The impact of obesity on the pharmacokinetics of drugs in adolescents and adults

Rongen, A. van; Rongen A. van

Chapter 10

The impact of obesity on the pharmacokinetics of drugs in adolescents and adults:

Summary, conclusions and perspectives

SUMMARY AND CONCLUSIONS

In the last three decades, worldwide prevalence rates of overweight (BMI > 25 kg/m^2) and obesity (BMI > 30 kg/m^2) have increased substantially for both adults and children [1]. Obesity and morbid obesity (BMI > 40 kg/m^2) are associated with several (patho)physiological changes, such as an increase in both fat and lean body mass, increased cardiac output and blood volume and increased glomerular filtration rate. In addition, obesity can have an influence on the activity of hepatic enzymes responsible for metabolism of drugs and the expression or activity of renal transporters, responsible for tubular secretion and reabsorption of drugs. These physiological changes can all have an impact on the pharmacokinetic (PK) parameters of drugs, thereby potentially necessitating the adaptation of dosing regimens. Despite the increased number of obese patients and the increased use of pharmacotherapy in these patients, evidence based dosing guidelines are largely lacking, particularly for morbidly obese adults and obese children and adolescents. For both these populations, particularly PK studies are needed to provide a basis for evidence based dosing guidelines. Ultimately these studies may also provide insight in the physiological changes caused by obesity.

As a first step, **Chapter 2** provides a literature overview of pharmacometric studies in the obese adult population. In this overview, we report published equations quantifying obesity related changes for the primary PK parameters total body clearance and volume of distribution. Based on this overview, it can be concluded that a large number of body size descriptors (i.e. total body weight [TBW], lean body weight [LBW], BMI, ideal body weight [IBW] or %IBW) in different equations (i.e. linear, power or allometric) are available to characterize the influence of obesity on PK parameters. Moreover, the model equations also include a large number of other covariates, such as creatinine clearance, serum creatinine, age or sex, resulting in complex equations. The included studies varied largely in degree of obesity, obesity definitions, age and race. Except for CYP3A-mediated clearance, which seems to decrease with obesity, most metabolic and elimination pathways seem to increase with obesity or remain unchanged. The parameter volume of distribution was found to increase or remain unchanged as well, even though there was limited information to identify the exact relation with the different weight measures. As more than half of the included pharmacometric models were not adequately internally validated, five evaluation criteria were proposed for the evaluation of the covariate model in the obese population, in line with the approach for paediatric covariate models by Krekels et al. [2]. From this overview, we can conclude that more pharmacometric studies in obese individuals should be performed before evidence based dosing guidelines for specific drugs can be developed. For these future studies, suggestions for the design of these studies were made, i.e. stratification for body

weight to structure the wide range in body weights, inclusion of non-obese patients in the analysis, and restrictions in age (a non-elderly population) and race to prevent a biased covariate analysis. In addition, we advise to focus on model drugs that represent a metabolic or elimination pathway to aid the development of physiologically based PK models for the (morbidly) obese population in these future studies.

Chapter 3 focussed on the current knowledge of the physiological changes in obesity and the resulting changes in drug disposition. From the small number of studies included in this review on drug absorption, it seems that drug absorption is rather unaltered, although this may be a premature conclusion warranting further study. Distribution is reported to be highly variable in obese patients and difficult to predict, particularly for lipophilic drugs. For example, no influence of obesity on volume of distribution of propofol, a highly lipophilic drug, was identified. Changes in clearance are typically found to be smaller than in volume of distribution, and can be predicted on the basis of reported changes in the involved metabolic or elimination pathways. For renal clearance higher or similar values can be anticipated in obese subjects. For clearance mediated by liver blood flow, higher values in obese individuals were reported for a small number of high extraction ratio drugs. Finally, Chapter 3 reported that very limited pharmacokinetic and dosing information is available for obese children. Particularly in children where dosing guidelines are typically expressed in mg/kg, evidence on the influence of obesity is needed as this mg/kg dosing algorithm may potentially lead to an overdose in obese individuals. In these studies in obese children, attention should be paid to the interrelation between growth, age and obesity that may exist in this population, as with increasing age body weight may increase as a result of growth, obesity or both. To address this issue, a covariate model in obese adolescents is proposed in which total body weight is divided in weight resulting from growth and development and from obesity-related changes. This distinction between these different weight measures can be further explored in future pharmacokinetic studies in the (obese) paediatric population.

In this thesis we studied the pharmacokinetics of various drugs in obese adolescents and morbidly obese adults. For obese adolescents we studied the pharmacokinetics of the CYP3A substrate midazolam and the renally eliminated drug metformin. In morbidly obese adults we also studied midazolam, together with acetaminophen, which is metabolized by glucuronidation, sulphation and CYP2E1-mediated oxidation. Finally, for midazolam a combined analysis was performed to study simultaneously the influence of age and obesity on midazolam clearance in obese adolescents and morbidly obese adults.

Studies in obese adolescents

In this thesis, we evaluated the influence of obesity on the pharmacokinetics of midazolam and metformin in adolescents. To address the previously mentioned issue of the interrelation between age and obesity in obese adolescents, the covariate model proposed in the literature review of Chapter 3 was further explored. In this so called (over)weight covariate model or excess weight model, total body weight is divided in developmental weight ($WT_{for\ age\ and\ length}$) and excess body weight (WT_{excess}), with total body weight = $WT_{for\ age\ and\ length} + WT_{excess}$.

In **Chapter 4**, the pharmacokinetics of the CYP3A substrate midazolam and its metabolites were studied in obese adolescents. Midazolam is a commonly used lipophilic benzodiazepine for preoperative sedation in paediatric anaesthesia because of its potent sedative, amnesic and anxiolytic properties. It is used as a CYP3A probe drug, since it is extensively metabolized by CYP3A to its metabolites [3]. CYP3A is an important metabolic enzyme system as it is responsible for the primary metabolism of 25% of all drugs [4]. In this study, 19 overweight and obese patients with a mean total body weight (TBW) of 102.7 kg (62-149.8 kg), BMI of 36.1 kg/m^2 (24.8-55 kg/m^2) and age of 15.9 years (range 12.5-18.9 years) received 2 or 3 mg intravenous midazolam. In the model for midazolam and metabolites, peripheral volume of distribution of midazolam (154 L (RSE of 11.2%)) increased substantially with TBW ($P < 0.001$), while a positive trend was found between midazolam clearance (0.66 L/min (8.3%)) and TBW or lean body weight (LBW) [5]. The (over)weight covariate model showed that the increase in peripheral volume could be explained by excess body weight (WT_{excess}) instead of body weight related to growth ($WT_{for\ age\ and\ length}$). To conclude, the pharmacokinetics of midazolam and its metabolites in overweight and obese adolescents showed a marked increase in peripheral volume of distribution without a significant influence on clearance. The findings may imply a need for a higher initial infusion rate upon initiation of a continuous infusion in obese adolescents to decrease the time to reach steady state.

