

Pharmacokinetics of Metformin during Pregnancy

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ABSTRACT:

Our objective was to evaluate the pharmacokinetics of metformin during pregnancy. Serial blood and urine samples were collected over one steady-state dosing interval in women treated with metformin during early to late pregnancy ($n = 35$) and postpartum ($n = 16$). Maternal and umbilical cord blood samples were obtained at delivery from 12 women. Metformin concentrations were also determined in breast milk samples obtained over one dosing interval in 6 women. Metformin renal clearance increased significantly in mid (723 ± 243 ml/min, $P < 0.01$) and late pregnancy (625 ± 130 ml/min, $P < 0.01$) compared with postpartum (477 ± 132 ml/min). These changes reflected significant increases in creatinine clearance (240 ± 70 ml/min, $P < 0.01$ and 207 ± 56 ml/min, $P < 0.05$ versus 165 ± 44 ml/min) and in metformin net secretion clearance

(480 ± 190 ml/min, $P < 0.01$ and 419 ± 78 ml/min, $P < 0.01$ versus 313 ± 98 ml/min) in mid and late pregnancy versus postpartum, respectively. Metformin concentrations at the time of delivery in umbilical cord plasma ranged between nondetectable (<5 ng/ml) and 1263 ng/ml. The daily infant intake of metformin through breast milk was 0.13 to 0.28 mg, and the relative infant dose was $<0.5\%$ of the mother's weight-adjusted dose. Our results indicate that metformin pharmacokinetics are affected by pregnancy-related changes in renal filtration and net tubular transport and can be roughly estimated by the use of creatinine clearance. At the time of delivery, the fetus is exposed to metformin concentrations from negligible to as high as maternal concentrations. In contrast, infant exposure to metformin through the breast milk is low.

Metformin is an effective oral hypoglycemic agent that improves insulin sensitivity (Krentz and Bailey, 2005). The introduction of metformin for the treatment of polycystic ovary syndrome (PCOS) provided evidence supporting its use in pregnant women (Lord et al., 2003). Subsequently, metformin has been used during pregnancy for the treatment of gestational as well as preexisting diabetes mellitus (Guideline Development Group, 2008; Wensel, 2009). Metformin is a small, basic compound that does not bind to plasma proteins (Scheen, 1996) and is a substrate for organic cation transporters (OCTs) (Wang et al., 2002; Kimura et al., 2005; Tanihara et al., 2007; Zhou et al., 2007). At doses of 0.5 to 1 g, metformin bioavailability is 40 to 60%, and bioavailability decreases with increasing dose (Tucker et al., 1981; Scheen, 1996). The drug is eliminated primarily by the kidneys

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without significant metabolism. The renal clearance of metformin in men and nonpregnant women correlates with creatinine clearance but exceeds glomerular filtration rate, indicating active net tubular secretion (Pentikäinen et al., 1979; Tucker et al., 1981; Scheen, 1996). An important role for renal OCT2 (encoded by *SLC22A2*) in the pharmacokinetics (PK) of metformin has been proposed based on a 30 to 60% change in metformin secretion clearance and renal clearance and up to a 74% change in its area under the concentration-time curve (AUC) in carriers of variant *SLC22A2* alleles (Song et al., 2008; Wang et al., 2008; Chen et al., 2009). Metformin crosses the placenta readily, and its umbilical cord concentrations at the time of delivery are at least half of the maternal concentrations and in some cases even exceed them (Hague et al., 2003; Vanky et al., 2005; Charles et al., 2006). When metformin is used during breast-feeding, the infant exposure is low, with the estimated relative infant dose reported to be 0.11 to 1.08% of the mother's weight-adjusted dose (Hale et al., 2002; Gardiner et al., 2003; Briggs et al., 2005).

Despite the increasing number of pregnant women being prescribed metformin for treatment of diabetes or PCOS and the recognition that physiological changes occurring during pregnancy may affect metformin disposition, data on the PK of the drug during gestation are limited. A study in seven women with type 2 diabetes demonstrated that metformin AUC_{0-4h} decreases nonsignificantly by 20% in late pregnancy, compared with postpartum (Hughes et al., 2006). How-

ABBREVIATIONS: PCOS, polycystic ovary syndrome; OCT/Oct, organic cation transporter; PK, pharmacokinetic(s); AUC, area under the concentration-time curve; LC, liquid chromatography; MS, mass spectrometry.

ever, only three postdose blood samples were collected and the full AUC and PK parameters were not reported. Furthermore, serum creatinine was used as the indicator of renal function during pregnancy. Charles et al. (2006) suggested that metformin pharmacokinetics do not change during pregnancy, but this conclusion was based on a population PK study with maternal blood samples from only 12 late pregnancies (with a median of two blood samples per pregnancy) and comparison to historic, nonpregnant controls. Thus, our main objective was to characterize the PK of metformin during pregnancy. We also measured umbilical cord and breast milk metformin concentrations.

Materials and Methods

Subjects. The study was approved by the institutional review boards at the University of Washington, Georgetown University, and the University of Texas Medical Branch in Galveston and was conducted in accordance with their guidelines. All subjects gave written informed consent. We examined the steady-state PK of oral metformin in the plasma of 35 pregnant and postpartum women who were receiving the drug for therapeutic reasons that included preexisting diabetes, gestational diabetes, and PCOS. Women were excluded from the study if their hematocrit was less than 28%. Blood and urine samples were collected during early (10–14 weeks' gestation), mid (22–26 weeks' gestation), and late pregnancy (34–38 weeks' gestation), as well as >3 months postpartum. The sample sizes of the participants at each time point varied due to the different times of enrollment and subject availability. Nine of the subjects participated in 2 study days, 6 participated in 3 study days, and 2 participated in all 4 study days. Of the women who were studied postpartum, 4 participated in the early pregnancy study, 10 participated in the mid pregnancy study, and 10 participated in the late pregnancy study. Umbilical cord plasma samples were obtained at delivery from 12 subjects. Six women participated in the breast milk sample collections for measurement of metformin concentrations.

