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

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Chapter 5

Increased metformin clearance in overweight and obese adolescents

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Ready to be submitted for publication

ABSTRACT

Introduction

In view of the increased use of metformin in obese adolescents, the aim of this study was to determine the pharmacokinetics of metformin in overweight and obese adolescents.

Methods

In overweight (BMI-SDS > 1.1) and obese (BMI-SDS > 2.3) adolescents receiving 500 or 1000 mg of oral metformin twice daily for 37 weeks during a clinical trial, blood samples were collected over 8 hours post-dose during an oral glucose tolerance test. Population pharmacokinetic modelling and systemic covariate analysis was performed using NONMEM 7.2.

Results

Twenty-two overweight and obese adolescents with a mean total body weight (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 years) participated in the study. In the model, oral clearance (CL/F) of metformin (1.17 L/min (RSE of 6%)) increased significantly with TBW ($P < 0.01$). More specifically, oral clearance increased with both developmental weight ($WT_{\text{for age and length}}$) and excess body weight (WT_{excess}) for which an excess weight covariate model was proposed in which $WT_{\text{for age and length}}$ scaled allometrically to the power of 0.75 and the influence of WT_{excess} was estimated separately.

Conclusion

The oral clearance of metformin in overweight and obese adolescents we report here (1.17 L/min) is larger than literature values of non-obese children (0.55 L/min) and similar to adult values (1.3 L/min). This increase may potentially be explained by increased tubular secretion of metformin resulting from an increased activity of renal transporters in obese adolescents.

INTRODUCTION

Worldwide prevalence rates of obesity are increasing, which is not only restricted to adults. In the United States, 34.5% of the adolescents (12-19 years) were overweight (BMI for age ≥ 85th percentile) and 20.5% obese (BMI for age ≥ 95th percentile) in 2011-2012 [1]. In Western-Europe prevalence rates of obesity (based on International Obesity Task Force (IOTF) cut-offs) ranged from 3.8-13.5%, while percentages in North Africa and the Middle East ranged from 4.2-23.3% in boys and girls (age < 20 years) in 2013 [2]. Obesity is the most common cause of insulin resistance in children and adolescents [3] and a known risk factor for type 2 diabetes mellitus (T2DM) [4]. Metformin is a registered drug for the treatment of T2DM in paediatric patients above 10 years of age. Recently, a large increase in metformin or other oral anti-diabetic drugs prescriptions was observed in children [5-10], suggesting that metformin is not only prescribed in children with type II diabetes, but also for obese children with type I diabetes in addition to insulin and for obesity with or without insulin resistance [5]. Until today, the pharmacokinetics of metformin have only been studied in non-obese children who received metformin for early-normal onset of puberty [11], except for one abstract in obese T2DM adolescents [12]. While tubular secretion is the primary route of elimination, conducted by organic cation transporters (OCT2) and multidrug and toxin extrusion transporters (i.e. MATE1 and MATE2K) [13,14], the influence of obesity on the expression or activity of these renal transporters is largely unknown. The aim of this study was therefore to determine the pharmacokinetics of metformin in overweight and obese adolescents who were part of a multicentre randomized double blind controlled trial on short- and long-term efficacy and safety outcomes of metformin [15].

METHODS

Patients

The clinical trial protocol of the multicentre randomized double blind controlled study on short- and long-term efficacy and safety outcomes of metformin are described elsewhere [15] and repeated in short as relevant to this pharmacokinetic sub-study. Obese adolescents were recruited at the pediatric outpatient clinic of the St. Antonius Hospital Nieuwegein and Jeroen Bosch Hospital in 's Hertogenbosch, the Netherlands. Patients were eligible for inclusion if they were between 10-16 years of age, obese (defined as BMI-SDS > 2.3 at pre-study screening), insulin resistant (defined as HOMA-IR ≥ 3.4 at pre-study screening), and of Caucasian descent. Patients were excluded if they had T2DM, polycystic ovary syndrome or endocrine disorders for which treatment with corticosteroids was indicated. In addition, patients with a height < -1.3 SD of target height, (history of) alcohol abuse, impaired renal function (glomerular filtration rate < 80 mL/

min), impaired hepatic function (alanine aminotransferase (ALT) > 150% of normal value for age) and patients who used anti-hyperglycemic drugs or were pregnant were excluded. Before participation, parents and patients provided written informed consent and assent, respectively. The study was approved by the local human research and ethics committee of the St. Antonius Hospital (VCMO, NL34611.100.11) and was conducted in accordance with the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO) of the Netherlands.

Study design

In this multicentre randomized double blind trial (NCT01487993 and EudraCT 2010-023980-17), 23 overweight and obese adolescents of the metformin arm participated in the pharmacokinetic study which was performed during an oral glucose tolerance test (OGTT). At the time of the pharmacokinetic sub-study, patients had received metformin twice daily in a 500 mg or 1000 mg dose for 37 weeks [15]. Patients were asked to fast overnight and postpone their morning metformin dose until arrival at the outpatient clinic. After insertion of a venous cannula, patients received a solution of 1.75 gram/kg body weight of glucose (max 75 gram), dissolved in 200 or 300 mL water. Directly after ingestion of the glucose solution the metformin tablet was ingested (500 or 1000 mg). Blood samples were collected before the metformin dose (trough concentration) and at 60, 120, 240, 360 and 480 minutes, centrifuged at 4000 RPM (g) for 5 minutes and stored at -20 ˚C until analysis. In addition, blood samples for creatinin, alanine aminotransferase (ALT) and DNA to determine the genetic variation in the OCT1 and MATE1 transporters were collected.

