# Amisulpride: Real-World Evidence of Dose Adaptation and Effect on Prolactin concentrations and Body Weight Gain by Pharmacokinetics-Pharmacodynamics Analyses

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## INTRODUCTION

Second-generation antipsychotics are the cornerstone of pharmacological treatment for schizophrenia. Among them, amisulpride has been shown to be an effective treatment with a moderate and low propensity for extrapyramidal symptoms and weight gain, respectively [Mauri_2018, Muller_2007, Leucht_2013]. In adults and older individuals, a concentration-therapeutic response relationship has been demonstrated leading to the determination of a reference range of amisulpride trough concentrations of 100-320 mg/L[Muller_2007]. Although some patients may need amisulpride plasma concentrations above the recommended therapeutic range, high plasma levels are associated with increased risks of extra-pyramidal symptoms. Because of all abovementioned reasons, therapeutic drug monitoring of amisulpride is strongly recommended [Hiemke_2017].

Amisulpride has a high propensity to raise prolactin [Peuskens_2014]. Indeed, by D2 receptor antagonism amisulpride blocks the dopamine transmission on the lactotroph cells in the anterior pituitary gland which lies outside the blood-brain barrier [Iwata_2016]. Due to the low capacity of the drug to penetrate the blood-brain barrier, amisulpride has a low ratio of central/peripheral concentration which yield to high dopamine blockade in the pituitary gland and decrease the inhibitory effect of dopamine on prolactin secretion [Besnard_2014, Peuskens_2014, Juruena_2010]. More than 90% of patients treated by amisulpride have hyperprolactinemia, [Holt_2011, During_2019] which remains asymptomatic in some cases but may also lead to troublesome fast onset adverse events on gonadal function such as amenorrhea, galactorrhea, infertility, loss of libido, erectile dysfunction and ejaculation deficiency [Iwata_2016]. These clinical manifestations can hamper the adherence of patient to the treatment. To date, a population pharmacokinetic model has been described and a pharmacokinetic-pharmacodynamic analysis combining this model with prolactin data has been published in elderly patients with Alzheimer's disease only [Reeves_2016, Reeves_2017]. Such information is not yet available in adult psychiatric population.

While body weight gain is the major adverse effect of second-generation antipsychotics, amisulpride has a low propensity to raise body weight [Leucht_2004, Leucht_2013]. However, description of the relationship between amisulpride concentration and body weight gain is useful as body weight gain can still be observed during treatment with amisulpride.

The objectives of this work were first to characterize the pharmacokinetic profile of amisulpride and to detect sources of variability in order to suggest optimal dosing for reaching the therapeutic reference range of concentrations in each patient. Secondly, we aimed to describe the relationship between amisulpride concentrations and prolactin levels and body weight data in an adult and elderly psychiatric population of a real-world clinical setting.

## MATERIALS AND METHODS

### Study population and design

The present study included 242 inpatients and outpatients from the Department of Psychiatry of the Lausanne University Hospital who had at least one amisulpride plasma concentration measurement performed between 2007 and 2016. Antipsychotic plasma concentration measurement are requested for therapeutic drug monitoring (TDM) by the treating psychiatrist for clinical purpose or by the department guideline for metabolic follow-up of patients starting a psychotropic treatment on a routine basis (PsyClin) [Choong_2008]. In the latter case, written informed consent from an ongoing longitudinal clinical and pharmacogenetic study (PsyMetab) as previously described was obtained from patients [Choong_2013]. PsyMetab study and retrospective analysis of PsyClin data were both approved by the Ethics Committee of Vaud (CER-VD). Exclusion criteria were undetectable amisulpride plasma concentrations suggestive of non-adherence to treatment, and non-reliable time information about blood sampling or last dose intake. When the blood sampling was not under steady-state conditions (at least of 5 days with the same dose), the dosing information history (dose, date and time of administration) were added in the dataset.

In addition to the accurate time of last drug intake and blood sampling, the following data were recorded at the same time as the blood samples were drawn for pharmacokinetic (PK) measurements: sex, age, body weight (BW), height, serum creatinine concentration and concomitant medications with permeability-glycoprotein inhibitors (iPGP) and lithium that might have influenced amisulpride therapy (Table 1). Lean body weight (LBW) was calculated as described elsewhere [Janmahasatian_2005] as well as body mass index (BMI). Creatinine clearance was estimated by the Cockcroft-Gault formula (CLCRCG) [Cockcroft_1976] if BMI< 25 kg/m$^2$ and the Cockcroft-Gault formula integrating the LBW if BMI≥ 25 kg/m$^2$ [Pai_2010]. For the PK-prolactin model purpose, the following data were also reported: menopause if women were aged from 55 years on and concomitant antipsychotics that might have increased prolactin levels such as zuclopenthixol, risperidone, paliperidone, haloperidol and levomepromazine.

### Amisulpride and prolactin concentration measurements

All blood samples were collected in EDTA-containing tubes. After centrifugation, plasma samples were stored at -20 °C until routine analysis. Quantification of amisulpride in plasma was performed by high performance liquid chromatography coupled to mass spectrometry until 2012 (detailed method

available on request), and then by ultra-high performance liquid chromatography coupled to tandem mass spectrometry [Ansermot_2013]. The lower limit of quantification was 1 ng/ml for both methods. Prolactin concentrations were determined by immunoassay on an Abbott Axsym system (Abbott GmbH, Wiesbaden, Germany) before 2014 and by electrochemiluminescence on a Cobas (Roche Diagnostics GmbH, Mannhein, Germany) from 2014. The prolactin concentration equivalence was obtained using the following equation based on internal validation processes: y = 1.05x – 0.56 (y = Abbott AxSym, x = Cobas Roche ; 0 à 268 ng/ml)

**Genotyping**

Genomic DNA was extracted from EDTA blood sample at the baseline visit using the FlexiGene DNA extraction kit (Qiagen Instruments AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol. The SNPs analysed in this work were obtained using the Cardio-MetaboChip; a custom Illumina iSelect genotyping array designed to test DNA variation of over 200,000 SNPs from regions identified by large scale meta-analyses of genomewide association studies for metabolic and cardiovascular traits: 3642 customized SNPs covering pharmacokinetic genes were added in the Cardio-MetaboChip [Lubomirov_2007, Lubomirov_2010]. The following SNPs of genes coding for the OCT1 and OCT2 transporters, the P-gp and some nuclear factors were selected based on previously published pharmacogenetic studies and on their minor allele frequency (MAF) in the Caucasian population [Kim_2009, Koren-Michowitz_2014, Bergen_2014, Christensen_2013, Knops_2015, Hodges_2011, Dobrinas_2013, Schipani_2010, Choong_JClinPsychopharmaco_2013, Oliver_2010, ElSharawy_2006, Oh_2009, Shi_2009]: *SLC22A1* (rs683369), *SLC22A1* (rs628031) , *SLC22A2* (rs316003), *SLC22A2* (rs316019), *ABCB1* (rs2235048), *ABCB1* (rs4148738), *NR1*|*2* (rs1523130), *NR1*|*2* (rs7643645), *NR1*|*2* (rs2461817), *NR1*|*3* (rs2307424), *NR1*|*3* (rs4073054), *NR1*|*3* (rs2502815), *RXRA* (rs3132297), *PPARG* (rs3856806), *PPARG* (rs2197423), *PPARG* (rs2920502), *PPARGC1A* (rs8192678). Quality control excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96 or Gene Call score less than 0.15. All these SNPs were in Hardy Weinberg Equilibrium (p>0.05). GenomeStudio Data Analysis Software was used to export results generated by Illumina Cardio-MetaboChip.

**Pharmacokinetic-pharmacodynamic analysis**

Non-linear mixed effect modelling was performed using NONMEM® version 7.4.1 program [Beal_NMusersguide_2009] with the PsN-Toolkit (version 4.2.0) [Lindbom_2005]. Statistical analyses and figures were performed using R (v. 3.3.3, http://www.r-project.org).

*Pharmacokinetic base model*

Log-transformed concentrations were used in the model. A stepwise procedure was used to identify models that best fitted the data. Multi-compartment models with first or zero-order absorption and linear elimination were first fitted to the data to determine the appropriate structural model, which was finally identified as a one-compartment model with first order absorption. Owing to very limited measurements at early time points after drug intake, the absorption parameter $k_a$ could not be estimated to obtain a reasonable value of Tmax. Thus, $k_a$ values ranging from 0.4 to 1.8 $h^{-1}$ were tested in the model. The final value was chosen to obtain a Tmax between 3-4 h corresponding to the second peak of absorption and compared to the $k_a$ value previously published for elderly patients [Reeves_2016, Reeves_2017].

Since amisulpride was administered orally, clearance (CL) and volume of distribution (V) were estimated with F, the absolute oral bioavailability fixed to 0.48 [Bergemann_2004]. Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the parameters. Proportional, additive and combined proportional-additive error models were finally compared to describe the residual variability.

