to contain about 0.07 nmol of P450 per mg microsomal protein. From the first trimester of gestation to 1 year of age, the total P450 content remained nearly constant at 25-50% of the adult values (0.07-0.15 nmol/mg) [33].

The major enzyme found in fetal livers was CYP3A7 (97 pmol per mg microsomal protein)., which accounted for about 32% of total P450 The content of

CYP1A1 in fetal liver microsomes was roughly about 6 pmol per mg protein. Immunoblotting failed to detect other P450 enzymes such as CYP2A6, 2C, 2D6 and 2E1 in fetal liver microsomes [26].

CYP1A1

CYP1A1 is the most abundant enzyme of CYP1A family in the adult lung tissue and is mainly involved in the biotransformation of environmental pollutants and carcinogens like benzopyrene in the lung. It is highly inducible by exposure to polycyclic aromatic hydrocarbons derived from cigarette smoking. The information regarding the ontogenesis of lung CYP1A1 is limited.

CYP1A1 does not appear to be inducible or expressed constitutively in the adult human liver. There is some evidence for the presence of CYP1A1 in human fetal liver. CYP1A1 mRNA and catalytic activity were found in human embryonic hepatic tissues at very various stages of gestation (50-60 days) [36]. Immunoreactive CYP1A1 protein has been detected in liver microsomes of human embryos at 11 to13 weeks of gestation [26]. Consistent with these reports, functional CYP1A1 may be involved in the demethylation of imipramine in human embryonic hepatic tissues at early stages of gestation (days 52-59) [5]. However, CYP1A1 mRNA and protein were not detected in the fetal liver samples in the middle or late gestation [4,11]. There are no other reports on the expression or function of CYP1A1 in fetal liver samples during the second and third trimester of gestation. The recent evidence appears to support the theory that CYP1A1 is constitutively expressed at very low levels in fetal liver during the first trimester, and then declines to nondetectable levels after the early gestation period and beyond.

CYP1A2

The reported CYP1A2 content in adult human liver accounts for about 13% of the total P450 protein expression and varies from 19 to 67 pmol per mg microsomal protein, (Rowland 2003; Zhang 2007). CYP1A2 is mainly involved in the metabolism of wide variety drugs and chemicals including theophylline, imipramine, caffeine and methoxyresorufin.

Several studies suggest an absence of quantifiable hepatic CYP1A2 mRNA and protein expression and negligible enzyme activity during fetal stages [5,7,11,23,26].

Tateishi demonstrated that CYP1A2 liver expression is about 10% of adult levels in infants younger than 1 year of age [31]. Two corroborating studies [4,28] provided convincing evidence of a maturational delay of CYP1A2 enzymatic activity. CYP1A2 protein rose in the group of infants aged 1-3 months and increased progressively to reach 50% of the adult value at one year. The ontogenic profile of the demethylation of methoxyresorufin (MEROD), a specific marker of CYP1A2, and the CYP1A2 mediated demethylation of caffeine in liver microsomes paralleled the evolution of the protein and confirmed the delayed development of CYP1A2.

CYP2A6

CYP2A6 constitutes about 4% of total P450 in the adult human liver and has a very limited role in drug metabolism [25]. CYP2A6 does not appear to be expressed in fetal liver [26].

CYP2B6

CYP2B6 is considered primarily as a hepatic enzyme and accounts for <1% of the total hepatic P450 content in adults. However, the relative abundance of CYP2B6

exhibited about a 45-fold range from 1.0 to 45 pmol per mg microsomal protein [37,38], which is much larger than that of the other cytochrome P450s. This large interindividual variability may be partially explained by the induced expression levels regulated by both the constitutive androstane (CAR) and pregnane X (PXR) receptors and their activating ligands, such as phenytoin and alcohol. CYP2B6 plays a role in the metabolism of drugs used in the pediatric population including cyclophosphamide, ifosfamide and efavirenz. CYP2B6 is highly polymorphic but null alleles are rare. CYP2B6*6 is the most clinically relevant allele associated with reduced protein expression and enzyme activity.

There is limited information about CYP2B6 expression during development. An early study found significantly low levels of CYP2B6 in 2 of 10 perinatal/infant samples [31]. In a recent study [9], detectable CYP2B6 protein was observed in 64% of the fetal samples from 10 weeks to 40 weeks gestation, which suggests it is expressed in the fetal stage.

After parturition, the percentage of samples with detectable CYP2B6 protein increased with the postnatal age from 64% in the neonatal samples to 75% in samples between 1 to 6 months, and approached 95% in samples from donors 11-17 years of age. The same trend was observed for CYP2B6 protein levels in which the median amount of CYP2B6 was 0.6 pmol/ mg microsomal protein in fetal/neonatal samples (10 weeks gestation to 30 postnatal days), and increased to 1.3 pmol/mg microsomal protein in samples after the neonatal period (>30 days to 17 years). The CYP2B6 protein levels in both groups varied 28-fold and 74-fold, respectively. The detailed

developmental course for CYP2B6 expression is demonstrated in Fig. 1c based on data from a recent publication [9].

Although CYP2B6 is highly inducible, induction contributed little or none to the differences in CYP2B6 expression observed in Croom et al's study (9), suggesting that a change in constitutive CYP2B6 was involved in the age-related increase in median CYP2B6 levels not sure I understand.

CYP2C

The four members of the human CYP2C family: CYP2C8, 2C18, 2C9 and 2C19, account for 18% of the total cytochrome P450 in the adult liver [25] and metabolize about 29% of clinically used drugs [10]. CYP2C9 is the major CYP2C enzyme, followed by 2C19, 2C8 and 2C18 [19]. The CYP2C family is highly polymorphic and 11 genetic polymorphisms of CYP2C9 and 15 genetic polymorphisms of CYP2C19 have been identified. Allelic variants of CYP2C9 and 2C19 are responsible for poor metabolizer phenotypes.

Very little is known about the ontogeny of CYP2C8 and 2C18. Because many CYP2C9 and 2C19 substrates are clinically used in pediatric patients, there is considerable interest in the ontogeny of the two enzymes. CYP2C9 substrates include warfarin, phenytoin, diclofenac, ibuprofen, tolbutamide and losartan. Most proton pump inhibitors, such as omeprazole and lansoprazole are CYP2C19 substrates.

Total CYP2C proteins and RNAs were found to be very low in the fetal liver [23,26] and increased dramatically during the first week after birth. After 1 week, the level of CYP2C remained fairly stable up to age 1 year but did not exceed around 30% of the adult level. Two enzyme activities that depend on CYP2C - the hydroxylation of

tolbutamide and demethylation of diazepam – were measured in these samples and were found to parallel the evolution and rise in the level of protein content after birth [33]. The bulk of the CYP2C content in this study was represented by 2C8, 2C9, 2C18 and 2C19.

A recent study from a different liver bank suggested that the ontogeny of CYP2C9 and 2C19 was different [12,16]. In fetal samples, CYP2C9-specific content and catalytic activity was about 1% of adult values during the first and second trimester, with a substantial increase (about 10% of mature values) during the third trimester. CYP2C19-specific protein and catalytic activities were detectable as early as 8 weeks of gestation, but unlike CYP2C9, CYP2C19 expression was similar throughout the prenatal period (10~20% of mature values).

The CYP2C9 and 2C19 developmental expression patterns were also quite different after birth. CYP2C9 levels increased dramatically at birth to reach about 25% of the reported adult values in the neonatal samples (first 30 days after birth). After this, CYP2C9 expression remained constant with little or no change until age 1 year. Between 1 to 2 years of postnatal age, CYP2C9 exhibited (achieved?) mature protein levels in most liver samples. In contrast, CYP2C19 expression did not change at birth and during the neonatal period, but increased about 2 fold in infants and children and by puberty achieved levels that were nearly 50% of those observed in adults. Adult CYP2C19 protein concentration and activity values were observed in samples from children after puberty.

The age-related changes in CYP2C protein and activities were presented in Fig. 1d and 1e.

CYP2D6

Although it only accounts for <2% of the human cytochrome P450 proteins, CYP2D6 is important for the oxidative metabolism of approximately 12% of therapeutic drugs. These include β-blockers, tricyclic antidepressants, antitussives drugs. Since several of these drugs are widely administered to newborns and neonates, the ontogenesis of this enzyme is important and has been investigated.

CYP2D6 is highly polymorphic with over 80 different alleles identified to date, including several complete loss-of function, reduced function, and multiple copy number alleles. A wide range of metabolic capacities result from the inheritance of different allele combinations. In the adult Caucasian population, approximately 7% of individuals are poor metabolizers. Debrisoquine, spartein, bufuralol and dextromethorphan are used as probes to evaluate 2D6 activity.

Low levels of CYP2D6 protein and activity (less than 3~5% of adult levels) were observed in the fetal liver samples [30]. In newborns, CYP2D6 protein or activity remained similar to third trimester fetal levels, but increased significantly thereafter. This postnatal increase was independent of gestational age and appeared to be controlled by time after birth. CYP2D6 protein content was about 25% of adult levels in neonates aged 1-7 days and steadily increased to 50% in neonates of 7-28 days and reached about two-thirds the adult values in infants over 1 month to 5 years (Fig. 1f). The rise of the CYP2D6 protein was associated with the rise in dextromethorphan O-demethylation, a sensitive and selective CYP2D6 probe reaction [34]. No significant differences in protein levels of CYP2D6 were found between infants greater than 1 year of age and less than 1 year of age, which suggests that CYP2D6 development was

completed by 1 year of age [31]. Increasing CYP2D6 protein and activity was significantly associated with postnatal age for those less than 1 year old, but large interindividual variations existed.

The high degree of CYP2D6 polymorphism known to be present in children was likely to contribute to the inter-individual variability in early neonatal life. Almost 8% of Causasian children and 2% of Afirican-american children under 18 years of age were poor metabolizers. These data were consistent with known adult rates of this polymorphism (7~10% of whites and 1~2% African-Americans). Furthermore, Stevens et al. found both age and genotype to be significantly associated with increasing dextromethorphan *O*-demethylase activity in the postnatal age groups.

CYP2E1

CYP2E1 is an ethanol inducible enzyme and accounts for 7% of the total hepatic P450 enzymes. Despite its limited contribution to drug metabolism, CYP2E1 was considered to be toxicologically important because of its ability to convert a variety of agents, including environmental procarcinogens to reactive intermediates that can lead to organ damage and/or tumorigenesis. CYP2E1 has multiple polymorphisms identified to date, but only one variant of CYP2E1*1D has been linked to altered function.

The fetal expression of CYP2E1 was undetectable in early/mid gestation [11,26,35] but exhibited readily detectable levels in the third trimester [15]. CYP2E1 protein and its associated activity rose immediately after birth, independently of the gestational age, and increased gradually thereafter to reach the adult values by the first year of age. The detailed developmental time course of CYP2E1 is summarized in Fig.

1g based on two in vitro studies by Vieria et al. (1996) and Johnsrud et al. (2003). Generally, Neonates up to 30 days have approximately 25% of adult levels of CYP2E1 protein, and this increases with postnatal age. Infants 31 to 90 days of age may exhibit up to 50% of adult levels. By 1 year of age, the expression approached adult values. CYP2E1*1D variant was identified with a low allelic frequency in a large pool of pediatric liver samples but it did not contributed significantly to the differences in CYP2E1 expression in different age groups.

CYP3A

The CYP3A is the most abundant and clinically important cytochrome P450 subfamily in the liver. It accounts for 33% of the total hepatic P450s and is responsible for the metabolism of almost 50% clinically used drugs. The subfamily consists of three major isoforms: CYP3A4, CYP3A5, and CYP3A7. They are structurally closely related, the amino acid sequence similarity between CYP3A4 and CYP3A5 is 84%, and it's 88% between CYP3A4 and CYP3A7. However, the isoforms differ in terms of their development with age, their tissue distributions and enzymatic properties. CYP3A7 is the major isoenzyme expressed in the fetal liver, whereas CYP3A4 is the major isoform present in the adult liver. CYP3A5 is detectable in only 25-30% of liver microsomes of adults and is the primary CYP3A isoform expressed extrahepatically (e.g. kidney, intestine) (Wrighton 1990). Total hepatic CYP3A protein levels remain nearly constant from the early stage of gestation to adulthood [17], although the expression of specific isoforms change differently. Characterization of the developmental expression of CYP3A4, 3A5 and 3A7 has historically been difficult by the lack of CYP3A isoform-specific antibodies or marker enzyme activity.

CYP3A7 is the dominant cytochrome P450 expressed in the fetal liver, accounting for approximately 50% (30-85%) of total P450 in human fetal hepatic micrsosomes that contain only traces of CYP3A4 and CYP3A5 [17]. The CYP3A7 present in fetal liver microsomes was able to carry out the 4-hydroxylation of retinoic acid, the N-demethylation of dextromethorphan, codeine and ethylmorphine, as efficiently as those of CYP3A4/5 in adult liver microsomes [6,14,18]. CYP3A7 activity was highly detectable in human fetal microsomes as early as 8 weeks of gestation.

The 16α-hydroxylation of dehydroepiandrosterone (DHEA), a selective marker for CYP3A7-dependent activities, was actively catalyzed by the fetal liver microsomes (aged 14-40 weeks). It displayed its maximal level during the first week following birth before progressively declining to reach a very low level in adult livers (Fig. 1h). The CYP3A7 content calculated from the amount of produced metabolite, 16α-hydroxydehydroepiandrosterone (16α –OH-DHEA), also revealed an age-dependent decrease from early gestation to reach the extremely low level after1 year of age [12,29].

In contrast to the sharp decline of CYP3A7 from early gestation to infancy, CYP3A4 protein levels (based on measurements of 7β DHEA-hydroxylase activities) increased slowly with age [12,29]. The 6 β-hydroxylation of testosterone, a probe reaction for CYP3A4 activity, was extremely low in the fetal liver (less than 10% of the adult level) and began to rise after birth. The activity reached 30~40% of the adult value after 1 month of birth and approached the adult value after 1 year of age [17].

The same developmental tend was also observed based on CYP3A4 catalyzed imipramine demethylation and amprenavir oxidation [28,32], as shown in Fig. 1i.

The microsomal content of CYP3A5 was determined by immunoblotting with a selective polycolonal antibody to CYP3A5 isoform. The expression of CYP3A5 was independent of age, and remained nearly constant from the early gestational age (12 week of gestation) to adulthood. However, CYP3A5 protein expression was found highly variable and the large interindividual variability was most likely subject to the polymorphisms dictated by the frequency of *CYP3A5*1/*1* or *CYP3A5*3/*3* in certain age groups [29].

Ontogeny functions

The development of CYP1A2, 2C19, 2D6, 2E1 and 3A4 was best described by hyperbolic function in the age range of observations. CYP2C9 development was reasonably fitted with hyperbolic function (Eq.1).

$$MF = MF_0 + \frac{(MF_{max} - MF_0) \times AGE(day)}{TM_{50} + AGE(day)} \qquad \text{Eq.1}$$

Table 2 listed ontogeny parameter estimates. The individual fitting plots were summarized in Fig. 2. Parameter MF_0 was the fraction of enzyme activity at birth (at postnatal age of 0 day) against adult level, or the quantities for the postnatal onset of enzyme expressions. MF_{max} was the maximal ratio of enzyme activity (to adult level) during the age range of investigated samples. TM_{50} was the postnatal age (day) to reach 50% of the maximal enzyme activity (MF_{max}) observed in the investigated age range, suggesting the development rate of each individual isoenzymes to attain the maximum values. Because of the lack of data on when the adult values are reached,

the ontogeny models for CYP2C9, 2C19, 2D6 and 1A2 can't be used to describe the enzymatic development beyond the specified age ranges.

The high MF_0 values for CYP2E1 suggested its fetal expression or prenatal onset, while the extremely low values of MF_0 for CYP2C9 and 1A2 suggested the two isoenzymes expression were triggered by birth effect.

CYP2C9, 2C19 and 2D6 appeared to mature faster than CYP2E1, 3A4 and CYP1A2, as shown by the short half-life values within days compared to those in months.

Conclusion

Overall, qualitative and quantitative analysis of these literature data allows the ontogeny of individual CYPs to be categorized into four groups:

A first group of P450 enzymes (fetal enzymes) includes CYP3A7 and CYP1A1, which are expressed at highest level during gestation and are silenced in the postnatal period.

A second group of P450 enzymes (early neonatal enzymes) includes CYP2D6, CYP2C9 and CYP2C19, which are not expressed or expressed at low levels in the fetus but expression increases substantially within hours or days after birth.

A third group of P450 enzymes (neonatal enzymes) includes CYP3A4, CYP2E1, and CYP1A2, which expression increases slowly within months or years after birth.

A fourth group includes the enzymes with a relatively constant level of expression during hepatic development such as CYP3A5.

The ontogeny functions of individual P450s can be further incorporated with other age-related physiological parameters to predict drug clearance in children at different ages and estimate pediatric doses for drugs metabolized by cytochrome P450 enzymes.

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Table 1 Summary of literature data on the ontogeny of hepatic cytochrome P450 enzymes

Enzyme	Assay	Fetus											
		<30 weeks	>30 weeks	<24 hours	1-7 days	8-28 days	1-3 months	3-12 months	1-10 years	10-18 years	Adults	Unit	Ref.
Total P450	protein		0.079	0.085	0.09	0.094	0.134	0.112			0.283	nmol/mg protein	[33]
	protein		307								540	pmol/mg protein	[26]
	protein						390			645		pmol/mg protein	[31]
	protein										344	pmol/mg protein	[25]
	protein										446.5	pmol/mg protein	[27]
CYP1A2	Caffeine N-3-demethylation	0.206	0.083	0.057	0.098	1.283	1.018	1.906			3.73	pmol/min/mg protein	[4]
	Methoxyresorufin demethylation	N.D.	N.D.	0.666	1.753	2.502	5.981	13.446	34.793		98.7	nmol/min/mg protein	[28]
	protein	N.D.	N.D.	0.007	0.021	0.035	0.1190	0.236	0.501		0.964	unit/mg protein	[28]
	Ethoxyresorufin O-deethylation		7.3								290	pmol/min/mg protein	[26]
	protein		6.3								55	pmol/mg protein	[26]
	protein						3.3			24.9		pmol/mg protein	[31]
	protein										52	pmol/mg protein	[37]
	protein										42	pmol/mg protein	[25]
	protein										42.4	pmol/mg protein	[27]
CYP2A6	Coumarin 7-hydroxylation		0.26								44	pmol/min/mg protein	[26]
	protein		N.D.								25	pmol/mg protein	[26]
	protein						6.38			6.75		pmol/mg protein	[31]
	protein										14	pmol/mg protein	[25]
	protein										36	pmol/mg protein	[37]
CYP2B6	protein			1.815	1.633	0.935	2.004	2.042	2.166	4.191	4	pmol/mg protein	[9]
	protein						2.65			19.36		pmol/mg protein	[31]

Enzyme	Assay	Fetus		<24 hours	1-7 days	8-28 days	1-3 months	3-12 months	1-10 years	10-18 years	Adults	Unit	Ref.
		<30 weeks	>30 weeks										
	protein										1	pmol/mg protein	[25]
	protein										11	pmol/mg protein	[37]
CYP2C	protein		N.D.	0.05	0.251	0.359	0.439	0.366			1.134	AU/mg protein	[33]
	protein		N.D.								83	pmol/mg protein	[26]
	protein										63.6	pmol/mg protein	[27]
	protein										60	pmol/mg protein	[25]
CYP2C8	protein						6.4			22.8		pmol/mg protein	[31]
	protein										24	pmol/mg protein	[37]
CYP2C9	Tolbutamide hydroxylation	N.D.	N.D.		0.37	11.20	8.23	7.3			27.05	nmol/min/mg protein	[33]
	protein	N.D.	0.4		11.3		11.3		18		40	pmol/mg protein	[12,16]
	protein						79.8			74.4		pmol/mg protein	[31]
	protein										73	pmol/mg protein	[37]
CYP2C19	Diazapam N-demethylation		1.5	7.2	21.4	18.6	28.2	46.6			56.6	nmol/min/mg protein	[33]
	protein	1.7000	4.0		4.1			8.9	11.7	14.9	25	pmol/mg protein	[12,16]
	protein										14	pmol/mg protein	[37]
CYP2D6	Dextromethorphan O-demethylation			0.046	0.018	0.073	0.058	0.045			0.168	nmol/min/mg protein	[30]
	Dextromethorphan O-demethylation	0.09	0.39	0.39	0.86			2.52			10.44	nmol/hr/mg protein	[34]
	protein	0.28	0.54	0.1	1.02	2.1		4.1			7.22	AU/mg protein	[34]
	Bufuralol 1-hydroxylation		N.D.								58	pmol/min/mg protein	[26]
	protein		N.D.								10	pmol/mg protein	[26]
	protein						43.7			53.5		pmol/mg protein	[31]
	protein										5	pmol/mg protein	[25]
	protein										8	pmol/mg protein	[37]

Enzyme	Assay	Fetus		Neonate			Infant		Child		Adults	Unit	Ref.
		<30 weeks	>30 weeks	<24 hours	1-7 days	8-28 days	1-3 months	3-12 months	1-10 years	10-18 years			
CYP2E1	Chlorzoxazone 6-hydroxylation	0.032	N.D.	0.205	0.3	0.358	0.45	0.374	0.786		0.968	nmol/min/mg protein	[35]
	7-Ethoxycoumarin demethylation		0.7								37	pmol/min/mg protein	[26]
	protein		N.D.								33	pmol/mg protein	[26]
	protein	N.D.	N.D.	0.52	0.74	1.24	1.65	1.87	4.31		5.39	pmol/mg protein	[35]
	protein	N.D.	5.8		13.4			36.2	43.1		48.7	pmol/mg protein	[15]
	protein						94.3			136.9		pmol/mg protein	[31]
	protein										22	pmol/mg protein	[25]
	protein										61	pmol/mg protein	[37]
CYP3A4	amprenavir oxidation			3.4	9	19.1	57.9				126.8	pmol/min/mg protein	[32]
	imipramine demethylation	0.017	0.014	0.048	0.037	0.085	0.177	0.197	0.461		0.411	nmol/min/mg protein	[28]
	Testosterone (6beta-hydroxylation)	0.006	0.004	0.010	0.015	0.033	0.039	0.049	0.121		0.113	nmol/min/mg protein	[17]
	Testosterone (6beta-hydroxylation)		173								2756	pmol/min/mg protein	[26]
	protein	4.5000	10.5		6.5			8.4	12.9		128.9	pmol/mg protein	[12,29]
CYP3A5	protein	5.2	9.7		5.8			3.6			1.2	pmol/mg protein	[12,29]
CYP3A7	16alpha_dehydroepiandrosterone	0.761	0.495	0.987	1.295	0.567	0.362	0.187	N.D.		0.084	nmol/min/mg protein	[17]
	protein	260.5	200.9		100.5			27.3	3.1		10	pmol/mg protein	[12,29]
	protein						538.7			96.90		pmol/mg protein	[31]
CYP3A	protein	0.658	0.847	0.720	0.675	0.721	0.741	0.745	0.666		0.809	OD unit/mg protein	[17]
	protein		97								147	pmol/mg protein	[26]
	protein						173.3			239.4		pmol/mg protein	[31]
	protein										96	pmol/mg protein	[25]

Enzyme	Assay	Fetus		<24 hours	1-7 days	8-28 days	1-3 months	3-12 months	1-10 years	10-18 years	Adults	Unit	Ref.
		<30 weeks	>30 weeks										
	protein										155	pmol/mg protein	[37]
	protein										141.82	pmol/mg protein	[27]

Table 2 Maturation parameter estimates for individual cytochrome P450 (CYP) isoforms

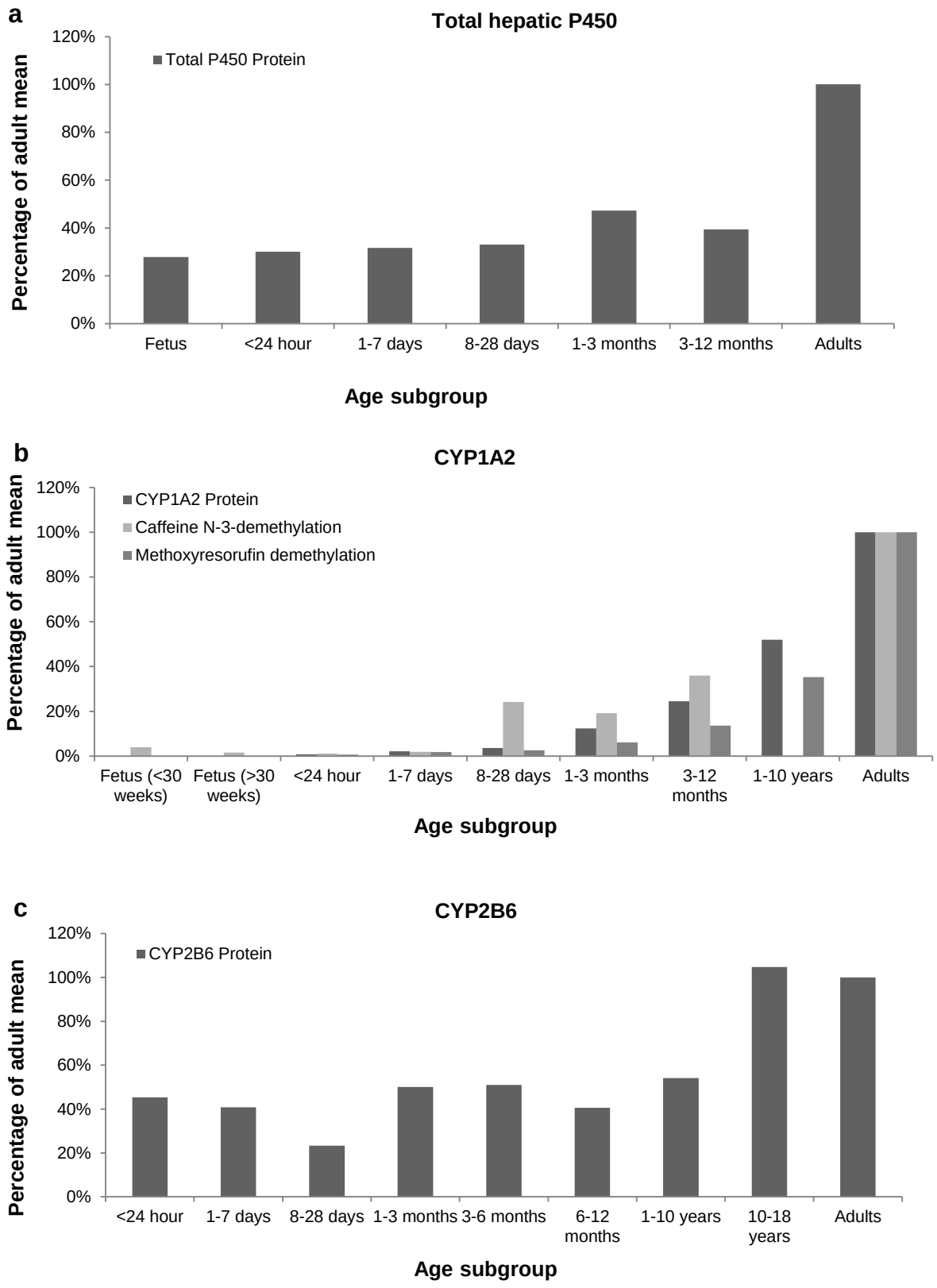
Enzyme	Age range (day)	MF_0	MF_{max}	TM_{50}
CYP1A2	0.5-2273	0.0078	0.539	100
CYP2C9	0.5-1788	0	0.308	4.8
CYP2C19	0.5-1788	0.0856	0.538	6.1
CYP2D6	0.5-355	0.0128	0.461	15.1
CYP2E1	0.5-2273	0.216	0.869	183
CYP3A4	0.5-2273	0.0835	0.978	139

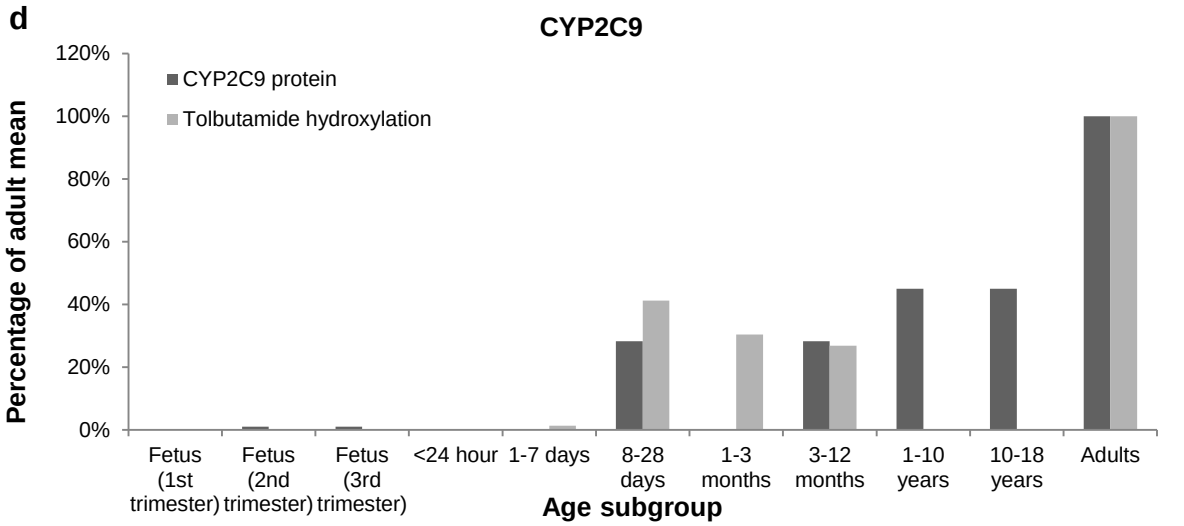
Figure legends:

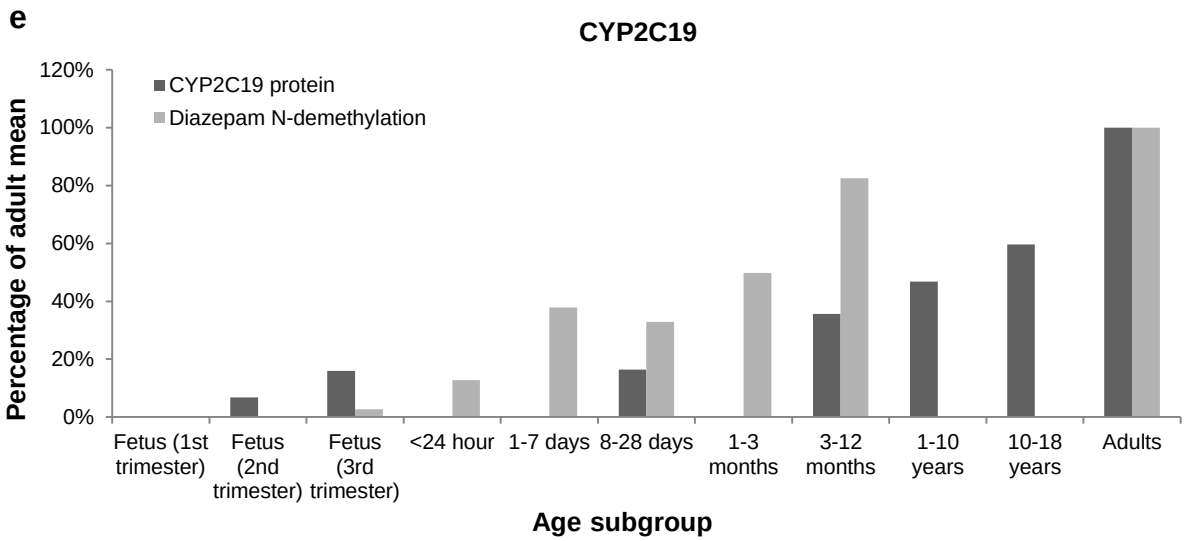
Fig. 1 Developmental changes of total hepatic P450 enzymes and individual isoenzymes. **a** Microsomal content of total P450 was spectrally determined by its absorbance at 450 nm after reduction in the presence of carbon monoxide. **b** The microsomal content of CYP1A2 protein was determined by immunoblotting. The microsomal activity of CYP1A2 was probed with the demethylation of methoxyresorufin (MEROD) or caffeine N-3-demethylation in two different studies. **c** The microsomal content of CYP2B6 protein was determined by immunoblotting. **d** The microsomal content of CYP2C9 protein was determined by immunoblotting. The microsomal activity of CYP2C9 was probed with hydroxylation of tolbutamide. **e** The microsomal content of CYP2C19 protein was determined by immunoblotting. The microsomal activity of CYP2C19 was probed with N-demethylation of diazepam. **f** The microsomal content of CYP2D6 protein was determined by immunoblotting. The microsomal activity of CYP2D6 was probed with O-demethylation of dextromethorphan. **g** The microsomal content of CYP2E1 protein was determined by immunoblotting in two different studies. The microsomal activity of CYP2E1 was probed with Chlorzoxazone 6-hydroxylation. **h** The microsomal content of CYP3A7 protein was determined by immunoblotting. The microsomal activity of CYP3A7 was measured by the production of 16α-hydroxydehydroepiandrosterone (16α –OH-DHEA). **i** The microsomal content of CYP3A4 activity was measured by three different probe reactions: 6 β-hydroxylation of testosterone, imipramine demethylation and amprenavir oxidation.