Dosing Regimen. Oral metformin therapy was not altered for study purposes. Dosage ranged from 500 to 3000 mg/day. Two of the subjects were treated with extended-release metformin tablets. The duration of the PK sampling was based on the subject's dosage interval, which ranged from 8 to 24 h. Oral metformin tablets [immediate release from Caraco Pharmaceutical Laboratories (Detroit, MI) and extended release from Ivax Pharmaceuticals (Miami, FL) or Apotex (Weston, FL)] were provided by the investigators for the 3 days before each study, and pill counts were conducted in 29 of the 35 subjects. The metformin supplier information was not recorded for the 6 other subjects. Subjects were asked to complete dosing calendars for documentation of administration times. They were further instructed to avoid alcohol, grapefruit, and grapefruit juice for 3 days before each study day and to fast starting at 5 h before study drug administration until 1 h after the metformin dose on each study day. Clear liquids were allowed during the fasting portion of the study.

Sample Collection. On each study day during pregnancy and postpartum, serial blood samples were collected: predose; then 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after metformin dosing; or truncated to correspond to the subject's dosing interval. Urine collections were performed over one dosing interval, 0 to 8, 0 to 12, or 0 to 24 h after metformin administration, for evaluation of metformin renal and secretion clearances as well as creatinine clearance. Umbilical cord (arterial and venous) blood samples were collected immediately after delivery. Breast milk collections were performed at 2- to 3-h intervals using the Medela Classic double electric breast pump, over one dosing interval. Both breasts were completely emptied of milk during each collection. The subjects were not allowed to breast-feed their infants during the study days. Buccal swabs were collected from each of the subjects.

Plasma and Breast Milk Metformin Analysis. Plasma and breast milk samples were stored at -80°C until analysis. One hundred microliters of sample was mixed with $20\ \mu\text{l}$ of $10\ \text{ng}/\mu\text{l}$ deuterated metformin (d_6 -metformin HCl, the internal standard, a total amount of 200 ng) and $250\ \mu\text{l}$ of acetonitrile. Calibration standards (50–5000 ng/ml) and quality-control samples were prepared along with each set of samples. The samples were vortexed vigorously for 10 s and centrifuged at 15,000 rpm (16,831g) for 6 min. The protein-free supernatant was transferred to a clean tube, and $1\ \mu\text{l}$ was injected onto the

high-performance liquid chromatography (LC) system with mass spectrometry (MS) detection.

The LC-MS system consisted of an 1100 Series high-performance liquid chromatograph coupled to a Series 1100 model G1946B mass spectrometer (Agilent Technologies, Palo Alto, CA). The column was a Discovery F5 ($150\ \text{mm} \times 4.6\ \text{mm} \times 3\ \mu\text{m}$; Supelco, Bellefonte, PA). Composition of the isocratic mobile phase was 60% 10 mM ammonium formate (pH 4.0) and 40% acetonitrile, and the flow rate was 0.50 ml/min. The column was maintained at 25°C , and the autosampler tray was maintained at 4°C . The mass spectrometer was operated in the positive electrospray selected-ion monitoring mode for ion signals at $130\ m/z$ for metformin and $136\ m/z$ for d_6 -metformin. The dwell time for both ions was set at 289 ms. The respective voltages for the fragmentor and the capillary were set at 1000 and 40 V. The retention time of metformin was 7.7 min, and the run time was 9.5 min. The limit of quantification was 50 ng/ml, i.e., the lowest metformin concentration that could be measured at a signal/noise ratio ≥ 10 with a coefficient of variation $< 10\%$ and an accuracy of 85 to 115%. Metformin recovery was not estimated because no extraction was performed; other than protein removal from plasma and breast milk samples by precipitation with acetonitrile, sample preparation was minimal. The intraday coefficients of variation determined on the basis of 10 replicate samples for both low (100 ng/ml) and high (2000 ng/ml) metformin concentrations were 1.2 and 3.2%, respectively. The interday coefficients of variation determined from successive runs over 7 days for both low and high concentrations were 2.9 and 7.2%, respectively. Representative LC-MS chromatograms of metformin in plasma are shown in Fig. 1.

Urine Metformin Analysis. Ten microliters of urine sample was mixed with $990\ \mu\text{l}$ of 10 mM ammonium formate (pH 4.0) containing 400 ng of internal standard, followed by vortexing and centrifugation as described above for plasma samples. Calibration standards (50–500 $\mu\text{g}/\text{ml}$) and quality-control samples were prepared along side each other in the same manner. One-half microliter of the sample was injected onto the high-performance liquid chromatography column. The LC-MS system and instrumentation settings were the same as those described for plasma samples. The limit of quantification in urine was 200 ng/ml. The intraday coefficients of variation for low and high concentrations were 0.8 and 1.8%, respectively. The corresponding interday coefficients of variation were 1.5 and 2.1%.

Genotyping Methods. Buccal cell DNA was isolated using a Puregene Buccal Cell Kit (Gentra Systems, Minneapolis, MN) and the *SLC22A2* c.808G>T polymorphism (rs316019) was determined using validated TaqMan assays from Applied Biosystems (Foster City, CA) as described previously (Hebert et al., 2008). An internal control of sequence-verified genotype was included for each assay.

