The role of glomerular filtration and active tubular secretion in predicting renal clearance of drugs in children using population pharmacokinetic and physiology-based pharmacokinetic modeling approaches: unspinning the yarn

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Section III. PBPK-based dosing of drugs cleared by glomerular filtration in children

Chapter 4

The predictive value of GFR-based scaling of pediatric clearance and doses for drugs eliminated by glomerular filtration with varying protein binding properties

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4.1 Abstract

For drugs eliminated by glomerular filtration (GF), clearance (CL) is determined by GF rate (GFR) and the unbound fraction of the drug. When predicting CL of GF-eliminated drugs in children, instead of physiologically-based (PBPK) methods which consider changes in both GFR and protein binding, empiric bodyweight-based methods are often used. Here we explore the predictive value of scaling using a GFR function and compare the results to linear and allometric scaling methods for drugs with different protein binding properties.

First, different GFR maturation functions were compared to identify the GFR function that would yield the most accurate GFR predictions across the pediatric age-range as compared to published pediatric inulin/mannitol CL values. Subsequently, the accuracy of pediatric CL scaling using this GFR maturation function was assessed and compared to PBPK CL predictions for hypothetical drugs binding to varying extends to serum albumin or α-acid glycoprotein across the pediatric age range. Additionally, empiric bodyweight-based methods were assessed.

The published GFR maturation functions yielded comparable maturation profiles, with the function of Salem et al. leading to the most accurate predictions. On the basis of this function, GFR-based scaling yields reasonably accurate (percentage prediction error ≤ 50%) pediatric CL values for all drugs, except for some drugs highly bound to AGP in neonates. Overall, this method was more accurate than linear or 0.75 allometric bodyweight-based scaling.

When scaling CL and dose by GFR function, maturational changes in plasma proteins concentrations impact GF minimally, making this method a superior alternative to empiric bodyweight-based scaling.

4.2 Introduction

Clearance (CL) is the driving parameter for dosing as it determines steady-state and trough concentrations. For children, precise scaling of clearance without bias across the pediatric age range is paramount to reach both an effective and safe (starting) dose. This is of relevance for defining (first-in-child) doses in clinical studies particularly of drugs for which differences in dose requirements between adults and children can be attributed entirely to differences in pharmacokinetics (PK) and/or for which target concentrations in children are known [1].

CL of drugs eliminated through glomerular filtration (GF) is dependent on GF rate (GFR) and plasma protein binding. GFR maturation across the pediatric population has been described by different functions based on data either from CL of endogenous (e.g. creatinine, cystatin C) or from exogenous (e.g. inulin, iohexol, aminoglycosides) compounds, used as markers for GFR function [2–7]. With respect to plasma protein binding, changes in the unbound drug fraction (f_u) with age need to be taken into account when predicting pediatric CL via GF, as only the drug fraction that is not bound to plasma proteins can be eliminated through GF. The unbound fraction across age is dependent on the protein the drug binds to (i.e. human serum albumin or α-acid glycoprotein) and the changes in the concentrations of these proteins with age[8]. As physiologically-based pharmacokinetic (PBPK) models include drug properties (i.e. f_u) and physiological differences between adults and children (i.e. maturation of plasma proteins concentrations and GFR), these models are considered the 'gold standard' for pediatric CL predictions [9].

The application of PBPK approaches is however constrained by the availability of both drug-specific data, skilled personnel and resources needed to access and use different modeling platforms. Therefore, empirical bodyweight-based scaling methods such as linear scaling or allometric scaling with a fixed exponent of 0.75 are still often used to derive pediatric CL from adult CL values. However, empirical scaling methods disregard information about maturation of both GFR and protein binding. Previous work has shown that these approaches are inaccurate for certain pediatric age-groups for drugs cleared by GF [10, 11], suggesting that more mechanistic information may be needed for accurate scaling. For

this, it has been proposed to adjust the allometric scaling with a maturation function for GFR, especially in the very young [12]. Here we assess the accuracy of scaling based on GFR maturation without taking into account maturational changes in f_u. We compare this approach to two relatively straight forward scaling methods based on bodyweight alone, since these methods are still often used and perhaps even preferred because of their ease.

To this end, we first identify the GFR maturation function that yields the most accurate GFR predictions across the pediatric age-range. Subsequently, we assess the accuracy of pediatric CL and dose scaling obtained with the GFR maturation function as compared to PBPK predictions for hypothetical drugs binding to varying extends to human serum albumin (HSA) or α-acid glycoprotein (AGP) across the pediatric age range. Additionally, the results are compared to those of the two-empiric bodyweight-based methods, i.e. linear and allometric scaling with a fixed exponent of 0.75.

4.3 Methods

4.3.1 Establishing the most accurate pediatric GFR maturation function

Functions that quantify GFR maturation throughout the pediatric age-range for children with a normal renal functionality and that only used demographic characteristics as input, were collected from the literature by searching the PUBMED data base with the search term “glomerular filtration maturation children human ” or from Simcyp v18 resources. Seven [7, 13–17] functions were identified, of which six [13–17] were developed based on exogenous markers for GFR (i.e. inulin,-Cr-EDTA, mannitol, iohexol) and one [7] was derived from CL values of antibiotics that are predominantly eliminated through GF. To visually compare the different GFR maturation profiles, age-appropriate body surface area (BSA), height, and weight values were derived from the NHANES database [18] and used for GFR predictions with each of the seven functions.

In this analysis, inulin and mannitol CL values were considered the ‘gold standard’ for GF function [19, 20], hence, they were used to select the most accurate pediatric GFR maturation function. GFR predictions with each of the seven maturation functions were compared to inulin [3–6] and mannitol [2] CL values published for children, for whom the necessary demographic characteristics were reported. Individual data were either digitized with WebPlotDigitizer (https://apps.automeris.io/wpd/) or extracted directly from the publications. When inulin and mannitol CL values were reported relative to the standard adult BSA (i.e. normalized by 1.73 m2), they were converted to absolute values. When gestational age was missing, a gestational period of 38 weeks was imputed. Missing BSA values were calculated based on age and bodyweight with the Haycock [21] and Dubois [22] formulas for children under and over 15 kg, respectively.

For the seven GFR maturation functions, the demographic characteristics corresponding to the individuals for whom inulin [3, 4, 6] and mannitol [2] CL values were available, were used as input and the resulting predictions were compared with the reported measurements. For this, a percentage prediction error ($\%PE_{GFR}$) between the predicted GFR with each function and the inulin [3, 4, 6] and mannitol [2] CL values was calculated according to equation 1. In addition, the root mean square percentage error ($\%RMSPE_{GFR}$) was calculated using equation 2 for the entire pediatric population as well as for selected age-groups to show the stratified accuracy of the GFR functions for preterm neonates, term neonates at the first day, newborns between 1 day and 1 month, and children between 1 and 6 months, between 6 months and 1 year, between 1 and 5 years, and between 5 and 15 years. In equations 1 and 2, the predicted GFR are values obtained with each of the published GFR maturation functions and observed $CL_{inulin/mannitol}$ are the published values for inulin or mannitol CL.

$$\%PE_{GFR} = \frac{predicted\ GFR - observed\ CL_{inulin/manitol}}{observed\ CL_{inulin/manitol}} \times 100 \quad [1]$$

$$\%RMSPE_{GFR} = \sqrt{\frac{1}{n} \times \sum_{i=1}^{n} \left(\frac{predicted\ GFR - observed\ CL_{inulin/manitol}}{observed\ CL_{inulin/manitol}} \right)^2} \quad [2]$$

As the predictions do not include variability or uncertainty in any of the terms, only point estimates of $\%PE_{GFR}$ and $\%RMSPE_{GFR}$ are obtained. To compensate for this, rather than applying the 2-fold rule that is commonly used in assessing the accuracy of PBPK model prediction we designated values within ±30% to be "accurate predictions", values outside the ±50% interval to be inaccurate, and with values in between to be reasonably accurate for $\%PE_{GFR}$. For $\%RMSPE_{GFR}$, values within 0% - 30% indicate "accurate predictions", values >50% indicate "inaccurate predictions", and values within 30% - 50% are "reasonably accurate". The GFR maturation function that would lead to the narrowest range in $\%PE_{GFR}$ predictions and the smallest $\%RMSPE_{GFR}$ overall and per age-group was selected and used in the PBPK-based approach as well as in the evaluation of pediatric CL scaling.

The results here do not include findings for preterm neonates as only four [7, 13, 15] of the seven GFR maturation functions were also developed for preterm neonates. Inulin and mannitol data collected from preterm neonates [3, 5, 23] were analyzed separately together with these four functions, and the results can be found in the supplemental material.

4.3.2 Evaluation of pediatric clearance scaling

To evaluate the accuracy of pediatric CL scaling using the selected GFR function or empiric functions, a 'true' CL value is needed as reference. As PBPK-based approaches are considered the "gold standard" for pediatric CL predictions, the renal PBPK model in equation 3 was used to derive 'true' CL values. 'True' CL of hypothetical drugs was predicted for typical pediatric individuals at the age of 1 day, 1, 3, 6 and 9 months, 2, 5, 10, and 15 years and a 35-year-old typical adult.

$$'true'CL = GFR \times f_u \quad [3]$$

In equation 3, pediatric GFR values were obtained with the best maturation function selected above. Demographic values needed to predict pediatric GFR values with the best GFR maturation function were derived from the NHANES database [18] and from the ICRP annals [24] for children and adults, respectively.

For f_u in equation 3, a total of 20 hypothetical drugs was evaluated. For these drugs, f_u values in adults ($f_{u,adult}$) of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1 were used and each drug was assumed to exclusively bind to either HSA or AGP. Pediatric f_u values ($f_{u,ped}$) at each pediatric age were obtained based on the ratios between relevant binding proteins concentrations and the $f_{u,adult}$, according to equation 4 [8]:

$$f_{u,ped} = \frac{1}{1 + \frac{[P]_{ped} \times (1 - f_{u,adult})}{[P]_{adult} \times f_{u,adult}}} \quad [4]$$

in which [P] stands for the plasma concentration of the relevant binding protein (i.e. HSA or AGP).

Equations 5 and 6 [15] were used to calculate the plasma concentrations ([P]) of HSA and AGP, respectively, for typical children of different ages, with age expressed in days. Visual representations of the maturation profiles of the plasma proteins as well as of the resulting fu,ped values are presented in supplemental Figure S4.1.

$$[HSA(g/L)] = 1.1287 \times \ln(Age) + 33.746 \quad [5]$$

$$[AGP(g/L)] = \frac{0.887 \times Age^{0.38}}{8.89^{0.38} + Age^{0.38}} \quad [6]$$

where [HSA(g/L)] and [AGP(g/L)] represent the plasma protein concentrations and Age is the age of the typical child expressed in days[15].

