Pharmacodynamics of Metformin in Pregnant Women With Gestational Diabetes Mellitus and Nonpregnant Women With Type 2 Diabetes Mellitus

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Abstract

Gestational diabetes mellitus is a condition similar to type 2 diabetes mellitus (T2DM) in that patients are unable to compensate for the degree of insulin resistance, and both conditions are often treated with metformin. The comparative pharmacodynamic response to metformin in these 2 populations has not been studied. This study characterized insulin sensitivity, β-cell responsivity, and disposition index following a mixed-meal tolerance test utilizing a minimal model of glucose, insulin, and C-peptide kinetics before and during treatment with metformin. The study included women with gestational diabetes mellitus (n = 34), T2DM (n = 14), and healthy pregnant women (n = 30). Before treatment, the gestational diabetes mellitus group had significantly higher baseline (45%), dynamic (68%), static (71%), and total β-cell responsivity (71%) than the T2DM group. Metformin significantly increased insulin sensitivity (51%) as well as disposition index (97%) and decreased mixed-meal tolerance test peak glucose concentrations (8%) in women with gestational diabetes mellitus after adjustment for gestational age–dependent effects; however, in women with T2DM metformin only significantly affected peak glucose concentrations (22%) and had no significant effect on any other parameters. Metformin had a greater effect on the change in disposition index (Δ disposition index) in women with gestational diabetes mellitus than in those with T2DM ($P = .01$). In conclusion, response to metformin in women with gestational diabetes mellitus is significantly different from that in women with T2DM, which is likely related to the differences in disease severity.

Keywords

β-cell responsivity, disposition index, gestational diabetes mellitus, insulin sensitivity, metformin, pregnancy, pharmacodynamics, type 2 diabetes mellitus

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Gestational diabetes mellitus is a common complication during pregnancy and is associated with significant adverse effects for the mother, fetus, and neonate.[1–4] The underlying abnormalities and risk factors are similar in women with gestational diabetes mellitus and those with type 2 diabetes mellitus (T2DM), although, in general, disease severity is greater with T2DM.[5,6] In both gestational diabetes mellitus and T2DM, patients have marked insulin resistance and an inability to compensate for the degree of insulin resistance. Metformin is a drug that lowers glucose concentrations primarily by decreasing insulin resistance through increasing peripheral glucose uptake and utilization. Metformin also decreases intestinal glucose absorption and hepatic glucose production. Metformin is commonly used in the treatment of women with T2DM and in women with gestational diabetes mellitus.[7] During pregnancy, the majority of medications, including oral glucose-lowering drugs such as metformin, are utilized in a similar manner to their Food and Drug Administration–approved indication in the nonpregnant population, without comparative clinical pharmacological studies being conducted during pregnancy to determine if this approach is appropriate.[5–7] Physiological, biochemical, and hormonal changes during pregnancy are known to alter the pharmacokinetics (PK) of drugs throughout gestation.[8] The renal clearance of metformin is 49% higher in midpregnancy and 29% higher in late pregnancy compared with to the nonpregnant state.[9] In addition, we know that pregnancy is associated with insulin resistance, hyperinsulinemia, and changes in glucose handling.[7] With differences in metformin exposure, glucose handling, and disease severity between gestational diabetes mellitus and T2DM,[7] it is likely that the pharmacodynamics (PD) response to metformin will also differ between pregnant women with gestational diabetes mellitus and nonpregnant women with T2DM. No data exist on how the PD of metformin in the treatment of women with gestational diabetes mellitus compares to that in women with T2DM. The objectives of this study were to describe and provide preliminary data comparing the PD response to metformin in women with gestational diabetes mellitus and nonpregnant women with T2DM. Mathematical modeling of glucose, insulin, and C-peptide dynamics following a mixed-meal tolerance test was used to estimate insulin sensitivity, β-cell responsivity, and disposition index in pregnant women with gestational diabetes mellitus and nonpregnant women with T2DM.[5,10] Insulin sensitivity and β-cell responsivity are hyperbolically related and informative in understanding the underlying pathology of gestational diabetes mellitus and T2DM as well as understanding metformin's pharmacology.[10,11]

Methods

Subjects

The study was approved by the institutional review boards at the University of Washington, Madigan Army Medical Center, University of Texas Medical Branch in Galveston, University of Pittsburgh, Indiana University, University of Utah Health Care, University of Alabama at Birmingham, and RTI International and was conducted in accordance with their guidelines. All subjects gave written, informed consent. This was a multicenter, prospective, phase 1/2 longitudinal PD study (clinicaltrials.gov identifier NCT01329016). There were 3 groups of women recruited for this study: pregnant women with a diagnosis of gestational diabetes mellitus (n = 34), gestational age-matched healthy pregnant women (n = 30), and nonpregnant women with a new diagnosis of T2DM (n = 14). Only women who completed the study and adhered to the protocol were included in the results. Subjects were determined to be nonadherent based on unacceptable study pill counts (any doses missed or time of dosage >1 hour different from expected time of dosing in the 3 days before study day 2), physician clinical impression, or subject's admittance to not having following study protocol.