*PK-Prolactin model*

The final pharmacokinetic model was combined with a direct $E_{max}$ model [Reeves_2017] to describe the prolactin data as follows:

$$PRL = PRL_{base} + (E_{max} \times CP)/(EC_{50} + CP) \quad (1)$$

where PRL is the prolactin level; $PRL_{base}$, the prolactin level measured at baseline; CP, the amisulpride concentration predicted by the PK model; $EC_{50}$, the amisulpride concentration at which the prolactin level reaches 50% of the maximal achievable value ($E_{max}$).

As prolactin level are markedly different in men and women [Peuskens_2014], the gender effect on Emax was included since the structural model development as follows:

$$E_{max} = a \times (1 - b \times MALE) \quad (2)$$

where MALE=1 if male patients and 0 if female patients.

Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the three parameters ($PRL_{base}$, $E_{max}$ and $EC_{50}$). Individual final PK/PD parameter estimates were used to calculate prolactin and amisulpride concentrations at time of amisulpride trough concentrations, i.e 12h and 24h after last dose intake for two or one administration per day, respectively. This allowed comparing the prolactin levels variation across the recommended reference range of amisulpride trough concentrations [Hiemke_2017].

*Exposure-Body Weight model*

Average amisulpride concentration over 24 hours ($C_{av}$) were derived from the final PK model using:

$$C_{av} = AUC_{0\text{-}24}/24 \quad (3)$$

where $AUC_{0\text{-}24}$ were computed by integration in NONMEM® based on the individual dose history and pharmacokinetic parameters. Linear mixed-effects models were fitted on the longitudinal body weight values to estimate the effect of time under treatment and amisulpride $C_{av}$ by using the nlme package in R.

*Covariate analysis*

Empirical Bayesian estimates (EBEs) of the pharmacokinetic parameters were derived and plotted against the available subject characteristics (age, sex, BW, LBW, height, BMI, CLCRCG, iPGP and several genetic polymorphisms (see Table 1)). Due to substantial eta-shrinkage on V (68%) in the base model, the graphically exploration was interpreted cautiously between EBEs of V and covariates [Savic_2009]. For the PK-prolactin model, EBEs of $E_{max}$ parameter were derived and plotted against relevant factors (age, menopause, BW, LBW, season and concomitant antipsychotics that might have the highest potential to increase prolactin level such as zuclopenthixol, risperidone, paliperidone, haloperidol, levomepromazine [Peuskens_2014]). Potentially influencing covariates were then incorporated sequentially in the model and tested for significance on the parameters. The covariate analysis was performed using a stepwise insertion/deletion approach testing linear or non-linear functions as appropriate (categorical covariates coded as 0 and 1, continuous covariates centered on their median value). Missing values for BW, LBW, BMI and CLCRCG were imputed to the population median value. Parameter values were estimated for each genotypic group (rich model), defined as the reference allele group (Ref) and alternative allele groups: heterozygote alternative (Het-Alt) and homozygous alternative (Hom-Alt) or for further regrouped (reduced model) subpopulations.

*Parameter estimation and model selection*

All models were fitted using the first-order conditional estimation method with interaction (FOCEI) with the subroutines ADVAN2 TRANS2 for the pharmacokinetic model and the subroutine ADVAN6 for the PK-prolactin model. The log likelihood ratio test, based on changes in the objective function value (ΔOFV), was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a $\chi^2$ distribution, a 3.8- (p=0.05) point change of OFV was considered statistically significant for one additional parameter in model building. To account for multiple testing in the covariate model the p-value was corrected by the number of tests. A change in OFV > 6.6 (p= 0.05/4= 0.0125) was considered statistically significant for one additional parameter during backward deletion steps in the pharmacokinetic model. The Akaike's information criterion (AIC) was instead employed to choose between non-hierarchical models. Diagnostic goodness-of-fit plots, precision and plausibility of the model parameters were also used to assess the reliability of the results.

*Model evaluation*

The final PK and PK-prolactin models stability was assessed by non-parametric bootstrap method implemented in PsN, generating 2000 datasets by re-sampling from the original dataset. Median parameters values with their 95%CI were thus derived and compared with the final model estimates. Visual predictive checks (VPCs) of final PK and PK-prolactin models were performed with PsN toolkit by simulations (n=1000).

*Simulations of dosage regimens*

Trough concentrations were simulated in 500 individuals per strata of age and LBW values with the final PK model including inter- and intra-individual variability after administration of several doses recommended in guidelines: 300 mg q.d, 200 mg b.i.d and 400 mg b.i.d in adults (4 combinations of age (20 and 60 years) and LBW (40 and 85 kg)); 50 mg q.d, 200 mg q.d. and 200 mg b.i.d. [Taylor_The Maudsley_2018] in elderly individuals (4 combinations of age (75 and 85 years) and LBW (30 and 60 kg)). The distribution of the through concentrations ($Cmin_{SS}$) per strata were plotted with the recommended reference range of trough concentrations (100-320 ng/mL) [Hiemke_2017], [Muller_2007].

## RESULTS

### Study population and data

The 242 patients provided a total of 513 concentrations for the pharmacokinetic analysis. Blood samples for pharmacokinetic measurements were collected at a median time of 13.3 h (range, 0.05h-58h) after last dose intake. A median of two samples (range, 1–12) of amisulpride was collected per patient. Amisulpride plasma concentrations ranged from 5 to 1514 ng/mL across a range of daily doses from 50 to 2000 mg (median = 600 mg). Subject's characteristics are presented in Table 1. For the PK-prolactin model, a total of 101 prolactin plasma concentrations from 68 patients were available (median = 73 ng/mL, range = 4 - 311 ng/mL) and plotted against the observed amisulpride concentrations in Figure 1. Nineteen prolactin concentrations were available before the beginning of amisulpride treatment (from - 40 days to the starting treatment day). Among the 82 prolactin measurements under treatment, only 3 were under the threshold for hyperprolactinemia set over 20 and 25 ng/mL for males and females, respectively [Citrome_2008]. For the exposure-body weight analysis, 284 body weight values (median = 73 kg, range = 45 -114 kg) from 113 patients were available for analysis. Data until 101 days of treatment were included in analysis. Ninety patients had a baseline body weight value measured from 15 days before treatment beginning.

### Pharmacokinetic-pharmacodynamic analysis

*Pharmacokinetic analysis*

A one-compartment model with first-order absorption and elimination described adequately the data. No improvement to the fit was observed using a two-compartment model (ΔOFV = -2.8). Parameter $k_a$ was fixed to 0.9 $h^{-1}$ corresponding to a calculated Tmax of 3.6 h. Finally, residual variability was described by an additive error model on the log scale. The estimates and the variability (CV%) of the base pharmacokinetic model were a CL of 39.6 L/h (47%) and a V of 954 L (53%).

Univariate analyses showed that the effect of age (ΔOFV = -71.7, $p < 0.001$), CLCRCG (ΔOFV = -50.3, $p < 0.001$), BW (ΔOFV = -19.1, $p < 0.001$), LBW (ΔOFV = -34.0, $p < 0.001$) and sex (ΔOFV = -21.6, $p < 0.001$) on CL improved significantly the description of the data. According to AIC, the LBW effect was chosen upon BW effect (AIC = 133.2 and AIC = 148.0, respectively) on CL. In contrast, BMI, iPGP and lithium was not associated to CL (ΔOFV = -2.2 for 1 additional parameter, ΔOFV ≥ -5.4 for 2 additional parameters, $p > 0.07$), neither the genetic covariates (ΔOFV = -1.6 for 2 additional parameters, ΔOFV ≥

-5.7 for 3 additional parameters, $p > 0.4$). No covariates tested showed any significant influence on V ($\Delta OFV = -3.36$, $p = 0.07$).

In multivariate analyses, age, LBW and CLCRCG remained as significant covariates in the forward insertion step ($\Delta OFV \leq -5.8$, $p \leq 0.01$). CLCRCG did not remain statistically significant after the backward deletion ($\Delta OFV = 2.7$, $p = 0.1$). Our final results suggest that CL is increased by 1.5 in an individual of 40 y with a LBW = 100 kg *vs.* 50 kg and decreased by 0.5 in a 80-year compared to a 40-year 50 kg individual. Age and LBW explained 42% and 22% of the variability in amisulpride clearance, respectively.

Structural, final pharmacokinetic model and bootstrap results are summarized in Supplementary Table 1. The model was considered reliable since the parameter estimates differed less than 10% from the bootstrap medians. The prediction-corrected VPC indicates that the final model described the data adequately (Supplementary Figure 1).