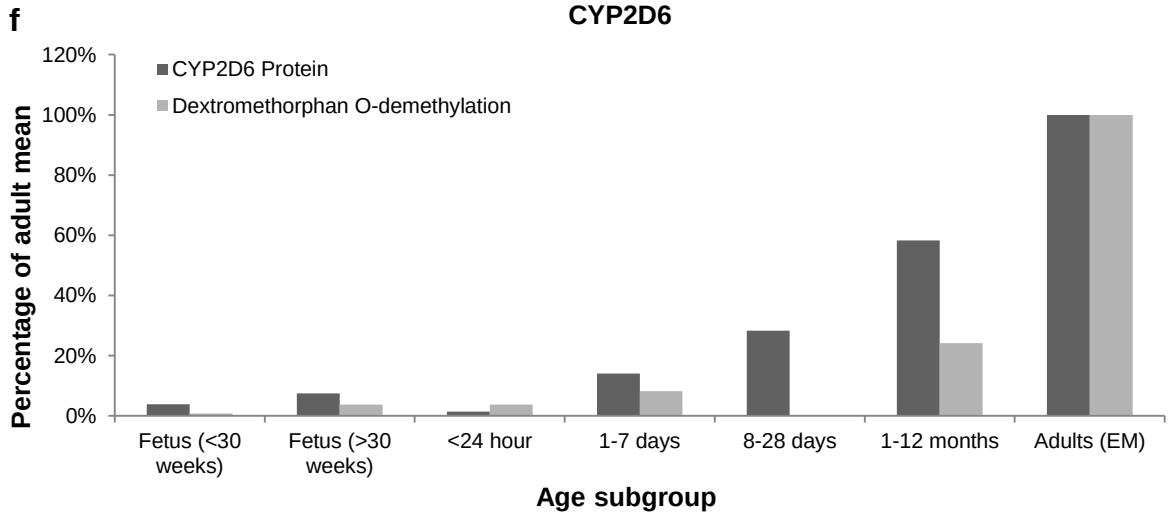
Fig. 2 Fitted curves of ontogeny data expressed as a fraction of adult values as a function of age for individual cytochrome P450 isoenzymes. The model given in equation 1 best described the data as shown. Model parameters for each CYP were given in Table 2.

Figure 1

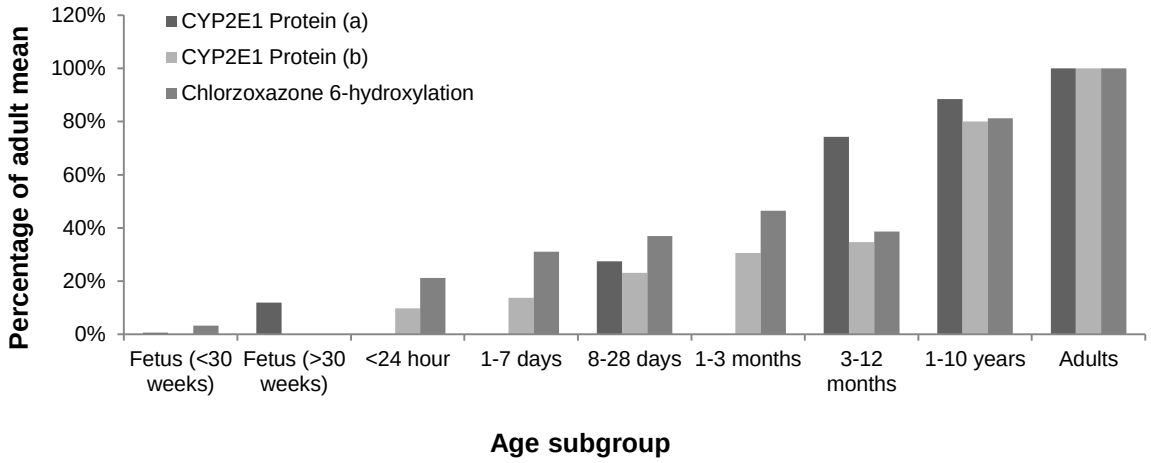

a
Total hepatic P450
Total P450 Protein
Percentage of adult mean
120%
100%
80%
60%
40%
20%
0%
Fetus
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
Adults
Age subgroup
b
CYP1A2
CYP1A2 Protein
Caffeine N-3-demethylation
Methoxyresorufin demethylation
Percentage of adult mean
Fetus (<30 weeks)
Fetus (>30 weeks)
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
Adults
Age subgroup
c
CYP2B6
CYP2B6 Protein
Percentage of adult mean
<24 hour
1-7 days
8-28 days
1-3 months
3-6 months
6-12 months
1-10 years
10-18 years
Adults
Age subgroup


d
CYP2C9
120%
100%
80%
60%
40%
20%
0%
CYP2C9 protein
Tolbutamide hydroxylation
Percentage of adult mean
Fetus (1st trimester)
Fetus (2nd trimester)
Fetus (3rd trimester)
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
10-18 years
Adults
Age subgroup

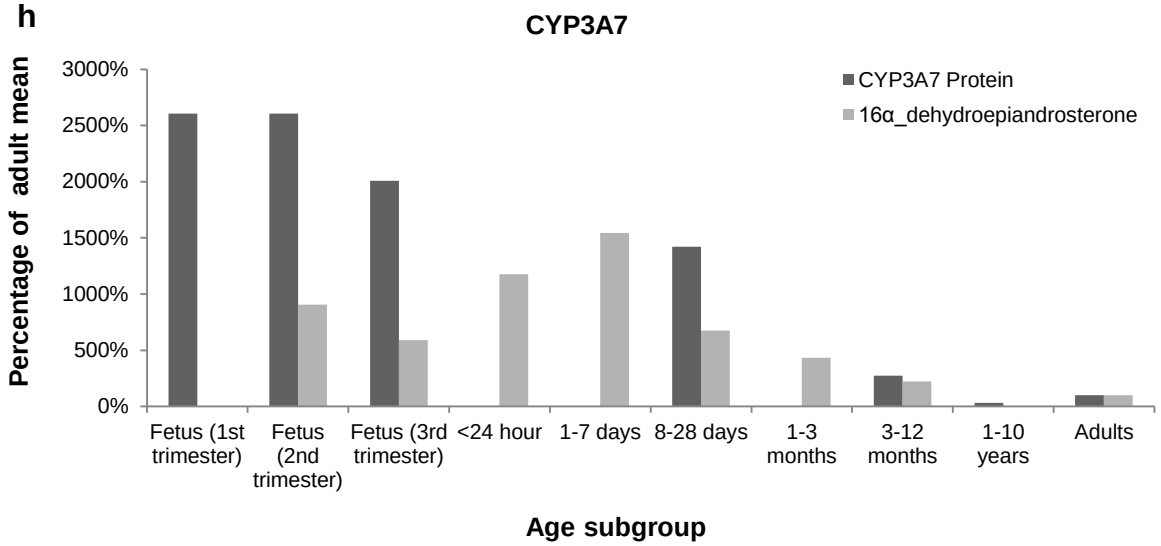

e
CYP2C19
120%
100%
80%
60%
40%
20%
0%
CYP2C19 protein
Diazepam N-demethylation
Percentage of adult mean
Fetus (1st trimester)
Fetus (2nd trimester)
Fetus (3rd trimester)
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
10-18 years
Adults
Age subgroup


f
CYP2D6
120%
100%
80%
60%
40%
20%
0%
CYP2D6 Protein
Dextromethorphan O-demethylation
Percentage of adult mean
Fetus (<30 weeks)
Fetus (>30 weeks)
<24 hour
1-7 days
8-28 days
1-12 months
Adults (EM)
Age subgroup

g


CYP2E1
Percentage of adult mean
120%
100%
80%
60%
40%
20%
0%
CYP2E1 Protein (a)
CYP2E1 Protein (b)
Chlorzoxazone 6-hydroxylation
Fetus (<30 weeks)
Fetus (>30 weeks)
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
Adults
Age subgroup

h


CYP3A7
Percentage of adult mean
3000%
2500%
2000%
1500%
1000%
500%
0%
CYP3A7 Protein
16α_dehydroepiandrosterone
Fetus (1st trimester)
Fetus (2nd trimester)
Fetus (3rd trimester)
<24 hour
1-7 days
8-28 days
1-3 months
3-12 months
1-10 years
Adults
Age subgroup

i

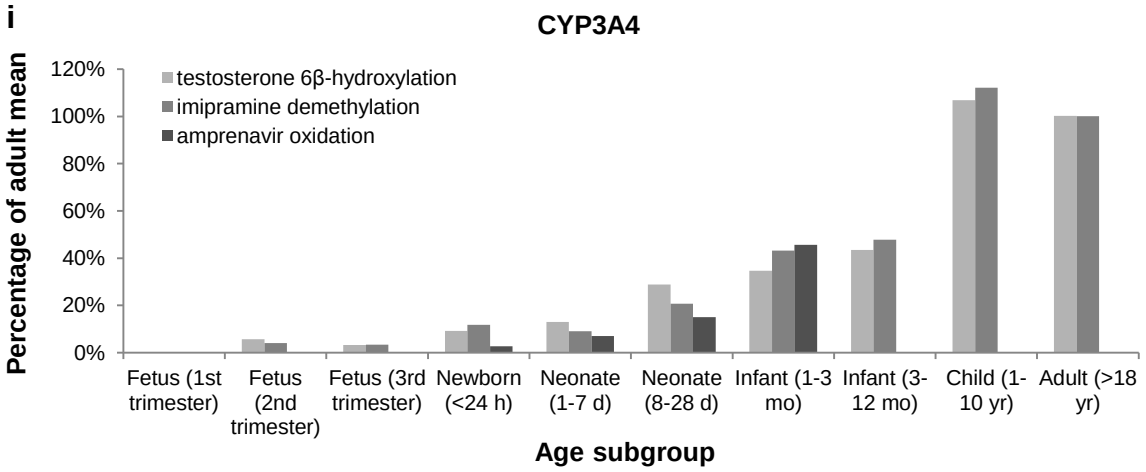
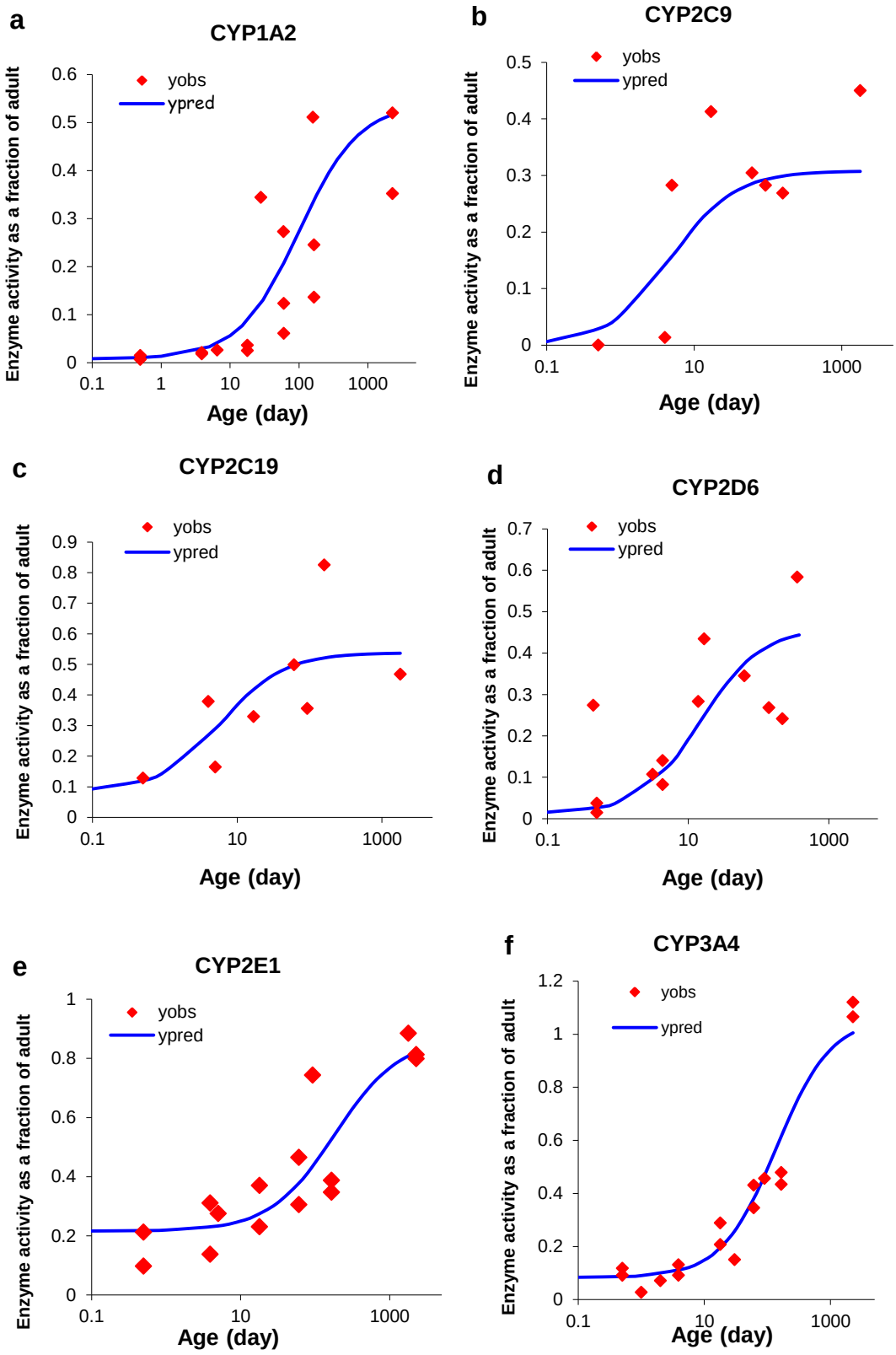

CYP3A4
Percentage of adult mean
120%
100%
80%
60%
40%
20%
0%
testosterone 6β-hydroxylation
imipramine demethylation
amprenavir oxidation
Fetus (1st trimester)
Fetus (2nd trimester)
Fetus (3rd trimester)
Newborn (<24 h)
Neonate (1-7 d)
Neonate (8-28 d)
Infant (1-3 mo)
Infant (3-12 mo)
Child (1-10 yr)
Adult (>18 yr)
Age subgroup

Figure 2


a
CYP1A2
yobs
ypred
Enzyme activity as a fraction of adult
Age (day)
b
CYP2C9
c
CYP2C19
d
CYP2D6
e
CYP2E1
f
CYP3A4

CHAPTER 3

Predicting the renal and hepatic clearance in pediatric populations using physiological and biochemical development knowledge

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is intended to submit to In Silico Pharmacology

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Abstract

Objectives: The apparent drug clearance (CL/F) is an important pharmacokinetic concept in dosing regimen determination. In pediatrics, drug clearance mechanism matures quickly during the postnatal period and leads to rapid changes with age and body size. Accurate clearance in pediatrics requires considering the developmental physiological and biochemical processes that govern drug elimination. In this study, mathematical models describing the maturation of hepatic cytochrome P450 enzyme-mediated clearance and renal clearance due to glomerular filtration was developed and assessed.

Methods: Using in vivo adult clearance data of 6 probe compounds that are primarily eliminated by one process, models for the ontogeny of renal clearance and hepatic clearance of CYP3A4 and CYP1A2 was developed from developmental physiology and the ontogeny information of specific enzymes, with an open software program R. The systemic clearance values of 6 model drugs ((alfentanil, midazolam, caffeine, theophylline, gentamycin and vancomycin) were predicted and compared with the observations from the literature. The clearance values of 6 drugs were also simulated for 500 virtual pediatric subjects from birth to 18 year old using the models.

Results: The model performs reasonably well for CYP3A4 substrates with predictions within 2-fold of the observed values in 82% of alfentanil and 78% of midazolam. The predictions of CYP1A2 substrates were 77% of caffeine and 68% of theophylline within 2-fold error. For renal clearance due to glomerular filtration, the model provides reasonable predictions for gentamicin and vancomycin with 62% and

66% within the 2-fold error, respectively. Overall, there was strong correlation between observed and predicted clearances for the model development ($R > 0.85$).

Conclusions: The physiology based scaling model predicts an age-and elimination pathway-specific clearance for the ontogeny of renal clearance, and metabolic clearance of CYP1A2 and CYP3A4. As developed with an open program R, the model provides a valuable source for informed individuals to understand the physiological based clearance scaling approach and to use it, without having to use the "black-box" kind of simulation software.

Key words: physiological based modeling, pharmacokinetics, pediatric, maturation, clearance

Introduction

From birth onward, neonates, young infants and children develops with important age-dependent changes in body composition, in size (weight and height) and in maturation of hepatic and renal function (1). These processes all have a major impact on the pharmacokinetic (PK) profile of a drug from its absorption and distribution properties to metabolism and elimination. As a result, the developmental changes in pharmacokinetics require an age-dependent adjustment of dosing regimens in children to achieve the target systemic exposure of a drug (2), which is measured by the area-under-the-plasma-concentration, AUC, or steady state plasma concentration, C_{ss}. The total exposure of a drug is determined by the efficiency of the elimination processes (Eq.1). The apparent drug clearance (CL/F) is the principle PK process determining age-dependent differences in drug dosage regimens.

$$AUC_{SS} = \frac{Dose}{(CL/F)} \quad or \quad C_{SS} = \frac{(Dose/\tau)}{(CL/F)} \qquad Eq.1$$

Simple allometric approaches can be applied to the estimation of pediatric clearance based on the adult clearance and the power function of body weight (Eq. 2). $CL_{child} = CL_{adult} \times (\frac{BW_{child}}{BW_{adult}})^b \qquad Eq.2$

The allometric exponent, b, typically assumes a value of 1 (the per kg model), 3/4 (the allometric ¾ power model), or 2/3 (the body surface area model) (3). These models derived from body size are simple to use in clinical practice. However, they are often failed to predict clearance in neonates and young infants because drug elimination pathways in the first year of life is not matured even after size adjustment (4, 5). Hence, a mechanism-based approach considering the underlying physiological and biochemical processes that govern drug elimination has been proposed. The

advantage of this approach over other size models is the ability to incorporate the ontogeny information of the various anatomical, physiological and biochemical processes in drug elimination, although a tremendous input of physiological data is required (6). Such a model has been applied to predict clearance of model drugs for different pediatric age groups using commercial software such as Simcyp or PKSim (7, 8). The objective of this research is to develop a mechanistic model in R to estimate "population mean clearance value" in any age of child for selected model drugs, on the basis known compound-specific information in literature and published studies on the development physiology and enzyme ontogeny in children. For this study, the age-dependence of renal clearance via glomerular filtration and hepatic clearance via the metabolism of CYP3A4 and CYP1A2 was examined.

Methods

Selection of model drugs

Probe substrates for cytochrome P450 phase I metabolism, and renal excretion are selected according to the following criteria:

- The primary pathway of elimination due to one process in healthy adults was >80% of an oral dose.
- Complete absorption (or >90%) from gastrointestinal tract after oral administration (po); for compounds showing incomplete absorption, only IV data were used.
- Probe choice is routinely administered for clinical indications in ill neonates, infants and children.

Model drugs also need to have the established clinical use in adults and pediatric patients of all ages, the availability of published data on in vivo clearance for different age groups and, adequate published data on their in vivo absorption, distribution, metabolism and excretion (ADME) study, namely the contribution of each clearance pathway to total clearance. The list of drugs and major clearance mechanisms were shown in Table 1. Clearance mechanisms were identified from pharmacology textbook (9), a key review article (10), drug label, as well as primary literature for individual compounds as shown in the table.

The table provides an estimate of the percentage of parent compound processed by the major fate pathways. These compounds are grouped according to the primary process of clearance, which include the process of renal, CYP3A4 and CYP1A2 elimination.

Alfentanil and midazolam are metabolized by CYP3A as the primary route of disposition. Alfentanil is extensively oxidized via two major N-dealkylation pathways both of which are mediated via CYP3A4 in human liver microsomes. Human in vivo studies have shown that more than 80% of an iv dose is recovered in the urine of healthy adult volunteers as CYP3A4 metabolites (11). Midazolam metabolism is mediated by CYP3A4 to 1-hydroxy and 4-hydroxy derivative corresponds to the main metabolite and a minimum of 70% of an oral dose and 77% of an iv dose is recovered in the urine within 24 h as this metabolite (11).

The predominant biotransformation of theophylline and caffeine are catalyzed by CYP1A2. Caffeine is primarily transformed via three major N-demethylation reactions (1-, 3- or 7-N-demethylation) producing theobromine, paraxaanthine or theophylline. N3-demthylation pathway is catalyzed by CYP1A2 with high affinity and accounts for 80% of the metabolism of caffeine in humans (12). Theophylline undergoes C-8-oxidation as the major metabolite route accounting for 49.1% of the total urinary excretion, together with oxidative 1- and 3-N-demethylation (17.5% and 24.5%, respectively). These reactions are mediated by the CYP1A2 isoform at pharmacological concentrations.

Over 90% of gentamicin is predominantly excreted unchanged in urine through glomerular filtration (13). Similarly, over 80% of vancomycin is mainly eliminated into the urine as unchanged (9).

Compilation of child clearance database

Then computerized literature searches (PubMed, 1970-present) were conducted to find references or publications describing pharmacokinetics of probe substrates in children, using words such as, newborn, neonate, infant, children and crossing these with terms such as probe drug names, pharmacokinetics. Additionally, a variety of pediatric pharmacology reviews (14-19) were examined to identify drugs for which PK datasets exists for children. Next, the primary PK studies in published literature were evaluated to extract key data including weight, gender, age, drug administration route, the number of does (single or multiple doses), the number of subjects, and PK

findings such as total clearance or apparent volume of distribution. Through these sources, a database (Appendix I) of age-dependent observed clearances for 6 therapeutic probes were presented, based upon the availability of data for pertinent age groups (especially very early life stages), and being able to obtain the primary data sources (CL and body weight), having a reasonable number of subjects (at least 3 per age group).

A scan of the database shows that for these model compounds the weight normalized clearance in neonates and young infants appears different from the adults. Table 2 was extracted from the database to illustrate this pattern and to show how the data has been compiled and organized. The table shows the mean and SE of a single drug, midazolam, a CYP3A4 substrate. The observed adult clearance value was the weighted mean and only the mean adult clearance value was regarded in this study.

Physiologically based hepatic clearance scaling

The approach involves an in vitro-in vivo extrapolation of enzyme activity data determined in hepatic microsomal preparations from different pediatric age groups as well as the adult. Briefly, the adult intrinsic clearance ($CL_{int,adult}$) is back calculated from in vivo hepatic drug clearance (CL_H), the free fraction in plasma (f_u), the blood to plasma drug concentration ratio (C_B/C_P), and hepatic blood flow (Q_H), using well-stirred model (Eq. 3) (20, 21). The generated adult intrinsic clearance value is then multiplied by scaling factor that represents the activity of the specific enzyme in relation to the age of the child (Eq. 4). This new child-scaled intrinsic clearance ($CL_{int,child}$) is used to generate an age-specific hepatic clearance calculated from the re-arranged equation (Eq. 5) using age-specific body weight, liver weight, liver blood

flow and predicted fraction unbound (scaled from adults based binding protein concentrations in blood). Figure 1A describes an overview of the process involved in scaling clearances from adult to children.

Because the elimination of midazolam and alfentanil are primarily due to CYP3A4 metabolism, their hepatic clearance is assumed to be close to their plasma clearance. The assumption was also applicable to theophylline and caffeine, which eliminations are primarily due to CYP1A2 metabolism.

$$CL_{int,adult} = \frac{Q_{H,adult} \times CL_{H,adult}}{f_{u,adult} \times (Q_{H,adult} - CL_{H,adult}/(\frac{C_B}{C_P}))} \qquad Eq.3$$

$$CL_{int,child} = CL_{int,adult} \times SF \qquad Eq.4$$

$$CL_{H,child} = \frac{Q_{H,child} \times f_{u,child} \times CL_{int,child}}{Q_{H,child} + f_{u,child} \times CL_{int,child}/(\frac{C_B}{C_P})} \qquad Eq.5$$

Physiologically based renal clearance scaling

For renal eliminated drugs, it is well accepted that the renal clearance is proportional to glomerular filtration rate (GFR). For example, surrogate measures of GFR are often used to adjust the dosing rate in adults with impaired renal function (22). Extension of these concepts to adaption of the adult regimen for the child leads to the proposition that the renal clearance in child, expressed as a fraction of the adult values, is adjusted proportionally by GFR and free fractions in plasma (Eq. 6) (8). Figure 1B describes an overview of the process involved in scaling clearances from adult to children.

$$\frac{CL_{GFR,child}}{CL_{GFR,adult}} = \frac{GFR_{child}}{GFR_{adult}} \times \frac{f_{u,child}}{f_{u,adult}} \qquad Eq.6$$

Where CL_{GFR} is the compound specific renal clearance (mL/min), GFR is glomerular filtration rate (mL/min). Gentamicin and vancomycin, are used to evaluate

this renal clearance model because they are excreted exclusively via filtration in the kidney and CL_{GFR} is close to the plasma clearance.

Drug and system specific input

Table 3 listed the drug specific parameters that are obtained from the literature (9, 11-13, 23), such as CL_{adult}, f_u, C_B/C_P. The adult plasma clearance values are geometric means from different PK studies in which drugs were administered by i.v. injections.

The CL of alfentanil in healthy adult volunteers was obtained from total 241 healthy adult volunteers in 9 studies after intravenous administration and the geometric mean CL was 4.7 ml/min/kg (SD: 2.3) (11). The typical clearance of midazolam in adults was estimated from total 198 healthy adult volunteers of 4 studies, which was was 7.7 ml/min/kg (SD: 3.7) (11).

The mean clearance of caffeine in adults after iv administration was estimated from 20 subjects of 2 studies and the value was 1.97 mL/min/kg (SD: 0.92) (12). The theophylline clearance in healthy adults after iv administration was estimated from 100 subjects of 12 studies and was 1.0 mL/min/kg (SD: 0.29) (12).

The total clearance of gentamicin in healthy adults after iv administration is estimated from 219 subjects of 6 studies, which was 1.3 mL/min/kg (SD: 0.5) (13). The total clearance of vancomycin in healthy adults after iv administration was estimated from 121 subjects of 6 studies and the mean value was 1.22 mL/min/kg (SD: 0.5) (24).

The physiological parameters such as plasma protein binding level, hepatic blood flow, liver volume and enzyme activity are variable with age. The empirical regression functions that can generate age appropriate parameters and account for the

developmental differences between infants and adults are shown in Table 4. The mean physiological inputs were listed in Table 5 for a typical normal male adult from ICRP (25).

Age-dependent plasma protein binding

In general, plasma proteins are lower in the infant than in the adult. Human serum albumin (HSA) concentrations are 75 to 80% of adult values at birth and gradually increase with age. Alpha1-acid glycoprotein (AAG) is initially half the adult concentration in the infant and matures slowly with age. Alcorn et al. (26) proposed a model that was best fitted to albumin and alpha1-AG protein concentration as a function of age (in days) (Table 4). Changes in the unbound fraction (f_u) of drugs in the plasma of pediatric subjects ($f_{u,child}$) were estimated using Equation 7.

$$f_{u,child} = \frac{1}{1+\frac{P_{child}}{P_{adult}}\times(\frac{1}{f_{u,adult}}-1)} \qquad Eq.7$$

Where P_{child} is the binding protein concentration in the child and P_{adult} is the binding protein concentration in the adult.

Age-dependent physiological changes

Body weight, height and body surface area

Original data on age-related changes in human body weight and height were obtained collected from reference in Appendix 2 (27). The mean weight or height changes with age in each study were pooled and fitted with the linear regression, natural spline function or polynomial functions. Polynomial equations (Table 4) best described the age-related changes in weight and height in the age band of day 1 to 18 year. Body surface area (BSA, cm^2) at a certain age was estimated using Dubois and

Dubois function with age appropriate weight and height (Eq. 8) (28). The fitting plots of projected body weight, height and body surface area changes with age were displayed in Figure 2.

$$BSA\ cm^2 = BW\ kg^{0.425} \times HT\ cm^{0.725} \times 71.84 \qquad Eq.8$$

In child pharmacokinetic study database, age-associated weight or mean weight values for individual or subgroups were not always reported in each study. The missing weight values were then calculated from the known age using the regression function listed in Table 4. The height values were not reported for subjects in most studies and therefore calculated values were used.

Liver weight

Johnson et al. (27) studied liver volume (LV) in 5036 children and young adults (ages from birth to 18 years) and derived a model (Table 4). The value of liver weight (LW) can be converted from liver volume by multiplying the density of liver of 1.08 kg/L. The predicted liver weight was validated against the independent data (Figure 2).

Cardio output and hepatic blood flow

Hepatic blood flow (Q_H) in adult was derived as the sum of the pre-portal organ blood flows, which includes the intestines, stomach, pancreas and spleen plus the arterial liver blood flow. The adult liver blood flow is scaled to children by maintaining the same percentage of cardiac output (CO) to the total sum of arterial blood flow and the portal blood flow, representing 25.5-27% for adults in the International Commission on Radiological Protection (ICRP) publication (25).

Age related change in cardiac output was modeled from Williams et al. (29) for their investigated CO in more than 50 studies involving normotensive children, adolescents and healthy adults. For children age 0.2 to 4 y, linear regressions of CO on age seemed appropriate with r^2=0.75. For older children (age 5 to 19 y), a nonlinear regression using body weight as the predictor is better with r^2=0.81. The predicted cardiac output rate was validated against independent data (30). The hepatic blood flow was calculated using Equation 9 by assuming the same portion of blood flow to the liver between children and adults. The simulated changes in hepatic blood flow with age were presented in Figure 3.

$$Q_H \ L/min \ = CO \times 0.27 \qquad Eq.9$$

Age-dependent drug metabolism

In vitro intrinsic clearance (CL_{int}) is determined either by drug depletion or metabolite kinetics reported for human liver microsomes. In vitro intrinsic clearance values can be scaled to the in vivo equivalent in the whole liver using average liver weight (LW; g of liver weight) with a microsomal recovery factor (MPPGL; mg of microsomal protein per g liver) (Eq. 10) (31). If the assumptions hold true that the enzyme affinity value (K_m) value for a particular enzyme isoform remains constant with adult values throughout infant development and that infants and adults express the same complement of hepatic enzymes, the in vivo intrinsic clearance for the infant is calculated using Equation 11. Thus, functional immaturity of the specific CYP enzyme and age-dependent liver growth (MPPGL and LW) explained the observed differences in CL_{int} between the child and adult.

$$CL_{int,child} = \frac{V_{max}}{K_m} \times MPPGL \times LW \qquad Eq.10$$

$$CL_{int,child} = CL_{int,adult} \times \frac{V_{max,child}}{V_{max,adult}} \times \frac{MPPGL_{max,child}}{MPPGL_{max,adult}} \times \frac{LW_{max,child}}{LW_{max,adult}} =$$

$$CL_{int,adult} \times OSF \times LSF \quad Eq.11$$

Ontogeny scaling factor (OSF) represents the elimination capacity of a specific enzyme in the infant relative to the adult. OSF is a unitless fraction of adult activity and is a function of both age and the particular enzyme. Liver scaling factor (LSF) represents the product of microsomal protein yield per gram liver and liver mass. Therefore, based on the age of child and the specific hepatic metabolic pathway, the prediction of intrinsic clearance in child is made based on the scaling factor for liver content and ontogenesis of a specific enzyme, in combination with the adult intrinsic clearance.

The human microsomal protein yield for adult livers is 40 mg per g liver, the most commonly used value (32) . Age was identified as a statistically significant covariate of MPPGL (33). However, the relationship between age and MP observed was difficult to be assessed. Consequently, the adult value of 40 mg microsomal protein/g of liver was used as the MPPGL recovery factor in all the age groups. The liver scaling factor (LSF) was then reduced to the ratio of liver mass between child and adult.

In vitro literature values of age-dependent CYP1A2 and CYP3A4 activity (34, 35), based on hepatic microsome studies using probe substrates or enzyme protein expression, were initially used to describe the enzyme-specific ontogeny. For each experimental study, the ratio of child to adult liver enzyme activity was sought so that absolute values and units were not an issue when comparing different studies. The

functions to describe changes with age summarized in Table 4 and model fitting was displayed in Figure 4.

Age-dependent glomerular filtration rate (GFR)

The glomerular filtration value, as measured by the mannitol clearance, was reported from 63 children between the ages of 2 days and 12 years (36). A model that characterized the maturation and growth of glomerular filtration was developed by Hayton (Equation 12) (37). The predicted changes of GFR were validated against the data (Figure 5).

$$GFR_{child} = 2.6 \times BW^{0.662} \times e^{-0.0822 \times age} + 8.14 \times BW^{0.662} \times \left(1 - e^{-0.0822 \times age}\right) \quad Eq.12$$

Where GFR_{child} was the calculated glomerular filtration rate in children (mL/min), BW was the body weight in kg, and age in month.

Model compilation and evaluation

The model was compiled as a function package in R (38). Clearance predictions were compared against literature values. To determine the ability of the ontogeny models to predict the observed clearances, the correlation between observed and predicted clearances for the model compounds was determined, as well as a measure of precision (the percentage of prediction values within 2-fold of the observed values). The Person's correlation coefficient between observations and predictions were calculated with R.

Simulation

Simulations were performed using 500 virtual pediatric subjects with age ranging from birth to 18 years. The cubic spline curves of predicted clearance versus age were generated and evaluated against observed in vivo clearance values for probe substrates in children.

Results

Clearance predictions for each of the model compounds were plotted against the observations in Figure 6. For CYP3A4 substrates, 82% (89/108) of predicted values were within 2-fold of the observed values of alfentanil while 78% (26/38) were within 2-fold of the observed values of midazolam. For CYP1A2 probe substrate, 77% (41/53) of predicted values were within 2-fold of the observed values of caffeine and 68% (125/183) were within 2-fold of the observed values of theophylline. About 62% of predicted values were within 2-fold of the observed values of gentamicin (58/94) and 66% for vancomycin (47/77). There were good correlation between the observed versus predicted values for each of the model drugs (Table 6). The overall coefficients of correlation were 0.883, 0.909 and 0.962, respectively, for CYP1A2, CYP3A4 metabolized elimination and GFR-mediated renal excretion.

