The Primary Glucose-Lowering Effect of Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic and 12-Week Dose-Ranging Studies

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OBJECTIVE

Delayed-release metformin (Met DR) is formulated to deliver the drug to the lower bowel to leverage the gut-based mechanisms of metformin action with lower plasma exposure. Met DR was assessed in two studies. Study 1 compared the bioavailability of single daily doses of Met DR to currently available immediate-release metformin (Met IR) and extended-release metformin (Met XR) in otherwise healthy volunteers. Study 2 assessed glycemic control in subjects with type 2 diabetes (T2DM) over 12 weeks.

RESEARCH DESIGN AND METHODS

Study 1 was a phase 1, randomized, four-period crossover study in 20 subjects. Study 2 was a 12-week, phase 2, multicenter, placebo-controlled, dose-ranging study in 240 subjects with T2DM randomized to receive Met DR 600, 800, or 1,000 mg administered once daily; blinded placebo; or unblinded Met XR 1,000 or 2,000 mg (reference).

RESULTS

The bioavailability of 1,000 mg Met DR b.i.d. was ~50% that of Met IR and Met XR (study 1). In study 2, 600, 800, and 1,000 mg Met DR q.d. produced statistically significant, clinically relevant, and sustained reductions in fasting plasma glucose (FPG) levels over 12 weeks compared with placebo, with an ~40% increase in potency compared with Met XR. The placebo-subtracted changes from baseline in HbA$_{1c}$ level at 12 weeks were consistent with changes in FPG levels. All treatments were generally well tolerated, and adverse events were consistent with Glucophage/Glucophage XR prescribing information.

CONCLUSIONS

Dissociation of the glycemic effect from plasma exposure with gut-restricted Met DR provides strong evidence for a predominantly lower bowel-mediated mechanism of metformin action.

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Although metformin was introduced as a treatment for type 2 diabetes (T2DM) >50 years ago, the mechanism of metformin action is still debated (1). Historically, the glucose-lowering actions of metformin have been attributed to its effects on mitochondrial function, AMPK, and glucagon receptor–stimulated adenylate cyclase in the liver and skeletal muscle, albeit at suprapharmacological doses (2,3). A recent study (4) in rodents suggests that intravenous metformin inhibits the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose, and decreased hepatic gluconeogenesis at lower doses than are required to affect AMPK. However, reports (5,6) that short-term intravenous metformin administration is less effective than oral administration in rats and humans have suggested that the gut may be important for the glucose-lowering action of metformin. Gut effects include secretion of the enteroendocrine L-cell products glucagon-like peptide 1 (GLP-1) and peptide YY, bile acid metabolism, and the gut microbiome (7,8).

When currently available metformin formulations (immediate-release metformin [Met IR] and extended-release metformin [Met XR]) are orally administered, the absolute bioavailability is ~50% of the total dose with the majority of absorption occurring in the duodenum and jejunum (9–11). Importantly, as metformin is not metabolized in the gut (11), ~50% of a typical therapeutic dose is delivered to the distal small intestine where it accumulates in the gut mucosa at concentrations up to 300 times greater than concentrations in plasma (12). After a single dose of orally administered Met IR, ~30% of the dose is recovered in the feces (10). Given that metformin absorption is transporter rate limited, lower doses (<1,000 mg) have higher bioavailability (11) but are less effective (13). Thus, we speculated that higher doses of metformin (≥1,500 mg) are necessary to "overwhelm" the transporters in the proximal small intestine and deliver optimally effective doses of metformin to the lower bowel. The fact that there is a clear dose response for metformin while the pharmacokinetic (PK)/pharmacodynamic relationship is weak (14) also supports the concept that presystemic mechanisms

may be important to its glucose-lowering effect.

We tested the hypothesis that gut exposure to metformin predominantly accounts for its glucose-lowering effect by using a delayed-release metformin (Met DR) formulation that targets the ileum, a region of the gut where the absorption of metformin is low (9,11). Met DR targets the ileum through pH-dependent dissolution of the tablet without modifying the structure of the metformin molecule. In this report, we describe two studies demonstrating that Met DR has lower bioavailability compared with Met IR and Met XR in otherwise healthy subjects (study 1) and that the delivery of low doses of metformin (600–1,000 mg) to the lower bowel is at least as effective as similar doses of Met XR in lowering plasma glucose levels over 12 weeks in subjects with T2DM (study 2).

