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Pharmacokinetics of lopinavir/ritonavir and efavirenz in food insecure HIV-infected pregnant and breastfeeding women in Tororo, Uganda

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Abstract

Pregnancy and food insecurity may impact antiretroviral (ART) pharmacokinetics (PK), adherence and response. We sought to quantify and characterize the PK of lopinavir/ritonavir (LPV/r) and efavirenz (EFV) by pregnancy and nutritional status among HIV-infected women in Tororo, Uganda. In 2011, 62/225 ante-partum/post-partum single dried blood spot samples DBS and 43 post-partum hair samples for LPV/r were derived from 116 women, 51/194 ante-/post-partum DBS and 53 post-partum hair samples for EFV from 105 women. 80% of Ugandan participants were severely food insecure, 26% lost weight ante-partum, and median BMI post-partum was only 20.2 kg/m^2. Rich PK-data of normally nourished (pregnant) women and healthy Ugandans established prior information. Overall, drug exposure was reduced (LPV −33%, EFV −15%, ritonavir −17%) compared to well-nourished controls [$p < 0.001$], attributable to decreased bioavailability. Pregnancy increased LPV/r clearance 68% [$p < 0.001$], whereas EFV clearance

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Conflict of Interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: Dr. Havlir had support from National Institutes of Health, non-financial support from Abbott in the previous 3 years. Dr. Charlebois had support from National Institutes of Health – NICHD in the previous 3 years. Dr. Gandhi had support from National Institutes of Health in the previous 3 years. Dr. Aweeka had support from National Institutes of Health in the previous 3 years. The other authors report no support from any organisation for the submitted work

remained unchanged. Hair concentrations correlated with plasma-exposure [$p < 0.001$], explaining 29% PK-variability.

In conclusion, pregnancy and food insecurity were associated with lower ART exposures in this cohort of predominantly underweight women, compared to well-nourished women. Much variability in plasma-exposure was quantified using hair concentrations. Addressing malnutrition as well as ART-PK in this setting should be a priority.

Background

In sub-Saharan Africa, the region most severely affected by HIV, women represent 58% of the people living with HIV.[1] In some areas, up to 40% of pregnant women are infected with HIV and all are eligible to receive some form of antiretroviral therapy (ART).[1] The pharmacokinetics (PK) of antiretroviral therapy (ART) in pregnant women residing in sub-Saharan Africa is not well characterized. ART exposure may be affected by alterations in absorption or metabolism due to pregnancy and underweight status,[2–4] the latter being of epidemic proportions in rural Uganda.[5] Food insecurity– an upstream determinant of underweight,– is associated with decreased adherence to ART,[6;7] and therefore may contribute to variability in exposure to ART beyond the effect of nutritional status on drug absorption and metabolism.[8–10] PK variability has major implications for outcomes of HIV-treatment, as erratic exposure to efavirenz (EFV) or lopinavir/ ritonavir (LPV/r) is associated with an increased risk of virological failure and drug resistance[11–21] and thus, in part explains the observation that underweight worsens the clinical course of HIV infection.[6;22–26] Reduced ARV exposure may additionally increase the risk of perinatal transmission of HIV infection.[27]

Quantifying and characterizing variability in drug exposure during pregnancy in underweight women can help determine whether adjustments in ART dosing, beyond those currently recommended,[28] are warranted in severely underweight pregnant women who initiate ART while the larger problem of food insecurity is being addressed at multiple levels. Previous studies have shown that dried blood spots (DBS)[29] and hair concentrations[30–32] are useful tools for studying ART exposure in resource limited settings. As ART accumulate in hair over a prolonged period of time (weeks to months), hair concentrations may have added value when studying the PK of ART. Therefore, this study sought to characterize the PK of EFV or LPV/r in a cohort of pregnant and post partum women residing in Uganda using DBS, explore whether variability in nutritional status impacts PK, and evaluate whether incorporating hair concentrations into the plasma PK-model would improve our understanding of inter-individual variability in pharmacokinetics.

Methods

Clinical trial

From December 2009 to September 2012 380 women between 12 and 28 weeks of gestation were enrolled in a prospective randomized clinical trial in Tororo, Uganda, “Novel strategies to prevent malaria and improve HIV outcomes in Africa” or the “PROMOTE study” (NCT00993031). Details and procedures for the clinical study have been described

previously.[33] Briefly, the purpose of this study was to evaluate differences in malaria and HIV outcomes among women randomized to receive either an HIV protease inhibitor based-(LPV/r) or non-nucleoside reverse transcriptase inhibitor-based (EFV) combination ART regimen. Inclusion criteria included being naïve to combination ART All women gave informed consent. The study protocol was approved by the Faculty of Medicine's Research and Ethics Committee at Makerere University, the Uganda National Council of Science and Technology, and the Committee on Human Research at the University of California San Francisco.