Entry Criteria

Gestational diabetes mellitus entry criteria included pregnant women before 32 weeks of gestation, singleton pregnancy, 18-45 years of age, failed diet therapy, and required drug treatment. Gestational diabetes mellitus diagnosis was made in 1 of 3 ways based on serum or plasma glucose concentrations: (1) 3-hour oral glucose tolerance test (100 g glucose orally with 2 or more values meeting or exceeding targets: fasting glucose ≥95 mg/dL, 1 hour ≥180 mg/dL, 2 hours ≥155 mg/dL, and 3 hours ≥140 mg/dL), (2) 2-hour oral glucose tolerance test (75 g glucose orally with 1 or more values meeting or exceeding targets, fasting glucose ≥92 mg/dL, 1 hour glucose ≥180 mg/dL, 2 hours glucose >153 mg/dL), or (3) 1-hour oral glucose tolerance test (50 g glucose orally with 1 hour glucose ≥185 mg/dL). The women with gestational diabetes mellitus who received metformin for treatment in this study came from another study in which women with gestational diabetes mellitus were randomized to 1 of 3 treatment arms (metformin, glyburide, or combination therapy with metformin and glyburide).[10] Only the women assigned to metformin monotherapy were included in this study. T2DM entry criteria included nonpregnant women, 18-45 years of age, new diagnosis of T2DM, hemoglobin A1C >7%, and expected to receive metformin treatment. Exclusion criteria for women with gestational

diabetes mellitus or T2DM included medications expected to interact with metformin, medications expected to alter blood glucose concentrations, serum creatinine >1.2 mg/dL, hematocrit <28%, allergy to metformin, significant liver disease (diagnosis of liver disease other than Gilbert syndrome), congestive heart failure or history of myocardial infarction, moderate to severe pulmonary disease (any pulmonary disease requiring drug treatment other than mild exercise-induced asthma requiring only intermittent pharmacologic treatment for which the patient was not currently receiving drug therapy), and adrenal or pituitary insufficiency. Healthy pregnant women's entry criteria included singleton pregnancy, 18-45 years of age, 20-32 weeks of gestation, and a normal 1-hour or 2-hour oral glucose tolerance test. Exclusion criteria for healthy pregnant women included hematocrit <28% or known kidney, liver, heart, pulmonary, adrenal, or pituitary disease as well as those receiving glucose-lowering agents or corticosteroids.

Treatment

All subjects with gestational diabetes mellitus and T2DM in this study were treated with metformin. Titration schematic for subjects with gestational diabetes mellitus can be found in the previously published parent study.[10] In brief, metformin was started at 500 mg orally twice daily and titrated to clinical control or treatment failure. For women with gestational diabetes mellitus, blood glucose concentrations were considered controlled when ≥75% of fasting glucose concentrations were ≤95 mg/dL and ≥75% of either 1-hour postprandial glucose concentrations were <140 mg/dL or 2-hour postprandial glucose concentrations were <120 mg/dL. For women with T2DM, clinical control was based on hemoglobin A1C (target A1C < 7%). Provider discretion was allowed for dosage titration. Dosage initiation and titration for women with T2DM was entirely based on clinical need without regard to the study. Metformin was provided to all subjects with gestational diabetes mellitus and T2DM for the 3 days preceding study day 2. Metformin was at steady state (consistent dosage for a minimum of 1 week) before evaluation of insulin sensitivity, β-cell responsivity, and disposition index during therapy (study day 2). Healthy pregnant subjects did not receive metformin.

Mixed-Meal Tolerance Test

Insulin sensitivity, β-cell responsivity, and disposition index were estimated before (study day 1) and during treatment (study day 2) utilizing a mixed-meal tolerance test, which involved consuming 1 can of Boost Plus energy drink and 2 slices of whole wheat toast with two teaspoons of margarine and consumed within 10 minutes. Serial blood samples were collected before the mixed-meal tolerance test (time = 0) and 10, 20, 30, 60, 90, 120, 150, 180, 210, and 240 minutes following the initiation of the mixed-meal tolerance test to measure serum glucose, insulin, and C-peptide concentrations. Plasma samples were collected at times 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hours postdose, truncated to the dosing interval, for measurement of metformin concentrations. Blood glucose concentrations were also measured at each time point in real time on each study day for safety assessment. Study day 2 took place once subjects achieved clinical control or before switching therapy if they failed to achieve glycemic control. On study day 2, the metformin morning dose was given at time 0 (pre–mixed-meal tolerance test), and blood sampling was conducted as during study day 1. Glucose concentrations were measured using a glucose oxidase/peroxidase assay.[12] Insulin and C-peptide concentrations were measured using previously described radioimmunoassays.[13,14]

Mixed-Meal Tolerance Test Parameter Estimation

Insulin sensitivity; total, baseline, static, and dynamic β-cell responsivity; and disposition index were estimated pretreatment and during metformin treatment as previously described utilizing SAAM II software (version 2.3, The Epsilon Group, Charlottesville, Virginia).[5,10,15–20] In brief, insulin sensitivity, defined as the ability of insulin to normalize glucose concentrations by stimulating uptake of glucose and suppressing its production, was estimated from glucose and insulin concentrations using the minimal model of glucose kinetics after the mixed- meal tolerance test. Model structure and equations have been previously published.[15] β-cell responsivity during the mixed-meal tolerance test was estimated from serum glucose and C-peptide concentrations using the minimal model of C-peptide kinetics. C-peptide was used in the model to provide an accurate reconstruction of prehepatic secretion. The equations for the model have been published previously.[19,20] Disposition index was calculated for each individual subject as the product of insulin sensitivity and total β-cell responsivity. It allows evaluation of an individual's glucose tolerance as a function of both insulin sensitivity and β-cell function.[10,15–20]

The area under the concentration-time curves for glucose, C-peptide, insulin, and metformin were calculated using the trapezoidal rule. PD response to metformin was based on an expected increase in insulin sensitivity. Gestational age-matched healthy pregnant subjects were studied to estimate gestational age-dependent changes in insulin sensitivity and β-cell responsivity between study day 1 and study day 2. Since the PD parameters are gestational age dependent and to evaluate specifically the drug effect, we subtracted out the gestational age effect from study day 2 in the women

with gestational diabetes mellitus. The correction for gestational age-dependent effect was accomplished by subtracting the average difference between study day 2 and study day 1 in the healthy pregnant subjects from individual study day 2 parameters for the subjects with gestational diabetes mellitus.[10] Changes in PD parameters (Δ) were determined by subtracting PD parameters estimated pretreatment on study day 1 from those estimated posttreatment on study day 2 corrected for gestational age-dependent effects in women with gestational diabetes mellitus.

