Published in final edited form as:

Clin Pharmacol Ther. 2009 June ; 85(6): 607–614. doi:10.1038/clpt.2009.5.

Are we optimizing gestational diabetes treatment with glyburide?

The pharmacologic basis for better clinical practice

MF Hebert, PharmDFCCP^{1,2}, X Ma, MS³, SB Naraharisetti, PhD⁴, KM Krudys, PhD³, JG Umans, MDPhD⁵, GDV Hankins, MD⁶, SN Caritis, MD⁷, M Miodovnik, MD⁵, DR Mattison, MD⁸, JD Unadkat, PhD⁴, EJ Kelly, PhD⁴, D Blough, Ph.D.¹, C Cobelli, PhD⁹, MS Ahmed, PhD⁶, WR Snodgrass, MDPhD⁶, DB Carr, MDMS², TR Easterling, MD², and P Vicini, PhD^{3,*}
for the Obstetric-Fetal Pharmacology Research Unit Network

¹ Department of Pharmacy, University of Washington

² Department of Obstetrics and Gynecology, University of Washington

³ Department of Bioengineering, University of Washington

⁴ Department of Pharmaceutics, University of Washington

⁵ MedStar Research Institute, Washington Hospital Center, and Georgetown University Medical Center

⁶ Galveston Department of Obstetrics and Gynecology, University of Texas Medical Branch

⁷ Department of Obstetrics and Gynecology, University of Pittsburgh

⁸ NICHD, NIH, University of Pittsburgh

⁹ Dipartimento di Elettronica e Informatica, Università di Padova Italy

Abstract

Glyburide's PK and PD have not been studied in women with gestational diabetes mellitus (GDM). The objective was to assess steady-state PK of glyburide as well as insulin sensitivity, beta-cell responsivity and overall disposition indices following a mixed meal tolerance test (MMTT) in GDM (n=40), non-pregnant type 2 diabetic (T2DM) (n=26) and healthy pregnant (n=40, MMTT only) women. At equivalent doses, glyburide plasma concentrations were ~50% lower in pregnancy compared to non-pregnant women. Average glyburide umbilical cord to maternal plasma concentration ratio at the time of delivery was 0.7 ± 0.4 . Insulin sensitivity was ~5-fold lower in women with GDM compared to healthy pregnancy. Despite comparable beta-cell responsivity index, average beta-cell function corrected for insulin resistance was >3.5- fold lower in women with glyburide-treated GDM than healthy pregnancy. Women with GDM that fail glyburide may benefit from alternate medication selection or dosage escalation, though fetal safety should be considered.

Corresponding Author and Reprint Requests: Mary F. Hebert, Pharm.D., FCCP, University of Washington, Department of Pharmacy, 1959 NE Pacific St., H-375 Health Science Center, Box 357630, Seattle WA 98195-7630, Phone: 206-616-5016, Fax: 206-543-3835, Email: E-mail: mhebert@u.washington.edu.

*Current address: Pfizer Global Research and Development, San Diego CA

CONFLICT OF INTEREST/DISCLOSURE

At the time of study conduct and analysis, the authors declare no conflict of interest. However, since completion of the study, Dr. Vicini's affiliation has become Pfizer Global Research and Development.

Keywords

glyburide; gestational diabetes; pregnancy; pharmacokinetics; CYP2C9; fetal exposure; umbilical cord; protein binding; mixed meal tolerance test; insulin sensitivity; beta-cell responsivity index; disposition index

Gestational diabetes mellitus (GDM) complicates 5–12% of pregnancies and is associated with adverse pregnancy outcomes. Insulin has been the mainstay of pharmacotherapy for GDM. Glyburide's (GLY) advantages include easier route of administration and schedule as well as improved patient satisfaction and adherence. GLY's acceptance has been due to its comparable efficacy with insulin and limited transfer to the fetus.(1,2) However, the pharmacokinetics (PK) of GLY have not been studied in pregnancy and dosage strategies generally follow those used in non-pregnant patients with type 2 diabetes mellitus (T2DM). We hypothesized that the PK of GLY would be different during pregnancy, due to the expected changes in metabolism, protein binding, and body composition. In addition, the effects of GLY on the insulin sensitivity and secretion parameters have not been systematically studied. Our objectives were to compare GLY PK in women with GDM and non-pregnant T2DM, to measure fetal exposure to glyburide at delivery and to evaluate insulin sensitivity (SI), beta-cell responsivity index (Φ_{total}) and disposition index (DI) following a mixed meal tolerance test (MMTT) in women with GDM on GLY therapy, compared with gestational age-matched healthy pregnant women and non-pregnant women with T2DM on GLY.

RESULTS

Subjects

Subject demographics are described in Table 1.

Pharmacokinetics

Noncompartmental—Estimated steady-state GLY PK parameters are reported in Table 2. Mean dose-normalized GLY area under the plasma concentration-time curve (AUC) and maximum concentration (C_{max}) were lower during pregnancy than in the non-pregnant controls (Figure 1). The gestational increase in apparent oral clearance (CL/F) was independent of CYP2C9 genotype (*1*1 - GDM: 17.6 ± 12.6 L/hr vs. T2DM: 9.0 ± 5.6 L/hr, $p = 0.01$; and combined *1*2, *1*3, and *2*2 - GDM: 13.7 ± 6.0 L/hr vs. T2DM: 6.9 ± 3.0 L/hr, $p = 0.01$). There were no significant differences in plasma 4-trans-hydroxyglyburide (M1) AUC (GDM: 52.4 ± 36.9 ng•hr/mL, T2DM: 77.8 ± 112.7 ng•hr/mL, $p = 0.2$) or dose adjusted AUC (GDM: 17.7 ± 16.5 ng•hr/mL per mg GLY dose, T2DM: 18.8 ± 17.4 ng•hr/mL per mg GLY dose, $p = 0.8$) during pregnancy. The percent of dose excreted in the urine as M1 did not differ significantly with pregnancy (GDM: $30 \pm 15\%$, T2DM: $27 \pm 12\%$, $p = 0.4$).