LPV/r (Aluvia®, Abbvie, USA) was dosed 400/100 mg twice daily. Doses were increased to 600/150 mg twice daily at 30 weeks gestation and were decreased to 400/100 mg twice daily immediately following delivery. EFV (efavirenz, Eurobindo, India) was dosed 600mg per day in all patients during all time periods.

Sampling and drug analysis

DBS for LPV, ritonavir (RTV) and EFV measurements were collected during regularly scheduled monthly visits between May and August 2011. At each visit to the clinic, a single DBS was obtained and the time of sampling was registered in the clinical report. Based on the time of prescribed ART, all women were expected to have reached steady state ART concentrations at the time of plasma or hair sampling. The dose and time of the last dose was determined by self-report. Whatman Classic cards, Whatman 903 Cards, and Chromatography paper were all used for collecting DBS. Differences in recoveries for all three cards were evaluated analytically and accounted for in the PK analyses.

In 2011, DBS Samples of the first 221 women enrolled in the PROMOTE-study were analyzed using a validated liquid chromatography-mass spectrometry (LC/MS/MS) method. Inter- and intra-day accuracy (% CV) of the medium standard ranged between 1.1–13%, 1.8–12.9% and 3.3–17.9% for LPV, RTV and EFV, respectively. The lower limits of quantification (LLOQ) for LPV, RTV and EFV were 0.025 mg/L, 0.025 mg/L and 0.1 mg/L, respectively. DBS concentrations below the limit of quantification were excluded from pharmacokinetic analysis because it was assumed that ART was not taken as prescribed the day prior to DBS collection.[34] DBS concentrations were adjusted to represent plasma concentrations using the formula [DBS concentration / (1 – 3 * hemoglobin (mg/l)] × fraction bound to plasma proteins = plasma concentration. This method was validated for EFV by Kromdijk *et al.*[35]

Scalp hair samples were collected at a post-partum visit in 96 women at a median of 84 days post-partum (range 59–91 days). The relevant ART was extracted from the hair sample and the samples were then analyzed using LC/MS/MS as described previously.[31;32;36] Intra- and inter-day variability CV was <12% for EFV, < 11% for LPV and < 12% RTV. The LLOQ was 0.01 ng/mg hair for RTV and 0.05 ng/mg hair for both LPV and EFV, as previously described.[32;36]

Covariates of adherence and nutritional status

Covariates of maternal anthropometry included body mass index (BMI), gestational weight gain (GWG), and Mid-Upper Arm Circumference (MUAC). Maternal weight was measured to the nearest 500 g using a Seca 876 mechanical scale at each hospital visit of the patient. These parameters were measured at enrollment, at 8 months of pregnancy (range 26.4–34.7 weeks), antepartum and at a post-partum visit (range 7–395 days post-partum). Height was measured to the nearest 0.1 cm using a Seca 206 wall-mounted measuring tape at enrollment. Mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm using measuring tape at the post partum visit.

Weekly gestational weight gain was calculated in patients who were pregnant during the period of sampling and was calculated by dividing the weight change between enrollment and 8 months pregnancy by the number of elapsed weeks between the two weight measurements.[33] Weight loss was defined as a rate of weight change <0 kg/wk during pregnancy and gestational weight gain for patients who did not lose weight were categorized by tertiles.[27] BMI and MUAC were categorized into tertiles. In addition, post-partum BMI values were clustered into four time-groups based on the period post pregnancy.

Food insecurity was measured using the household food insecurity access scale (HFIAS) [37] and household hunger scale (HHS) [38]. Using the HFIAS, households were categorized into four levels of household food insecurity access: food secure, and mild, moderately and severely food insecure (the Household Food Insecurity Access Prevalence status indicator).

Using the HHS, patients were categorized as having little to no household hunger, moderate household hunger or severe household hunger. Adherence based on self report was registered once during the PK-study period and was categorized into 5 categories: <75%, ≥75–85%, ≥85–95%, ≥95–99% and ≥99% adherence.

Pharmacokinetic analyses

To obtain a measure of the influence of food insecurity and underweight on the PK of EFV, LPV and RTV, nonlinear mixed-effects modeling (NONMEM VI, Globomaxx LLC, Hanover, MD, USA) was used. To enhance PK-model development, rich datasets - containing dense sampled ART plasma concentration-profiles - from other studies evaluating ART pharmacokinetics in (pregnant) women were identified and were used to establish prior PK-information(Table 1). To control for ethnicity in the models evaluating EFV, PK parameters from Ugandan individuals were used as prior information,[39] while for LPV a population PK-model derived from parameters obtained in French pregnant women was used.[40]

Simulations—After extrapolating PK parameters from other datasets, our sparse data were introduced using visual predictive checks (VPC) and numerical predictive checks (NPC). 1000 simulations were based on dosing regimen and pregnancy status of the patients in the Uganda dataset, and with the parameter values (including the inter- and intra-individual variability as reported in these other studies) obtained from the prior models. The

differences in pharmacokinetic profiles between the prior models and our dataset were studied for the three drugs.