Plasma Metformin Concentration Analysis

Serial blood samples were collected predose and then 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hours postdose, truncated to the dosing interval, for measurement of metformin plasma concentrations utilizing a validated liquid chromatography–tandem mass spectrometric assay as previously described.[21] Metformin area under the concentration-time curve over 1 dosing interval was determined utilizing a trapezoidal rule.

Statistical Analyses

Statistical comparisons of PD parameters between study day 1 and study day 2 utilized a paired Student t test. The mean percentage difference from study day 1 to study day 2 was calculated as the mean change divided by the mean value on study day 1. Statistical comparisons between gestational diabetes mellitus and T2DM utilized an unpaired Student t test. *P*-values were not adjusted for multiple comparisons. Statistical results are reported where appropriate as mean ± SD (95%CI). All statistical analyses were done using R-based programs,[22] and graphs were generated in R using the package ggplot2.[23]

Power Analysis

This study is 1 component of a larger study evaluating the effects of oral glucose-lowering agents in the treatment of women with gestational diabetes mellitus and T2DM. Thirty subjects were anticipated to be needed to detect a 50% change in insulin sensitivity.[10]

Results

Demographics

Demographics for adherent subjects with gestational diabetes mellitus, those with T2DM, and healthy pregnant subjects who completed the study are reported in Table 1. Demographics for all gestational diabetes mellitus and T2DM subjects can be found in Supplementary Table S1. No significant differences were found in race or ethnicity between study arms ($P \approx 1$). Age, weight, and body mass index were not significantly different between gestational diabetes mellitus and T2DM groups. Similar race and ethnicity distributions were reported for healthy pregnant subjects who completed the study. There was an average of 5 weeks between study days for the subjects with gestational diabetes mellitus and 15 weeks for the subjects with T2DM.

Table 1. Demographics, Metformin Dosing, and Area Under the Concentration-Time Curve for Subjects With Gestational Diabetes Mellitus, Nonpregnant Subjects With Type 2 Diabetes Mellitus, and Healthy Pregnant Subjects

	GDM	T2DM	HP
n	25	12	28
Age SD1 (y)	31 ± 5 (22 to 39)	33 ± 7 (23 to 45)	25 ± 5 (18 to 38)
Height SD1 (cm)	163 ± 6 (155 to 179)	160 ± 8 (147 to 175)	162 ± 8 (147 to 178)
Body weight SD1 (kg)	90 ± 20 (70 to 100)	100 ± 30 (60 to 100)	80 ± 10 (50 to 109)
BMI prepregnancy (kg/m^2)	31 ± 6 (21 to 43)	36 ± 7 (24 to 46)	27 ± 5 (20 to 40)
GA, SD1 (wk)	31 ± 2 (20 to 33)	NA	30 ± 1 28 to 33)
GA, SD2 (wk)	35 ± 1 (32 to 38)	NA	36 ± 1 (34 to 38)
Metformin dose SD2 (mg/day)	1400 ± 500 (1000 to 2000)	800 ± 200 (500 to 1000)	NA
Metformin AUC (μg•h/mL)	11 ± 4	12 ± 3	NA
White	80%	75%	82%
Black	16%	25%	18%
Asian	4%	0%	0%
Hispanic/Latina	36%	67%	32%
Baseline C-peptide AUC (pmol•h/L)	12 000 ± 4000	8000 ± 4000	9000 ± 3000
Baseline glucose AUC (mg•h/L)	500 ± 70	1000 ± 400	380 ± 40
Baseline insulin AUC (μU•h/mL)	300 ± 100	200 ± 200	190 ± 80

AUC indicates area under the concentration-time curve; BMI, body mass index; GA, gestational age; GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus; HP, healthy pregnant subjects; SD1, study day 1; SD2, study day 2.

Results reported as mean ± SD.

Gestational Age Correction

In the subjects with gestational diabetes mellitus, the correction for gestational age–dependent changes between study days 1 and 2 can be found in Supplementary Table S2.

PD Parameters Within Group Comparisons

In the women with gestational diabetes mellitus, metformin increased insulin sensitivity 51% ($P = .005$), total β-cell responsivity 26% ($P = .04$), static β-cell responsivity 29% ($P = .04$), and disposition index 97% ($P = .003$) compared with baseline (ie, study day 2 versus study day 1), and decreased baseline β-cell responsivity 24% ($P = .004$), and mixed-meal tolerance test peak glucose concentration 8% ($P = .006$) but had no significant effect on dynamic β-cell responsivity. In

Table 2. Pharmacodynamic Parameters Following a Mixed-Meal Tolerance Test on Study Day 1 (Pretreatment) and Study Day 2 (With Metformin Treatment) in Nonpregnant Control Subjects With Type 2 Diabetes Mellitus and Women With Gestational Diabetes Mellitus, Corrected for Gestational-Age–Dependent Changes