Drug assay

Serum metformin concentrations were quantified by high performance liquid chromatography (HPLC). For sample preparation, 50 µL of internal standard (Phenformin 500 mg/L in methanol) and 250 µL of acetonitrile were added to 100 µL of the sample. After vortex mixing, this mixed sample was centrifuged at 4000 g for 10 minutes. Ten µL of the supernatant was injected into the HPLC column (Zorbax SB-CN 5 µm 4.6x150 mm (Agilent)), protected by a precolumn (H3-10C5 (Hichrom) which was kept at 30 °C and detected by photodiode array detector (234 nm). Isocratic elution was performed with a mobile phase consisting of sodium sulphate (pH of 2.3) and acetonitrile in a 770:230 ratio at a flow rate of 1.2 mL/min. The assay was linear over 0.2 to 5.0 mg/L and 0.2 mg/L was the limit of quantification (LOQ). Intra- and inter-assay coefficients of variation were within 0.8 - 3.6% and 4 - 8.5%, respectively.

DNA analysis

DNA was isolated from whole blood on the MagNA Pure LC 2.0 instrument (Roche®). Ready-made TaqMan assays were used for determination of *OCT1* (rs72552763, rs12208357,

rs34130495, rs34059508 and rs622342) and *MATE1* (rs2289669, rs10735) polymorphisms on the ABI PRISM® 7500 Real-Time PCR system (Applied Biosystems®, Bleiswijk, the Netherlands). Violation of Hardy-Weinberg (HW) equilibrium was tested with the Chi-squared test. In addition, observed minor allele frequencies (MAF) were compared with the frequencies found in the SNP database of NCBI. The *OCT1* haplotype, defined by the presence of one or more of the following genetic variants; rs72552763, rs12208357, rs34130495 and rs34059508, was estimated with the haplo.stats package (R, version 3.1.1), which uses the expectation-maximization (EM) logarithm and a posterior probability > 0.98.

Statistical analysis

The area under the curve concentrations from 0 to 8 hours after dose ($AUC_{0\text{-}8h}$) and AUC until infinity (AUC_{inf}) for metformin were calculated for each patient separately using the linear trapezoidal rule in R software (version 3.0.1) [16]. Seven patients had metformin concentrations measured until 7 hours post-dose and for these patients the $AUC_{0\text{-}8h}$ was calculated based on extrapolation until 8 hours. The calculated $AUC_{0\text{-}8h}$ and AUC_{inf} were corrected for the administered dose (1000 mg), as patients received 500 or 1000 mg of metformin. The Pearson correlation test was applied to test the correlation between total body weight (TBW) and dose-corrected $AUC_{0\text{-}8h}$ and $AUC_{inf.}$ The Mann-Whitney test or Kruskal-Wallis test was applied to test statistical differences in dose-corrected $AUC_{0\text{-}8h}$ or dose-corrected AUC_{inf} and different genotype groups of the OCT1 and MATE1 transporter. Statistical analyses were performed using IBM SPSS software, version 22.

Population pharmacokinetic analysis and internal model validation

Metformin data was analysed using non-linear mixed effects modelling with NONMEM (version 7.2; ICON Development Solutions, Hanover, MD, USA) [17]. Pirana (2.9.1) [18], R (3.0.1) [16], Xpose (4.5.0) [18] and Psn (3.6.2) [18] were used to evaluate and visualize the data. Of the 129 metformin samples, 7 trough samples (5.4%) and 1 post-dose sample (0.8%) were below the LOQ. One trough sample below the LOQ of 0.2 mg/L (i.e. 0.12 mg/L) was kept in the dataset, whereas for the other below LOQ samples no metformin could be detected upon which these samples were subsequently removed from the analysis [19,20]. The first order conditional estimation method with interaction was used for model development. Discrimination between different models was guided by Likelihood Ratio Test, by comparison of the objective function value (OFV, i.e. -2 log likelihood (-2LL)) between nested models. A *P* value of < 0.05, representing a decrease of 3.84 in OFV for one degree of freedom, was considered statistically significant. In addition, goodness-of-fit plots for metformin (observed vs. individual-predicted concentrations, observed vs. population-predicted concentrations, conditional weighted residuals vs. time after dose, and conditional weighted residuals vs. population-predicted concentrations plots) were used for diagnostic purposes. Furthermore, precision of parameter estimates, the correlation

matrix and visual improvement in the individual plots were used to evaluate the model. Pharmacokinetic models incorporating either one or two compartments with first-order, zero-order or combined first- and zero order oral absorption were tested. Furthermore, the addition of one or more transit compartments [21] or an oral absorption lag time was evaluated. Interindividual variability (IIV) was assumed to follow a log normal distribution. Residual variability was tested using proportional, additive or a combined proportional and additive error models for metformin. For internal model evaluation, a bootstrap resampling method using 1000 replicates and prediction-corrected visual predictive check (pVPC) [22] using 1000 simulated datasets of individuals from the original dataset were used.

Covariate model

Tested covariates were total body weight (TBW), BMI, BMI-SDS, lean body weight (LBW) according to the equation of Janmahasatian et al. [23], Forster et al. [24] and Peters et al. [25], waist-hip ratio, age, gender, creatinin and eGFR according to the bedside Schwartz formula [26]. In addition, the influence of genetic polymorphisms of the OCT1 and MATE1 transporter were tested. Covariates were plotted independently against the eta (η) estimates of the pharmacokinetic parameters to visualize potential relations. Continuous covariates were tested using linear and power equations (Equation 1 and 2):

$$P_i = P_p \times (1 + Y \times (COV - COV_{median})) \quad \text{(Eq. 1)}$$

$$P_i = P_p \times (COV/COV_{median})^X \quad \text{(Eq. 2)}$$

where P_i and P_p represent individual and population parameter estimates, respectively, *COV* represents the covariate, COV_{median} represents the median value of the covariate for the population, *Y* represents a correlation factor between the population pharmacokinetic parameter and the change in covariate value for a linear function and *X* represents the exponent for a power function. The categorical covariates gender and genetic polymorphisms of the *OCT1* and *MATE1* transporter were examined by calculating a separate parameter for each category of the covariate. Based on *OCT1* (rs622342) and *MATE1* (rs2289669 and rs10735) genotype, subjects were categorized into two groups: variants (homozygous and heterozygous variants) or wild types. For *OCT1* haplotype, subjects were categorized in normal transporter activity or decreased and absent transporter activity. In addition, different combinations of the genotypes were tested, for example between the OCT1 (rs622342) and MATE1 (rs2289669) transporter [27].