In adults, simulations of doses of 300 mg q.d., 200 mg b.i.d. and 400 mg b.i.d. indicated that 64%, 31% and 9% of $Cmin_{SS}$ were under the therapeutic reference range, 35%, 62% and 50% were in the reference range and 1%, 7% and 41% were over the reference range, respectively. Especially, 65% were over the reference range if age was 60 years or more with the maximum dose (see Figure 2.a.). Concerning elderly individuals, simulations of doses of 50 mg q.d., 200 mg q.d. and 200 mg b.i.d. showed that 97%, 16% and 1% were under the reference range, 3%, 74% and 33% were in the reference range and 0%, 10% and 66% were over the reference range. Especially, 82% were over the reference range if LBW was 30 kg at 200 mg b.i.d. (see Figure 2.b.).

*PK-Prolactin model*

Univariate analyses showed that none of the factors had a significant effect on $E_{max}$ parameter ($p>0.06$), besides the already included sex effect. Final PK-prolactin model parameters and the bootstrap results are presented in Table 2. All parameters were estimated with good precision (RSE ≤ 36%). The model was considered reliable since the parameter estimates differed less than 10% from the bootstrap. The prediction-corrected VPC indicates that the final model described the data adequately (Supplementary Figure 2). $E_{max}$ parameter was estimated to decrease by 53% in males compared to females. Prolactin values predicted by the final PK-Prolactin model at time of amisulpride trough concentrations were plotted against amisulpride trough concentrations in Figure 3. This showed that when amisulpride trough concentrations are in the therapeutic reference range, prolactin

levels are over the normal values in males (model-predicted median= 71 ng/mL, range= 41-135 ng/mL), and in pre- (147 ng/mL, 78-309 ng/mL) and postmenopausal (128 ng/mL, 75-186 ng/mL) women.

*Exposure-Body Weight analysis*

Univariate models showed that time under treatment and $C_{av}$ had a significant effect on body weight measures. Body weight increased by 0.16 kg for 10 additional days of treatment (p=0.008) and by 0.2 kg for 100 additional ng/mL of amisulpride plasma concentrations (p=0.04). In multivariate analysis, none of these variables were significant ($p \geq 0.09$, see Table 3).

## DISCUSSION

A guide for optimizing amisulpride dose adjustments with regard to therapeutic response and adverse events based on pharmacokinetic-pharmacodynamics analysis has been previously proposed in a particular population of elderly with Alzheimer's disease but such a population approach was not used so far in adult and older psychotic patients. The present study provides a description of a pharmacokinetic profile of amisulpride which, combined with prolactin and body weight data, could be used for amisulpride treatment optimization.

The pharmacokinetic analysis showed that amisulpride concentrations were well described by a one-compartment model with parameters consistent with previously reported pharmacokinetic model parameters [Reeves_2016]. Amisulpride clearance was close to the reported value of 54.3 L/h. The volume of distribution estimated was concordant with the steady-state volume of distribution published for a two-compartment model (Vss = 1191 L) [Reeves_2016]. Since 95% of the drug concentrations available in our study population were collected less than 24 hours after dose administration, the characterization of the second compartment could not be done. Moreover, determination of the initial elimination phase is not always accurately determined in pharmacokinetic modelling due to the complexity in modelling the absorption phase with the two plasma concentration peaks [Rosenzweig_2002]. The $k_a$ value was fixed to 0.9 in order to obtain a calculated Tmax of 3.6 h. This Tmax value corresponds to the second peak of absorption which lies between 3 and 4 h [Bergemann_2004]. The first peak at 1 h can be estimated only in cases of rich pharmacokinetic data sampling. Age and body weight also significantly contributed to amisulpride clearance variability in the previously published pharmacokinetic model and creatinine clearance effect on amisulpride clearance was not present in the final model [Reeves_2016]. Amisulpride is mainly eliminated by renal clearance at about 330 mL/min, meaning that glomerular filtration and tubular secretion are involved [Rosenzweig_2002]. In this work, the pharmacokinetic model including only creatinine clearance effect on amisulpride clearance was less statistically significant than the model including the effect of age and lean body weight (AIC = 117 and 87, respectively) meaning that the contribution of age and lean body weight to the variability of amisulpride clearance was higher than the contribution of creatinine clearance alone. Creatinine clearance depends on age and body weight and age effect may also be a surrogate of the biliary elimination, the other major elimination pathway of amisulpride [Compendium]. Indeed, biliary elimination decreases with ageing. Moreover, the part of variability on amisulpride clearance explained by age was much higher than the one explained by lean body weight meaning that age may be involved in several elimination pathways of amisulpride. In the present study lean body weight had a more significant effect on clearance than body weight, supposedly because half of the patients were overweight or obese (median BMI = 25 kg/m$^2$). The physiologic explication

may be that renal elimination is correlated with lean body weight rather than body weight in overweight and obese patients because fat mass is not implicated in renal function. Of note, in a study including 85 patients, the dose-corrected plasma concentrations of amisulpride were higher in patients taking lithium [Bergemann_2004]. In our work, a trend (p=0.08) for a 6% decrease of amisulpride clearance was observed in the presence of lithium possibly by a competition of both compounds for renal elimination.

Simulations highlighted high variability in plasma concentrations of patients receiving the same dose. In elderly patients, the maximum recommended dose of 200 mg b.i.d. lead to concentrations over the reference range especially in oldest patients and should be cautiously used considering the potential occurrence of adverse effects such as hypotension and/or sedation [Compendium, Hiemke_2007]. In this frail population amisulpride should be prescribed with a slow titration from the 50 mg q.d. starting dose. In some cases, the therapeutic doses of 100 or 200 mg q.d. could results in plasma levels under the reference range of concentrations. In elderly patients the threshold of striatal dopamine D2/3 receptor occupancy to obtain a therapeutic effect has been showed to be lower when compared to younger patients (50 to 60% and 65-80%, respectively) [Graff-Guerrero_2015]. Thus lower doses are sufficient to reach therapeutic effect in elderly patients. In adults, the maximum dose of 800 mg/d recommended in Switzerland (up to 1200 mg/d in other countries [Taylor_TheMaudsley_2018]) can lead to plasma concentrations largely over the reference range especially when age increases and/or for low body weight patients. On the other hand, a dose of 300 mg q.d, which is considered as therapeutic [Taylor_TheMaudsley_2018], can be under the reference range especially in very young adults or overweight patients. In the study of Muller et al. the reference range of 100-320 ng/mL was determined based on the best predictive probabilities of avoiding non-response with minimal extra-pyramidal symptoms. But inherently to statistical methods and to the variability of clinical response some patients are non-responders with plasma concentrations in this range (9% in the study of Muller et al.) and need amisulpride concentrations > 320 ng/mL to obtain a therapeutic response [Muller_2007, Hiemke_2017]. Thus, therapeutic drug monitoring appears to be very useful especially when adverse effects are more likely (i.e in elderly individuals or in low body weight patients) and when clinical response is poor in order to discriminate a lack of adherence or a non-response with recommended doses.

The pharmacokinetic-prolactin model was in accordance with previously reported model in elderly individuals [Reeves_2017]. In males and in females, our Emax estimates were close to the reported values of 52 and 124 ng/mL, respectively. EC50 value (i.e. 42 ng/ml) was slightly higher than the reported value of 18 ng/mL but in the same order of magnitude than amisulpride therapeutic

concentrations (> 100 ng/mL). Gender effect was introduced from the beginning of the structural model development as this effect is very important on prolactin plasma levels, explaining 41% of the Emax inter-individual variability. Estrogen is involved in the regulation of prolactin secretion and females are very sensitive to prolactin increase due to the action of estrogens at central and peripheral levels. It has been recommended to monitor prolactin, based on gender but also on menopausal status, especially in premenopausal patients in whom antipsychotic-induced hyperprolactinemia can provoke early menopause [Lange_2017].We therefore also investigated menopause effect on Emax parameter in the model. As compared to premenopausal women, a decrease of Emax parameters was found, which was however non-significant in postmenopausal women and in males (21% and 58%, respectively, ΔOFV= -1.6, p= 0.2).

Prediction of prolactin levels at time of trough concentrations showed that an amisulpride exposure at therapeutic concentrations result in prolactin levels over the normal values (see Figure 3). This is in accordance with previous studies showing that amisulpride is a prolactin-raising antipsychotic at relatively low dose and that there is no dose-effect [Peuskens_2014, Besnard_2014]. This is also supported by studies on dopamine receptor occupancy in adults showing that therapeutic response for antipsychotics occurs at a range of striatal $D_2$ receptor occupancy of 65-80%, while hyperprolactinemia is observed from 73% of occupancy of the same receptors [Sparshatt_2009, Lako_2013, Tsuboi_2013]. Thus, the threshold for receptor occupancy leading to hyperprolactinemia lies in the same range of receptor occupancies for therapeutic effect. Hyperprolactinemia decreases gonadotropins secretion which provoke at short-term gonadal dysfunctions in males and females [Iwata_2016, Holt_2011]. Moreover, hyperprolactinemia over a long period of time could cause breast cancer particularly in postmenopausal women and loss of bone mineral density, which can lead to osteoporosis and an increase risk of falls in elderly patients [Besnard_2014, Iwata_2016]. Moreover, metabolic disturbances such as insulin resistance, weight gain and cardio-vascular diseases could also be induced via a lack of oestrogen during hyperprolactinemia [Iwata_2016, Lange_2017, Riecher-Rössler_2016].