	GDM (n = 25)			T2DM (n = 12)		
Parameter	SD1	SD2	Δ	SD1	SD2	Δ
SI (10^{-4} min^{-1} μU^{-1} mL)	3 ± 2	4 ± 2	2 ± 2 (0.5 to 3) ($P = .005$)*	5 ± 8	6 ± 5	1 ± 5 (–2 to 4) ($P = .4$)
Φ_{total} (10^{-9} min^{-1})	100 ± 50	130 ± 70	30 ± 60 (1 to 50) ($P = .04$)*	30 ± 30	40 ± 30	10 ± 20 (–0.4 to 30) ($P = .06$)
Φ_{static} (10^{-9} min^{-1})	90 ± 40	120 ± 70	30 ± 60 (1 to 50) ($P = .04$)*	30 ± 30	40 ± 30	10 ± 20 (–0.6 to 30) ($P = .06$)
$\Phi_{dynamic}$ (10^{-9})	2000 ± 1000	2000 ± 1000	100 ± 1000 (–300 to 600) ($P = .5$)	600 ± 900	600 ± 700	50 ± 700 (–400 to 500) ($P = .8$)
$\Phi_{baseline}$ (10^{-9} min^{-1})	11 ± 5	9 ± 6	–3 ± 4 (–4 to –0.9) ($P = .004$)*	6 ± 4	7 ± 4	1 ± 2 (–0.3 to 2) ($P = .1$)
DI (10^{-13} min^{-2} μU^{-1} mL)	300 ± 300	600 ± 400	300 ± 500 (100 to 500) ($P = .003$)*	200 ± 300	200 ± 200	40 ± 100 (–50 to 100) ($P = .4$)
MMTT peak glucose (mg/dL)	150 ± 20	140 ± 20	–10 ± 20 (–20 to –4) ($P = .006$)*	280 ± 80	220 ± 80	–60 ± 90 (–100 to –4) ($P = .04$)*

DI indicates disposition index; GDM, gestational diabetes mellitus group; MMTT, mixed-meal tolerance test; SD1/SD2, study day 1/2; SI, insulin sensitivity; T2DM, type 2 diabetes mellitus group; Δ, average change between SD1 and SD2; Φ, β-cell responsivity.
Results reported as mean ± SD (95%CI).
*Statistically different ($P \leq .05$) comparing SD2 to SD1.

the subjects with T2DM there was a 22% decrease in peak glucose concentration ($P = .04$, Table 2) with metformin, but none of the estimated PD parameters were significantly altered by metformin in this group.

Gestational Diabetes Mellitus Versus T2DM Disease Severity

Table 3 provides mean difference data for mixed-meal tolerance test baseline parameters between pregnant women with gestational diabetes mellitus and nonpregnant women with T2DM. Mean β-cell responsivity parameters were higher in the women with gestational diabetes mellitus at baseline than in the nonpregnant women with T2DM. Specifically, baseline β-cell responsivity was an average of 45% higher ($P = .002$), dynamic β-cell responsivity 68% higher ($P = .0009$), static β-cell responsivity 71% higher ($P = .000007$), and total β-cell responsivity 71% higher ($P = .000003$) in the women with gestational diabetes mellitus as compared with the nonpregnant women with T2DM. In addition, mean mixed-meal tolerance test peak glucose concentrations were on average 48% lower ($P = .0002$) in women with gestational diabetes mellitus than in women with T2DM. The pretreatment mean disposition index curves for subjects with T2DM and gestational diabetes mellitus are depicted in Figure 1.

Table 3. Disease Severity: Mean Difference in Pretreatment, Baseline Mixed-Meal Tolerance Test Parameters for Women With Gestational Diabetes Mellitus and Nonpregnant Women With Type 2 Diabetes Mellitus

Parameter	Mean Difference	*P* Value
SI (10^{-4} min^{-1} μU^{-1} mL)	−2 (−7 to 3)	.4
Φ_{total} (10^{-9} min^{-1})	73 (47 to 99)	.000003
Φ_{static} (10^{-9} min^{-1})	63 (39 to 87)	.000007
$\Phi_{dynamic}$ (10^{-9})	1302 (597 to 2007)	.0009
$\Phi_{baseline}$ (10^{-9} min^{-1})	5 (2 to 8)	.002
DI (10^{-13} min^{-2} μU^{-1} mL)	157 (−52 to 366)	.1
MMTT peak glucose (mg/dL)	−136 (−190 to −82)	.0002

DI indicates disposition index; MMTT, mixed-meal tolerance test. Δ, average change between SD1 and SD2; Φ, β-cell responsivity.
Results reported as mean (95%CI). Refer to Table 2 for baseline actual parameter values.

Although the mean disposition index curve for subjects with T2DM relative to those with gestational diabetes mellitus on study day 1 appears shifted down and to the left, because of intersubject variability, there were