The log ratio of predicted to observed clearances versus age indicated that model performance was both age and process-dependent (Figure 7). For CYP1A2 metabolic elimination, the data blow the identity line across the age range indicated the under estimation of the model for both CYP1A2 substrates. For CYP3A4 metabolic elimination, the under estimation trend for alfentanil was not changed until 2 year old

of age. For GFR-mediated renal elimination, the clearances of both gentamicin and vancomycin was over estimated in children less than 1 year of old.

500 virtual pediatric subjects from born to age 18 with simulated boy weight and height were created for each of the model compounds. The simulated clearance values (normalized by weight) versus age were compared against observed in vivo clearance values for probe substrates in children (Figure 8). Most observations were within the 2-fold confidence interval.

Removal of premature data points did not improve the overall model performance. The prediction precisions of model for preterm neonate subpopulation decreased except for CYP1A2 substrates (Table 6).

Discussion

Clearance is an important pharmacokinetic concept for scaling dosage, understanding the risks of drug-drug interactions and environmental risk assessment in children. Body weight normalized clearance values often exceed those of standard adult values (39, 40). Clinicians often interpret these findings as if children had greater enzyme activities in a unit weight (or volume) of the liver than adults. This might be true if hepatic metabolic enzymes mature at birth. However, drug metabolism enzymes activity often express low at birth and develop quickly in a few months after birth, and by 1 year of old most enzymes reach the adult activity. The physiology-based scaling approach not only incorporates the ontogeny of enzymes and other physiological functions but also the growth in size. Theoretically, it should be a better model to predict the clearance across the age range from neonates to adults, especially in young infants than other methods based only on size.

Recently, the importance of age to drug clearance has been assessed by some pivotal papers from Bjorkman and Alcorn and MacNamara, and commercial software PK-sim and Simcyp. In Alcorn and McNamara's paper, an exponential growth function was used to fit the in vitro microsomal ontogeny data from birth to 6 month old and clearance was thereafter scaled for term neonates to 6 month of age (18). Furthermore, Bjorkman used the same in vitro enzyme ontogeny function to predict clearance of alfentanil and midazolam as a function of age from birth to 20 years of age (15). Both studies suggested that additional data are required and it is critical to know at what age the enzyme activities reach the maturation level. Simcyp successfully predicted clearance for 11 drugs in children from birth to adults with inter-subject variability incorporated using a population algorithm but the software and source data are not open (7). Edginton et al. used both in vitro activity measures and in vivo clearance in children from model compounds to gap the age discrepancy with PKSim (8), but the model validation is not convincing because the observed in vivo data used in model development were also used for validation purpose. Our study provided a reliable mechanistic scaling method based on a large scale in vitro enzyme ontogeny database and in vivo child-adult clearance database. Furthermore, it is an open algorithm with models written as R function package.

The metabolic clearance of theophylline was under estimated in young infants <1 year of age, compared to caffeine. It could be the alternative pathways to contribute the overall clearance of theophylline or caffeine in neonates. In the adult, CYP1A2 eliminates >70% of a theophylline dose and the remaining 20% is through renal elimination, while 90% of caffeine elimination is mediated by CYP1A2 in adult with

1-1.5% excreted renally (14). The lower predicted clearance values may lie in the enhanced contribution of renal clearance to theophylline elimination in young infants, which was not considered in the model. It had been reported that renal excretion accounts for the majority of theophylline elimination in the very young infants, with limited N-demethylation by another CYP enzyme (41). In the neonate –infant group, the N-3-demethylation of caffeine was reported to correlate with CYP3A4, CYP1A2 activity, and CYP3A7. Thepresence of CYP3A7 has been detected in neonatal liver but absent in adult liver may contribute to its overall under predictions for caffeine clearance in this age group. In the current model, alternative elimination pathways are not taken into account and caused additional uncertainty in the prediction.

The interindividual variability of in vitro ontogeny observed in pediatric populations is remarkably large and is, in most cases, greater than the variability observed in adults (42). If the microsomal experiments could study a wide age range with data analyzed in small enough age groups to examine the age-related changes, especially for metabolically cleared compounds from birth to 1 year of age, the resolution of clearance prediction would be greatly improved.

There are many other parameters and assumptions in the model that require further investigation and validation. For example, it is not sure whether the amount of MPPGL is similar in pediatric and adult livers. Studies have found that microsomal recovery did not change with time. However, a recent study suggest a decrease in MPPGL with an average of 40 $mg \cdot g^{-1}$ for a 30 year old individual and 31 $mg \cdot g^{-1}$ for a 60 year old individual (33). This study has been extended using a set of pediatric samples and suggested an increase in microsomal protein content from birth to the

maximum observed at approximately 30 years of age. The average neonate exhibits a microsomal protein content of only 26 mg per gram liver.

Many other enzymes exist that are responsible for drug elimination. These include many more CYPs (e.g. CYP2D6, CYP2E1 and CYP2C) and non-cytochrome P450 enzymes such as esterase, alcohol dehydrogenases. Other phase II processes include glutathione and glycine conjugation, UGT and sulfotransferase are not discussed in this study. Liver and renal transporters are not discussed either due to the lack of ontogeny data. Further experimentations to increase the ontogeny database will be needed to increase the scope of PB-based clearance scaling.

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Table 1 Elimination pathways for probe substrates in healthy adult volunteers.

Drug	Route	% Excreted	Metabolic Pathways	Reference
Alfentanil	IV	>90% metabolized	CYP3A4 (>80%)	Dorne, 2003
Midazolam	IV	>90% metabolized	CYP3A4 (>80%)	Dorne, 2003
Caffeine	IV	>90% metabolized	CYP1A2 (>90%)	Dorne, 2001
Theophylline	IV	>90% metabolized	CYP1A2 (>90%)	Dorne, 2001
Gentamicin	IV	82±10% in urine	Glomerular filtration	Dorne, 2004
Vancomycin	IV	>90% in urine	Glomerular filtration	Bertz, 1997

Table 2 A section of children's clearance database.

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	midazolam	Rey 1991	6	39	2.5	y	15.2	13.30	4.3
CYP3A4	midazolam	Muchohi 2008	13	39	27	m	10.4	14.40	2.7
CYP3A4	midazolam	Mathews 1988	6	39	5.2	y	18.4	11.98	6.68
CYP3A4	midazolam	Mathews 1988	6	39	4.7	y	15.9	8.53	1.8
CYP3A4	midazolam	Mathews 1988	5	39	1.3	y	8.8	9.07	3.35
CYP3A4	midazolam	Reed 2001	5	39	0.96	y	7.88	11.33	6.33
CYP3A4	midazolam	Reed 2001	13	39	5.2	y	19.2	10.00	3.83
CYP3A4	midazolam	Reed 2001	2	39	15.4	y	62	9.33	3.83
CYP3A4	midazolam	Payne 1989	8	39	5.52	m	17.3	9.11	1.21
CYP3A4	midazolam	Mulla 2003	20	39.5	3.8	d	3.4	1.40	0.15
CYP3A4	midazolam	Lee 1999	60	27	4.5	d	0.965	9.11	1.21
CYP3A4	midazolam	Jacqz-Aigrain 1992	15	32.8	3	d	1.9	1.70	1.8
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	35	3.5	d	2.3	1.39	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	34	3.5	d	2.7	1.41	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	35	3.5	d	2.7	1.07	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	37	3.5	d	2.7	0.74	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	39	3.5	d	3	0.87	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	41	3.5	d	3.2	2.69	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	38	3.5	d	3.2	1.16	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	37	3.5	d	3.6	3.50	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	38	3.5	d	3.8	3.76	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	40	3.5	d	3.9	3.72	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	10	37	3.5	d	3.1	2.03	1.24
CYP3A4	midazolam	de Wildt 2001	24	29	5.5	d	1.02	1.80	n.a.
CYP3A4	midazolam	Salonen 1987	3	39	7.33	y	32.8	6.67	5.5

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	midazolam	Salonen 1987	6	39	6.72	y	28	4.83	2.33
CYP3A4	midazolam	Salonen 1987	6	39	6.07	y	21.6	8.67	2.5
CYP3A4	midazolam	Salonen 1987	6	39	6.11	y	22.3	11.20	1.33
CYP3A4	midazolam	Harte 1997	10	27.9	3	d	1.045	1.66	1.37
CYP3A4	midazolam	Tolia 1991	20	39	13.5	y	43.75	10.00	5
CYP3A4	midazolam	Clausen 1988	8	39	27	y	68	6.28	1.03
CYP3A4	midazolam	Pentikainen 1989	7	39	44.3	y	79.4	5.63	0.43
CYP3A4	midazolam	Mandema 1992	8	39	22	y	69	7.50	1.26
CYP3A4	midazolam	Greenblatt 1984	10	39	27.9	y	68.8	7.75	0.41
CYP3A4	midazolam	Greenblatt 1984	10	39	28.5	y	58.5	9.39	0.86
CYP3A4	midazolam	Dorne 2003	198	39	18	y	70	7	1.5

n=number of subjects in study; GA= gestational age; PNA=postnatal age; BW=body weight; CL=plasma clearance

wk=week; y=year; m=month; d=day; n.a.=not available

Table 3 Summary of drug specific input in adults

Drug	Major binding plasma protein	Unbound fraction in plasma, f_u	Blood:plasma partition ratio (C_b/C_p)	Adult drug CL (mL/min/kg)
Midazolam	Albumin	0.02	0.55	7.7
Alfentanil	alpha1-acid glycoprotein	0.1	0.63	4.7
Theophylline	Albumin	0.44	0.82	1.0
Caffeine	Albumin	0.68	1	1.97
Gentamicin	Albumin	0.95	n.a.	1.3
Vancomycin	Albumin	0.7	n.a.	1.22

Table 4 A summary of regression equations to calculate age-specific physiology and biochemical input.

Parameter	Unit	Age range of observation	Regression equations	Source
Body weight (BW)*	kg	day 1 – 18 yr	$BW = 4.2986 + 5.4396a - 0.9175a^2 + 0.091a^3 - 0.0026a^4$	derived
Height (HT)*	cm	day 1 – 18 yr	$HT = 53.674 + 22.304a - 3.759a^2 + 0.386a^3 - 0.0179a^4 + 0.0003a^5$	derived
Body surface area (BSA)	cm^2		$BSA = BW\ kg\ ^{0.425} \times HT\ cm\ ^{0.725} \times 71.84$	Lack, 1997
Glomerular filtration rate (GFR)**	mL/min	day 2– 12 yr	$GFR_{child} = 2.6 \times BW^{0.662} \times e^{-0.0822 \times age} + 8.14 \times BW^{0.662} \times \ 1 - e^{-0.0822 \times age}$	Hayton, 2000
Binding protein concentration ratio (HSA)	-		$\frac{P_{child}}{P_{adult}}_{HSA} = 0.00005627 \times age\ (day) + 0.767$	McNamara, 2002
Binding protein concentration ratio (AAG)	-		$\frac{P_{child}}{P_{adult}}_{AAG} = 0.0001137 \times age\ (day) + 0.534$	McNamara, 2002
Liver volume (LV)	L	day 0– 18 yr	$LV\ L\ = 0.722 \times BSA(m^2)^{1.176}$	Johnson, 2005
Cardiovascular output (CO)	L/min	0.2 yr- 4 yr	$CO = 0.734 + 0.563 \times age\ \ y$	Williams, 1994
		5 yr- 19 yr	$CO = 3.107 + 0.012 \times BW\ (kg)^{1.369}$	Williams, 1994
CYP3A4 ontogeny scaling factor (OSF)	-		$OSF_{CYP3A4} = 0.0835 + \frac{0.894 \times age\ \ day}{139 + age\ \ day}$	derived
CYP1A2 ontogeny scaling factor (OSF)	-		$OSF_{CYP1A2} = 0.0078 + \frac{0.531 \times age\ \ day}{100 + age\ \ day}$	derived
*a is the postnatal age in year				
**BW is body weight in kg, and age is the postnatal age in month				
"-" is the unitless fraction				

Table 5 Physiological input used in the physiologically based clearance scaling model for a normal male adult

Parameter	Unit	Input
Body weight (BW)	kg	70
Body surface area (BSA)	m^2	1.9
Cardiac output (CO)	mL/min	6500
Liver blood flow rate (% cardiac output)	fraction	25.5%
Liver blood flow (Q_H)	mL/min	1657.5
Microsomal protein per gram liver (MPPGL)	mg/gram	45
Liver weight (LW)	gram	1800

Table 6 Percentage of clearance predictions within 2-fold of the observed values (success rate) and correlation coefficient between observations and predictions

Drug	Success Rate		Pearson's Correlation Coefficient	
	overall	preterm neonate	overall	preterm neonate
Alfentanil	82% (89/108)	67% (4/6)	0.85	0.73
Midazolam	78% (26/38)	43% (3/7)	0.96	-0.183
Caffeine	77% (41/53)	78% (28/36)	0.93	0.99
Theophylline	68% (125/183)	71% (34/48)	0.88	0.948
Gentamicin	62% (58/94)	40% (21/53)	0.96	0.757
Vancomycin	66% (47/71)	40% (16/40)	0.97	0.933

Figure legends:

Figure 1 A summary of workflow for scaling clearance from adults to children for enzymatic hepatic clearance pathway (A) and renal elimination pathway (B).

Figure 2 Fitting plots for mean changes in body weight, height, body surface area and liver weight with age.

Figure 3 Age-related changes in cardiac output (A) and hepatic blood flow (B).

Figure 4 Fitted curves of ontogeny data expressed as a fraction of adult values as a function of age for CYP3A4 and CYP1A2.

Figure 5 Simulated changes in glomerular filtration rate (GFR) with age

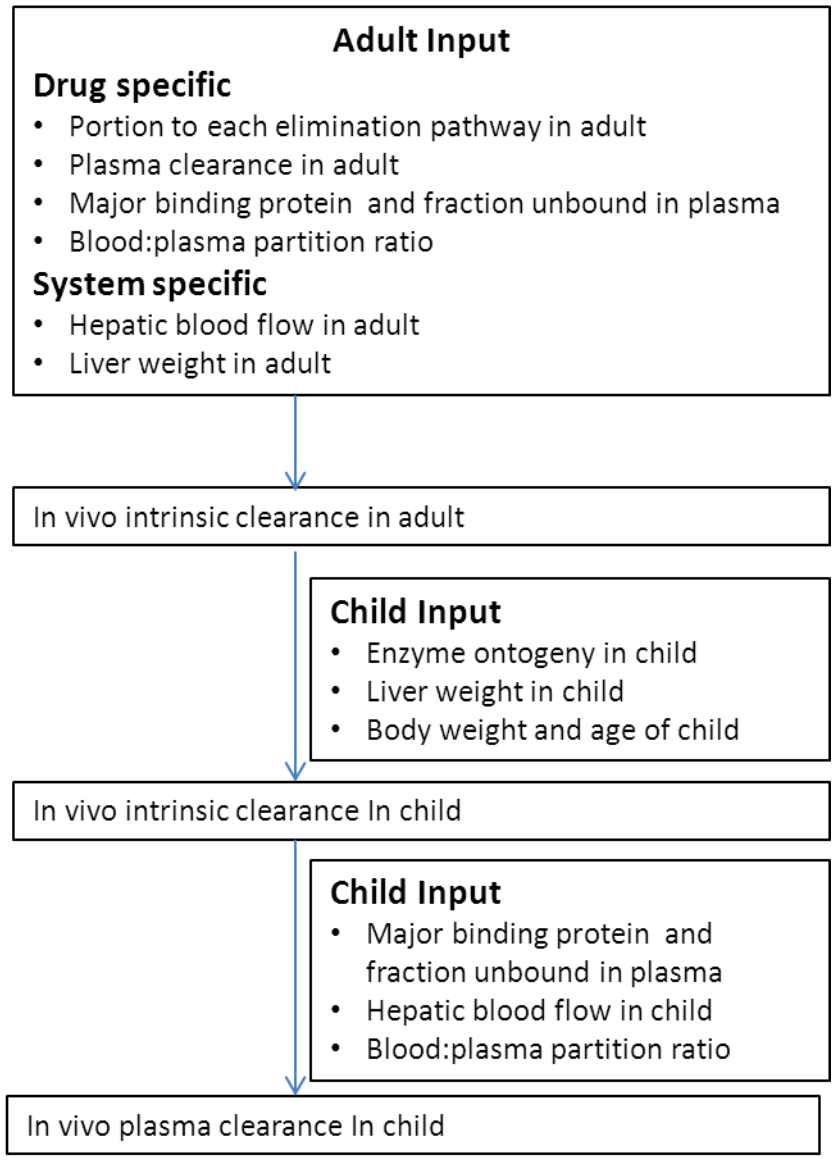
Figure 6 Predicted clearance values versus observed in vivo clearance values (solid lines represent unity and dashed lines represent 2-fold error).

Figure 7 Ratio (log) of predicted to observed clearance versus age. Solid lines represent lines of unity, and the area between the dashed lines represents an area within 2-fold error.

Figure 8 Predicted and observed age-dependence of clearance for (a) caffeine; (b) theophylline; (c) alfentanil; (d) midazolam; (e) gentamicin; (f) vancomycin.

Figure 1

A


Adult Input
Drug specific
• Portion to each elimination pathway in adult
• Plasma clearance in adult
• Major binding protein and fraction unbound in plasma
• Blood:plasma partition ratio
System specific
• Hepatic blood flow in adult
• Liver weight in adult
In vivo intrinsic clearance in adult
Child Input
• Enzyme ontogeny in child
• Liver weight in child
• Body weight and age of child
In vivo intrinsic clearance In child
Child Input
• Major binding protein and fraction unbound in plasma
• Hepatic blood flow in child
• Blood:plasma partition ratio
In vivo plasma clearance In child

B

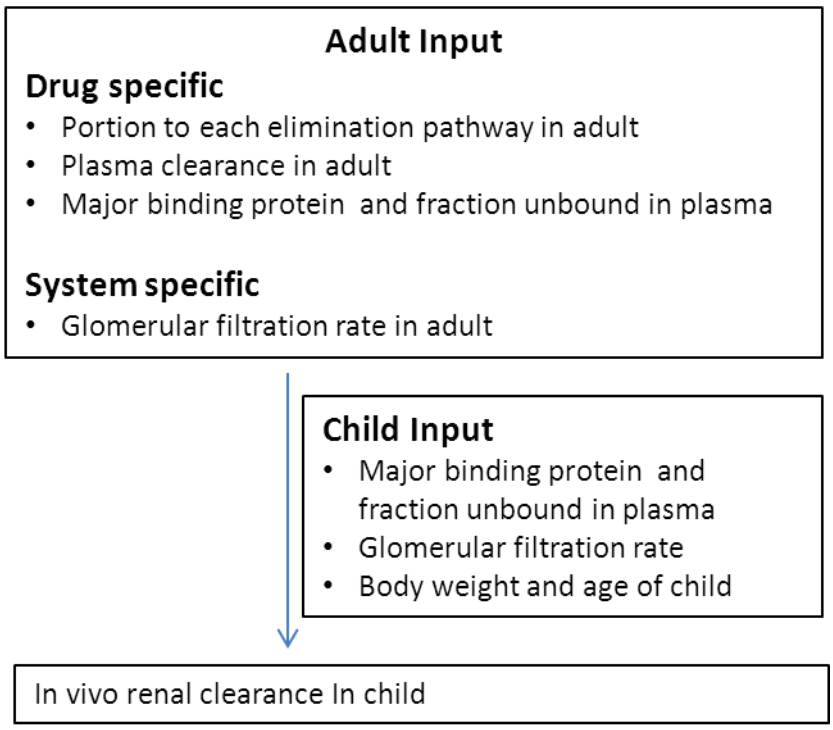

Adult Input
Drug specific
• Portion to each elimination pathway in adult
• Plasma clearance in adult
• Major binding protein and fraction unbound in plasma
System specific
• Glomerular filtration rate in adult
Child Input
• Major binding protein and fraction unbound in plasma
• Glomerular filtration rate
• Body weight and age of child
In vivo renal clearance In child

Figure 2

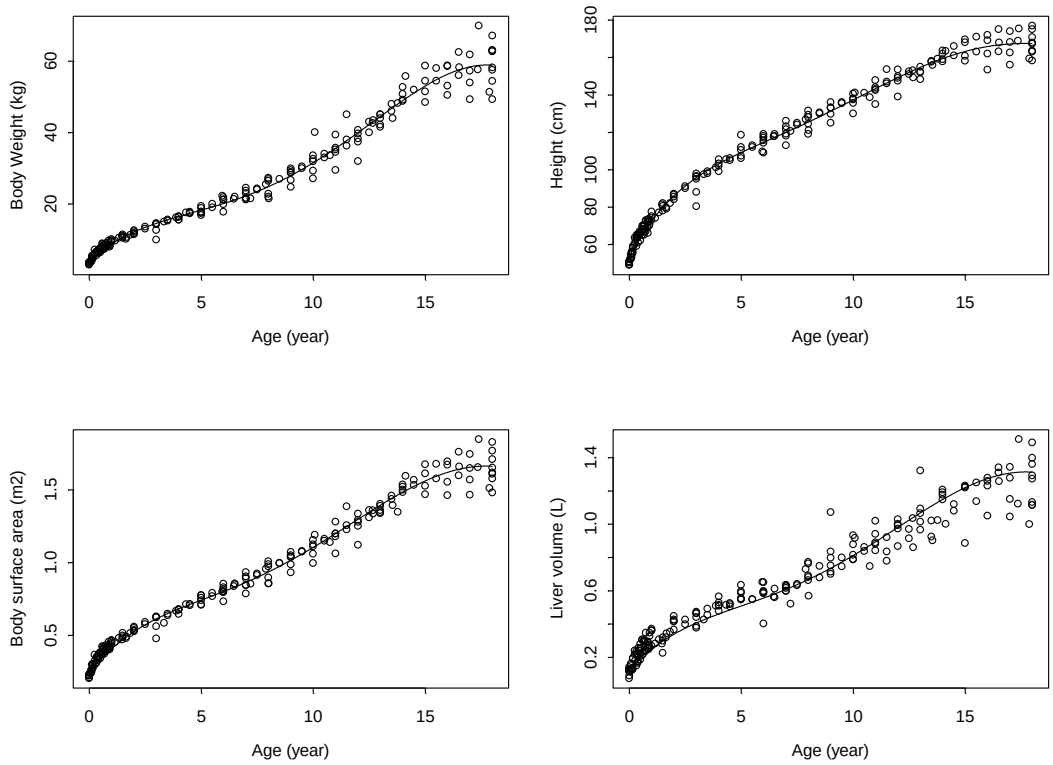

Body Weight (kg)
20
40
60
0
5
10
15
Age (year)
Height (cm)
60
80
100
140
180
0
5
10
15
Age (year)
Body surface area (m2)
0.5
1.0
1.5
0
5
10
15
Age (year)
Liver volume (L)
0.2
0.6
1.0
1.4
0
5
10
15
Age (year)

Figure 3

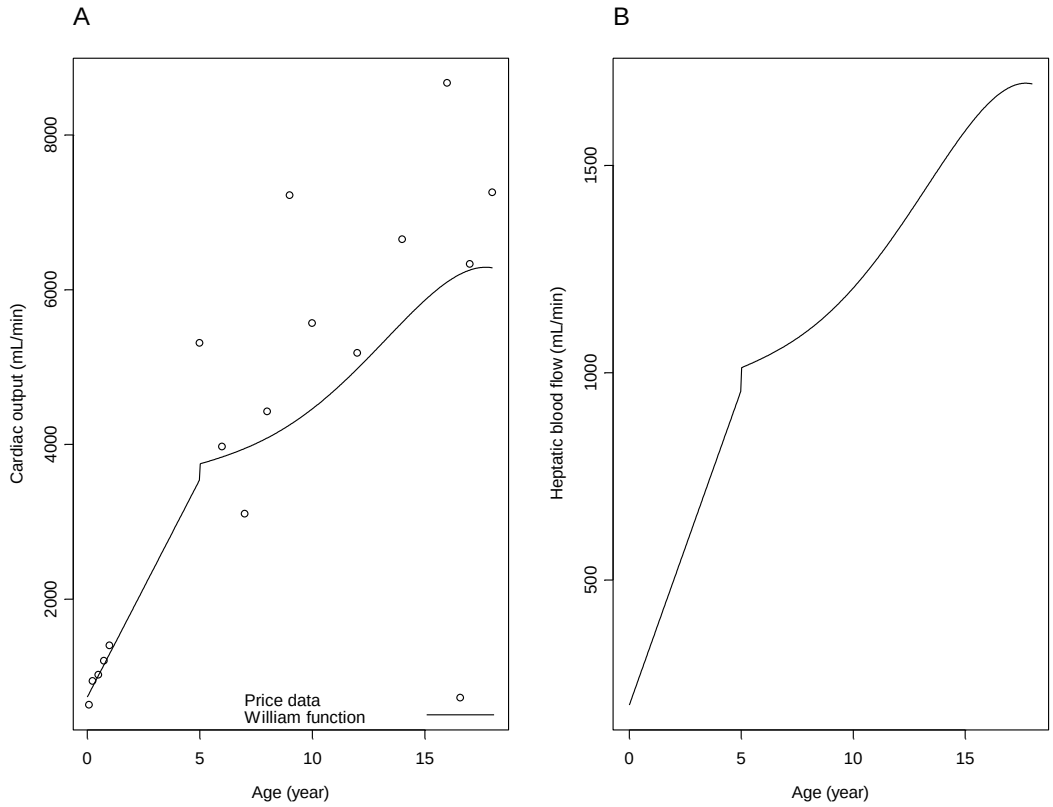

A
B
Cardiac output (mL/min)
2000
4000
6000
8000
Price data
William function
0
5
10
15
Age (year)
Heptatic blood flow (mL/min)
500
1000
1500
0
5
10
15
Age (year)

Figure 4

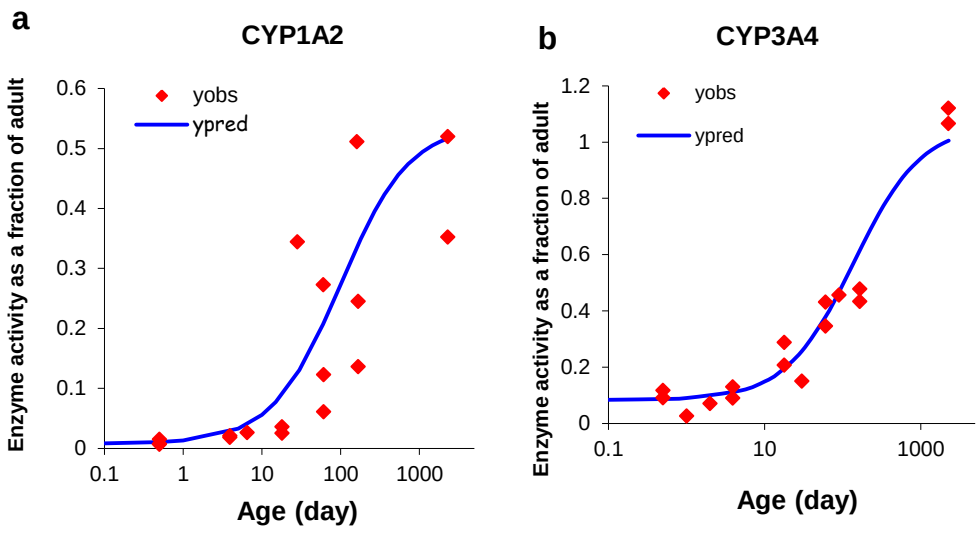

a
CYP1A2
yobs
ypred
Enzyme activity as a fraction of adult
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
1
10
100
1000
Age (day)
b
CYP3A4
yobs
ypred
Enzyme activity as a fraction of adult
1.2
1
0.8
0.6
0.4
0.2
0
0.1
10
1000
Age (day)

Figure 5

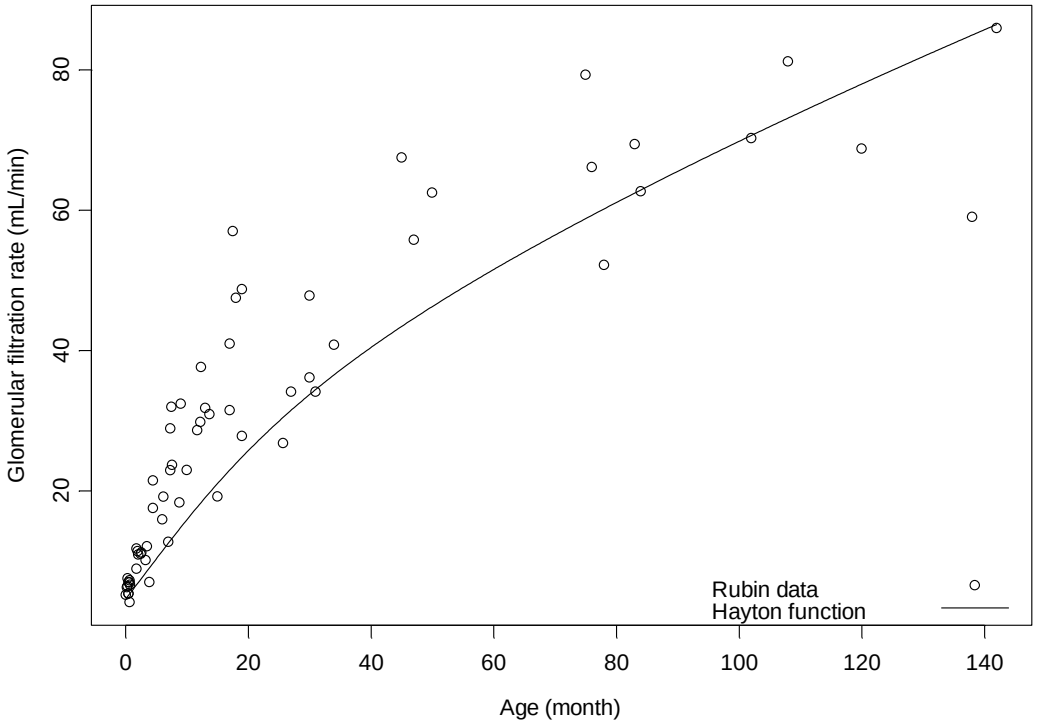

Glomerular filtration rate (mL/min)
20
40
60
80
0
20
40
60
80
100
120
140
Age (month)
Rubin data
Hayton function

Figure 6

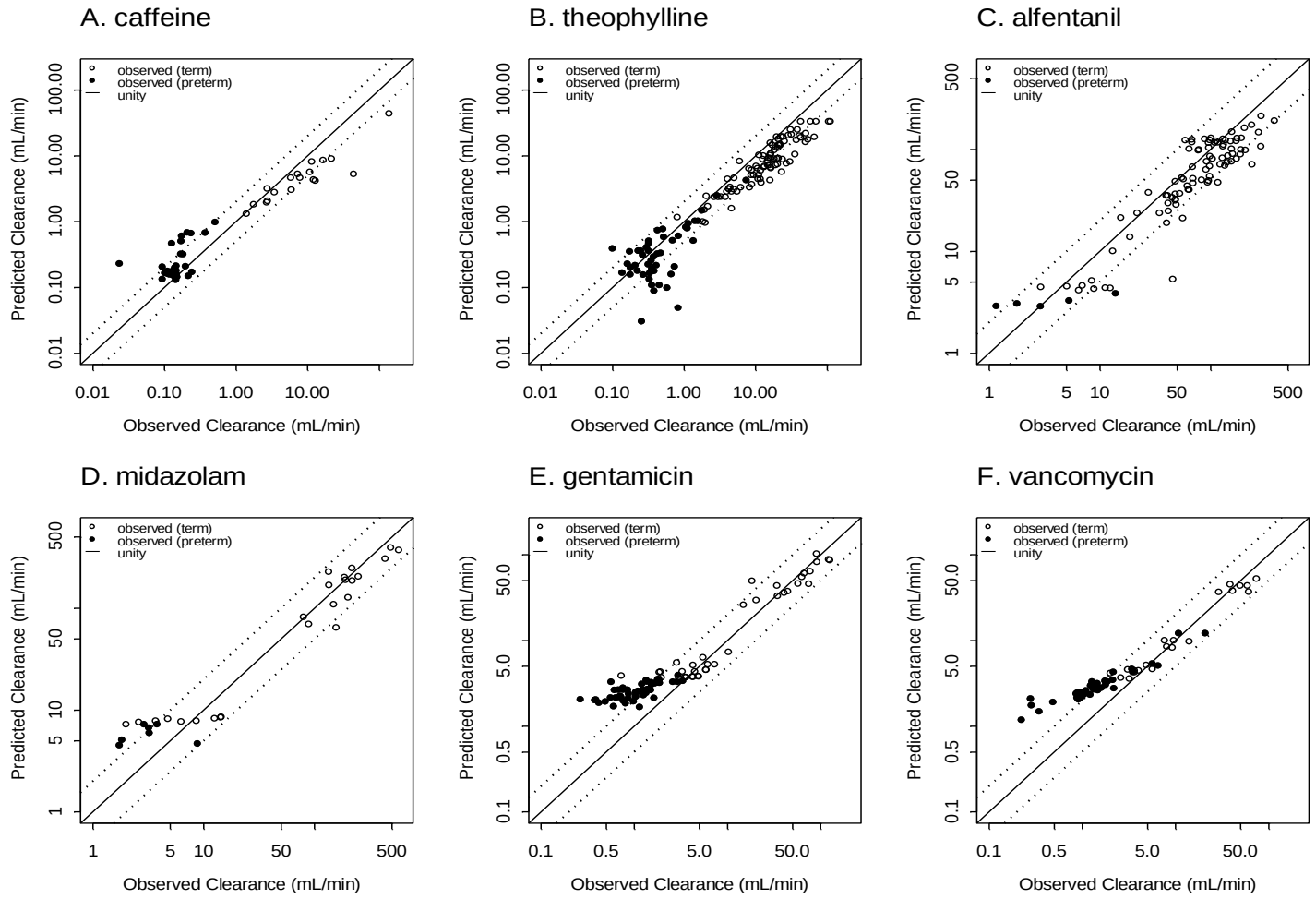

A. caffeine
B. theophylline
C. alfentanil
D. midazolam
E. gentamicin
F. vancomycin
observed (term)
observed (preterm)
unity
Predicted Clearance (mL/min)
Observed Clearance (mL/min)