RESEARCH DESIGN AND METHODS

Met DR tablets were produced according to current good manufacturing practices and comprise a Met IR hydrochloride (HCl) core overlaid with a proprietary enteric coat. The enteric coat, which includes Eudragit polymers and other commonly used excipients, delays disintegration and dissolution of the tablet until it reaches a pH of 6.5 in the distal small intestine and beyond. Tablet strengths used included 300 and 500 mg metformin HCl. Placebo tablets were visually identical but contained no active ingredient. Met IR and Met XR tablets used as comparator treatments were commercially available products (Glucophage; Bristol-Myers Squibb, New York, NY). Both study protocols were performed in accordance with good clinical practice and were approved by the institutional review boards. All subjects provided written informed consent prior to study enrollment.

Study 1 (clinical trial reg. no. NCT02291510, clinicaltrials.gov) was a randomized, four-period, crossover PK study in 20 otherwise healthy male and female subjects who were between 19 and 65 years of age and had a BMI of 25–35 kg/m^2. All subjects received 1 day of dosing for each of four treatments (500 mg Met DR b.i.d., 1,000 mg Met DR b.i.d., 1,000 mg Met IR b.i.d., and 2,000 mg Met XR q.d.) in a randomized (1:1:1:1)

sequence, separated by a washout period of 3–7 days. Met XR was administered once with the evening meal per the prescribing information (15), and twice-daily doses of Met DR and Met IR were administered 12 h apart after meals. Plasma metformin concentrations were measured over a 36.5-h period that included five standardized meals. PK parameters were determined using noncompartmental analysis methods based on the individual data for plasma metformin concentration over time for each subject. PK parameters were analyzed in the evaluable population (randomized subjects who completed all treatment periods consistent with protocol procedures) using a mixed-effects model on a natural log scale with fixed effects for treatment, period, and sequence and subject within sequence as a random effect. Results were exponentiated to obtain the geometric least squares (LS) mean ratios and corresponding 90% CIs on the original scale. Using two one-sided t tests with an $\alpha = 0.05$ and an assumption that the within-subject variation would be at least 25%, this sample size provided 90% power to detect a difference in the area under the curve (AUC) of at least 25% between 1,000 mg b.i.d. Met DR and 2,000 mg q.d. Met XR.

Study 2 (clinical trial reg. no. NCT01819272, clinicaltrials.gov) was a phase 2, 12-week, randomized, placebo-controlled, dose-response study conducted in 240 subjects with T2DM and an estimated glomerular filtration rate (eGFR) of ≥60 mL/min/1.73 m^2 based on the Modification of Diet in Renal Disease equation. Subjects were male or female, between 18 and 65 years of age, had a BMI of 25–45 kg/m^2, and had their T2DM treated with diet and exercise alone or with metformin and/or a DPP-4 inhibitor (DPP-4i) only. Subjects were washed out of these medications for 14–17 days. Other inclusion criteria included an HbA$_{1c}$ level of 7.0–9.5% if treated with diet and exercise alone or 6.0–9.5% if treated with metformin/DPP-4i, serum creatinine <1.5 mg/dL (male) or <1.4 mg/dL (female), and an eGFR of ≥60 mL/min/1.73 m^2 prior to randomization. Subjects were randomized (1:1:1:1:1:1) to one of six treatment arms in a balanced manner stratified by screening HbA$_{1c}$ level (<8% vs. ≥8%).

The double-blind treatment consisted of indistinguishable placebo, or 600, 800, or 1,000 mg Met DR once daily in the morning. Dosing with the morning meal was selected based on a previous trial demonstrating that once-daily dosing in the morning resulted in lower bioavailability with equivalent fasting plasma glucose (FPG) lowering than the same total daily dose (1,000 mg) administered with the evening meal or split between the morning and evening meals (clinical trial reg. no. NCT01804842, clinicaltrials.gov). Active treatment arms of 1,000 and 2,000 mg Met XR administered once daily in the evening per prescribing information (15) were included for reference. The 2,000 mg Met XR dose was titrated over 3 weeks; no other treatments were titrated.