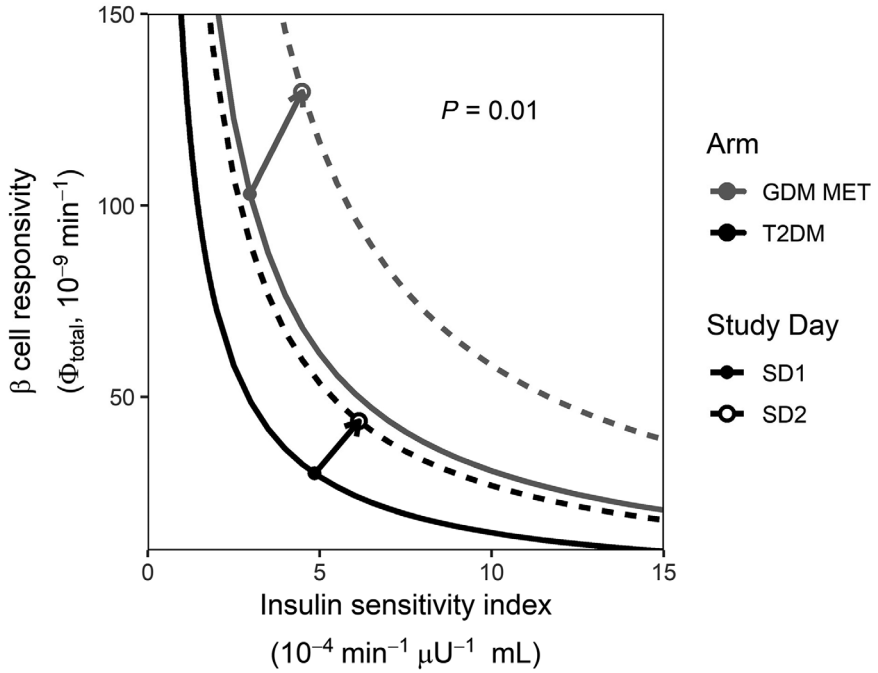

Figure 1. Pharmacodynamic effects of metformin in women with gestational diabetes mellitus (GDM) and type 2 diabetes mellitus (T2DM). Mean disposition index for all adherent subjects who completed the study in the GDM metformin (MET) arm at baseline (solid gray line) and on study day 2 (dashed gray line) as well as for subjects with T2DM at baseline (solid black line) and on study day 2 (dashed black line). The vectors depict the mean pharmacodynamic effect of metformin in subjects with GDM (gray arrow) and subjects with T2DM (black arrow). Solid dots represent mean baseline disposition index (study day 1), and open circles represent study day 2 mean disposition index (GDM corrected for mean gestational age-dependent change). Φ_{total} indicates total beta-cell responsivity.

no significant differences in disposition index or insulin sensitivity between groups at baseline.

Metformin Dose per Day

Average metformin dose per day on study day 2 for subjects with gestational diabetes mellitus and T2DM can be seen in Table 1. The mean metformin dose per day in the women with gestational diabetes mellitus was higher than that for the women with T2DM (difference 607 [95%CI 369 to 844] mg, $P < .001$). However, due to differences in PK during pregnancy as compared with the nonpregnant state, mean metformin area under the concentration-time curve was 14% smaller in the women with gestational diabetes mellitus as compared with the women with T2DM. This difference is not statistically significant (gestational diabetes mellitus 11 ± 4 μg•h/mL versus T2DM 12 ± 3 μg•h/mL, $P = .2$).

PD Effect of Metformin in Subjects With Gestational Diabetes Mellitus Versus Nonpregnant Subjects With T2DM

Figure 1 depicts the average disposition index curves for each group on study day 1 and study day 2. Figure 1 also depicts the mean metformin pharmacologic PD response vectors for subjects with gestational diabetes mellitus and nonpregnant subjects with T2DM starting at their pretreatment average disposition index on study day 1 and extending to the concomitant treatment average disposition index on study day 2. With metformin treatment the disposition index curve shifted up and to the right in women with T2DM but reached the pretreatment disposition index curve only for the women with gestational diabetes mellitus. When the change from study day 1 to study day 2 with metformin was compared (Table 4), the mean Δ disposition index was greater for the women with gestational diabetes mellitus (300 ± 500 10^{-13} min^{-2} μU^{-1} mL) than for the women with T2DM (40 ± 100 10^{-13} min^{-2} μU^{-1} mL, $P = .01$). There was no significant difference in Δ insulin sensitivity ($P = .9$) or $\Delta\beta$-cell responsivity ($P = .4$) with metformin in women with gestational diabetes mellitus versus nonpregnant women with T2DM. Mean peak glucose concentrations decreased with metformin treatment in both T2DM (mean difference 60 ± 90 mg/dL, $P = .04$) and gestational diabetes mellitus (mean difference 10 ± 20 mg/dL, $P = .006$) groups. Eighty-four percent of the subjects with gestational diabetes mellitus and 83% of the subjects with T2DM had some pharmacologic response to metformin (ie, increase in insulin sensitivity).

Mean differences in PD parameters following the mixed-meal tolerance test on study day 2 while on treatment with metformin in subjects with gestational

Table 4. Mean Difference in Mixed-Meal Tolerance Test Parameters During Treatment With Metformin (Study Day 2 and Change From Study Day 1 to Study Day 2) for Women With Gestational Diabetes Mellitus and Nonpregnant Women With Type 2 Diabetes Mellitus

Parameter	Mean Difference on SD2 Between Study Arms	*P* Value Comparing Mean Value on SD2 Between Study Arms	*P* Value Comparing Mean Change From SD1 to SD2 Between Study Arms
SI (10^{-4} min^{-1} μU^{-1} mL)	−1.6 (−5.2 to 1.86)	.3	.9
Φ_{total} (10^{-9} min^{-1})	86 (51 to 121)	.00001	.4
Φ_{static} (10^{-9} min^{-1})	76 (43 to 109)	.00005	.4
$\Phi_{dynamic}$ (10^{-9} min^{-1})	1400 (779 to 2020)	.00006	.7
$\Phi_{baseline}$ (10^{-9} min^{-1})	1.4 (−2.3 to 5.1)	.4	.001
DI (10^{-13} min^{-2} μU^{-1} mL)	422 (227 to 617)	.0001	.01
Peak glucose (mg/dL)	−85 (−133 to −37)	.002	.08

DI indicates disposition index; GDM, gestational diabetes mellitus; SD1/SD2, study day 1/2; SI, insulin sensitivity; Φ_{total}, total β-cell responsivity; Φ_{static}, static β-cell responsivity; $\Phi_{dynamic}$, dynamic β-cell r; $\Phi_{baseline}$, baseline β-cell responsivity.