Figure 7

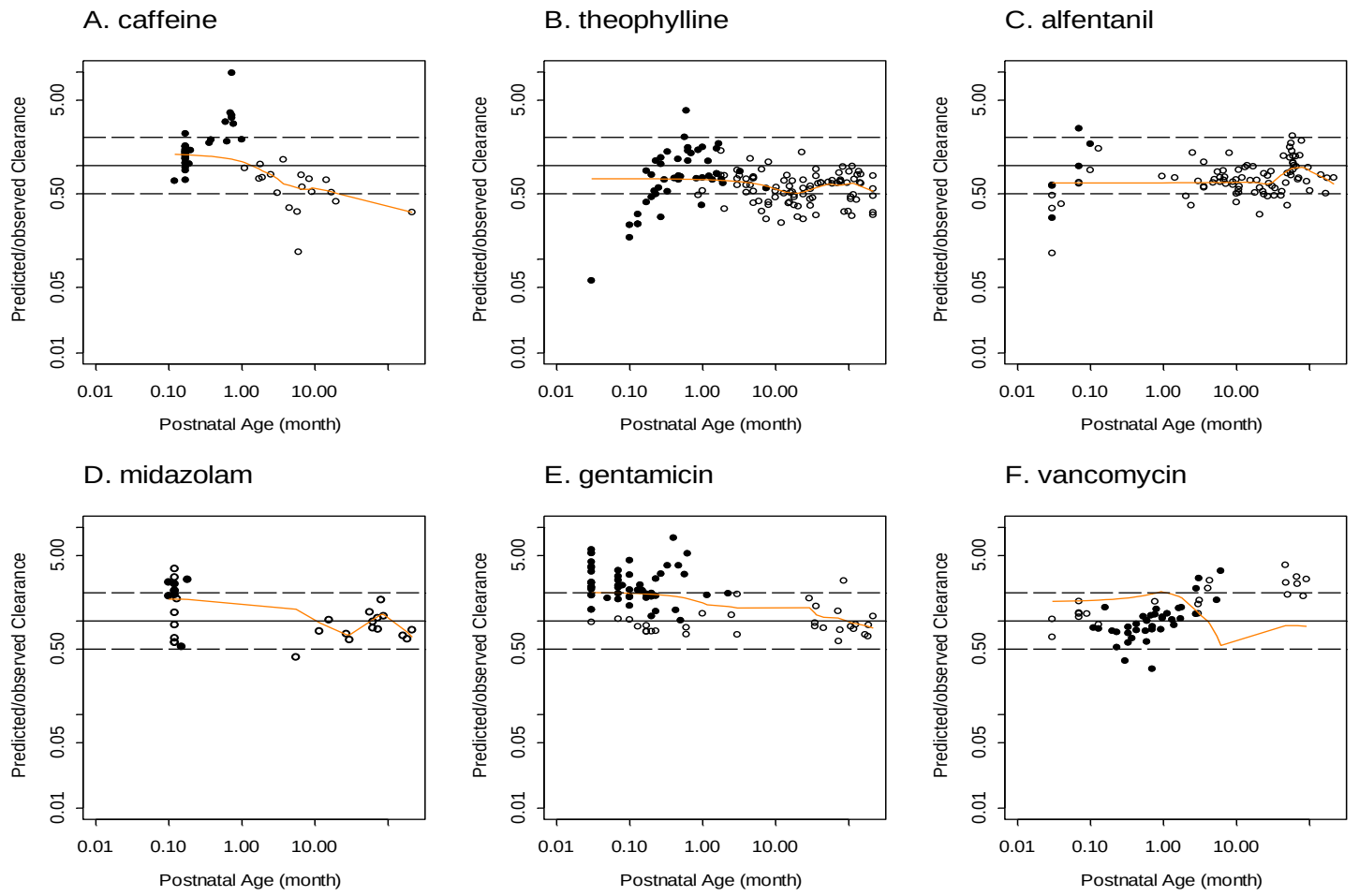
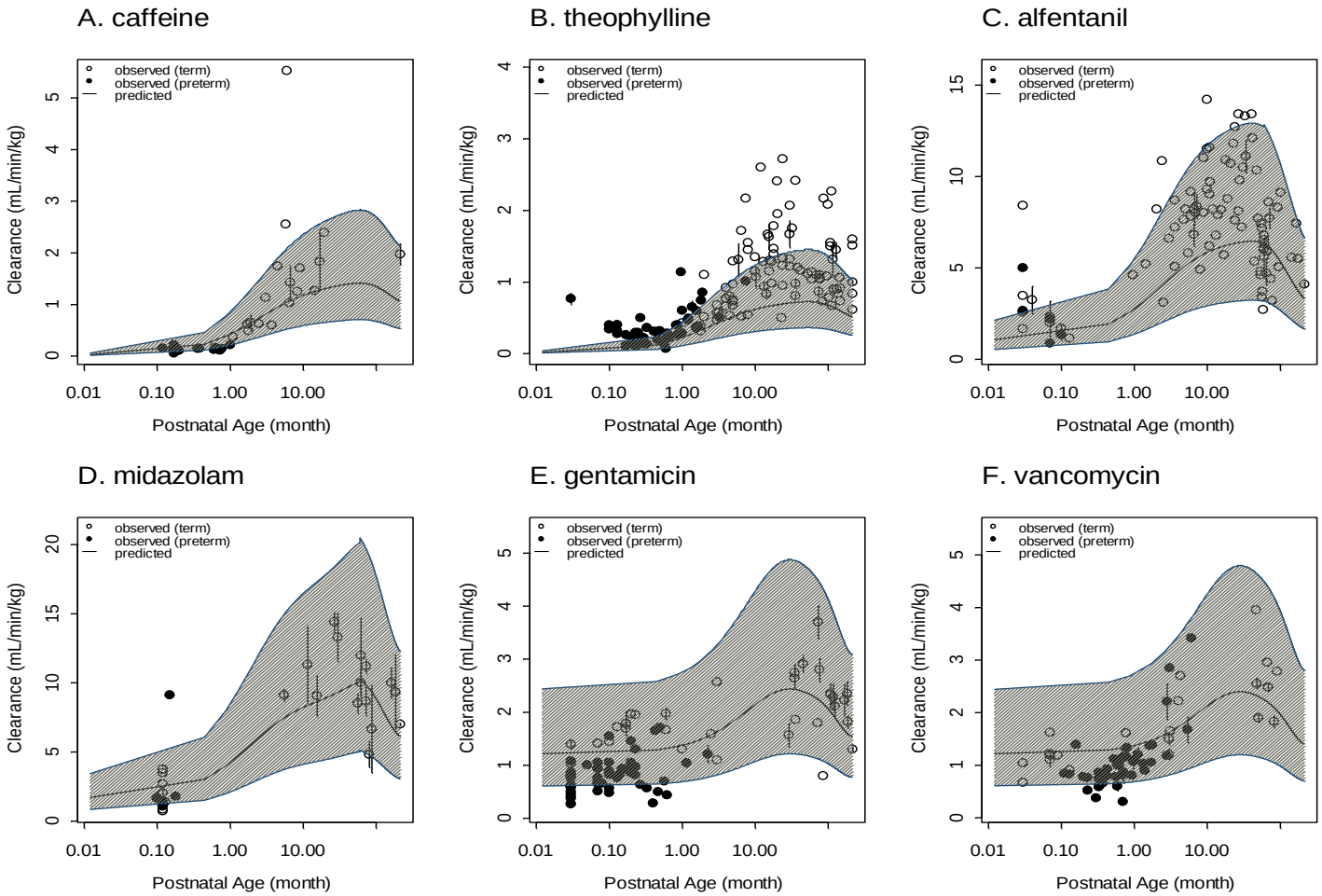

A. caffeine
B. theophylline
C. alfentanil
D. midazolam
E. gentamicin
F. vancomycin
Predicted/observed Clearance
0.01
0.05
0.50
5.00
0.01
0.10
1.00
10.00
Postnatal Age (month)

Figure 8


A. caffeine
B. theophylline
C. alfentanil
D. midazolam
E. gentamicin
F. vancomycin
observed (term)
observed (preterm)
predicted
Clearance (mL/min/kg)
Postnatal Age (month)
0.01
0.10
1.00
10.00

CHAPTER 4

Predicting renal and hepatic clearance in pediatric populations: physiologically based modeling versus allometric scaling

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Abstract

Objectives: In pediatric population, developmental changes in clearance can be predicted by age and size (body weight or body surface area). Clearance in children is commonly scaled from adults by size using either the per kilogram, body surface area or allometric ¾ power models. But the size model does not account for the maturation process of the elimination organ and therefore may not be appropriate to scale clearance to the very young children. The physiology-based approach accounts for maturity but requires detailed knowledge regarding developmental changes. In this study, these approaches were compared and appropriate use of these approaches is dependent on the age and clearance pathway.

Methods: A child PK database was developed and the dataset of experimentally obtained clearance values was used. Predicted clearance values in children were calculated based on the adult clearance, age and weight data using the four approaches. The ratio of predicted to observed values was graphed separately for probe substrates of three predominant clearance pathways: CYP1A2, CYP3A4 and renal clearance due to glomerular filtration.

Results: Allometric ¾ power model and body surface area approach systemically over estimated clearance in children below 1 year of old for all the compounds. Physiology-based approach accurately predicted clearance at all ages for compounds eliminated via CYP3A4. The clearance of CYP1A2 substrates within the first year were under predicted by the physiology-based approach but over estimated by the other approaches. In the case of renal clearance in children below 1 year of age, the per kilogram and physiology-based model performed similar with a few overestimations.

Conclusions: Physiology-based clearance scaling accurately predicted clearance in children from birth to 18 years. The allometric ¾ power and body surface area model are only accurate when the major clearance pathway is fully matured.

Key words: clearance, allometric scaling, physiology-based modeling, pediatrics

Introduction

The pharmacokinetic (PK) profiles of many drugs are different between children and adults (1, 2). A principle PK parameter determining age-dependent difference is drug clearance. Predicting clearance in children based on the clearance in adult has been the topic of recent publications (3, 4). Empirical approaches that focus on linear body weight (W) or body surface area (BSA) have traditionally been used. The third size model, using an exponent of weight ($W^{3/4}$), is termed the "allometric ¾ power model." This allometric equation can be predictive of clearance in children following a specific age (5). It becomes invalid when used below a certain age where the activities of the eliminating processes are not fully developed. This is because the size based approaches do not account for the maturation of hepatic and renal elimination (6).

A physiology-based approach has been proposed to incorporate maturation of CL for several major enzymatic (e.g. CYP3A4) and physiological (e.g. glomerular filtration) elimination processes (7-9). The drawback of this approach is that detailed knowledge about eliminating processes in adults is required and further, the ontogeny of these clearance processes is needed.

The objective of this study is to retrospectively compare the accuracy of the three size scaling approaches (linear weight, body surface area and 3/4 power model) with the physiology-based approach using compounds eliminated via various enzymatic or physiological processes (glomerular filtration). Furthermore, by using probe substrates for which the clearance process in adults is dominated (>85%) by one clearance pathway after intravenous administration, the pathway-specific age range of appropriate use for each scaling approach will be determined.

Methods

Selection of test drugs

The drugs to evaluate the prediction approaches were selected according to the following criteria: (1) The drug is routinely administered for clinical indications in ill neonates, infants and children. (2) The drug mainly binds to human serum albumin (HAS) or alpha-glycol acid protein (AGP). (3) The drug is primarily eliminated due to one process in healthy adults, which accounts >85% of an oral or i.v. dose. (4) The selected elimination processes include hepatic CYP3A4, CYP1A2 metabolism and renal excretion. (5) The drug shows complete absorption (or >90%) from gastrointestinal tract after oral administration (po). If the compound is showing incomplete absorption, only IV data are used. (6) Mean or individual clearance data for children and adults have been published.

Using the criteria described in (1)-(6), alfentanil and midazolam, theophylline and caffeine, gentamicin and vancomycin were selected separately, for CYP3A4 metabolism, for CYP1A2 metabolism and for renal clearance due to glomerular filtration.

Children PK database and age-dependent clearance dataset

The pharmacokinetics of the 6 drugs has been evaluated in children to determine the appropriate dose levels for specific ages. A search of the literature produced a limited number of studies that reported systemic clearance values of these probe substrates in neonate, infants and children. Appendix I provided the observed systemic clearances of selected probe substrates for children aged from premature to 18 years.

The observed adult clearance value was the weighted mean as preciously presented (10-12) and, for vancomycin, was the adult clearance value taken from Guay et al. (13).

Physiology-based clearance scaling

This approach used information regarding clearance pathways in adults and scaled them to children. Briefly, the adult intrinsic clearance ($CL_{int,adult}$) is back calculated from in vivo hepatic drug clearance (CL_H), the free fraction in plasma (f_u), the blood to plasma drug concentration ratio (C_B/C_P), and hepatic blood flow (Q_H), using the well-stirred model (Eq. 1). The generated adult intrinsic clearance value is then multiplied by scaling factors (ontogeny scaling factor [OSF] and liver scaling factor [LSF]) that represents the activity of the specific enzyme in relation to the age of the child (Eq. 2). This new child-scaled intrinsic clearance ($CL_{int,child}$) is used to generate an age-specific hepatic clearance calculated from the re-arranged equation (Eq. 3) using age-specific body weight, liver weight, liver blood flow and predicted fraction unbound (scaled from adults based binding protein concentrations in blood).

$$CL_{int,adult} = \frac{Q_{H,adult} \times CL_{H,adult}}{f_{u,adult} \times (Q_{H,adult} - CL_{H,adult}/(\frac{C_B}{C_P}))} \qquad Eq.1$$

$$CL_{int,child} = CL_{int,adult} \times OSF \times LSF \qquad Eq.2$$

$$CL_{H,child} = \frac{Q_{H,child} \times f_{u,child} \times CL_{int,child}}{Q_{H,child} + f_{u,child} \times CL_{int,child}/(\frac{C_B}{C_P})} \qquad Eq.3$$

Ontogeny scaling factor (OSF) represents the elimination capacity of a specific enzyme in the infant relative to the adult; and it is a unitless fraction of adult activity with a function of both age and the particular enzyme. Liver scaling factor (LSF) represents the product of microsomal protein yield per gram liver and liver mass.

The clearance of a drug due to glomerular filtration rate in children was calculated by the following equation (Eq. 4):

$$\frac{CL_{GFR,child}}{CL_{GFR,adult}} = \frac{GFR_{child}}{GFR_{adult}} \times \frac{f_{u,child}}{f_{u,adult}} \qquad Eq.4$$

The age dependence of renal clearance in children by the process of glomerular filtration was studied by Hayton (14).

The age-dependent regression functions including ontogeny functions, the maturation function of glomerular filtration rate (GFR) were shown in Table 1.

Allometric scaling

In a child, the clearance can be predicted based on the known adult clearance and the power function of body weight (Eq. 4):

$$CL_{child} = CL_{adult} \times \left(\frac{BW_{child}}{BW_{adult}}\right)^{b} \qquad Eq.4$$

b=1, per kg model;

b= ¾, power model

Where BW_{child} is the body weight of the child and BW_{adult} is the body weight of the adult. The mean body weight of the pediatric population from each study was used in the equation. If this value was not reported in the study, the mean weight value was taken for the mean age (or middle of the age range in some cases) using information from the International Commission on Radiological Protection (ICRP) (15). The adult body weight was set at 70 kg for all calculations. Both CL_{child} and CL_{adult} are given in flow units, e.g. mL/min).

The clearance in a child can also be calculated based on the adult clearance and body surface area (Eq. 5):

$$BSA\ model \qquad CL_{child} = CL_{adult} \times \frac{BSA_{child}}{BSA_{adult}} \qquad Eq.5$$

Where BSA_{child} is the body surface area of the child and BSA_{adult} is the body surface area of the adult. The surface area is calculated from body weight and height using Dubios and Dubios equation (16). The mean body weight of the pediatric population from each study was used in the equation. If this value was not reported in the study, the mean weight value was taken for the mean age (or middle of the age range in some cases) using information from the International Commission on Radiological Protection (ICRP). Most pediatric studies did not report height. The height was taken for the mean age (or middle of the age range in some cases) using information from the International Commission on Radiological Protection (ICRP).The adult body surface area was set at 1.9 m^2 for all calculations.

Approach comparison

The ratios of experimentally predicted to observed clearance values (ml/min), using allometric, linear, and body surface area and physiology-based scaling, were calculated and plotted against age for each compound. The compounds were grouped according to their primary elimination pathways, which included the process of CYP3A4, CYP1A2 and renal clearance. The line of unity at which the predicted values was equal to the observed values was plotted together with the lines for where predicted clearance was either twice or half the observed clearance. To determine the age range for which allometric scaling was appropriate, and to determine if this age range depended on the process of clearance, the ratios of experimentally predicted to observed clearances (ml/min), using different scaling approaches, were calculated and plotted against age for each compound that is cleared via one prominent pathway.

To determine the ability of the defined scaling models to predict observed clearances, the correlation between observed and predicted clearances for each scaling method was determined, as well as bias and success rate (the percentage of prediction values within 2-fold of the observed values) . The bias (accuracy) was assessed by the geometric mean of the ratio of predicted and observed values or the average fold error (afe) metric using Equation 6:

$$afe = 10^{\frac{1}{n}\sum_{i=1}^{n}\left|log\frac{predicted}{observed}\right|} \qquad Eq.6$$

(under-predition=1/afe)

Where N represents the number of data inputs used for the calculation. “i” in the above equations represents the age points in the age brackets defined in this study (17, 18).

This approach prohibited poor overpredictions from being canceled out by equally poor underpredictions; underpredictions were of equal value to overpredictions (19). It also did not allow any single outlier prediction from biasing conclusions concerning a particular prediction method. A method that predicted all actual values perfectly would have a value of 1; one that made predictions that were on average 2-fold off (100% above or 50% below) would have a value of 2 and so forth.

In addition, the success rate was measured for each predefined age group. These groups were preterm neonates (0-30 days, <30 weeks of gestation), full term neonate (1-30 days, >=30 week of gestation), infant (1-12 months), children 1 - 5 years, children 6-11 years, adolescents 12-17 years, and adults (> =18 years old). The success rate was simply the number of successful predictions using the method

divided by the total number of predictions made using the method and then multiplied by 100. A prediction within an average -fold error of 2 was considered successful (19).

Results

For each elimination process, there were 2 drugs that had adequate clinical data available for assessment of prediction.

Figure 1-3 summarized the prediction results of four scaling approaches, divided by the process responsible (>85%) for clearance. For each process; CYP3A4 (Figure 1), CYP1A2 (Figure 2) and renal (Figure 3), the clearance was scaled via physiology-based approach (A), per kg body weight (B), ¾ allometric power model (C) and body surface area (D).

For hepatic enzymes CYP3A4, physiology-based approach (method A) predicted clearance values within 2-fold of observed values for 53 of 66 data inputs (Table 2). The accuracy of prediction using method A was better than the other methods as indicated by the lowest geometric mean accuracy value of 2.9 (Table 2).

For hepatic enzymes CYP1A2, the most predictive method was physiology-based method, in which 98 of 143 predictions were within 2-fold of observed, and the geometric mean prediction accuracy was 3.8. Allometric approaches (power model and BSA model) resulted in significant overestimations of CL for the low observed values and thereby decreased the predictive power of these approaches. Thus, only ~40% of the predictions were within 2-fold of observations for methods C and D, and geometric mean prediction accuracy was poor, with values of 36.4 and 56.8.

Overall for hepatic eliminated drugs, predicted clearance values resembled the observed values more closely in the physiology-based model. The accuracy of the predictions in each model was confirmed by comparing the average fold error (AFE) (Table 2). The prediction accuracy decreased in the order physiology-based > linear weight > ¾ allometric power > BSA.

In the case of renal elimination, the simplest approach, per kg body weight (method B) predicted clearance within 2-fold of observed for 101 of 135 data inputs (75%; Table 2). The geometric mean prediction accuracy value for method B was 3.0. Physiology-based approach (method A) predicted clearance within 2-fold of observed for 81 of 135 data inputs (60% of success). The geometric mean accuracy value for method A was 4.3. The BSA scaling (method D) was the poorest predictor in that only 35 of 135 predictions were within 2-fold of observed. Corresponding rankings of decreasing prediction accuracy were linear weight > physiology-based > ¾ allometric power > BSA.

Figure 4-6 summarized the ratios of predicted to observed clearance values across the entire age range, for the renal eliminated and hepatic metabolized (CYP3A4 and 1A2) drugs. The consistency of data above the lines of 2-fold error appeared to be age and model-dependent.

For hepatic enzyme metabolized drugs, there was evidence that physiology-based approach was more appropriate at young age. Physiology-based method produced little bias around the line of unity in neonates less than 1 month (Figure 5 and 6). The 3/4 allometric power equation and BSA scaling consistently overestimated clearance

in neonates. Success rates for the prediction methods by age groups were shown in Figure 7. For children greater than 1 year of age, the four prediction approaches were comparable, with values ranging from 68% to 100% success.

In the case of renal elimination, the linear scaling method appeared to be the most predictive method in neonates and infants under 1 year old, in which more than 75% of predictions were within 2 fold of observed values (Figure 7). The 3/4 allometric power equation and BSA scaling resulted in poor predictions in neonates and infants under 1 year old. The two methods consistently overestimated clearance for children under 1 year old.

Overall, the linear scaling, 3/4 allometric power equation and BSA scaling systemically overestimated clearance in the young age groups as evidenced by the lack of data points at or below the 2-fold error line. The physiology-based approach appeared not to be biased towards either over or under estimation of clearance in neonates or infants under 1 year age. When assessed by success rate criteria, physiology-based approach appeared to be the most appropriate prediction method across the age range of neonate to 18 years.

Discussion

The body surface area model is widely used to scale drug dose in children out of infancy (16, 20). This model requires the measure of height, as well as body weight, to estimate size and is usually determined from nomograms, which introduce the possibility of additional errors. Surface area can also be estimated from an allometric model with a power parameter of 2/3. However, the surface area model does not fit known observations. The body area of animals rises more slowly than the surface law

would suggest, as larger animals are stockier. The surface law refers to an animal's skin. The mass of empirical evidence suggests that the appropriate scaling factor is significantly different from 0.67 and is actually 0.75 (21).

Thus, the ¾ power model has been proposed to scale metabolic and physiological processes among species based on body size, including scaling to human. This easy-to-use equation presents significant improvement over the surface area model. When the clearance is calculated using the allometric surface area and compared to the ¾ power model, the two models are in close agreement for the human weight range above 20 kg (5). The surface area model overpredicts clearance by more than 10% at body weights below 20 kg (an approximate age of 5 year old child's mean weight) (6).

In our study, both body surface area model and ¾ power model tend to systemically overestimate clearance in neonates and infants under 1 year old of age, when the maturity of the process responsible for clearance are immature. This was likely because allometric scaling approaches account for development of body size but not for the ontogeny of hepatic and renal elimination pathways. Thereby, the allometric equations (BSA model and ¾ power model) are not appropriate for predicting clearance in neonates and young infants, where the intrinsic activity of the elimination process has not yet reached the activity in adults. This was also demonstrated in a study that compared the allometric approach to the physiology-based approach for the two CYP3A4 substrates, midazolam and alfentanil (22).

The per kilogram model is the poorest model but remains the most commonly used in human. In humans, an under-prediction of clearance of more than 10% occurs at bodyweights less than 47 kg compared to allometric ¾ power model. This error

increases as size decreases and approaches 50% for a newborn of 3.4 kg. However, this gross under prediction is not seen for neonates and young infants in our study because of immaturity of enzyme systems or glomerular filtration (the right answer for the wrong reason!). In our study, the per kilogram model resulted in better predictions for neonates and young infants compared to allometric approaches. In the case of renal elimination, the per kg model appeared to yield more accurate predictions than physiology-based approach for neonates. The significant underpredictions of CL using per kilogram model was seen in children greater than 1 year old for renal eliminated or CYP3A4 metabolized drugs, where CYP3A4 enzyme activity and glomerular filtration has reached adult activity (23).

As demonstrated in Figure 7, the physiology-based approach, which explicitly accounts for the age dependence of hepatic enzyme activity or renal function, appears to be the most predictive in neonates and young infants under 1 year old. For children greater than 5 years, allometric methods (BSA model and ¾ power model) were appropriate for clearance prediction.

Premature neonates represent a specific subgroup, with variable clearances dependent on weight, gestational and postnatal age. For example, the fluconazole population clearance in young infants required adjustment for gestational age at birth (BGA) and postnatal age (PNA) (24, 25). The disposition of theophylline in premature neonate presented a much longer elimination half-life (26). Furthermore, premature neonates generally receive drugs for varying clinical conditions, which may alter the neonate's physiology and the drug's pharmacokinetics. Despite the enzyme ontogeny divergence in premature neonates was not considered in the physiology –based

approach due to the lack of data in enzyme ontogeny and physiological factors, our study demonstrated that physiology-based approach more accurately predicted premature clearances in comparison to allometric approach and per kilogram model.

For hepatic enzymes, different isoenzymes develop at different rate. For example, the development of CYP1A2 is relatively slow compared with CYP3A4. It takes about 3 years for CYP1A2 to reach 80% of its activity in adults. At 1 year after birth, only 50% of the adult CYP1A2 activity was reached, while about 80% of the adult CYP3A activity was reached at the same time. The physiology-based approach under predicted the theophylline clearance in the children greater than 1 year old. The possible explanation could be the enhanced renal excretion to theophylline elimination before CYP1A2 develops. The contribution of CYP1A2 to theophylline clearance increases with CYP1A2 development. This phenomenon is not unusual that the neonate use alternative routes for certain drugs' elimination in very early life before its primary pathway develops. The sulphate pathway is the dominant metabolite route for paracetamol (acetaminophen) in infancy before glucuronidation enzyme pathways mature.

Clearance scaling is the first step towards the scaling of pharmacokinetics profiles from adults to children. The next step is to determine the age-dependent of distribution of volumes using allometric scaling or physiological approach. Together, the two parameters or a physiology-based pharmacokinetic (PBPK) simulation can lead to a reasonable prediction of a child's PK profile and decisions regarding dosing and potential therapeutic or adverse effect events can be informed.

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Table 1 A summary of regression equations to calculate age-specific physiology and biochemical input.

Parameter	Unit	Age range of observation	Regression equations	Source
Body weight (BW)*	kg	day 1 – 18 yr	$BW = 4.2986 + 5.4396a - 0.9175a^2 + 0.091a^3 - 0.0026a^4$	derived
Height (HT)*	cm	day 1 – 18 yr	$HT = 53.674 + 22.304a - 3.759a^2 + 0.386a^3 - 0.0179a^4 + 0.0003a^5$	derived
Body surface area (BSA)	cm^2		$BSA = BW\ kg\ ^{0.425} \times HT\ cm\ ^{0.725} \times 71.84$	Lack, 1997
Glomerular filtration rate (GFR)**	mL/min	day 2– 12 yr	$GFR_{child} = 2.6 \times BW^{0.662} \times e^{-0.0822 \times age} + 8.14 \times BW^{0.662} \times \ 1 - e^{-0.0822 \times age}$	Hayton, 2000
Binding protein concentration ratio (HSA)	-		$\frac{P_{child}}{P_{adult}}_{HSA} = 0.00005627 \times age\ (day) + 0.767$	McNamara, 2002
Binding protein concentration ratio (AAG)	-		$\frac{P_{child}}{P_{adult}}_{AAG} = 0.0001137 \times age\ (day) + 0.534$	McNamara, 2002
Liver volume (LV)	L	day 0– 18 yr	$LV\ L\ = 0.722 \times BSA(m^2)^{1.176}$	Johnson, 2005
Cardiovascular output (CO)	L/min	0.2 yr- 4 yr	$CO = 0.734 + 0.563 \times age\ \ y$	Williams, 1994
		5 yr- 19 yr	$CO = 3.107 + 0.012 \times BW\ (kg)^{1.369}$	Williams, 1994
CYP3A4 ontogeny scaling factor (OSF)	-		$OSF_{CYP3A4} = 0.0835 + \frac{0.894 \times age\ \ day}{139 + age\ \ day}$	derived
CYP1A2 ontogeny scaling factor (OSF)	-		$OSF_{CYP1A2} = 0.0078 + \frac{0.531 \times age\ \ day}{100 + age\ \ day}$	derived
*a is the postnatal age in year				
**BW is body weight in kg, and age is the postnatal age in month				
"-" is the unitless fraction				

Table 2 Prediction accuracy and successful rate using four prediction methods, divided by the process responsible for clearance.

Elimination process	Physiology-based	Linear body weight	3/4 allometric weight	Body surface area
Accuracy				
CYP3A4 (n=66)	2.9	5.3	4.7	5.1
CYP1A2 (n=143)	3.8	10.7	36.4	56.8
Renal (n=135)	4.3	3.0	12.1	22.6
Success rate				
CYP3A4 (n=66)	81%	52%	71%	69%
CYP1A2 (n=143)	69%	51%	40%	41%
Renal (n=135)	60%	75%	28%	26%

Figure legends

Figure 1 Predicted versus observed clearance values (mL/min) for alfentanil and midazolam eliminated via hepatic CYP3A4 metabolism, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. Solid lines represent lines of unity, and the area between the dashed lines represents an area within 2-fold error.

Figure 2 Predicted versus observed clearance values (mL/min) for caffeine and theophylline eliminated via hepatic CYP1A2 metabolism, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. Solid lines represent lines of unity, and the area between the dashed lines represents an area within 2-fold error.

Figure 3 Predicted versus observed clearance values (mL/min) for gentamicin and vancomycin eliminated via renal clearance due to glomerular filtration, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. Solid lines represent lines of unity, and the area between the dashed lines represents an area within 2-fold error.

Figure 4 The ratio (natural log) of predicted to observed clearance plotted against age for alfentanil and midazolam eliminated via hepatic CYP3A4 metabolism, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. The solid line of 0 represents the line of identity and the dashed lines indicates where predicted clearance was twice or half the observed clearance.

Figure 5 The logarithmic ratio (base 10) of predicted to observed clearance plotted against age for caffeine and theophylline eliminated via hepatic CYP1A2 metabolism, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. The solid line of 0 represents the line of identity and the dashed lines indicates where predicted clearance was twice or half the observed clearance.

Figure 6 The logarithmic ratio (base 10) of predicted to observed clearance plotted against age for gentamicin and vancomycin eliminated via renal clearance due to glomerular filtration, using the physiology-based (A), per kg body weight (B), allometric body weight (C) and BSA (D) scaling approach. The solid lines of 0 represent the lines of identity and the dashed lines indicate where predicted clearance was twice or half the observed clearance.

Figure 7 Histogram of success rates for clearance predictions obtained by four prediction methods for different age groups of preterm neonates, term neonates, infants 1-12 months, children 1-5 year, children 6-12 year, adolescents 12-17 year or adults >=18 year. Graph A is for CYP3A4 metabolized drugs. Graph B is for CYP1A2 metabolized drugs. Graph C is for renally eliminated drugs.