In **Chapter 5**, the pharmacokinetics of metformin in overweight and obese children and adolescents are described. Metformin is a biguanide compound which is registered for the treatment of type 2 diabetes mellitus in paediatric patients above 10 years. Even though a large increase in metformin prescriptions in children and adolescents was observed in the Netherlands between 1998 and 2011 [6], the pharmacokinetics of metformin is not extensively studied in this population. For metformin, active tubular secretion is the primary route of renal elimination conducted by organic cation transporters (OCT) and human multidrug and toxin extrusion transporters (MATE) [7-10]. In this study, 22 overweight and obese adolescents with a mean TBW of 79.3 kg (range 54.7-104.9 kg), BMI of 29.1 kg/m^2 (22.9-39.3 kg/m^2) and age of 15.9 years (11.1-17.5) participated in the study. In these patients receiving 500 or 1000 mg oral metformin twice daily for 37 weeks as part of a randomized controlled placebo study [11], blood samples were collected over 8

hours post-dose during an oral glucose tolerance test (OGTT). In the covariate analysis, we found that oral clearance (CL/F) of metformin (1.17 L/min (RSE of 6%)) significantly increased with TBW ($P < 0.01$). More specifically, oral clearance increased with both developmental weight ($WT_{for\ age\ and\ length}$) and excess body weight (WT_{excess}) for which an excess weight covariate model was proposed in which $WT_{for\ age\ and\ length}$ scaled allometrically to the power of 0.75 and the influence of WT_{excess} was estimated separately. The oral clearance of metformin in overweight and obese adolescents that we reported (1.17 L/min) is higher than literature values obtained in non-obese children (0.55 L/min) [12] and similar to values found in adults (1.3 L/min) [13]. This increase may potentially be explained by increased tubular secretion of metformin resulting from an increased activity of renal transporters in obese adolescents.

Studies in morbidly obese and non-obese adults

To place the results of morbidly obese patients in perspective, a pharmacometric analysis of the influence of obesity on the PK of a drug preferably includes data from non-obese patients as well. For midazolam, these non-obese adults are, apart from the combined pharmacometric analysis on the influence of obesity, first described as part of a separate study in **Chapter 6**. This study was designed to detect the 24-hour variation in the PK parameters of the CYP3A substrate midazolam. Oral (2 mg) and intravenous (1 mg) midazolam was administered in a semi-simultaneous manner (separated by 2.5 hours) at six different time points throughout a 24-hour period in 12 healthy volunteers. In the analysis, 24-hour variation was identified for oral bioavailability (0.28 (RSE of 7.1%)) which was best parameterized using a cosine function with an amplitude of 0.04 (17.3%) and a peak at 12:14 in the afternoon. The absorption rate constant was 1.4 (4.7%) times higher when midazolam was administered at 14:00. Clearance (0.38 L/min (4.8%)) showed only a minor 24-hour variation parameterized as a cosine function with an amplitude of 0.03 L/min (14.8%) and a peak at 18:50. Based on the model, it was shown that the time of dosing hardly affects time concentration profiles after intravenous administration, while after oral dosing the concentrations are higher during the day compared to the night, reflecting considerable 24-hour variation in intestinal processes.

Using the same semi-simultaneous study-design of Chapter 6, **Chapter 7** described the pharmacokinetics of midazolam in morbidly obese patients in a combined analysis with data from the healthy volunteers from Chapter 6 (10:00 hour administration time, n=12). In this study, 20 morbidly obese patients participated with a mean TBW of 144 kg (range 112-186 kg) and a mean BMI of 47 kg/m^2 (range 40-68 kg/m^2). All patients received 7.5 mg of midazolam orally and 5 mg intravenously (separated by 159 ± 67 min). The covariate analysis showed that clearance (0.36 L/min (4%)) was similar in morbidly obese patients compared to healthy volunteers. However, oral bioavailability was higher in morbidly obese patients compared to healthy volunteers (60% (13%) vs. 28% (7%),

respectively) ($P < 0.001$). Central and peripheral volumes of distribution increased substantially with body weight (both $P < 0.001$). In conclusion, in morbidly obese patients, systemic clearance of midazolam is unchanged, while oral bioavailability and volume of distribution are increased. Given the large increase in volumes of distribution, dose adaptations for intravenous midazolam should be considered.

In **Chapter 8** the pharmacokinetics of acetaminophen and its metabolites (glucuronide, sulphate, cysteine and mercapturate) in morbidly obese and non-obese patients are reported. Acetaminophen is mainly metabolized via glucuronidation and sulphation, while the minor pathway through CYP2E1 is held responsible for hepatotoxicity. In obese patients, CYP2E1 activity is reported to be induced, thereby potentially worsening the safety profile of acetaminophen. In this study, 20 morbidly obese patients (median TBW of 140.1 kg (range 106-193 kg) and BMI of 45.1 kg/m^2 (40-55.2 kg/m^2) and 8 non-obese patients (TBW of 69.4 kg (53.4-91.7 kg) and BMI of 21.8 kg/m^2 (19.4-27.4 kg/m^2)) received 2 g of intravenous acetaminophen. In morbidly obese patients, the median $AUC_{0\text{-}8h}$ of acetaminophen was significantly lower ($P = 0.009$), while the $AUC_{0\text{-}8h}$ ratio of the glucuronide, sulphate and cysteine metabolite to acetaminophen were significantly higher ($P = 0.043$, 0.004 and 0.010, respectively). In the model, acetaminophen CYP2E1-mediated clearance (cysteine and mercapturate) increased with LBW (0.0185 L/min (15%), $P < 0.01$). Moreover, an accelerated formation of the cysteine and mercapturate metabolites was found with increasing LBW ($P < 0.001$). Glucuronidation clearance (0.219 L/min (5%)) and sulphation clearance (0.0646 L/min (6%)) also increased with LBW ($P < 0.001$). In conclusion, obesity leads to lower acetaminophen concentrations and earlier and higher peak concentrations of acetaminophen cysteine and mercapturate. While a higher dose may be anticipated to achieve adequate acetaminophen peak and AUC concentrations, this requires further study in terms of efficacy and safety.

Combined populations of obese adolescents and morbidly obese adults

CYP3A is known to be influenced by age, inflammation and obesity [14-18]. As clearance is typically lower in (non-obese) children compared to (non-obese) adults, allometric scaling with a factor of 0.75 on the basis of body weight is often applied [19-21]. Particularly for (lean) adolescents there is agreement on this approach [22]. However, this approach that uses body weight as a proxy for size, may be questionable in obese adolescents. **Chapter 9** may give an appropriate answer to this question. In this chapter, the clearance of the CYP3A substrate midazolam was studied in obese adolescents and morbidly obese adults in a combined analysis. Data from studies in 19 overweight and obese adolescents (Chapter 4) and 20 morbidly obese adults (Chapter 7) were analysed. The population mean of midazolam clearance was significantly higher in obese adolescents compared to morbidly obese adults (0.71 (7%) vs. 0.44 (11%) L/min, $P < 0.01$). Moreover, in obese adolescents clearance increased mainly with WT_{excess} (= TBW - $WT_{for\ age\ and\ length}$) for

which a model was proposed with 0.75 allometric scaling on the basis of $WT_{for\ age\ and\ length}$ and a separate function for WT_{excess}. In conclusion, a higher midazolam clearance in obese adolescents compared to morbidly obese adults was reported. We hypothesize that the higher midazolam clearance in obese adolescents is explained by less obesity-induced suppression of CYP3A activity, and the increase with WT_{excess} is explained by increased liver flow. The approach characterizing the influence of obesity in the paediatric population may be of value for use in future studies in obese adolescents.

PERSPECTIVES

Can doses for obese adolescents be predicted on the basis of data obtained in morbidly obese adults?

Since pharmacokinetic studies in obese children are largely lacking, various reviews conclude when dosing information is not available for obese children, data can be extrapolated from obese adults as long as the effects of growth and development on the pharmacokinetics relevant to the child's age are considered [23-27]. However, the results of Chapter 5 and 9 of this thesis show that the prediction of changes in clearance in obese adolescents may be more complex.