Compartmental—Estimates of GLY CL/F obtained with compartmental modeling (GDM: 18.3 ± 10.3 L/hr and T2DM: 9.6 ± 6.9 L/hr, $p < 0.001$) were similar to those from noncompartmental analysis. Excellent correlations were found between CL/F estimates from the two methods for women with GDM ($r = 0.9$, data not shown) and T2DM ($r = 0.9$, data not shown). V/F was significantly higher in women with GDM (83 ± 65 L) than in non-pregnant women with T2DM (53 ± 36 L, $p = 0.04$).

Monte Carlo Simulations—Based on the PK parameters, steady-state GLY plasma concentration-time profiles were generated utilizing Monte Carlo simulations for women with GDM and non-pregnant with T2DM. Simulated concentration-time profiles were similar when twice daily oral doses ranged from 1.25–10.0 mg in non-pregnant women and 1.25–23.75 mg

in pregnant women (Figure 2). Simulated median GLY steady-state concentration at a dose of 23.75 mg orally twice daily in women with GDM was 98.4 ng/mL.

Umbilical cord—At delivery, maternal plasma GLY concentrations were < limit of assay quantitation (LOQ)-32.7 ng/mL and umbilical cord venous concentrations were < LOQ-12.5 ng/mL. Cord:maternal GLY plasma concentration ratios were similar for venous and arterial cord samples. Mean GLY cord:maternal plasma concentration ratio was 1.0 ± 1.2 . Excluding one outlier, the mean ratio was 0.7 ± 0.4 . GLY percent unbound was the same in maternal and cord venous plasma ($1.0 \pm 0.2\%$ and $1.0 \pm 0.3\%$ respectively).

Mixed Meal Tolerance Test

Glucose, insulin and C-peptide concentration-time profiles are shown in Figure 3. MMTT response parameter estimates are reported in Table 3. The overall metabolic state (i.e. beta-cell function corrected for the degree of insulin resistance) as described by the DI, was similar in women with GDM and non-pregnant with T2DM, with both values lower than in healthy pregnant women. The hyperbolic relationship between Φ_{total} and SI are shown in Figure 4. Although the results show considerable between-subject variability, the responses in women with either GDM or T2DM were characterized by a hyperbolic curve shifted down and to the left relative to the healthy pregnant controls, consistent with these conditions.

DISCUSSION

Our results have significant dosing and medication selection implications for optimizing GDM pharmacotherapy. The large gestational increase in the CL/F (> 2-fold) suggests that higher dosages may be needed during pregnancy to achieve glycemic control. The GLY CL/F in our non-pregnant controls was similar to that previously reported in non-pregnant populations (3) who typically receive GLY 1.25–20 mg/day for T2DM treatment.(4) GLY dosage for GDM has traditionally been the same (i.e. max 20 mg/day). However, given the large PK changes in pregnancy, we used simulations to determine what dosage range in pregnancy would give comparable concentrations to those in the non-pregnant women with T2DM. As shown in Figure 2, simulated GLY concentration time profiles for non-pregnant women receiving 1.25 to 10 mg twice daily were comparable to those in pregnant women receiving 1.25 to 23.75 mg twice daily. GLY failure rates for GDM treatment have been reported to be 14–21%.(1,5–8) Some of the women who fail GLY therapy may improve glycemic control by increasing GLY dosage above that used in non-pregnant patients.

GLY appears to be fairly safe for the fetus at maternal doses of 1.25–10 mg orally twice daily. Prior to our study, GLY was generally believed to have limited ability to cross the placenta based on placental perfusion (2) and umbilical cord serum GLY concentrations <10 ng/mL (n =12, HPLC with UV detection) at delivery (1). In our study, utilizing an LC/MS assay (LOQ 18.75 pg on column), umbilical cord plasma GLY concentrations averaged 70% of maternal concentrations. In approximately 20% of the umbilical cord venous samples collected at delivery, GLY plasma concentrations were $\geq$ the mean maternal steady-state trough concentration. This observation should be considered if the current maximal GLY dosage were exceeded in pursuit of increased hypoglycemic effect.

The GLY concentration-response relationship remains uncertain, even in non-pregnant patients. One single dose study reported no increase in insulin secretion in 9 healthy, non-pregnant volunteers utilizing continuous GLY infusion at concentrations above 200 nM (~100 ng/mL).(9) While this cannot be extrapolated directly to chronic oral GLY administration, our simulated median GLY steady-state concentration in the GDM subjects at a dose of 23.75 mg orally twice daily was comparable (98.4 ng/mL). Two other studies in T2DM patients showed little benefit in glucose response with single dose GLY > 5 mg (n=12)(10) and chronic GLY

dosing > 15 mg/day (n=14)(11). Given the insulin resistance and limited beta cell reserve in patients with GDM and T2DM, a ceiling to glyburide's therapeutic efficacy is not surprising.

Theoretically, the gestational differences in GLY PK could be explained by alterations in bioavailability, hepatic blood flow, fraction unbound and/or intrinsic clearance. However, our data are most suggestive of increased intestinal and/or hepatic metabolism since unbound GLY CL/F and M1 formation CL were increased during pregnancy. Other factors are less likely the cause of GLY's PK changes [e.g. change in hepatic blood flow (would not change oral AUC), change in absorption (no change seen in percent of dose recovered in the urine as M1) and change in fraction unbound (no change in protein binding)]. While intestinal blood flow may be increased as a result of the gestational increase in cardiac output, this would likely result in an increase in bioavailability and higher concentrations rather than the lower concentrations seen in our study.