GFR-based scaling of clearance

For GFR-based scaling of CL from adults to children of different ages, equation 7 is used. Here 'true' adult CL values of the drug, i.e. GFR_{adult} multiplied by $f_{u,adult}$ (for 20 hypothetical drugs, see equation 3), were scaled by the ratio between GFR_{ped} and GFR_{adult}, with GFR_{ped} calculated according the selected function (see results, Salem [17], equation 12). Note that $f_{u,adult}$ is included for obtaining the 'true' adult CL values, however, changes in fu with age are not included when applying GFR-based scaling (equation 7).

$$GFR\ scaled\ CL_{ped} = {'true'}CL_{adult} \times \left(\frac{GFR_{ped}}{GFR_{adult}}\right) \quad [7]$$

Empiric and linear body-weight based scaling methods

For comparative purposes, the accuracy of GFR-based scaling was evaluated together with linear bodyweight-based scaling (equation 8) and bodyweight-based allometric scaling with a fixed exponent of 0.75 (equation 9), which are two commonly used empirical pediatric CL scaling methods.

$$Linear\ scaled\ CL_{ped} = {'true'}CL_{adult} \times \left(\frac{WT_{ped}}{WT_{adult}}\right) \quad [8]$$

$$Allometric\ scaled\ CL_{ped} = {'true'}CL_{adult} \times \left(\frac{WT_{ped}}{WT_{adult}}\right)^{0.75} \quad [9]$$

Comparison of different scaling methods

The accuracy of GFR-based, linear and allometric scaling with a fixed exponent of 0.75 of clearance was assessed by calculating the $\%PE_{CL}$ compared to 'true' Cl_{ped} according to equation 10. Note that in 'true' CL_{ped} (equation 3) the changes in f_u with age are considered according to equations 4-6.

$$\%PE_{CL} = \frac{scaled\ CL_{ped} - {'true'}CL_{ped}}{{'true'}CL_{ped}} \times 100 \quad [10]$$

4.3.3 Assessment of pediatric dose scaling

As CL scaling is commonly used as the basis for dose scaling, the implications of the different CL scaling methods on the accuracy of the dose-adjustments derived from them were also assessed. For each of the 20 hypothetical drugs for which 'true' adult CL values (equation 3) were calculated, equation 11 was used to derive the pediatric dose.

$$dose_{ped} = dose_{adult} \times \left(\frac{CL_{ped}}{{'true'}CL_{adult}}\right) \times 100 \quad [11]$$

in which CLped refers to CL values obtained with either of the three simplified scaling methods (GFR–based scaling, linear scaling or allometric scaling with a fixed exponent of 0.75) according to equations 7, 8 and 9, respectively. This method assumes steady-state conditions (i.e. drug exposure is only dependent on dose and CL) and that the same drug target exposure (i.e. AUC) is applicable in children and adults. As relative dose adjustments were assessed, the adult dose was expressed as 1.

The 'true' reference doses were obtained by replacing the CL_{ped} value in equation 11 by the 'true' CL_{ped} value (equation 3). The accuracy of the scaled doses was assessed by calculating the $\%PE_{dose}$ according to equation 10.

4.4 Results

4.4.1 Establishing the most accurate pediatric GFR maturation function

Figure 4.1 shows the seven published GFR maturation profiles [7, 13–17]. All profiles are comparable

with the steepest maturation occurring in the first two years of life and plateau values being reached beyond the age of 15 years.

Figure 4.2 depicts the $\%PE_{GFR}$ between GFR predictions according to the seven different functions versus inulin [3, 4, 6] or mannitol [2] CL measurements. In addition, Table 4.1 presents the $\%RMSPE_{GFR}$ and the range in $\%PE_{GFR}$ per age-group as well as for the entire pediatric age-range. The results show that all functions tend towards over-prediction of GFR in the very young. In newborns, inter-individual variability is higher than in older children which yields the largest spread in $\%PE_{GFR}$ for all GFR functions, with values ranging between -112% and 484%. Furthermore, $\%RMSPE_{GFR}$ in newborns can reach values of 158% compared to values below 50% in older children. For all functions, the $\%PE_{GFR}$ range becomes narrower with increasing age and above 5 years most functions lead to accurate predictions ($\%PE_{GFR}$ within ±30%). The function of Salem [17] had the best predictive performance per age-group and across all pediatric ages. These GFR predictions were similar to the ones obtained with the function of Rhodin [14], as indicated by the $RMSPE_{GFR}$% values and $\%PE_{GFR}$ ranges for the entire population as for the different age groups. Results for preterm neonates are presented in the supplemental material (Figure S4.2, Table S4.1).

From these results, the GFR maturation function published by Salem [16] (equation 12) was selected and used in the renal PBPK model (equation 3) to determine the 'true' renal CL of the 20 hypothetical drugs for the typical adult and the typical pediatric individuals. These GFR values are also used in equation 7 to calculate GFR based scaled clearance values across the pediatric range.

$$GFR_{ml/min} = 112 \times \left(\frac{Weight(kg)}{70}\right)^{0.63} \times \frac{PMA^{3.3}}{PMA^{3.3}+TM_{50}^{3.3}} \quad [12]$$

with PMA defined as postmenstrual age in weeks and TM50 as the PMA at which GFR reaches half of the adult levels.

4.4.2 Evaluation of pediatric clearance scaling

Figure 4.3 shows the $\%PE_{CL}$ for GFR-based scaling and for the two empirical bodyweight-based scaling methods, none of which take changes in plasma protein concentrations into account. The figure illustrates how scaling accuracy of CL with each of the three methods is impacted by f_u (color intensifies with increased f_u) and plasma protein concentrations at every investigated age. Overall, GFR-based scaling is

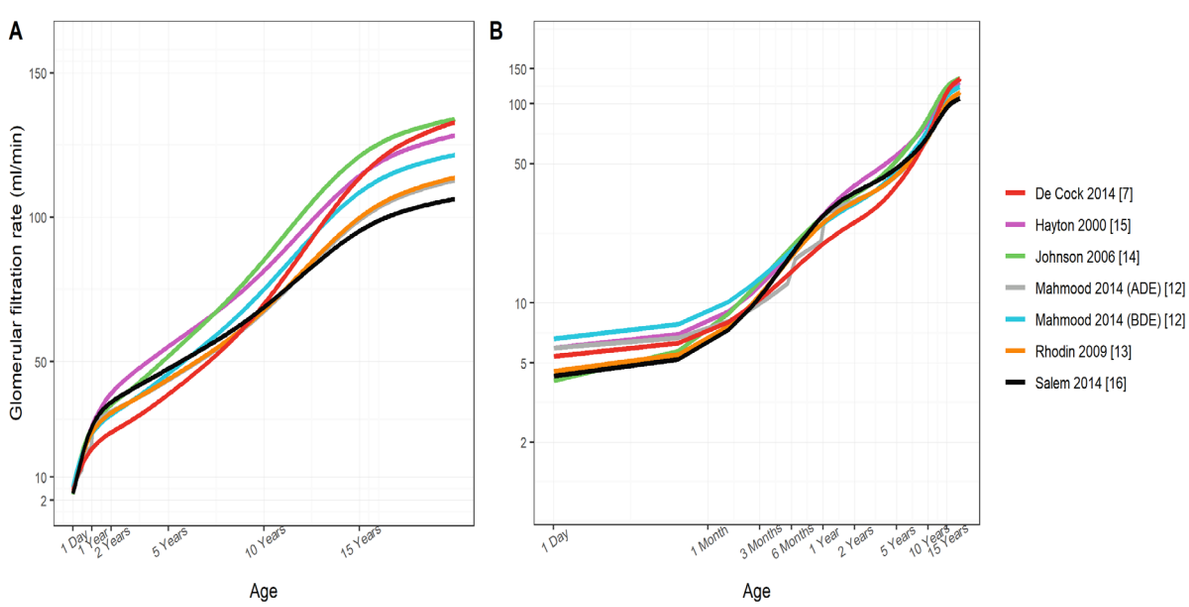

Figure 4.1 – Pediatric glomerular filtration rate (GFR) according to published GFR maturation functions [7, 13–17] throughout the pediatric age range. Panel A – semi-logarithmic scale; Panel B – double logarithmic scale.

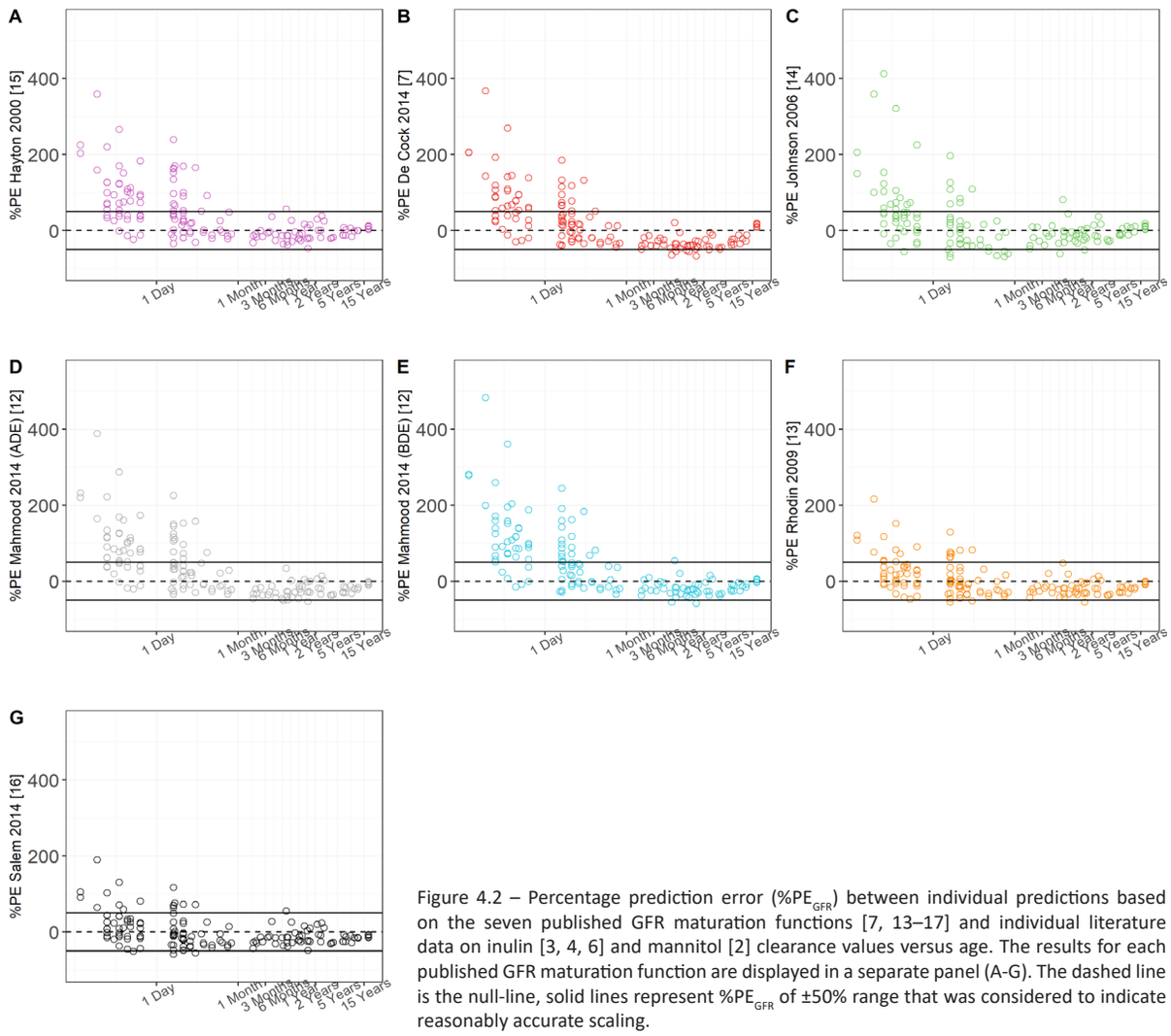

Figure 4.2 – Percentage prediction error ($\%PE_{GFR}$) between individual predictions based on the seven published GFR maturation functions [7, 13–17] and individual literature data on inulin [3, 4, 6] and mannitol [2] clearance values versus age. The results for each published GFR maturation function are displayed in a separate panel (A-G). The dashed line is the null-line, solid lines represent $\%PE_{GFR}$ of ±50% range that was considered to indicate reasonably accurate scaling.

more accurate than the two empirical bodyweight-based methods, leading to $\%PE_{CL}$ values within ±50% (f_u adult < 0.3). Bodyweight-based allometric scaling with a fixed exponent of 0.75 is mostly inaccurate for individuals below 3 months. GFR-based scaling and linear scaling outperform allometric scaling for these subjects. For children between 6 months and 15 years of age, linear scaling is reasonably accurate albeit with a trend in $\%PE_{CL}$ values indicating systematic bias towards under-prediction. In this age-range similar, yet less strong, trends are seen for allometric scaling with a fixed exponent of 0.75, while GFR-based scaling is generally the most accurate of the three (Figure 4.3).