In Chapter 5 we showed that the oral clearance of metformin in obese adolescents (1.17 L/min) is similar to values reported in healthy adults and type 2 diabetes mellitus patient (T2DM) adults (1.3 L/min) [13] and lower compared to obese adults (1.5 L/min, calculated by dose/AUC) [28]. Although these results may be expected, the fact that similar results were obtained for obese adolescents and T2DM patients may be somewhat surprising given the fact that the adolescents are at a lower stage of growth and development and the T2DM adults may suffer from a certain level of obesity. In any case, the fact that oral clearance was higher in obese adolescents compared to non-obese children [12] and comparable to healthy adults and T2DM adult patients [13], may lead to an advice to increase the maximum recommended dose of 2 g of metformin for children to the maximum dose in adults (i.e. 3 g per day) in case of insufficient clinical response.

In contrast, in Chapter 9, a higher midazolam clearance compared to morbidly obese adults was found in obese adolescents. From the perspective that PK values for obese adolescents can be predicted from obese adults taking into account growth and development, these results are highly unexpected since lower values would have been anticipated. With respect to this CYP3A substrate, it is hypothesized that another aspect, i.e. the duration of obesity, seems to be of relevance concerning these populations. From Chapter 7, we know that the previously reported lower CYP3A protein expression or activity [29-32] caused by an increased inflammation state [31,33-35], is probably counteracted by a higher liver volume and/or liver blood flow in morbidly obese patients, resulting in

a similar midazolam clearance compared to non-obese individuals [36]. This hypothesis is supported by the results of a follow-up study in these morbidly obese patients one year after bariatric surgery when these patients had an average weight loss of 44.5 ± 10.2 kg [37]. In this study, patients after bariatric surgery showed a 1.7 higher midazolam clearance compared to values before their surgery [37]. This increase was explained by the recovery of hepatic CYP3A activity due to a decreased inflammation status after bariatric surgery while liver size and/or flow remained yet unchanged [37]. This was confirmed by a semi-physiologically based pharmacokinetic model (PBPK) including the 1-OH-midazolam metabolite, where the intrinsic hepatic clearance of midazolam was 1.5 times higher in patients after bariatric surgery compared to morbidly obese patients before their surgery, tested against different hepatic flow scenarios [38]. As such, the higher total midazolam plasma clearance in obese adolescents compared to morbidly obese adults, can in our opinion, be explained by the hypothesis that the CYP3A activity in obese adolescents is not (yet) suppressed to the same extent as in morbidly obese adults. Therefore it seems that the duration of obesity influences the extent of (patho)physiologic changes caused by obesity, in this case inflammatory processes in the liver.

An interesting question to consider would be whether the changes in glucuronidation, sulphation and CYP2E1-mediated oxidation we reported in Chapter 8 for acetaminophen in morbidly obese adults would also apply to obese children. In Chapter 8 we showed that all three metabolic pathways of acetaminophen, i.e. glucuronidation, sulphation and CYP2E1 oxidation, increased with obesity. Non-alcoholic fatty liver disease (NAFLD) may be the underlying cause of the increased CYP2E1 oxidation and sulphation of acetaminophen in morbidly obese patients [39,40]. Considering the above hypothesis that the duration of obesity may influence the (patho)physiological changes caused by obesity, it is possible that NAFLD may not have the same impact on metabolic pathways in obese children as in morbidly obese adults, since NAFLD exists for a shorter time period or is not as severe as in morbidly obese adults. Moreover, the influence of obesity on glucuronidation, sulphation and CYP2E1 oxidation is in contrast with its influence on CYP3A oxidation (i.e. induction vs. suppression), which is another reason why it is difficult to predict how glucuronidation, sulphation and CYP2E1 oxidation may change in obese adolescents vs. obese adults. Clinical studies in obese children and adolescents in which the different metabolites are evaluated both in plasma and urine to prevent identifiability issues [41] should provide the answers to these questions. Based on the results of Chapter 9, we conclude that the (patho)physiologic changes in obese adolescents may differ from those in morbidly obese adults resulting in a different influence on the pharmacokinetics of a drug in obese adolescents vs. morbidly obese adults.

How to analyse pharmacokinetic data in obese children and adolescents?

As outlined in the previous paragraph, clinical studies and subsequent pharmacometric analyses are crucial to determine the right dose for obese children and adolescents. The evaluation of the influence of weight on the pharmacokinetics in obese children and adolescents may be complicated by the interrelation between growth, age and obesity [42]. Moreover, the influence of a kg of excess weight in children on pharmacokinetic parameters may not be equal to a kg of weight due to growth and development. Therefore, when analysing pharmacokinetic data in obese adolescents, it is important to consider the distinction between developmental weight (i.e. weight related to growth and development) and excess weight and to evaluate how these covariates each influence the PK parameters.

In the Chapters 5 and 9 we proposed an excess weight covariate model in which total body weight of obese adolescents was considered to be composed of two parts, i.e. developmental weight ($WT_{for\ age\ and\ length}$) and excess body weight (WT_{excess}), which are calculated by:

$$WT_{for\ age\ and\ length} = BMI_{without\ overweight} \times length^2 \quad \text{(Eq. 1)}$$

$$WT_{excess} = TBW - WT_{for\ age\ and\ length} \quad \text{(Eq. 2)}$$

in which $BMI_{without\ overweight}$ is the BMI derived from the BMI for age CDC growth chart at a BMI z score of 0 together with the age of the patient [43] and TBW the total body weight of the patient. In addition, relative WT_{excess} was calculated by:

$$\%WT_{excess} = (WT_{excess} / WT_{for\ age\ and\ length}) \times 100\% \quad \text{(Eq. 3)}$$

Using this approach the relation between $WT_{for\ age\ and\ length}$, WT_{excess} and $\%WT_{excess}$ and the pharmacokinetic parameter is visualized first. In case a trend is observed for the pharmacokinetic parameter clearance and $WT_{for\ age\ and\ length}$ in obese adolescents, allometric scaling with an exponent of 0.75 can be applied for $WT_{for\ age\ and\ length}$ (Equation 4) as is generally applied for scaling from adults to adolescents [22]. An advantage of this approach is that data from non-obese adolescents are not necessarily needed and results of (non-obese or 70 kg) adults can be used instead. After implementation of the function for $WT_{for\ age\ and\ length}$, the influence of WT_{excess} on clearance can subsequently be estimated using a separate function (Equation 5).

$$CL_{non\text{-}obese\ adolescent} = CL_{70\ kg\ adult} \times (WT_{for\ age\ and\ length} / 70)^{0.75} \quad \text{(Eq. 4)}$$

$$CL_{(obese)\ adolescent} = CL_{non\text{-}obese\ adolescent} + (Z \times WT_{excess}) \quad \text{(Eq. 5)}$$

in which $CL_{non\text{-}obese\ adolescent}$ represents the clearance estimate of adolescents without overweight with $CL_{70\ kg\ adult}$ representing the population clearance of a 70 kg adult, $WT_{for\ age\ and\ length}$ representing developmental weight for adolescents according to Equation 1 and 0.75 being the scaling factor that was previously proposed by the US FDA for scaling clearance in (non-obese) adolescents [22]. $CL_{(obese)\ adolescent}$ represents the individual clearance estimates of (obese) adolescents; *Z* represents the linear relationship between clearance estimates of adolescents without overweight ($CL_{non\text{-}obese\ adolescent}$) and the change in clearance with $WT_{excess.}$

Using this approach it was found that the increase in midazolam clearance in obese adolescents with TBW seemed to be mainly explained by $WT_{excess,}$ with a less obvious trend with $WT_{for\ age\ and\ length}$ (Figure 3, Chapter 9). However, as allometric scaling from (non-obese) adults to adolescents is generally accepted [22], the latter may result from the limited data available and therefore the model scaling $WT_{for\ age\ and\ length}$ to the power of 0.75 and a separate function for WT_{excess} was applied. The increase of clearance with WT_{excess} is most likely explained by an increase in liver flow that can be anticipated with increasing weight in obese adolescents and which may be of relevance for midazolam clearance as it is an intermediate extraction ratio drug. For metformin, it was found that the increase in oral clearance of metformin with TBW in obese adolescents was explained by both $WT_{for\ age\ and\ length}$ and $WT_{\ excess}$ (Figure 3, Chapter 5). In agreement with the model used for midazolam, $WT_{for\ age\ and\ length}$ was allometrically scaled on the basis of 70 kg to the power of 0.75, after which the influence of WT_{excess} on clearance was estimated. Both excess weight models resulted in a similar description of the data in comparison with the final model using one weight measure (i.e. TBW) only. It can be anticipated however, that the model distinguishing between $WT_{for\ age\ and\ length}$ and WT_{excess} may have more predictive value when more (extreme) individuals are concerned.