Pharmacokinetic Analysis. Steady-state PK parameters were estimated using standard noncompartmental techniques, as described previously (Hebert et al., 2008). Creatinine clearance (CrCL) was estimated by $\text{CrCL} = [(\text{urine volume})(\text{urine creatinine concentration})]/[(\text{serum creatinine concentration})(\text{duration of the collection interval})]$. Because metformin was orally administered, its clearance and volume of distribution could not be estimated independent of its oral bioavailability (F). Hence, the parameters are reported as apparent oral clearance (i.e., $\text{CL}/F = \text{dose}/\text{AUC}$) and apparent oral volume of distribution (i.e., $V_d/F = (\text{CL}/F)/k_{\text{elim}}$, respectively, where k_{elim} was the terminal elimination rate constant as estimated by log-linear regression. Metformin renal net secretion clearance was estimated by $\text{clearance}_{\text{secretion}} = \text{clearance}_{\text{renal}} - f_u \times \text{creatinine clearance}$, in which f_u was the unbound fraction of metformin in plasma and was assumed to be 1 (Scheen, 1996). One subject who was treated with extended-release metformin during mid pregnancy, but not postpartum, was not included in the analysis of apparent oral clearance and fraction excreted unchanged in the urine. Actual body weights were used for weight-adjusted parameters.

The amount of metformin excreted in the breast milk for each collection interval was summed over the dosing interval (breast milk volume \times concentration for each interval). The breast milk/plasma ratio was determined by breast milk AUC/maternal plasma AUC over one dosing interval. The percentage of maternal dose excreted in breast milk was determined by (amount of metformin excreted in the breast milk over the dosing interval/maternal dose) $\times 100$. Infant daily exposure to metformin via breast milk was calculated by (amount of metformin excreted in the breast milk over one dosing interval \times number of maternal doses per day)/body weight of an age-matched 50th

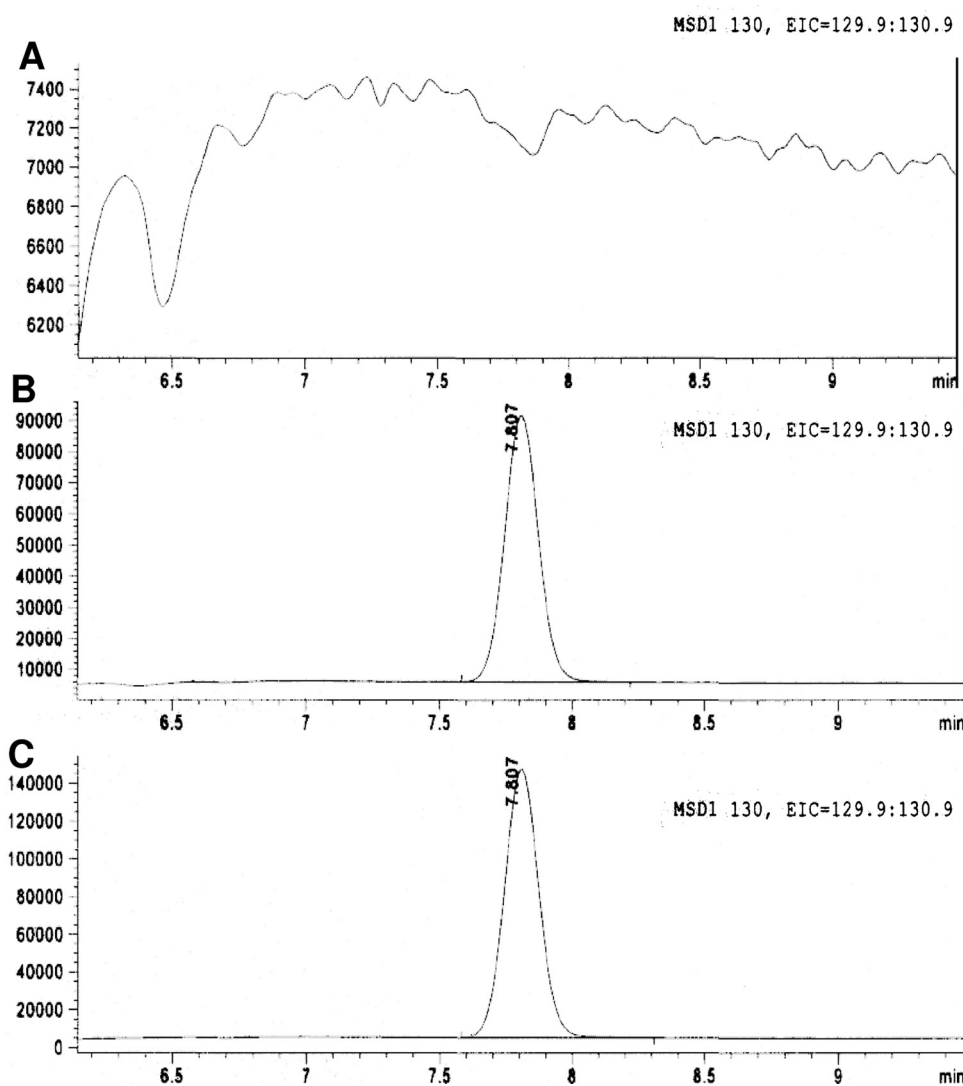


FIG. 1. LC-MS chromatograms of metformin, including a blank sample (A), a blank sample spiked with 100 ng of metformin (B), and a sample obtained at 3 h postdose from a patient in late gestation treated with 850 mg of metformin every 8 h (C).

percentile infant girl (www.cdc.gov/growthcharts). Relative infant dose was calculated by [(infant daily exposure)/(maternal daily dose/maternal actual weight)] \times 100.

Statistical Analysis. A Mann-Whitney test was used to compare the estimated PK parameters between early, mid, or late pregnancy and the postpartum study days. A Wilcoxon signed-rank test was used for within-subject comparisons of metformin PK parameters obtained in mid or late gestation with those obtained postpartum. Results are reported as means \pm S.D., unless otherwise indicated. $P \leq 0.05$ was considered significant.