4.4.3 Assessment of pediatric dose scaling

Figure 4.4 and Table 4.2 show pediatric doses (expressed as percentage of adult dose) obtained with 'true' CL values versus those obtained with CL values by the three scaling methods in typical patients for 20 hypothetical drugs differing in unbound drug fraction in adults and binding to either HSA or AGP. Both the figure and table show that the 'true' doses predicted based on 'true' pediatric CL values are dependent on f_u whereas the scaled doses derived from CL values scaled with the three different scaling methods (i.e. GFR scaling, linear scaling and allometric scaling) are not. Overall, the results show that doses obtained with GFR-based scaling are lower than the 'true' reference doses for drugs highly bound (i.e. f_u =0.1) to HSA or AGP (up to 20 to 60%, respectively). For drugs with low protein binding (i.e. f_u = 0.9), the differences between the 'true' reference dose and GFR-based scaled doses are small throughout the pediatric age-range (<5%). Using linear bodyweight-based scaling doses are also lower than the 'true' reference doses for children with ages between 6 months and 10 years (up to 25.5%

Table 4.1 – Root mean square percentage error (%RMSPE$_{GFR}$) and percentage prediction error (%PE$_{GFR}$) ranges for the GFR predictions by the different published GFR maturation functions, stratified by age groups.

Age groups	**Salem 2014[16]**			**Rhodin 2002 [13]**			**Hayton 2000 [15]**			**De Cock 2014 [7]**			**Johnson 2006 [14]**			**Mahmood 2016 (ADE) [12]**			Mahmood 2016 (BDE) [12]		
	%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$	
		[min-max]			[min-max]			[min-max]			[min-max]			[min-max]			[min-max]			[min-max]	
At first day	54	-51	190	62	-47	216	123	-23	360	113	-29	367	123	-55	413	69	-44	235	158	-15	484
Between 1 day and 1 month	36	-58	117	36.5	-55	129	181	-34	482	58	-44	185.5	62	-112	197	52	-53	141	71	-34	245
Between 1 and 6 months	26	-43	28	25	-43	20	36	-32	117	32	-49	15	26	-49	13	29	-47	7	21	-37	28
Between 6 months and 1 year	30	-38	55	32	-42	48.5	30	-38	57.5	42	-63	21	38	-60	81.5	34	-48	33	35	-55	54
Between 1 and 5 years	23	-49	24	27	-55	13	22	-47	39	41	-67	-4.3	23	-51	37	26	-55	22	29	-58	16
Between 5 and 15 years	16	-28	-6	18	-31	0.82	9	-16	13	23	-37	19	10	-12	19	13	-21	17	14	-26	7
Entire pediatric age-range	38	-58	190	41	-55	216	141	-47	482	68	-67	367	77	-112	413	50	-55	235	88.5	-58	484

to 49% lower). For younger children, the difference between doses becomes smaller (less than 30% difference). Doses obtained using bodyweight-based allometric scaling with a fixed exponent of 0.75 are generally higher than the 'true' reference doses for children younger than 6 months. For this method, the highest difference of >150% was obtained for drugs with high fraction unbound in children younger than 1 month (Figure 4.4, Table 4.2).

4.5 Discussion

This study aimed to identify the GFR maturation function that yields the most accurate GFR predictions across the entire pediatric age-range and subsequently to assess what the accuracy of GFR-based scaling of CL and dose is as compared to the gold standard (i.e. PBPK-based predictions) and to two commonly used empiric bodyweight-based scaling methods. By comparing scaled CL values to PBPK CL predictions, we studied the influence of the maturation of plasma proteins concentrations on CL and dose scaling and showed at what ages this maturation is of relevance for each scaling method. The assessed scaling methods are typically used to guide pediatric dosing when little or no information is available on a

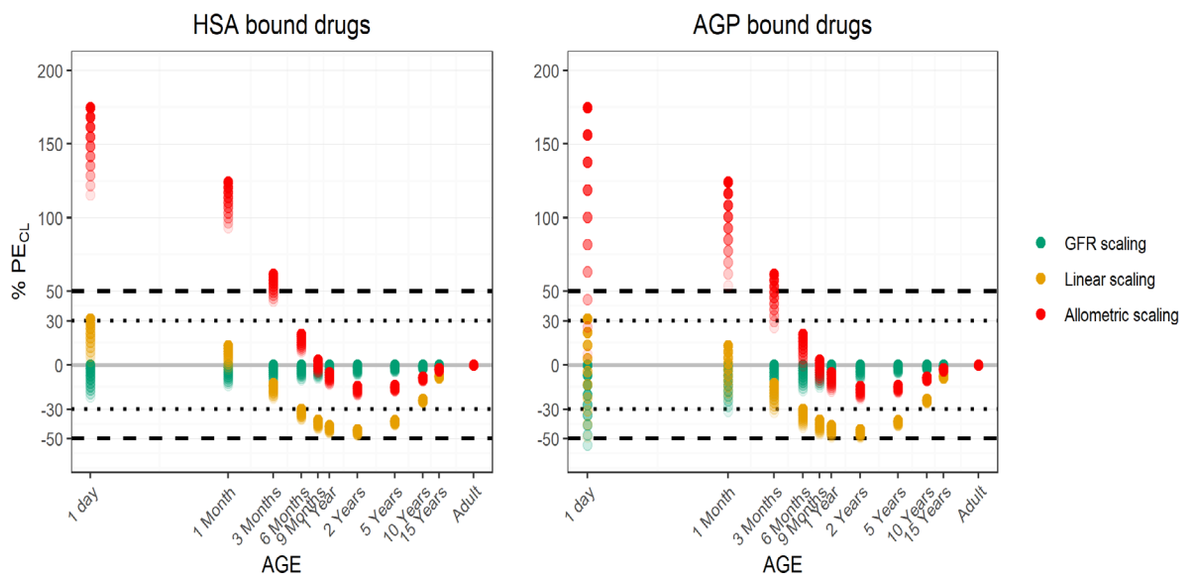

Figure 4.3 – Percentage prediction error (%PE_{CL}) between 'true' clearance (CL) values and CL values obtained with three different simplified scaling methods in typical pediatric patients for 20 hypothetical drugs differing in unbound drug fraction in adults and binding to either HSA (left panel) or AGP (right panel). Green dots indicate GFR-based scaling, orange dots indicate linear bodyweight-based scaling, red dots indicate bodyweight-based scaling with a fixed allometric exponent of 0.75. Colors intensify with increasing f_u. The grey solid line is the null-line, black dashed lines and black dotted lines represent the %PE_{CL} range of ±30% and ±50%, respectively, that indicate accurate and reasonably accurate scaling, respectively.

drug in this population. As such, this work identifies drug properties (i.e. f_u) and patient characteristics (i.e. age) for which bodyweight-based scaling methods suffice and when more mechanistic information is necessary by means of either GFR-based scaling or PBPK for accurate CL and dose scaling. Our findings provide guidance for (first-in-child) clinical studies on what scaling method to use when deriving pediatric doses from adult doses of small molecules drugs that are mainly eliminated by GF.

The published GFR maturation functions we evaluated were found to have comparable profiles while the functions published by Salem [17] and by Rhodin [14] had similar accuracy in predicting inulin [3, 4, 6] and mannitol [2] CL measures, with the function by Salem [17] being overall slightly more accurate. This function (equation 12) was used in PBPK-based predictions of 'true' pediatric CL values (equation 3) and it was directly used for simplified GFR-based scaling (equation 7).

Drug CL by GF depends on GFR and plasma protein binding, which are taken into account by PBPK modeling approaches. However, the extent of protein binding and the proteins the drugs bind to may not

always be known, especially for the pediatric population. The simplified scaling functions, which include GFR-based scaling (equation 7), bodyweight-based linear scaling (equation 8), and bodyweight-based

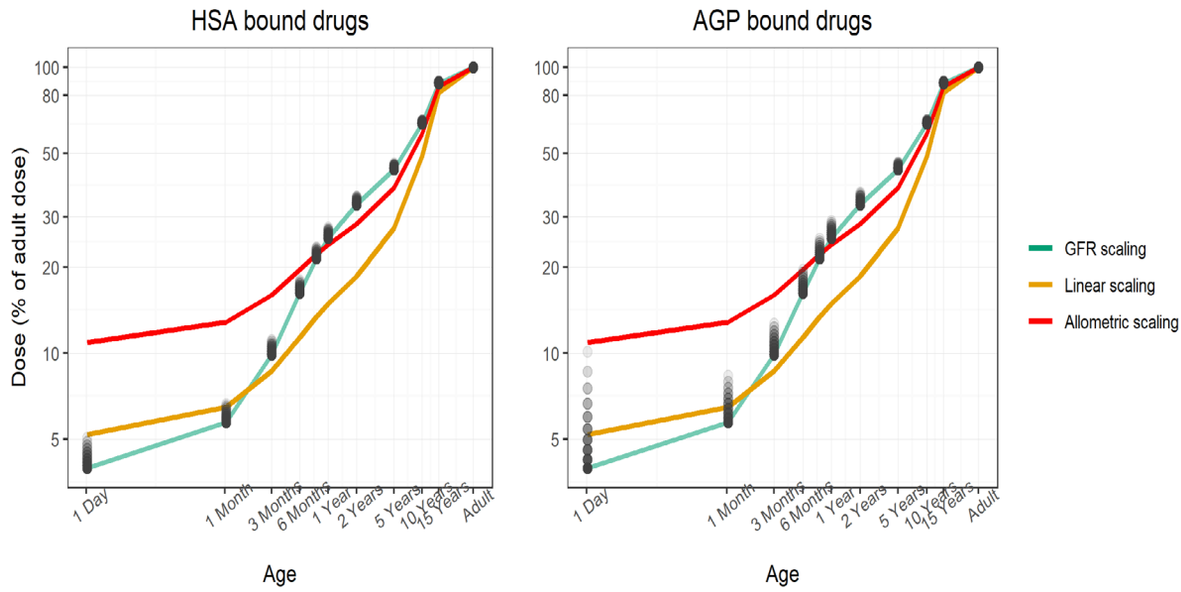

Figure 4.4 – Pediatric doses (percentage of adult dose) obtained with 'true' clearance (CL) values (black dots) and CL values obtained with three different simplified scaling methods (lines) in typical pediatric patients for 20 hypothetical drugs differing in unbound drug fraction in adults and binding to either HSA (left panel) or AGP (right panel). Green line indicates dose values obtained with GFR-based scaling, orange line indicates dose values obtained with linear bodyweight-based scaling, red line indicates dose values obtained with bodyweight-based scaling with a fixed allometric exponent of 0.75. The black dots indicate dose values obtained with 'true' CL. Color intensifies with increasing f_u

allometric scaling with a fixed exponent of 0.75 (equation 9), typically do not take changes in plasma protein binding with age into account. The difference between GFR-based scaled pediatric CL values and 'true' pediatric CL values reflects the impact of ignoring maturation in plasma protein concentrations on CL scaling. The current analysis showed that with GFR-based scaling this impact can be disregarded throughout the entire pediatric age-range, except in neonates for a few drugs highly bound to AGP (Figure 4.3). Prediction errors in scaled CL values are largest in neonates, especially for drugs that bind to AGP, possibly due to its steep maturation in early life (Figure S4.1). GFR-based scaling leads to under-prediction of CL in neonates and drug doses, as compared to 'true' CL and 'true' reference doses, which will result in a reduced risk of developing toxic effects, but also in an increased risk of treatment failure. Bodyweight-based allometric scaling with a fixed exponent of 0.75 tends to over-predict CL in children younger than 6 months, even though for drugs with a low f_u maturational changes in the expression of drug binding plasma proteins can still partially correct this bias. Bodyweight-based linear scaling leads to reasonably accurate CL predictions in this young population. After the age of 6 months the influence of plasma protein binding on CL scaling decreases as shown by a smaller deviation of GFR-based scaled CL from PBPK-based CL predictions. In this age-range reasonably accurate CL predictions are obtained using bodyweight-based scaling, irrespective of whether the exponent is 1 (linear scaling), 0.75 (allometric scaling), or 0.62 (GFR function from Salem et al.). As scaled CL values drive the scaled dose values, the same patters are observed for this variable.