When comparing the results of the midazolam excess weight model of Chapter 9 with the results of Chapter 4, where midazolam concentrations together with its metabolites in obese adolescents were analysed, some differences are found. First, in Chapter 4, an increase in peripheral volume of distribution of midazolam in obese adolescents was found which was explained by WT_{excess} [44]. Moreover, in the regular covariate analysis of Chapter 4 only a positive trend between clearance of midazolam to 1-OH-midazolam and TBW or LBW could be identified ($P < 0.01$), but did not fulfil the criteria of the backward deletion step ($P < 0.001$) [44]. This difference may perhaps be explained by a statistical difference between a metabolite model (Chapter 4) and parent model (Chapter 9), since a metabolite model has more variability and as a result less power. From these results, it seems that these type of analyses with data from a small number of individuals with varying characteristics (i.e. degree of obesity and age) may benefit from more data from clinical studies together with approaches to characterize obesity in relation to growth and development as we propose here.

The excess weight covariate model we applied in Chapters 5 and 9 was previously used in a slightly different approach by Bartelink et al. [45] for busulfan in under-, normal and overweight children, adolescents and adults (median age of 4 (0.1-35) years). In this model, the body weight of each patient was considered to be composed of body weight related to growth (body weight-for-age, $BW_{for\ age}$) and body weight related to under- or overweight (body weight Z score, $BW_{z\ score}$) [45]. In our model, we used $WT_{for\ age\ and\ length}$ instead of $BW_{for\ age}$, since the influence of length is thought to be of more relevance for adolescents. Moreover, in contrast to Bartelink et al. who included very young children (median age of 4 (0.1-35) years), we could use 0.75 allometric scaling for $WT_{for\ age\ and\ length}$ as we included adolescents only for which this 0.75 allometric scaling approach is generally accepted [22].

The excess weight covariate model we propose here may provide guidance on how to analyse data in obese children or adolescents for clearance in future paediatric studies. This is particularly of relevance because of the increased prevalence of childhood obesity. When analysing paediatric PK data, it is important to check whether obese individuals are part of the studied population. In that case, a distinction needs to be made between developmental weight and overweight upon which an evaluation needs to be performed on how these different covariates each influence the pharmacokinetic parameters. For adolescents, the model then can be simplified by application of 0.75 allometric scaling for $WT_{for\ age\ and\ length}$, while this may be controversial for younger children. In conclusion, the excess weight covariate model may provide insight into the extent of the influence of the different weight covariates on the pharmacokinetic parameters and can be used for evidence based dosing guidelines in obese children and adolescents. Until then, more clinical studies in obese children are needed for which our approach may be of use for the pharmacometric analysis.

How to achieve safe and effective acetaminophen dosing for morbidly obese patients?

In Chapter 8 we found an increased total body clearance of acetaminophen in morbidly obese patients, which could be explained by an increase in all three metabolic pathways, i.e. glucuronidation, sulphation and oxidation (mainly CYP2E1). In addition, also an accelerated formation of the CYP2E1-mediated metabolites (cysteine and mercapturate) was found in morbidly obese patients. As beside the increased total clearance, also an increased volume of distribution for acetaminophen was reported, both acetaminophen peak concentrations and area under the plasma concentration-time curves (AUCs) were substantially lower in morbidly obese patients. This implies that, when peak concentrations and/or AUC are predictive for the analgesic effect of acetaminophen, the acetaminophen dose should be increased in morbidly obese patients. However, as stated in Chapter 8, when elevating the dose, the question is whether the earlier and higher formation of CYP2E1-mediated metabolites may contribute to acetaminophen hepatotoxicity in the

obese population. When considering the different clearance values from Chapter 8, the glucuronidation and sulphation clearances are increased to a greater extent than CYP2E1-mediated clearance, resulting in a larger proportion of glucuronidation and sulphation clearance in relation to total clearance in morbidly obese patients than in non-obese patients, and a lower proportion of CYP2E1-mediated clearance (Table 2, Chapter 8). This implies that the fraction of the dose that is metabolized through the potentially toxic CYP2E1 pathway is slightly lower in morbidly obese patients compared to non-obese patients. Figure 1 illustrates these findings by showing the results of simulations on the basis of the final model of Chapter 8 upon a multiple dosing regimen of 1 g every 6 hours in a non-obese patient and different morbidly obese patients (n=3). Figure 1 shows that upon multiple dosing, acetaminophen, acetaminophen glucuronide and cysteine and mercapturate concentrations are overall lower in the obese, while sulphate concentrations are rather similar. This plot also shows that the higher cysteine and mercapturate concentrations that are initially observed after the first dose do not remain at this higher level. This figure should however be considered with care because even though extensive

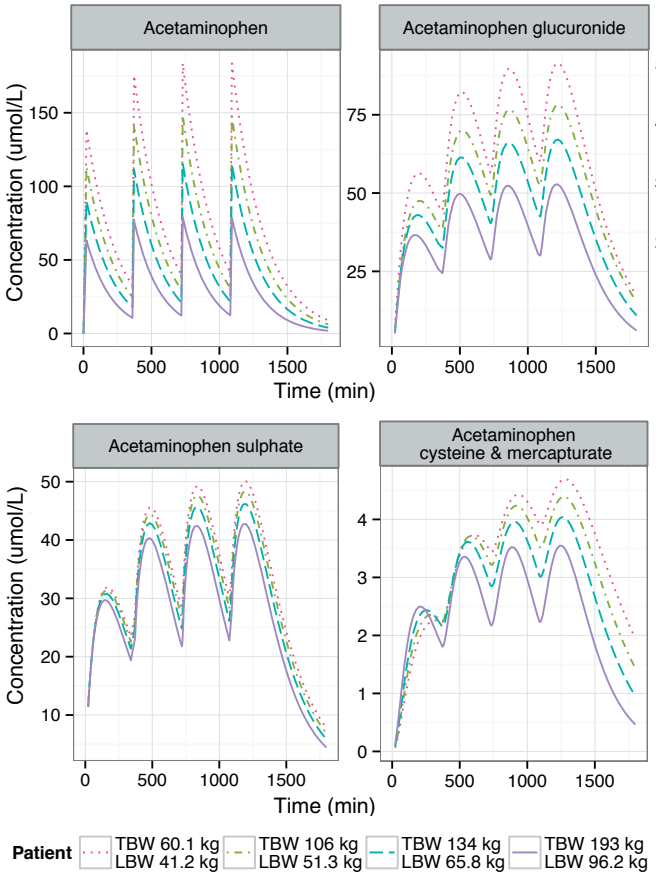

Figure 1 Population predicted acetaminophen (left upper panel), acetaminophen glucuronide (right upper panel), acetaminophen sulphate (left lower panel) and acetaminophen cysteine & mercapturate (right lower panel) concentrations over time in one non-obese patient (60.1 kg with 41.2 kg LBW) and three morbidly obese patients (106, 134, 193 kg and with 51.3, 65.8 and 96.2 kg LBW, respectively) after multiple dosing of 1 g intravenous acetaminophen every 6 hours.

sampling was performed until 8 hours post-dose, only one sample was obtained at 24 hours after multiple dosing. Moreover, for this figure, it is emphasized that concentrations of metabolites are not only determined by the fraction of the dose eliminated through the specific pathway, but also by the volume of distribution of the metabolite, and the elimination clearance of the metabolite, which all may change with obesity. Finally, the metabolites are – as far as we know – all inactive, while for the potentially toxic pathway through CYP2E1, it is anticipated that not the concentrations of these metabolites determine the hepatotoxicity, but the percentage of the dose metabolised by CYP2E1.