Increased GLY CL/F may be a result of induction of CYP2C9, CYP3A and/or CYP2C19 since these are the enzymes involved in GLY metabolism *in vitro*.(12,13) However, GLY appears to be a CYP2C9 substrate *in vivo* in the non-pregnant population.(14–17) Our study revealed a gestational increase in GLY CL/F with both poor and extensive CYP2C9 genotypes, which most likely reflect induction of CP2C9 and CYP3A, since these activities were previously shown to be increased (and CYP2C19 activity decreased) during pregnancy.(18–20)

Since pregnancy appears to alter GLY metabolism, we assessed its effects on GLY's primary metabolite (M1). Two small studies in non-pregnant subjects demonstrated significant, but highly variable, hypoglycemic activity with 4-trans-hydroxyglyburide (M1) and 3-cis-hydroxyglyburide.(21,22) In addition to the 2 previously identified GLY metabolites, 4 other metabolites are formed by human liver and placental microsomes.(23) In our study, there was no difference in M1 AUC during pregnancy. However, the other metabolites may or may not be clinically important depending on their concentrations and pharmacologic activities. One of the metabolites (M5) is of particular interest, because of its high formation by human placenta and potential for fetal circulation access.(23)

While euglycemic hyperinsulinemic and hyperglycemic clamp studies remain the “gold standard” for assessment of SI and Φ_{total} , the oral glucose tolerance test (OGTT) has been validated in normal subjects against the euglycemic hyperinsulinemic clamp, with good correlation between SI, Φ_{total} and DI estimates from the OGTT and MMTT.(24) Normal pregnancy is characterized by insulin resistance with compensatory augmentation of insulin production, particularly in the 3rd trimester.(25–29) Results from our healthy pregnant women are consistent with expected changes during pregnancy. GDM is characterized by more severe insulin resistance and impaired beta-cell compensation, as reflected by our results.(30–32) In response to the MMTT, SI was lower in women with GDM compared to healthy pregnant women. The Φ_{total} (beta-cells' ability to secrete insulin in response to glucose concentrations) was similar in GLY-treated GDM and healthy pregnant women reflecting the pharmacologic activity of GLY. The static component (Φ_s) was similar in healthy pregnant women and in those with GDM, each being higher than in non-pregnant women with T2DM. In contrast, the dynamic component (Φ_d) was similarly impaired in women with GDM and T2DM compared with healthy pregnant women. Although insulin secretion was significantly enhanced, the effects of GLY were still not enough to compensate for the degree of insulin resistance exhibited by the women with GDM, as demonstrated by the significantly lower DI (beta-cell response to glucose concentration corrected for the prevailing insulin sensitivity). This can also be seen in the higher glucose C_{max} and AUC despite higher insulin and C-peptide concentrations in GDM relative to the healthy pregnant women (Figure 3).

Utilizing the MMTT with model-based analysis, Basu et al.(33) reported Φ_{total} ($43.0 \pm 13.5 \times 10^{-9} \text{ min}^{-1}$), SI ($8.7 \pm 4.9 \times 10^{-4} \text{ min}^{-1} \text{ per } \mu\text{U/mL}$) and DI ($378 \pm 263 \times 10^{-13} \text{ min}^{-2} \text{ per } \mu\text{U/mL}$) in non-pregnant, non-diabetic women (n=28, age 23 ± 3 years, BMI $25 \pm 3 \text{ kg/m}^2$). Φ_{total} was higher ($p < 0.0001$), and SI lower ($p < 0.0001$) in our GLY-treated women with GDM than in these non-pregnant healthy women. The DI in our women with GDM was significantly lower ($p = 0.003$) than these non-pregnant subjects.(33) This suggests that glyburide failed to adequately augment insulin secretion in the women with GDM, given their degree of insulin resistance. In contrast, the overall DI in our healthy pregnant women was much higher than previously reported in Basu et al., nonpregnant, non-diabetic women ($p < 0.005$), reflecting the adequacy of their compensatory increase in insulin secretion to compensate for the insulin resistance which accompanies normal pregnancy. Buchanan et al. analyzed data from a limited number of subjects (n=7 GDM and n=8 healthy pregnant) and reported that the DI for pregnant women (healthy and those with GDM) was the same during and after pregnancy.(32,34)

GDM is a heterogeneous disorder with a wide range of beta-cell dysfunction and insulin resistance. Mechanistically individualized therapy may optimize glucose control. While GLY dose titration may be adequate in some women, others may benefit from additional or alternative therapy to improve the insulin resistance, which characterizes GDM (Figure 4). In addition, therapy may be further optimized by the use of agents, which improve postprandial rather than fasting hyperglycemia by normalizing the dynamic component of beta cell response.

Current GDM treatment improves fetal and neonatal outcomes;(25) although, normalization of postprandial hyperglycemia may further improve outcomes. When blood glucose concentrations are: fasting $< 95 \text{ mg/dL}$, 1-hour postprandial $< 140 \text{ mg/dL}$ and 2-hour postprandial $< 120 \text{ mg/dL}$, adverse neonatal outcomes are decreased.(35) However, these targets are not based on normalizing glycemic physiology nor is there a consensus on optimal glucose targets in GDM. The HAPO Study demonstrated a strong continuous relationship between maternal glucose concentrations (fasting and post-glucose challenge) and complications.(36) In addition, they reported continuous association between maternal glucose concentration and risk of increased birth weight even for concentrations below those meeting criteria for GDM.(36) It is unclear whether attempting to further tighten glycemic control in women with GDM will compensate for the increased risk of hypoglycemia and whether this could be achieved using drugs which merely increase maternal insulin rather than addressing insulin resistance or postprandial glucose excursions.