Results

A total of 37 pregnant subjects (23 white, 3 Hispanic/Latina, 1 Native American, 5 black, 2 Asian, 1 Pacific Islander, 1 Asian/Pacific Islander, and 1 Pacific Islander/white) participated in this study. The subject characteristics are described in Table 1. Of the 37 subjects, 2 were not included in our analysis (one withdrew before completing the sampling over one dosage interval and the other's dosage schedule did not allow determination of metformin PK parameters with confidence). Of the other 35 subjects, 25 subjects were OCT2 genotype G/G, 9 subjects were G/T, and 1 subject was T/T at the G808T locus. Umbilical cord plasma samples were collected in 12 of the women at the time of delivery. Of the subjects who were studied postpartum, 6 were breast-feeding and 3 of them were studied again 4 to 12 weeks after cessation of lactation. Because the PK parameters did not seem to differ between the lactation and postcessation of lactation study

days (data not shown), we report for these subjects the means of the parameters obtained during the 2 study days.

Because of the high intersubject variation in PK parameters observed in our study and in previous reports (Pentikäinen et al., 1979; Tzvetkov et al., 2009), we focused our analysis on those subjects who participated in both pregnancy and postpartum study days to achieve the advantage of paired statistical comparison. The estimated PK parameters of metformin in those subjects are reported in Table 2. In the mid and late pregnancy study days, metformin renal clearance increased on average by 49% ($P < 0.01$) and 29% ($P < 0.01$), respectively, compared with postpartum (723 ± 243 and 625 ± 130 ml/min versus 477 ± 132 ml/min, respectively), regardless of the subject's OCT2 genotype (Table 2; Fig. 2). The changes in renal clearance paralleled the 29% ($P < 0.01$) and 21% ($P < 0.05$) increases in creatinine clearance during mid and late pregnancy (240 ± 70 and 207 ± 56 ml/min versus 165 ± 44 ml/min, respectively). Metformin secretion clearance was on average 45% ($P < 0.01$) and 38% ($P < 0.01$) higher in mid and late pregnancy than postpartum (480 ± 190 and 419 ± 78 ml/min versus 313 ± 98 ml/min, respectively). Similar changes were seen in metformin renal clearance and secretion clearance when comparisons between early, mid, or late pregnancy period and postpartum period were made using group means for all subjects within a study period ($n = 8, 18, 14,$ and 16 in early, mid, and late pregnancy, as well as postpartum, respectively) (data not shown).

TABLE 1

Characteristics of study subjects

Results are expressed as means \pm S.D. unless otherwise indicated. The subject characteristics were compared between the early, mid, or late pregnancy study days and the postpartum study day. The age (yr) and height (cm) were 32.5 ± 5.9 and 167.0 ± 7.4 , respectively, for the first study day of each subject.

Characteristics	Early Pregnancy (10–14 Weeks, $n = 8$)	Mid Pregnancy (22–26 Weeks, $n = 18$)	Late Pregnancy (34–38 Weeks, $n = 14$)	Postpartum (≥ 12 Weeks, $n = 16$)
Actual body weight (kg)	100.8 \pm 21.9 (N.S.)	108.1 \pm 27.3 (N.S.)	117.8 \pm 22.6 ($P < 0.01$)	97.7 \pm 18.6
Creatinine clearance (ml/min)	189 \pm 46 (N.S.)	234 \pm 61 ($P < 0.01$)	215 \pm 60 ($P < 0.05$)	164 \pm 44
No. of subjects treated for preexisting diabetes	6 ^a	11	10	11 ^a
No. of subjects treated for gestational diabetes	0	3	3	1
No. of subjects treated for PCOS	2	4	1	3
No. of subjects treated for unknown indications	0	0	0	1
Median metformin dose (mg/day)	1000 (N.S.)	1250 (N.S.)	2000 (N.S.)	1250
Median dosing interval (h)	12	12	12	12

N.S., not significant.

^a One subject treated for diabetes and PCOS.

TABLE 2

Estimated metformin plasma steady-state pharmacokinetic parameters throughout gestation in subjects studied both during pregnancy and postpartum

The estimated pharmacokinetic parameters were compared between the mid or late pregnancy study days and the postpartum study day. Data are means \pm S.D.

Parameter	Early Pregnancy (10–14 Weeks, $n = 4$) ^a	Mid Pregnancy (22–26 Weeks, $n = 10$)	Late Pregnancy (34–38 Weeks, $n = 10$)	Postpartum ($n = 15$)
Renal clearance (ml/min)	456 \pm 192	723 \pm 243 ($P < 0.01$)	625 \pm 130 ($P < 0.01$)	477 \pm 132
Renal clearance (ml/min/kg)	5.0 \pm 1.9	7.0 \pm 2.3 ($P < 0.01$)	5.5 \pm 1.0 ($P < 0.05$)	4.9 \pm 1.3
Secretion clearance (ml/min)	266 \pm 160	480 \pm 190 ($P < 0.01$)	419 \pm 78 ($P < 0.01$)	313 \pm 98
Secretion clearance (ml/min/kg)	2.8 \pm 1.1	4.7 \pm 1.8 ($P < 0.01$)	3.7 \pm 0.6 ($P < 0.01$)	3.2 \pm 0.9
Apparent oral clearance (ml/min) ^b	1935 \pm 1662	2038 \pm 848 (N.S.)	1759 \pm 960 (N.S.)	1590 \pm 864
Apparent oral clearance (ml/min/kg) ^b	18.8 \pm 10.9	19.5 \pm 7.5 (N.S.)	15.2 \pm 7.1 (N.S.)	15.6 \pm 6.5
Half-life (h)	4.3 \pm 0.4	4.3 \pm 0.9 ($P < 0.05$)	4.1 \pm 0.6 (N.S.)	3.8 \pm 1.0
Percent dose recovered in the urine unchanged (%) ^b	32 \pm 18	39 \pm 12 (N.S.)	40 \pm 11 ($P < 0.01$)	35 \pm 11
Apparent oral volume of distribution (liters) ^b	743 \pm 714	768 \pm 364 (N.S.)	632 \pm 396 (N.S.)	534 \pm 364
Apparent oral volume of distribution (l/kg) ^b	7.2 \pm 4.9	7.1 \pm 3.2 (N.S.)	5.1 \pm 2.1 (N.S.)	5.1 \pm 2.4
Creatinine clearance (ml/min)	191 \pm 70	240 \pm 70 ($P < 0.01$)	207 \pm 56 ($P < 0.05$)	165 \pm 44