This present study and previous studies [Holt_2011, During_2019] suggest that patients receiving amisulpride will have hyperprolactinemia. Thus even if patient do not report clinical symptoms, treating hyperprolactinemia will be beneficial for them in order to avoid such adverse events. Some recommendations have been already proposed and should be chosen given that amisulpride has been shown to be one of the most efficacious antipsychotic with a low potential to induce weight gain [Leucht_2013]. Switch to a prolactin sparing antipsychotic such as aripiprazole, olanzapine, quetiapine and clozapine if possible with regard to therapeutic benefit [Haddad_2004]. Adding aripiprazole is controversial. It has been shown that 10 mg/day seems to be sufficient to decrease significantly the

antipsychotic-induced hyperprolactinemia. However, mixed results are reported concerning efficacy and safety of aripiprazole in this context due to different study design but also because aripiprazole can provoke decompensation of the psychotic disease due to its partial agonist activity [Chen_2015, Grigg_2017]. In a case report, topiramate was shown to surprisingly decreased prolactin level and prolactin rebound was observed after discontinuation of topiramate treatment [Huang_2017]. Adding a dopaminergic agonist (such as bromocriptine and cabergoline) would have an inhibition action on prolactin secretion but may worsen psychotic symptoms and this is thus not recommended by recent guidelines [Haddad_2004, Grigg_2017]. Finally, it has been proposed to add oral contraceptive in premenopausal women to treat symptoms of oestrogen deficiency [Haddad_2004].

In the present study patients gained approximately 1.5 kg for a 3-month treatment period, and weight increase was not dependent on amisulpride concentrations. A low body weight gain as found in the present study is comparable to the value reported for a 100-day treatment period in a meta-analysis of randomized studies conducted by the manufacturer [Leucht_2004].

The present study has to be interpreted with some limitations. The sparse sampling in the pharmacokinetic analysis prevented to estimate inter-occasion variability in amisulpride clearance which may contribute to the relatively high residual variability (53%) in the final model. Oestrogen-based contraceptives increases prolactin levels but intake of contraceptives was not available in the medical records and was thus not taken into account in the pharmacokinetic-prolactin analysis. However, the consequences of such intake on the model are probably low considering a probable low adherence of psychiatric patients for contraceptives during acute psychotic episode. Furthermore, physiological pulsatile secretion of prolactin varies notably within waking hours, depending also on meals and menstrual cycle [Veldhuis_1988], [Rosenbloom_2010] and such fluctuations were not taken into account in the present work. However, to our knowledge it is not known if, and in such cases, to what extent, the physiological pulsatile secretion of prolactin is maintained during antipsychotic treatment due to the permanent reduction of inhibitory effect of dopamine on prolactin secretion.

In conclusion, the results of the present study supports therapeutic drug monitoring of amisulpride and dose adjustment based on age and body weight or lean body weight in overweight and obese patients. Hyperprolactinemia was not dependent on amisulpride concentrations and thus amisulpride dose reduction is not appropriate when aiming to reduce prolactin levels. Further studies are needed to evaluate the best solutions to avoid short and long-term adverse consequences of antipsychotic-induced hyperprolactinemia.

## References


Ansermot N, Brawand-Amey M, Kottelat A, Eap CB. Fast quantification of ten psychotropic drugs and metabolites in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring. J Chromatogr A. 2013;1292:160-72.

Beal S, Sheiner, L.B., Boeckmann, A., & Bauer, R.J. NONMEM User's Guides. (1989-2009). Icon Development Solutions, Ellicott City, MD, USA. 2009.

Bergemann N, Kopitz J, Kress KR, Frick A. Plasma amisulpride levels in schizophrenia or schizoaffective disorder. Eur Neuropsychopharmacol. 2004;14(3):245-50.

Bergen AW, Javitz HS, Krasnow R, Michel M, Nishita D, Conti DV, et al. Organic cation transporter variation and response to smoking cessation therapies. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco. 2014;16(12):1638-46.

Besnard I, Auclair V, Callery G, Gabriel-Bordenave C, Roberge C (2014) [Antipsychotic-drug-induced hyperprolactinemia: physiopathology, clinical features and guidance]. Encephale 40 (1):86-94. doi:10.1016/j.encep.2012.03.002

Chen JX, Su YA, Bian QT, Wei LH, Zhang RZ, Liu YH, et al. Adjunctive aripiprazole in the treatment of risperidone-induced hyperprolactinemia: A randomized, double-blind, placebo-controlled, dose-response study. Psychoneuroendocrinology. 2015;58:130-40.

Choong E, Solida A, Lechaire C, Conus P, Eap CB (2008) [Follow-up of the metabolic syndrome induced by atypical antipsychotics: recommendations and pharmacogenetics perspectives]. Rev Med Suisse 4 (171):1994-1996, 1998-1999

Choong E, Quteineh L, Cardinaux J-R, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, Bondolfi G, Etter M, Holzer L, Magistretti P, von Gunten A, Preisig M, Vollenweider P, Beckmann JS, Pralong FP, Waeber G, Kutalik Z, Conus P, Bochud M, Eap CB (2013) Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. JAMA psychiatry 70:1011-1019. doi:10.1001/jamapsychiatry.2013.187

Choong E, Polari A, Kamdem RH, Gervasoni N, Spisla C, Jaquenoud Sirot E, et al. Pharmacogenetic study on risperidone long-acting injection: influence of cytochrome P450 2D6 and pregnane X receptor on risperidone exposure and drug-induced side-effects. J Clin Psychopharmacol. 2013;33(3):289-98.

Christensen MM, Pedersen RS, Stage TB, Brasch-Andersen C, Nielsen F, Damkier P, et al. A gene-gene interaction between polymorphisms in the OCT2 and MATE1 genes influences the renal clearance of metformin. Pharmacogenet Genomics. 2013;23(10):526-34.

Citrome L. Current guidelines and their recommendations for prolactin monitoring in psychosis. Journal of psychopharmacology (Oxford, England). 2008;22(2 Suppl):90-7.

Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16 (1):31-41. doi:10.1159/000180580

Dobrinas M, Cornuz J, Eap CB. Pharmacogenetics of CYP1A2 activity and inducibility in smokers and exsmokers. Pharmacogenet Genomics. 2013;23(5):286-92.

ElSharawy A, Manaster C, Teuber M, Rosenstiel P, Kwiatkowski R, Huse K, et al. SNPSplicer: systematic analysis of SNP-dependent splicing in genotyped cDNAs. Hum Mutat. 2006;27(11):1129-34.

Graff-Guerrero A, Rajji TK, Mulsant BH, Nakajima S, Caravaggio F, Suzuki T, et al. Evaluation of Antipsychotic Dose Reduction in Late-Life Schizophrenia: A Prospective Dopamine D2/3 Receptor Occupancy Study. JAMA Psychiatry. 2015;72(9):927-34.

Grigg J, Worsley R, Thew C, Gurvich C, Thomas N, Kulkarni J. Antipsychotic-induced hyperprolactinemia: synthesis of world-wide guidelines and integrated recommendations for assessment, management and future research. Psychopharmacology (Berl). 2017;234(22):3279-97.

Haddad PM, Wieck A. Antipsychotic-induced hyperprolactinaemia: mechanisms, clinical features and management. Drugs. 2004;64(20):2291-314.

Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K, Eckermann G, Egberts K, Gerlach M, Greiner C, Grunder G, Haen E, Havemann-Reinecke U, Hefner G, Helmer R, Janssen G, Jaquenoud E, Laux G, Messer T, Mossner R, Muller MJ, Paulzen M, Pfuhlmann B, Riederer P, Saria A, Schoppek B, Schoretsanitis G, Schwarz M, Gracia MS, Stegmann B, Steimer W, Stingl JC, Uhr M, Ulrich S, Unterecker S, Waschgler R, Zernig G, Zurek G, Baumann P (2017) Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry. doi:10.1055/s-0043-116492

Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, Klein TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics. 2011;21(3):152-61.

Holt RI, Peveler RC (2011) Antipsychotics and hyperprolactinaemia: mechanisms, consequences and management. Clin Endocrinol (Oxf) 74 (2):141-147. doi:10.1111/j.1365-2265.2010.03814.x

Huang Y, Ma H, Wang Y, Peng M, Zhu G. Topiramate add-on treatment associated with normalization of prolactin levels in a patient with schizophrenia. Neuropsychiatr Dis Treat. 2017;13:1395-7.