Figure 1

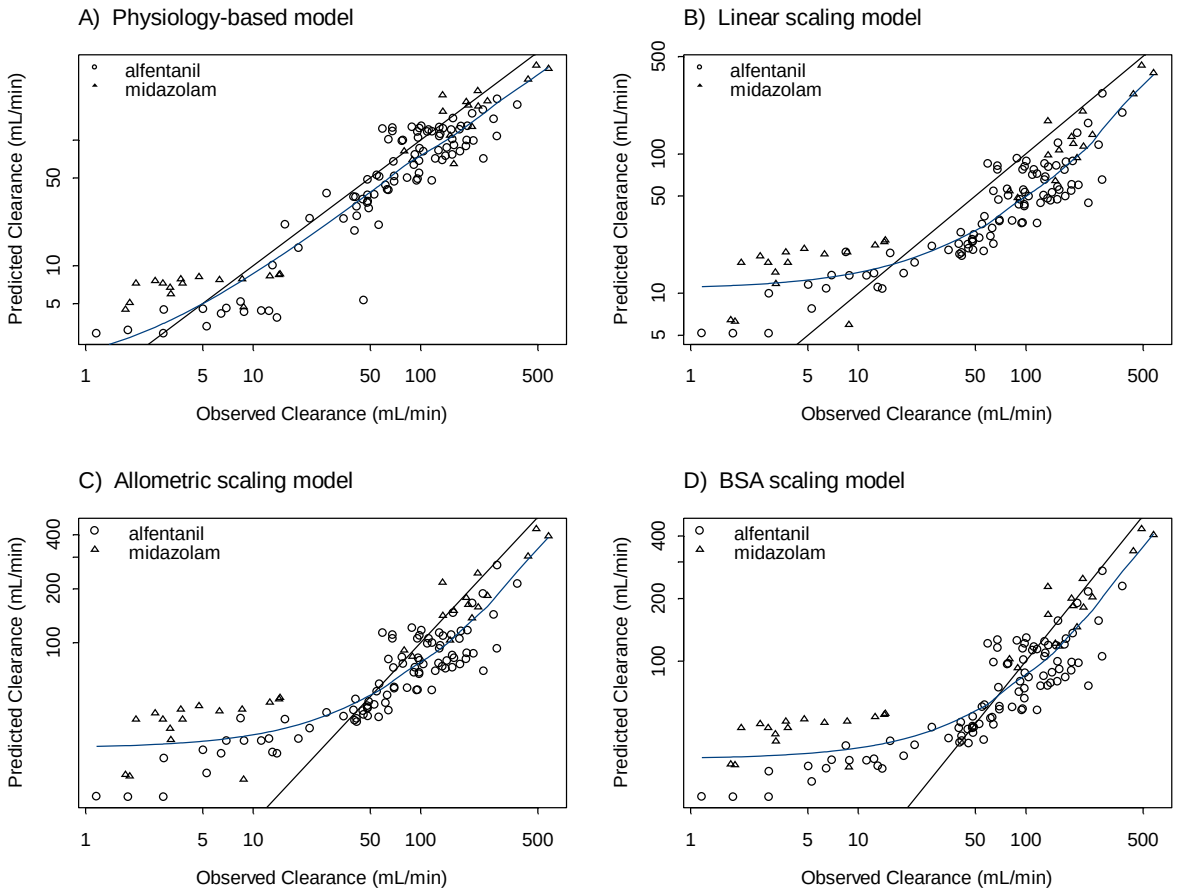

A) Physiology-based model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
alfentanil
midazolam
Predicted Clearance (mL/min)
Observed Clearance (mL/min)
1
5
10
50
100
500
5
10
50
100
200
400

Figure 2

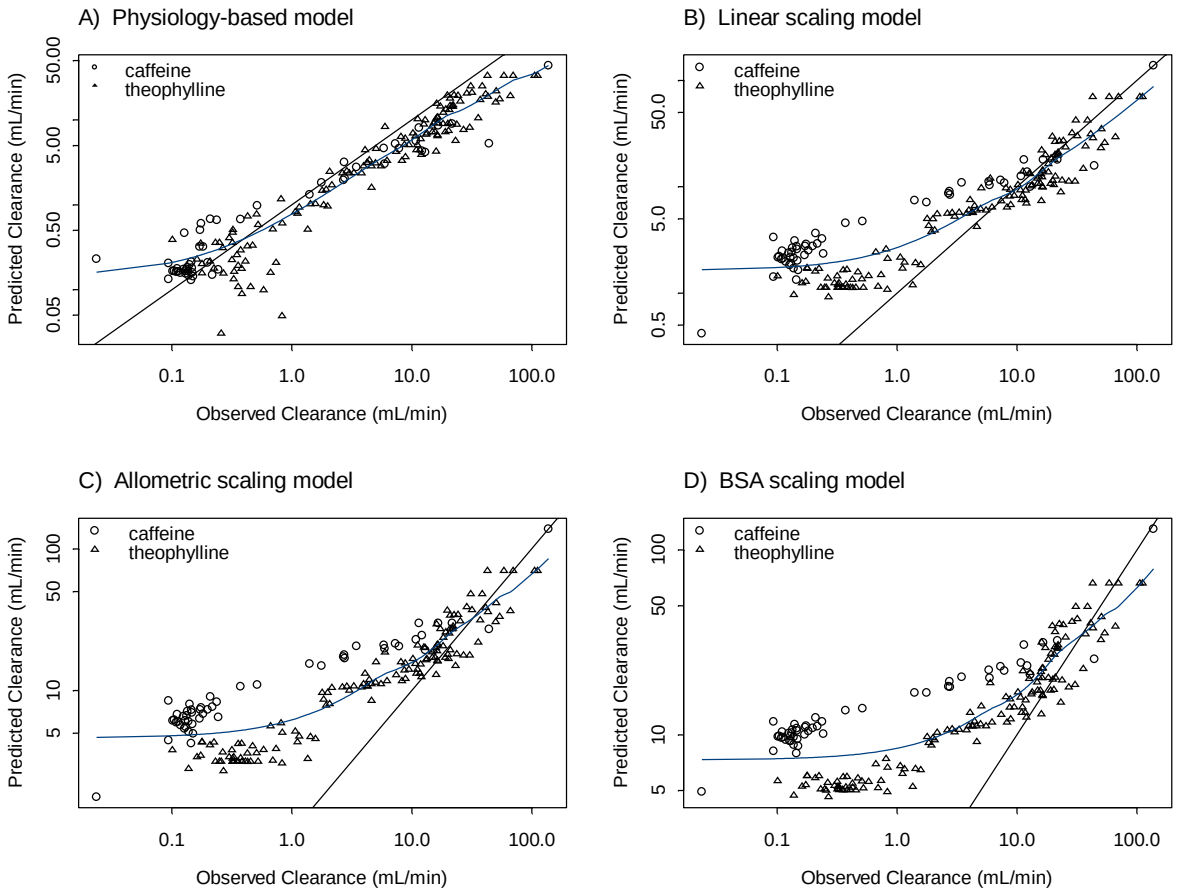

A) Physiology-based model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
caffeine
theophylline
Predicted Clearance (mL/min)
Observed Clearance (mL/min)
0.05
0.50
5.00
50.00
0.5
5.0
50.0
5
10
50
100
0.1
1.0
10.0
100.0

Figure 3

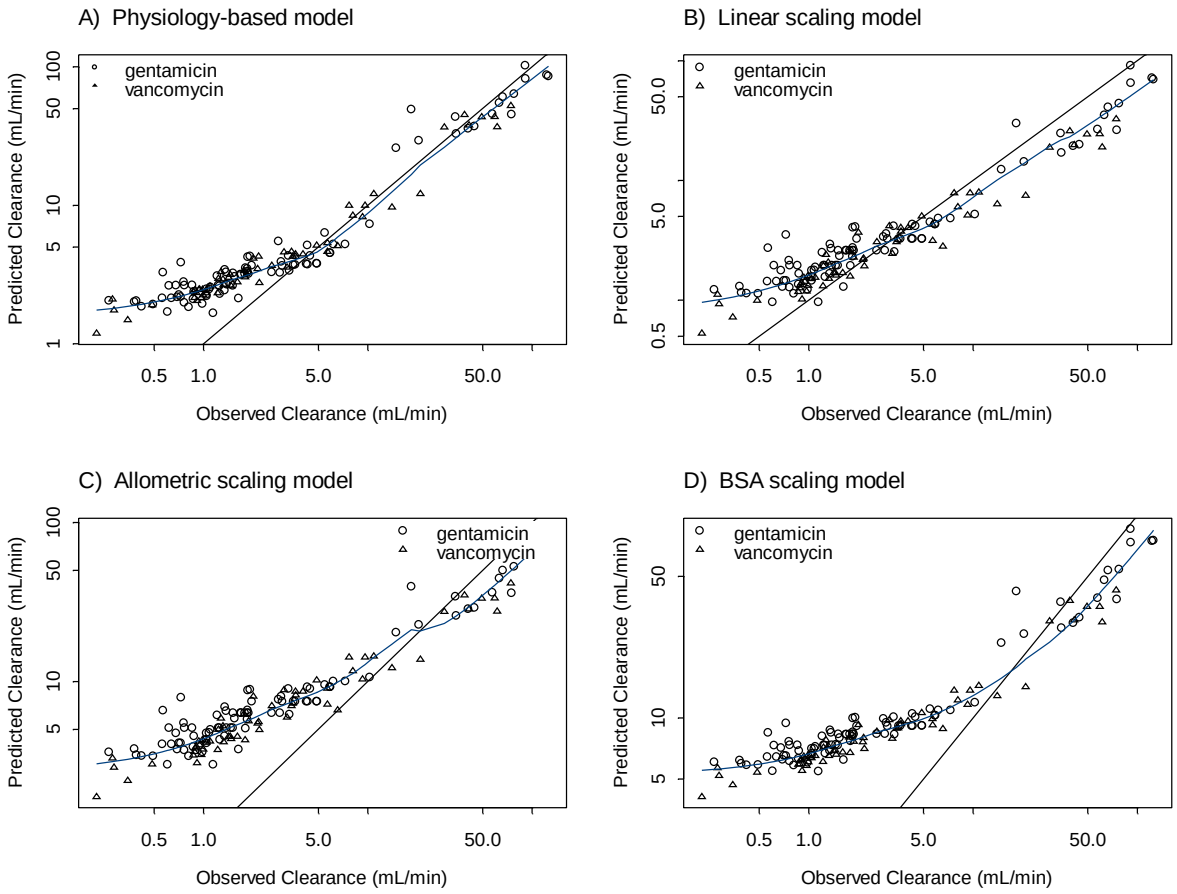

A) Physiology-based model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
gentamicin
vancomycin
Predicted Clearance (mL/min)
Observed Clearance (mL/min)
0.5
1.0
5.0
50.0
1
5
10
50
100
0.5
5.0
50.0

Figure 4

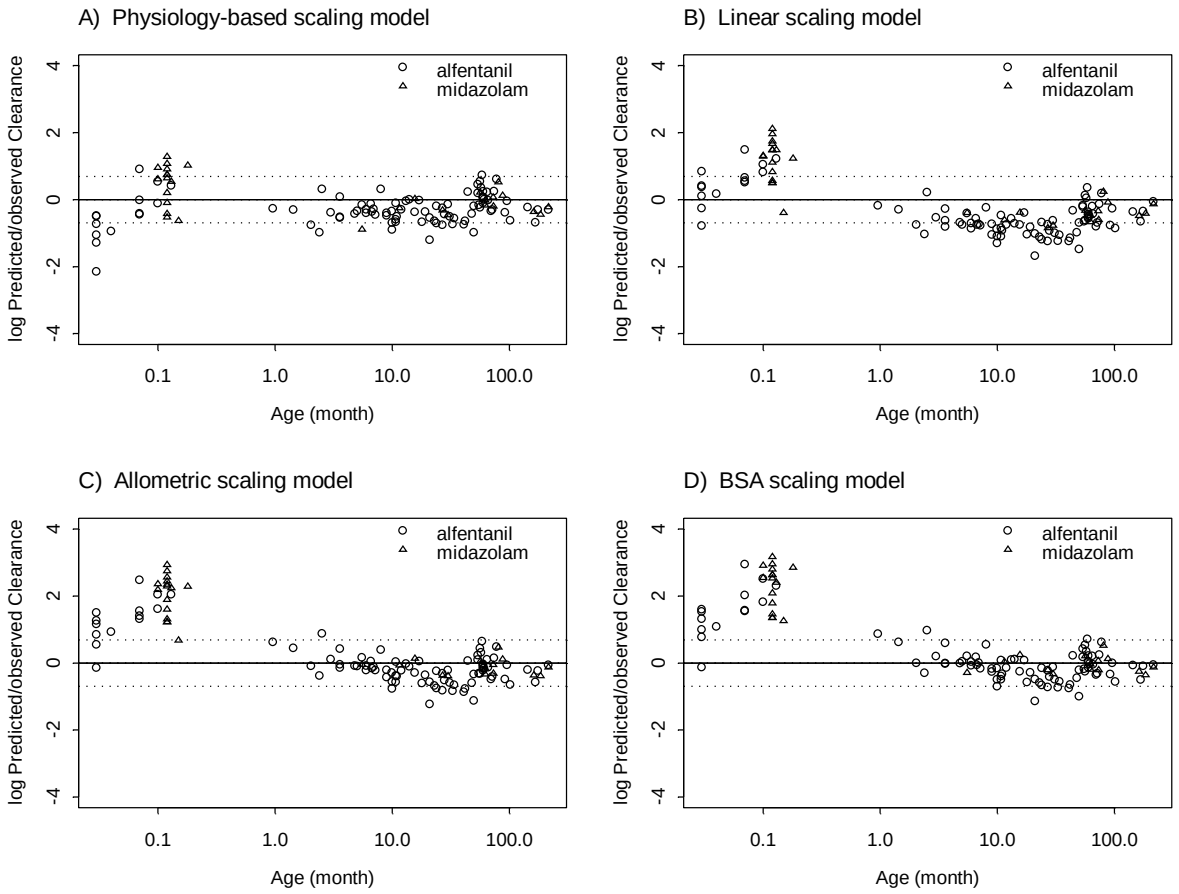

A) Physiology-based scaling model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
alfentanil
midazolam
log Predicted/observed Clearance
Age (month)
0.1
1.0
10.0
100.0
-4
-2
0
2
4

Figure 5

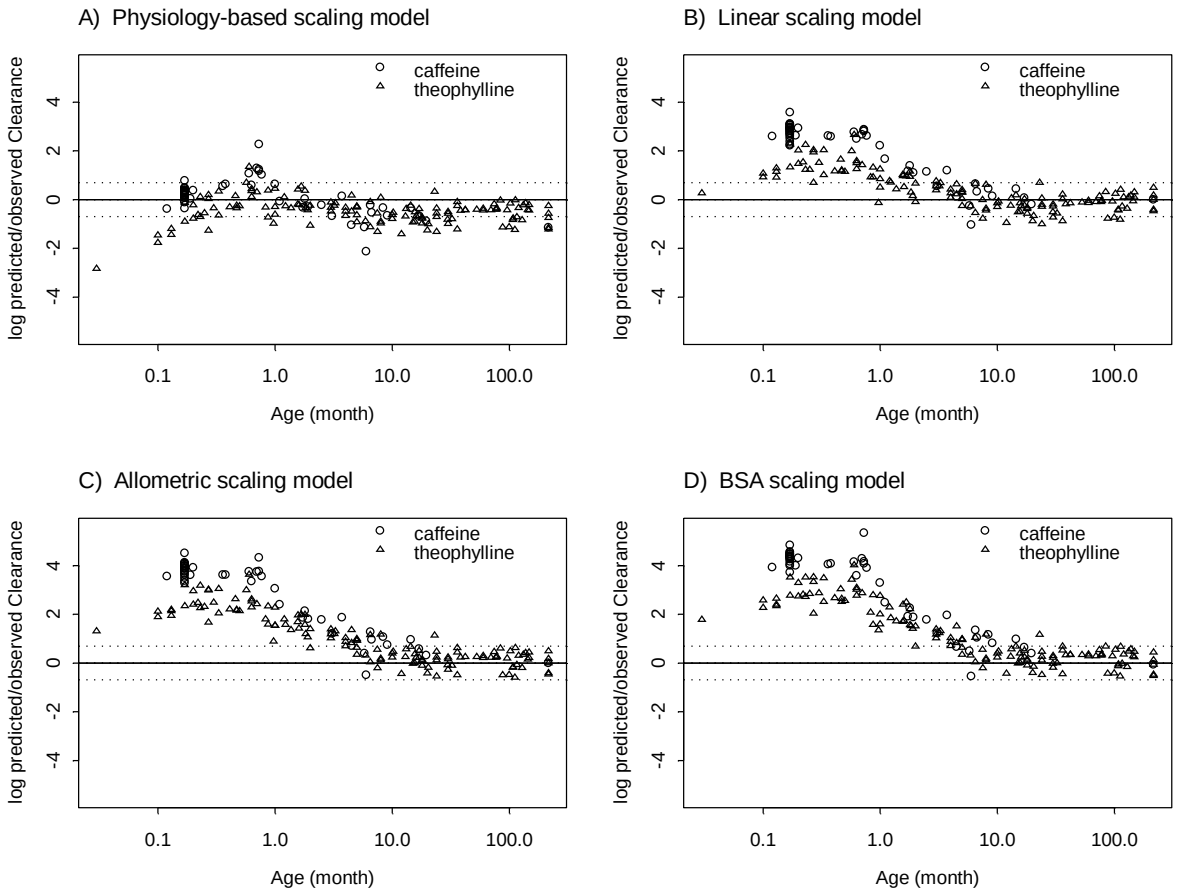

A) Physiology-based scaling model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
caffeine
theophylline
log predicted/observed Clearance
Age (month)
0.1
1.0
10.0
100.0
-4
-2
0
2
4

Figure 6

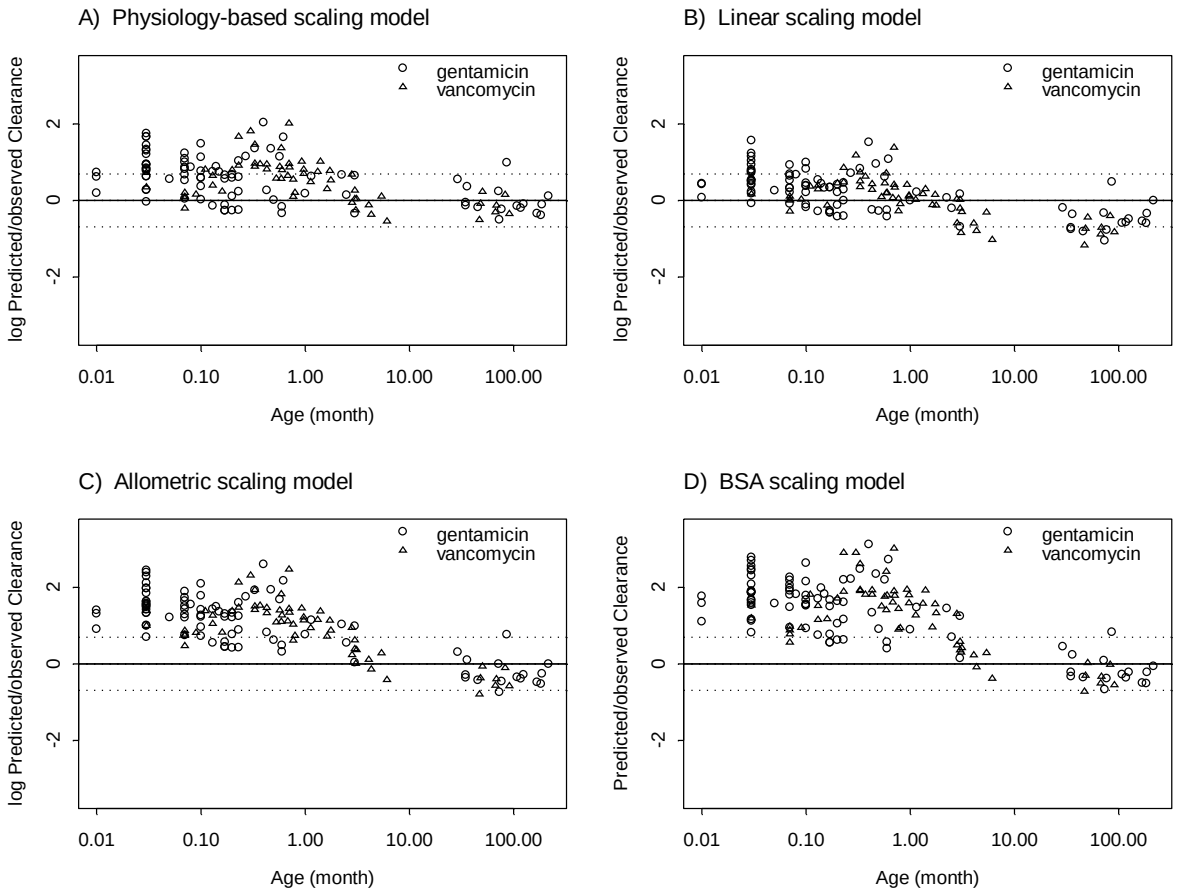
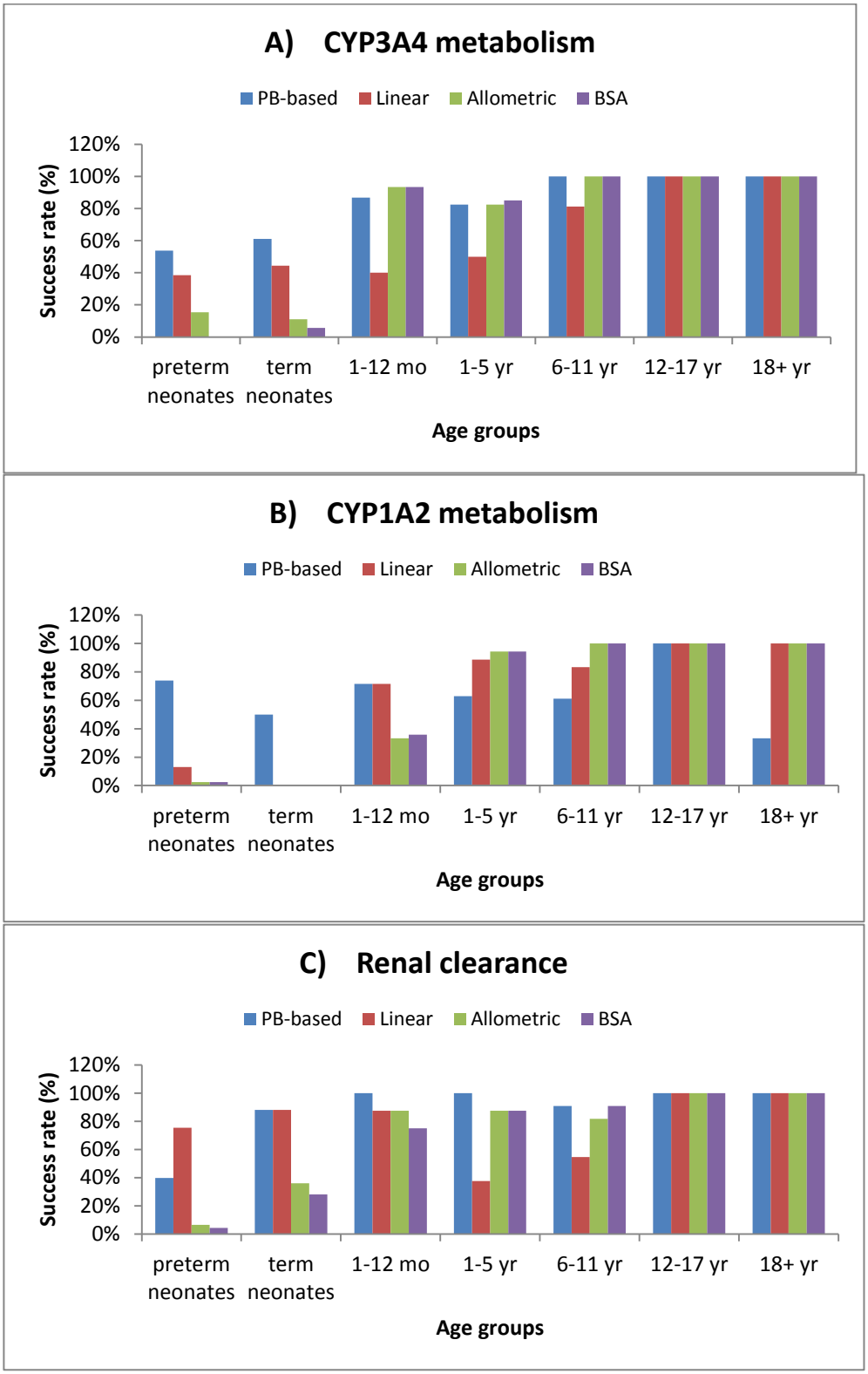

A) Physiology-based scaling model
B) Linear scaling model
C) Allometric scaling model
D) BSA scaling model
gentamicin
vancomycin
log Predicted/observed Clearance
Predicted/observed Clearance
Age (month)
0.01
0.10
1.00
10.00
100.00
-2
0
2

Figure 7


A) CYP3A4 metabolism
PB-based
Linear
Allometric
BSA
Success rate (%)
120%
100%
80%
60%
40%
20%
0%
preterm neonates
term neonates
1-12 mo
1-5 yr
6-11 yr
12-17 yr
18+ yr
Age groups
B) CYP1A2 metabolism
PB-based
Linear
Allometric
BSA
Success rate (%)
120%
100%
80%
60%
40%
20%
0%
preterm neonates
term neonates
1-12 mo
1-5 yr
6-11 yr
12-17 yr
18+ yr
Age groups
C) Renal clearance
PB-based
Linear
Allometric
BSA
Success rate (%)
120%
100%
80%
60%
40%
20%
0%
preterm neonates
term neonates
1-12 mo
1-5 yr
6-11 yr
12-17 yr
18+ yr
Age groups

CHAPTER 5

Maturation of hepatic and renal clearance in pediatric populations

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Abstract

Population modeling in pediatric studies often uses size and age as covariates. Covariate-parameter correlations are described in an exponential relationship used by allometric scaling. However, extrapolations based on such parameter estimates have limited value due to differences in the impact of developmental growth across populations. The quantitative models used to describe the clearance maturation processes across the age range may be required to improve extrapolation and predictive performance.

Previously published pharmacokinetic parameters from probe substrates of hepatic and renal elimination processes are used to develop clearance pathway-specific maturation models. The postconceptional age (PCA) or postnatal age (PNA) are used as covariates in the modeling practice. Clearance maturation of hepatic and renal elimination, after standardized to a 70-kg adult, is appropriately described with a Hill function of postconceptional age. The two renal excreted compounds due to glomerular filtration, gentamicin and vancomycin, presented similar maturation half-life and Hill coefficient, suggesting a system-specific maturation of glomerular filtration function. Similar results were attained with CYP1A2 metabolic probes theophylline and caffeine. Midazolam and alfentanil demonstrated different clearance maturation profiles suggesting a complexity in CYP3A4 metabolism maturation.

Key words: maturation, clearance, CYP, renal GFR

Introduction

The pharmacokinetic (PK) profiles of many drugs are different between children and adults (1). Organ maturation, body composition and ontogeny of drug elimination pathways have marked effects on pharmacokinetics parameters in the first few years of life (2). A principle PK parameter determining age-dependent difference is drug clearance. Understanding the relationship between clearance (CL) and age is useful for drug dosing.

Predicting pediatric CL of drugs used in children has been approached by using physiologically based pharmacokinetic (PBPK) simulation (3). PBPK simulation required detailed physiology data such as in vivo portions of individual elimination pathways contributed to CL, enzyme ontogeny information derived from measurements of enzyme expression and activity in postmortem livers, continuous input on age-related protein binding and physiological data into the simulation algorithm (4, 5). The predicted results have been used to assist with first-time dosing in children when the complete pharmacokinetic data is absent in children (6).

An alternative approach is to use the population PK modeling to obtain age-associated parameters when sparse drug concentration data in children is available. Population modeling in pediatric pharmacokinetic (PK) studies often uses size and age as covariates (7). Covariate-parameter correlations are often described in simple exponential relationships used by allometric scaling (8). However, extrapolations based on such parameter estimates have limited value due to the differences in the impact of developmental growth across populations and the complexity of maturation process (9). The quantitative models that can describe the clearance maturation

processes across the age range, therefore, are required to improve extrapolation and predictive performance. In this study, we collected individual or summary clearance estimates from pediatric PK studies in literature for drugs that are primarily eliminated through CYP3A4 or CYP1A2 metabolism, and renal glomerular filtration. These studies are often restricted to a particular age band e.g. neonates, infants or prepubescent children. It is possible to use CL estimates from these different age groups to develop models for the maturation of renal clearance, and hepatic clearance for CYP3A4 or CYP1A2.

Methods

Age-dependent clearance dataset

A MEDLINE (Medical Literature Analysis and Retrieval System Online) search (1976-present) were, using words such as, *neonate, infant, children* and crossing these with terms such as drug names, pharmacokinetics. Additionally, a variety of pediatric pharmacology reviews were examined. A database of age-dependent observed clearances (Appendix I), in neonates, young infants, children, adolescents and adults was created for therapeutic probes, which alfentanil and midazolam representing CYP3A4 metabolism, caffeine and theophylline representing CYP1A2 metabolism, gentamicin and vancomycin representing renal glomerular filtration. Table 1 was extracted from the database to illustrate this pattern and to show how the data has been compiled and organized.

Modeling CL maturation

Two developmental models were evaluated for all the six model compounds, using nonlinear regression with NONMEM VI (Globomax LLC, Hanover, MD, USA). The first model used post conception age (PCA) as a covariate (Eq. 1). In the second model, postnatal age (PNA) was used (Eq. 2).

$$CL_i = CL_{std} \times (\frac{W_i}{70})^{0.75} \times \frac{PCA^{\theta}}{Tcl^{\theta}+PCA^{\theta}} \qquad Equation\ 1$$

$$CL_i = CL_{std} \times (\frac{W_i}{70})^{0.75} \times (1 + \beta cl \times EXP \ -PNA \times \frac{ln2}{Tcl}\ ) \qquad Equation\ 2$$

Where CL_i is the observed clearance (mL/min) in the individual person of weight W_i, CL_{std} (mL/min/70 kg) is the typical clearance value in a standard size adult of 70 k; θ is the Hill coefficient of for clearance; βcl is a parameter estimating the fraction above or below clearance; Tcl is a parameter describing the maturation half-lives of the age-related changes of CL. These functions have been used in the developmental population PK analysis in pediatric clinical studies. Ages (PNA and PCA) were expressed in months by suing the following rules: 1 year = 12 months and 1 month = 30 days = 4.33 weeks, so that 1 year = 52 weeks. For all subjects older than age 3 months, PCA was calculated as PNA + 9.

The allometric weight scaling describes the size effect and the Hill function in this equation is used as an empirical capacity limited model for the maturation of renal function. The descriptive performance was compared between two models for all the model compounds, using visual inspection of fits and precision of estimates. The predictive performance was assessed by comparing with the observed data set to the 5 and 95^{th} percentiles of model-simulated data (n=500).

Results

Gentamicin and vancomycin clearance maturation was better described using PCA model (model 1) than PNA model (model 2). The descriptive performance of model 1 was slightly better than the model 2, based on evaluation of objective function, basic goodness-of-fit plots (Figure 1) and the precision of parameter estimates (Table 2). The predictive performance of the model using model 1 was similar compared to the model 2 considering the visual predictive check results (Figure 2).

The adult clearance (Clstd) of gentamicin and vancomycin predicted by the two models were similar. In the first model, the time to reach 50% of adult clearance was 11 months (PCA) for gentamicin and 9.5 month (PCA) for vancomycin. By 1 year of age, both vancomycin and gentamicin clearance had attained more than 90% of the adult level.

PCA sigmoidal hyperbolic model produced better prediction for alfentanil and midazolam clearance data than PNA model, according to performance plots (Figure 3 and 4) and the precision of parameter estimates (Table 3). Alfentanil CL at birth in a term neonate was 50% of an adult value and reached the adult level by 6 month. Midazolam clearance at birth in a term neonate was 27% of an adult, reaching 64% by 1 year of old and 80% of the matured CL at the age of 2 years. The half-life estimates (Tcl) indicated that alfentanil metabolism developed faster than midazolam metabolism.

The maturation of caffeine and theophylline clearance was better fitted using PCA sigmoidal model (Figure 5 and 6). The parameters were presented in Table 4. In a term neonate, the caffeine clearance at birth was 8% of an adult, increasing to 50% at 6 months and by 2 years of age achieved 95% of matured activity. Theophylline clearance developed in a similar rate, reaching 50% level at 4 month of age and 95% by 2 years.

Discussion

Growth and development are two major aspects of children not seen in adults. These aspects can be investigated by using size and age as covariates in pediatric PK studies. To identify other covariates other than body size, it is highly desirable to standardize the PK parameters to an appropriate body size measure. Once size is standardized, age or other physiological factors (i.e. GFR) can be investigated within a given dataset describing time-concentration profiles in a population. The quantitative models used to describe the maturation process vary depending on the range of the ages under investigation. An exponential age model is commonly used for population samples limited to a defined age band (10). However, this exponential function extrapolates badly beyond the range of observations. An exponential asymptotic model, similar to models based on first-order processes in biology systems, has been previously used to investigate acetaminophen age-related clearance maturation (11). A sigmoidal Emax model has been used to investigate acyclovir clearance in neonates and infants (12).

In our study, we found a sigmoidal E_{max} model is a robust model to describe clearance maturation during infancy for different hepatic and renal elimination processes.

A plot of maturation profiles of 6 drugs representing 3 clearance processes is displayed in Figure 7. The alfentanil clearance matures more rapid, compared to the clearance of renal probes and theophylline and caffeine. The results are consistent with the in vitro development profiles of CYP3A4 and CYP1A2 enzymatic activities (13-15).

While maturation profile of midazolam in our study is similar to that predicted by Anderson et al. (16), it is slower than that observed in other drugs cleared by CYP3A4 such as alfentanil in our study and sildenafil. It has been proposed that CYP3A4 possesses three substrate binding types (Kenworthy, et al., 1999). Three CYP3A4 marker activities: testosterone 6β-hydroxylase, midazolam 1'-hydroxylase, and felodipine dehydrogenase activities represent the aforementioned three substrate binding types. Metabolism of midazolam by 1- and 4-hydroxylation closely resembles the probe reactions utilizing testosterone and diazepam. The predominating metabolic reactions for alfentanil are piperidine and amid N-dealkylation, catalyzed by CYP3A4, CYP3A5 and CYP3A7. CYP3A5 and CYP3A7 may have contributed significantly to the high clearance of alfentanil in neonates at birth and the rapid maturation profile, in comparison with midazolam. The substrate dependency of CYP3A4 maturation produced multiple developmental models for CYP3A4 metabolized drugs.

Renal function is influenced by physiological changes such as glomerular filtration rate (GFR) and tubular secretion/absorption processes at different stages of

development. Gentamicin and vancomycin are almost entirely eliminated by kidney (17). In our study, the maturation rates of clearance values of the two antibiotics are similar. Thus, a developmental model for GFR was developed from clearance values of the two drugs throughout pediatric life until adults. It is anticipated that the renal developmental model can be used to describe maturation in clearance of other renally excreted antibiotics in neonates and infants. This approach will make a distinction between system specific and drug specific information in pediatric pharmacokinetics models (Eq. 3).

$$CL_i = CL_p \times (\frac{W_i}{70})^{0.75} \times MF \qquad Equation\ 3$$

Using this approach, CL_p is considered a drug specific property and is therefore estimated for each of drugs separately. The remaining information in this equation is considered system specific information that can be applied for all renally excreted drugs.