In our study, fasting glucose concentrations in women on GLY with clinically controlled GDM were, by design, not significantly different from those in the gestational age-matched healthy pregnant controls. The main difference between these two groups was the pronounced postprandial glucose excursion revealed by the MMTT in the women with GDM (Figure 3). Since macrosomia has been associated with fasting hyperglycemia as well as poor postprandial glucose control,(22,37,38) it is possible that further normalization of the overall metabolic state in women with GDM will improve postprandial glycemic control as well as fetal and neonatal outcomes. For insulin-requiring GDM patients, dosage adjustment targeted to maintain 1 hour postprandial glucose concentrations $< 140 \text{ mg/dL}$ resulted in decreased risk of complications when compared with adjustments only targeted to maintain fasting glucose between 60–105 mg/dL.(39) However, tighter control of fasting glucose concentrations in that study may have eliminated the difference. Our data suggest that increasing insulin secretion with higher GLY dosage will not shift the overall DI for many women with GDM to normal values in pregnancy. This is particularly true for those with severe insulin resistance whose insulin concentrations have already been greatly and perhaps maximally augmented by GLY (Figures 3 & 4). Improved metabolic control in many patients will require combination therapy in part directed at their underlying insulin resistance. It is possible that such a strategy will alleviate beta-cell

stress and preserve beta-cell function. Recently, the MiG Trial investigators reported that metformin alone or with supplemental insulin did not increase perinatal complications as compared to insulin alone in the treatment of GDM.(40)

In summary, we observed striking gestational augmentation of GLY CL/F, suggesting that patients with inadequate glucose control might benefit from increased GLY dosage. The results show comparable concentrations achieved with 1.25–10 mg twice daily in the non-pregnant subjects as with 1.25–23.75 twice daily in GDM. However, GLY dosage increases above the current maximum (10 mg twice daily) should be evaluated with attention to fetal and neonatal safety, given the demonstrated ability of GLY to cross the placenta. Due to inadequate data, particularly with respect to fetal safety, we are unable to recommend clinical guidelines for glyburide dosage at this time. In addition, our results suggest that alternate medication selection may improve overall metabolic control beyond results achieved with GLY monotherapy in women with GDM. Patients achieving inadequate glucose control with GLY monotherapy may benefit from combination therapy including medication targeted at the severe insulin resistance, such as metformin. Additional research is needed to determine if more aggressive or mechanistically based management of women with GDM will further improve maternal, fetal and neonatal outcomes with acceptable risk. If tighter glucose control becomes desirable, it is likely that many patients will not achieve glycemic control with GLY monotherapy.

METHODS

Subjects

The study was approved by the IRBs at the University of Washington; University of Texas Medical Branch, Galveston; University of Pittsburgh; Georgetown University and MedStar Research Institute and conducted in accordance with their guidelines. This study included 40 GDM (28–38 weeks gestation), 40 healthy pregnant and 26 non-pregnant T2DM women. The GDM women were eligible if they had GDM as defined by Carpenter and Coustan,(41) receiving GLY monotherapy on a stable dose for ≥ 1 week and clinically controlled on GLY with fasting glucose concentration of < 95 mg/dL. Women were excluded for hematocrit $< 28\%$, drug interactions with GLY or drugs that alter blood glucose concentrations. Healthy pregnant women were included if they had a normal OGTT and singleton pregnancy. They were gestational age-matched to the women with GDM and receiving no medications expected to alter blood glucose concentrations. Subjects with T2DM were eligible if they were female, not pregnant and receiving GLY for therapeutic reasons with a stable dose for ≥ 1 week. Women with T2DM were excluded for drug interactions with GLY, receiving corticosteroids, age > 55 years or hematocrit $< 28\%$.

The GLY dosage for GDM and T2DM subjects were determined by their prescribing physicians based on clinical need. All 40 women with GDM and 25 women with T2DM were receiving GLY twice daily. One woman with T2DM was receiving GLY once daily. Investigators provided GLY to all subjects for the week prior to the pharmacokinetic study and subjects were asked to complete dosing calendars for documentation of administration times.

Serial blood samples (6 mLs each) were collected for GLY (total and unbound) and M1 plasma concentrations: pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours postdosing. An additional 24-hour post-dosing sample was collected from the subject taking GLY once daily. Urine was collected over 1 dosing interval to determine M1 concentration.

Sample Analysis

Plasma concentrations of GLY as well as plasma and urine concentrations of M1 were determined using a validated HPLC-MS assay described previously (CV% <15% and LOQ 0.25 ng/mL).(42)

Protein binding—The plasma protein binding of glyburide was determined by ultrafiltration using Millipore Centrifree® ultrafiltration cartridges. Briefly, ³H-GLY (2.25 µCi; 26.5 ng; 42 Ci/mmol; PerkinElmer Life and Analytical Sciences) was aliquoted into tubes and evaporated to dryness. Then, 450 µL of C_{max} and 12 h plasma sample of each subject were added. Tubes were incubated at 37° C for at least 30 minutes and then 400 µL of the plasma sample was centrifuged at 1000 g for 15 minutes (~30 µL of filtrate collected). An aliquot (30 µL) of the unfiltered plasma and the filtrate was counted on a scintillation counter. Preliminary experiments demonstrated constant GLY protein binding over the range of 25–750 ng/mL. ³H-GLY spiked and filtered phosphate buffer saline percent recovery was 81 ± 1.6% (n=10).

Buccal cell DNA was isolated using a PUREGENE™ buccal cell kit (Gentra Systems, Minneapolis, MN). The CYP2C9*2 and *3 genotypes (430C>T and 1075A>C, respectively) were determined using Drug Metabolism Genotyping Taqman® allelic discrimination assays (Applied Biosystems). For CYP2C9*5/*11 genotyping (1080C>G and 1003C>T, respectively), primer sequences from Higashi et al.(43) were utilized and genomic DNA was amplified by PCR followed by dye-terminator sequencing of the amplicons. The CYP2C9*6 (818deleteA) genotype was determined by DNA sequencing of amplified DNA using the same conditions developed for CYP2C9*5/*11. The PCR primers were CYP2C9*6-4F.FOR (5'-TTAGATCTGCAATAATTTTCTCCTATCA- 3') and CYP2C9*6-301R.REV (5'-AAGTGCTTCTCAAGCATTACTGATTG-3') and the CYP2C9*6-4F.FOR primer was used for product sequencing and genotype determination.