Potential covariates were entered into the model one at a time and statistically tested by the Likelihood Ratio Test. In addition, if applicable, reduction in interindividual variability (IIV, omega (ω)) of the parameter was evaluated upon inclusion of the covariate on the parameter. Further, trends in the random effects of the parameter vs. the covariate

involved were observed. Finally, after forward inclusion ($P < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($P < 0.01$). The choice of the final covariate model was further evaluated as discussed in the Population pharmacokinetic analysis and internal model validation section.

Excess weight covariate model

To further analyse the influence of (over)weight on the pharmacokinetics of metformin, an excess weight model was tested for the parameters for which total body weight proved a covariate given the criteria described under Population pharmacokinetic analysis and internal model validation. Using this covariate model, total body weight of each individual patient was considered to be composed of two parts: developmental weight ($WT_{for\ age\ and\ length}$) and excess body weight (WT_{excess}) [28] while separate functions for each of these weights were used.

For each individual patient of the study, $WT_{for\ age\ and\ length}$ was derived from the Dutch TNO growth calculator [29] on the basis of the length and age of the patient and the BMI-SDS score of 0 (no overweight) [29]. WT_{excess} and relative WT_{excess} ($\%WT_{excess}$) were calculated using equation 3 and 4, respectively for each individual patient :

$$WT_{excess} = TBW - WT_{for\ age\ and\ length} \quad \text{(Eq. 3)}$$

$$\%WT_{excess} = (WT_{excess} / WT_{for\ age\ and\ length})*100\% \quad \text{(Eq. 4)}$$

in which TBW is the total body weight of the patient. First, $WT_{for\ age\ and\ length}$, WT_{excess} and $\%WT_{excess}$ were all plotted independently against the eta estimate of the pharmacokinetic parameter of interest to visualize the relation.

The separate impact of $WT_{for\ age\ and\ length}$ and WT_{excess} on clearance in adolescents was evaluated using Equations 5 and 6:

$$CL_{non\text{-}obese\ adolescent} = CL_{70\ kg\ adult} * (WT_{for\ age\ and\ length}/70) \quad \text{(Eq. 5)}$$

$$CL_{(obese)\ adolescent} = CL_{non\text{-}obese\ adolescent} + (Z*WT_{excess}) \quad \text{(Eq. 6)}$$

in which $CL_{non\text{-}obese\ adolescents}$ 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 and 0.75 being the scaling factor that was previously proposed by the US FDA for scaling clearance from adults to adolescents [30], $CL_{(obese)\ adolescent}$ represents the individual clearance estimates of (obese) adolescents; and *Z* represents the linear influence of WT_{excess} in this function.

RESULTS

Patients and data

Twenty-three patients participated in the pharmacokinetic study. One patient was excluded from the analysis, due to interference of co-medication at the metformin peak of the chromatogram of the drug assay. In total 22 patients were included in the analysis from which a total of 122 metformin serum samples were available. Three patients received a 500 mg oral dose of metformin and 19 patients received a 1000 mg oral dose. A summary of all patient characteristics are presented in Table 1. Supplementary Table 1 presents the allele frequencies of the *OCT1* and *MATE1* polymorphisms, showing that all polymorphisms were in line with the Hardy-Weinberg equilibrium.

Table 1 Demographic parameters of 22 overweight and obese adolescents.

	Overweight and obese adolescents (n=22)
Female/male (n)	16/6
Overweight/obese	5/17
Age (years)	14.5 ± 1.8 (11.1-17.5)
Body weight (kg)	79.3 ± 13.9 (54.7-104.9)
BMI (kg/m^2)	29.1 ± 4.4 (22.9-39.3)
BMI-SDS	2.8 ± 0.6 (1.7-4.0)
Waist-hip ratio	1.0 ± 0.06 (0.9-1.1)
LBW (kg) eq. Janmahasatian et al. [23]	49.0 ± 8.7 (35.3-71.1)
LBW (kg) eq. Foster et al. [24]	48.6 ± 8.6 (35.4-72.8)
LBW (kg) eq. Peters et al. [25]	55.5 ± 7.5 (41.7-71.0)
Creatinine (µmol/L)	54.5 ± 7.0 (44-69)
eGFR (mL/min/1.73 m^2) [26]	111.8 ± 12.2 (94.1-135.8)

Values are expressed as mean ± standard deviation (range) unless specified otherwise
BMI= body mass index, eGFR= estimated glomerular filtration rate, eq.= equation, LBW= lean body weight

Observed metformin concentrations

The absorption of metformin in overweight and obese adolescents proved variable with a median T_{max} of 120 minutes (range 60-240) and a median C_{max} of 1.80 mg/L (range 0.79-3.45). In 9 patients fast absorption was observed with a maximum concentration at 60 minutes. The median dose-corrected (1000 mg) AUC_{0-8} and AUC_{inf} of metformin in 22 overweight and obese adolescents were 603.5 mg*min/L (range 286.7-1118.2) and 802.7 mg*min/L (range 322.8-2568.8), respectively. Dose-corrected AUC_{0-8} and AUC_{inf} of metformin significantly decreased with TBW (r = -0.46, P = 0.032 and r = -0.47 P = 0.027, respectively). Genetic variation in the OCT1 and MATE1 transporter did not have a significant influence on the dose-corrected AUC_{0-8} and AUC_{inf} of metformin ($P > 0.05$).