A critical question in the prediction of CL using physiology-based approach is when the activity of the applicable CYP isoforms attains adult levels. There are not enough data on this from in vitro studies. CYP1A2 develops relatively slow compared with CYP2C, 2D6 and 3A. For example, about half of the adult CYP1A2 activity was reached at 1-year after birth, while about 80% the adult CYP3A activity was reached at 1 year of age (18). The in vitro ontogeny data is still not available for CYP1A2 of which activity reaches 80% of the adult level (15). Thus, using bottom-up PBPK approach to predict clearance in children from birth to 18 year old led to under

predictions in older children. In addition, in vitro assays used to determine the age-dependency of enzyme activity can vary greatly depending on the substrates, which led to variable clearance predictions. Therefore, maturation rates based on in vivo PK studies can be used as a systemic maturation function to obtain more accurate predictions in children.

It may not always be necessary to repeat PK studies in children when similar examples exist in literature. Literature information from similar compounds that mechanistically describes the maturation process and changes in organ functions can be used in the covariate analysis for the other compounds, or be extrapolated to a new population. The current study, using published CL values, demonstrated that size and age models yields a reasonable maturation profiles for CYP1A2 probe drugs and renal elimination. The maturation models and rate estimates can be generalized as systemic information in the PK simulations of drugs that are cleared via the two pathways.

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Table 1 A section of children's clearance database.

pathway	drug	study	subjn	GA	GA.unit	PNA	PNA.unit	BW	BW.unit	CL	CL.sd	CL.unit
renal	gentamicin	Rodvold 1993	19	28.7	wk	34.6	d	1.78	kg	1.04	0.37	mL/min/kg
renal	gentamicin	Kildoo 1984	15	28.4	wk	1	d	1	kg	0.38	0.14	mL/min/kg
renal	gentamicin	Kildoo 1984	15	28.4	wk	18.7	d	0.887	kg	0.44	0.18	mL/min/kg
renal	gentamicin	Kildoo 1984	6	28.4	wk	67.5	d	1.49	kg	1.21	0.39	mL/min/kg
renal	gentamicin	Rocha 2000	68	29.5	wk	3	d	1.2	kg	1.05	0.29	mL/min/kg
renal	gentamicin	Rocha 2000	34	27	wk	3	d	0.875	kg	0.48	0.13	mL/min/kg
renal	gentamicin	Rocha 2000	34	32	wk	3	d	1.5	kg	0.57	0.16	mL/min/kg
renal	gentamicin	Ho 1995	14	39	wk	3.8	y	15.3	kg	2.91	0.60	mL/min/kg
renal	gentamicin	Ho 1995	7	39	wk	2.9	y	13.1	kg	2.64	0.54	mL/min/kg
renal	gentamicin	Ho 1995	13	39	wk	2.9	y	14.9	kg	2.74	0.63	mL/min/kg
renal	gentamicin	Ho 1995	8	39	wk	9.8	y	33.9	kg	2.29	0.72	mL/min/kg
renal	gentamicin	Ho 1995	6	39	wk	10.4	y	31.5	kg	2.11	0.53	mL/min/kg
renal	gentamicin	Ho 1995	5	39	wk	9	y	27	kg	2.34	0.46	mL/min/kg
renal	gentamicin	Ho 1995	7	39	wk	14	y	55.1	kg	2.23	0.77	mL/min/kg
renal	gentamicin	Ho 1995	10	39	wk	15.6	y	50.2	kg	1.82	0.37	mL/min/kg
renal	gentamicin	Ho 1995	7	39	wk	15.3	y	53.5	kg	2.35	0.57	mL/min/kg
renal	gentamicin	Pons 1988	15	37	wk	1	d	3.2	kg	1.03	0.37	mL/min/kg
renal	gentamicin	Pons 1988	8	37	wk	5	d	3.3	kg	1.79	0.64	mL/min/kg
renal	gentamicin	Pons 1988	1	37	wk	18	d	3.7	kg	1.67	0.00	mL/min/kg
renal	gentamicin	Pons 1988	27	39	wk	1	d	3.2	kg	1.40	0.46	mL/min/kg
renal	gentamicin	Pons 1988	16	39	wk	5	d	3.3	kg	1.78	0.39	mL/min/kg
renal	gentamicin	Pons 1988	14	39	wk	18	d	3.7	kg	1.97	0.44	mL/min/kg

Table 2 Gentamicin and vancomycin maturation clearance parameter estimates.

Parameter	Gentamicin		Vancomycin	
Model 1				
OBJ	582		423	
CL_{std} (ml/min/70 kg)	109	(6.5%)	103	(8.4%)
Tcl (PCA, month)	10.9	(5.9%)	9.93	(5.0%)
θ	3.13	(11.6%)	4.31	(10.5%)
RUV	0.137	(13.0%)	0.08	(16.6%)
Model 2				
OBJ	629		478	
CL_{std} (ml/min/70 kg)	101	(7.4%)	98.8	(8.5%)
Tcl (PNA, month)	1.54	(93.5%)	4.79	(19.8%)
βcl	-0.78	(4.5%)	-0.76	(4.1%)
RUV	0.209	(12.7%)	0.159	(15.2%)

OBJ: objective function value; RUV: residual unexplained variability

Table 3 Alfentanil and midazolam maturation clearance parameter estimates.

Parameter	Alfentanil		Midazolam	
Model 1				
OBJ	861		278	
CL_{std} (ml/min/70 kg)	371	(5.4%)	452	(6%)
Tcl (PCA, month)	10.4	(7.6%)	15.4	(16.4%)
θ	4.7	(20.2%)	1.82	(25.8%)
RUV	0.165	(16.2%)	0.318	(24.6%)
Model 2				
OBJ	873		246	
CL_{std} (ml/min/70 kg)	371	(5.2%)	459	(5.1%)
Tcl (PNA, month)	3.5	(25.9%)	0.14	(26.3%)
βcl	-0.69	(12.6%)	-1.51	(13.3%)
RUV	0.18	(16.5%)	0.154	(24.7%)

OBJ: objective function value; RUV: residual unexplained variability

Table 4 Caffeine and theophylline maturation clearance parameter estimates.

Parameter	Caffeine		Theophylline	
Model 1				
OBJ	690		656	
CL_{std} (ml/min/70 kg)	138	(22.8%)	59.7	(4%)
Tcl (PCA, month)	15.2	(.1%)	12.8	(5.3%)
θ	4.81	(8.9%)	2.88	(8.7%)
RUV	0.289	(28.4%)	0.196	(11.7%)
Model 2				
OBJ	760		631	
CL_{std} (ml/min/70 kg)	65.6	(23.5%)	60	(3.7%)
Tcl (PNA, month)	0.532	(40.9%)	5.27	(1.3%)
βcl	-1.21	(8.4%)	-0.892	(2%)
RUV	0.706	(28.8%)	0.176	(3%)

OBJ: objective function value; RUV: residual unexplained variability

Figure legends

Figure 1 Observed vs predicted clearance for gentamicin and vancomycin using sigmoidal hyperbolic model (model 1: A and C) and first order model (model 2: B and D)

Figure 2 Observed and Predicted clearance values vs age (PCA and PNA) for gentamicin and vancomycin.

Figure 3 Observed vs predicted clearance for alfentanil and midazolam using sigmoidal hyperbolic model (model 1: A and C) and first order model (model 2: B and D)

Figure 4 Observed and Predicted clearance values vs age (PCA and PNA) for alfentanil and midazolam.

Figure 5 Observed vs predicted clearance for caffeine and theophylline using sigmoidal hyperbolic model (model 1: A and C) and first order model (model 2: B and D)

Figure 6 Observed and Predicted clearance values vs age (PCA and PNA) for caffeine and theophylline.

Figure 7 Predicted clearance maturation profiles using model 1 for renal GFR excreted drugs-gentamicin and vancomycin (A), CYP3A4 substrates-midazolam and alfentanil (B) and CYP1A2 substrates-caffeine and theophylline (C).

Figure 1

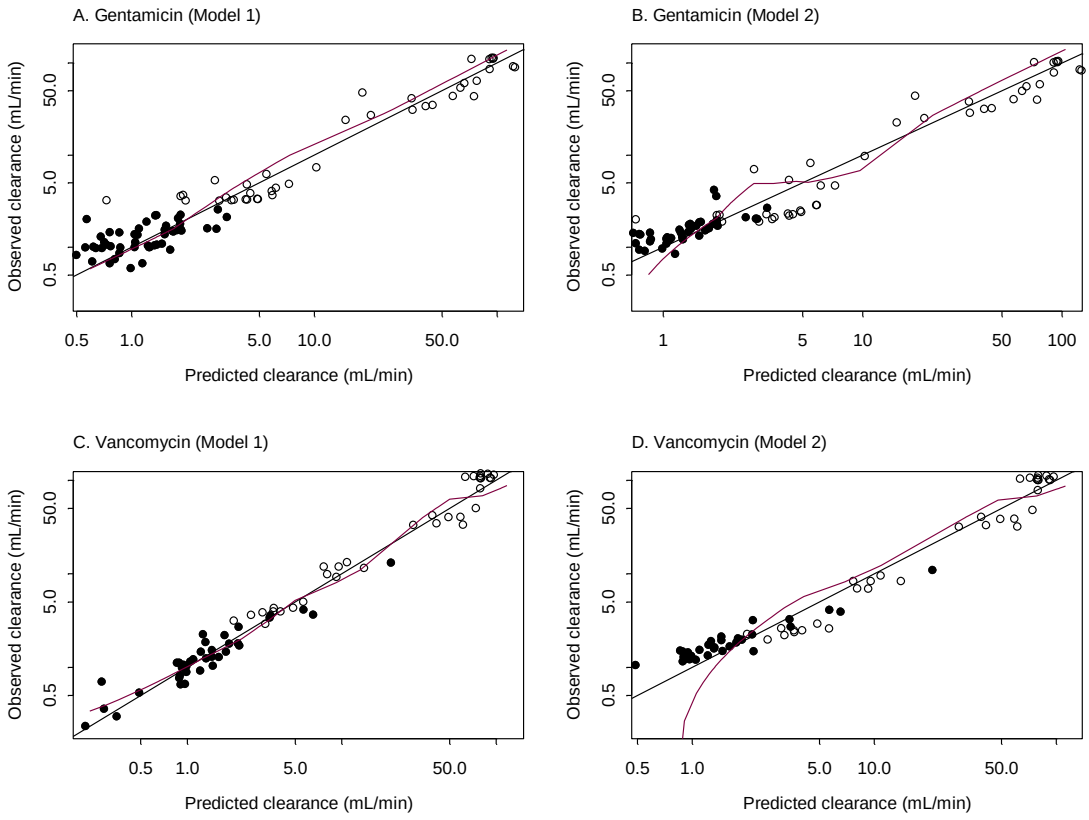

A. Gentamicin (Model 1)
B. Gentamicin (Model 2)
C. Vancomycin (Model 1)
D. Vancomycin (Model 2)
Observed clearance (mL/min)
Predicted clearance (mL/min)
0.5
5.0
50.0
1.0
10.0
1
10
100

Figure 2

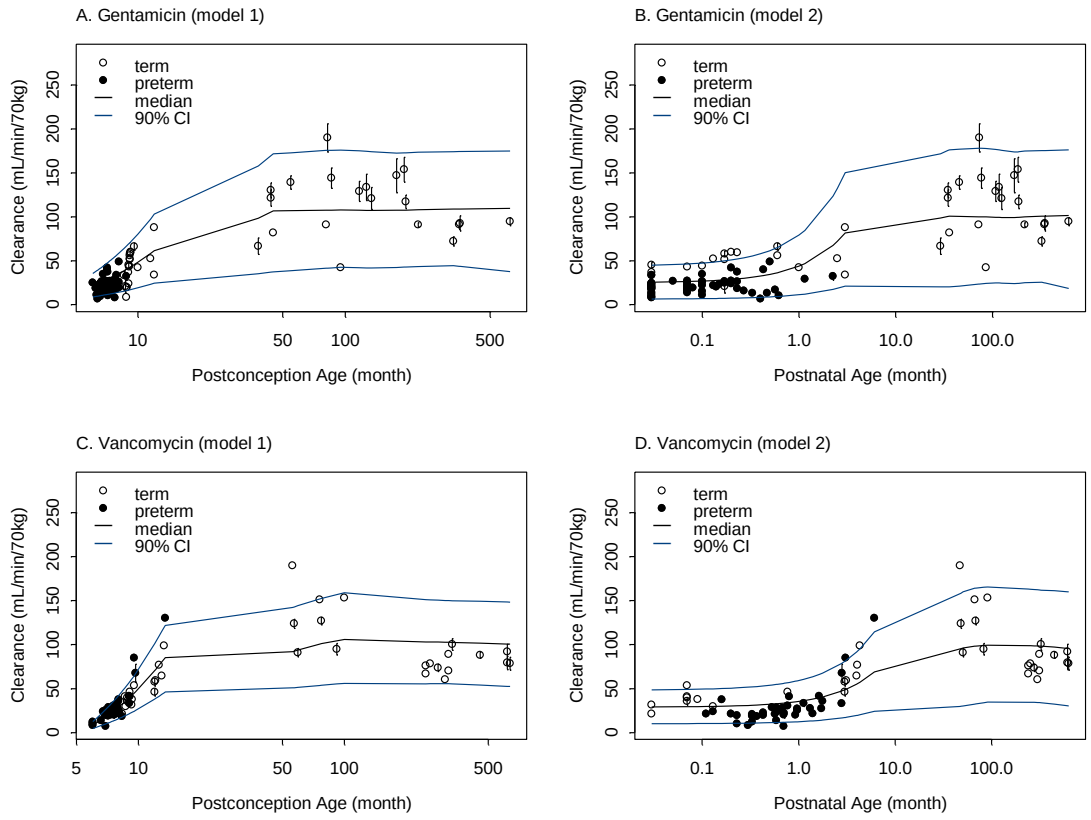

A. Gentamicin (model 1)
B. Gentamicin (model 2)
C. Vancomycin (model 1)
D. Vancomycin (model 2)
term
preterm
median
90% CI
Clearance (mL/min/70kg)
0
50
100
150
200
250
10
50
100
500
5
0.1
1.0
10.0
100.0
Postconception Age (month)
Postnatal Age (month)

Figure 3

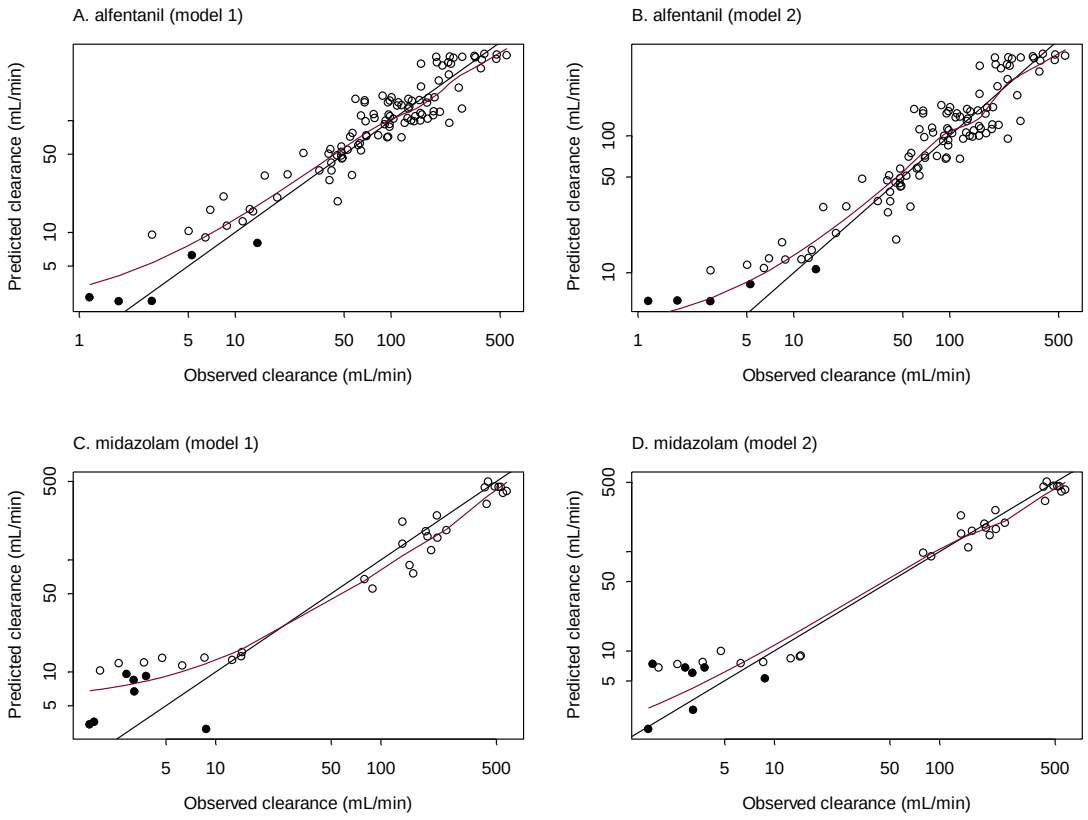

A. alfentanil (model 1)
Predicted clearance (mL/min)
Observed clearance (mL/min)
1
5
10
50
100
500
B. alfentanil (model 2)
Predicted clearance (mL/min)
Observed clearance (mL/min)
C. midazolam (model 1)
Predicted clearance (mL/min)
Observed clearance (mL/min)
D. midazolam (model 2)
Predicted clearance (mL/min)
Observed clearance (mL/min)

Figure 4

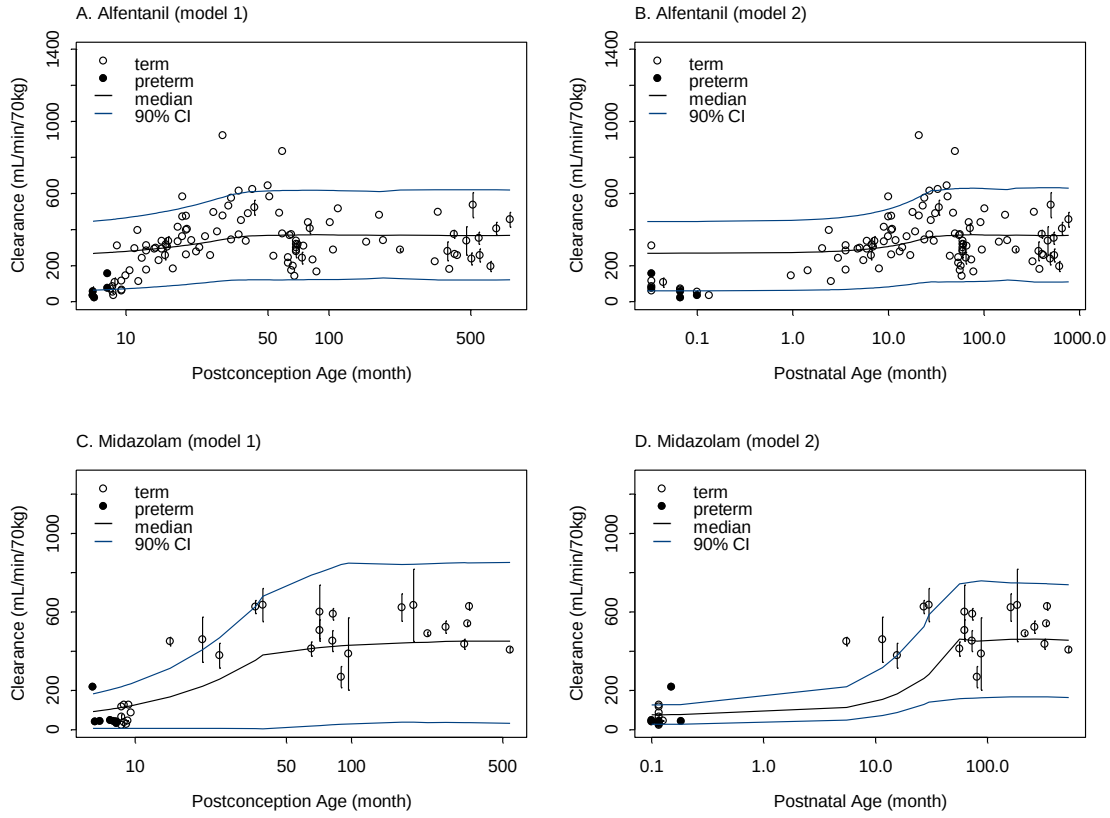

A. Alfentanil (model 1)
B. Alfentanil (model 2)
C. Midazolam (model 1)
D. Midazolam (model 2)
term
preterm
median
90% CI
Clearance (mL/min/70kg)
0
200
600
1000
1400
10
50
100
500
0.1
1.0
10.0
100.0
1000.0
Postconception Age (month)
Postnatal Age (month)

Figure 5

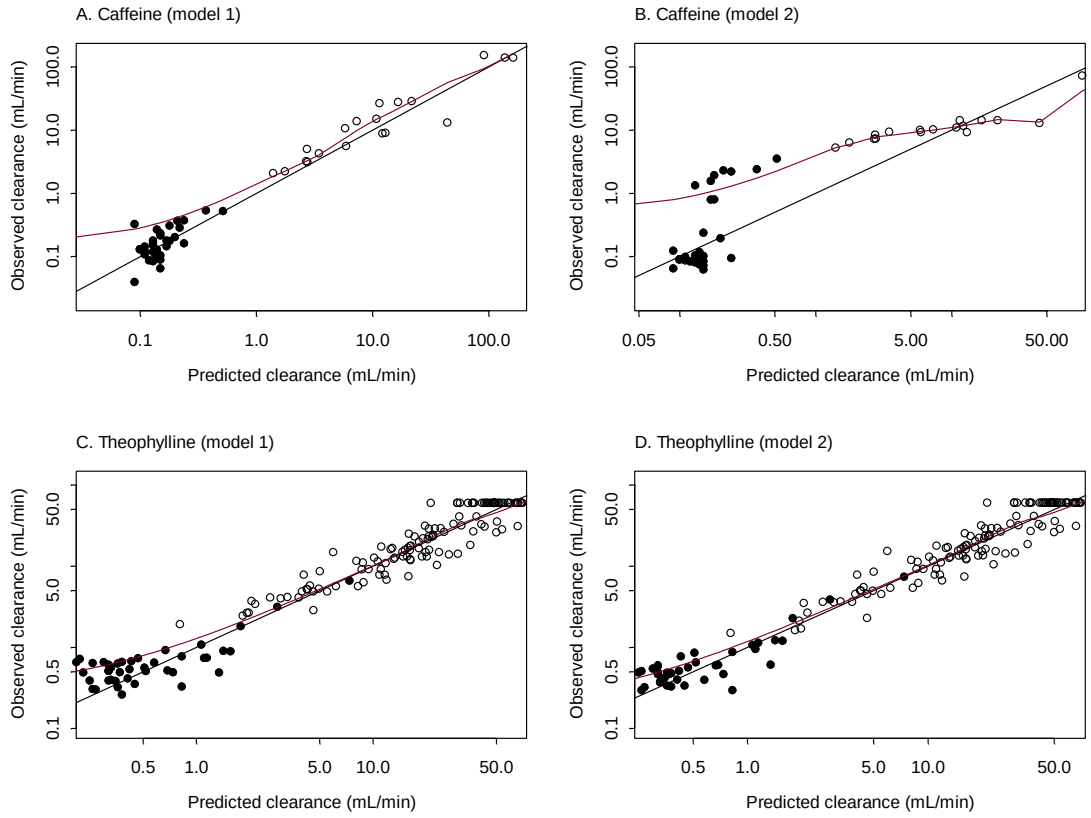

A. Caffeine (model 1)
Observed clearance (mL/min)
0.1
1.0
10.0
100.0
0.1
1.0
10.0
100.0
Predicted clearance (mL/min)
B. Caffeine (model 2)
Observed clearance (mL/min)
0.1
1.0
10.0
100.0
0.05
0.50
5.00
50.00
Predicted clearance (mL/min)
C. Theophylline (model 1)
Observed clearance (mL/min)
0.1
0.5
5.0
50.0
0.5
1.0
5.0
10.0
50.0
Predicted clearance (mL/min)
D. Theophylline (model 2)
Observed clearance (mL/min)
0.1
0.5
5.0
50.0
0.5
1.0
5.0
10.0
50.0
Predicted clearance (mL/min)

Figure 6

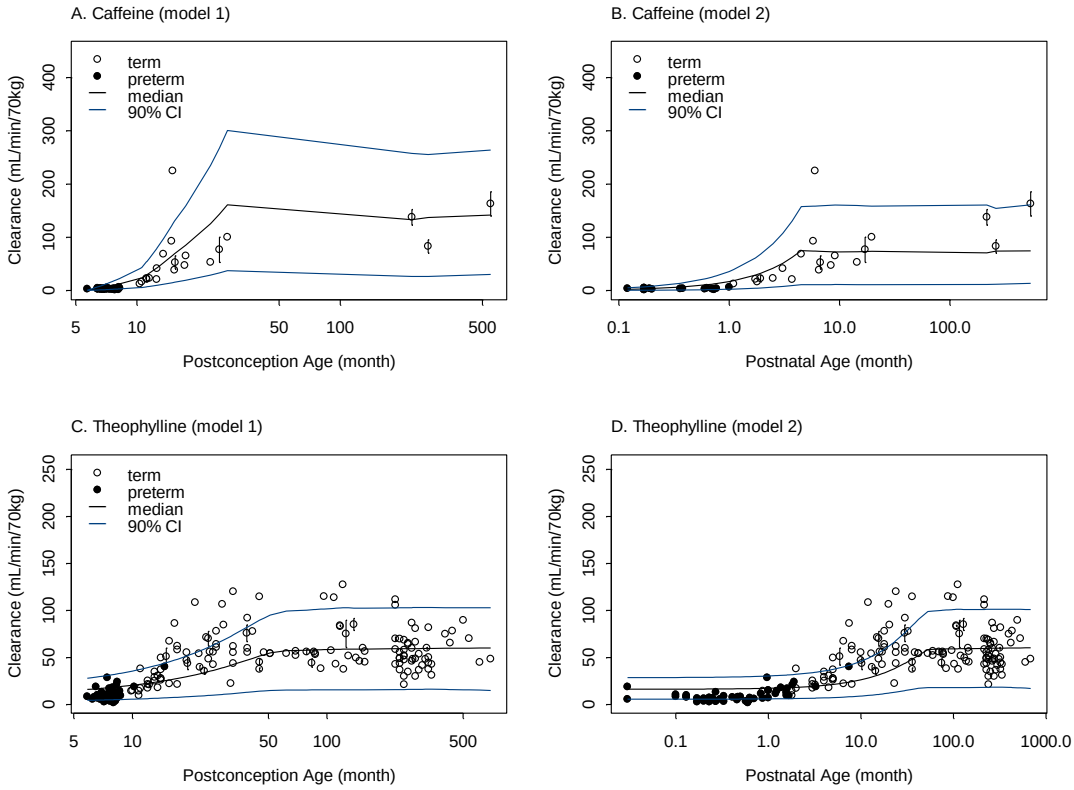
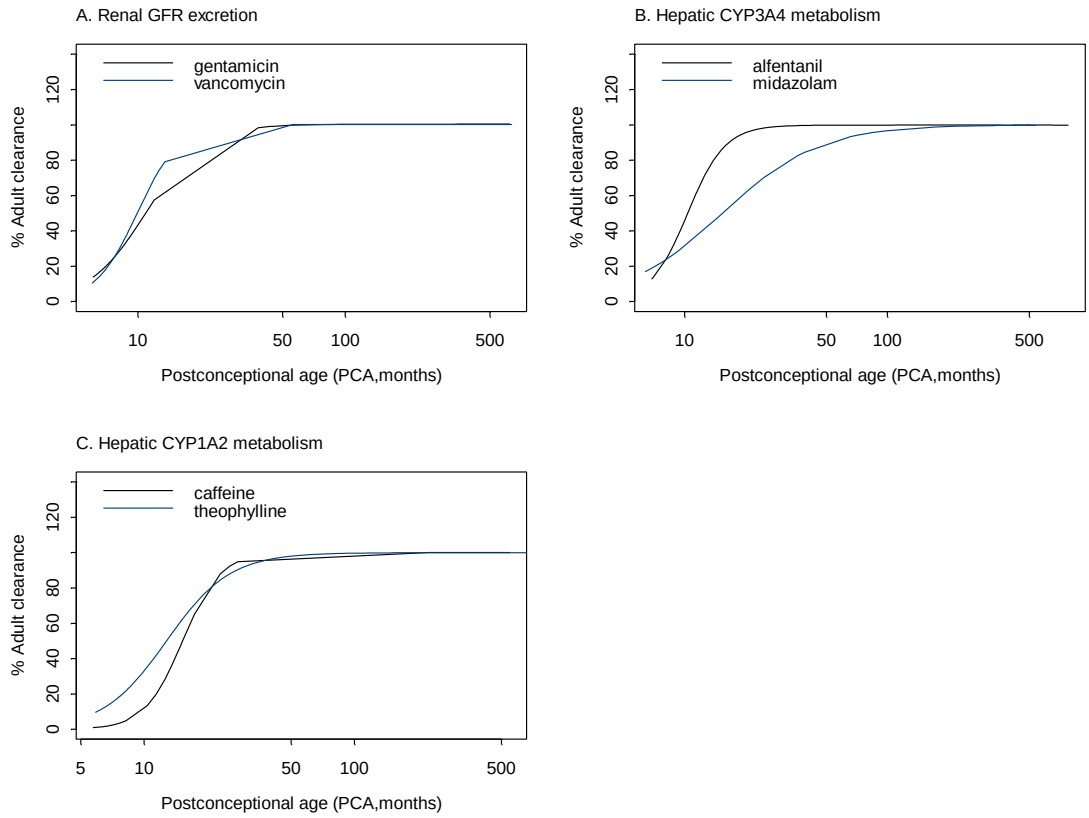

A. Caffeine (model 1)
B. Caffeine (model 2)
C. Theophylline (model 1)
D. Theophylline (model 2)
term
preterm
median
90% CI
Clearance (mL/min/70kg)
Postconception Age (month)
Postnatal Age (month)

Figure 7


A. Renal GFR excretion
gentamicin
vancomycin
% Adult clearance
0
20
40
60
80
120
10
50
100
500
Postconceptional age (PCA,months)
B. Hepatic CYP3A4 metabolism
alfentanil
midazolam
% Adult clearance
0
20
40
60
80
120
10
50
100
500
Postconceptional age (PCA,months)
C. Hepatic CYP1A2 metabolism
caffeine
theophylline
% Adult clearance
0
20
40
60
80
120
5
10
50
100
500
Postconceptional age (PCA,months)

APPENDICES

Appendix I Age-dependent clearance dataset

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	alfentanil	Wiest 1991(1)	1	35	1	d	2	2.65	0
CYP3A4	alfentanil	Wiest 1991	1	41	1	d	3.6	3.47	0
CYP3A4	alfentanil	Wiest 1991	1	41	1	d	5.1	1.66	0
CYP3A4	alfentanil	Wiest 1991	1	35	1	d	2.79	5.00	0
CYP3A4	alfentanil	Wiest 1991	1	37	1	d	3.47	2.56	0
CYP3A4	alfentanil	Wiest 1991	1	39	1	d	5.44	8.40	0
CYP3A4	alfentanil	Wiest 1991	1	37	4	d	2.58	1.14	0
CYP3A4	alfentanil	Wiest 1991	1	36	2	d	2.8	2.31	0
CYP3A4	alfentanil	Wiest 1991	1	41	2	d	3.48	2.00	0
CYP3A4	alfentanil	Wiest 1991	9	38	1.33	d	3.47	3.24	2.23
CYP3A4	alfentanil	Marlow 1990	22	30	2	d	1.34	0.87	0.00
CYP3A4	alfentanil	Killian 1990	5	29.3	3	d	1.33	1.35	0.69
CYP3A4	alfentanil	Davis 1989	6	29.5	2	d	1.33	2.20	2.4
CYP3A4	alfentanil	Davis 1989	9	39	5	y	20	5.60	2.4
CYP3A4	alfentanil	Killian 1990	5	36.8	3	d	2.97	1.70	0.47
CYP3A4	alfentanil	Bower 1982	7	39	41.3	y	70.3	3.40	8.01
CYP3A4	alfentanil	Bower 1982	5	39	39	y	71.6	4.80	13.95
CYP3A4	alfentanil	Bovill 1982	6	39	42	y	73	7.58	2.39
CYP3A4	alfentanil	Bovill 1982	5	39	45	y	69	5.06	1.06
CYP3A4	alfentanil	Scott 1987	17	39	51.5	y	70	2.80	1.6
CYP3A4	alfentanil	Persson 1988	10	39	45.5	y	68.2	3.70	1.6
CYP3A4	alfentanil	Van den Nieuwenhuyzen 1993	20	39	33.8	y	75.8	5.25	1.3
CYP3A4	alfentanil	Hudson 1991	11	39	64.3	y	74.7	6.40	1.9
CYP3A4	alfentanil	den Hollander 1988	6	39	0.56	y	6.45	8.21	2.13