Pharmacokinetic analysis

Noncompartmental analysis—Steady state pharmacokinetic parameters were estimated using standard non-compartmental techniques. Area under the concentration-time curve (AUC) was estimated using linear trapezoidal rule. Apparent oral clearance was estimated by $CL/F = \text{Dose}/AUC_{0-\tau}$ in which τ was the duration of the dosing interval. Formation clearance of M1 was estimated as $CL_{\text{form, 4 trans OH-gly}} = Ae/AUC_{\text{glyburide}}$ where Ae was the amount of M1 (after deconjugation) in the urine collection. C_{max} and time to maximum concentration (T_{max}) were determined from the measured concentrations. Unbound apparent oral clearance of GLY (CL/F_{unbound}) and the unbound formation clearance (CL_{unbound form, 4 trans OH-gly}) of M1 were estimated as the ratio of the respective clearance values and fraction unbound of GLY in plasma. AUC and C_{max} were dose-normalized to 1 mg GLY dose by dividing by the actual dose the subject received every 12 hours.

Compartmental modeling and simulations—GLY PK was described by a one-compartment model with first order elimination.(44) The nonlinear absorption was best described by an inverse Gaussian density function (14)

$$f_A(t)=F\sqrt{\frac{MAT}{2\pi CV^2 t^3}}\exp\left[-\frac{(t-MAT)^2}{2CV^2 MAT t}\right].$$

Model parameters fitted were volume of distribution (V/F), clearance (CL/F), mean absorption time (MAT) and normalized variance (CV²). Bioavailability (F) is fixed to 1 during estimation,

so that the clearance and volume of distribution are both apparent estimates. GLY measurement error was assumed to be a constant, but unknown coefficient of variation. To explore expected variability in GLY concentrations for various dosage regimens in women with GDM and T2DM, Monte Carlo simulation was performed using the sample statistics of the individual estimates.(45) In particular, we simulated 1.25–10 mg of GLY every 12 h for non-pregnant women with T2DM, and 1.25–25 mg of GLY every 12 h for women with GDM. Simulations were performed for dosage increments of 1.25 mg within the dosage range. A hundred sets of parameters were obtained for every dosage level. From these, the concentration-time curves were computed. Measures of interest included minimum concentration, C_{max} and 99% confidence interval for the simulated GLY AUC at steady-state.

Mixed meal tolerance test

A MMTT (1 can of Boost Plus® energy drink, 2 slices of whole wheat toast and 2 teaspoons of margarine consumed within 10 minutes) was administered to all study subjects with serial blood samples collected for measurement of serum insulin, glucose and C-peptide concentrations at: pre-MMTT, 10, 20, 30, 60, 90, 120, 150, 180 and 240 minutes post-initiation of the MMTT.

Mixed Meal Tolerance Test Modeling

Insulin sensitivity (SI)—Insulin sensitivity was estimated from glucose and insulin concentrations using the minimal model of glucose kinetics following a MMTT. The model structure and equations have been previously published.(46) SI is defined as the ability of insulin to normalize glucose levels by stimulating uptake of glucose and suppressing its production.

Beta-cell Responsivity Index (Φ_{total})— Φ_{total} during the MMTT was estimated from serum glucose and C-peptide concentrations using the minimal model of C-peptide kinetics.(47,48) C-peptide was used in the model to provide an accurate reconstruction of pre-hepatic secretion. The equations for the model have been published previously (48).

Disposition Index (DI)—The DI is calculated for each individual subject as the product of SI and Φ_{total} . It allows for evaluation of an individual's glucose tolerance level as a function of both insulin sensitivity and beta-cell function.

Model identification and parameter estimation—To make the glucose minimal model *a priori* uniquely identifiable; glucose effectiveness, volume of distribution of glucose, and insulin action parameter were fixed to their expected population means 0.014 min^{-1} , 1.45 dl/kg , and 0.011 min^{-1} respectively, reported previously.(46,47) The area under glucose absorption rate $R_a(t)$ was constrained to equal the total amount of ingested glucose, multiplied by the fraction that is actually absorbed, f (fixed to 0.9).(47,49) Insulin concentrations were used as linearly interpolated forcing functions to the glucose subsystem. Glucose measurement error was characterized with an unknown standard deviation proportional to the concentrations. For the purpose of *a priori* identifiability of C-peptide minimal model, kinetic parameters are fixed to standard population values. C-peptide measurement error was assumed to be independent, Gaussian with a mean of zero and a constant, but unknown variance. Glucose concentration and its time-derivative were linearly-interpolated and assumed as error-free inputs to the model.(49,50) Model parameters for individual subject and their precisions were estimated with nonlinear least squares regression using the SAAM II software system (University of Washington, Seattle, WA). The AUCs for glucose, C-peptide and insulin were calculated using the trapezoidal rule. In some individuals, maximum *a posteriori* Bayesian priors were required for some of the parameters.

Statistical Analysis

Data are presented as means $\pm$ SD. Wilcoxon signed rank test was used to compare parameters. Pharmacokinetic parameters were adjusted using actual body weight. Significance level was set at 5%.

Acknowledgments

This work was supported by grants from NIH/NICHD Grants #U10HD047892, #U10HD047905, #U10HD047890 and #U10HD047891 and well as NIH/NCRR Grants #UL1RR025014, #M01RR00037, #M01RR023942 and P41EB001975.