Iwata Y, Nakajima S, Caravaggio F, Suzuki T, Uchida H, Plitman E, Chung JK, Mar W, Gerretsen P, Pollock BG, Mulsant BH, Rajji TK, Mamo DC, Graff-Guerrero A (2016) Threshold of Dopamine D2/3 Receptor Occupancy for Hyperprolactinemia in Older Patients With Schizophrenia. J Clin Psychiatry 77 (12):e1557-e1563. doi:10.4088/JCP.15m10538

Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B (2005) Quantification of lean bodyweight. Clin Pharmacokinet 44 (10):1051-1065. doi:10.2165/00003088-200544100-00004

Juruena MF, de Sena EP, de Oliveira IR (2010) Safety and tolerability of antipsychotics: focus on amisulpride. Drug, healthcare and patient safety 2:205-211. doi:10.2147/dhps.s6226

Kim DH, Sriharsha L, Xu W, Kamel-Reid S, Liu X, Siminovitch K, et al. Clinical relevance of a pharmacogenetic approach using multiple candidate genes to predict response and resistance to imatinib therapy in chronic myeloid leukemia. Clin Cancer Res. 2009;15(14):4750-8.

Knops N, van den Heuvel LP, Masereeuw R, Bongaers I, de Loor H, Levtchenko E, et al. The functional implications of common genetic variation in CYP3A5 and ABCB1 in human proximal tubule cells. Mol Pharm. 2015;12(3):758-68.

Koren-Michowitz M, Buzaglo Z, Ribakovsky E, Schwarz M, Pessach I, Shimoni A, et al. OCT1 genetic variants are associated with long term outcomes in imatinib treated chronic myeloid leukemia patients. Eur J Haematol. 2014;92(4):283-8.

Lako IM, van den Heuvel ER, Knegtering H, Bruggeman R, Taxis K. Estimating dopamine D(2) receptor occupancy for doses of 8 antipsychotics: a meta-analysis. J Clin Psychopharmacol. 2013;33(5):675-81.

Lange B, Mueller JK, Leweke FM, Bumb JM. How gender affects the pharmacotherapeutic approach to treating psychosis - a systematic review. Expert Opin Pharmacother. 2017;18(4):351-62.

Leucht S, Wagenpfeil S, Hamann J, Kissling W (2004) Amisulpride is an "atypical" antipsychotic associated with low weight gain. Psychopharmacology (Berl) 173 (1-2):112-115. doi:10.1007/s00213-003-1721-6

Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, Kissling W, Stapf MP, Lässig B, Salanti G, Davis JM (2013) Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet (London, England) 382:951-962. doi:10.1016/S0140-6736(13)60733-3

Lindbom L, Pihlgren P, Jonsson EN, Jonsson N. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005;79:241-57.

Lubomirov R, Csajka C, Telenti A. ADME pathway approach for pharmacogenetic studies of anti-HIV therapy. Pharmacogenomics. 2007;8(6):623-33.

Lubomirov R, di Iulio J, Fayet A, Colombo S, Martinez R, Marzolini C, et al. ADME pharmacogenetics: investigation of the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir. Pharmacogenet Genomics. 2010;20(4):217-30.

Mauri MC, Paletta S, Di Pace C, Reggiori A, Cirnigliaro G, Valli I, Altamura AC (2018) Clinical Pharmacokinetics of Atypical Antipsychotics: An Update. Clin Pharmacokinet. doi:10.1007/s40262-018-0664-3

Muller MJ, Regenbogen B, Hartter S, Eich FX, Hiemke C (2007) Therapeutic drug monitoring for optimizing amisulpride therapy in patients with schizophrenia. J Psychiatr Res 41 (8):673-679. doi:10.1016/j.jpsychires.2005.10.003

Oh S-H, Park S-M, Lee YH, Cha JY, Lee J-Y, Shin EK, et al. Association of peroxisome proliferator-activated receptor-gamma gene polymorphisms with the development of asthma. Respir Med. 2009;103:1020-4.

Oliver P, Lubomirov R, Carcas A. Genetic polymorphisms of CYP1A2, CYP3A4, CYP3A5, pregnane/steroid X receptor and constitutive androstane receptor in 207 healthy Spanish volunteers. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2010;48:635-9.

Pai MP. Estimating the glomerular filtration rate in obese adult patients for drug dosing. Adv Chronic Kidney Dis. 2010;17:e53-62.

Peuskens J, Pani L, Detraux J, De Hert M (2014) The effects of novel and newly approved antipsychotics on serum prolactin levels: a comprehensive review. CNS drugs 28 (5):421-453. doi:10.1007/s40263-014-0157-3

Reeves S, Bertrand J, D'Antonio F, McLachlan E, Nair A, Brownings S, Greaves S, Smith A, Taylor D, Howard R (2016) A population approach to characterise amisulpride pharmacokinetics in older people and Alzheimer's disease. Psychopharmacology (Berl). doi:10.1007/s00213-016-4379-6

Reeves S, Bertrand J, McLachlan E, D'Antonio F, Brownings S, Nair A, Greaves S, Smith A, Dunn JT, Marsden P, Kessler R, Uchida H, Taylor D, Howard R (2017) A Population Approach to Guide Amisulpride Dose Adjustments in Older Patients With Alzheimer's Disease. J Clin Psychiatry 78 (7):e844-e851. doi:10.4088/JCP.16m11216

Riecher-Rossler A. Oestrogens, prolactin, hypothalamic-pituitary-gonadal axis, and schizophrenic psychoses. The lancet Psychiatry. 2017;4(1):63-72.

Rosenbloom AL. Hyperprolactinemia with antipsychotic drugs in children and adolescents. Int J Pediatr Endocrinol. 2010;2010.

Rosenzweig P, Canal M, Patat A, Bergougnan L, Zieleniuk I, Bianchetti G. A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. Human psychopharmacology. 2002;17:1-13.

Savic RM, Karlsson MO. Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. Aaps j. 2009;11(3):558-69.

Schipani A, Siccardi M, D'Avolio A, Baietto L, Simiele M, Bonora S, et al. Population pharmacokinetic modeling of the association between 63396C->T pregnane X receptor polymorphism and unboosted atazanavir clearance. Antimicrob Agents Chemother. 2010;54:5242-50.

Shi H, Yu X, Li Q, Ye X, Gao Y, Ma J, et al. Association between PPAR-gamma and RXR-alpha gene polymorphism and metabolic syndrome risk: a case-control study of a Chinese Han population. Arch Med Res. 2012;43(3):233-42.

Solian® comprimés [14.01.2019]. Available from: http://www.swissmedicinfo.ch/.

Sparshatt A, Taylor D, Patel MX, Kapur S. Amisulpride - dose, plasma concentration, occupancy and response: implications for therapeutic drug monitoring. Acta Psychiatr Scand. 2009;120(6):416-28.

Taylor DM, Barnes TRE, Young AH. The Maudsley Prescribing Guidelines in Psychiatry. 13th Edition ed2018 2018. 872 p.

Tsuboi T, Bies RR, Suzuki T, Mamo DC, Pollock BG, Graff-Guerrero A, et al. Hyperprolactinemia and estimated dopamine D2 receptor occupancy in patients with schizophrenia: analysis of the CATIE data. Prog Neuropsychopharmacol Biol Psychiatry. 2013;45:178-82.

Veldhuis JD, Johnson ML. Operating characteristics of the hypothalamo-pituitary-gonadal axis in men: circadian, ultradian, and pulsatile release of prolactin and its temporal coupling with luteinizing hormone. J Clin Endocrinol Metab. 1988;67(1):116-23.

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**Author contribution**

Anaïs Glatard wrote manuscript, analyzed data.

Monia Guidi wrote manuscript, analyzed data.

Aurélie Delacrétaz wrote manuscript, collected data.

Céline Dubath wrote manuscript, collected data.

Claire Grosu wrote manuscript, collected data.

Nermine Laaboub wrote manuscript, collected data.

Franziska Gamma wrote manuscript, collected data.

Armin von Gunten wrote manuscript, collected data.

Philippe Conus wrote manuscript, collected data, obtained fundings.

Chantal Csajka wrote manuscript, analyzed data.

Chin B. Eap wrote manuscript, designed research, obtained fundings.