Results reported as mean ± SD. The values for women with GDM were adjusted to account for normal gestational-age–dependent. Refer to Table 2 for actual parameter values.

diabetes mellitus and subjects with newly diagnosed T2DM are shown in Table 4. While receiving metformin treatment, the average disposition index for women with gestational diabetes mellitus was ~3-fold higher than the average disposition index for women with T2DM (mean difference 421×10^{-13} min^{-2} μU^{-1} mL, $P = .0001$). In addition, while on metformin treatment, the average peak mixed-meal tolerance test glucose concentration was 85 mg/dL lower in the women with gestational diabetes mellitus than in those with T2DM ($P = .002$).

Safety

The adverse effects associated with the mixed-meal tolerance test were limited to occasional bruising from phlebotomy. No hypoglycemia was noted during the mixed-meal tolerance test with either group.

Discussion

For the most part, clinical trials for new drugs have been conducted in nonpregnant individuals. Our understanding of the clinical pharmacology of medications during pregnancy is growing,[5,9,10,24] but most medication prescribing during pregnancy is based on data collected from the nonpregnant population. Normal pregnancy is associated with lower fasting and preprandial glucose concentrations and much higher peak insulin concentrations than are seen in the nonpregnant population.[7] This is likely due in part to the insulin resistance that occurs during normal pregnancy.[6] Metformin is approved by the Food and Drug Administration for treatment of patients with T2DM. There has been discussion in the literature as to whether or not gestational diabetes mellitus and T2DM are the same condition.[25] Both conditions are associated with insulin resistance and decreased pancreatic β-cell compensation ability.[6,26]

Insulin sensitivity, β-cell responsivity, and disposition index with glyburide have been reported for patients with gestational diabetes mellitus and T2DM.[5] With glyburide treatment, women with gestational diabetes mellitus have greater total (ie, overall insulin response) and static (ie, second-phase insulin response) β-cell responsivity than women with T2DM.[5] In this study, before metformin treatment, women with gestational diabetes mellitus demonstrated not only better total and static β-cell responsivity, but also dynamic (ie, first-phase insulin response) and baseline (ie, basal, nonstimulated index of insulin secretion) β-cell responsivity compared with those with T2DM. This is consistent with more severe pancreatic β-cell dysfunction in women with T2DM than in those with gestational diabetes mellitus. However, overall disposition index at baseline for women with gestational diabetes mellitus and nonpregnant women with T2DM was not significantly different in either this study or our previous work.[6] With these underlying differences in disease severity before treatment, it is reasonable to question whether the clinical pharmacological response to treatment will also differ during pregnancy. Data are extremely limited on the PD effects of medications during pregnancy, including metformin. This study provides preliminary data comparing the PD of metformin treatment in women with gestational diabetes mellitus and nonpregnant women with T2DM.

Women with gestational diabetes mellitus have a 7-fold increased risk for developing T2DM later in life than women without gestational diabetes mellitus during pregnancy.[27] This is not surprising given the similar risk factors, such as obesity, as well as similar pathology, including increased insulin resistance and decreased ability to compensate for the degree of insulin resistance in women with gestational diabetes mellitus and those with T2DM. Metformin is the most commonly utilized treatment for patients with T2DM. The difference in metformin response in women with gestational diabetes mellitus as compared with nonpregnant women with T2DM is complicated

by baseline differences in pregnancy status, which increases insulin resistance, as well as disease state. That being said, we report for the first time that a similar percentage of women receiving metformin experienced some positive effect on insulin sensitivity if they had gestational diabetes mellitus (84%) or T2DM (83%). Despite this similarity, the magnitude of response to metformin differed between women with gestational diabetes mellitus and those with T2DM for some PD parameters. On average, metformin treatment in women with gestational diabetes mellitus had a greater effect on the change in disposition index than women with T2DM ($P = .01$), but there was no significant difference in the change in peak glucose concentrations between women with gestational diabetes mellitus and T2DM ($P = .08$). Of note, although average metformin dosage was higher in the women with gestational diabetes mellitus, due to the PK differences in pregnancy as compared with the nonpregnant state,[9] there was no significant difference in metformin exposure as measured by area under the concentration-time curve compared with the nonpregnant women with T2DM in this study.

Metformin decreases insulin resistance through decreased glycogenolysis, lipolysis, and activity of hepatic glucose-6-phosphatase as well as increased glycogen synthesis, insulin receptor tyrosine kinase activity, and glucose transporter type 4 (GLUT4) activity.[28,29] Our data with metformin in the treatment of women with gestational diabetes mellitus demonstrate improvement in insulin sensitivity, consistent with its primary mechanism of action. Metformin can also increase insulin secretion through increasing the release of glucagon-like-peptide-1 (GLP-1).[29] GLP-1 is known to improve β-cell activity, which should translate into increased β-cell responsivity.[30,31] Interestingly, in contrast to expectations for a drug that has been reported to increase release of GLP-1, metformin significantly decreased the baseline β-cell responsivity in women with gestational diabetes mellitus, which likely reflects the changes seen in insulin sensitivity. Consistent with expectations, metformin increased total β-cell responsivity by 26% and static β-cell responsivity by 29%, although there was no significant change in dynamic β-cell responsivity. Most importantly, metformin improved the disposition index, a measure of the overall metabolic state, by 97%, shifting the disposition index curve up and to the right in women with gestational diabetes mellitus. In contrast to the results in women with gestational diabetes mellitus, the women with T2DM had no significant change in any of the PD parameters with metformin. This is to some extent a result of the smaller number of subjects studied with T2DM, a limitation of this preliminary work.