Estimation of effect of food insecurity and pregnancy—Next, we sought to elucidate patient characteristics that explained the variability in exposure in our population. The PK-parameters derived from the models in the literature were used as priors and were fixed to the typical values from the literature, while intra-individual variability was estimated using a proportional residual error, and inter-individual variability was estimated as variability between the sampling occasions on bioavailability. In order to characterize covariates related to the adherence of ART (self reported adherence and hair measurements as described in the results section), a joint ART model was developed to increase the power to detect these covariate relations. Using these priors first an apparent bioavailability (F1) was estimated and second the oral clearance (CL/F) was re-estimated and the difference in objective function between these two models was compared. Nutritional status, pregnancy and hair concentrations were evaluated in association with ART F1 and CL/F. Missing hair concentrations were replaced by the median concentration in the analysis and a subgroup analysis was performed of patients who had hair concentration data available.

Visualization—The final PK model for LPV/r and EFV, the dosing-history data from the Ugandan women and the model-estimated population parameters, were used to visualize and predict concentration-time curves in pregnant, non-pregnant underweight and well nourished patients.

Results

Patients and data

Of the 380 women enrolled in PROMOTE, the first 221 women enrolled were included in this PK-analysis. These 221 women are representative of the whole study group. Patient characteristics are summarized in Table 2. Food security scores indicate that many women experienced severe household food insecurity (80%) and 50% experienced moderate to severe household hunger. Maternal anthropometry suggests grossly inadequate weight gain during pregnancy (median 0.15 kg/week, measured between 12–28 and 34 weeks of pregnancy) and 26% experienced weight loss during pregnancy. 19% of patients had a post partum BMI below 18.5 kg/m^2. The patient characteristics (inadequate weight gain during pregnancy, low BMI post-partum) show that the severe food insecurity lead to underweight in a sizeable proportion.

Population PK model

Simulations—Prior literature-based models using nourished HIV infected pregnant and non-pregnant women were compared with the sparse data collected for this particular analysis from the PROMOTE study using simulations (VPC) as shown in Figure 1. These visual inspections indicate that the observed data were consistently below the median of the simulated concentrations for all three drugs. Numerical comparison (NPC) of the observed and simulated data shows that 75% 76% 64% of the observed data were below the median

of the simulated concentrations in prior datasets of EFV, LPV and RTV, respectively (Table 3).

Quantification of F1 in severely food insecure women—In order to quantify the difference between the pharmacokinetic data in prior models and the data from this study, an apparent bioavailability (F1) or CL/F in our data was estimated, while using all other PK-parameters of the prior models. The introduction of apparent bioavailability resulted in a decrease in objective function value of −67, −21 and −6.9 for LPV, EFV and RTV respectively ($p < 0.001$), which was superior to estimating altered CL/F (ΔOFV +6, +3.5 and +5 for LPV, EFV and RTV respectively [$p < 0.005$]). LPV exposure was reduced by 33% (RSE 5.8%), EFV by 15% (RSE 7.4%), and RTV by 17% (RSE 7.1%) in our group of predominantly underweight women compared to exposure measures in well-nourished pregnant women in prior reports, attributed to a decrease in bioavailability. Incorporation of F1 explained 14% of the observed variability in ART-exposures of all drugs (from 30 to 27% [$p < 0.001$]).

Pregnancy—The effect of pregnancy on CL/F was estimated and resulted in an increase in CL/F ante-partum compared to post-partum by +63% (RSE 8.3%) for LPV and +17% (RSE 14%) for RTV. Accounting for the effect of pregnancy improved the model (OFV −21 [$p < 0.001$] and OFV −3 [$p<0.05$] for LPV and RTV respectively). EFV CL/F was not significantly altered by pregnancy ($P>0.5$). Incorporation of pregnancy on CL/F explained 20% of the observed variability in LPV-exposures and 3% of the observed variability in LPV-exposures [$p < 0.001$].

Nutritional status—To determine if ART absorption or metabolism were affected by underweight, we investigated the correlation between the estimated inter-patient variability on CL/F in the dataset and all measures of nutritional status (BMI ante-partum and post-partum, MUAC, weekly gestational weight gain, HFIAS and HHS). The covariate analyses showed no correlations between PK parameters of all three drugs (CL/F, F1, inter-occasion and residual error of the model) and any of the measures of nutrition in these women experiencing severe food insecurity.

ART adherence and hair concentrations—The models discussed thus far assumed perfect drug adherence. Self-reported adherence was not associated with the pharmacokinetics of these agents (CL/F, F1, inter-occasion and residual error of the model).