The CL predictions of selected drugs (>80% renal elimination) in neonates and children using the GFR maturation function of Rhodin [14], was recently described [25]. Our results are in line with these published findings, with the added advantage that our analysis captures the entire hypothetical parameter space regarding the relevant drug-specific parameters (i.e. extent and type of plasma protein binding). As such the presented analysis covers both drugs that are currently in clinical use as well as all small molecule drugs that are still to be developed. Therefore, this framework can be used to make a priori assessments on the accuracy of the pediatric CL and dose scaling methods for new drugs.

Table 4.2 – Pediatric doses presented as % of adult dose for drugs eliminated through GFR with varying f_u values. The 'true' doses predicted based on 'true' pediatric CL values are dependent on f_u whereas the scaled doses derived from CL values scaled with the three different scaling methods (i.e. GFR scaling, linear scaling and allometric scaling) are not.

Demographic Characteristics of Typical Individuals			**'True' dose (% of adult dose) obtained based on 'true' CL**				**Scaled dose (% of adult dose) obtained using three CL scaling methods**		
Age	Weight* (kg)	GFR** (ml/min)	Drugs binding to HSA		Drugs binding to AGP		GFR scaling	Linear scaling	Allometric scaling
			f_u = 0.1	f_u = 0.9	f_u = 0.1	f_u = 0.9			
1 Day	3.4	4.3	5%	4.1 %	10.1 %	4.2 %	4 %	5.2%	11 %
1 Month	4.3	6.2	6.6 %	5.8 %	8.3 %	5.9 %	5.7 %	6.5 %	13 %
3 Months	5.8	10.7	11.1 %	10 %	12.7 %	10.1 %	9.9 %	8.6 %	16 %
6 Months	7.5	17.6	17.9 %	16.4 %	19.6 %	16.5 %	16.2 %	11.4 %	20 %
9 Months	8.9	23.2	23.5 %	21.6 %	25.1 %	21.8 %	21.4 %	13.4 %	22 %
1 Year	9.9	27.4	27.5 %	25.5 %	29.1 %	25.6 %	25.3 %	14.9 %	24 %
2 Years	12.3	35.9	35.4 %	33.3 %	36.5 %	33.4 %	33.1 %	18.6 %	28 %
5 Years	18.2	47.7	46 %	44.2 %	46.6 %	44.3 %	44 %	27.4 %	38 %
10 Years	32.5	68.9	65.4 %	63.8 %	65.6 %	63.8 %	63.6 %	48.9 %	58 %
15 Years	54.2	95.3	89.7 %	88.1 %	89.7 %	88.1 %	87.9 %	81.6 %	86 %
Adult	66.5	108.4	100 %	100 %	100 %	100 %	100 %	100 %	100 %

*weights from the NHANES database [17] for children and from the ICRP annals [23] for adults
** GFR values were predicted with Salem [16]

The current results are also in line with previous findings from our group comparing 'true' PBPK-based CL predictions to CL values scaled by both methods however small differences in numerical results are present. These differences are caused by two different GFR maturation functions being used in the PBPK model for the predictions of the 'true' CL values. For the current analysis we used the function published by Salem [17], which we now found to be most accurate, whereas, in the previous analyses the function by Johnson [15] was used.

The conclusions from our analysis are based on typical individuals and do not take inter-individual variability into account. For preterm and term neonates younger than 1 month, high variability in the inulin [3, 4, 6] and mannitol [2] CL data is observed, which poses a challenge when scaling CL and doses to this age–range. This suggests that variables other than the demographics used in GFR maturation functions are predictive of GFR-based clearance. For this special population, dosing recommendations that rely on empiric PK models of the same drug, even in slightly older children or of a similar drug that is mainly eliminated through GF in the same population, may therefore offer a better alternative [26, 27]. We emphasize that all published GFR maturation functions included in our analysis describe GFR maturation in pediatric individuals with normal renal function. These functions should therefore not be used for CL or dose scaling for pediatric patients with renal deficiencies. To account for renal impairment, functions that require a biomarker for renal function (e.g., creatinine, cystatin C, etc.) as input are more reliable and suitable to predict GFR. These functions can be implemented in the renal PBPK model in equation 3 and can also be used for GFR-based scaling. The impact of ignoring plasma protein binding in these scenarios may not be the same as observed in the current analysis, as plasma protein binding may also be altered in patients with renal deficiencies.

4.6 Conclusion

The maturation function by Salem [17] (equation 12) describes GFR most accurately throughout the pediatric age-range as compared to data on inulin and mannitol clearance. GFR-based CL and dose

scaling for drugs eliminated through GFR yields reasonably accurate pediatric CL and dose values, despite ignoring the influence of maturational changes in protein binding, except for drugs highly bound to AGP in neonates.

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4.9 Supplementary material

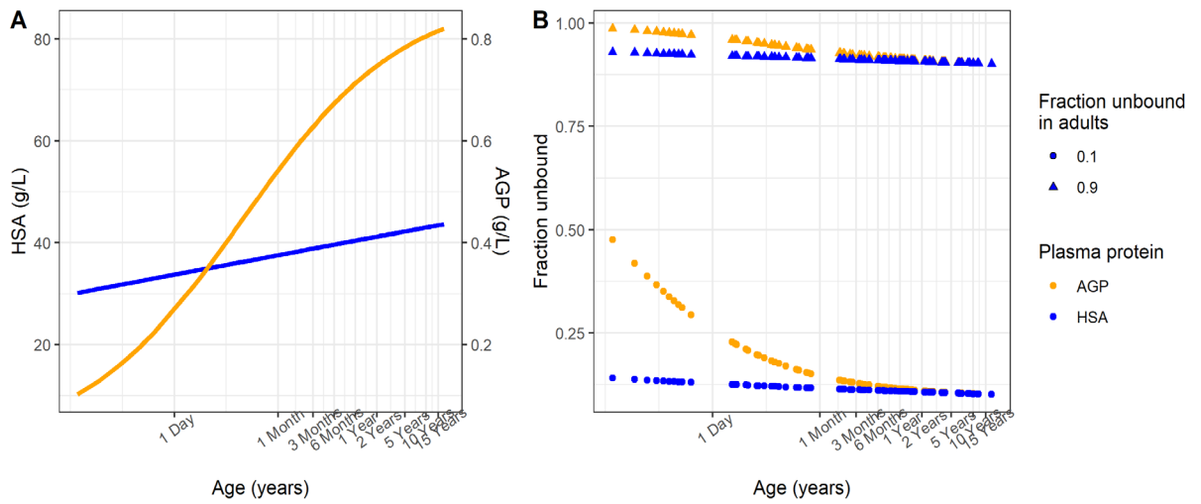

Figure S4.1 – Maturation profiles for plasma protein expression and plasma protein binding. Left panel (A) shows plasma concentrations of the plasma proteins human serum albumin (HSA) in blue and α-acid glycoprotein (AGP) in orange with age. Right panel (B) shows the changes in protein binding with age for each of the plasma proteins (AGP in orange, HSA in blue) when the fraction unbound measured in adults is either 0.1 (circles) and 0.9 (triangles).

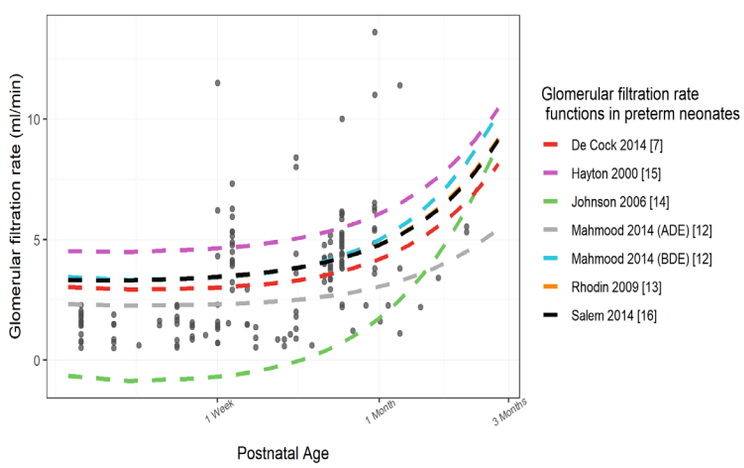

Figure S4.2 – GFR predictions using published maturation function [7, 12–16] for typical preterm neonates born at 35 weeks and a weight of 2330 g during the first 12 weeks of life [27] (dashed lines) overlaid with observed inulin clearance measurements collected from literature [3, 5, 22] (dots). Rhodin [13] and Salem [16] are overlapping.

Establishing the most accurate GFR maturation function in preterm neonates

As only four [7, 12, 14] of the published GFR maturation functions assessed in this manuscript were developed including data from preterm neonates and as maturation functions for physiological parameters in PBPK models are often not known in preterm neonates, the assessment of the accuracy of the published GFR maturation predictions in preterm neonates was performed separately. For this, a typical preterm neonate born at 35 weeks with a birthweight of 2330 g followed for the first 12 weeks of life was used [27]. The demographics for the typical preterm neonate were selected as they most resembled the data collected from literature[3, 5, 22]. For the remaining three [13, 15, 16] GFR maturation functions that were not based on data from preterm neonates extrapolations were made. Furthermore, extrapolations were made for the functions used to characterize the maturation of plasma proteins concentrations and to obtain the unbound fractions in preterm neonates.

In Figure S4.2 we show the GFR predictions with the seven published functions for the typical preterm neonate overlaid with literature data collected for preterm neonates [3, 5, 22]. By using the demographics of the published data, we found that in preterm neonates, the prediction accuracy of the maturation function of Mahmood [12] had the lowest $\%RMSPE_{GFR}$ value of 37%, but it had a similar $\%PE_{GFR}$ range compared to Salem [16] and Rhodin [13] (Table S4.1).

TaTable S4.1 – Root mean square percentage error (%RMSPE$_{GFR}$) and percentage prediction error% (%PE$_{GFR}$) ranges of the GFR predictions for preterm neonates, obtained with each of the studied GFR maturation functions.