The relevance of the contribution of the different pathways was illustrated by Xie et al. who suggested, based on different studies, that patients with a higher glucuronidation capacity might have more protection against liver injury after acetaminophen overdose [46]. A study with Gilbert's syndrome patients showed a decreased proportion of glucuronidation and an increased proportion of oxidation of acetaminophen, which might imply an increased risk of liver injury after acetaminophen overdose in these patients [47]. In addition, another study showed that the prevalence of polymorphisms in UGT, that results in a higher glucuronidation capacity of acetaminophen, was lower in acute liver failure patients after unintentional acetaminophen overdose than in patients with acute liver failure from other causes [48]. As such, it seems that a higher glucuronidation capacity is protecting indirectly against liver injury, since a higher glucuronidation clearance together with an increased total clearance may lead to a decreased CYP2E1 fraction of total clearance. As morbidly obese patients showed a slightly lower CYP2E1 fraction, the risk of liver injury does, theoretically, not need to be increased in morbidly obese patients, even though morbidly obese patients have a higher CYP2E1 metabolism.

Given the fact that both the acetaminophen peak concentrations and AUC values seem about two times lower in severely obese patients compared to non-obese patients, a higher dose may be warranted in the obese population. In a prospective study as a follow up to an initial population PK study [49,50], an individualized dosing scheme of acetaminophen in morbidly obese patients is to be studied aiming for similar acetaminophen concentrations in morbidly obese patients compared to non-obese patients. Based on the results of Chapter 8, model based simulations showed that morbidly obese patients with a LBW < 65 kg should receive the regular dose of 1 g four times daily, while morbidly obese patients with a LBW of 65-95 kg should receive 1.5 g of acetaminophen per dose and patients with a LBW ≥ 95 kg 2 g of acetaminophen to achieve similar acetaminophen plasma concentrations as non-obese patients. Figure 2 shows the results of this individualized dosing scheme of acetaminophen on the basis of LBW, that resulted as most predictive parameter for the pharmacokinetics of acetaminophen in Chapter 8. The four non-obese and four morbidly obese patients in this figure represent the extremes in LBW of the dataset for the female and male patients (i.e. the lowest and highest LBW for both female and male non-obese and morbidly obese patients).

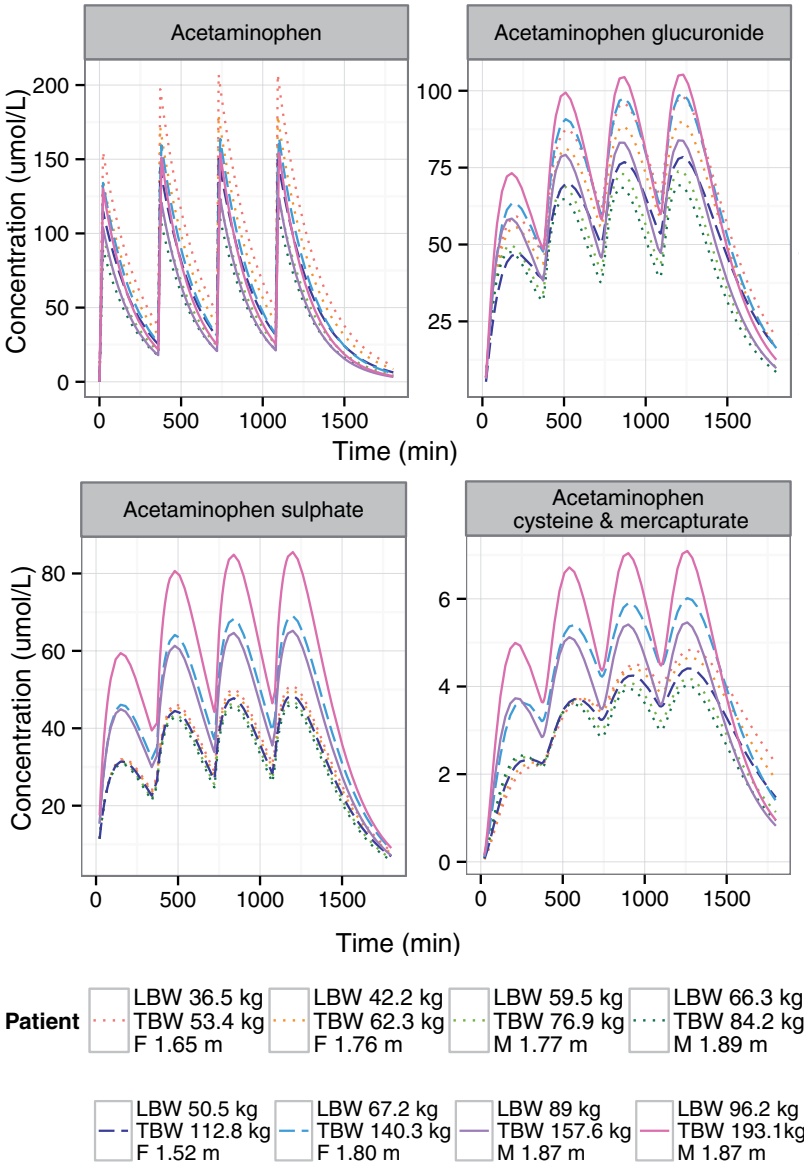

Figure 2 Population predicted acetaminophen (left upper graph), acetaminophen glucuronide (right upper graph), acetaminophen sulphate (left lower graph) and acetaminophen cysteine & mercapturate (right lower graph) concentrations over time in four non-obese patients (dotted lines 36.5, 42.2, 59.5, 66.3 kg LBW, two female and two male) and four morbidly obese patients (long dash lines two females of 50.5, 67.2 kg LBW and solid lines two males 89 and 96.2 kg LBW). The four non-obese patients and morbidly obese patient of 50.5 kg LBW received 1 g of acetaminophen four times daily, the morbidly obese patient of 67.2 kg and 89 kg LBW received 1.5 g of acetaminophen per dose and the morbidly obese patient of 96.2 kg LBW received 2 g of acetaminophen four times daily to reach similar acetaminophen concentrations across the entire population of morbidly obese and non-obese individuals.

When LBW is indeed used to guide dosing in morbidly obese patients when it is the most predictive covariate in the model, it is important to realize that LBW is influenced by weight, gender and height [51]. As a consequence men will have a higher LBW than women with the same height and body weight (Figure 3). Figure 3 shows that for instance only women with a height above 1.70 m and a body weight above 160 kg, reach a LBW of 70 kg. As a consequence, morbidly obese men are more prone to receive a higher dose of acetaminophen than morbidly obese women when dosing on LBW. This is further illustrated by Figure 4 in which the results on total clearance of acetaminophen (CL_{tot}) for the obese and non-obese individuals of Chapter 8 are presented with indication of their gender. This figure shows that morbidly obese women have the same LBW and CL_{tot} as non-obese men (Figure 4a), while having a higher TBW (Figure 4b). Morbidly obese men have a substantially higher LBW and CL_{tot} than morbidly obese women (Figure 4a), with only a limited difference in TBW (Figure 4b). The figure also illustrates the finding that LBW was more predictive for clearance than TBW (Chapter 8).