Since hair concentrations of ART can serve as an objective biomarker of adherence over time, hair levels were tested as a surrogate for adherence in subsequent models. Hair concentrations ranged between 1.1–13, 0.06–1.35 and 0.4–34 ng/mg hair for LPV, RTV and EFV, respectively and were associated strongly with PK measures of exposures (F1 and values below LLOQ) as assessed in dried blood spots for all three drugs: In 16 out of 17 patients who had dried blood spot concentrations below the LLOQ, hair concentrations were below the median of the hair distribution ($p<0.001$). The model overestimated exposure (as assessed by F1) when hair concentrations were below the median value for all patients and underestimated exposure (F1) in patients with hair concentration above the median value (Figure 2). Incorporation of hair concentrations on F1 explained 29% of the variability in

ART-exposure between patients (from 26% to 20% of inter-occasion variability, OFV −50 [p<0.001]) across the three drugs. In the three drugs the correlation between hair concentrations and F1 was the same; inclusion of a parameter on F1 for each drug versus one parameter for all three drugs showed no significant differences.

Final model—All parameter estimates of the final PK-models are presented in Table 4. Pregnancy explained 20% of the observed interpatient variability in LPV exposures and 3% of variability in RTV-exposures. Underweight and hair concentration data explained 14%, and 29% of the relative observed inter-individual variability in ART-exposure in these pregnant and breastfeeding women experiencing severe food insecurity and being predominantly underweight. The final model was used to predict concentration-time profiles in pregnant and non-pregnant women to show the difference between antiretroviral exposure in well nourished and underweight patients, and the results are shown in Figure 3. Food insecurity and underweight leads to low EFV and LPV/r exposure, an effect that for LPV/r is even more pronounced during pregnancy (Figure 3b). A prediction regarding the standard 400/100mg LPV/r dose in pregnant women with severe food insecurity was included to show the effect of dose adjustments.

Exploration of effect of food insecurity/underweight in pregnant and non-pregnant women—Finally, we performed an exploratory analysis to distinguish between differences in exposure as a result of pregnancy and of food insecurity. We estimated F1 in ante-partum and post-partum women while again fixing CL/F, as these values represent the effect of pregnancy on all three drugs (based on the literature values). In this model, apparent bioavailability ante-partum and post-partum was adequately characterized using one parameter (−36%, −33%, −17% for EFV, LPV and RTV respectively). Two parameter-estimates of F1, for pregnant and for breastfeeding women per drug, did not significantly improve the fit of the PK-model. This analysis showed that the lower exposure estimates in our dataset were most probably related to a hampered bioavailability of all drugs both in pregnant women and post-partum women and underweight did not significantly impact the effects of pregnancy on antiretroviral CL/F.

Discussion

This study in 221 pregnant and breastfeeding HIV-infected women with severe food insecurity in Uganda showed that food insecurity leading to underweight and pregnancy all influence exposure parameters to ART. Compared to previously published data from well-nourished women, food insecurity is associated with decreases in ART exposure ranging from 15 to 41% in this group of pregnant and breastfeeding women, irrespective of pregnancy status. Pregnancy alone comparing antepartum to postpartum estimates increased LPV CL/F by 57%, while no significant impact of pregnancy on EFV exposure was detected in our population. In addition, ART hair concentrations (serving both as a biomarker of adherence and measure of exposure) explained 29% of the variability in ART exposure between patients as estimated by plasma measures. Combining DBS and hair concentration measurements significantly improved the fit of the models and may be employed for other investigations attempting to characterize ART exposure and adherence in the context of treatment trials.

Our estimate that ART bioavailability was decreased by 15–41% in these women with severe food insecurity compared to historical data from normally nourished women, suggests that food insecurity may have a direct effect on ART disposition, beyond its effects on nutritional status. This phenomenon has been observed in studies involving other drugs. [41–45] Eighty percent of the women in this Ugandan cohort experienced severely limited access to food. Food insecurity leads to general undernutrition (protein and energy deficiencies) as well as micronutrient deficiencies, which can all have important effects on small intestinal mucosal structure and transport function, influencing drug absorption. [46;47] The lack of nutrition and/or a diet low in fat may impact absorption, although previous studies did not show consistent differences in exposures of EFV, LPV and RTV in combination with food.[48–52] Potentially underweight and malnutrition impact drug transport.[44;45;53]. Protease inhibitors, interact with numerous transport proteins.[54] Potentially underweight and malnutrition impact drug transport proteins in the intestine or in the liver or kindey, or it may impact gastro-intestinal transit time. In addition, reduced protein binding in malnourished pregnant and breast feeding women may have altered the free fraction of these highly protein bound drugs. Previous studies showed that during pregnancy LPV free fraction increased by averaged 18%.[55] Malnutrition may increase the free fraction further, as albumine or proteins like alfa acid glycoprotein concentrations may be reduced due to malnutrition. [42;56]This may partially mitigate the effect of the reduction in total LPV concentrations observed in this study. Thus, lack of food or reduced drug transport or protein binding due to underweight may partially explain the diminished bioavailibility of ART in this cohort of women.