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**Table 1: C**haracteristics and genetic polymorphism of the study population

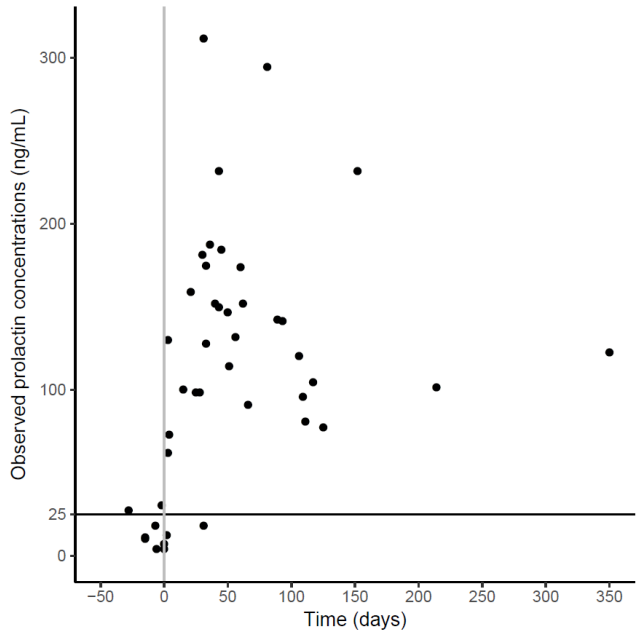
| **Characteristics** | **Value** | **% missing data** | |
|---|---|---|---|
| **Clinical characteristics** | | | |
| Sex (male), n (%) | 132 (55%) | 0 | |
| Age (years), median (range) | 37 (18-91) | 0 | |
| Body weight (kg), median (range) | 75 (43-185) | 5.6 | |
| Lean body weight (kg), median (range) | 52 (29-98) | 14.2 | |
| Body mass index (kg/m$^2$), median (range) | 25 (15-59) | 14.2 | |
| Serum creatinine concentration (µmol/L), median (range) | 76 (44-167) | 36 | |
| CLCRCG[1] (mL/min), median (range) | 93 (20-180) | 38.4 | |
| **Concomitant medications** | | | |
| P-gp inhibitors[2] , n (%) | 56 (11) | 6 | |
| Lithium, n (%) | 42 (8) | 45 | |
| **Genetic polymorphisms[3]** | **Genotype** | **Value** | **Frequencies** |
| *SLC22A1* | | | |
| rs683369 | CC/CG/GG | 58/21/6 | 68/25/7 |
| rs628031 | AA/AG/GG | 10/32/42 | 12/38/50 |
| *SLC22A2* | | | |
| rs316003 | TT/CT/CC | 45/32/6 | 54/39/7 |
| rs316019 | AA/AC/CC | 1/19/63 | 1/23/76 |
| *ABCB1* | | | |
| rs2235048 | AA/AG/GG | 24/42/19 | 28/50/22 |
| rs4148738 | CC/CT/TT | 13/41/31 | 15/48/37 |
| *NR1\|2* | | | |
| rs1523130 | CC/CT/TT | 38/31/16 | 45/36/19 |
| rs7643645 | AA/AG/GG | 32/43/10 | 38/50/12 |
| rs2461817 | AA/AC/CC | 16/46/23 | 19/54/27 |
| *NR1\|3* | | | |

| | | | |
|---|---|---|---|
| rs2307424 | AA/AG/GG | 9/42/34 | 11/49/40 |
| rs4073054 | AA/AC/CC | 48/28/9 | 56/33/11 |
| rs2502815 | AA/AG/GG | 9/36/40 | 11/42/47 |
| *RXRA* | | | |
| rs3132297 | AA/AG/GG | 3/19/63 | 4/22/74 |
| *PPARG* | | | |
| rs3856806 | TT/CT/CC | 3/18/64 | 4/21/75 |
| rs2197423 | GG/AG | 24/61 | 28/72 |
| rs2920502 | GG/GC/CC | 44/35/6 | 52/41/7 |
| *PPARGC1A* | | | |
| rs8192678 | TT/TC/CC | 7/36/42 | 8/42/50 |

1. CLCRCG, creatinine clearance estimated by the Cockcroft-Gault formula if BMI< 25 kg/m$^2$ and estimated by the Cockcroft-Gault formula integrating lean body weight if BMI≥ 25 kg/m$^2$. [Pai_2010]
2. Inhibitors of P-gp were ritonavir, darunavir, ketoconazole, simvastatin, candesartan, hydrochlorothiazide, omeprazole, esomeprazole, cetirizine, levocetirizine [Ivanyuk_2017]
3. Genetic data available for n=85 individuals except for rs628031, n=84; rs316003, n=83; rs316019, n=83

P-gp: permeability-glycoprotein

a.




b.

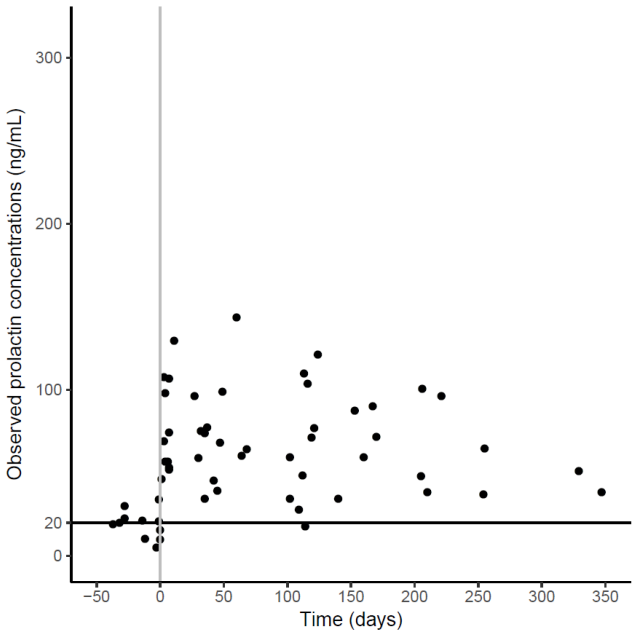
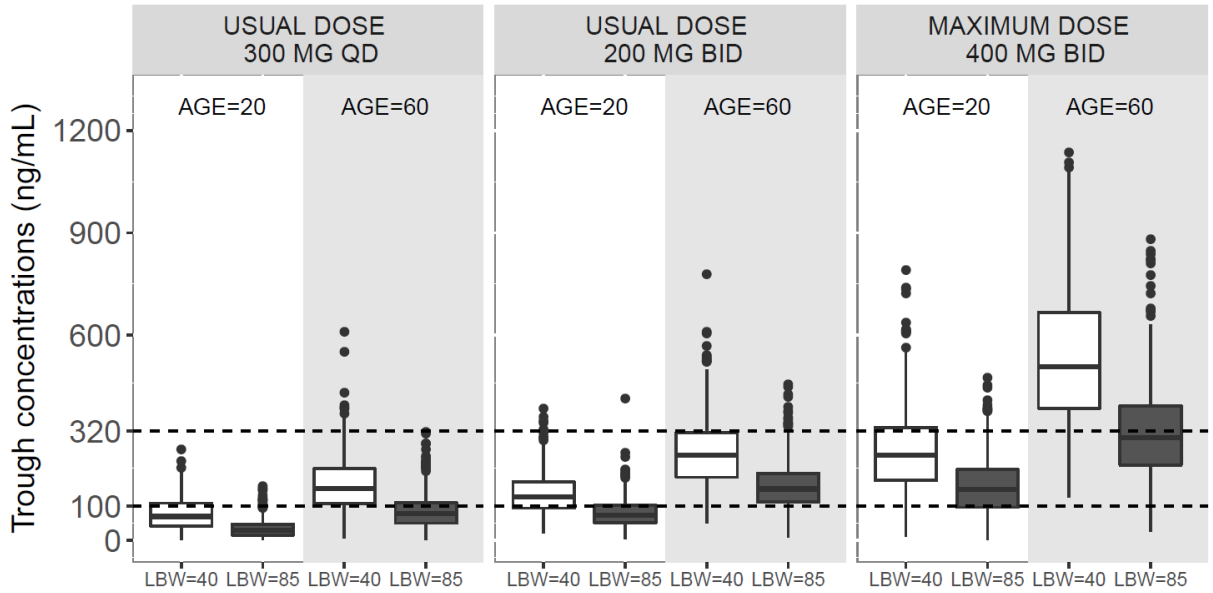



**Figure 1:** Observed prolactin concentrations versus time in females (a.) and males (b.). Vertical grey line represents the beginning of amisulpride treatment. Horizontal line represents threshold for hyperprolactinemia defined over 25 ng/mL in females and 20 ng/mL in males [Citrome_2008].

a.




b.