Age groups	**Salem 2014 [16]**			**Rhodin 2002 [13]**			**Hayton 2000 [15]**			**De Cock 2014 [7]**			**Johnson 2006 [14]**			**Mahmood 2016 (ADE) [12]**			**Mahmood 2016 (BDE) [12]**		
	%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$		%RMSPE$_{GFR}$	%PE$_{GFR}$	
		[min-max]			[min-max]			[min-max]			[min-max]			[min-max]			[min-max]			[min-max]	
Preterm	46	-72	26	45	-72	26	60	-56	299	42	-77	39	238	-1215	-73	41	-68	48	37	-78	28

4.10 Supplementary references

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4.11 R code

```
# title: "Dose scaling using GFR maturation functions"
# author: "SC"
# date: "29 Apr 2019"
# output: data to be used with scripts:
# list of scripts to be added here
#'@abbrev: [GA - gestational age]
# ==============================

# set wd:
loc1 <- "1.Data/1.LitData/"
wd <- paste0(wd1, loc1)

# small function to read in all csv files:

  data_name <- function(wd, file_name, ext = ".csv") {paste(wd,"\\", file_
name, ext, sep = "")}

# preterm datasets with comments:
# ------------------------------

  # preterm female babies, healthy otherwise (?)
  mydata1 <- read.csv(data_name(wd = wd, file_name = "Barnett 1948"), header
= T, sep=";", stringsAsFactors = F)
  mydata1$status <- "preterm"

# preterm babies - healthy at the time of study

  mydata5 <- read.csv(data_name(wd = wd, file_name = "Leak 1976"), sep=";",
header = T, stringsAsFactors = F)
  mydata5$status <- "preterm"


# "healthy kidneys" babies datasets with comments:
# ------------------------------

  mydata2 <- read.csv(data_name(wd = wd, file_name = "Coulthard 1975"),
header = T, sep=";", stringsAsFactors = F)
  mydata2$status <- ifelse(mydata2$Gestation..wks. < 37, "preterm",
"healthy") # cut-off of 37 weeks for GA was used.


# term babies with meningo-myelocoeles with low life expectancy; postmortem
analysis of kidneys
# didn't reveal any renal impairment

  mydata4 <- read.csv(data_name(wd = wd, file_name = "Dean 1947"), sep=",",
header = T, stringsAsFactors = F)
  mydata4$status <- "healthy" # MM changed to healthy


# (near)term healthy neonates: 38-42 weeks of gestation;
mydata6 <- read.csv(data_name(wd = wd, file_name = "Oh 1966"), sep=";",
header = T, stringsAsFactors = F)
names(mydata6) <- as.character(mydata6[1,])
mydata6 <- mydata6[c(-1,-45),]
mydata6$status <- "healthy"

mydata7 <- read.csv(data_name(wd = wd, file_name = "Oh 1966-2"), sep=";",
header = T, stringsAsFactors = F)
mydata7$status <- "healthy"
```

```
# healthy babie, Rubin 1965:
# input by hand (not proud)

mydata8 <- data.frame(
  AGED = c(2,7,10,10,14,14,15,19,19,20,22,54,55,61,63,75,81,101,108,118,137
,138,181,190,216,223,225,229,232,268,275,314,356,371,374,395,417,456,517,51
7,532,547,578,578,782,821,912,912,943,1034,1368,1429,1521,2281,2311,2372,25
24,2554,3102,3284,3649,4197,4318,6569),
  AGEY = c(0.005,0.019,0.027,0.027,0.038,0.038,0.041,0.052,0.052,0.055,0.06
0,0.148,0.151,0.167,0.173,0.205,0.222,0.277,0.296,0.323,0.375,0.378,0.496,0
.521,0.592,0.611,0.616,0.627,0.636,0.734,0.753,0.860,0.975,1.016,1.025,1.08
3,1.141,1.250,1.416,1.416,1.458,1.500,1.583,1.583,2.141,2.250,2.499,2.499,2
.583,2.833,3.749,3.916,4.166,6.249,6.332,6.499,6.915,6.998,8.498,8.998,9.99
8,11.497,11.831,18),
  AGEM = c(0.066,0.230,0.329,0.329,0.460,0.460,0.493,0.625,0.625,0.658,0.72
3,1.776,1.809,2.006,2.072,2.466,2.664,3.321,3.551,3.880,4.505,4.538,5.952,6
.248,7.103,7.333,7.399,7.530,7.629,8.813,9.043,10.326,11.707,12.2,12.3,13,1
3.7,15,17,17,17.5,18,19,19,25.7,27,30,30,31,34,45,47,50,75,76,78,83,84,102,
108,120,138,142,216),
  PMA = c(40.286,41.000,41.429,41.429,42.000,42.000,42.143,42.714,42.714,42
.857,43.143,47.714,47.857,48.714,49.000,50.714,51.571,54.429,55.429,56.857,
59.571,59.714,65.857,67.143,70.857,71.857,72.143,72.714,73.143,78.286,79.28
6,84.857,90.857,93.000,93.435,96.476,99.517,105.164,113.853,113.853,116.025
,118.197,122.541,122.541,151.648,157.296,170.329,170.329,174.673,187.706,23
5.493,244.181,257.214,365.821,370.166,378.854,400.576,404.920,483.117,509.1
83,561.314,639.511,656.889,978.366),
  BWg = 1000 * c(2.4,3.35,3,3.2,2.7,3.8,3.7,2.8,3,3.1,3,3.75,3.5,5.3,4.3,5.
4,4.15,3.8,5.1,3.6,7.2,7.1,5,4.2,8,7.9,8.35,7.7,8,8.9,8.8,7.7,7.3,9.25,11,7
.5,9.6,6.5,10.5,11.4,9.5,13.6,10,13.2,10.3,10,10.6,11.8,15.5,17,17.8,13.6,1
6.7,25.9,19.5,18.9,20.9,22.7,28.9,24.9,25,20,35.3,70),
  BWkg = c(2.4,3.35,3,3.2,2.7,3.8,3.7,2.8,3,3.1,3,3.75,3.5,5.3,4.3,5.4,4.15
,3.8,5.1,3.6,7.2,7.1,5,4.2,8,7.9,8.35,7.7,8,8.9,8.8,7.7,7.3,9.25,11,7.5,9.6
,6.5,10.5,11.4,9.5,13.6,10,13.2,10.3,10,10.6,11.8,15.5,17,17.8,13.6,16.7,25
.9,19.5,18.9,20.9,22.7,28.9,24.9,25,20,35.3,70),
  BSA = c(0.208,0.231,0.212,0.224,0.2,0.251,0.242,0.205,0.214,0.222,0.208,0
.244,0.237,0.312,0.274,0.307,0.266,0.246,0.299,0.243,0.388,0.388,0.292,0.26
,0.423,0.421,0.436,0.413,0.423,0.459,0.452,0.413,0.399,0.467,0.524,0.405,0.
479,0.387,0.508,0.516,0.477,0.607,0.491,0.593,0.505,0.492,0.503,0.544,0.679
,0.69,0.737,0.606,0.67,0.94,0.83,0.67,0.87,0.9,1.035,0.96,0.99,0.8,1.24,1.8
3),
  GFR = c(5.16,6.28,6.44,7.51,5.32,5.37,7,7.23,6.93,4.11,6.61,11.8,8.9,11.4
,10.9,11,11.2,10.1,12.1,7,21.5,17.5,15.9,19.2,12.7,22.9,28.9,32,23.7,18.3,3
2.4,22.9,28.6,29.8,37.6,31.8,30.9,19.2,40.8,31.4,57.1,47.4,27.8,48.6,26.8,3
4.1,36.2,47.8,34.1,40.7,67.5,55.8,62.5,79.3,66.2,52.2,69.5,62.7,70.3,81.1,6
8.8,59.2,86,120)
  )
mydata8$status <- "healthy"

# diseased datasets:
# ------------------------------

# dose was given in mg/kg and the children often needed 50-100% more of the
drug than the adults to achieve
# equivalent plasma concentrations; a higher clearance of amikacin in pro-
portion with BW
# dose based on BSA resulted in uniform requirements and predictable plasma
concentrations
# aplastic anemia; pelvic inflamatory disease; cystic fibrosis; acute lympho-
cytic leukemia; acute nonlymphatic
# leukemia; appendicitis; ovarian teratoma; wilms tumor;They also say that
the value they found was 20-25% higher
```

```
# than the value reported in adults

mydata9 <- read.csv(data_name(wd = wd, file_name = "Vogelstein 1977"),
sep=";",  header = T, stringsAsFactors = F)
mydata9$status <- "diseased"

# standardize datasets to merge into one:
# ------------------------------
# changes in mydata1:

mydata1$PMA.wk <- (mydata1$Age.days. / 7) + 33.4 # healthy preterm, based
on Table 1 of Anchieta et al. 2003, this assumption should be good.

# changes in mydata6:
# there are 168 hours in a week

mydata6$PMA.wk <- (as.numeric(mydata6$`Age (h)`) / 168) + 38 # term GA = 38
weeks

#changes to mydata7
mydata7$PMA.wk <- (mydata7$Age..days./7) + 38 # term GA = 38 weeks

# merge datasets into one:
# ------------------------------------------
# OrID has the number of the dataset pasted in front of the original ID
# for mydataset1 made all of them factors and put 1 in front
# mydata2 and mydata3 were excluded because they produced negative clear-
ances or was data from older adults
# with decreased renal function

options(stringsAsFactors = FALSE)

all.data <- data.frame(
  OrID = c(paste("1", as.numeric(as.factor(mydata1$ID)), sep=""),
paste("4", mydata4$ID, sep=""),
           paste("5", mydata5$study, sep=""), paste("6", mydata6$-
Case,sep=""), paste("7", mydata7$Case, sep=""),
           paste("9", mydata9$Patient.No., sep=""), paste("8", c(1:n-
row(mydata8)), sep="")),
  BW.g = c(mydata1$WT.g., mydata4$Weight, mydata5$WT.at.study..g., mydata-
6$`Birth wt (g)`, mydata7$Study.weight..g.,
            (mydata9$Weight..kg.*1000),mydata8$BWg),
  PMA.wk = c(mydata1$PMA.wk,mydata4$PMA..wks.,mydata5$PMA.wks.,mydata6$PMA.
wk,
           mydata7$PMA.wk,mydata9$PMA.with.assumed.38.weeks.of.gestation,
mydata8$PMA),
  AGE.y = c((mydata1$Age.days./365), (mydata4$AGE.days./365), (mydata-
5$weeks.at.study/52), (as.numeric(mydata6$`Age (h)`)/8760),
             (mydata7$Age..days./365), mydata9$Age.yrs., mydata8$AGEY),
  BSA = c(mydata1$BSA.sqm., mydata4$BSA, mydata5$Calc.BSA, mydata6$`BSA
(sqm)`, mydata7$BSA..sqm.,
          mydata9$BSA.sqm., mydata8$BSA),
  GFR = c(mydata1$CL.ml.min., mydata4$Inulin.clearance.ml.min, mydata5$Cin.
ml.min., mydata6$`Cin(ml/min)`,
          mydata7$Cin.ml.min., mydata9$Inul.RC, mydata8$GFR),
  STATUS=c(mydata1$status, mydata4$status, mydata5$status, mydata6$status,
mydata7$status,
           mydata9$status, mydata8$status)
)

# Extra changes:
# -------------
```

```
# mydata2
names(mydata2)<-c("ID","GA.wk","BrW.kg","FPA","PMA.wk","GFR","PW.kg","SW.
kg","status")
mydata2$AGE.y<-(mydata2$PMA.wk-mydata2$GA.wk)/52
mydata2$BW.g<-mydata2$PW.kg*1000
mydata2$BSA<-NA # made this change as BSA in this dataset returned negative
CL values for Johnson 2006


# added mydata2 to the whole dataset:

mydata2a<-mydata2[,c(1,11,5,10,12,6,9)]
names(mydata2a)<-names(all.data)
all.in<-rbind(all.data,mydata2a)
all.in$GFR<-as.numeric(all.in$GFR) # the observed GFR column made numeric
all.in$BW.g<-as.numeric(all.in$BW.g)
all.in$BSA<-as.numeric(all.in$BSA)
remove(list = c("mydata1", "mydata2", "mydata2a", "mydata4", "mydata5",
"mydata6", "mydata7", "mydata8", "mydata9", "all.data"))