Population pharmacokinetic model and internal model validation

An one compartment model with first order absorption of metformin best described the data. The pharmacokinetic model was parameterized in terms of oral absorption rate constant (Ka), oral volume of distribution (V/F) and oral clearance (CL/F) from the central compartment. Residual variability was best described by a proportional error model. Table 2 shows the parameter estimates of the base model without covariates.

Table 2 Population pharmacokinetic parameters of the base model, excess weight covariate model and final covariate model for metformin in 22 overweight and obese adolescents and results from a bootstrap analysis of the final model (995/1000 resamples successful).

Parameter	Base model (RSE%)	Excess weight model (RSE%)	Final covariate model (RSE%)	Bootstrap (95% confidence interval)
CL/F (L/min)	1.21 (7)	-	-	-
$CL/F = CL/F_{70\,kg} \times (WT_{for\ age\ and\ length}/70)^{0.75} + (Z \times WT_{excess})$				
$CL/F_{70\,kg}$	-	1.16 (16)	-	-
Z	-	0.011 (52)	-	-
$CL/F = CL/F_{75.8\,kg} \times (1 + Y \times (TBW - 75.8)$				
$CL/F_{75.8\,kg}$	-	-	1.17 (6)	1.18 (1.04-1.36)
Y	-	-	0.0138 (44)	0.0126 (0.0035-0.0291)
V/F (L)	488 (10)	486 (10)	485 (10)	481.8 (396.8-607.8)
Ka (min^{-1})	0.0253 (25)	0.025 (25)	0.0248 (25)	0.0252 (0.0159-0.0563)
Interindividual variability (%)				
CL/F	31.6 (58)	26.5 (64)	26.8 (61)	24.8 (5.5-40.3)
V/F	37 (34)	36.5 (34)	36.2 (33)	33.8 (18.2-46.4)
Ka	74.2 (41)	71.4 (43)	70.3 (44)	65.4 (26.7-132.9)
Residual variability (%)				
Proportional error	21.7 (25)	21.8 (25)	21.8 (25)	22.0 (16.1-27.5)
OFV	-134.4	-140.8	-141.0	-150.7 (-204 .4 - -108.0)

CL/F= oral clearance, Ka= absorption rate constant, OFV= objective function value, RSE= relative standard error, TBW=total body weight, V/F= oral volume of distribution, WT_{excess}= excess body weight, $WT_{for\ age\ and\ length}$= developmental weight

In the covariate analysis, a significant influence of TBW and LBW according to the equation of Peters et al. [25] were found for oral clearance of metformin (CL/F) in a linear manner ($P < 0.01$, -6.6 ΔOFV and -7.6 ΔOFV) upon which the covariate model with TBW was chosen as final model. Figure 1 shows the Empirical Bayes estimates (EBEs) for oral clearance of metformin together with the covariate function of the final model. No other covariates were identified as significant covariate for any of the pharmacokinetic parameters ($P > 0.05$). Genetic polymorphisms of the *OCT1* and *MATE1* transporter or any combination of

genotypes were not of significant influence on any of the pharmacokinetic parameters ($P > 0.05$). *OCT1* haplotype (the four SNPs rs72552763, rs12208357, rs34130495, rs34059508)

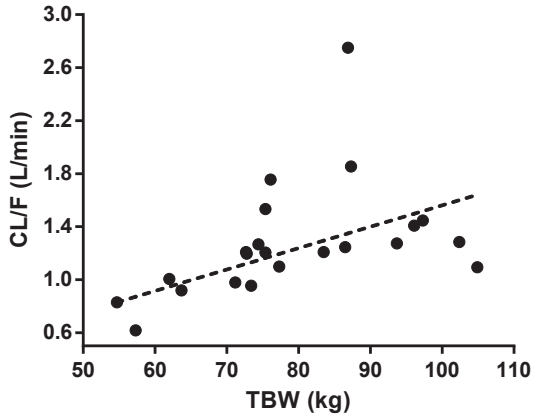


Figure 1 Empirical Bayes estimates (EBEs) for oral clearance (CL/F) of metformin vs. total body weight (TBW) with the covariate relation between oral clearance and TBW for the final covariate model.

showed a trend with oral absorption rate constant (Ka), however problems occurred with the minimization and the Ka could not be estimated with adequate precision.

The model parameters of the final model are summarized in Table 2 and goodness of fit plots of metformin are shown in Supplementary Figure 1. The bootstrap analysis confirms the results of the model with parameter estimates and eta estimates within 8.5% and 14%, respectively, compared to those obtained within the original dataset (Table 2). In addition, prediction-corrected VPC (pcVPC) for metformin indicated good predic-

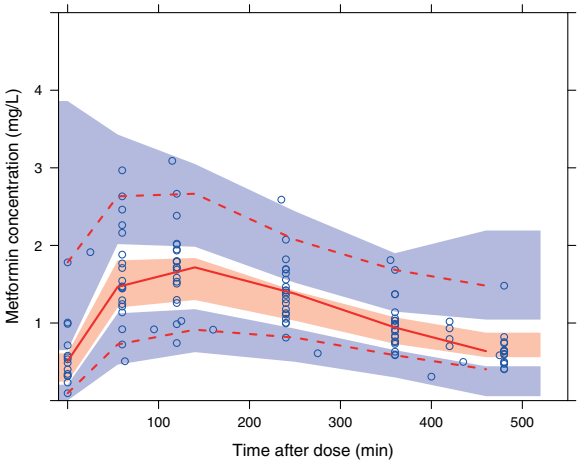


Figure 2 Prediction-corrected visual predictive check of the final model for metformin. Observed concentrations are shown as blue circles with solid, lower and upper dashed red lines showing the median, 2.5th and 97.5th percentiles of the observed data, respectively. The shaded areas represent 95% confidence intervals for the median, 2.5th and 97.5th percentiles of simulated concentrations (n=1000), based on the original dataset.

tive performance with good agreement between observed data and model simulated confidence intervals for the median, 2.5th and 97.5th percentiles (Figure 2).