References

1. Langer O, Conway DL, Berkus MD, Xenakis EMJ, Gonzales O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. *N Engl J Med* 2000;343:1134–1138. [PubMed: 11036118]
2. Elliot BD, et al. Comparative placental transfer of oral hypoglycemic agents in humans: a model of human placental drug transfer. *Am J Obstet Gynecol* 1994;171:653–660. [PubMed: 8092211]
3. Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2002;72:326–332. [PubMed: 12235454]
5. Groop LC, et al. Comparison of pharmacokinetics, metabolic effects and mechanisms of action of glyburide and glipizide during long-term treatment. *Diabetes Care* 1987;10:671–678.
6. Jacobson GF, Ramos GA, Ching JY, Kirby RS, Ferra A, Field DR. Comparison of glyburide and insulin for the management of gestational diabetes in a large managed care organization. *Am J Obstet Gynecol* 2005;193:118–124. [PubMed: 16021069]
7. Kremer CJ, Duff P. Glyburide for the treatment of gestational diabetes. *Am J Obstet Gynecol* 2004;190:1438–1439. [PubMed: 15167862]
8. Chmait R, Dinise T, Moore T. Prospective observational study to establish predictors of glyburide success in women with gestational diabetes mellitus. *J Perinatol* 2004;24:617–622. [PubMed: 15152273]
9. Glyburide Product Labeling. Teva Pharmaceuticals USA. Sellersville PA. Revised 2/2005
10. Groop LC, et al. Dose-dependent effects of glyburide on insulin secretion and glucose uptake in humans. *Diabetes Care* 1991;14:724–727. [PubMed: 1954808]
11. Coppack SW, Lant AF, McIntosh CS, Rodgers AV. Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus. *Br J Clin Pharmac* 1990;29:673–684.
11. Rochon M, Rand L, Roth L, Gaddipati S. Glyburide for the management of gestational diabetes: risk factors predictive of failure and associated pregnancy outcomes. *Am J Obstet Gynecol* 2006;195:1090–1094. [PubMed: 17000241]
12. Naritomi Y, Terashita S, Kagayama A. Identification and relative contributions of human cytochrome P450 isoforms involved in the metabolism of glibenclamide and lansoprazole: evaluation of an approach based on the in vitro substrate disappearance rate. *Xenobiotica* 2004;34:415–417. [PubMed: 15370958]
13. Van Giersbergen PLM, Treiber A, Clozel M, Bodin F, Dingemans J. In vivo and in vitro studies exploring the pharmacokinetic interaction between bosentan, a dual endothelin receptor antagonist and glyburide. *Clin Pharmacol Ther* 2002;71:253–262. [PubMed: 11956508]
14. Kirchheiner J, et al. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002;71:286–296. [PubMed: 11956512]
15. Yin OQP, Tomlinson B, Chow MSS. CYP2C9 but not CYP2C19 polymorphisms affect the pharmacokinetics and pharmacodynamics of glyburide in Chinese subjects. *Clin Pharmacol Ther* 2005;78:370–377. [PubMed: 16198656]

16. Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ, Kivisto KT. Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther* 2005;78:90–92. [PubMed: 16003298]
17. Zhang YF, Chen XY, Guo YJ, Si DY, Zhou H, Zhong DF. Impact of cytochrome P450 CYP2C9 variant allele CYP2C9*3 on the pharmacokinetics of glibenclamide and lornoxicam in Chinese subjects. *Yao Xue Xue Bao* 2005;40:796–799. [PubMed: 16342679]
18. Hebert MF, et al. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther* 2008;84:248–253. [PubMed: 18288078]
19. Yerby MS, et al. Pharmacokinetics of anticonvulsants in pregnancy: alterations in plasma protein binding. *Epilepsy Res* 1990;5:223–228. [PubMed: 2384078]
20. McGready R, et al. Pregnancy and use of oral contraceptives reduces the biotransformation of progesterone to androstenedione. *Eur J Clin Pharmacol* 2003;59:553–557. [PubMed: 12955370]
21. Rydberg T, Jonsson A, Karlsson MO, Melander A. Concentration-effect relations of glibenclamide and its active metabolites in man: modeling of pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 1997;43:373–381. [PubMed: 9146849]
22. Rydberg T, Jonsson A, Roder M, Melander A. Hypoglycemic activity of glyburide (glibenclamide) metabolites in humans. *Diabetes Care* 1994;17:1026–1030. [PubMed: 7988301]
23. Ravindran S, Zharikova OL, Hill RA, Nanovskaya TN, Hankins GDV, Ahmed MS. Identification of glyburide metabolites formed by hepatic and placental microsomes of humans and baboons. *Biochem Pharmacol* 2006;72:1730–1737. [PubMed: 17011523]
24. Dalla Man C, et al. Insulin sensitivity by oral glucose minimal models: validation against clamp. *Am J Physiol Endocrinol Metab* 2005;289:954–959.
25. Crowther CA, Hillier JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. for the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005;352:2477–2486. [PubMed: 15951574]
26. Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims FA. Longitudinal changes in insulin release and insulin resistance in non obese pregnant women. *Am J Obstet Gynecol* 1991;165:1667–1672. [PubMed: 1750458]
27. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes. *Am J Obstet Gynecol* 1999;180:903–916. [PubMed: 10203659]
28. Buchanan TA, Metzger BE, Freinkel N, Bergman RN. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. *Am J Obstet Gynecol* 1990;162:1008–1014. [PubMed: 2183610]
29. Di Cianni C, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. *Diabetes Metab Res Rev* 2003;19:259–270. [PubMed: 12879403]
30. Buchanan TA, Catalano PM. The pathogenesis of GDM: implications for diabetes after pregnancy. *Diabetes Rev* 1995;3:584–610.
31. Kuhl C. Insulin secretion and insulin resistance in pregnancy and GDM: implications for diagnosis and management. *Diabetes* 1991;40:18–24. [PubMed: 1748255]
32. Homko C, Sivan E, Chen X, Reece EA, Boden G. Insulin secretion during and after pregnancy in patients with gestational diabetes mellitus. *J Clin Endocrinol Metab* 2001;86:568–573. [PubMed: 11158010]
33. Basu R, et al. Effects of age and sex on postprandial glucose metabolism. Differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. *Diabetes* 2006;55:2001–2014. [PubMed: 16804069]
34. Buchanan TA. Pancreatic B-cell defect in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. *J Clin Endocrinol Metab* 2001;86:989–993.
35. Gonzalez-Quintero VH, et al. The impact of glycemic control on neonatal outcome in singleton pregnancies complicated by gestational diabetes. *Diabetes Care* 2007;30:467–470. [PubMed: 17327306]