# add data for the AUC, dose, cl at maintenance dose analysis:

# Generate typical demographics dataframe:
demo <- data.frame(
  lab = c("1 day","1 Month","3 Months", "6 Months", "9 Months", "1 Year",
"2 Years", "5 Years", "10 Years", "15 Years", "Adult"),
  age = c(1/365,30/365,0.25,0.5,0.75,1,2,5,10,15,35), # in years
  wt = c(3.45,4.3,5.75,7.55,8.9,9.9, 12.35, 18.25, 32.5,54.25, (73+60)/2),
# in kg
  kw = c(0.6,0.7,0.7,0.7,0.7,0.7,0.73,0.65,0.56,0.51,0.42)/100, # percen-
tege of body weight
  ht = c(49.75,54.25,60,66,70.75,74.75,86,108.25, 138.25,166,(163+176)/2),
# in cm; added to calculate the BSA needed for CO%
  mat = round(x = c(20.3, 14.9, 31.3, 46.5, 45.3, 44.2, 66.5, 73.7, 73.7,
73.7, 79.8)/79.8,digits = 3), # % of adult maximal capacity (assumption for
ages >1yr) De Woskin 2009
  hemat = c(56, 44, 35.5, 36, 36, 36, 36.5, 37, 40, 42, 44) / 100
      # Hematocrite in percentage for each age (AGE), from Am Fam Physi-
cian. 2001 Oct 15;64(8):1379-86.Anemia in children.Irwin JJ
)
demo$bsa <- BSA(ht = demo$ht, age = demo$age, wt = demo$wt)
demo$wt.g <- demo$wt*1000

# end-of-script #
```

```
# title: "Dose scaling using GFR maturation functions"
# author: "SC"
# date: "29 Apr 2019"
# output: functions to be used with scripts:
#'@abbreviations: [bw - bodyweight][cl - clearance][bsa - body surface
area][bde/ade - bodyweight/age dependent exponent][pma - postmenstrual age]
#'[pe - prediction error][RMSE - root mean square error]
# ============================================================

# 1. read in data from literature with Rmd
data_name <- function(wd, file_name, ext = ".csv") {paste(wd,"\\", file_name,
ext, sep = "")}

# GFR functions:
# -----------------------------

  # all GFR predictions are in ml/min

  # Roosmarijn de Cock 2014: RED
  # normalized to bw of 4000 g

  CL_RdC <- function(bw, cl4kg = 0.39) { # bw in g; vancomycin value;
    bde <- 2.23 * bw ^ (-0.065)
    cl <- cl4kg * (bw / 4000) ^ bde
    return(cl * 1000 / 60)
  }

  # 3. Mahmood BDE 2016: CYAN
  # normalized to 70000 g; based on inulin clearance

  CL_Mah_BDE <- function(bw) {
    bde <- 1.199 * (bw/1000) ^ (-0.157)
    cl <- 128 * (bw / 70000) ^ bde
    return(cl)
  }

  # 4. Mahmood ADE 2016:
  CL_Mah_ADE_pre <- function(bw, age) cl <- 120 * (bw / 70000) ^ 1.15  #
function with exponent for preterm neonates

  CL_Mah_ADE <- function(bw, age) {
    ade <- ifelse(age < 0.5 , 1,
                  ifelse(age >= 0.5 & age <= 1, 0.9, 0.75))
    cl <- 120 * (bw / 70000) ^ ade
  } # function with exponents for the term babies

  # 5. Johnson 2006: GREEN

  CL_J <- function(bsa) (-6.16 * bsa ^ 2) + (99.054 * bsa) - 17.74 # bsa in
m^2

  # 6. Salem 2014: BLACK

  CL_S <- function(bw, pma) { # bw in g; pma in weeks

       cl <- 112 * (((bw / 70000) ^ 0.632) * (pma ^ 3.3) / ((55.4 ^ 3.3) +
pma ^ 3.3))

       return(cl)
  }
  # 7. Rhodin 2009: ORANGE
```

```
  CL_R <- function(bw, pma) { # bw in g; pma in weeks

    cl <- 121 * ((bw / 70000) ^ 0.75) * (pma ^ 3.4) / (47.7 ^ 3.4 + pma ^
3.4)
    return(cl)
  }

  # 8. Hayton 2000: MAGENTA

  CL_H <- function(bw, age) { # bw in g; age in years
    age_mo <- age * 12 # age converted to months to use as input in funtion
    cl <- 2.6 * ((bw / 1000) ^ 0.662) * exp(-0.0822 * age_mo) + (8.14 * (bw
/ 1000) ^ 0.662) * (1 - exp(-0.0822 * age_mo))
    return(cl)
  }

# Functions to calculate prediction errors:
  # ----------------------------------------


# 10. PE% function:

pe <- function(a, b) { # a = prediction; b = observation; directly in %
  err <- 100 * (a - b) / b
  return(err)
}

# 11. RMSE:

rmse <- function(x) re <- sqrt(sum(x^2, na.rm = TRUE)/length(x[!is.na(x)]))


# Physiological maturation functions:
# --------------------------

# 12. fraction unbound maturation functions:

# human serum albumin (HSA) (g/l): [Johnson 2006 - pg. 9 - eq. 5 & 7]

HSA <- function(age) {hsa <- 1.1287 * log(age * 365) + 33.746 ; return(h-
sa)}

fu_paed_hsa <- function(age_ad = 35, age, fu) {return(1 / (1 + (((1 - fu) *
HSA(age)) / (HSA(age_ad) * fu))))}

# alpha1-acid glycoprotein (AGP) (g/L): [Johnson 2006 - pg. 9 - eq. 6 & 7]

AAG <- function(age) {aag <- 0.887 * (age * 365) ^ 0.38 / ((8.89 ^ 0.38) +
(age * 365) ^ 0.38) ; return(aag)}

fu_paed_aag <- function(age_ad = 35, age, fu) {return(1 / (1 + (((1 - fu) *
AAG(age)) / (AAG(age_ad) * fu))))}


# Derive BSA for preterms and children of different ages:
# -------------------------------------

# 13. BSA function:

BSA <- function(ht, age, wt) { # age in years
  haycock <- 0.024265 * ht ** 0.3964 * wt ** 0.5378
  dubois <- 0.007184 * ht ** 0.725 * wt ** 0.425
```

```
  return(ifelse(wt < 15, haycock, dubois)) # in m^2
  # for wt <15kg use Haycock et al. for children < or =15kg, else Dubois
and Dubois
}

# 14. BSA function for preterms

BSApre <- function(wt)  # Furqan & Haque, 2009 m^2 (WT in kg)
{
  bsa <- (4 * wt + 7)/(90+wt) # m^2
  return(bsa)
}

# Functions for plots:

# 14. PE% plots :

pe_plot <- function(data, x, y, ylabel = "Your function name", col_name =
"black") {
  ggplot(data, aes_string(x = x, y = y))+
    geom_point(aes(shape = as.factor(STATUS)), col = col_name, size = 2) +
    scale_shape_discrete(solid = F)+
    scale_x_log10(breaks = c(0.0027,  0.08,  0.25,  0.5,  1,  2,  5, 15,
35),
                  labels = c("1 Day ", "1 Month ", "3 Months ", "6 Months
", "1 Year ", "2 Years ", "5 Years ", "15 Years ", "Adult "))+
    geom_hline(yintercept = c(-50, 0 , 50), linetype = c("solid", "dashed",
"solid"))+
    ylim(c(-100, 550))+
    ylab(paste("%PE", ylabel))+
    xlab("")+
    theme(axis.text.x = element_text(angle = 30, size = 10),
          axis.text.y = element_text(size = 14))+
    guides(shape = F)
}
```

```
##############################################################################
# Run-Time Environment:       R version 3.3.5
# Author:                     SC
# Project number:             1
# Short title:                GFR Dosing
# Purpose:                    Final figures & tables GFR dosing manuscript
#
# Date:                       2018-10-05
# Version:                    V.2.0
# Changes with prev.:         separate figures for dosing for AGP and HSA
# bound drugs
##############################################################################
# Remove all objects
rm(list=ls(all=TRUE))

# Load library
  library(lattice)
  library(stats)
  library(ggplot2)
  library(dplyr)
  library(cowplot)
  library(gridExtra)

# set ggplot white background theme:
  theme_set(theme_bw())

# work dir
  wd1 <-"D:/sinzi/work/GFR_manuscript/Code_review_Linda/"
  setwd(wd1)

# call scripts with data and functions
# ------------------------------------
  loc0 <- "2.Rscripts/"

  source(paste0(wd1, loc0, "SC01_GFR.PBPK_v08_Func.R")) # script to load
all functions
  source(paste0(wd1, loc0, "SC01_GFR.PBPK_v08_Data.R")) # script to load
data from literature

  loc0 <- "1.Data/2.GrowthCharts/"

  source(paste0(wd1,loc0,"nhanes-dump.R")) # script with nhanes data from
literature

# growth charts data transformation:
# ----------------------------------

# nhanes data transformations (only weight-age datasets used):

# combine datasets and get average weight between males and females from
birth till 20 yo
  nhanes.wt.kg <- c(NHANES.LT.3ys[NHANES.LT.3ys$Agemos<24&NHANES.
LT.3ys$Sex==1,4]/2+NHANES.LT.3ys[NHANES.LT.3ys$Agemos<24&NHANES.LT.3ys$-
Sex==2,4]/2,
                    NHANES.GT.2ys[NHANES.GT.2ys$Sex==1,4]/2+NHANES.GT.2ys[N-
HANES.GT.2ys$Sex==2,4]/2)

# ages for the combined ds
  nhanes.age.yrs <- c(NHANES.LT.3ys[NHANES.LT.3ys$Agemos<24&NHANES.LT.3ys$-
Sex==1,2],NHANES.GT.2ys[NHANES.GT.2ys$Sex==1,2])/12

# format dataframe:
```

```
# ---------------

  grow_data <- data.frame(
    typ_age = nhanes.age.yrs, # age in years
    typ_wt = nhanes.wt.kg * 1000, # wt in grams
    status = "healthy"
  )

  grow_data[grow_data$typ_age == 0,]$typ_age <- 1/365 # set min age to 1
day


# typical preterm neonates:
# -------------------

# preterm data from paper from Achieta2003 - typical ID with GA = 35 weeks:

  preterm_35wks <- read.csv(file = paste0(wd1, loc0, "Preterms_GA_35wks.
csv"), header = FALSE) # age in days; wt in g
  names(preterm_35wks) <- c("typ_age", "typ_wt") # change names
  preterm_35wks$status <-"preterm"
  preterm_35wks$typ_age <- preterm_35wks$typ_age/365 # age from days to
years

# split literature ds collection in "healthy" and "preterm"

  all_preterm <- all.in[all.in$STATUS == "preterm",]
  all.in <- all.in[all.in$STATUS == "healthy",]

# Figure 1: Qualitative assessment - GFR maturation profiles based on growth
charts data as input; no preterm
  # --------------------------------
  cols <- c("Hayton 2000 [15]"="#C95BBD","Johnson 2006
[14]"="#6FC95B","Mahmood 2014 (ADE) [12]"="#ADAFAD",
            "Mahmood 2014 (BDE) [12]"="#2AC7DD", "Rhodin 2009 [13]" =
"#F98708", "De Cock 2014 [7]" = "#EA3027", "Salem 2014 [16]" = "#080808")
  ggplot(data = grow_data)+
    geom_line(aes(x = typ_age, y = CL_H(bw = typ_wt, age = typ_age), col =
"Hayton 2000 [15]"), size = 1.25)+ # hayton (magenta)