To further analyse the influence of (over)weight on the oral clearance of metformin, Figure 3 shows clearance vs. developmental weight ($WT_{for\ age\ and\ length}$) and vs. excess body weight (WT_{excess}) (Figure 3a and 3b) with positive trends for both weight measures (ΔOFV -5.5 and -4.2, respectively $P < 0.05$). No significant trend was observed for $\%WT_{excess.}$($P > 0.05$).

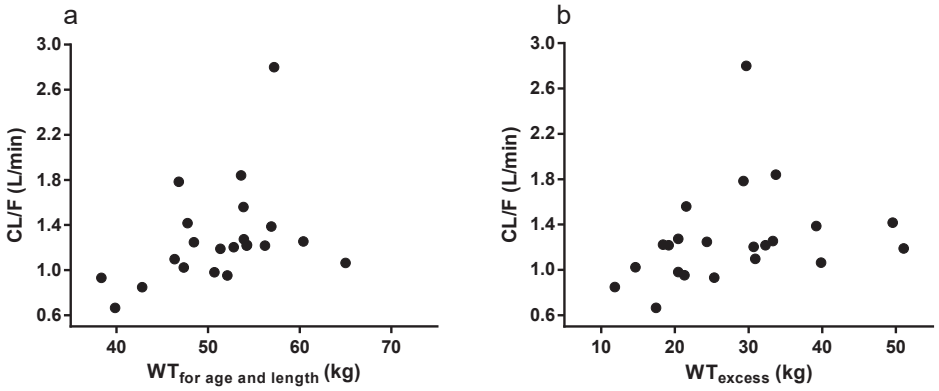


Figure 3 Empirical Bayes estimates (EBEs) for (a) oral clearance (CL/F) of metformin vs developmental weight ($WT_{for\ age\ and\ length}$) and (b) excess body weight (WT_{excess}) of the base model.

To capture the contribution of these different weight measures in obese adolescents, an excess weight covariate model (Equations 5 and 6, Methods section) was applied, in which $WT_{for\ age\ and\ length}$ was scaled on the basis of 70 kg to the power of 0.75 (Equation 5) [30], while for WT_{excess} a separate function was estimated (Equation 6). Figure 4 and Table 2 shows the results of this approach in which the final covariate model for clearance was replaced by the excess weight covariate model. It illustrates that $WT_{for\ age\ and\ length}$ scales allometrically to the power of 0.75, with an additional increase in clearance depending on

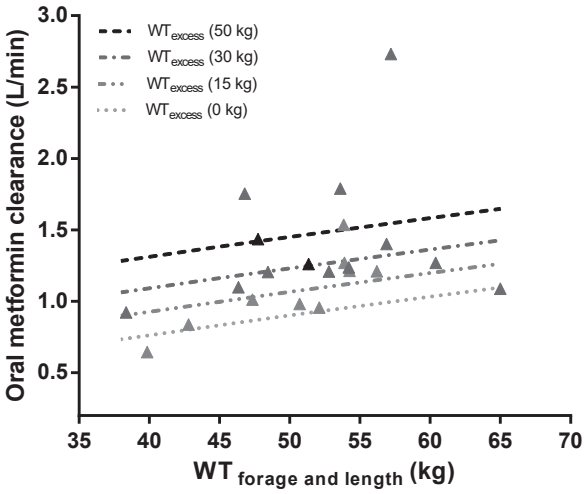


Figure 4 Oral metformin clearance (population prediction (line) and Empirical Bayes estimates (triangles)) from 22 overweight and obese adolescents vs. $WT_{for\ age\ and\ length}$ for different WT_{excess} levels (i.e. 15, 30 and 50 kg, (dark) grey/black lines). Population values for oral clearance are composed of the clearance of non-obese adolescents ($WT_{excess\ 0\ kg}$, light grey dotted line) plus an increase related to WT_{excess} according Equations 5 and 6. Observed individual values of obese adolescents are represented by triangles with grey colours varying according to the degree in WT_{excess}.

WT_{excess} (varying between 0 and 50 kg). Both the excess weight covariate model (Figure 4 and Table 2) and the final covariate model (Table 2) performed similarly in describing the data in terms of OFV (-140.8 vs -141.0, $P > 0.05$) and goodness of fit plots.

DISCUSSION

This study aimed to determine the pharmacokinetics of metformin in overweight and obese adolescents in view of the increased use of metformin for obesity in children in which often adult dosages are used. This study shows that the AUC decreases and oral clearance of metformin (CL/F) increases significantly with total body weight (TBW) in obese adolescents (Figure 1). The increase in oral clearance can be explained by both $WT_{for\ age\ and\ length}$ and WT_{excess} (Figure 3a and b) 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 a separate function for WT_{excess} was estimated. This excess covariate weight model was applied because the interrelation between growth, age and obesity in obese children and adolescents complicates a systematic covariate analysis [31, 28].