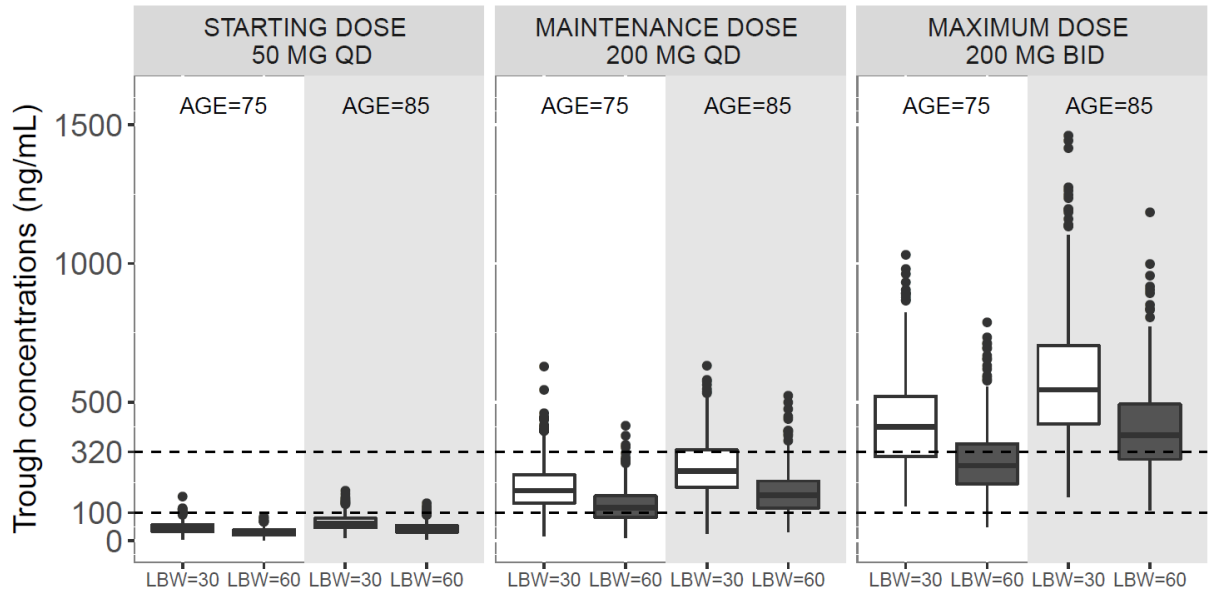



**Figure 2:** Distribution of the simulated amisulpride trough concentrations after administration of amisulpride dose at steady state in 500 adult (a.) and elderly (b.) individuals in each of the following strata of age and lean body weight: a. 20 or 60 years old (white and grey background, respectively) and 40 or 85 kg (white and grey fill, respectively) ; b. 75 or 85 years old (white and grey background, respectively) and 30 or 60 kg (white and grey fill, respectively). Boxes represent 25$^{th}$, 50$^{th}$ and 75$^{th}$ percentile of trough concentrations; data higher than 75$^{th}$ percentile plus (1.5 x IQR) and lower than 25$^{th}$ percentile minus (1.5 x IQR) are plotted individually; IQR is the interquartile range defined by 75$^{th}$

minus 25$^{th}$ percentile. Dashed lines represent the reference range of trough concentrations [Hiemke_2017, Muller_2007].

**Table 2** Parameter estimates of the final pharmacokinetic-prolactin model with bootstrap results.

| Parameter | Final population parameters | | Bootstrap evaluation (n=2000 samples) | |
|---|---|---|---|---|
| | Estimate | RSE[a] (%) | Median | $CI_{95\%}$ |
| **PK** | | | | |
| CL (L/h) | 44 | 4 | 44 | 41 ; 48 |
| V (L) | 956 | 11 | 961 | 705 ; 1258 |
| $k_a$ ($h^{-1}$) | 0.9 fixed | - | - | - |
| $\theta_{AGE\_CL}$ | -0.46 | 10 | -0.47 | -0.54 ; -0.36 |
| $\theta_{LBW\_CL}$ | 0.54 | 36 | 0.54 | 0.27 ; 0.85 |
| $IIV_{CL}$ (CV%)[b] | 33 | 12 | 33 | 24 ; 42 |
| $IIV_V$ (CV%)[b] | 65 | 24 | 63 | 35 ; 92 |
| Proportional residual error (%) | 53 | 4 | 53 | 46 ; 60 |
| **PRL Emax** | | | | |
| PRLbase (ng/mL) | 16 | 35 | 16 | 11 ; 20 |
| Emax (ng/mL) | 141 | 13 | 142 | 116 ; 182 |
| EC50 (ng/mL) | 42 | 34 | 43 | 4.6 ; 110 |
| $\theta_{MALE\_EMAX}$ | -0.53 | 13 | -0.54 | -0.65 ; -0.38 |
| $IIV_{Emax}$ (CV%)[b] | 50 | 16 | 49 | 36 ; 62 |
| Additive residual error (ng/mL) | 15 | 13 | 15 | 10 ; 20 |

Final model:

CL (L) = 44 x (1 - 0.46 x ((age - median age)/median age)) x (1 + 0.54 x ((lean body weight – median lean body weight)/median lean body weight)

Emax (ng/mL) = 141 x (1-0.53*MALE) with MALE=1 if male patients and MALE=0 if female patients.

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Interindividual variability defined as CVs (%).

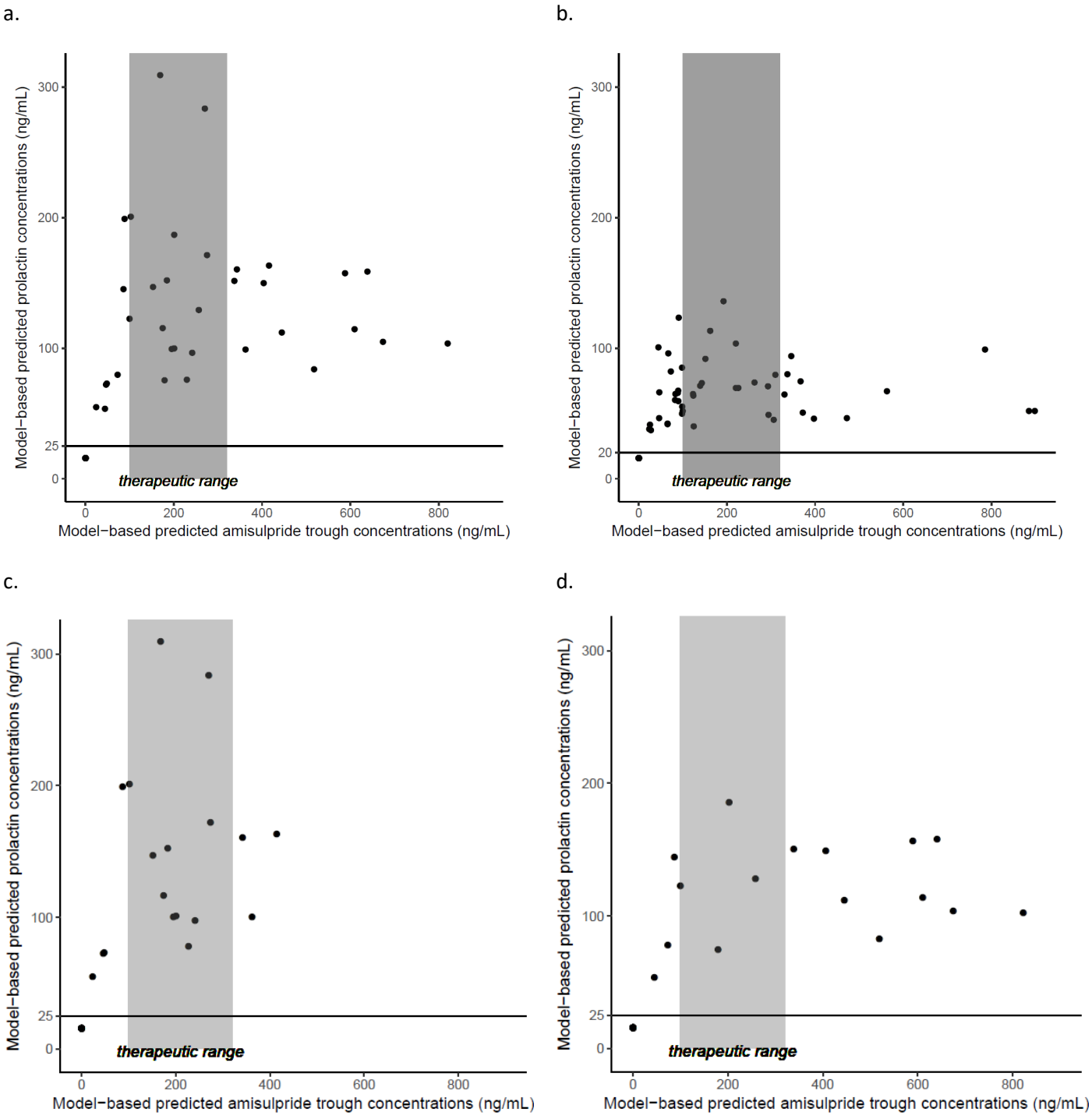



**Figure 3:** Model-based predicted prolactin plasma levels at time of amisulpride trough concentrations in females (a.), males (b.), premenopausal females (c.) and postmenopausal females (d.). Prolactin and amisulpride concentrations were predicted with the final PK-Prolactin model. Horizontal line represents threshold for hyperprolactinemia defined over 25 ng/mL in females and 20 ng/mL in males [Citrome_2008].