36. The HAPO Study Cooperative Research Study Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 1991;358:2001–2008.
37. Javanovic-Peterson L, et al. and the National Institute of Child Health and Human Development – Diabetes in Early Pregnancy Study. Maternal postprandial glucose levels and infant birth weight: the diabetes in early pregnancy study. *Am J Obstet Gynecol* 1991;164:103–111. [PubMed: 1986596]
38. Combs CA, Gunderson E, Kitzmiller JL, Gavin LA, Main EK. Relationship of fetal macrosomia to maternal postprandial glucose control during pregnancy. *Diabetes Care* 1992;15:1251–1257. [PubMed: 1425084]
39. De Veciana M, et al. Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. *N Engl J Med* 1995;333:1237–1241. [PubMed: 7565999]
40. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP. for the MiG Trial Investigators. Metformin versus insulin for the treatment of gestational diabetes. *N Engl J Med* 2008;358:2003–2015. [PubMed: 18463376]
41. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 1982;144:768–773. [PubMed: 7148898]
42. Naraharisetti SB, Kirby BJ, Hebert MF, Easterling TR, Unadkat JD. Validation of a sensitive LC-MS assay for quantification of glyburide and its metabolite 4-transhydroxy glyburide in plasma and urine: an OPRU Network study. *J Chromatog B* 2007;860:34–41.
43. Higashi MK, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002;287:1690–1698. [PubMed: 11926893]
44. Tracewell WG, Stalker DJ, Maloley PA, Gallagher TF, Gwilt PR. Population pharmacokinetics of glyburide in patients with well-controlled diabetes. *Pharmacotherapy* 1998;18:51–56. [PubMed: 9469681]
45. Andrew MA, et al. Amoxicillin Pharmacokinetics in pregnant women: Modeling and simulations of dosage strategies. *Clin Pharmacol Ther* 2007;81:547–556. [PubMed: 17329990]
46. Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: estimation of insulin sensitivity from a meal test. *IEEE Trans Biomed Eng* 2002;49:419–429. [PubMed: 12002173]
47. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. *Am J Physiol Endocrinol Metab* 2004;287:637–643.
48. Toffolo G, Breda E, Cavaghan MK, Ehrmann DA, Polonsky KS, Cobelli C. Quantitative indexes of beta-cell function during graded up & down glucose infusion from C-peptide minimal models. *Am J Physiol Endocrinol Metab* 2001;280:2–10.
49. Dalla Man C, et al. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsiveness and insulin sensitivity in nondiabetic individuals. *Diabetes* 2005;54:3265–3273. [PubMed: 16249454]
50. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. *Diabetes* 2001;50:150–158. [PubMed: 11147781]

Figure 1.

Dose-normalized, steady state, mean glyburide plasma concentration-time profiles in gestational diabetic subjects during pregnancy and non-pregnant type 2 diabetic control subjects. Error bars represent standard deviations.

Figure 2.

Semi logarithmic plot of simulated steady-state glyburide plasma concentration-time curves in gestational diabetic subjects denoted by red lines (1.25 mg to 23.75 mg glyburide every 12 hours) and non-pregnant type 2 diabetic control subjects (1.25 mg to 10 mg of glyburide every 12 hours) with the 99% confidence interval bordered by the blue lines. The simulations are based on a hundred sets of parameters at each dosage, with dosage increased in increments of 1.25 mg within the dosage range for both groups.

Figure 3. Mean glucose (panel A), insulin (panel B) and C-peptide (panel C) concentration time profiles following mixed meal tolerance test in gestational diabetic subjects considered to be clinically controlled with glyburide (diamonds), gestational age-matched healthy pregnant subjects (triangles) and non-pregnant type 2 diabetic subjects (squares). Error bars represent standard deviations.

Hyperbolic Relationships and Averaged Values for GDM, Type2 DM Controls and Healthy Pregnancy Controls

Figure 4.

Hyperbolic relationship between beta-cell responsivity index and insulin sensitivity for normal healthy pregnant subjects (mean: dotted line, individual subjects: open triangles), gestational diabetic subjects (mean: solid line, individual subjects: open diamonds), type 2 diabetic subjects (mean: dashed line, individual subjects: open squares). Large solid circle on each line represents the mean for the population. The hyperbolas for each group have been calculated not by fitting curves to the points, but rather by plotting the mean disposition index for each group (GDM: 193.1 min⁻² per μ U/mL; Healthy pregnant: 698.8 min⁻² per μ U/mL; T2DM: 165.6 min⁻² per μ U/mL). Therefore, the plot lines describe all the possible pairs of insulin sensitivity index and beta-cell responsivity index for a typical gestational diabetic, healthy pregnant and type-2 diabetic subject.