Table 3 Pharmacokinetic parameters of oral metformin in overweight and obese adolescents from this study compared to literature values from different patient populations.

	Study design	**TBW (kg)**	**Age (y)**	**AUC [a] (mg*min/L)**	**CL/F (L/min)**	**V/F (L)**	**Ka (min^{-1})**
Obese adolescents from this study	Fasted	79.3±13.9	14.5±1.8	$AUC_{0\text{-}inf}$ 460.5±223 $AUC_{0\text{-}8}$ 316.5±101.6	1.17[b]	485[b]	0.0248[b]
Non-obese children [11]	Dinner	33.3±2.7	9.5±0.1	$AUC_{0\text{-}12}$ 816.2±57.8	0.55±0.55[c]	185±11.7[d]	0.0175 ±0.0045
Obese T2DM adolescents [12]	Breakfast	98±25	(12-16)	$AUC_{0\text{-}inf}$ 378.7±1.6	-	-	-
Healthy adults [12]	Breakfast	91±21	(20-45)	$AUC_{0\text{-}inf}$ 398±1.7	-	-	-
Healthy adults and T2DM adult patients [32]	-	71±14 89±16	27±6 56±5	-	1.27±0.27 1.32±0.11	559±163 648±13.8	-
Obese adults [33]	Fasted	114.6±26.1	43.5± 11.7	$AUC_{0\text{-}inf}$ 342±108	1.46[e]	114.6±45.8[f]	-

Values are expressed as mean ± standard deviation (range) unless specified otherwise
[a] dose corrected (500 mg) AUC
[b] population mean
[c] reported CL of 0.33 ± 0.033 with fixed F of 60%
[d] reported V of 111 ± 7 with fixed F of 60%
[e] calculated by Cl/F= dose/AUC (1000 mg / 684 mg*min/L)
[f] reported weight normalized V/F of 1.0 ± 0.4 L/kg

When the current pharmacokinetic parameters of metformin in overweight and obese adolescents are compared with literature values of non-obese children and (obese) adults (Table 3), the dose-corrected AUC value we report here in overweight and obese adolescents proved about two times lower than in non-obese children [11]. However, this AUC is around the same value compared to obese T2DM adolescents [12], healthy adults [12], and similar or higher than obese adults [33] (Table 3). From these results its seems that obese adolescents have a higher clearance than their non-obese counterparts [11] (Table 3), while within the obese adolescent population oral clearance increased even further in more severely obese individuals (Figure 1). The oral clearance value in obese adolescents seems comparable to the value in non-obese adults [32] and similar or lower in obese adults [32,33] (Table 3).

The higher oral clearance in overweight and obese adolescents vs. non-obese children may have different explanations. First, a higher tubular secretion of metformin by induced OCT2 and MATE1/MATE2K transporters in the kidney [13,14] can be postulated in obese patients. This hypothesis is supported by our excess weight model which shows within the population of obese adolescents an increase in oral clearance with WT_{exess} (Figure 3b). Moreover, studies with intravenous procainamide, ciprofloxacin and cisplatin, which are all primarily eliminated by tubular secretion, show also a higher clearance in obese adults compared with non-obese adults [34]. These drugs are also renally transported by the OCT system, i.e. procainamide by OCT1-3 [35], ciprofloxacin by OCT and OAT (organic anion transporter) [35], and cisplatin by OCT2 [36]. In contrast, obese rodent models provide no basis for this hypothesis. High fat diet induced mice do show a trend in increased OCT2 renal mRNA expression, but no difference in OCT1 expression [37]. Ob/Ob and db/db mice show also no difference in renal mRNA expression of the OCT1 transporter and even a decrease of the renal OCT2 and MATE1 transporter [38-40]. These ob/ob and db/db mice models may, however, not be representative for obese patients, since these mice have no production of leptin or no functional leptin receptor and leptin may act on renal cells to regulate gene expression [37]. It is difficult to determine the net result of the various effects of obesity on the regulation of transporter expression in these rodent models [41], as pro-inflammatory cytokines, insulin, leptin and cholesterol all have opposing effects on mRNA expression of the transporters [41]. In addition, renal transporters (OCT1-2 and MATE1/MATE2K) should be considered as a whole as they work together in the excretion of metformin and one transporter can compensate the other [38]. Hence, our suggested increase in active tubular secretion by increased activity of the renal transporters in obese adolescents warrants further study. Another explanation for the higher oral clearance (CL/F) in obese adolescents could be a decreased bioavailability (F) in obese adolescents as a result of a decreased expression or activity of the intestinal transporters of metformin (i.e. OCT1, PMAT, OCT3). There is however no literature available about changes in intestinal transporter expression or activity with

obesity, so this hypothesis remains rather speculative. Lastly, the higher oral clearance in obese adolescents vs. non-obese children may also to some extent be explained by difference in age (11-17.5 years vs. 9 years, respectively). However, as this difference in age is small, this study suggests in our opinion that the increase in oral clearance (CL/F) of metformin in obese adolescents may be explained by an increase in clearance or a decrease in oral bioavailability resulting from an increase or decrease in the activity in renal or intestinal transporters, respectively. Since the value for oral clearance we report in obese adolescents is higher compared to non-obese children [11] and comparable to the value in healthy adults and T2DM adult patients [32], the maximum recommended dose of 2 g of metformin for children may be considered to be increased to the maximum dose in adults (i.e. 3 g per day) in case of (lack of) clinical response.