**Table 3:** Effect estimates of time since beginning of amisulpride treatment and amisulpride average concentration on body weight.

| | Estimate | 95%CI | p-value |
|---|---|---|---|
| **Univariate model** | | | |
| Time (days) | 0.016 | 0.004; 0.027 | 0.008 |
| **Univariate model** | | | |
| Cav (ng/mL) | 0.002 | 0.0001; 0.004 | 0.04 |
| **Multivariate model** | | | |
| Time (days) | 0.013 | -0.002; 0.028 | 0.09 |
| Cav (ng/mL) | 0.001 | -0.002; 0.003 | 0.64 |

Cav: amisulpride average concentrations

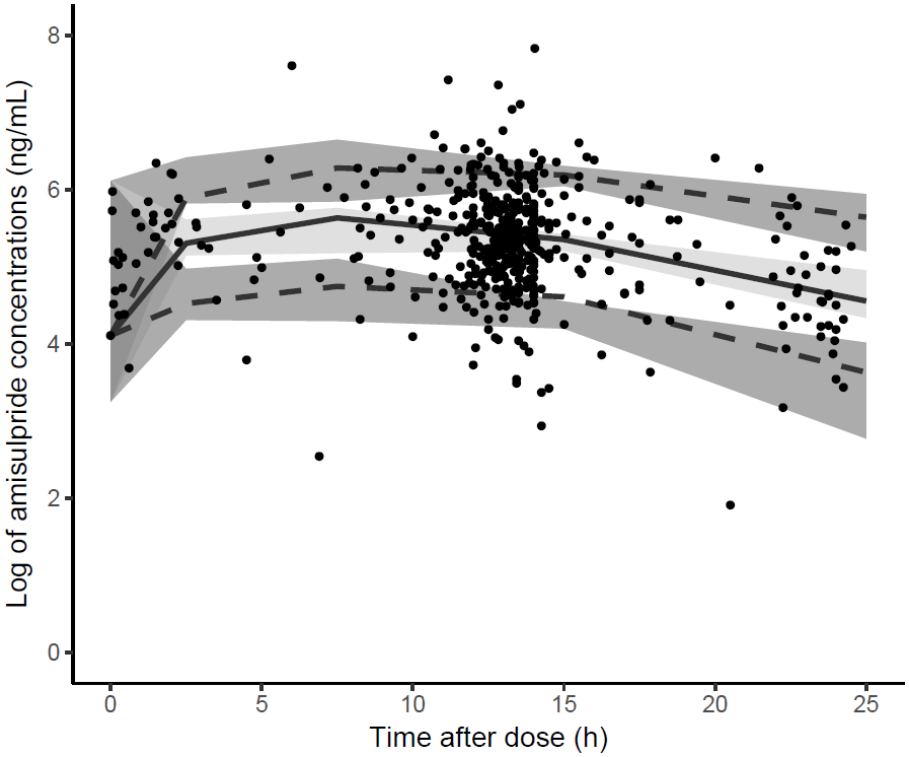
**Supplementary Table 1** Parameter estimates of the structural and final pharmacokinetic models with bootstrap results of the final model.

| Parameter | Structural population parameters | | Final population parameters | | Bootstrap evaluation (n=2000 samples) | |
|---|---|---|---|---|---|---|
| | Estimate | RSE[a] (%) | Estimate | RSE[a] (%) | Median | $CI_{95\%}$ |
| CL (L/h) | 39.6 | 4 | 43.9 | 4 | 43.8 | 39.9 ; 48.1 |
| V (L) | 954 | 11 | 926 | 10 | 923 | 695 ; 1249 |
| $k_a$ ($h^{-1}$) | 0.9 fixed | - | 0.9 fixed | - | - | - |
| $\theta_{AGE\_CL}$ | - | - | -0.47 | 10 | -0.46 | -0.54 ; -0.35 |
| $\theta_{LBW\_CL}$ | - | - | 0.53 | 37 | 0.53 | 0.25 ; 0.83 |
| $IIV_{CL}$ (CV%)[b] | 47 | 8 | 34 | 11 | 33 | 24 ; 42 |
| $IIV_V$ (CV%)[b] | 53 | 40 | 58 | 28 | 57 | 32 ; 84 |
| Proportional residual error (%) | 54 | 4 | 53 | 3 | 53 | 46 ; 60 |

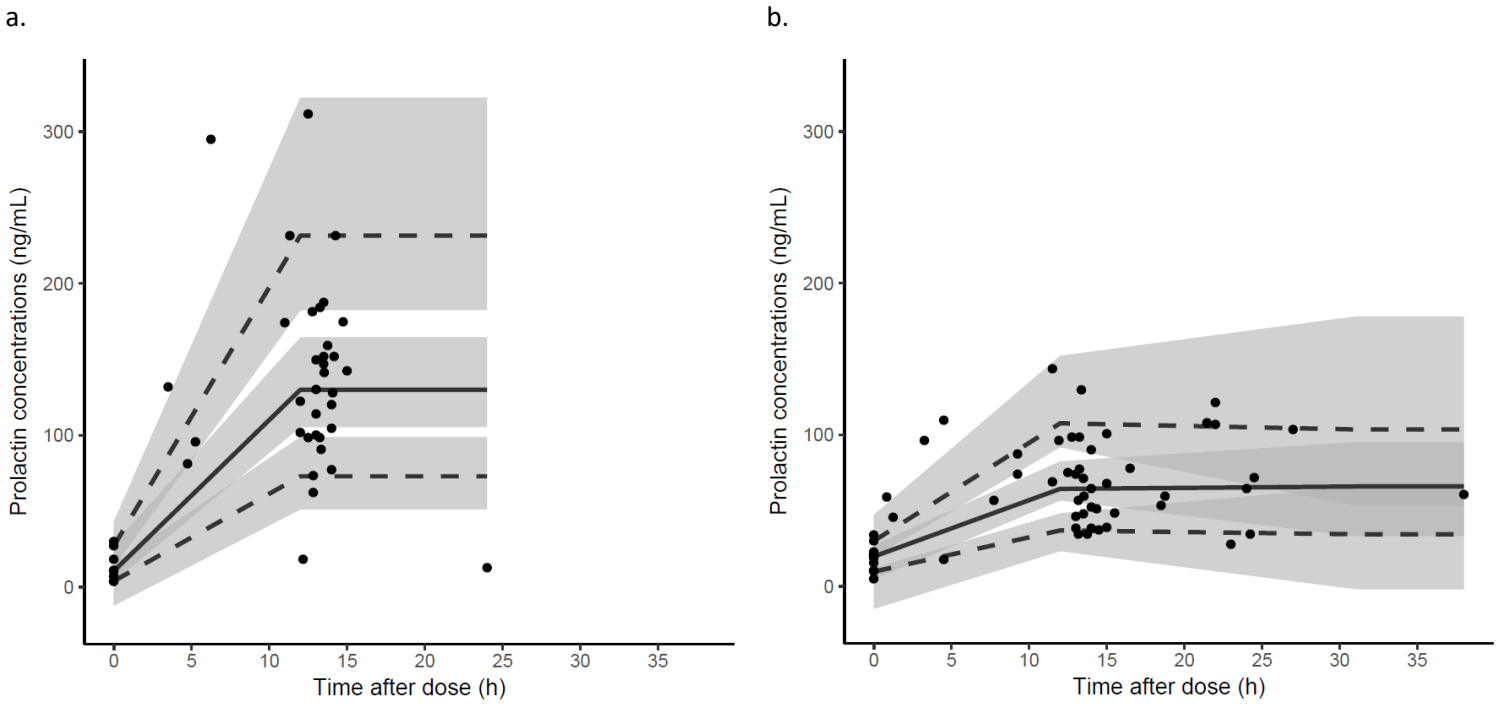
Final model: CL (L) = 43.9 x (1 - 0.47 x ((age - median age)/median age)) x (1 + 0.53 x ((lean body weight – median lean body weight)/median lean body weight)

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Interindividual variability defined as CVs (%).




**Supplementary Figure 1:** Prediction-corrected visual predictive check of the pharmacokinetic final model (n=513 amisulpride concentrations). Open circles represent amisulpride plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Shaded areas represent a simulation-based 95% confidence interval for the median, the 5% and 95% percentiles.




**Supplementary Figure 2:** Visual predictive check of the final PK-Prolactin model (n=101 prolactin plasma levels) in a. females and b. males. Black dots represent prolactin plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Shaded areas represent a simulation-based 95% confidence interval for the median, the 5% and 95% percentiles.