    geom_line(aes(x = typ_age, y = CL_J(bsa = BSApre(wt = typ_wt/1000)),
col = "Johnson 2006 [14]"), size = 1.25)+ #johnson 2006 (green)
    geom_line(aes(x = typ_age, y = CL_Mah_ADE(bw = typ_wt, age = typ_age),
col = "Mahmood 2014 (ADE) [12]"), size = 1.25)+ # mahmood 2016 ade (grey)
    geom_line(aes(x = typ_age, y = CL_Mah_BDE(bw = typ_wt), col = "Mahmood
2014 (BDE) [12]"), size = 1.25)+ # mahmood 2016 bde (cyan)
    geom_line(aes(x = typ_age, y = CL_R(bw = typ_wt, pma = (typ_age*52 +
40)), col = "Rhodin 2009 [13]"), size = 1.25)+ # rhodin 2005 (orange)
    geom_line(aes(x = typ_age, y = CL_RdC(bw = typ_wt, cl4kg = 0.39), col =
"De Cock 2014 [7]"), size = 1.25)+ # RdC 2012 (red)
    geom_line(aes(x = typ_age, y = CL_S(bw = typ_wt, pma = (typ_age*52 +
40)), col = "Salem 2014 [16]"), size = 1.25)+ # Salem 2015 (black)

    xlab("Age")+
    scale_colour_manual(name=" ",values=cols) +
    theme(axis.text.x = element_text(angle = 30, size = 8),
          axis.text.y = element_text(size = 8))+
    background_grid(major = "xy") -> base_plot1



  base_plot1 +
    scale_y_continuous(breaks = c(2, 10, 50, 100, 150), limits= c(1, 160))+
```

```
    scale_x_continuous(breaks = c(0.0027, 1,  2,  5, 10, 15, 35),
                       labels = c("1 Day ", "1 Year ", "2 Years ", "5 Years
", "10 Years ", "15 Years ", "Adult "))+
    ylab("Glomerular filtration rate (ml/min)") +
    theme(legend.position="none") ->gph1

  base_plot1 +
    scale_y_continuous(trans= "log10", breaks = c(2, 5, 10, 50, 100, 150),
limits= c(1, 200))+
    scale_x_log10(breaks = c(0.0027,  0.08,  0.25,  0.5,  1,  2,  5, 10,
15, 35),
                  labels = c("1 Day ", "1 Month ", "3 Months ", "6 Months
", "1 Year ", "2 Years ", "5 Years ", "10 Years ", "15 Years ", "Adult "))+
    ylab("")-> gph2

# save plot:

loc0 <- "3.Results/"

tiff(filename = paste0(wd1, loc0, "Figure_1_GFR_maturation.tiff"), width = 30,
height = 11.6, units = 'cm', res = 300)

plot_grid(gph1, gph2, labels = c('A', 'B'), nrow = 1, ncol = 2, rel_widths
= c(1, 1.5))

dev.off()


# Figure 2: Quantitative assessment - Prediction error between literature
data and the GFR functions predictions for each maturation function
# ------------------------

all.in %>% mutate(pe = pe(a = CL_H(age = AGE.y, bw = BW.g), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Hayton 2000 [15]", col_name =
"#C95BBD")+
  background_grid(major = "xy")-> gg1

all.in %>% mutate(pe = pe(a = CL_RdC(bw = BW.g), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "De Cock 2014 [7]", col_name =
"#EA3027")+
  background_grid(major = "xy")-> gg2

all.in %>% mutate(pe = pe(a = CL_J(bsa = BSA), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Johnson 2006 [14]", col_name =
"#6FC95B")+
  background_grid(major = "xy") -> gg3

all.in %>% mutate(pe = pe(a = CL_Mah_ADE(bw = BW.g, age = AGE.y), b = GFR))
%>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Mahmood 2014 (ADE) [12]", col_
name = "#ADAFAD")+
  background_grid(major = "xy") -> gg4

all.in %>% mutate(pe = pe(a = CL_Mah_BDE(bw = BW.g), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Mahmood 2014 (BDE) [12]", col_
name = "#2AC7DD")+
  background_grid(major = "xy") -> gg5

all.in %>% mutate(pe = pe(a = CL_R(bw = BW.g, pma = PMA.wk), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Rhodin 2009 [13]", col_name =
"#F98708")+
  background_grid(major = "xy") -> gg6
```

```
all.in %>% mutate(pe = pe(a = CL_S(bw = BW.g, pma = PMA.wk), b = GFR)) %>%
  pe_plot(x = "AGE.y", y = "pe", ylabel = "Salem 2014 [16]", col_name =
"#080808")+
  background_grid(major = "xy")-> gg7

# save plot:
tiff(filename = paste0(wd1, loc0, "Figure_2_PE.tiff"), width = 30, height =
28, units = 'cm', res = 300)
plot_grid(gg1, gg2, gg3, gg4, gg5, gg6, gg7, labels = c('A', 'B', 'C', 'D',
'E', 'F','G'), nrow = 3, ncol = 3)
dev.off()


# Dosing based on the best GFR function (Salem et al. 2014)

# ------------------------------------------------------

# create the required dataset:

dose <- 100 # assume aduld dose is 100 for direct output of %

demo_fu <- left_join(demo, expand.grid(age = demo$age, fu = seq(0.1, 1, by
= 0.1))) # add fraction unbound to all ages
demo_fu$fu_ped_hsa <- fu_paed_hsa(age = demo_fu$age, fu = demo_fu$fu) # fu
of HSA pediatric
demo_fu$fu_ped_agp <- fu_paed_aag(age = demo_fu$age, fu = demo_fu$fu) # fu
of AGP pediatric
demo_fu$gfr <- CL_S(bw = demo_fu$wt * 1000, pma = (demo_fu$age * 52 + 40))
# dosing is based on the GFR Salem 2015 function

# The adult demographics and dose
adult <- demo_fu %>% filter(age == 35) %>% select(fu, gfr)
names(adult) <- c("fu", "gfr_ad")
demo_fu_ext <- left_join(demo_fu, adult, by = "fu") # add this as a new
column to ease calculations

# dose scaled by PBPK clearance (based on GFR and fu maturation)
demo_fu_ext$dose_calc_hsa <- dose * (demo_fu_ext$gfr / demo_fu_ext$gfr_ad)
* (demo_fu_ext$fu_ped_hsa / demo_fu_ext$fu) # dose as % of adult dose
demo_fu_ext$dose_calc_agp <- dose * (demo_fu_ext$gfr / demo_fu_ext$gfr_ad)
* (demo_fu_ext$fu_ped_agp / demo_fu_ext$fu)

# save clearance values in dataframe:

demo_fu_ext$cl_adult <- demo_fu_ext$gfr_ad*demo_fu_ext$fu
demo_fu_ext$cl_ped_hsa <- demo_fu_ext$gfr*demo_fu_ext$fu_ped_hsa
demo_fu_ext$cl_ped_agp <- demo_fu_ext$gfr*demo_fu_ext$fu_ped_agp

# function to scale dose and clearance with AS0.75 and linear scaling

dose_ped <- function(dose_ad = 100, wt_ped, wt_ad, ex = 1) {
  ddose <- dose_ad * (wt_ped / wt_ad) ^ ex
  return(ddose)
} # dose scaling

# ----------

cl_ped <- function(cl_ad = 120, wt_ped, wt_ad, ex = 1) {
  clped <- cl_ad * (wt_ped / wt_ad) ^ ex
  return(clped)
```

```
} # cl scaling


# add the doses calculated with the function above to the data frame
  demo_fu_ext$dose_lin <- dose_ped(dose_ad = 100, wt_ped = demo_fu_ext$wt,
wt_ad = demo[demo$age == 35, "wt"], ex = 1)
  demo_fu_ext$dose_as <- dose_ped(dose_ad = 100, wt_ped = demo_fu_ext$wt,
wt_ad = demo[demo$age == 35, "wt"], ex = 0.75)
  demo_fu_ext$dose_r <- dose * (demo_fu_ext$gfr / demo_fu_ext$gfr_ad) #
dose based on GFR fraction

# add the scaled clearances to the data frame:

  demo_fu_ext$cl_lin <- cl_ped(cl_ad = demo_fu_ext$cl_adult, wt_ped = demo_
fu_ext$wt, wt_ad = demo[demo$age == 35, "wt"], ex = 1)
  demo_fu_ext$cl_as <- cl_ped(cl_ad = demo_fu_ext$cl_adult, wt_ped = demo_
fu_ext$wt, wt_ad = demo[demo$age == 35, "wt"], ex = 0.75)
  demo_fu_ext$cl_gfr <- demo_fu_ext$cl_adult * (demo_fu_ext$gfr / demo_fu_
ext$gfr_ad) # dose based on GFR fraction

# Figure 3: Pediatric dose as a % of the adult dose.
# ----------------------------------------

  # lines <- c("GFR scaling" = "solid", "Linear scaling" = "dotted", "Allo-
metric scaling" = "dashed")
  col2 <- c("GFR scaling" = "#009E73", "Linear scaling" = "#E69F00", "Allo-
metric scaling" = "#FB0101")
  base_plot2 <-
    demo_fu_ext %>%
    ggplot()+
    geom_line(aes(x = age, y = dose_r, col = "GFR scaling"), size = 1.2,
alpha = 0.55)+
    geom_line(aes(x = age, y = dose_lin, col = "Linear scaling"), size =
1.3)+
    geom_line(aes(x = age, y = dose_as, col = "Allometric scaling"), size =
1.2)+
    scale_y_continuous(breaks = c(0.1, 5, 10, 20, 30, 50, 80, 100), trans =
"log10")+
    xlab("Age")+
    background_grid(major = "xy")+
    guides(alpha = FALSE)+
    theme(plot.title = element_text(hjust = 0.5),
          axis.text.x = element_text(angle = 35))

  base_plot2 +
    geom_point(aes(x = age, y = dose_calc_agp, alpha = factor(fu)), colour
= "#404040", shape = 19,  fill = "#C0392B", size = 2.3)+
    ylab(" ")+
    theme(plot.title = element_text(hjust = 0.5))+
    scale_color_manual(name = " ", values = col2,
                       breaks = c("GFR scaling", "Linear scaling", "Al-
lometric scaling"))+
    scale_x_continuous(breaks = c(0.0027, 0.08, 0.25, 0.5, 1, 2, 5, 10, 15,
35),
                       trans = "log10",
                       labels = c("1 Day", "1 Month", "3 Months", "6
Months", "1 Year",
                                  "2 Years", "5 Years", "10 Years", "15
Years", "Adult" ))+
    ggtitle("AGP bound drugs") -> gph_b

  base_plot2 +
```

```
    geom_point(aes(x = age, y = dose_calc_hsa, alpha = factor(fu)), colour
= "#404040", shape = 19, fill = "#2B88C0", size = 2.3)+
    ylab("Dose (% of adult dose)")+
    ggtitle("HSA bound drugs")+
    theme(plot.title = element_text(hjust = 0.5))+
    scale_color_manual(name = " ", values = col2)+
    scale_x_continuous(breaks = c(0.0027, 0.08, 0.25, 0.5, 1, 2, 5, 10, 15,
35),
                       trans = "log10",
                       labels = c("1 Day", "1 Month", "3 Months", "6
Months", "1 Year",
                                  "2 Years", "5 Years", "10 Years", "15
Years", "Adult" ))+
    theme(legend.position="none") -> gph_a


tiff(filename = paste0(wd1, loc0, "Figure_3_Pediatric_Dose.tiff"), width = 25,
height = 10, units = 'cm', res = 300)
plot_grid(gph_a, gph_b, nrow = 1, ncol = 2, rel_widths = c(1, 1.4))
dev.off()


# Figure 4_scaled CL: %PE PBPK clearance vs. scaled clearance.
# ------------------------------------------------------------

col2 <- c("GFR scaling" = "#009E73", "Linear scaling" = "#E69F00", "Allo-