Factors other than food insecurity, such as genetic polymorphisms, may also contribute to the low apparent bioavailability when comparing our data to previously published parameters, a factor that is especially important in the pharmacokinetics of EFV.[57–59] To control for variability in exposure based on ethnicity, a model of Ugandan individuals was used for EFV as prior information. In the study of Bouillon-Pichault *et al.* [40] and Molto *et al.*[60], the original soft gel capsules of LPV/r were used, whereas in the current study, LPV/r Maltrex-formulation tablets were used. Both formulations provide similar exposure, with a maximum increase in difference between exposures of 18% in favor of the tablet.[51] Therefore, the effect of food insecurity on the bioavailability of LPV/r may even be higher than the −32% in the current analysis.

Food insecurity leading to underweight alone did not seem to influence the association between pregnancy and ART exposure. Although, the increase in LPV-clearance of 68% during pregnancy that we observed was larger than the 39% reported by Bouillon-Pichault *et al.* or the 48% reported by Stek *et al.*,[40;61], two additional analyses stemming from the latter research team, that included 23 and 27 patients, reported that CL/F more than doubled in pregnant versus post-partum women.[28;62]. The lack of significant association between EFV clearance and pregnancy in this current study is consistent with previous observations. [63], justifying the lack of dose adjustments of EFV throughout pregnancy. In contrast current recommendations are to increase LPV/r doses during pregnancy (as shown in Figure 3b), but by only 33% from week 30 until the time of delivery which may not fully

compensate for ante-partum/post-partum differences. Thus, this study supports previous results that the clearance of LPV, but not of EFV, is altered during pregnancy.

As we were interested to assess the impact of nutritional status on ART exposure, multiple measurements were used to characterize the maternal nutritional status of pregnant and breastfeeding women. However, for this cohort of predominantly malnourished women, no association between varying degrees of nutritional status and ART exposure was detected, which may be largely driven by the lack of variation in nutritional status. Prior studies suggest that underweight and malnutrition may impair metabolism of some drugs, reduce the apparent drug clearance (CL/F) and increase exposure, a phenomenon inconsistent with our findings. Both LPV/r and EFV are metabolized by cytochrome P450 pathways. Prior papers report that microsomal cytochrome P450 content and activity are lowered after restricting protein in rat diets.[64] Antipyrine, as a probe of CYP metabolism, showed reduced clearance in children suffering from malnutrition.[65] Rat studies evaluating the impact of malnutrition, show a reduced clearance to itraconazole and clarithromycin (mainly metabolized using CYP3A) and reduced clearance of phenylbutazone (mainly metabolized via CYP2B6), resulting in increased exposure to these agents.[64] In contrast with these animal data, but consistent with our results, exposure to phenylbutazone was decreased in undernourished patients.[42] Another possible explanation for the lack of association between PK and malnutrition was the study design. Time of dosing was recorded by the patient and not observed by clinic staff. As many households lack clocks, the actual time between dose and blood draw for plasma antiretroviral levels may not have been accurately recorded on all occasions. Moreover, denser sampling techniques at specific times during pregnancy would increase the understanding of PK variability due to malnutrition and to pregnancy. In addition, more accurate monitoring of adherence is needed. In this study we were not able to look at the free fractions of drugs. This increased free fraction may partially mitigate the reduction in total LPV concentrations observed with standard dosing. Incorporating more objective biomarkers of antiretroviral adherence and measurements of the free fraction would improve PK-models and increase to ability to detect differences in PK due to malnourishment.

Previous studies report that food insecurity decreases adherence to ART.[6] Poor adherence is in turn associated with ART PK variability [44–46]. Savic et al recently showed that adherence explained the major part of the variability in the pharmacokinetics of atazanavir.[8] Poor adherence is also clearly associated with incomplete HIV RNA suppression, immunologic decline, progression to AIDS and death,[66–70]. In the current study, self-reported adherence did not influence plasma PK and 83% of patients said that they were 100% adherent, which is not consistent with population-level data on adherence rates. In contrast, hair concentrations were identified as the most critical factor explaining variability in DBS exposure for these women with severe food insecurity. As antiretrovirals accumulate in hair weeks to months prior to sampling,[71] compared to measures in dried blood spots (e.g. LPV has a half life approximately 5–6 hours), hair concentrations of antiretrovirals reflect both behavior (adherence) and biology (pharmacokinetics).[71] Therefore, the hair concentrations of antiretrovirals in our study reflected adherence patterns, as well as exposure measures, in the cohort. Gandhi *et al.* have shown that hair concentrations are a

powerful independent predictor of virological outcomes.[30;32] Our study adds to the growing literature on the utility of measuring hair concentrations of antiretrovirals in patients on chronic HIV treatment of ART, by illuminating the role of hair concentrations to better understand inter-individual variability in plasma exposure.