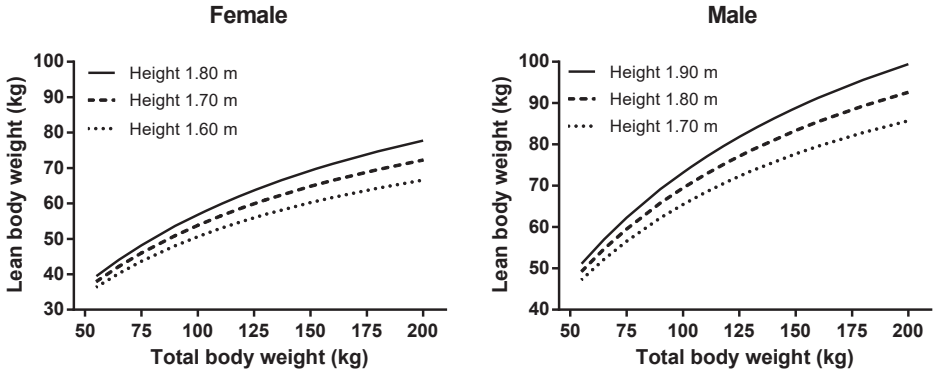

Figure 3 Lean body weight [51] vs. total body weight of various heights for females (left graph) and males (right graph).

The proposed individualized dosing scheme should be evaluated in a multiple dosing scheme (every 6 hours) for 3 days in morbidly obese patients. In our opinion, the higher dose of 1.5 or 2 g is justified as in a study with non-obese patients (n=37), AST and ALT levels did not exceed the upper limit of the reference range (< 1 time ULN) after three days administering 4, 6 or 8 g of acetaminophen per day [52]. In the prospective study, acetaminophen and metabolite concentrations (glucuronide, sulphate and cysteine and mercapturate) and liver function markers (AST, ALT, PT, bilirubin, creatinine, γ-GT) should be measured each day until three days after the last acetaminophen dose. This prospective study will provide answers to the remaining question of Chapter 8 whether it is possible to increase the dose of acetaminophen in morbidly obese patients in terms of safety of acetaminophen in this group.

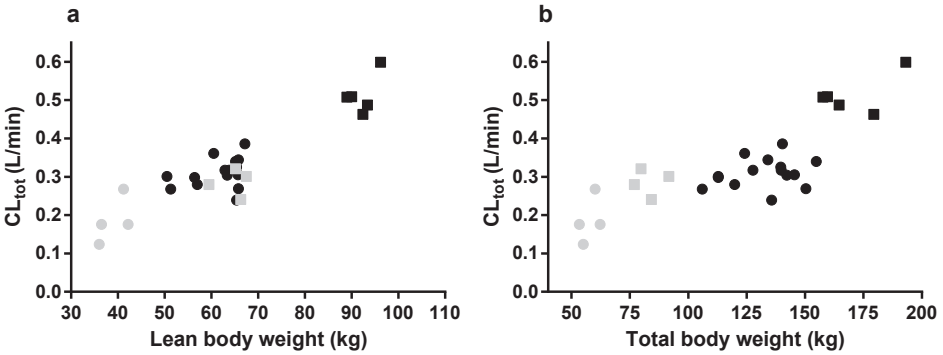

Figure 4 Total clearance of acetaminophen (CL_{tot}) vs. lean body weight (a) and total body weight (b) for non-obese (grey figures) and morbidly obese patients (black figures) including females (circles) and males (squares) of Chapter 8.

Although AST and ALT are commonly used to determine liver injury after acetaminophen overdose, the use of these liver function markers to measure the safety of acetaminophen has some disadvantages. Changes in AST and ALT levels may not per se be specific for acetaminophen induced liver injury as changes in AST and ALT can, for example, also occur with a wide range of liver pathologies, myocardial damage or extreme exercise [53-55]. Also in Chapter 8, we encountered slightly higher AST and ALT levels in our morbidly obese population, both before and after bariatric surgery in comparison to non-obese patients. Another disadvantage of AST and ALT is the delay in appearance in serum, since increases in serum are not usually observed until 12 hours after acetaminophen ingestion with an interval of at least 24 hours to confidently exclude the development of liver injury [53,55].

Recently, a considerable amount of studies has been conducted in the search for the ultimate biomarker to identify liver injury after acetaminophen overdose at the earliest possible time point [46,56-60]. Potential biomarkers are microRNA-122 (miRNA-122), high-mobility group box-1 (HMGB1) and keratin-18 (K-18). It is also thought that acetaminophen protein adducts (APAP protein adducts) could be a rational and useful biomarker for acetaminophen induced liver injury. APAP protein adducts are formed during acetaminophen metabolism when NAPQI covalently binds to cytosolic and mitochondrial proteins leading to hepatocellular necrosis [53]. A study in mice showed that the concentration of APAP protein adducts increased rapidly after acetaminophen overdose and peaked before the ALT peak [61]. However, in a recent study with overdose patients, APAP protein adduct concentrations were measured at the beginning of the study, but the peak was observed around the time of peak ALT [46]. Protein adducts are, based on these results, probably not eligible as an early marker for the clinic to detect liver injury.

The potential of miRNA-122, HMGB1 and K18 to serve as a biomarker for acetaminophen induced liver injury was investigated in patients with established liver injury [57,62],

but also in patients at first presentation to the hospital after acetaminophen overdose [58]. MiRNAs are small non-protein coding RNA species involved in posttranscriptional gene product regulation [53]. MiRNA-122 is highly liver specific and plays an important role in the physiology of the liver [53,55]. The release of miRNA-122 from hepatocytes after acetaminophen overdose is probably biphasic. Early after overdose, it is released in exosomes by active mechanisms from the hepatocytes aiming for hepatocyte proliferation and regeneration. Later on, in the phase of hepatocellular necrosis, it may be released in a similar mechanism as ALT and AST, when cell membrane integrity is lost [55]. In addition to miRNA-122, HMGB1 and K-18 are also high potential biomarkers for acetaminophen-induced hepatotoxicity. HMGB1 is a chromatin-binding protein and is passively released by cells undergoing necrosis and works as a damage-associated molecular pattern (DAMP) molecule, alerting the immune system to dying cells [53]. A hyper-acetylated form of HMGB1 is actively secreted by monocytes and macrophages functioning as an inflammatory mediator [62]. K-18 is an intermediate filament protein expressed in epithelial cells of the liver, responsible for cell structure. During apoptosis, cleavage of K-18 occurs for structural rearrangement of cells. Cleaved K-18 fragments are released from apoptotic cells, while full length K-18 is passively released form necrotic cells [53 54].

In patients with established acetaminophen induced liver injury, increased levels of HMGB1, K-18 and miRNA-122 were found in comparison with healthy controls and overdose patients without liver injury and correlated with peak serum ALT levels ($P <$ 0.01-0.0001) [57,62]. Elevated total and acetylated HMGB1, full length K-18 and a lower percentage of cleaved K18 were associated with patients that had a worse prognosis or died or required liver transplant compared to patients who survived ($P < 0.05$-0.001) [62]. In a study in patients in which ALT activity was still normal at first presentation to the hospital after a single overdose of acetaminophen, mi-RNA-122, HMGB1 and full length K-18 could accurately distinguish between patients with and without acute liver injury early in time [58]. The importance of the early rise of miRNA-122, HMGB1 and K-18 after overdose is described in a case report, where a patient did not receive acetylcysteine treatment based on the Rumack-Matthew nomogram after acetaminophen overdose (acetaminophen concentration was 107 mg/L at 4.5 h) and returned to the hospital 43 hours after overdose [63]. Retrospectively, at 4.5 hours after overdose, miRNA-122 was elevated 50 fold above the upper limit of normal (ULN) and HMGB1 and K-18 8 fold [63].