In study 2, the primary end point was the change in FPG level from baseline to week 4. Secondary end points included changes in HbA$_{1c}$ and FPG levels from baseline to week 12 and changes in FPG from baseline to weeks 4, 8, and 12. The baseline-corrected AUC of the FPG concentration-time curve at steady state (AUC$_{4–12wk}$) was also calculated for each evaluable subject to integrate the FPG data collected through 12 weeks into a single value, with week 4 chosen as the first value because 2,000 mg Met XR was titrated over the first 3 weeks. Fasting (premorning dose) PK and plasma lactate concentrations were also measured.

Changes in FPG level from baseline to subsequent study visits were assessed using an ANCOVA model with treatment and baseline HbA$_{1c}$ levels (<8% and ≥8%) as factors and baseline FPG level as a covariate. For the change in HbA$_{1c}$ level, an ANCOVA model with treatment as a factor and baseline HbA$_{1c}$ as a covariate was used. Notable departures from the Gaussian assumption were detected for the change in FPG level for all active treatment groups. Therefore, the main analyses used the Kruskal-Wallis test for comparisons to placebo and the Hodges-Lehmann method for CIs around the median differences from placebo. Analyses of HbA$_{1c}$ used parametric methods, as the departure from the Gaussian assumption for these did not require alternative methods to be used.

All analyses for the primary end point were conducted using the intent-to-treat (ITT) population (randomized subjects who received at least one dose of the study drug) and the evaluable populations (evaluable populations for weeks 4 and 12 consisted of subjects who completed the corresponding treatment period without any major protocol violations and with nonmissing FPG data at baseline and the corresponding end point). A sample size of 40 subjects per treatment group provided ~80% power to detect a statistically significant difference in week 4 FPG values between at least one Met DR treatment group and the placebo group. The calculation was based on a two-sided two sample t test and $\alpha = 0.05$ with balanced randomization, a placebo-subtracted change from baseline equal to −20 mg/dL, a common SD of 30 mg/dL, and a missing data rate of 10%.

RESULTS

Metformin DR PK (Study 1)

Study 1 randomized 20 subjects, and all subjects received at least one dose of the study treatment. Most subjects were male (70%), white (65%), and not Hispanic or Latino (85%). The mean age was 32.2 years, the mean weight was 89.0 kg, and the mean BMI was 29.6 kg/m^2. One subject did not complete all four treatments because of an adverse event (AE) unrelated to study medication (vessel puncture site hematoma). All other subjects completed the study protocol procedures and were included in the evaluable population.

Mean plasma concentration-time profiles of metformin following single daily dose administration (up to two doses) were markedly lower for Met DR than Met IR and Met XR (Fig. 1A). A delay in absorption with Met DR was observed; the median time prior to the first nonzero concentration ranged from 6 to 7 h after the first (evening) doses of Met DR compared with <1 h for Met IR and Met XR. The time to reach peak concentrations was greater after the evening dose than the subsequent morning dose for both Met DR (10 and 6–7 h, respectively) and Met IR (5 and 3 h, respectively). Peak concentrations of Met DR twice daily were higher following evening doses than morning doses (1,000 mg b.i.d.: 880 vs 604 ng/mL; 500 mg b.i.d.: 538 vs 476 ng/mL). Collectively, these data suggest a diurnal effect in rate and extent of absorption that is consistent with slowed transit during the evening and sleeping hours.