Young et al. described previously that there was no perinatal transmission of HIV in the PROMOTE cohort but that food insecurity/underweight did affect birth outcomes [33]. Infants whose mothers gained less than 0.1 kg/week were at increased risk for low birth weight (LBW), preterm delivery, and composite adverse birth outcomes.[33], but it is unclear whether these outcomes are related to combination ART, due to the nutritional status of the mothers or other parameters. However, previous studies of HIV-outcomes in malnourished patients suggest that food insecurity and underweight may increase the risk of treatment failure.[25–27] For our cohort, 20 of 221 patients (9%) contributing PK had viral load measurements >400 copies/mL at the time of delivery; results distinct from those reported in normally nourished women.[28;40;61–63;72;73] Any increased risk of treatment failure in patients with severe food insecurity may be explained by poor ART adherence and/or alterations in the bioavailability of LPV/r and EFV due to food insecurity and underweight.

In summary, the current study shows that pregnancy and severe food insecurity are associated with lower ART exposure in these predominantly underweight HIV-infected pregnant and breastfeeding women, compared to normally nourished women. Hair concentrations of antiretrovirals explained a major part of the inter-individual variability in ART plasma exposure, an effect which may be associated with the fact that hair levels serve as independent and objective surrogates of antiretroviral adherence. Refinement of dosage guidelines for LPV/r, specifically in the context of malnutrition, is potentially needed. For EFV, results from this study indicate that exposure is impacted by food insecurity but not by pregnancy as reported previously. Further studies to assess the impact of malnutrition on ART pharmacokinetics and clinical outcomes in pregnant women residing in resource-limited settings are warranted.

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What is already known about this subject

- Both underweight and pregnancy may affect the pharmacokinetics of ARTs
- Food insecurity is associated with decreased adherence to ARTS and increased risk of treatment failure
- Dried blood spots and hair concentrations have independently shown to be useful tools for studying ART-PK in resource limited settings.

What this study adds

- Pregnancy and food insecurity leading to underweight affects LPV, RTV and EFV exposures, in the Ugandan pregnant and breast feeding women.
- Hair concentrations as independent and objective surrogates of antiretroviral adherence, explained a major part of the variability in plasma exposures between patients.

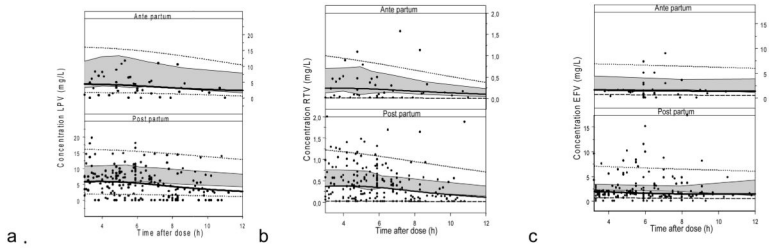

Figure 1.

VPC-based Simulations of LPV (a), RTV (b) and EFV (c) in ante and post partum, breastfeeding women.

Black dots represent the concentrations (dried blood spot adjusted to represent plasma concentrations). The solid lines indicate the median of the observed data, the dark gray shaded area is the simulated median with uncertainty and the dotted lines are 90th percentiles of simulated data.