Above studies suggest that miRNA-122, HMGB1 and K-18 are promising markers for early detection of liver injury and should therefore be further studied to confirm at which time after acetaminophen overdose the biomarker is able to predict liver injury, the sensitivity for excluding injury and establishing whether there is a quantitative relationship between biomarker level and outcome [53]. In addition, the development of quick point of care tests performed in the standard hospital laboratory are needed [53].

Hence, to answer the question whether it is possible to increase the dose of acetaminophen in morbidly obese patients thereby to reach similar acetaminophen concentrations as in non-obese patients, the above described biomarkers and liver function markers should be considered in a prospective study. In this prospective study, according to Figure 2, morbidly obese patients should receive an individualized dosing scheme based on LBW with morbidly obese patients < 65 kg LBW receiving a dose of 1 g of acetaminophen, morbidly obese patients with a LBW between 65 and 90 kg 1.5 g and patients with LBW > 90 kg 2 g of acetaminophen 4 times daily. In this study, both efficacy and safety measures should be implemented.

In conclusion, with this thesis we aimed to contribute to existing gaps in knowledge regarding the development of evidence based dosing in obese adolescents and morbidly obese adults. Studies in morbidly obese patients showed that glucuronidation, sulphation and CYP2E1-mediated oxidation of acetaminophen is increased. That implies that, in order to achieve similar acetaminophen concentrations in morbidly obese patients compared to non-obese patients, an individualized dosing scheme of acetaminophen based on LBW should be explored with adequate monitoring of existing and novel biomarkers for liver toxicity. Surprisingly, CYP3A midazolam clearance was similar to non-obese adults, probably because the obesity related suppression of CYP3A activity in the liver is counteracted by increase liver size and/or liver flow. Moreover, obese adolescents showed a higher CYP3A midazolam clearance compared to morbidly obese adults. We hypothesized that CYP3A activity in obese adolescents was not (yet) suppressed to the same extent as in morbidly obese adults. The duration of obesity thereby seems a factor to consider as it is of influence on the (patho)physiological changes of obesity. Metformin clearance in obese adolescents was similar to literature values of type 2 diabetes adults implying that if necessary similar doses compared to adults may be appropriate. The studies described in this thesis also indicate that data of morbidly obese adults cannot be extrapolated to obese children with only taking growth and development into account. This underscores the importance of conducting pharmacokinetic studies in obese adolescents and children. For the pharmacometric analysis of the data of these future paediatric studies, we have proposed an excess weight covariate model to study the influence of obesity on pharmacokinetic parameters in children, by dividing total body weight in developmental body weight ($WT_{\text{for age and length}}$) and excess body weight (WT_{excess}). Using this approach both the influence of size resulting from development/growth (i.e. $WT_{\text{for age and length}}$) and size resulting from obesity (WT_{excess}) can be separately studied and accounted for. Moreover, this approach can be used for scaling for adults to (obese) adolescents. Ultimately these efforts should lead to evidence based guidelines in both obese adults and obese adolescents.

LITERATURE

1. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766-81.
2. Krekels EH, van Hasselt JG, Tibboel D, Danhof M, Knibbe CA. Systematic evaluation of the descriptive and predictive performance of paediatric morphine population models. Pharm Res 2011;28:797-811.
3. Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. Clin Pharmacol Ther 2007;81:270-83.
4. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138:103-41.
5. Peters AM, Snelling HL, Glass DM, Bird NJ. Estimation of lean body mass in children. Br J Anaesth 2011;106:719-23.
6. Fazeli Farsani S, Souverein PC, Overbeek JA, et al. Long term trends in oral antidiabetic drug use among children and adolescents in the Netherlands. Br J Clin Pharmacol 2015;80:294-303.
7. Clarke JD, Dzierlenga AL, Nelson NR, et al. Mechanism of Altered Metformin Distribution in Nonalcoholic Steatohepatitis. Diabetes 2015;64:3305-13.
8. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820-7.
9. Goswami S, Yee SW, Stocker S, et al. Genetic variants in transcription factors are associated with the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacol Ther 2014;96:370-9.
10. Yacovino LL, Aleksunes LM. Endocrine and metabolic regulation of renal drug transporters. J Biochem Mol Toxicol 2012;26:407-21.
11. van der Aa MP, Elst MA, van Mil EG, Knibbe CA, van der Vorst MM. METFORMIN: an efficacy, safety and pharmacokinetic study on the short-term and long-term use in obese children and adolescents - study protocol of a randomized controlled study. Trials 2014;15:207,6215-15-207.
12. Sanchez-Infantes D, Diaz M, Lopez-Bermejo A, Marcos MV, de Zegher F, Ibanez L. Pharmacokinetics of metformin in girls aged 9 years. Clin Pharmacokinet 2011;50:735-8.
13. Graham GG, Punt J, Arora M, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50:81-98.
14. Kotlyar M, Carson SW. Effects of obesity on the cytochrome P450 enzyme system. Int J Clin Pharmacol Ther 1999;37:8-19.
15. Brill MJ, Diepstraten J, van Rongen A, van Kralingen S, van den Anker JN, Knibbe CA. Impact of obesity on drug metabolism and elimination in adults and children. Clin Pharmacokinet 2012;51:277-304.
16. Vet NJ, Brussee JM, de Hoog M, et al. Inflammation and Organ Failure Severely Affect Midazolam Clearance in Critically Ill Children. Am J Respir Crit Care Med 2016.
17. Carcillo JA, Doughty L, Kofos D, et al. Cytochrome P450 mediated-drug metabolism is reduced in children with sepsis-induced multiple organ failure. Intensive Care Med 2003;29:980-4.
18. Ince I, Knibbe CA, Danhof M, de Wildt SN. Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from in vitro and in vivo investigations. Clin Pharmacokinet 2013;52:333-45.
19. Anderson BJ, McKee AD, Holford NH. Size, myths and the clinical pharmacokinetics of analgesia in paediatric patients. Clin Pharmacokinet 1997;33:313-27.