The relative bioavailability and exposure resulting from single daily doses of Met DR twice daily versus Met IR twice daily and Met XR once daily are shown in Fig. 1B. The mean maximal drug concentration after dosing was higher for Met XR once daily (1,688 ng/mL) and Met IR twice daily (1,328 ng/mL) than for 1,000 and 500 mg Met DR b.i.d. doses (905 and 607 ng/mL). The mean AUC from time of dosing to the last measurable concentration was also higher for the Met XR once-daily dose (16,990 ng × h/mL) and the Met IR twice-daily dose (18,710 ng × h/mL) than for the 1,000 and 500 mg Met DR b.i.d. dose (9,010 and 6,160 ng × h/mL). The rate and extent of exposure (AUC from time of dosing to the last measurable concentration and maximal drug concentration after dosing) from the 1,000 mg Met DR b.i.d. dose were ~52% and 33% lower, respectively, than with the same dose of Met IR dose, and ~48% and 47% lower, respectively, than with 2,000 mg Met XR q.d. dose. The rate and extent of exposure from 500 mg Met DR b.i.d. were ~68% and 55% lower, respectively, than with the 1,000 mg Met IR b.i.d. dose, and ~65% and 64% lower, respectively, than with the 2,000 mg Met XR q.d. dose. The reduction in plasma exposure with both doses of Met DR was statistically significant compared with Met IR and Met XR ($P < 0.0001$). The PK of Met DR was not dose proportional, which is consistent with the known increased bioavailability at lower doses. As expected, the comparison of Met XR to Met IR demonstrated bioequivalence based on total exposure.

Metformin DR Dose Response (Study 2)

Study 2 randomly assigned 240 subjects (39–41 per group) to six treatment groups. Twenty-eight (11.7%) subjects discontinued from the study early with discontinuation rates of 14.6%, 7.7%, 7.5%, 12.5%, 10.0%, and 17.5%, respectively, for placebo, 600 mg Met DR, 800 mg Met DR, 1,000 mg Met DR, 1,000 mg Met XR, and 2,000 mg Met XR doses (Supplemental Fig. 1) The most common reasons for discontinuation were lost to follow-up (2.9%), protocol violation (2.5%), and loss of glucose control (2.5%). There were no statistically significant differences in demographics