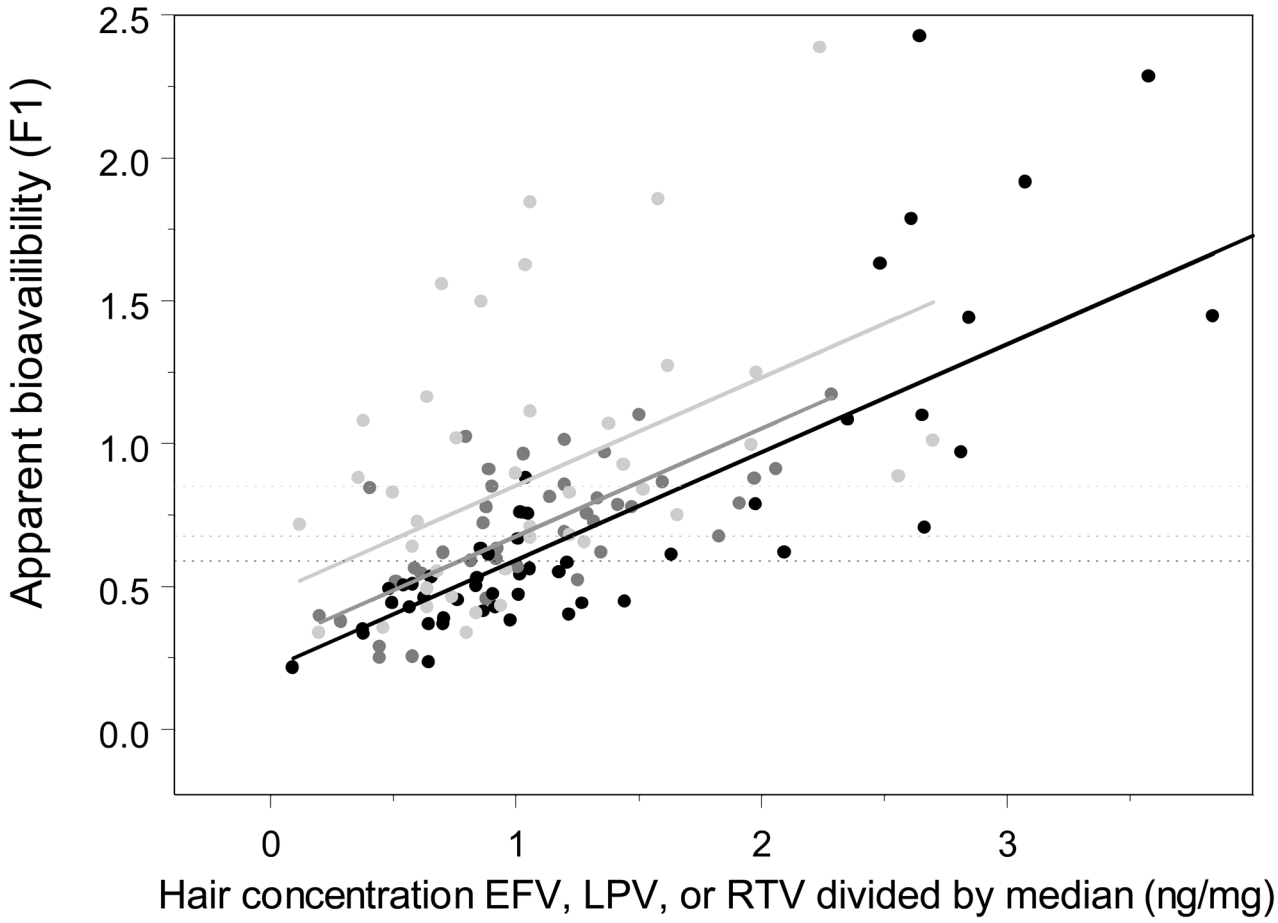

Figure 2.

Association of apparent bioavailability (F1) and hair concentrations.

Individual predictions of F1 of EFV (black dots), LVP (dark grey dots) and RTV (light grey dots) are shown. The association between F1 and hair concentrations was described using a linear function (straight black line). For comparison, the models without a measure of adherence are presented as a dotted lines (F1= 0.60, 0.67 and 0.85 for EFV, LPV and RTV respectively).

EFV efavirenz, LPV lopinavir, RTV ritonavir

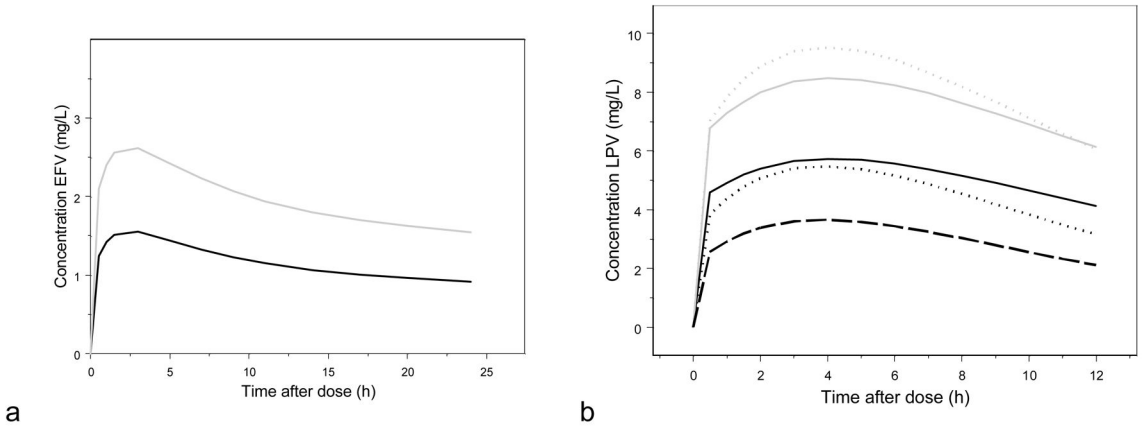

Figure 3.

Predicted plasma concentration-time profiles for well nourished and malnourished pregnant and post pregnant women.

Predicted plasma concentration-time profiles for EFV (a) and LPV (b) of well nourished women are (gray lines), and malnourished women (black lines). For LPV, plasma concentration-time profiles are shown for post-partum women (straight lines), after 35% dose increase during pregnancy (dotted lines). The dashed black lines show what would happen without dose adjustment in malnourished women during pregnancy.