20. Momper JD, Mulugeta Y, Green DJ, et al. Adolescent dosing and labeling since the Food and Drug Administration Amendments Act of 2007. JAMA Pediatr 2013;167:926-32.
21. Mahmood I. Dosing in children: a critical review of the pharmacokinetic allometric scaling and modelling approaches in paediatric drug development and clinical settings. Clin Pharmacokinet 2014;53:327-46.
22. Food and Drug Administration Center for Drug Evaluation and Research. Advisory Committee for Pharmaceutical Science and Clinical Pharmacology (ACPS-CP) meeting, Summary minutes and FDA transcript. March 14, 2012.
23. Mulla H, Johnson TN. Dosing dilemmas in obese children. Arch Dis Child Educ Pract Ed 2010;95:112-7.
24. Kendrick JG, Carr RR, Ensom MH. Pharmacokinetics and drug dosing in obese children. J Pediatr Pharmacol Ther 2010;15:94-109.
25. Kendrick JG, Carr RR, Ensom MH. Pediatric Obesity: Pharmacokinetics and Implications for Drug Dosing. Clin Ther 2015;37:1897-923.
26. Rowe S, Siegel D, Benjamin DK,Jr, Best Pharmaceuticals for Children Act - Pediatric Trials Network Administrative Core Committee. Gaps in Drug Dosing for Obese Children: A Systematic Review of Commonly Prescribed Emergency Care Medications. Clin Ther 2015;37:1924-32.
27. Harskamp-van Ginkel MW, Hill KD, Becker K, et al. Drug Dosing and Pharmacokinetics in Children With Obesity: A Systematic Review. JAMA Pediatr 2015;169:678-85.
28. Padwal RS, Gabr RQ, Sharma AM, et al. Effect of gastric bypass surgery on the absorption and bioavailability of metformin. Diabetes Care 2011;34:1295-300.
29. Kolwankar D, Vuppalanchi R, Ethell B, et al. Association between nonalcoholic hepatic steatosis and hepatic cytochrome P-450 3A activity. Clin Gastroenterol Hepatol 2007;5:388-93.
30. Yoshinari K, Takagi S, Yoshimasa T, Sugatani J, Miwa M. Hepatic CYP3A expression is attenuated in obese mice fed a high-fat diet. Pharm Res 2006;23:1188-200.
31. Ghose R, Omoluabi O, Gandhi A, et al. Role of high-fat diet in regulation of gene expression of drug metabolizing enzymes and transporters. Life Sci 2011;89:57-64.
32. Woolsey SJ, Mansell SE, Kim RB, Tirona RG, Beaton MD. CYP3A Activity and Expression in Nonalcoholic Fatty Liver Disease. Drug Metab Dispos 2015;43:1484-90.
33. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007;132:2169-80.
34. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911,9; quiz 920.
35. Renton KW. Regulation of drug metabolism and disposition during inflammation and infection. Expert Opin Drug Metab Toxicol 2005;1:629-40.
36. Brill MJ, van Rongen A, Houwink AP, et al. Midazolam pharmacokinetics in morbidly obese patients following semi-simultaneous oral and intravenous administration: a comparison with healthy volunteers. Clin Pharmacokinet 2014;53:931-41.
37. Brill MJ, van Rongen A, van Dongen EP, et al. The Pharmacokinetics of the CYP3A Substrate Midazolam in Morbidly Obese Patients Before and One Year After Bariatric Surgery. Pharm Res 2015;32:3927-36.
38. Brill M, Valitalo P, Darwich AS, et al. Semiphysiologically based pharmacokinetic model for midazolam and CYP3A mediated metabolite 1-OH-midazolam in morbidly obese and weight loss surgery patients. CPT Pharmacometrics Syst Pharmacol 2016;5:20-30.

39. Aubert J, Begriche K, Knockaert L, Robin MA, Fromenty B. Increased expression of cytochrome P450 2E1 in nonalcoholic fatty liver disease: mechanisms and pathophysiological role. Clin Res Hepatol Gastroenterol 2011;35:630-7.
40. Hardwick RN, Ferreira DW, More VR, et al. Altered UDP-glucuronosyltransferase and sulfotransferase expression and function during progressive stages of human nonalcoholic fatty liver disease. Drug Metab Dispos 2013;41:554-61.
41. Ahlers SJ, Valitalo PA, Peeters MY, et al. Morphine Glucuronidation and Elimination in Intensive Care Patients: A Comparison with Healthy Volunteers. Anesth Analg 2015;121:1261-73.
42. Knibbe CA, Brill MJ, van Rongen A, Diepstraten J, van der Graaf PH, Danhof M. Drug disposition in obesity: toward evidence-based dosing. Annu Rev Pharmacol Toxicol 2015;55:149-67.
43. CDC. Centers for Disease Control and Prevention, Clinical Growth Charts. (Accessed December 14, 2014, at http://www.cdc.gov/growthcharts/clinical_charts.htm).
44. van Rongen A, Vaughns JD, Moorthy GS, Barrett JS, Knibbe CA, van den Anker JN. Population pharmacokinetics of midazolam and its metabolites in overweight and obese adolescents. Br J Clin Pharmacol 2015;80:1185-96.
45. Bartelink IH, van Kesteren C, Boelens JJ, et al. Predictive performance of a busulfan pharmacokinetic model in children and young adults. Ther Drug Monit 2012;34:574-83.
46. Xie Y, McGill MR, Cook SF, et al. Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells. Xenobiotica 2015;45:921-9.
47. Esteban A, Perez-Mateo M. Heterogeneity of paracetamol metabolism in Gilbert's syndrome. Eur J Drug Metab Pharmacokinet 1999;24:9-13.
48. Court MH, Freytsis M, Wang X, et al. The UDP-glucuronosyltransferase (UGT) 1A polymorphism c.2042C>G (rs8330) is associated with increased human liver acetaminophen glucuronidation, increased UGT1A exon 5a/5b splice variant mRNA ratio, and decreased risk of unintentional acetaminophen-induced acute liver failure. J Pharmacol Exp Ther 2013;345:297-307.
49. Smits A, De Cock RF, Allegaert K, Vanhaesebrouck S, Danhof M, Knibbe CA. Prospective Evaluation of a Model-Based Dosing Regimen for Amikacin in Preterm and Term Neonates in Clinical Practice. Antimicrob Agents Chemother 2015;59:6344-51.
50. Krekels EH, DeJongh J, van Lingen RA, et al. Predictive performance of a recently developed population pharmacokinetic model for morphine and its metabolites in new datasets of (preterm) neonates, infants and children. Clin Pharmacokinet 2011;50:51-63.
51. Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet 2005;44:1051-65.
52. Temple AR, Lynch JM, Vena J, Auiler JF, Gelotte CK. Aminotransferase activities in healthy subjects receiving three-day dosing of 4, 6, or 8 grams per day of acetaminophen. Clin Toxicol (Phila) 2007;45:36-44.
53. Vliegenthart AD, Antoine DJ, Dear JW. Target biomarker profile for the clinical management of paracetamol overdose. Br J Clin Pharmacol 2015;80:351-62.
54. Dear JW, Antoine DJ. Stratification of paracetamol overdose patients using new toxicity biomarkers: current candidates and future challenges. Expert Rev Clin Pharmacol 2014;7:181-9.
55. Hornby RJ, Starkey Lewis P, Dear J, Goldring C, Park BK. MicroRNAs as potential circulating biomarkers of drug-induced liver injury: key current and future issues for translation to humans. Expert Rev Clin Pharmacol 2014;7:349-62.
56. Vliegenthart AD, Shaffer JM, Clarke JI, et al. Comprehensive microRNA profiling in acetaminophen toxicity identifies novel circulating biomarkers for human liver and kidney injury. Sci Rep 2015;5:15501.

57. Starkey Lewis PJ, Dear J, Platt V, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 2011;54:1767-76.
58. Antoine DJ, Dear JW, Lewis PS, et al. Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology 2013;58:777-87.
59. Antoine DJ, Sabbisetti VS, Francis B, et al. Circulating Kidney Injury Molecule 1 Predicts Prognosis and Poor Outcome in Patients With Acetaminophen-Induced Liver Injury. Hepatology 2015;62:591-9.
60. Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A 2009;106:4402-7.
61. McGill MR, Lebofsky M, Norris HR, et al. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. Toxicol Appl Pharmacol 2013;269:240-9.
62. Antoine DJ, Jenkins RE, Dear JW, et al. Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity. J Hepatol 2012;56:1070-9.
63. Dear JW, Antoine DJ, Starkey-Lewis P, Goldring CE, Park BK. Early detection of paracetamol toxicity using circulating liver microRNA and markers of cell necrosis. Br J Clin Pharmacol 2014;77:904-5.