A

B
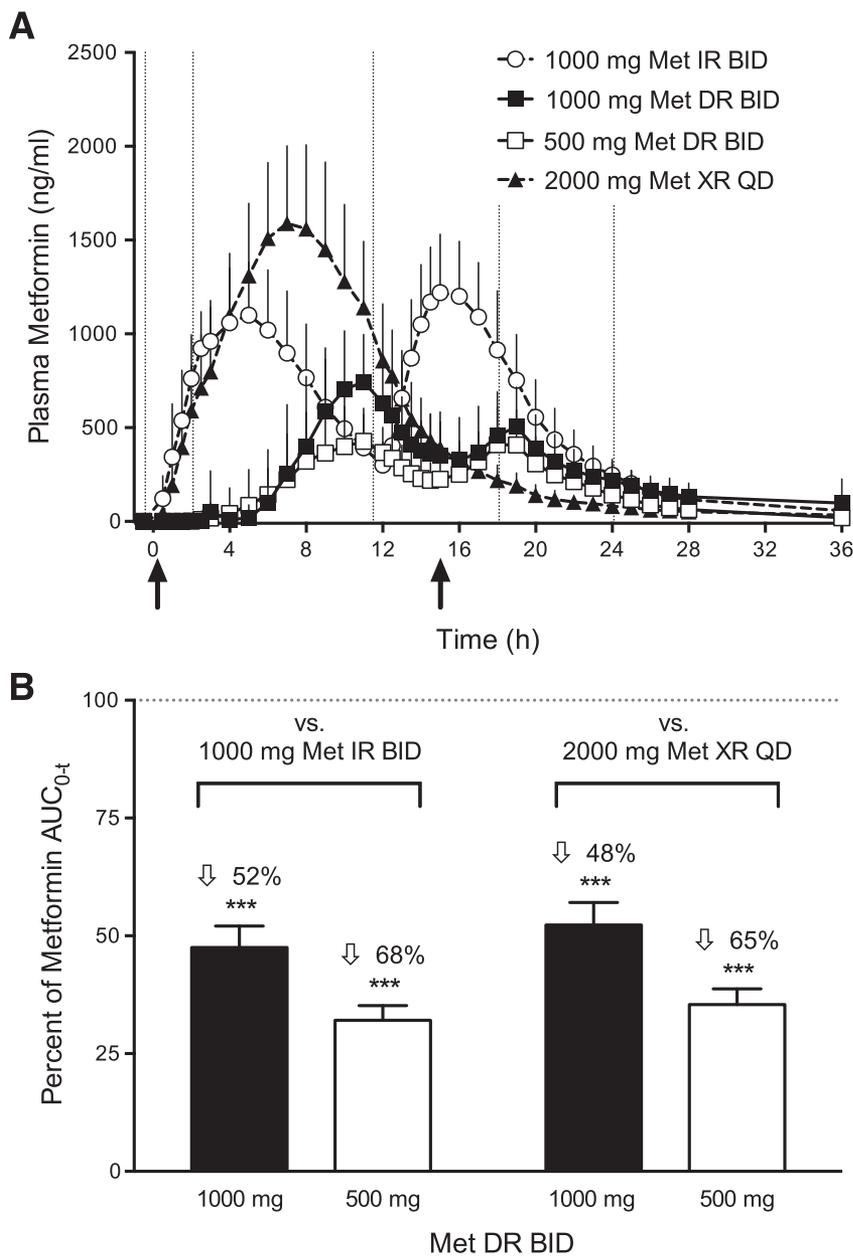
Figure 1—Plasma metformin concentrations and bioavailability after administration of a single daily dose (study 1). *A*: Mean (SD) plasma metformin concentrations by treatment and time point. Evaluable population ($N = 19$). Treatments were administered at $t = 0$ h (8:00 P.M.) and at $t = 12$ h (8:00 A.M.) except for Met XR, which was administered as a single dose at $t = 0$ (black arrows). Meals were administered at $t = -0.42$, 2.08, 11.5, 18.08, and 24.08 h relative to the first dose (dotted vertical lines). *B*: Relative bioavailability and exposure of single daily doses of Met DR b.i.d. vs. Met IR b.i.d. and Met XR q.d. Evaluable population ($N = 19$). Data are expressed as the percentage geometric LS mean ratio and the corresponding 90% upper confidence limit. ***$P < 0.0001$ vs. Met IR or Met XR. *t*, last quantifiable concentration following dose administration.

between treatment groups. Subjects exhibited relatively good glycemic control at baseline (Table 1). All other characteristics related to diabetes were generally similar across treatment arms. Twenty-five subjects (10.4%) and 44 subjects (18.3%) were excluded from the week 4 and week 12 evaluable populations,

respectively, prior to unblinding for not completing the treatment period without major protocol violations or for having missing values. The percentage of subjects included in the week 12 evaluable population for each treatment group ranged from 73.2% for the placebo group to 92.5% for the 800 mg Met DR group.

All active treatment groups had improvements in FPG level compared with placebo at week 4 (Fig. 2*A*). There were dose-dependent reductions in median FPG level; the reduction produced by the 800 mg Met DR dose was statistically significant compared with that by placebo. Median reductions for 1,000 and 2,000 mg Met XR at week 4 were statistically significant. The administration of 1,000 mg Met DR resulted in a 50% greater median reduction in plasma glucose levels than 1,000 mg Met XR and ~72% of the 2,000 mg Met XR effect. The baseline-corrected AUC_{4-12wk} for FPG (Fig. 2*B*) also decreased in a dose-dependent fashion (4.0, −96.0, −108.0, −156.0, −98.0, and −215.0 mg/dL * week, respectively, for placebo, 600, 800, and 1,000 mg Met DR and 1,000 and 2,000 mg Met XR doses) with statistically significant reductions for all Met DR and Met XR groups compared with placebo (all $P < 0.05$). A dose response in FPG levels was evident for both Met DR and Met XR with a left-shifted profile for Met DR compared with Met XR of ~40%.