Table 1

Demographics for gestational diabetic subjects (GDM), gestational age-matched healthy pregnant subjects (healthy pregnant) and non-pregnant type 2 diabetic subjects (T2DM)

	GDM (n=40)	Healthy Pregnant (n=40)	T2DM (n=26)
Age (years)	32 ± 6	27 ± 7	41 ± 8
Actual Body Weight (kg)	96 ± 22	80 ± 18	100 ± 19
Height (cm)	164 ± 8	164 ± 8	163 ± 8
BMI (kg/m ²)	36 ± 6	30 ± 6	37 ± 4
Gestational Age (weeks)	34.6 ± 2.9	34.7 ± 2.9	Non-pregnant
Average Glyburide Dose (mg orally twice daily)	3.4 ± 2.4 (Range 1.25–10)	0	3.8 ± 3.4 (Range 1.25–10)
Race/Ethnicity	17 White	15 White	9 White
	11 Hispanic/Latina	8 Hispanic/Latina	10 Hispanic/Latina
	9 Black	14 Black	3 Black
	1 Black/White	1 Asian	2 Asian
	1 Native American/White	1 Pacific Islander	1 Pacific Islander
	1 Hispanic/Latina/White	1 Black/White	1 Pacific Islander/White
CYP2C9 Genotype	34 subjects *1*1		19 subjects *1*1
	4 subjects *1*2		4 subjects *1*2
	2 subjects *1*3		1 subject* 1*3
			1 subject *1*2/* 1*3
			1 subject *2*2

Table 2

Estimated steady-state glyburide pharmacokinetic parameters (mean $\pm$ SD) in gestational diabetic subjects (n=40) and non-pregnant type 2 diabetic subjects (n=26)

Parameter	GDM	T2DM	Percent Change in Mean with Pregnancy	p value
AUC _{0→12} , dose-normalized (ng/hr/mL)	72 $\pm$ 27	153 $\pm$ 69 *	↓53%	0.0001
C _{max} , dose-normalized (ng/mL)	15 $\pm$ 8	33 $\pm$ 21 *	↓55%	< 0.0001
CL/F (L/hr)	17.1 $\pm$ 11.5	8.3 $\pm$ 4.8	↑106%	< 0.0001
CL/F (mL/hr/kg)	170 $\pm$ 88	85 $\pm$ 53	↑100%	< 0.0001
CL/F _{unbound} (L/hr)	1103 $\pm$ 749	537 $\pm$ 313	↑105%	< 0.0001
CL/F _{unbound} (L/hr/kg)	10.9 $\pm$ 5.8	5.5 $\pm$ 3.4	↑98%	< 0.0001
Percent unbound (%)	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1	↔	0.2
T _{max} (hours)	3.0 $\pm$ 1.5	3.5 $\pm$ 1.9	↔	0.4
CL _{form} , 4 trans OH-gly (L/hr)	4.7 $\pm$ 2.6	2.0 $\pm$ 0.9	↑135%	< 0.0001
CL _{form} , 4 trans OH-gly (mL/hr/kg)	48.9 $\pm$ 28.7	20.1 $\pm$ 8.6	↑143%	< 0.0001
CL _{unbound form} , 4 trans OH-gly (L/hr)	303 $\pm$ 184	128 $\pm$ 59	↑137%	< 0.0001
CL _{unbound form} , 4 trans OH-gly (L/hr/kg)	3.2 $\pm$ 2.0	1.3 $\pm$ 0.6	↑146%	< 0.0001
AUC _{4 trans OH-gly} /AUC _{gly}	0.26 $\pm$ 0.22	0.14 $\pm$ 0.12	↑89%	0.005

AUC_{0→12}, dose normalized, dose-normalized area under the glyburide plasma concentration-time curve from time zero to 12 hours;

C_{max}, dose normalized, dose-normalized maximum glyburide plasma concentration; CL/F, apparent oral glyburide clearance; CL/F_{unbound}, unbound apparent oral glyburide clearance; T_{max}, time to maximum glyburide concentration, CL_{form}, 4 trans OH-gly, 4-trans-hydroxyglyburide formation clearance, CL_{unbound form}, 4 trans OH-gly, 4-trans-hydroxyglyburide unbound formation clearance and AUC_{4 trans OH-gly}/AUC_{gly}, ratio of area under the concentration-time curves for 4-trans-hydroxyglyburide and glyburide.

* Excludes subject receiving glyburide once daily.

Table 3

Response to the mixed meal tolerance test (MMTT) in gestational diabetic subjects, gestational age-matched healthy pregnant subjects and non-pregnant type 2 diabetic subjects

Parameter	GDM	Healthy Pregnant	T2DM
Insulin Sensitivity SI (10^{-4} min^{-1} per $\mu\text{U/mL}$)	1.2 ± 0.9	5.8 ± 3.9 ($p < 0.0001$)	3.0 ± 3.3 ($p < 0.01$)
Beta-cell Responsivity Index (Total) Φ_{total} (10^{-9} min^{-1})	140.6 ± 99.9	124.0 ± 53.1 ($p = 0.7$)	54.8 ± 48.6 ($p < 0.0001$)
Beta-cell Responsivity Index (Static) Φ_s (10^{-9} min^{-1})	119.2 ± 87.1	95.4 ± 54.2 ($p = 0.5$)	41.4 ± 37.3 ($p < 0.0001$)
Beta-cell Responsivity Index (Dynamic) Φ_d (10^{-9} min^{-1})	1720 ± 1319	3120 ± 1602 ($p < 0.0001$)	1535 ± 1845 ($p = 0.2$)
Disposition Index DI ($10^{-13} \text{ min}^{-2}$ per $\mu\text{U/mL}$)	193.1 ± 225.5	698.8 ± 540.7 ($p < 0.0001$)	165.6 ± 232.6 ($p = 0.3$)
Peak post-MMTT glucose concentration	167 ± 33	122 ± 16 ($p < 0.0001$)	187 ± 70 ($p = 0.1$)

Φ_{total} : global index, the average increase above basal beta-cell secretion divided by the average glucose stimulus above the threshold level. Φ_s : static index, the effect of steady state glucose concentration on beta-cell insulin secretion. Φ_d : dynamic index, the stimulatory effect of the rate of glucose increasing on the insulin secretion. Statistical comparisons with GDM subjects.