However, when our data in patients with T2DM were compared with previously reported effects of metformin in nonpregnant patients with T2DM, our subjects with T2DM were similar but had less disease severity based on mean insulin sensitivity (this study 5×10^{-4} min^{-1} μU^{-1} mL versus previous work 2.8-2.1 $\times 10^{-4}$ min^{-1} μU^{-1} mL), total β-cell responsivity (this study 30×10^{-9} min^{-1} versus previous work 6.4-7.4 $\times 10^{-9}$ min^{-1}), static β-cell responsivity (this study 30×10^{-9} min^{-1} versus previous work 12.2-15.5 $\times 10^{-9}$ min^{-1}), dynamic β-cell responsivity (this study 600×10^{-9} min^{-1} versus previous work 418–480 $\times 10^{-9}$ min^{-1}), baseline β-cell responsivity (this study 6×10^{-9} min^{-1} versus previous work 4.6-5.5 $\times 10^{-9}$ min^{-1}), and disposition index (this study 200×10^{-13} min^{-2} μU^{-1} mL versus previous work 17.4-20 $\times 10^{-13}$ min^{-2} μU^{-1} mL).[32] Response to metformin was also similar in our subjects with T2DM to that reported in the literature based on insulin sensitivity (this study 1×10^{-4} min^{-1} μU^{-1} mL versus previous work 0.1-0.7 $\times 10^{-4}$ min^{-1} μU^{-1} mL), total β-cell responsivity (this study 10 $\times 10^{-9}$ min^{-1} versus previous work 1.2-2.4 $\times 10^{-9}$ min^{-1}), static β-cell responsivity (this study 10×10^{-9} min^{-1} versus previous work 5.9-13 $\times 10^{-9}$ min^{-1}), dynamic β-cell responsivity (this study 50×10^{-9} min^{-1} versus previous work 31-54 $\times 10^{-9}$ min^{-1}), baseline β-cell responsivity (this study 1×10^{-9} min^{-1} versus previous work 0.5-1.3 $\times 10^{-9}$ min^{-1}), and disposition index (this study 40×10^{-13} min^{-2} μU^{-1} mL versus previous work 5.2-11.6 $\times 10^{-13}$ min^{-2} μU^{-1} mL).[32]

On study day 2 (during metformin treatment) in a similar pattern to study day 1 (before metformin treatment), women with gestational diabetes mellitus had closer to normal (ie, significantly higher) β-cell responsivity (total, dynamic, and static) than women with T2DM. However, whereas there was a significant difference before treatment in baseline β-cell responsivity, with metformin treatment this difference was no longer observable. This appears to be driven by the decrease in baseline β-cell responsivity with metformin treatment in the women with gestational diabetes mellitus and no change from baseline in β-cell responsivity in the women with T2DM. In addition, the overall metabolic states (ie, disposition index) were not different between the 2 groups at baseline but became markedly different with metformin treatment. Women in the gestational diabetes mellitus group were much better able to compensate for the degree of insulin resistance than the women with T2DM. Because the ability to compensate for the degree of insulin resistance is key to maintaining glycemic control, this difference is critically important and is an apt illustration of the need not only for rigorous PK studies in pregnancy but also for PD studies comparing response to the nonpregnant population.

Limitations of Study

This study provides preliminary data on the PD of metformin during pregnancy as compared with nonpregnant patients with type 2 diabetes mellitus and should be interpreted with care. A major limitation of this study is the small sample size in the T2DM study group. This group was intended to be larger and equivalent in number to the gestational diabetes mellitus study group, but due to slow enrollment in this arm and the end of the funding cycle, the funding agency closed the study for further enrollment.

Another limitation of this study is the multiple testing performed without adjustment for multiple comparisons. This article focused on 2 arms of a larger study, and it is unclear what should be considered a "family" of tests for this report in analyzing and interpreting a subset of those data. Further, adjusting *P* values for comparisons and analyses not presented herein would be confusing for the reader and challenging to explain. Therefore, we did not make any adjustments for multiple testing but did make it clear to the reader that this was the case.

Last, the use of the mixed-meal tolerance test method to derive the PD parameters provides the advantages that it is well tolerated by patients, easy to administer, includes the effects of gastrointestinal incretins, and correlates well with the gold standard hyperglycemic clamp studies. However, it is limited because it does require assumption making regarding the rate of nutrient absorption and requires significant mathematical modeling.

Conclusions

This study describes and compares the clinical pharmacologic PD response to metformin in women with gestational diabetes mellitus and nonpregnant women with T2DM. The clinical pharmacological response to metformin treatment is significantly different in pregnant women with gestational diabetes mellitus than in nonpregnant women with T2DM, which likely reflects differences in disease severity. With similar metformin exposure, women with gestational diabetes mellitus have a greater improvement in overall disposition index with metformin treatment than nonpregnant women with T2DM.

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Author Contributions

D.L.S. was responsible for pharmacodynamic modeling and writing/editing of this manuscript; L.M.S. carried out data analysis and manuscript editing; X.M. and D.D.S. were responsible for study design, pharmacodynamic modeling, and manuscript editing; S.K.F.-N., S. Clark, S. Caritis, D.H., S.K.Q., L.S.H., A.T.T., T.M., and K.E.T. contributed to study conduct and manuscript editing; M.A. performed metformin sample analysis and manuscript editing; R.V. contributed to glucose, insulin, and C-peptide analysis and manuscript editing; Z.R. performed study protocol development and manuscript editing; Z.B. carried out study design and manuscript editing; T.R.E. was responsible for study design, study conduct, and manuscript editing; L.M.B. contributed to study protocol development and data compilation and coordination; and M.F.H. was responsible for study design, study conduct, data analysis, and manuscript writing and editing.