LS mean (SE) changes in HbA_{1c} level from baseline were negligible for all Met DR treatments and for 1,000 mg Met XR treatment, while placebo increased the HbA_{1c} level by 0.45% (0.14). Not surprisingly, the administration of 2,000 mg Met XR resulted in an LS mean (SE) reduction of 0.21 (0.13) from baseline, since that dose was higher than the mean dose of metformin that subjects were receiving prior to the washout (1,438 mg/day). The placebo-subtracted LS mean (SE) changes from baseline in HbA_{1c} level at 12 weeks were −0.48% (0.19), −0.45% (0.18), and −0.35% (0.19) for 600, 800, and 1,000 mg Met DR, respectively, and −0.45% (0.19) and −0.67% (0.19) for 1,000 and 2,000 mg Met XR, respectively. All differences were statistically different from placebo ($P < 0.05$), with the exception of 1,000 mg Met DR ($P = 0.061$).

Steady-state metformin concentrations were achieved by week 2 for all Met DR groups and the 1,000 mg Met XR group and by week 4 for the 2,000 mg Met XR group, which required dose titration through week 3 (Fig. 2*C*).

Safety and Tolerability
Consistent with the Glucophage/Glucophage XR prescribing information (15),

Table 1—Baseline characteristics of subjects with T2DM in the 12-week study (study 2)

	Placebo qAM (n = 41)	Met DR 600 mg qAM (n = 39)	Met DR 800 mg qAM (n = 40)	Met DR 1,000 mg qAM (n = 40)	Met XR 1,000 mg qPM (n = 40)	Met XR 2,000 mg qPM (n = 40)	All (N = 240)
Age, years	51 ± 10	54 ± 8	53 ± 10	52 ± 9	51 ± 10	52 ± 10	52 ± 9
Male sex, %	46	46	32	65	45	47	47
White, black, other,[a] %	68/27/5	77/23/0	58/33/10	75/23/3	60/35/5	68/30/3	68/28/4
BMI, kg/m^2	33.6 ± 5.3	33.1 ± 5.7	33.5 ± 5.9	33.3 ± 5.6	32.8 ± 5.3	33.7 ± 5.2	33.3 ± 5.4
Duration of T2DM, years	7.0	5.9	6.0	5.9	6.6	7.0	6.4
HbA$_{1c}$, %	7.4 ± 1.0	7.5 ± 0.9	7.3 ± 0.9	7.4 ± 1.0	7.4 ± 1.0	7.4 ± 1.0	7.4 ± 0.9
FPG at screening, mg/dL	147 ± 37	148 ± 43	142 ± 35	141 ± 30	139 ± 34	149 ± 41	144 ± 37
FPG at baseline, mg/dL	177 ± 55	180 ± 50	163 ± 40	172 ± 44	166 ± 50	180 ± 58	173 ± 50
FPG, change from screening to baseline, mg/dL	31 ± 45	33 ± 44	21 ± 26	31 ± 34	27 ± 44	31 ± 34	29 ± 38
Patients receiving therapy with Met[b] prior to study entry (% IR/% XR)	93 (87/13)	80 (84/16)	95 (84/16)	90 (92/8)	85 (82/18)	88 (80/20)	88 (85/15)
Mean dose of prior Met,[b] mg (median)	1,308 (1,000)	1,532 (1,500)	1,446 (1,500)	1,492 (1,600)	1,325 (1,000)	1,541 (2,000)	1,438 (1,500)
Patients with mild renal impairment,[c] %	34	26	40	33	43	35	35

Data are reported as mean ± SD or percentage of subjects for the ITT population (N = 240), unless otherwise indicated. Other, Asian, Pacific Islander/Native Hawaiian, American Indian/Alaska Native, or other; qAM, once daily in the morning; qPM, once daily in the evening. [a]Percentages may not add up to 100 because of rounding. [b]Includes Met IR and Met XR. [c]Mild renal impairment defined as eGFR ≥60 to <90 mL/min/1.73 m^2.

the most common treatment-emergent AEs (TEAEs) were gastrointestinal in nature. In study 1, the most commonly reported TEAEs in any treatment group included diarrhea, nausea, vomiting, and headache. Most TEAEs were assessed as being unrelated to study treatment and were mild in intensity; there were no deaths.