In this study, we could not identify an influence of genetic polymorphisms in the *OCT1* and *MATE1* transporter on the pharmacokinetics of metformin. Probably, a larger number of patients is needed to accurately test this influence. Stocker et al. was not able to identify an influence of the *MATE1* promoter variant on the pharmacokinetics of metformin [42], whereas two other studies did find an influence of genetic variation in the *OCT1* transporter on the pharmacokinetic parameters of metformin [43,44]. Stocker et al. concluded that MATE1 and MATE2 transporters work together in the renal elimination of metformin and that genetic variants in *MATE* and *OCT* transporters should be considered together when determining the genetic determinants of renal elimination of metformin [42]. For future studies, it would be interesting to evaluate the genetic variants of the OCT1-3 and MATE1-2 together. In addition, it is of special interest, to evaluate the genetic variants of the intestinal transporters of metformin (i.e. OCT1, OCT3 and PMAT) on the bioavailability [43] and oral absorption of metformin , since the oral absorption of metformin showed a large interindividual variability in our data.

There are some limitations to this study. First, no non-obese adolescents were included, because this pharmacokinetic study was part of a randomized double blind controlled trial on metformin in obese adolescents with insulin resistance [15], precluding a head to head comparison with the obese adolescents. Second, our patients received metformin in a fasted state because, for ethical reasons, the PK study was performed during an OGTT for which a venous line was inserted for clinical purposes. In clinical practice patients take metformin with food and as such our absorption rate constant may differ from other studies. Oral absorption varied largely between the obese adolescents. Nine adolescents had a very fast absorption with a maximum concentration at 60 minutes, while in non-obese children the mean T_{max} was reported to be 2.4 hours [11]. This difference may be explained by the fasted state of the obese adolescents or by the state of obesity.

In conclusion, we have shown that the oral clearance of metformin (CL/F) increases significantly with total body weight (TBW) in overweight and obese adolescents. The

increase in oral clearance is explained by both $WT_{for\ age\ and\ length}$ and WT_{excess} for which an excess weight model was proposed in which $WT_{for\ age\ and\ length}$ scaled allometrically to the power of 0.75 and a separate function for WT_{excess} was estimated. Compared to the literature, our oral clearance value in obese adolescents is increased compared to non-obese children, comparable to non-obese adults and similar or lower to the oral clearance value in obese adults. Future studies should focus on the protein expression and activity of renal transporters in obese patients vs. non-obese patients.

ACKNOWLEDGEMENTS

We thank Remko Harms for measuring the serum samples, Pyry Välitalo for supporting the VPC analysis and Willem van den Brink for his support calculating the AUC's with R software. We thank Marieke Elst for her help conducting the clinical trial.

REFERENCES

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 2014;311:806-14.
2. 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.
3. Cali AM, Caprio S. Obesity in children and adolescents. J Clin Endocrinol Metab 2008;93:S31-6.
4. Levy-Marchal C, Arslanian S, Cutfield W, et al. Insulin resistance in children: consensus, perspective, and future directions. J Clin Endocrinol Metab 2010;95:5189-98.
5. 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.
6. Hsia Y, Dawoud D, Sutcliffe AG, Viner RM, Kinra S, Wong IC. Unlicensed use of metformin in children and adolescents in the UK. Br J Clin Pharmacol 2012;73:135-9.
7. Hsia Y, Neubert AC, Rani F, Viner RM, Hindmarsh PC, Wong IC. An increase in the prevalence of type 1 and 2 diabetes in children and adolescents: results from prescription data from a UK general practice database. Br J Clin Pharmacol 2009;67:242-9.
8. Kostev K, Richter H. Unlicensed use of metformin in children and adolescents in Germany and France. Br J Clin Pharmacol 2012;73:307-8.
9. Liberman JN, Berger JE, Lewis M. Prevalence of antihypertensive, antidiabetic, and dyslipidemic prescription medication use among children and adolescents. Arch Pediatr Adolesc Med 2009;163:357-64.
10. Neubert A, Hsia Y, de Jong-van den Berg LT, et al. Comparison of anti-diabetic drug prescribing in children and adolescents in seven European countries. Br J Clin Pharmacol 2011;72:969-77.
11. 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.
12. Gao X, Christensen M, Burghen GA, et al. Pharmacokinetics of metformin in pediatric type2 diabetic and healthy adult subjects. Clin Pharmacol Ther 2003;abstract PII-65:46.
13. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820-7.
14. Gorboulev V, Ulzheimer JC, Akhoundova A, et al. Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 1997;16:871-81.
15. 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.
16. R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statatistical Computing, 2008.
17. Beal S, Sheiner LB, Boeckmann A, Bauer RJ. NONMEM User's Guides. (1989-2009). Ellicot City, MD, USA: Icon Development Solutions. 2009.
18. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for NONMEM: Tutorial on Pirana, PsN, and Xpose. CPT Pharmacometrics Syst Pharmacol 2013;2:e50.
19. Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn 2001;28:481-504.
20. Byon W, Fletcher CV, Brundage RC. Impact of censoring data below an arbitrary quantification limit on structural model misspecification. J Pharmacokinet Pharmacodyn 2008;35:101-16.