Table 1

Characteristics of the patients populations of which the population PK-parameters were used as prior information to describe our population of pregnant women in Uganda (PROMOTE)

Study			study drug	Cl/F (L/h)	patients (N)	Body weight (kg)
PROMOTE	HIV-infected Ugandan Women	2nd trimester	LPV/r, EFV		27	57 (43–82)
		3rd trimester			41	58 (45–98)
		post partum			153	53 (39–76)
Rich data sets						
Bouillon- Pichault [40]	HIV-infected French women	2nd trimester	LPV(/r)	6.1	30	69.8kg ±16
		3rd trimester		6.1	61	74.1 ±12
		non pregnant		4.4	105	62.2kg ± 13.6
Molto [60]	HIV-infected Spanish patients	men+ women	RTV (LPV/r)	18.6	78	67.7 +11.5
Mirocknick [62]#	HIV-infected US women	3rd trimester	RTV (LPV/r)	16	26	80.3 (60.5–122)
		post partum		27	23	77 (54–113)
Mukonzo [39]	Ugandan healthy women		EFV	4.0	69	57.5 ±5.9*
Cressey [63]#	HIV-infected Thai/US women	3rd trimester	EFV	11	25	71.5 (43–132)
		post partum	EFV	10$	25	60.0 (37–125)

#Comparison were made using non-compartmental analysis

$Effect of pregnancy on clearance LPV; CL/F*1.38, RTV: CL/F*1.68, EFV not significantly different

Table 2

Patient characteristics of the study population from Tororo, Uganda (N=221)

Parameter		NNRT-arm (N=105)	PI-arm (N=116)
Patients at time of first DBS: AP,PP		32 (30%), 73 (70%)	36 (31%), 80 (69%)
Patients with hair-measurements		53 (50%)	43 (37%)
Concentration measurements: AP,PP (mean number per patients)		61, 225 (2.7)	52, 194 (2.1)
		all women (N=221)	
Age (years), median (range)		30.5 (18–49)	
Bodyweight (kg), median (range)		54 (39–89)	
BMI (kg,m2), median (range): 8 months pregnancy, PP[$]		22.4 (17–39), 20.2 (15–30)	
Weight gain per week during pregnancy (kg/week) (N=64), median (range)		0.15 (−0.95–0.76)	
Mid-upper arm circumference (cm) PP (N=191), median (range)		25.5 (21–30)	
BMI PP: underweight, normal weigt, overweight[#]		19%, 72%,9%	
Food insecurity category (%)	Food secure	2 (1%)	
	Mild	6 (4%)	
	Moderate	24 (15%)	
	Severe	132 (80%)	
Household hunger (%)	None to limited hunger	82 (50%)	
	Moderate hunger	68 (41%)	
	Severe hunger	14 (9%)	
Adherence based on self report (%)	<75%	1 (0%)	
	≥75–85%	3 (1%)	
	≥85–95%	14 (6%)	
	≥95%–99%	20 (9%)	
	100% adherent	183 (83%)	

Values are expressed as counts unless specified otherwise.

AP ante-partum, PP post-partum DBS dried blood sample

[$]The measurement of PP BMI was performed at a median of 170 days after delivery, ranging between 7 days-- 395 days after delivery).

[#]BMI classification women BMI <18.5 underweight, 18.5–25 normal weight, >25 overweight.[74]

Table 3

NPC results, showing the percentage of 'malnourished-women' data generated from the prior models below the median and outside the 80th Prediction Interval simulated by the ART PK-models per time interval

	Expected (%)	Observed (%)	
		ante-partum	post-partum
EFV			
< median	50%	80%	73%
<10%	10%	16%	12%
>90%	10%	8%	9%
LPV			
< median	50%	78%	73%
<10%	10%	13%	22%
>90%	10%	0%	4%
RTV			
< median	50%	63%	64%
<10%	10%	2%	8%
>90%	10%	12%	8%

Table 4

Population pharmacokinetic parameter estimates of the final pharmacokinetic model of EFV and LPV

	Estimate					
Parameter	**EFV**	**RSE %**	**LPV**	**RSE %**	**RTV**	**RSE**
CL/F (L/h)	4.0	*$*	4.4	#	18.8	@
Vc/F (L)	19.1	*$*	58.4	#	54.7	@
Vp/F (L)	477	*$*				
Q (L/h)	13.7	*$*				
KA (h^{-1})	0.15	*$*	0.27	#	0.18	@
$\theta_{pregnancy}$ in: TVCL=CL/F*$\theta_{pregnancy}$	1		1.57	8.7	1.37	15.5
Parameter of adherence	All data					
θ_F in: F1=θ_F+$\theta_{Fadherence}$*(Hair/median−1)	41%	6.1	32%	5.3	15%	7.9
$\theta_{Fadherence}$ in: F1=θ_F+$\theta_{Fadherence}$*(Hair/median−1)	0.38	13.3				
Random variability						
Inter-individual variability (IOV on F1)	22%	13.8				
Intra-individual variability (proportiol residual error)	53%	7.5				

CL/F typical value of apparent clearance for non-pregnant women, V/F apparent volume of distribution in the whole population of the central compartment (c) and the peripheral compartment (p); Q inter-compartmental clearance, KA absorption rate constant; $\theta_{pregnancy}$ increase in CL/F during pregnancy; F1 typical value of apparent bioavailability, estimated using two parameters θ_F (overall bioavailability) and $\theta_{Fadherence}$ (factor correlating hair concentrations as a measure of adherence with bioavailability);

$ Parameter fixed on model Mukonzo [39],

Bouillon- Pichault [40] and

@ Molto [60].

IOV inter-occassion variability estimated on F1