metric scaling" = "#FB0101")
demo_fu_ext %>% #mutate(err = pe()) %>%
  ggplot()+
  geom_hline(aes(yintercept = 0), linetype = "solid", col = "grey", size =
1)+
  geom_hline(aes(yintercept = -50), linetype = "dashed", col = "black",
size =  1)+
  geom_hline(aes(yintercept = 50), linetype = "dashed", col = "black", size
=  1)+
  geom_hline(aes(yintercept = -30), linetype = "dotted", col = "black",
size =  1)+
  geom_hline(aes(yintercept = 30), linetype = "dotted", col = "black", size
=  1)+
  #facet_grid(.~fu)+

  ylim(-60, 200)+
  xlab("AGE")+
  guides(alpha = F)+
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        plot.title = element_text(hjust = 0.5))+
  scale_color_manual(name = " ", values = col2,
                     breaks = c("GFR scaling", "Linear scaling", "Allomet-
ric scaling"))+
  background_grid(major = "y") -> basis_plot3

basis_plot3+
  geom_point(aes(x = age, y = pe(cl_gfr, cl_ped_hsa), alpha = factor(fu),
col = "GFR scaling"), size = 2.25)+
  geom_point(aes(x = age, y = pe(cl_lin, cl_ped_hsa), alpha = factor(fu),
col = "Linear scaling"), size = 2.25)+
  geom_point(aes(x = age, y = pe(cl_as, cl_ped_hsa), alpha = factor(fu),
col = "Allometric scaling"), size = 2.25)+
  ggtitle("HSA bound drugs")+
  scale_x_continuous(breaks = demo$age, labels = demo$lab, trans =
'log10')+
  theme(legend.position="none")+
```

```
  scale_y_continuous(name = expression('% PE'['CL']), limits = c(-60, 200),
breaks = c(-50, -30, 0, 30, 50, 100, 150, 200)) -> gph6

basis_plot3+
  geom_point(aes(x = age, y = pe(cl_gfr, cl_ped_agp), alpha = factor(fu),
col = "GFR scaling"), size = 2.25)+
  geom_point(aes(x = age, y = pe(cl_lin, cl_ped_agp), alpha = factor(fu),
col = "Linear scaling"), size = 2.25)+
  geom_point(aes(x = age, y = pe(cl_as, cl_ped_agp), alpha = factor(fu),
col = "Allometric scaling"), size = 2.25)+
  scale_x_continuous(breaks = demo$age, labels = demo$lab, trans =
'log10')+
  scale_y_continuous(limits = c(-60, 200), breaks = c(-50, -30, 0, 30, 50,
100, 150, 200))+
  ylab(" ")+
  ggtitle("AGP bound drugs") -> gph7

tiff(filename =  paste0(wd1, loc0,"Figure_4_PE_clearance_after_rev.tiff") ,
width = 25, height = 10, units = 'cm', res = 300)
plot_grid(gph6, gph7, nrow = 1, ncol = 2, rel_widths = c(1, 1.4))
dev.off()

## for the reviewer comments:

demo_fu_ext %>%
  ggplot()+
  geom_line(aes(x = age, y = cl_as, alpha = factor(fu), col = "Allometric
scaling", group = fu))+
  geom_point(aes(x = age, y = cl_ped_hsa, alpha = factor(fu), col = "GFR
scaling"))+
  geom_point(aes(x = age, y = cl_ped_agp, alpha = factor(fu), col = "Linear
scaling"))+
  scale_color_manual(name = " ", values = col2, breaks = c("GFR scaling",
"Linear scaling", "Allometric scaling"))+
  scale_x_continuous(breaks = demo$age, labels = demo$lab)#+
  # scale_y_continuous(trans = 'log10')

demo_fu_ext %>% filter(lab %in% c("1 day", "1 Month", "3 Months", "6
Months")) %>%
  ggplot()+
  geom_line(aes(x = age, y = cl_as, alpha = factor(fu), col = "Allometric
scaling", group = fu))+
  geom_point(aes(x = age, y = cl_ped_agp, alpha = factor(fu), col = "GFR
scaling"))+
  geom_point(aes(x = age, y = cl_ped_hsa, alpha = factor(fu), col = "Linear
scaling"))+
  scale_color_manual(name = " ", values = col2, breaks = c("GFR scaling",
"Linear scaling", "Allometric scaling"))+
  scale_x_continuous(breaks = demo$age[demo$lab %in% c("1 day", "1 Month",
"3 Months", "6 Months")], labels = demo$lab[demo$lab %in% c("1 day", "1
Month", "3 Months", "6 Months")], trans = 'log10')#+
  # scale_y_continuous(trans = 'log10')

# Table with first-dose recommendation based on GFR scaling, AS0.75 and lin-
ear scaling
# These tables are combined to make Table 2 for the paper.

demo_fu_ext %>% filter(fu %in% c(0.1)) %>% # results for fu = 0.1
  select(Age = lab, "Weight (kg)" = wt, "GFR (ml/min)"= gfr, "GFR ratio
dose" = dose_r, "Linear dose scaling" = dose_lin,
         "Allometric Scaled Dose" = dose_as, "CLR scaling HSA" = dose_calc_
hsa, "CLR scaling AGP" = dose_calc_agp,
```

```
         "Unbound fraction HSA (pediatric)" = fu_ped_hsa, "Unbound fraction
AGP (pediatric)" = fu_ped_agp, "fu" = fu) -> tab2a
write.csv(x = tab2a, file = paste0(wd1, loc0, "Table_2a_dosing.csv"))

demo_fu_ext %>% filter(fu %in% c(0.9)) %>% # results for fu = 0.9
  select(Age = lab, "Weight (kg)" = wt, "GFR (ml/min)"= gfr, "GFR ratio
dose" = dose_r, "Linear dose scaling" = dose_lin,
         "Allometric Scaled Dose" = dose_as, "CLR scaling HSA" = dose_calc_
hsa, "CLR scaling AGP" = dose_calc_agp,
         "Unbound fraction HSA (pediatric)" = fu_ped_hsa, "Unbound fraction
AGP (pediatric)" = fu_ped_agp, "fu" = fu) -> tab2b

write.csv(x = tab2b, file = paste0(wd1, loc0, "Table_2b_dosing.csv"))


# Supplement Figure S1:
# ------------------------
# the preterm predictions only on log scale.

ggplot()+
  geom_point(data = all_preterm, aes(x = AGE.y, y = GFR), col = "#4E4D4D",
alpha = 0.75)+
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_H(bw = typ_wt, age = typ_age), col = "Hayton 2000 [15]"),
linetype = "dashed", size = 1.25)+ # hayton (magenta)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x
= typ_age, y = CL_J(bsa = BSApre(wt = typ_wt/1000)), col = "Johnson 2006
[14]"), linetype = "dashed", size = 1.25)+ #johnson 2006 (green)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_Mah_ADE_pre(bw = typ_wt, age = typ_age), col = "Mahmood
2014 (ADE) [12]"), linetype = "dashed", size = 1.25)+ # mahmood 2016 ade
(grey)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_Mah_BDE(bw = typ_wt), col = "Mahmood 2014 (BDE) [12]"),
linetype = "dashed", size = 1.25)+ # mahmood 2016 bde (cyan)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_R(bw = typ_wt, pma = (typ_age * 52 + 40)), col = "Rhodin
2009 [13]"), linetype = "dashed", size = 1.25)+ # rhodin 2005 (orange)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_RdC(bw = typ_wt), col = "De Cock 2014 [7]"), linetype =
"dashed", size = 1.25)+ # RdC 2012 (red)
  geom_line(data = preterm_35wks[preterm_35wks$typ_age > 0.005,], aes(x =
typ_age, y = CL_S(bw = typ_wt, pma = (typ_age * 52 + 40)), col = "Salem
2014 [16]"), linetype = "dashed", size = 1.25)+ # Salem 2015 (black)
  scale_x_log10(breaks = c(0.0027, 7/365, 0.08,  0.25,  0.5,  1,  2,  5,
10, 15, 35),
                labels = c("1 Day ", "1 Week","1 Month ", "3 Months ", "6
Months ", "1 Year ", "2 Years ", "5 Years ", "10 Years ", "15 Years ",
"Adult "))+
  # scale_y_log10(limits = c(0.5,150))+
  xlab("Postnatal Age")+
  ylab("Glomerular filtration rate (ml/min)")+
  theme(axis.text.x = element_text(angle = 30, size = 7))+
  background_grid(major = "xy")+
  scale_colour_manual(name="Glomerular filtration rate\n functions in pre-
term neonates",values=cols) -> gph2_preterm

tiff(filename = paste0(wd1, loc0, "Figure_S1_GFR_functions_preterm.tiff"),
width = 20, height = 10, units = 'cm', res = 300)
plot_grid(gph2_preterm, nrow = 1, ncol = 1)
dev.off()
```

```
# Figure with the fraction unbound and hsa/agp:
col3 <- c("HSA" = "blue", "AGP" = "orange")
ggplot(all.in)+
  geom_line(aes(x= AGE.y, y = HSA(age = AGE.y), col = "HSA"), size = 1.3)+
  geom_line(aes(x= AGE.y, y = AAG(age = AGE.y)*100, col = "AGP"), size =
1.3)+
  scale_x_continuous(trans = 'log10', name = "Age (years)",
                     breaks = c(0.0027,  0.08,  0.25,  0.5,  1,  2,  5, 10,
15, 35),
                     labels = c("1 Day ", "1 Month ", "3 Months ", "6
Months ", "1 Year ", "2 Years ", "5 Years ", "10 Years ", "15 Years ",
"Adult "))+
  scale_y_continuous(sec.axis = sec_axis(~ . / 100, name = "AGP (g/L)"),
name = "HSA (g/L)")+
  scale_color_manual(values = col3, name = "Plasma protein")+
  theme(axis.text.x = element_text(angle = 35))+
  guides(col = FALSE) -> s2a

shape1 <- c("0.1" = 21, "0.9" = 24)
ggplot(all.in)+
  geom_point(aes(x= AGE.y, y = fu_paed_aag(age = AGE.y, fu = 0.1), shape =
"0.1", col = "AGP"), fill = "orange")+
  geom_point(aes(x= AGE.y, y = fu_paed_aag(age = AGE.y, fu = 0.9), shape =
"0.9", col = "AGP"), fill ="orange")+
  geom_point(aes(x= AGE.y, y = fu_paed_hsa(age = AGE.y, fu = 0.1), col =
"HSA", shape = "0.1"), fill = "blue")+
  geom_point(aes(x= AGE.y, y = fu_paed_hsa(age = AGE.y, fu = 0.9), col =
"HSA", shape = "0.9"), fill = "blue")+
  scale_x_continuous(trans = 'log10', name = "Age (years)",
                     breaks = c(0.0027,  0.08,  0.25,  0.5,  1,  2,  5, 10,
15, 35),
                     labels = c("1 Day ", "1 Month ", "3 Months ", "6
Months ", "1 Year ", "2 Years ", "5 Years ", "10 Years ", "15 Years ",
"Adult "))+
  scale_y_continuous(name = "Fraction unbound")+
  scale_color_manual(values = col3, name = "Plasma protein")+
  scale_shape_manual(values = shape1, name = "Fraction unbound\nin
adults")+
  theme(axis.text.x = element_text(angle = 35)) -> s2b

tiff(filename = paste0(wd1, loc0, "Figure_S2_Prot_bind_and_prot_mat.tiff"),
width = 25, height = 10, units = 'cm', res = 300)
plot_grid(s2a, s2b, labels = c('A','B'), nrow = 1, ncol = 2, rel_widths =
c(1, 1.3))
dev.off()
```