21. Savic RM, Jonker DM, Kerbusch T, Karlsson MO. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J Pharmacokinet Pharmacodyn 2007;34:711-26.
22. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. Aaps J 2011;13:143-51.
23. Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet 2005;44:1051-65.
24. Foster BJ, Platt RW, Zemel BS. Development and validation of a predictive equation for lean body mass in children and adolescents. Ann Hum Biol 2012;39:171-82.
25. Peters AM, Snelling HL, Glass DM, Bird NJ. Estimation of lean body mass in children. Br J Anaesth 2011;106:719-23.
26. Bedside Schwartz and Counahan-Barratt Methods 2012. Fadem SZ, Rosenthal B. (Accessed August, 31, 2015, at http://nephron.com/bedside_peds_nic.cgi).
27. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. Pharmacogenet Genomics 2010;20:38-44.
28. 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.
29. De TNO groeicalculator voor professionals. TNO (Accessed November 14, 2015, at https://groei-web.pgdata.nl/calculator.asp).
30. 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.
31. 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.
32. Graham GG, Punt J, Arora M, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50:81-98.
33. 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.
34. 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.
35. Bauer LA, Black DJ, Lill JS, Garrison J, Raisys VA, Hooton TM. Levofloxacin and ciprofloxacin decrease procainamide and N-acetylprocainamide renal clearances. Antimicrob Agents Chemother 2005;49:1649-51.
36. Ciarimboli G, Lancaster CS, Schlatter E, et al. Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. Clin Cancer Res 2012;18:1101-8.
37. More VR, Slitt AL. Alteration of hepatic but not renal transporter expression in diet-induced obese mice. Drug Metab Dispos 2011;39:992-9.
38. Clarke JD, Dzierlenga AL, Nelson NR, et al. Mechanism of Altered Metformin Distribution in Nonalcoholic Steatohepatitis. Diabetes 2015;64:3305-13.
39. Canet MJ, Hardwick RN, Lake AD, et al. Renal xenobiotic transporter expression is altered in multiple experimental models of nonalcoholic steatohepatitis. Drug Metab Dispos 2015;43:266-72.
40. More VR, Wen X, Thomas PE, Aleksunes LM, Slitt AL. Severe diabetes and leptin resistance cause differential hepatic and renal transporter expression in mice. Comp Hepatol 2012;11:1,5926-11-1.

41. Jang EH, Kim HK, Park CS, Kang JH. Increased expression of hepatic organic cation transporter 1 and hepatic distribution of metformin in high-fat diet-induced obese mice. Drug Metab Pharmacokinet 2010;25:392-7.
42. Stocker SL, Morrissey KM, Yee SW, et al. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacol Ther 2013;93:186-94.
43. Shu Y, Brown C, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther 2008;83:273-80.
44. Tzvetkov MV, Vormfelde SV, Balen D, et al. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther 2009;86:299-306.

SUPPLEMENTARY MATERIAL

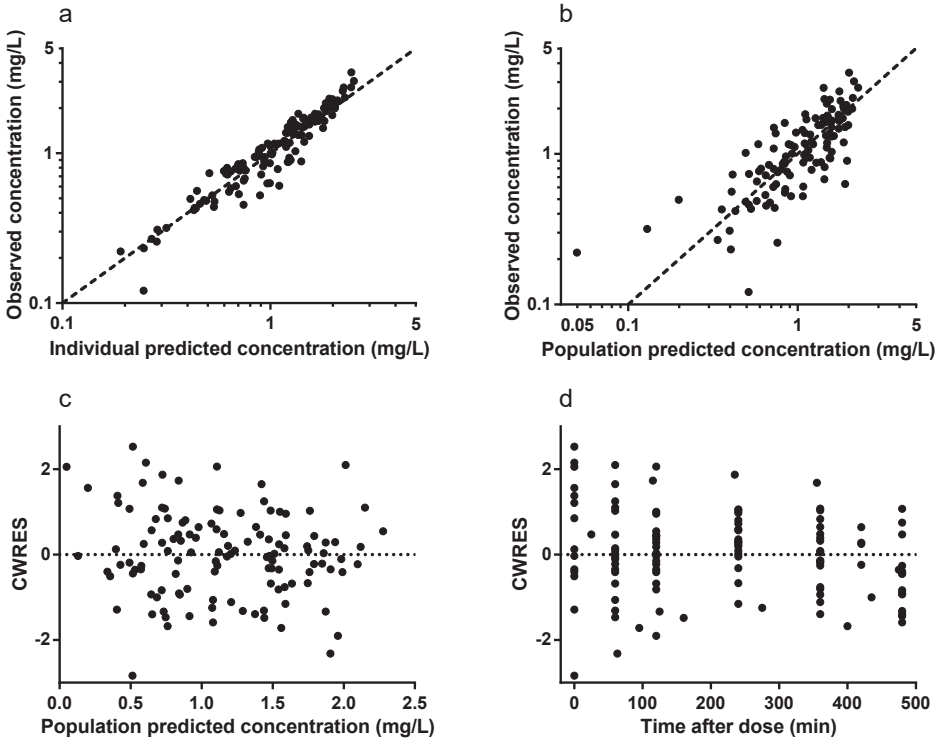
Supplementary Table 1 Genotype results of the *OCT1* and *MATE1* transporter.

Gene	Genetic marker	Allele change	WT (n)	HT (n)	HM (n)	MAF study (%)	MAF lit. [a] (%)	HW [b] (P)
SLC22A1 (OCT1)	rs72552763	GAT>del	16	6	0	14	15	0.46
	rs12208357	181C>T	17	5	0	11	7	0.55
	rs34130495	1201G>A	19	3	0	7	2	0.73
	rs34059508	1393G>A GAT>del	22	0	0	0	1	NA
	rs622342	A>C	7	10	5	45	41	0.70
SLC47A1 (MATE1)	rs2289669	G>A	9	10	3	36	46	0.93
	rs10735	G>A	14	8	0	18	16	0.30

HT= heterozygous, HM= homozygous, MAF= minor allele frequencies, MATE1= multidrug and toxin extrusion transporter 1, OCT1= organic cation transporter 1, SLC= solute carrier family, WT= wild type

[a] MAF literature European

[b] Hardy-Weinberg equilibrium



Supplementary Figure 1 (a) Observed vs. individual predicted concentration, (b) observed vs. predicted concentrations of metformin, (c) conditional weighted residuals (CWRES) vs. population predicted concentrations of metformin and (d) CWRES vs. time, of the final model for 22 overweight and obese adolescents.