In study 2, all active treatments were well tolerated, and TEAEs were consistent with Glucophage/Glucophage XR prescribing information (15). However, the incidence of gastrointestinal TEAEs was relatively low (compared with prescribing information) in all active treatment groups, with gastrointestinal TEAEs reported by 7.3%, 12.8%, 17.5%, 15.0%, 17.5%, and 12.5% of subjects, respectively, receiving placebo, 600 mg Met DR, 800 mg Met DR, 1,000 mg Met DR, 1,000 mg Met XR, and 2,000 mg Met XR. This suggests that the population was tolerant to metformin, which is consistent with 88% of subjects having received metformin prior to study enrollment and washout.

As metformin accumulation can result in increased lactate production, which, in turn, increases the risk of the rare but serious metabolic complication of lactic acidosis, the effects of Met DR on plasma lactate levels were also evaluated in study 2. Mean lactic acid values were within normal ranges throughout the study, but were elevated from baseline by 0.31–0.33 mmol/L (median 0.19–0.29 mmol/L) for Met XR compared with 0.09–0.12 mmol/L (median −0.22 to 0.22 mmol/L) for Met DR and 0.16 mmol/L (median 0.17 mmol/L) for placebo (Fig. 3). The lack of change from baseline in lactate levels for the Met DR groups most likely reflects lower metformin exposure. One subject treated with 2,000 mg Met XR experienced moderate blood lactate increases for 16 days (up to 5.9 mmol/L) without other AEs or changes to the treatment regimen.
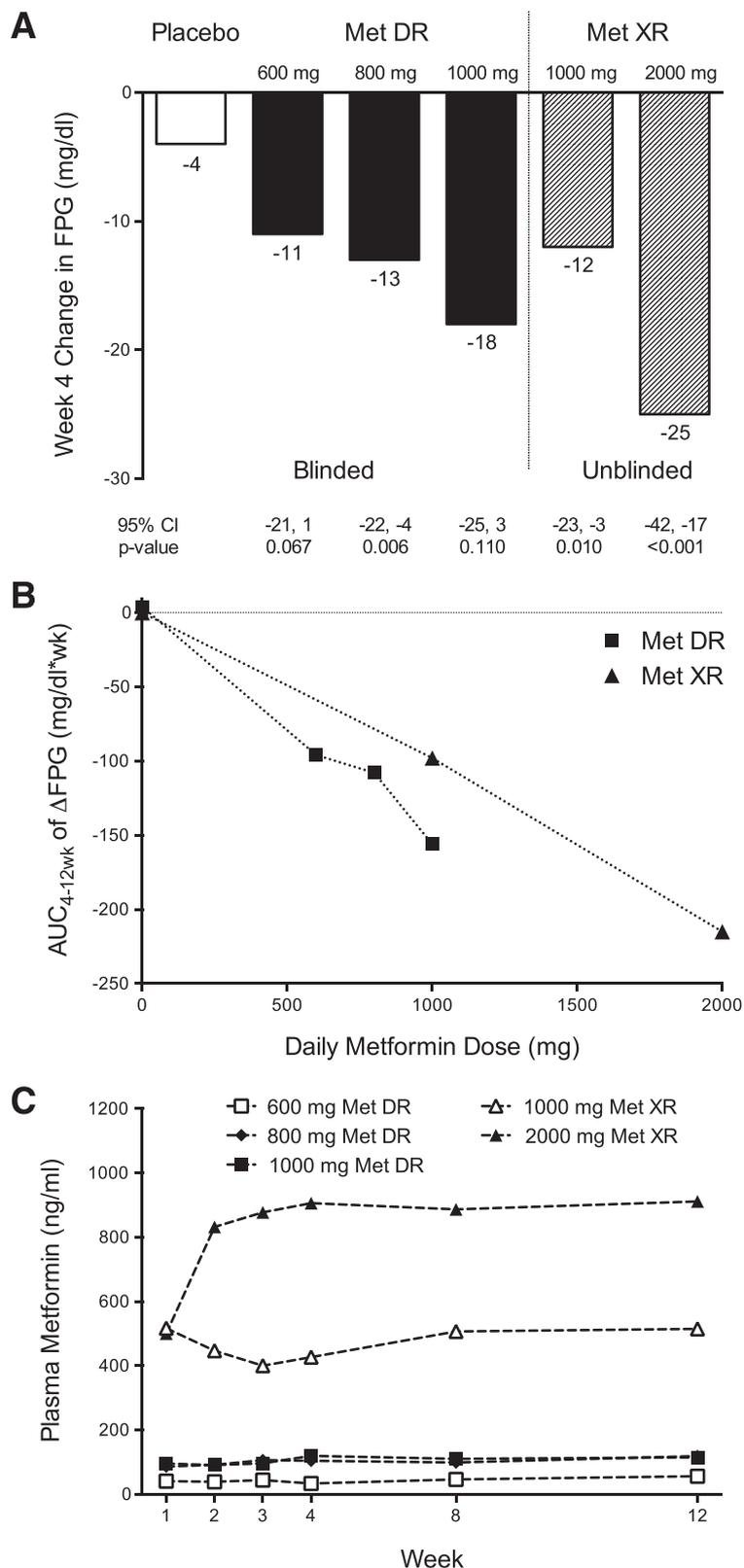
CONCLUSIONS