N.S., not significant.

^a Because of the small number of subjects in early pregnancy, statistical comparisons were not performed for this study day.

^b A subject who was treated with extended release metformin during mid pregnancy but not postpartum was not included in the analysis.

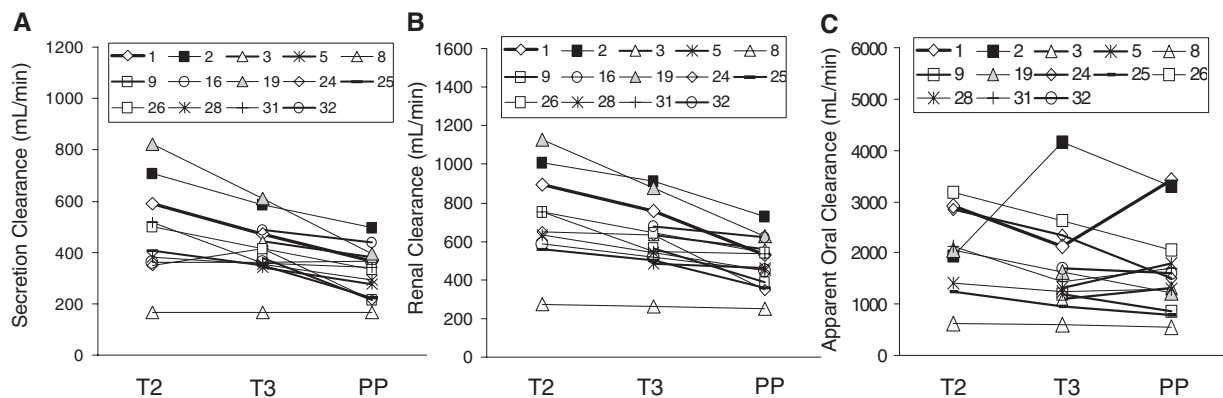


FIG. 2. Metformin secretion clearance (A), renal clearance (B), and apparent oral clearance (C) during mid (T2) and late pregnancy (T3) and postpartum (PP). White, gray, and black symbols indicate G/G (wild type), G/T, and T/T *SLC22A2* genotypes at loci G808T, respectively.

Metformin apparent oral clearance tended to increase during pregnancy, most likely reflecting the increase in its renal component, but this change was not statistically significant. In addition, the elimination half-life of metformin was slightly longer at mid pregnancy than postpartum (4.3 ± 0.9 h versus 3.8 ± 1.0 h, $P < 0.05$), and in late pregnancy the fraction of the drug recovered in the urine unchanged was greater ($40 \pm 11\%$ versus $35 \pm 11\%$, $P < 0.01$) than postpartum (Table 2). Metformin apparent oral volume of distribution was not significantly altered during pregnancy (Table 2).

Across all subjects and all study days, metformin renal clearance correlated with both its secretion clearance ($r = 0.97$, $P < 0.01$) and creatinine clearance ($r = 0.80$, $P < 0.01$) (Fig. 3). A modest correlation was also observed between metformin secretion clearance and

creatinine clearance ($r = 0.64$, $P < 0.01$, data not shown). The values of these parameters did not appear to differ between subjects treated for PCOS ($n = 8$) and those treated for prepregnancy or gestational diabetes ($n = 25$) (data not shown). In addition, the values for carriers of the variant *SLC22A2* G808T allele were within the range obtained for subjects with the G/G genotype (Fig. 3).

Because metformin bioavailability is dose-dependent (Tucker et al., 1981), we compared the PK parameters of the drug in a subset of women who received the same dose level of metformin, 500 mg twice daily (Table 3). In these subjects, metformin renal clearance and apparent oral clearance were significantly higher ($P < 0.05$) and the AUC ($P < 0.05$) and maximal concentration ($P < 0.05$) were signif-