Conflicts of Interest

D.L.S. declares that, at the time of the conduct and analysis of the study, she had no conflict of interest. However, since the completion of the study, D.L.S.'s affiliation has changed to PRA Health Sciences. X.M. is an employee of Eli Lilly and Company. All other authors declare that at the time of the conduct and analysis of the study, they had no conflicts of interest. A professional medical writing company was not used for preparation of this manuscript.

Data Sharing

Readers should contact Dr Mary Hebert (mhebert@uw.edu) for questions regarding data sharing.

References

1. Jovanovic L, Pettitt DJ. Gestational diabetes mellitus. *JAMA*. 2001;286:2516-2518.
2. Paglia MJ, Coustan DR. Gestational diabetes: evolving diagnostic criteria. *Curr Opin Obstet Gynecol*. 2011;23:72-75.
3. HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. *Int J Gynaecol Obstet*. 2002;78:69-77.
4. American College of Obstetricians and Gynecologists Committee on Practice Bulletins—Obstetrics, ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 30, September 2001 (replaces Practice Bulletin Number 180, July 2017). Gestational diabetes mellitus. *Obstet Gynecol.* 2018;131(2):e49-e64.
5. Hebert MF, Ma X, Naraharisetti SB, et al. Are we optimizing gestational diabetes treatment with glyburide? The pharmacologic basis for better clinical practice. *Clin Pharmacol Ther*. 2009;85:607-614.
6. Buchanan TA. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. *J Clin Endocrinol Metab.* 2001;86:989-993.
7. Phelps RL, Metzger BE, Freinkel N. Carbohydrate metabolism in pregnancy. XVII. Diurnal profiles of plasma glucose, insulin, free fatty acids, triglycerides, cholesterol and individual amino acids in late normal pregnancy. *Am J Obstet Gynecol*. 1981;140(7):730-736.
8. Klieger C, Pollex E, Kazmin A, Koren, G. Hypoglycemics: pharmacokinetic considerations during pregnancy. *Ther Drug Monit*. 2009;31:533-541.
9. Eyal S, Easterlin TR, Carr D, et al. Pharmacokinetics of metformin during pregnancy. *Drug Metab Dispos*. 2010;38:833-840.
10. Shuster DL, Shireman LM, Ma X, et al. Pharmacodynamics of glyburide, metformin and glyburide/metformin combination therapy in the treatment of gestational diabetes mellitus. *Clin Pharmacol Ther*. In press.
11. Bergman, R.N. Minimal model: perspective from 2005. *Horm Res*. 2005;64:8-15.
12. Bandi ZL, Fuller JB, Bee DE, James GP. Extended clinical trial and evaluation of glucose determination with the Eastman Kodak Ektachem GLU/BUN Analyzer. *Clin Chem*. 1981;27:27-34.
13. Haffner SM, Mykkanen L, Stern MP, Valdez RA, Heisserman JA, Bowsher RR. Relationship of proinsulin and insulin to cardiovascular risk factors in nondiabetic subjects. *Diabetes*. 1993;42:1297-1302.
14. Wiedmeyer HM, Polonsky KS, Myers GL, et al. International comparison of C-peptide measurements. *Clin. Chem.* 2007;53:784-787.
15. 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.
16. 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.
17. Cobelli C, Toffolo GM, Dalla Man C, et al. Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. *Am J Physiol Endocrinol Metab*. 2007;293:E1-E15.
18. Caumo A, Bergman RN, Cobelli C. Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. *J Clin Endocrinol Metab*. 2000;85:4396-4402.
19. 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:E637-E643.
20. Dalla Man C, Campioni M, Polonsky KS, et al. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. *Diabetes*. 2005;54:3265-3273.
21. Zhang X, Wang X, Vernikovskaya DI, et al. Quantitative determination of metformin, glyburide and its metabolites in plasma and urine of pregnant patients by LC-MS/MS. *Biomed Chromatogr*. 2015;29:560-569.
22. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2018.
23. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2016.
24. Yerby MS, Friel PN, McCormick K et al. Pharmacokinetics of anticonvulsants in pregnancy: alterations in plasma protein binding. *Epilepsy Res*. 1990;5(3):223-228.
25. Zajdenverg L, Negrato CA. Gestational diabetes mellitus and type 2 diabetes: same disease in a different moment of life? Maybe not. *Arch Endocrinol Metab*. 2017;61:208-210.
26. Xiang AH, Peters RK, Kjos SL, et al. Pharmacological treatment of insulin resistance at two different stages in the evolution of type 2 diabetes: impact on glucose tolerance and β-cell function. *J Clin Endocrinol Metab*. 2004;89:2846-2851.
27. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*. 2009;373:1773-1779.
28. Wiernsperger NF, Bailey CJ. Antihyperglycaemic effect of metformin: therapeutic and cellular mechanisms. *Drugs*. 1999;58:31-82.
29. Scarpello JH, Howlett HC. Metformin therapy and clinical uses. *Diabetes Vasc Dis Res*. 2008;5:157-167.
30. Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. *Diabetologia*. 2002;45:1111-1119.
31. Tran KL, Park YI, Pandya S, et al. Overview of glucagon-like peptide-1 receptor agonists or the treatment of patients with type 2 diabetes. *Am Health Drug Benefits*. 2017;10:178-188.
32. Williams-Herman D, Xu L, Teng R, et al. Effect of initial combination therapy with sitagliptin and metformin on β-cell function in patients with type 2 diabetes. *Diabetes Obes Metab.* 2012;14:67-76.