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	alfentanil	den Hollander 1988	5	39	5.1	y	18.6	6.30	0.8
CYP3A4	alfentanil	den Hollander 1988	1	39	0.3	y	4.3	5.07	0
CYP3A4	alfentanil	den Hollander 1988	1	39	0.4	y	6.3	7.64	0
CYP3A4	alfentanil	den Hollander 1988	1	39	0.5	y	5.8	7.80	0
CYP3A4	alfentanil	den Hollander 1988	1	39	0.5	y	5.3	9.16	0
CYP3A4	alfentanil	den Hollander 1988	1	39	0.75	y	8.7	8.02	0
CYP3A4	alfentanil	den Hollander 1988	1	39	0.9	y	8.3	11.58	0
CYP3A4	alfentanil	den Hollander 1988	1	39	2.5	y	13.7	7.22	0
CYP3A4	alfentanil	den Hollander 1988	1	39	5	y	20.9	6.53	0
CYP3A4	alfentanil	den Hollander 1988	1	39	5	y	13	6.05	0
CYP3A4	alfentanil	den Hollander 1988	1	39	5	y	14.3	6.78	0
CYP3A4	alfentanil	den Hollander 1988	1	39	8	y	31	5.04	0
CYP3A4	alfentanil	Goresky 1987	6	39	0.6	y	7.57	8.36	1.6
CYP3A4	alfentanil	Goresky 1987	12	39	5.98	y	22.67	7.70	1.83
CYP3A4	alfentanil	Goresky 1987	1	39	0.9	y	10.9	9.02	0
CYP3A4	alfentanil	Goresky 1987	1	39	0.2	y	5.2	10.84	0
CYP3A4	alfentanil	Goresky 1987	1	39	0.3	y	4.8	8.69	0
CYP3A4	alfentanil	Goresky 1987	1	39	0.9	y	9.2	6.18	0
CYP3A4	alfentanil	Goresky 1987	1	39	0.3	y	6.8	7.22	0
CYP3A4	alfentanil	Goresky 1987	1	39	4.6	y	17.7	7.42	0
CYP3A4	alfentanil	Goresky 1987	1	39	1	y	8.5	8.21	0
CYP3A4	alfentanil	Goresky 1987	1	39	1.6	y	11.2	8.77	0
CYP3A4	alfentanil	Goresky 1987	1	39	4.7	y	21.3	7.17	0
CYP3A4	alfentanil	Goresky 1987	1	39	3.7	y	14.5	5.35	0
CYP3A4	alfentanil	Goresky 1987	1	39	4	y	15	10.33	0
CYP3A4	alfentanil	Goresky 1987	1	39	8.5	y	30	9.10	0
CYP3A4	alfentanil	Goresky 1987	1	39	14.7	y	43	5.49	0

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	alfentanil	Goresky 1987	1	39	1.3	y	11.2	8.16	0
CYP3A4	alfentanil	Goresky 1987	1	39	1.5	y	12.4	10.90	0
CYP3A4	alfentanil	Goresky 1987	1	39	1.1	y	8.1	6.78	0
CYP3A4	alfentanil	Goresky 1987	1	39	14	y	51	7.42	0
CYP3A4	alfentanil	Goresky 1987	1	39	12	y	36.6	5.56	0
CYP3A4	alfentanil	Meistelman 1987	8	39	5.4	y	21	4.70	1.74
CYP3A4	alfentanil	Meistelman 1987	5	39	31.3	y	58	4.20	1.75
CYP3A4	alfentanil	Meistelman 1987	1	39	4.7	y	20	3.40	0
CYP3A4	alfentanil	Meistelman 1987	1	39	5.5	y	22	5.90	0
CYP3A4	alfentanil	Meistelman 1987	1	39	7.7	y	23	8.30	0
CYP3A4	alfentanil	Meistelman 1987	1	39	4.5	y	14	4.60	0
CYP3A4	alfentanil	Meistelman 1987	1	39	4.8	y	24	3.70	0
CYP3A4	alfentanil	Meistelman 1987	1	39	4.5	y	20	4.80	0
CYP3A4	alfentanil	Meistelman 1987	1	39	6.2	y	23	4.40	0
CYP3A4	alfentanil	Meistelman 1987	1	39	4.9	y	22	2.70	0
CYP3A4	alfentanil	Meistelman 1987	1	39	28	y	66	7.20	0
CYP3A4	alfentanil	Meistelman 1987	1	39	35	y	55	3.90	0
CYP3A4	alfentanil	Meistelman 1987	1	39	32	y	58	2.70	0
CYP3A4	alfentanil	Meistelman 1987	1	39	34	y	60	3.90	0
CYP3A4	alfentanil	Meistelman 1987	1	39	27	y	60	3.30	0
CYP3A4	alfentanil	Rautiainen 1991	13	39	0.55	y	5.86	6.81	2.19
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.08	y	2.85	4.60	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.12	y	3.6	5.20	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.17	y	4.93	8.20	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.21	y	5.02	3.10	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.25	y	5.27	6.60	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.42	y	5.07	8.20	0

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.46	y	7.08	5.80	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.58	y	6.04	8.00	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.67	y	5.63	4.90	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.75	y	5.85	11.00	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	0.83	y	6.65	9.30	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	1.17	y	6.12	7.90	0
CYP3A4	alfentanil	Rautiainen 1991	1	39	1.42	y	12.1	5.70	0
CYP3A4	alfentanil	Roure 1987	20	39	34	m	14.3	11.10	3.9
CYP3A4	alfentanil	Roure 1987	10	39	55	y	65	5.90	1.6
CYP3A4	alfentanil	Roure 1987	1	39	10	m	8.25	14.20	0
CYP3A4	alfentanil	Roure 1987	1	39	10	m	8.25	11.50	0
CYP3A4	alfentanil	Roure 1987	1	39	11	m	8.58	9.70	0
CYP3A4	alfentanil	Roure 1987	1	39	21	m	11.47	20.70	0
CYP3A4	alfentanil	Roure 1987	1	39	21	m	11.47	10.70	0
CYP3A4	alfentanil	Roure 1987	1	39	23	m	11.96	11.80	0
CYP3A4	alfentanil	Roure 1987	1	39	24	m	12.19	12.70	0
CYP3A4	alfentanil	Roure 1987	1	39	24	m	12.19	7.60	0
CYP3A4	alfentanil	Roure 1987	1	39	27	m	12.86	13.40	0
CYP3A4	alfentanil	Roure 1987	1	39	27	m	12.86	8.10	0
CYP3A4	alfentanil	Roure 1987	1	39	28	m	13.07	9.80	0
CYP3A4	alfentanil	Roure 1987	1	39	31	m	13.68	10.50	0
CYP3A4	alfentanil	Roure 1987	1	39	33	m	14.06	13.30	0
CYP3A4	alfentanil	Roure 1987	1	39	41	m	15.45	13.40	0
CYP3A4	alfentanil	Roure 1987	1	39	42	m	15.61	12.10	0
CYP3A4	alfentanil	Roure 1987	1	39	50	m	16.83	7.70	0
CYP3A4	alfentanil	Roure 1987	1	39	50	m	16.83	17.00	0
CYP3A4	alfentanil	Roure 1987	1	39	60	m	18.31	6.00	0

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	alfentanil	Roure 1987	1	39	70	m	19.86	8.60	0
CYP3A4	alfentanil	Roure 1987	1	39	78	m	21.24	3.20	0
CYP3A4	alfentanil	Dorne 2003	241	39	18	y	70	4.1	1.5
CYP3A4	midazolam	Rey 1991	6	39	2.5	y	15.2	13.30	4.3
CYP3A4	midazolam	Muchohi 2008	13	39	27	m	10.4	14.40	2.7
CYP3A4	midazolam	Mathews 1988	6	39	5.2	y	18.4	11.98	6.68
CYP3A4	midazolam	Mathews 1988	6	39	4.7	y	15.9	8.53	1.8
CYP3A4	midazolam	Mathews 1988	5	39	1.3	y	8.8	9.07	3.35
CYP3A4	midazolam	Reed 2001	5	39	0.96	y	7.88	11.33	6.33
CYP3A4	midazolam	Reed 2001	13	39	5.2	y	19.2	10.00	3.83
CYP3A4	midazolam	Reed 2001	2	39	15.4	y	62	9.33	3.83
CYP3A4	midazolam	Payne 1989	8	39	5.52	m	17.3	9.11	1.21
CYP3A4	midazolam	Mulla 2003	20	39.5	3.8	d	3.4	1.40	0.15
CYP3A4	midazolam	Lee 1999	60	27	4.5	d	0.965	9.11	1.21
CYP3A4	midazolam	Jacqz-Aigrain 1992	15	32.8	3	d	1.9	1.70	1.8
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	35	3.5	d	2.3	1.39	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	34	3.5	d	2.7	1.41	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	35	3.5	d	2.7	1.07	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	37	3.5	d	2.7	0.74	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	39	3.5	d	3	0.87	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	41	3.5	d	3.2	2.69	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	38	3.5	d	3.2	1.16	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	37	3.5	d	3.6	3.50	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	38	3.5	d	3.8	3.76	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	1	40	3.5	d	3.9	3.72	0
CYP3A4	midazolam	Jacqz-Aigrain 1990	10	37	3.5	d	3.1	2.03	1.24
CYP3A4	midazolam	de Wildt 2001	24	29	5.5	d	1.02	1.80	n.a.

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP3A4	midazolam	Salonen 1987	3	39	7.33	y	32.8	6.67	5.5
CYP3A4	midazolam	Salonen 1987	6	39	6.72	y	28	4.83	2.33
CYP3A4	midazolam	Salonen 1987	6	39	6.07	y	21.6	8.67	2.5
CYP3A4	midazolam	Salonen 1987	6	39	6.11	y	22.3	11.20	1.33
CYP3A4	midazolam	Harte 1997	10	27.9	3	d	1.045	1.66	1.37
CYP3A4	midazolam	Tolia 1991	20	39	13.5	y	43.75	10.00	5
CYP3A4	midazolam	Clausen 1988	8	39	27	y	68	6.28	1.03
CYP3A4	midazolam	Pentikainen 1989	7	39	44.3	y	79.4	5.63	0.43
CYP3A4	midazolam	Mandema 1992	8	39	22	y	69	7.50	1.26
CYP3A4	midazolam	Greenblatt 1984	10	39	27.9	y	68.8	7.75	0.41
CYP3A4	midazolam	Greenblatt 1984	10	39	28.5	y	58.5	9.39	0.86
CYP3A4	midazolam	Dorne 2003	198	39	18	y	70	7	1.5
CYP1A2	theophylline	Loughnan 1976	1	39.0	1.7	y	10.4	1.95	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	2.0	y	13.3	0.95	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	2.0	y	13.7	1.33	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	2.0	y	11.2	2.72	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	2.5	y	11.5	2.07	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	2.7	y	11.4	1.75	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	3.0	y	14.8	2.42	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	3.2	y	13.2	1.20	0.00
CYP1A2	theophylline	Loughnan 1976	1	39.0	4.4	y	16.4	1.12	0.00
CYP1A2	theophylline	Loughnan 1976	10	39.0	2.5	y	12.5	1.67	0.61
CYP1A2	theophylline	Giacoia 1976	1	28.0	29.0	d	1.2	1.14	0.00
CYP1A2	theophylline	Giacoia 1976	1	29.0	30.0	d	1.2	0.60	0.00
CYP1A2	theophylline	Giacoia 1976	1	28.0	57.0	d	1.8	0.86	0.00
CYP1A2	theophylline	Giacoia 1976	1	28.0	55.0	d	1.9	0.74	0.00
CYP1A2	theophylline	Giacoia 1976	1	26.0	48.0	d	1.9	0.60	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Giacoia 1976	1	26.0	47.0	d	1.4	0.38	0.00
CYP1A2	theophylline	Giacoia 1976	1	32.0	37.0	d	2.2	0.49	0.00
CYP1A2	theophylline	Giacoia 1976	1	29.0	25.0	d	2.1	0.40	0.00
CYP1A2	theophylline	Giacoia 1976	8	28.3	41.0	d	1.7	0.65	0.26
CYP1A2	theophylline	Simmons 1978	1	39.0	3.0	m	5.6	0.68	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	3.0	m	5.6	0.47	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	4.0	m	6.0	0.66	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	4.0	m	6.0	0.92	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	5.0	m	6.4	1.29	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	8.0	m	7.5	1.55	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	8.0	m	7.5	0.54	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	10.0	m	8.2	1.35	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	10.0	m	8.2	1.15	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	17.0	m	10.4	0.98	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	18.0	m	10.7	1.39	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	20.0	m	11.2	2.41	0.00
CYP1A2	theophylline	Simmons 1978	1	39.0	23.0	m	12.0	0.50	0.00
CYP1A2	theophylline	Simmons 1978	13	39.0	10.2	m	8.1	1.07	0.55
CYP1A2	theophylline	Rosen 1979	1	39.0	4.0	m	6.0	0.77	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	4.5	m	6.2	0.80	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	5.0	m	6.4	0.97	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	6.5	m	7.0	1.72	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	7.5	m	7.4	2.17	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	8.0	m	7.5	1.45	0.00
CYP1A2	theophylline	Rosen 1979	6	39.0	5.9	m	6.8	1.31	0.56
CYP1A2	theophylline	Rosen 1979	1	39.0	12.0	m	8.9	2.60	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	13.0	m	9.2	1.28	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Rosen 1979	1	39.0	15.0	m	9.8	1.67	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	16.0	m	10.1	1.30	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	17.0	m	10.4	1.28	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	18.0	m	10.7	1.78	0.00
CYP1A2	theophylline	Rosen 1979	1	39.0	18.0	m	10.7	1.47	0.00
CYP1A2	theophylline	Rosen 1979	7	39.0	15.6	m	10.0	1.63	0.47
CYP1A2	theophylline	Franko 1982	1	39.0	0.9	m	5.0	0.40	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	1.0	m	4.3	0.43	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	1.8	m	2.6	0.31	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	1.8	m	5.5	0.39	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	2.0	m	4.2	1.10	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	2.0	m	3.8	0.51	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	3.0	m	5.7	0.58	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	4.5	m	3.9	0.53	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	5.0	m	5.8	0.73	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	5.0	m	5.7	0.75	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	5.0	m	6.6	0.67	0.00
CYP1A2	theophylline	Franko 1982	1	39.0	6.5	m	9.7	0.52	0.00
CYP1A2	theophylline	Franko 1982	12	39.0	3.2	m	5.2	0.58	0.21
CYP1A2	theophylline	el Desoky 1997	1	39.0	2.0	y	12.0	1.23	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	2.5	y	12.5	1.22	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	2.5	y	12.3	1.32	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	3.5	y	14.0	1.16	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	3.5	y	14.2	1.16	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	5.0	y	18.5	1.04	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	5.0	y	18.0	1.14	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	6.5	y	20.0	1.04	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	el Desoky 1997	1	39.0	6.5	y	21.0	1.05	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	7.0	y	22.0	0.73	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	8.0	y	23.5	1.08	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	9.0	y	24.0	1.55	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	11.0	y	27.0	0.90	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	11.5	y	26.8	0.84	0.00
CYP1A2	theophylline	el Desoky 1997	1	39.0	12.0	y	30.0	1.06	0.00
CYP1A2	theophylline	el Desoky 1997	15	39.0	6.4	y	19.7	1.10	0.20
CYP1A2	theophylline	Igarashi 2009	28	39.0	1.1	y	9.4	0.94	0.26
CYP1A2	theophylline	Igarashi 2009	10	39.0	1.2	y	9.7	0.84	0.24
CYP1A2	theophylline	Igarashi 2009	23	39.0	3.0	y	13.2	0.98	0.31
CYP1A2	theophylline	Igarashi 2009	12	39.0	3.0	y	13.9	0.81	0.11
CYP1A2	theophylline	Igarashi 2009	16	39.0	5.8	y	20.1	1.10	0.20
CYP1A2	theophylline	Igarashi 2009	11	39.0	6.2	y	19.9	0.87	0.28
CYP1A2	theophylline	Jones 1979	1	28.0	4.0	d	1.1	0.40	0.00
CYP1A2	theophylline	Jones 1979	1	27.0	4.0	d	1.1	0.32	0.00
CYP1A2	theophylline	Jones 1979	1	25.0	3.0	d	1.1	0.34	0.00
CYP1A2	theophylline	Jones 1979	1	29.0	30.0	d	1.1	0.29	0.00
CYP1A2	theophylline	Jones 1979	1	27.0	0.0	d	1.1	0.23	0.00
CYP1A2	theophylline	Jones 1979	1	27.0	19.0	d	1.1	0.22	0.00
CYP1A2	theophylline	Jones 1979	1	28.0	36.0	d	1.1	0.47	0.00
CYP1A2	theophylline	Jones 1979	1	29.0	7.0	d	1.1	0.29	0.00
CYP1A2	theophylline	Jones 1979	1	29.0	10.0	d	1.1	0.36	0.00
CYP1A2	theophylline	Jones 1979	1	30.0	19.0	d	1.1	0.21	0.00
CYP1A2	theophylline	Jones 1979	1	27.0	19.0	d	1.1	0.28	0.00
CYP1A2	theophylline	Jones 1979	11	27.8	12.4	d	1.1	0.31	0.08
CYP1A2	theophylline	Kraus 1993	20	28.9	46.9	d	5.0	0.36	0.12

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Kraus 1993	15	30.1	98.0	d	5.7	0.51	0.17
CYP1A2	theophylline	Kraus 1993	17	31.2	224.0	d	7.3	1.01	0.24
CYP1A2	theophylline	Arnold 1981	1	39.0	9.3	y	29.3	2.27	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	7.3	y	23.1	2.17	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	8.3	y	25.9	2.08	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	12.3	y	42.2	0.73	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	8.5	y	26.6	0.78	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	10.0	y	31.9	0.90	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	9.4	y	29.6	0.67	0.00
CYP1A2	theophylline	Arnold 1981	1	39.0	12.3	y	42.2	0.92	n.a.
CYP1A2	theophylline	Arnold 1981	8	39.0	9.7	y	31.4	1.31	0.72
CYP1A2	theophylline	Islam 2004	42	30.0	15.0	d	1.3	0.32	0.10
CYP1A2	theophylline	Gilman 1986	179	30.0	14.0	d	1.2	0.31	0.10
CYP1A2	theophylline	Vichyanond 1994	13	39.0	9.0	y	28.7	1.50	0.03
CYP1A2	theophylline	Stile 1986	9	28.0	1.0	d	1.1	0.77	0.23
CYP1A2	theophylline	Hilligoss 1980	17	30.5	50.1	d	1.1	0.38	0.07
CYP1A2	theophylline	Brazier 1979	33	33.6	2.9	d	1.5	0.40	0.08
CYP1A2	theophylline	Aranda 1976	6	27.5	7.5	d	0.9	0.29	0.04
CYP1A2	theophylline	Ellis 1976	30	39.0	10.7	y	34.9	1.45	0.58
CYP1A2	theophylline	Latini 1977	1	33.0	4.0	d	2.4	0.28	0.00
CYP1A2	theophylline	Latini 1977	1	33.0	6.0	d	1.7	0.13	0.00
CYP1A2	theophylline	Latini 1977	1	32.0	6.0	d	1.7	0.23	0.00
CYP1A2	theophylline	Latini 1977	1	30.0	7.0	d	1.7	0.11	0.00
CYP1A2	theophylline	Latini 1977	1	32.0	8.0	d	1.6	0.13	0.00
CYP1A2	theophylline	Latini 1977	1	29.0	8.0	d	1.5	0.50	0.00
CYP1A2	theophylline	Latini 1977	1	30.0	8.0	d	1.0	0.14	0.00
CYP1A2	theophylline	Latini 1977	7	31.3	6.7	d	1.6	0.22	0.14

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Neese 1977	1	29.0	26.0	d	1.2	0.27	0.00
CYP1A2	theophylline	Neese 1977	1	33.0	5.0	d	1.2	0.26	0.00
CYP1A2	theophylline	Neese 1977	1	33.0	10.0	d	1.2	0.13	0.00
CYP1A2	theophylline	Neese 1977	1	33.0	21.0	d	1.2	0.24	0.00
CYP1A2	theophylline	Neese 1977	1	33.0	17.0	d	1.3	0.14	0.00
CYP1A2	theophylline	Neese 1977	1	32.0	9.0	d	1.4	0.22	0.00
CYP1A2	theophylline	Neese 1977	1	32.0	18.0	d	1.4	0.07	0.00
CYP1A2	theophylline	Neese 1977	1	33.0	14.0	d	1.6	0.30	0.00
CYP1A2	theophylline	Neese 1977	1	34.0	5.0	d	1.7	0.10	0.00
CYP1A2	theophylline	Neese 1977	9	32.4	13.9	d	1.4	0.19	0.08
CYP1A2	theophylline	Miller 1984	1	39.0	27.0	y	70.0	1.17	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	22.0	y	70.0	0.86	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	20.0	y	70.0	0.83	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	19.0	y	70.0	1.00	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	25.0	y	70.0	0.60	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	19.0	y	70.0	0.70	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	20.0	y	70.0	0.74	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	19.0	y	70.0	0.53	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	27.0	y	70.0	0.54	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	35.0	y	70.0	0.94	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	57.0	y	70.0	0.69	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	44.0	y	70.0	1.01	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	18.0	y	70.0	1.60	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	22.0	y	70.0	1.24	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	19.0	y	70.0	0.70	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	18.0	y	70.0	1.51	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	20.0	y	70.0	0.43	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Miller 1984	1	39.0	27.0	y	70.0	0.64	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	41.0	y	70.0	1.28	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	23.0	y	70.0	0.70	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	20.0	y	70.0	0.68	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	20.0	y	70.0	0.98	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	19.0	y	70.0	0.62	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	21.0	y	70.0	0.50	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	21.0	y	70.0	0.93	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	21.0	y	70.0	0.92	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	36.0	y	70.0	1.12	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	28.0	y	70.0	0.61	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	33.0	y	70.0	1.07	0.00
CYP1A2	theophylline	Miller 1984	1	39.0	50.0	y	70.0	0.64	0.00
CYP1A2	theophylline	Miller 1984	30	39.0	26.4	y	70.0	0.86	0.05
CYP1A2	theophylline	Chester 1982	1	39.0	27.0	y	70.0	0.72	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	23.0	y	70.0	0.95	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	23.0	y	70.0	1.16	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	26.0	y	70.0	0.69	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	20.0	y	70.0	0.75	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	20.0	y	70.0	0.31	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	22.0	y	70.0	0.66	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	18.0	y	70.0	0.61	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	24.0	y	70.0	0.77	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	28.0	y	70.0	0.45	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	23.0	y	70.0	0.43	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	24.0	y	70.0	0.52	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	19.0	y	70.0	0.80	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	theophylline	Chester 1982	1	39.0	18.0	y	70.0	0.84	0.00
CYP1A2	theophylline	Chester 1982	1	39.0	19.0	y	70.0	0.71	0.00
CYP1A2	theophylline	Chester 1982	15	39.0	22.3	y	70.0	0.69	0.21
CYP1A2	theophylline	Dorne 2001	100	39.0	18.0	y	70.0	1.00	0.29
CYP1A2	caffeine	Aranda 1979	12	28.5	11.5	d	1.2	0.15	0.09
CYP1A2	caffeine	Aranda 1979	5	39.0	2.5	m	5.6	0.63	n.a.
CYP1A2	caffeine	Aranda 1979	3	39.0	4.5	m	7.0	1.74	n.a.
CYP1A2	caffeine	Aranda 1979	2	39.0	6.0	m	8.0	5.53	n.a.
CYP1A2	caffeine	Gorodischer 1982	13	30.6	5.8	d	1.4	0.14	0.01
CYP1A2	caffeine	Gorodischer 1982	1	28.0	18.0	d	1.4	0.12	0.00
CYP1A2	caffeine	Gorodischer 1982	1	25.0	21.0	d	0.9	0.14	0.00
CYP1A2	caffeine	Gorodischer 1982	1	30.0	5.0	d	1.2	0.21	0.00
CYP1A2	caffeine	Gorodischer 1982	1	31.0	6.0	d	1.4	0.11	0.00
CYP1A2	caffeine	Gorodischer 1982	1	33.0	3.5	d	1.5	0.15	0.00
CYP1A2	caffeine	Gorodischer 1982	5	29.4	10.7	d	1.3	0.14	0.04
CYP1A2	caffeine	Pons 1988	1	33.0	19.0	d	2.3	0.16	0.00
CYP1A2	caffeine	Pons 1988	1	32.0	22.0	d	0.2	0.11	0.00
CYP1A2	caffeine	Pons 1988	1	31.0	22.0	d	1.5	0.12	0.00
CYP1A2	caffeine	Pons 1988	1	31.0	22.0	d	1.9	0.11	0.00
CYP1A2	caffeine	Pons 1988	1	31.0	30.0	d	2.4	0.22	0.00
CYP1A2	caffeine	Pons 1988	1	40.0	33.0	d	3.8	0.37	0.00
CYP1A2	caffeine	Pons 1988	1	41.0	52.0	d	4.4	0.61	0.00
CYP1A2	caffeine	Pons 1988	1	38.0	54.0	d	3.6	0.49	0.00
CYP1A2	caffeine	Pons 1988	1	39.0	174.0	d	5.1	2.56	0.00
CYP1A2	caffeine	Pons 1988	1	36.0	273.0	d	6.4	1.70	0.00
CYP1A2	caffeine	Pons 1988	1	37.0	434.0	d	9.1	1.26	0.00
CYP1A2	caffeine	Pons 1988	1	36.0	588.0	d	9.1	2.39	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	caffeine	Pons 1988	1	41.0	92.0	d	5.3	1.13	0.00
CYP1A2	caffeine	Pons 1988	1	38.0	112.0	d	4.6	0.60	0.00
CYP1A2	caffeine	Pons 1988	1	38.0	196.0	d	5.7	1.03	0.00
CYP1A2	caffeine	Pons 1988	1	38.0	252.0	d	5.8	1.25	0.00
CYP1A2	caffeine	Pons 1988	5	31.6	23.0	d	1.7	0.14	0.04
CYP1A2	caffeine	Pons 1988	4	40.0	57.8	d	4.3	0.65	0.34
CYP1A2	caffeine	Pons 1988	5	37.8	201.4	d	5.5	1.43	0.75
CYP1A2	caffeine	Pons 1988	2	36.5	511.0	d	9.1	1.83	0.80
CYP1A2	caffeine	Lee 2002	18	28.9	5.0	d	1.1	0.09	0.02
CYP1A2	caffeine	Lee 2002	10	28.8	5.0	d	1.1	0.10	0.02
CYP1A2	caffeine	Lee 2002	1	33.0	5.0	d	1.7	0.06	0.00
CYP1A2	caffeine	Lee 2002	1	27.0	5.0	d	0.7	0.21	0.00
CYP1A2	caffeine	Lee 2002	1	29.0	5.0	d	1.1	0.09	0.00
CYP1A2	caffeine	Lee 2002	1	24.0	5.0	d	0.7	0.13	0.00
CYP1A2	caffeine	Lee 2002	1	28.0	5.0	d	1.1	0.10	0.00
CYP1A2	caffeine	Lee 2002	1	27.0	5.0	d	1.0	0.14	0.00
CYP1A2	caffeine	Lee 2002	1	29.0	5.0	d	1.3	0.09	0.00
CYP1A2	caffeine	Lee 2002	1	29.0	5.0	d	1.1	0.13	0.00
CYP1A2	caffeine	Lee 2002	1	31.0	5.0	d	1.1	0.12	0.00
CYP1A2	caffeine	Lee 2002	1	27.0	5.0	d	1.0	0.12	0.00
CYP1A2	caffeine	Lee 2002	1	32.0	5.0	d	1.6	0.09	0.00
CYP1A2	caffeine	Lee 2002	1	28.0	5.0	d	1.2	0.10	0.00
CYP1A2	caffeine	Lee 2002	1	29.0	5.0	d	1.3	0.10	0.00
CYP1A2	caffeine	Lee 2002	1	32.0	5.0	d	1.3	0.11	0.00
CYP1A2	caffeine	Lee 2002	1	29.0	5.0	d	0.9	0.16	0.00
CYP1A2	caffeine	Lee 2002	1	30.0	5.0	d	1.2	0.11	0.00
CYP1A2	caffeine	Lee 2002	1	28.0	5.0	d	1.0	0.14	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
CYP1A2	caffeine	Lee 2002	1	28.0	5.0	d	0.8	0.18	0.00
CYP1A2	caffeine	Denaro 1996	12	39.0	45.0	y	70.0	2.33	1.12
CYP1A2	caffeine	Blanchard 1983	10	39.0	21.8	y	79.5	1.15	0.54
CYP1A2	caffeine	Dorne 2001	20	39.0	18.0	y	70.0	1.97	0.92
renal	gentamicin	Rodvold 1993	19	28.7	34.6	d	1.78	1.04	0.37
renal	gentamicin	Kildoo 1984	15	28.4	1	d	1	0.38	0.14
renal	gentamicin	Kildoo 1984	15	28.4	18.7	d	0.887	0.44	0.18
renal	gentamicin	Kildoo 1984	6	28.4	67.5	d	1.49	1.21	0.39
renal	gentamicin	Rocha 2000	68	29.5	3	d	1.2	1.05	0.29
renal	gentamicin	Rocha 2000	34	27	3	d	0.875	0.48	0.13
renal	gentamicin	Rocha 2000	34	32	3	d	1.5	0.57	0.16
renal	gentamicin	Ho 1995	14	39	3.8	y	15.3	2.91	0.60
renal	gentamicin	Ho 1995	7	39	2.9	y	13.1	2.64	0.54
renal	gentamicin	Ho 1995	13	39	2.9	y	14.9	2.74	0.63
renal	gentamicin	Ho 1995	8	39	9.8	y	33.9	2.29	0.72
renal	gentamicin	Ho 1995	6	39	10.4	y	31.5	2.11	0.53
renal	gentamicin	Ho 1995	5	39	9	y	27	2.34	0.46
renal	gentamicin	Ho 1995	7	39	14	y	55.1	2.23	0.77
renal	gentamicin	Ho 1995	10	39	15.6	y	50.2	1.82	0.37
renal	gentamicin	Ho 1995	7	39	15.3	y	53.5	2.35	0.57
renal	gentamicin	Pons 1988	15	37	1	d	3.2	1.03	0.37
renal	gentamicin	Pons 1988	8	37	5	d	3.3	1.79	0.64
renal	gentamicin	Pons 1988	1	37	18	d	3.7	1.67	0.00
renal	gentamicin	Pons 1988	27	39	1	d	3.2	1.40	0.46
renal	gentamicin	Pons 1988	16	39	5	d	3.3	1.78	0.39
renal	gentamicin	Pons 1988	14	39	18	d	3.7	1.97	0.44
renal	gentamicin	Watterberg 1987	16	29.9	4.5	d	1.12	0.93	0.33

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	gentamicin	Watterberg 1987	1	29	3	d	0.74	1.55	0.00
renal	gentamicin	Watterberg 1987	1	29	2	d	0.87	0.93	0.00
renal	gentamicin	Watterberg 1987	1	26	2	d	0.94	1.05	0.00
renal	gentamicin	Watterberg 1987	1	29	13	d	0.99	1.65	0.00
renal	gentamicin	Watterberg 1987	1	31	2	d	1.12	0.65	0.00
renal	gentamicin	Watterberg 1987	1	29	2	d	1.28	0.68	0.00
renal	gentamicin	Watterberg 1987	1	30	2	d	1.36	0.77	0.00
renal	gentamicin	Watterberg 1987	24	28.8	4.2	d	1.04	0.83	0.40
renal	gentamicin	Watterberg 1987	1	29	7	d	0.74	0.82	0.00
renal	gentamicin	Watterberg 1987	1	29	10	d	0.87	0.57	0.00
renal	gentamicin	Watterberg 1987	1	26	12	d	0.94	0.28	0.00
renal	gentamicin	Watterberg 1987	1	29	17	d	0.99	0.70	0.00
renal	gentamicin	Watterberg 1987	1	31	8	d	1.12	0.63	0.00
renal	gentamicin	Watterberg 1987	1	29	6	d	1.28	1.05	0.00
renal	gentamicin	Watterberg 1987	1	30	14	d	1.36	0.50	0.00
renal	gentamicin	Vervelde 1999	29	32	6	d	1.8	0.86	0.21
renal	gentamicin	Vervelde 1999	5	33	15	d	1.95	1.71	0.17
renal	gentamicin	Assael 1980	1	27	1	d	0.98	0.78	0.00
renal	gentamicin	Assael 1980	1	30.3	1	d	1.1	0.58	0.00
renal	gentamicin	Assael 1980	1	30.5	1	d	1.1	0.51	0.00
renal	gentamicin	Assael 1980	1	31.4	1	d	1.65	0.46	0.00
renal	gentamicin	Assael 1980	1	33.4	1	d	2.1	0.27	0.00
renal	gentamicin	Assael 1980	1	34	1	d	1.45	0.76	0.00
renal	gentamicin	Assael 1980	1	34	1	d	1.2	0.87	0.00
renal	gentamicin	Assael 1980	1	34	1	d	2.25	0.60	0.00
renal	gentamicin	Assael 1980	1	34	1	d	2.75	1.08	0.00
renal	gentamicin	Assael 1980	1	38.3	1	d	3.15	0.61	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	gentamicin	Assael 1980	1	32.4	1	d	1.37	0.79	0.00
renal	gentamicin	Assael 1980	1	38	1	d	2.7	0.27	0.00
renal	gentamicin	Assael 1980	1	38	1	d	3.1	0.60	0.00
renal	gentamicin	Assael 1980	13	33.5	1	d	1.92	0.63	0.23
renal	gentamicin	Assael 1980	1	39	1	m	3.3	1.30	0.00
renal	gentamicin	Assael 1980	1	39	2.5	m	3.45	1.59	0.00
renal	gentamicin	Assael 1980	1	39	3	m	2.6	1.10	0.00
renal	gentamicin	Assael 1980	1	39	3	m	4	2.57	0.00
renal	gentamicin	Assael 1980	1	39	36	m	11	1.86	0.00
renal	gentamicin	Assael 1980	1	39	72	m	19	1.80	0.00
renal	gentamicin	Assael 1980	1	39	86	m	23	0.80	0.00
renal	gentamicin	Assael 1980	7	39	29.07	m	9.48	1.57	0.58
renal	gentamicin	Koren 1985	48	31.6	1.5	d	1.5	1.01	0.23
renal	gentamicin	Gonzalez-Martin 1986	5	39	6.4	y	20.5	2.80	0.50
renal	gentamicin	Gonzalez-Martin 1986	5	39	6.1	y	20.3	3.70	0.70
renal	gentamicin	Giacoia 1986	9	34.7	3.1	d	2.11	0.88	0.25
renal	gentamicin	Giacoia 1986	10	34.8	2.4	d	2.08	0.66	0.14
renal	gentamicin	Sato 1997	3	39	0	d	2.5	1.21	n.a.
renal	gentamicin	Sato 1997	6	39	1	d	2.5	0.79	n.a.
renal	gentamicin	Sato 1997	4	39	2	d	2.5	1.41	n.a.
renal	gentamicin	Sato 1997	4	39	3	d	2.5	1.45	n.a.
renal	gentamicin	Sato 1997	3	39	4	d	2.5	1.72	n.a.
renal	gentamicin	Sato 1997	3	39	5	d	2.5	1.69	n.a.
renal	gentamicin	Sato 1997	4	39	6	d	2.5	1.97	n.a.
renal	gentamicin	Sato 1997	2	39	7	d	2.5	1.95	n.a.
renal	gentamicin	Sato 1997	3	30	0	d	2	0.85	n.a.
renal	gentamicin	Sato 1997	5	30	1	d	2	0.84	n.a.