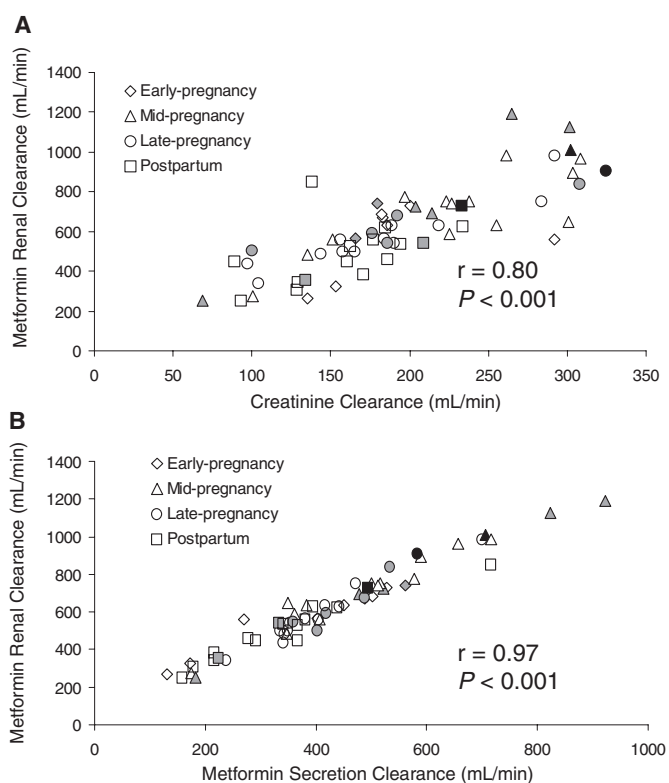


FIG. 3. Correlation between metformin renal clearance and creatinine clearance (A) or metformin net secretion clearance (B) for a total of 35 subjects (over 62 study days) during pregnancy and postpartum. White, gray, and black symbols indicate G/G, G/T, and T/T *SLC22A2* genotypes at loci G808T, respectively.

icantly lower during mid pregnancy than postpartum (Table 3; Fig. 4). Metformin apparent oral volume of distribution was larger during early and mid pregnancy than postpartum (Table 3). Metformin oral clearance correlated better in these subjects with its secretion clearance ($r = 0.80$, $P < 0.001$) than with creatinine clearance ($r = 0.65$, $P = 0.002$) (Fig. 5).

Of the 12 subjects who provided umbilical cord plasma samples at delivery, metformin cord plasma concentrations were below the limit of quantification in 3. Those subjects took their last metformin dose 10, 60, or 149 h before sample collection. Mean metformin concentrations in venous cord plasma of the other 9 subjects was 400 ± 387

ng/ml (ranging from 68 ng/ml at 2.0 h after a 500-mg dose to 1209 ng/ml at 9.2 h after a 2000-mg dose). Mean arterial cord plasma concentration was 417 ± 399 ng/ml (ranging from 55 to 1263 ng/ml for the same subjects, respectively). Arterial cord plasma concentrations of metformin were $103 \pm 13\%$ of venous concentrations (range 81–121%).

Metformin breast milk concentrations remained relatively unchanged over the dosing interval (Fig. 6). The breast milk/plasma AUC ratio in six women was 0.40 ± 0.11 . Because a breast milk sample was missing from one woman, and breasts were not completely emptied of milk in another, the actual amount of metformin excreted in the breast milk over one dosing interval was determined in only four women, treated with 1500 mg/day ($n = 1$) or 2000 mg/day ($n = 3$). The amounts excreted daily were 0.13, 0.15, 0.21, and 0.28 mg, corresponding to a relative infant dose of 0.21, 0.14, 0.21, and 0.43% of the mother's weight-adjusted dose, respectively.

Discussion

Despite the increasing number of women being prescribed metformin for improving insulin sensitivity in pregnancy, the data on the PK of the drug during gestation are incomplete and partially conflicting. Our study characterizes metformin PK during pregnancy and demonstrates that the physiological changes occurring during pregnancy indeed alter metformin PK.

In nonpregnant subjects, the protein binding of metformin in plasma is negligible, and the drug does not undergo significant metabolism (Scheen, 1996). Thus, pregnancy-induced changes in its PK can be primarily attributed to altered renal blood flow (which leads to secondary changes in renal glomerular filtration and tubular secretion rates) and/or up-regulation in organic cation transporter-mediated active tubular secretion. Metformin net tubular secretion is unlikely to be confounded by changes in its passive tubular reabsorption, because metformin is a strong base ($pK_a = 11.5$) (Scheen, 1996). During normal pregnancy, effective renal plasma flow increases on average 50 to 85%, with a corresponding 50% increase in glomerular filtration rate (Davison and Dunlop, 1980; Sturgiss et al., 1994). In line with these changes in renal function, the most significant effect of pregnancy in our subjects was on metformin renal clearance. In our paired analysis, we found 49 and 29% increases in metformin renal clearance during the mid and late pregnancy studies, along with corresponding 29 and 21% increases in creatinine clearance. Metformin renal clearance and the fraction excreted unchanged in the urine postpartum

TABLE 3

Estimated metformin plasma steady-state pharmacokinetic parameters throughout gestation in subjects treated with 500 mg of metformin twice daily

The estimated pharmacokinetic parameters were compared between the early, mid, or late pregnancy study days and the postpartum study day. Data are means \pm S.D.

Parameter	Early Pregnancy (10–14 Weeks, $n = 5$)	Mid Pregnancy (22–26 Weeks, $n = 7$)	Late Pregnancy (34–38 Weeks, $n = 4$) ^a	Postpartum ($n = 6$)
Area under the concentration-time curve (ng \cdot h/ml)	6544 \pm 2834 (N.S.)	6144 \pm 3381 ($P < 0.05$)	6937 \pm 1839	9804 \pm 3140
Time to maximal concentration (h)	1.4 \pm 0.2 (N.S.)	2.0 \pm 0.7 (N.S.)	2.0 \pm 0.7	1.8 \pm 0.3
Maximal concentration (ng/ml)	1218 \pm 465 (N.S.)	1063 \pm 485 ($P < 0.05$)	1135 \pm 304	1611 \pm 386
Apparent oral clearance (ml/min)	1457 \pm 560 (N.S.)	1629 \pm 648 ($P < 0.05$)	1283 \pm 418	928 \pm 285
Apparent oral clearance (ml/min/kg)	15.1 \pm 4.1 (N.S.)	16.6 \pm 5.4 ($P < 0.05$)	11.9 \pm 3.7	10.7 \pm 2.7
Renal clearance (ml/min)	524 \pm 221 (N.S.)	710 \pm 276 ($P < 0.05$)	549 \pm 63	415 \pm 147
Renal clearance (ml/min/kg)	5.5 \pm 1.9 (N.S.)	7.3 \pm 2.7 ($P < 0.05$)	5.1 \pm 0.5	4.7 \pm 1.4
Percentage of dose recovered in the urine unchanged (%)	37 \pm 12 (N.S.)	44 \pm 6 (N.S.)	46 \pm 14	45 \pm 5
Apparent oral volume of distribution (liters)	509 \pm 213 ($P < 0.05$)	542 \pm 229 ($P < 0.05$)	432 \pm 168	303 \pm 66
Apparent oral volume of distribution (l/kg)	5.3 \pm 1.8 (N.S.)	5.6 \pm 2.1 (N.S.)	4.0 \pm 1.5	3.6 \pm 1.2

N.S., not significant.