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	gentamicin	Sato 1997	4	30	2	d	2	0.94	n.a.
renal	gentamicin	Sato 1997	2	30	3	d	2	0.89	n.a.
renal	gentamicin	Sato 1997	2	30	4	d	2	0.76	n.a.
renal	gentamicin	Sato 1997	1	30	5	d	2	0.93	0.00
renal	gentamicin	Sato 1997	4	30	6	d	2	1.46	n.a.
renal	gentamicin	Sato 1997	1	30	7	d	2	1.30	0.00
renal	gentamicin	Sato 1997	1	28	0	d	1.5	0.83	0.00
renal	gentamicin	Sato 1997	1	28	1	d	1.5	0.41	0.00
renal	gentamicin	Sato 1997	1	28	2	d	1.5	0.51	0.00
renal	gentamicin	Sato 1997	1	28	3	d	1.5	0.82	0.00
renal	gentamicin	Sato 1997	1	28	5	d	1.5	0.92	0.00
renal	gentamicin	Sato 1997	1	28	7	d	1.5	0.98	0.00
renal	gentamicin	Walker 1979	10	39	27	y	70.5	1.03	0.23
renal	gentamicin	Bauer 1983	12	39	29.1	y	73.2	1.31	0.42
renal	gentamicin	Bauer 1983	51	39	28.8	y	72.8	1.29	0.52
renal	gentamicin	Bauer 1983	59	39	51.2	y	70.6	1.35	0.48
renal	gentamicin	Dorne 2004	219	39	18	y	70	1.30	0.50
renal	vancomycin	Jarret 1993	1	25.00	7.00	d	0.43	0.52	0.00
renal	vancomycin	Jarret 1993	1	24.57	10.00	d	0.59	0.59	0.00
renal	vancomycin	Jarret 1993	1	24.71	9.00	d	0.76	0.38	0.00
renal	vancomycin	Jarret 1993	1	28.57	10.00	d	1.03	0.86	0.00
renal	vancomycin	Jarret 1993	1	29.14	13.00	d	1.08	0.92	0.00
renal	vancomycin	Jarret 1993	1	30.00	7.00	d	1.30	0.76	0.00
renal	vancomycin	Jarret 1993	1	28.71	16.00	d	1.32	1.11	0.00
renal	vancomycin	Jarret 1993	1	30.57	17.00	d	1.58	0.78	0.00
renal	vancomycin	Jarret 1993	1	34.14	6.00	d	1.69	0.78	0.00
renal	vancomycin	Jarret 1993	1	34.43	4.00	d	2.14	0.82	0.00

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	vancomycin	Jarret 1993	1	34.43	11.00	d	1.96	0.65	0.00
renal	vancomycin	Jarret 1993	11	29.48	10.00	d	1.26	0.74	0.20
renal	vancomycin	Culter 1984	1	39.00	26.00	y	83.20	0.96	0.00
renal	vancomycin	Culter 1984	1	39.00	26.00	y	72.60	1.26	0.00
renal	vancomycin	Culter 1984	1	39.00	21.00	y	82.10	1.08	0.00
renal	vancomycin	Culter 1984	1	39.00	25.00	y	74.60	0.85	0.00
renal	vancomycin	Culter 1984	1	39.00	20.00	y	76.50	0.94	0.00
renal	vancomycin	Culter 1984	1	39.00	20.00	y	72.80	1.08	0.00
renal	vancomycin	Culter 1984	6	39.00	23.00	y	76.97	1.03	0.14
renal	vancomycin	Lisby-Sutch 1988	1	28.00	13.00	d	1.14	0.79	0.00
renal	vancomycin	Lisby-Sutch 1988	1	27.00	21.00	d	0.91	0.31	0.00
renal	vancomycin	Lisby-Sutch 1988	1	29.00	21.00	d	1.17	0.81	0.00
renal	vancomycin	Lisby-Sutch 1988	1	29.00	28.00	d	1.10	0.81	0.00
renal	vancomycin	Lisby-Sutch 1988	1	36.00	23.00	d	2.00	1.61	0.00
renal	vancomycin	Lisby-Sutch 1988	5	29.80	21.20	d	1.26	0.87	0.47
renal	vancomycin	Lisby-Sutch 1988	1	28.00	42.00	d	0.96	0.90	0.00
renal	vancomycin	Lisby-Sutch 1988	1	27.00	52.00	d	1.38	1.05	0.00
renal	vancomycin	Lisby-Sutch 1988	1	27.00	53.00	d	1.29	1.39	0.00
renal	vancomycin	Lisby-Sutch 1988	1	27.00	84.00	d	1.83	1.18	0.00
renal	vancomycin	Lisby-Sutch 1988	1	32.00	49.00	d	2.50	1.37	0.00
renal	vancomycin	Lisby-Sutch 1988	1	28.00	92.00	d	2.30	2.85	0.00
renal	vancomycin	Lisby-Sutch 1988	1	37.00	122.00	d	4.18	2.22	0.00
renal	vancomycin	Lisby-Sutch 1988	1	32.00	183.00	d	6.12	3.42	0.00
renal	vancomycin	Lisby-Sutch 1988	8	29.77	84.60	d	2.57	2.21	0.95
renal	vancomycin	Asbury 1993	19	29.30	33.90	d	1.78	1.20	0.53
renal	vancomycin	Asbury 1993	4	26.00	17.60	d	0.81	0.60	0.17
renal	vancomycin	Gous 1995	20	39.00	3.00	m	6.40	1.50	0.50

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	vancomycin	Gous 1995	15	39.00	3.00	m	6.40	1.20	0.40
renal	vancomycin	Wrishko 2000	6	39.00	6.90	y	21.20	1.83	0.30
renal	vancomycin	Kildoo 1990	15	29.00	29.00	d	1.24	1.07	0.34
renal	vancomycin	Schaad 1980	7	32.00	3.30	d	1.23	0.85	n.a.
renal	vancomycin	Schaad 1980	7	34.00	4.70	d	1.57	1.39	n.a.
renal	vancomycin	Schaad 1980	7	40.00	2.60	d	3.07	1.19	n.a.
renal	vancomycin	Rodvold 1997	3	25.86	29.00	d	0.83	1.10	0.29
renal	vancomycin	Rodvold 1997	6	27.06	39.50	d	1.38	1.05	0.21
renal	vancomycin	Reed 1987	15	28.40	20.50	d	1.07	1.14	0.65
renal	vancomycin	McDougal 1995	16	26.60	18.00	d	0.97	1.00	0.27
renal	vancomycin	McDougal 1995	15	29.40	23.00	d	1.38	1.17	0.32
renal	vancomycin	McDougal 1995	13	35.90	24.00	d	2.62	1.33	0.30
renal	vancomycin	Rodvold 1995	29	31.20	17.70	d	1.86	1.01	0.37
renal	vancomycin	Schaad 1980	12	39.00	0.26	y	4.90	1.65	n.a.
renal	vancomycin	Schaad 1980	4	39.00	0.36	y	5.20	2.70	n.a.
renal	vancomycin	Rodvold 1997	6	39.00	4.00	m	6.50	1.67	0.60
renal	vancomycin	Chang 1994	28	39.00	4.00	y	16.20	2.55	0.55
renal	vancomycin	Chang 1995	33	39.00	5.70	y	19.90	2.48	0.47
renal	vancomycin	Chang 1995	31	39.00	4.20	y	15.40	1.90	0.52
renal	vancomycin	Schaad 1980	5	39.00	3.92	y	15.50	3.95	n.a.
renal	vancomycin	Schaad 1980	7	39.00	5.58	y	20.00	2.95	n.a.
renal	vancomycin	Schaad 1980	6	39.00	7.58	y	26.70	2.78	n.a.
renal	vancomycin	Birt 1990	22	39.00	52.40	y	71.00	1.12	0.48
renal	vancomycin	Pou 1996	45	39.00	50.80	y	71.00	1.31	0.82
renal	vancomycin	Pou 1996	45	39.00	50.80	y	71.00	1.13	0.72
renal	vancomycin	Guay 1993	121	39.00	37.30	y	79.60	1.22	0.50
renal	vancomycin	Pleasants 1996	10	39.00	27.10	y	51.20	1.55	0.32

pathway	drug	study	subjn	GA (wk)	PNA	PNA.unit	BW (kg)	CL (mL/min/kg)	CL.sd
renal	vancomycin	Hoie 1990	1	37.00	2.00	d	4.10	1.19	0.00
renal	vancomycin	Hoie 1990	1	41.00	2.00	d	3.50	1.62	0.00
renal	vancomycin	Hoie 1990	1	38.00	2.00	d	3.30	1.22	0.00
renal	vancomycin	Hoie 1990	1	37.00	4.00	d	3.40	0.91	0.00
renal	vancomycin	Hoie 1990	1	36.00	1.00	d	3.00	0.67	0.00
renal	vancomycin	Hoie 1990	1	40.00	1.00	d	2.50	1.04	0.00
renal	vancomycin	Hoie 1990	6	38.00	2.00	d	3.30	1.10	0.32

n=number of subjects in study; GA= gestational age; PNA=postnatal age; BW=body weight; CL=plasma clearance

wk=week; y=year; m=month; d=day; n.a.=not available

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Appendix II Pediatric weight, height, body surface area and liver size database (Johnson et al)

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
ICRP, 1975	122	0.001	3.5	51	0.226	0.124	0.026	M	NA	Autopsy	1975
ICRP, 1975	93	0.125	5.4	56.2	0.297	0.132	0.027	M	NA	Autopsy	1975
ICRP, 1975	106	0.625	8.7	69.8	0.418	0.22	0.044	M	NA	Autopsy	1975
ICRP, 1975	69	0.875	9.7	73.6	0.453	0.271	0.053	M	NA	Autopsy	1975
ICRP, 1975	186	1.5	11.4	82	0.515	0.225	0.042	M	NA	Autopsy	1975
ICRP, 1975	114	2.5	13.6	91	0.59	0.425	0.074	M	NA	Autopsy	1975
ICRP, 1975	78	3.5	15.6	99	0.646	0.491	0.08	M	NA	Autopsy	1975
ICRP, 1975	62	4.5	17.5	106	0.713	0.525	0.082	M	NA	Autopsy	1975
ICRP, 1975	36	5.5	20	113	0.79	0.548	0.082	M	NA	Autopsy	1975
ICRP, 1975	22	6.5	22	119	0.854	0.612	0.089	M	NA	Autopsy	1975
ICRP, 1975	29	7.5	24.3	125	0.924	0.64	0.092	M	NA	Autopsy	1975
ICRP, 1975	20	8.5	27	130.5	0.997	0.748	0.11	M	NA	Autopsy	1975
ICRP, 1975	21	9.5	30	136	1.074	0.745	0.11	M	NA	Autopsy	1975
ICRP, 1975	27	10.5	33	141	1.148	0.862	0.13	M	NA	Autopsy	1975
ICRP, 1975	17	11.5	36.3	146	1.226	0.835	0.125	M	NA	Autopsy	1975
ICRP, 1975	12	12.5	40	151	1.309	0.914	0.14	M	NA	Autopsy	1975
ICRP, 1975	15	13.5	46	158	1.436	1.021	0.17	M	NA	Autopsy	1975
ICRP, 1975	16	14.5	52	166	1.568	1.08	0.18	M	NA	Autopsy	1975
ICRP, 1975	20	15.5	58	171	1.678	1.137	0.2	M	NA	Autopsy	1975
ICRP, 1975	24	16.5	62.5	175	1.761	1.341	0.25	M	NA	Autopsy	1975
ICRP, 1975	97	18	67.1	176.9	1.829	1.49	0.31	M	NA	Autopsy	1975
ICRP, 1975	93	0.001	3.4	50.2	0.221	0.126	0.027	F	NA	Autopsy	1975
ICRP, 1975	83	0.125	4.5	55	0.267	0.123	0.026	F	NA	Autopsy	1975
ICRP, 1975	102	0.375	6.7	63.5	0.35	0.165	0.033	F	NA	Autopsy	1975
ICRP, 1975	87	0.625	8.2	67.5	0.4	0.22	0.044	F	NA	Autopsy	1975

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
ICRP, 1975	88	0.875	9.1	72	0.433	0.247	0.048	F	NA	Autopsy	1975
ICRP, 1975	164	1.5	10.8	80.5	0.497	0.298	0.056	F	NA	Autopsy	1975
ICRP, 1975	105	2.5	13	90	0.574	0.397	0.069	F	NA	Autopsy	1975
ICRP, 1975	68	3.5	15.3	98	0.636	0.454	0.075	F	NA	Autopsy	1975
ICRP, 1975	32	4.5	17.3	105	0.705	0.518	0.081	F	NA	Autopsy	1975
ICRP, 1975	36	5.5	19	112	0.768	0.547	0.082	F	NA	Autopsy	1975
ICRP, 1975	29	6.5	22	118	0.849	0.559	0.082	F	NA	Autopsy	1975
ICRP, 1975	20	7.5	24	124	0.913	0.632	0.091	F	NA	Autopsy	1975
ICRP, 1975	13	8.5	27	130	0.994	0.678	0.1	F	NA	Autopsy	1975
ICRP, 1975	16	9.5	30.5	135.4	1.078	0.799	0.12	F	NA	Autopsy	1975
ICRP, 1975	11	10.5	34	141	1.163	0.838	0.12	F	NA	Autopsy	1975
ICRP, 1975	8	11.5	38	147	1.256	0.778	0.12	F	NA	Autopsy	1975
ICRP, 1975	9	12.5	43	152.5	1.36	0.97	0.15	F	NA	Autopsy	1975
ICRP, 1975	15	13.5	48	157.5	1.459	0.924	0.15	F	NA	Autopsy	1975
ICRP, 1975	13	14.5	52	161	1.533	1.119	0.19	F	NA	Autopsy	1975
ICRP, 1975	18	15.5	54.5	163	1.578	1.249	0.22	F	NA	Autopsy	1975
ICRP, 1975	21	16.5	56	163.2	1.598	1.309	0.24	F	NA	Autopsy	1975
ICRP, 1975	70	18	57.5	163.5	1.619	1.36	0.28	F	NA	Autopsy	1975
Altman, 1962	63	0.001	3.4	50.5	0.222	0.115	0.024	M	NA	Autopsy	1962
Altman, 1962	37	0.5	7.6	66	0.38	0.278	0.056	M	NA	Autopsy	1962
Altman, 1962	34	1	10.1	75	0.466	0.37	0.071	M	NA	Autopsy	1962
Altman, 1962	24	2	12.6	87	0.557	0.426	0.077	M	NA	Autopsy	1962
Altman, 1962	27	3	14.6	95	0.624	0.472	0.08	M	NA	Autopsy	1962
Altman, 1962	26	4	16.5	102	0.676	0.514	0.08	M	NA	Autopsy	1962
Altman, 1962	27	5	19.4	112	0.775	0.551	0.084	M	NA	Autopsy	1962
Altman, 1962	20	6	21.9	119	0.853	0.583	0.086	M	NA	Autopsy	1962

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Altman, 1962	16	7	24.5	126	0.932	0.616	0.089	M	NA	Autopsy	1962
Altman, 1962	13	8	27.3	131.5	1.007	0.662	0.095	M	NA	Autopsy	1962
Altman, 1962	16	9	29.9	136	1.072	0.713	0.1	M	NA	Autopsy	1962
Altman, 1962	8	10	32.6	138	1.124	0.787	0.11	M	NA	Autopsy	1962
Altman, 1962	4	11	35.2	144	1.198	0.88	0.13	M	NA	Autopsy	1962
Altman, 1962	14	12	38.3	150	1.279	0.972	0.15	M	NA	Autopsy	1962
Altman, 1962	8	13	42.2	152	1.346	1.065	0.17	M	NA	Autopsy	1962
Altman, 1962	12	14	48.8	162	1.5	1.148	0.19	M	NA	Autopsy	1962
Altman, 1962	7	15	54.5	168	1.613	1.218	0.21	M	NA	Autopsy	1962
Altman, 1962	19	16	58.8	172	1.695	1.278	0.23	M	NA	Autopsy	1962
Altman, 1962	14	17	61.8	174	1.746	1.343	0.26	M	NA	Autopsy	1962
Altman, 1962	24	18	63.1	175	1.768	1.398	0.28	M	NA	Autopsy	1962
Altman, 1962	36	0.001	3.4	50.2	0.221	0.116	0.024	F	NA	Autopsy	1962
Altman, 1962	5	0.5	7.3	65.5	0.37	0.222	0.044	F	NA	Autopsy	1962
Altman, 1962	14	1	9.8	75	0.458	0.361	0.07	F	NA	Autopsy	1962
Altman, 1962	11	2	12.3	87	0.549	0.417	0.075	F	NA	Autopsy	1962
Altman, 1962	12	3	14.4	96	0.622	0.463	0.078	F	NA	Autopsy	1962
Altman, 1962	13	4	16.4	102	0.674	0.509	0.081	F	NA	Autopsy	1962
Altman, 1962	9	5	18.8	110.5	0.757	0.546	0.084	F	NA	Autopsy	1962
Altman, 1962	8	6	21.1	117	0.829	0.588	0.087	F	NA	Autopsy	1962
Altman, 1962	6	7	23.7	123	0.903	0.634	0.092	F	NA	Autopsy	1962
Altman, 1962	9	8	26.4	128	0.973	0.69	0.099	F	NA	Autopsy	1962
Altman, 1962	5	9	28.9	133	1.04	0.75	0.11	F	NA	Autopsy	1962
Altman, 1962	5	10	31.9	137.5	1.111	0.815	0.12	F	NA	Autopsy	1962
Altman, 1962	8	11	35.7	143	1.199	0.889	0.13	F	NA	Autopsy	1962
Altman, 1962	8	12	39.7	149	1.292	1	0.15	F	NA	Autopsy	1962

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Altman, 1962	3	13	45	155	1.403	1.093	0.17	F	NA	Autopsy	1962
Altman, 1962	4	14	49.2	159	1.484	1.176	0.19	F	NA	Autopsy	1962
Altman, 1962	7	15	51.5	160.8	1.526	1.231	0.21	F	NA	Autopsy	1962
Altman, 1962	7	16	53.1	162	1.554	1.259	0.23	F	NA	Autopsy	1962
Altman, 1962	7	17	54	162.5	1.569	1.278	0.24	F	NA	Autopsy	1962
Altman, 1962	9	18	54.4	163	1.577	1.292	0.26	F	NA	Autopsy	1962
Ogiu, 1997	55	0.001	2.9	49.2	0.202	0.119	0.03	M	J	Autopsy	1996
Ogiu, 1997	14	0.083	4.2	54.7	0.257	0.161	0.033	M	J	Autopsy	1996
Ogiu, 1997	19	0.167	5.4	58.1	0.301	0.192	0.032	M	J	Autopsy	1996
Ogiu, 1997	16	0.25	7.2	63.6	0.364	0.24	0.042	M	J	Autopsy	1996
Ogiu, 1997	21	0.33	6.7	63.8	0.35	0.235	0.036	M	J	Autopsy	1996
Ogiu, 1997	12	0.417	7.1	65.4	0.365	0.256	0.04	M	J	Autopsy	1996
Ogiu, 1997	13	0.5	7.6	66.3	0.381	0.249	0.031	M	J	Autopsy	1996
Ogiu, 1997	11	0.583	8.9	69.9	0.423	0.302	0.03	M	J	Autopsy	1996
Ogiu, 1997	10	0.67	8.3	70.5	0.409	0.304	0.028	M	J	Autopsy	1996
Ogiu, 1997	8	0.75	9	72.2	0.431	0.346	0.07	M	J	Autopsy	1996
Ogiu, 1997	6	0.833	8.4	66.1	0.401	0.293	0.05	M	J	Autopsy	1996
Ogiu, 1997	5	0.92	8.3	72.3	0.41	0.326	0.03	M	J	Autopsy	1996
Ogiu, 1997	34	3	10	80.4	0.476	0.376	0.06	M	J	Autopsy	1996
Ogiu, 1997	16	2	11.5	85.5	0.526	0.447	0.06	M	J	Autopsy	1996
Ogiu, 1997	20	3	14.3	97.8	0.624	0.464	0.09	M	J	Autopsy	1996
Ogiu, 1997	13	4	15.6	105.4	0.676	0.564	0.09	M	J	Autopsy	1996
Ogiu, 1997	13	5	17.8	110.4	0.74	0.595	0.11	M	J	Autopsy	1996
Ogiu, 1997	8	6	19.9	115.6	0.802	0.65	0.11	M	J	Autopsy	1996
Ogiu, 1997	12	7	21.7	118.1	0.845	0.625	0.063	M	J	Autopsy	1996
Ogiu, 1997	10	8	22.8	124.5	0.896	0.763	0.11	M	J	Autopsy	1996

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Ogiu, 1997	11	9	29.4	133.2	1.049	0.835	0.11	M	J	Autopsy	1996
Ogiu, 1997	12	10	29.3	135.5	1.06	0.878	0.13	M	J	Autopsy	1996
Ogiu, 1997	10	11	34.5	142.5	1.179	0.94	0.15	M	J	Autopsy	1996
Ogiu, 1997	9	12	40.7	153.5	1.335	1.029	0.12	M	J	Autopsy	1996
Ogiu, 1997	9	13	41.6	152.3	1.339	0.966	0.14	M	J	Autopsy	1996
Ogiu, 1997	9	14	50.8	163.6	1.536	1.206	0.14	M	J	Autopsy	1996
Ogiu, 1997	19	15	58.7	169.6	1.676	1.224	0.28	M	J	Autopsy	1996
Ogiu, 1997	21	16	58.6	169.1	1.672	1.231	0.24	M	J	Autopsy	1996
Ogiu, 1997	25	17	57.3	168.1	1.649	1.15	0.2	M	J	Autopsy	1996
Ogiu, 1997	17	18	58.2	167.2	1.653	1.115	0.14	M	J	Autopsy	1996
Ogiu, 1997	44	18	62.7	167.9	1.711	1.272	0.2	M	J	Autopsy	1996
Ogiu, 1997	75	0.001	3.6	50.2	0.228	0.139	0.035	F	J	Autopsy	1996
Ogiu, 1997	12	0.083	3.8	53.2	0.24	0.15	0.03	F	J	Autopsy	1996
Ogiu, 1997	17	0.167	5	59	0.29	0.194	0.02	F	J	Autopsy	1996
Ogiu, 1997	10	0.25	6	60	0.322	0.216	0.026	F	J	Autopsy	1996
Ogiu, 1997	14	0.33	6.6	63.3	0.347	0.219	0.031	F	J	Autopsy	1996
Ogiu, 1997	15	0.417	6.4	64.8	0.344	0.219	0.037	F	J	Autopsy	1996
Ogiu, 1997	7	0.5	7.4	65.6	0.37	0.254	0.031	F	J	Autopsy	1996
Ogiu, 1997	5	0.583	7.1	68.3	0.371	0.306	0.048	F	J	Autopsy	1996
Ogiu, 1997	8	0.67	7.8	66.7	0.387	0.293	0.05	F	J	Autopsy	1996
Ogiu, 1997	10	0.75	8	72.8	0.406	0.295	0.049	F	J	Autopsy	1996
Ogiu, 1997	3	0.833	8.4	73.2	0.418	0.29	0.027	F	J	Autopsy	1996
Ogiu, 1997	3	0.92	8	71	0.402	0.347	0.02	F	J	Autopsy	1996
Ogiu, 1997	38	1	9.5	77.5	0.457	0.359	0.06	F	J	Autopsy	1996
Ogiu, 1997	21	2	11.7	86.5	0.534	0.414	0.083	F	J	Autopsy	1996
Ogiu, 1997	18	3	14.6	96.5	0.628	0.44	0.07	F	J	Autopsy	1996

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Ogiu, 1997	10	4	16.3	103.2	0.678	0.529	0.089	F	J	Autopsy	1996
Ogiu, 1997	7	5	16.8	107.4	0.707	0.586	0.13	F	J	Autopsy	1996
Ogiu, 1997	9	6	19.9	114.2	0.795	0.596	0.11	F	J	Autopsy	1996
Ogiu, 1997	10	7	21.2	119.1	0.842	0.6	0.1	F	J	Autopsy	1996
Ogiu, 1997	8	8	26.9	129.3	0.988	0.772	0.1	F	J	Autopsy	1996
Ogiu, 1997	10	9	26.7	129.7	0.987	0.797	0.14	F	J	Autopsy	1996
Ogiu, 1997	4	10	33.6	140.6	1.154	0.933	0.08	F	J	Autopsy	1996
Ogiu, 1997	10	11	39.4	147.8	1.281	1.02	0.13	F	J	Autopsy	1996
Ogiu, 1997	6	12	37.4	147.5	1.251	0.993	0.15	F	J	Autopsy	1996
Ogiu, 1997	4	13	45.1	148.3	1.36	1.014	0.1	F	J	Autopsy	1996
Ogiu, 1997	6	14	52.7	157.8	1.52	1.19	0.15	F	J	Autopsy	1996
Ogiu, 1997	8	15	48.5	158.1	1.469	0.885	0.2	F	J	Autopsy	1996
Ogiu, 1997	5	16	50.5	153.4	1.462	1.05	0.21	F	J	Autopsy	1996
Ogiu, 1997	9	17	49.3	156	1.465	1.045	0.13	F	J	Autopsy	1996
Ogiu, 1997	23	18	49.3	158.3	1.481	1.132	0.17	F	J	Autopsy	1996
Murry, 1995	3	3.88	16.2	101.2	0.666	0.508	0.036	U	NA	Radio	1997
Murry, 1995	5	7.89	25.48	126.9	0.955	0.729	0.16	U	NA	Radio	1997
Murry, 1995	5	12.66	42.65	153	1.358	1.035	0.041	U	NA	Radio	1997
Murry, 1995	4	16.52	58.3	167.9	1.66	1.258	0.27	U	NA	Radio	1997
Coppoletta, 1933	23	0.0041	3.1	49	0.209	0.072	0.03	U	NA	Autopsy	1933
Coppoletta, 1933	12	0.0109	3.1	49	0.209	0.089	0.03	U	NA	Autopsy	1933
Coppoletta, 1933	37	0.038	3.75	52	0.237	0.114	0.03	U	NA	Autopsy	1933
Coppoletta, 1933	34	0.083	3.75	52	0.237	0.118	0.03	U	NA	Autopsy	1933
Coppoletta, 1933	18	0.115	4.1	53	0.25	0.123	0.031	U	NA	Autopsy	1933
Coppoletta, 1933	41	0.153	4.6	55	0.27	0.126	0.035	U	NA	Autopsy	1933
Coppoletta, 1933	55	0.166	4.7	56	0.275	0.13	0.041	U	NA	Autopsy	1933

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Coppoletta, 1933	34	0.33	5.6	59	0.309	0.148	0.042	U	NA	Autopsy	1933
Coppoletta, 1933	36	0.41	6.2	61	0.33	0.174	0.035	U	NA	Autopsy	1933
Coppoletta, 1933	46	0.5	6.45	62	0.34	0.185	0.05	U	NA	Autopsy	1933
Coppoletta, 1933	36	0.58	7.3	65	0.37	0.21	0.04	U	NA	Autopsy	1933
Coppoletta, 1933	37	0.67	7.3	65	0.37	0.235	0.051	U	NA	Autopsy	1933
Coppoletta, 1933	20	0.75	7.8	67	0.388	0.241	0.062	U	NA	Autopsy	1933
Coppoletta, 1933	25	0.83	8.4	69	0.408	0.254	0.056	U	NA	Autopsy	1933
Coppoletta, 1933	28	0.91	8.6	70	0.416	0.256	0.059	U	NA	Autopsy	1933
Coppoletta, 1933	11	1	9.37	73	0.443	0.267	0.071	U	NA	Autopsy	1933
Coppoletta, 1933	34	1.16	9.65	74	0.452	0.281	0.065	U	NA	Autopsy	1933
Coppoletta, 1933	32	1.33	10.47	77	0.48	0.306	0.067	U	NA	Autopsy	1933
Coppoletta, 1933	30	1.5	10.5	78	0.484	0.319	0.087	U	NA	Autopsy	1933
Coppoletta, 1933	19	1.67	10.65	79	0.489	0.343	0.045	U	NA	Autopsy	1933
Coppoletta, 1933	14	1.83	11.3	82	0.513	0.352	0.06	U	NA	Autopsy	1933
Coppoletta, 1933	28	2	11.8	84	0.53	0.365	0.07	U	NA	Autopsy	1933
Coppoletta, 1933	53	3	12.65	88	0.56	0.387	0.08	U	NA	Autopsy	1933
Coppoletta, 1933	19	4	15.55	99	0.645	0.478	0.075	U	NA	Autopsy	1933
Coppoletta, 1933	19	5	17.4	106	0.711	0.552	0.095	U	NA	Autopsy	1933
Coppoletta, 1933	53	6	17.75	109	0.732	0.594	0.09	U	NA	Autopsy	1933
Coppoletta, 1933	42	7	21.45	113	0.786	0.63	0.09	U	NA	Autopsy	1933
Coppoletta, 1933	26	8	22	119	0.854	0.681	0.09	U	NA	Autopsy	1933
Coppoletta, 1933	29	9	24.75	125	0.931	0.7	0.08	U	NA	Autopsy	1933
Coppoletta, 1933	27	10	27.1	130	0.995	0.789	0.09	U	NA	Autopsy	1933
Coppoletta, 1933	12	11	29.5	135	1.061	0.842	0.09	U	NA	Autopsy	1933
Coppoletta, 1933	14	12	32	139	1.121	0.867	0.09	U	NA	Autopsy	1933
Urata, 1995	3	0.47	7.45	66.9	0.378	0.237	0.03	M	J	Radio	1995

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Urata, 1995	8	0.74	7.84	68.2	0.391	0.235	0.03	M	J	Radio	1995
Urata, 1995	8	1.65	10.19	79.7	0.478	0.318	0.08	M	J	Radio	1995
Urata, 1995	3	4.33	17.6	105.4	0.712	0.514	0.03	M	J	Radio	1995
Urata, 1995	4	6.46	21.35	117.7	0.837	0.565	0.06	M	J	Radio	1995
Urata, 1995	5	8.02	21.5	121.04	0.856	0.568	0.09	M	J	Radio	1995
Urata, 1995	3	10.75	33.6	138.7	1.14	0.747	0.08	M	J	Radio	1995
Urata, 1995	10	13.55	44.02	155.85	1.393	0.902	0.11	M	J	Radio	1995
Urata, 1995	10	17.36	57.65	168.9	1.656	1.122	0.13	M	J	Radio	1995
Urata, 1995	3	0.353	6.47	65.13	0.345	0.212	0.07	F	J	Radio	1995
Urata, 1995	7	0.67	8.57	69.64	0.414	0.296	0.05	F	J	Radio	1995
Urata, 1995	3	4.5	17.75	105	0.713	0.505	0.19	F	J	Radio	1995
Urata, 1995	3	7.21	21.5	120.5	0.853	0.52	0.011	F	J	Radio	1995
Urata, 1995	3	11.5	45.05	153.6	1.386	0.92	0.38	F	J	Radio	1995
Urata, 1995	5	14.134	55.8	163.38	1.595	1.001	0.14	F	J	Radio	1995
Urata, 1995	6	17.87	51.33	159.3	1.512	0.999	0.09	F	J	Radio	1995
Rylance, 1982	3	6	21.25	118	0.816	0.401	0.15	U	NE	Radio	1982
Rylance, 1982	3	9	28.65	133	1.036	1.071	0.2	U	NE	Radio	1982
Rylance, 1982	3	13	44	155	1.389	1.322	0.23	U	NE	Radio	1982
Noda, 1997	6	0.27	6.25	61	0.297	0.178	0.08	U	J	Radio	1997
Noda, 1997	6	1.475	11.1	81.25	0.469	0.281	0.05	U	J	Radio	1997
Noda, 1997	7	3.34	15.05	97.5	0.584	0.426	0.09	U	J	Radio	1997
Noda, 1997	10	7	23	121.5	0.891	0.597	0.22	U	J	Radio	1997
Noda, 1997	8	13.78	48.25	159.25	1.347	1.024	0.21	U	J	Radio	1997
Noda, 1997	17	18	63	170.75	1.61	1.114	0.19	U	J	Radio	1997
Heinemann, 1999	3	5	17.5	118.5	0.773	0.634	0.19	M	NE	Autopsy	1999
Heinemann, 1999	5	17.4	69.9	175.4	1.848	1.51	0.3	M	NE	Autopsy	1999

Study	N	Age (yr)	Weight (kg)	Height (cm)	BSA (m2)	Liver volume (L)	Liver volume SD (L)	Sex	Race	Method	Study year
Heinemann, 1999	4	12.68	43.42	149.2	1.336	0.86	0.23	F	NE	Autopsy	1999
Heinemann, 1999	5	5.949	22.25	109.65	0.8	0.651	0.51	F	NE	Autopsy	1999
Heinemann, 1999	6	10.08	40.08	141.19	1.19	0.917	0.47	F	NE	Autopsy	1999