^a Because of the small number of subjects in late pregnancy, statistical comparisons were not performed for this study day.

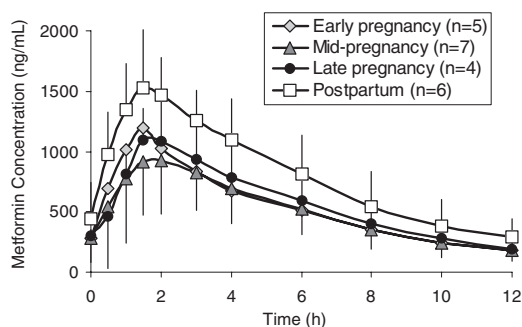


FIG. 4. Plasma concentrations (means \pm S.D.) versus time profile during early, mid, and late pregnancy as well as postpartum in women treated with 500 mg of metformin twice daily.

were similar to those previously reported in nonpregnant subjects (Pentikäinen et al., 1979; Tucker et al., 1981; Sambol et al., 1995).

Our findings also indicate 45 and 38% increases in metformin secretion clearance in mid and late pregnancy, respectively. Metformin renal clearance correlated better with its net tubular secretion clearance ($r = 0.97$) than with creatinine clearance ($r = 0.80$), which is not surprising given the high secretory clearance of metformin. Pregnancy has been reported to induce changes in tubular secretion of endogenous compounds such as glucose and amino acids (Davison and Dunlop, 1980). Although enhanced net tubular secretion has been reported for digoxin (Hebert et al., 2008) and amoxicillin (Andrew et al., 2007) during pregnancy, little is known about the impact of pregnancy on tubular handling of most drugs. In our subjects who

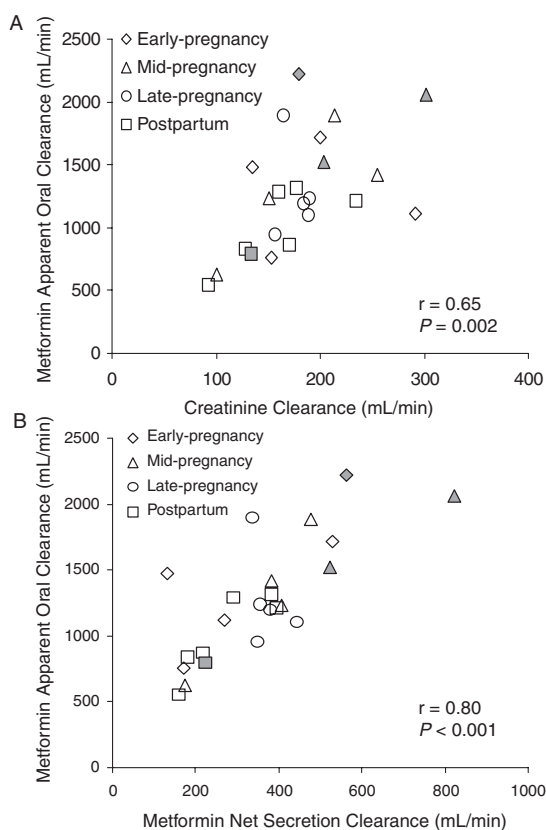


FIG. 5. Correlation between metformin apparent oral clearance and creatinine clearance (A) or metformin net secretion clearance (B) for a total of 14 subjects (over 24 study days), taking 500 mg of metformin twice daily, during pregnancy and postpartum. White and gray symbols indicate G/G and G/T *SLC22A2* genotypes at loci G808T, respectively.

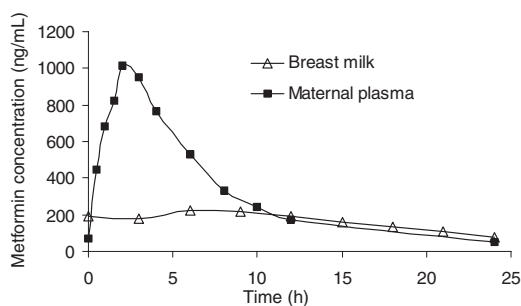


FIG. 6. Metformin concentration in maternal plasma and breast milk of a representative subject over one dosing interval at steady state. The subject was treated with 1500 mg of metformin once daily for type 2 diabetes.

were studied postpartum, the mean net tubular secretion clearance of metformin (313 ml/min) was approximately two-thirds of the mean effective renal plasma flow reported previously in nonpregnant women (482 ml/min, based on four studies in a total of 62 women) (Davison and Dunlop, 1980); i.e., metformin tubular secretion is a relatively high extraction process. Given the high body weight of our postpartum subjects and possibly higher effective renal plasma flow, we may be overestimating the extraction ratio of metformin in our subjects (Hebert et al., 2005). Because the estimated tubular extraction ratio for metformin is moderately high, the gestational changes in the net secretory clearance of the drug can potentially be explained by enhanced renal plasma flow. Alternately, the increase in net secretory clearance could signify an up-regulation in the renal tubular transport mechanism of metformin. Metformin is a substrate for renal OCTs, including OCT1, OCT2, the multidrug and excursion transporters (Becker et al., 2009), and the plasma membrane monoamine transporter (Zhou et al., 2007). In humans, OCT2 plays an important role in metformin renal clearance (Song et al., 2008; Wang et al., 2008; Chen et al., 2009). Several studies in vitro and in animal species suggest that Oct2 expression and activity in the kidney can be regulated by steroidal hormones (Urakami et al., 1999; Shu et al., 2001; Alnouti et al., 2006). However, little is known about OCT2 regulation in humans. Hence, altered tubular secretion clearance of metformin may be suggestive of pregnancy-induced enhancement of OCT2 expression and/or function.

Several studies have recently shown that metformin pharmacokinetics are dependent in part on OCT2 genotype/activity (Song et al., 2008; Wang et al., 2008; Chen et al., 2009). The variant 808G>T, which has a high allelic frequency in all the racial populations studied, was associated with decreased secretion clearance and renal clearance in Asian homozygous and heterozygous carriers (Wang et al., 2008). In contrast, the same variant resulted in opposite changes in European-Americans and African-Americans (Chen et al., 2009). This discrepancy may be due to a linkage disequilibrium of 808G>T and four other single nucleotide polymorphisms that are frequent in the Han Chinese population but not in European-Americans (Chen et al., 2009). The genotype-phenotype relationships of 808G>T were not evident in our study, even postpartum. This result can be attributed to the different study population, as the previous studies were performed in healthy volunteers of either Asian or non-Asian race under very controlled experimental conditions, whereas our subjects were pregnant, not healthy, and of diverse racial backgrounds, but predominantly white.

Because the association between OCT1 genotype and metformin renal clearance is weak and the multidrug and excursion 1 genotype did not affect metformin renal clearance (Tzvetkov et al., 2009), we did not genotype our subjects for polymorphisms in these transporters. Nevertheless, OCT1 affects metformin distribution and has been

suggested to contribute to the drug's limited, dose-dependent gastrointestinal absorption (Shu et al., 2008). Therefore, we cannot rule out the possibility that polymorphisms in OCTs other than OCT2 could affect metformin absorption and distribution. Furthermore, altered activity of these transporters, together with other physiological changes that occur during pregnancy, could lead to gestation-induced changes in metformin oral absorption and volume of distribution. Indeed, when we limited our analysis to a subset of patients treated with the same dosage regimen, 500 mg twice daily, metformin apparent oral clearance and apparent oral volume of distribution increased, whereas the drug's maximal concentration and AUC decreased during pregnancy (Table 3). In this patient subset, the fraction of metformin dose excreted in the urine was not affected by pregnancy. Given that metformin is almost entirely eliminated unchanged in the urine, this suggests that pregnancy does not alter metformin absorption.

In nonpregnant patients, plasma metformin concentrations are typically in the range of 0.5 to 2.0 $\mu\text{g/ml}$ (Scheen, 1996), but the therapeutic concentrations for metformin have not been established. Furthermore, metformin pharmacodynamics may change during pregnancy due to enhanced insulin resistance (Hebert et al., 2009). Our findings suggest that higher metformin doses may be required in pregnant patients with insufficient glycemic control, although the impact of dosages higher than 2500 mg/day on the fetus requires further evaluation (Glueck et al., 2004; Rowan et al., 2008; Wensel, 2009).

A previous report described metformin umbilical cord serum concentrations to be comparable with the maternal concentrations or even exceed them ($n = 13$) (Vanky et al., 2005). Likewise, our findings indicate significant placental transfer of metformin, although recent studies suggest that metformin is a safe option for the treatment of pregnant women. No significant increase in a composite measure of neonatal complications was found among women with gestational diabetes treated with metformin, compared with insulin (Rowan et al., 2008). A follow-up for 18 months showed no differences in the growth or motor and social development of 126 infants of mothers who took metformin at conception and throughout pregnancy compared with those of control infants (Glueck et al., 2004).

The amount of metformin excreted in breast milk was 0.13 to 0.28 mg/day (corresponding to 0.024–0.050 mg/kg/day), less than 0.5% of the mother's weight-adjusted dose. The data are in line with those reported in previous studies that measured the excretion of the drug in breast milk over a dosage interval (Hale et al., 2002; Gardiner et al., 2003) or estimated infant exposure based on single time point measurements in women treated with 1000 to 1500 mg of metformin daily (Briggs et al., 2005). More importantly, a daily dose of 2000 mg in our study did not result in greater infant exposure (absolute infant dose of 0.027, 0.036, and 0.050 mg/kg/day) than 1500 mg daily (0.024 mg/kg/day in our study and the previously described values of 0.023–0.064 mg/kg/day) (Hale et al., 2002). Although the data only represent a small number of women, they suggest a saturable mechanism of metformin transfer across mammary epithelial cells, probably through OCTs (Gardiner et al., 2008). Our data suggest that the infant is exposed to subtherapeutic doses of metformin during nursing. Furthermore, specific timing of metformin intake by the mother relative to nursing, as recommended by the general guidelines from the American Academy of Pediatrics Work Group on Breastfeeding (1997), is not expected to affect infant exposure to metformin.

Our results suggest that metformin pharmacokinetics change during pregnancy and that creatinine clearance can be used as a gross estimate of metformin PK in the pregnant patient. In our subject population, the effect of pregnancy on metformin PK was more

pronounced than the effect of the *SLC22A2* 808G>T genotype. In agreement with previous studies, we found that metformin crosses the placenta. Infant exposure to metformin through breast-feeding is low and is not expected to result in pharmacological effects.

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