Metformin is the oldest and most commonly prescribed oral glucose-lowering medication in the world and is considered a first-line therapy for patients in whom T2DM is newly diagnosed (16). Nevertheless, there is no consensus on its primary site of action, although it is generally agreed to have pleiotropic effects. The fact that metformin is not metabolized in vivo and is ~50% bioavailable (11) allows for almost equal exposures in the gut and plasma with typical dosing; however, until recently, most studies have focused on systemically based mechanisms only. The liver has been the main focus of study, owing to decreased hepatic glucose output with metformin (17) and observations of metformin accumulation in the liver at concentrations ~10 times greater than those in plasma (18,19). However, metformin also accumulates in the intestine at concentrations 300 times greater than in plasma (12). Thus, the gut is a major reservoir for metformin exposure and is potentially responsible for much of its glucose-lowering effects, including enhanced secretion of GLP-1 and peptide YY, which in turn affects systemic mechanisms including reducing hepatic glucose production through glucagon suppression and enhanced glucose-dependent insulin secretion (15,20–23).

While the effects of metformin on increasing GLP-1 secretion have been known for some time (24–27), its significance is debated. Interestingly, the increase in plasma GLP-1 levels resulting from metformin administration is similar to that of a DPP-4i (20) and thus could explain much of the glucose-lowering effect of metformin. In addition, unlike a DPP-4i that reduces GLP-1 degradation, metformin increases GLP-1 secretion and thus can significantly increase concentrations local to the L cell, which may in turn enhance neural signaling in the gut and portal vein to rapidly regulate glycemic control (28–30).

The current study demonstrates that metformin primarily restricted to the gut effectively lowers plasma glucose levels. The observation that low doses of Met DR appear to be more effective than similar doses of the more bioavailable Met XR suggests that the gut contribution to glucose lowering may be more important than systemic

A

Placebo   Met DR   Met XR

	600 mg	800 mg	1000 mg	1000 mg	2000 mg
95% CI	-21, 1	-22, -4	-25, 3	-23, -3	-42, -17
p-value	0.067	0.006	0.110	0.010	<0.001

Blinded   Unblinded

B

C

Figure 2—Change in FPG and fasting metformin concentrations in the 12-week study (study 2). *A*: Median change in FPG level at week 4. Week 4 evaluable population (*N* = 215). *P* value from Kruskal-Wallis test; 95% CI based on Hodges-Lehmann estimation of the median difference vs. placebo; baseline is defined as the median measurement at day 1. *B*: Median change in and FPG AUC$_{4-12wk}$ (mg/dL * week). Week 12 evaluable population (*N* = 196). *P* < 0.05 vs. placebo for pairwise comparison without adjustment. *C*: Median fasting plasma metformin concentrations. Week 12 evaluable population (*N* = 196).

mechanisms. The apparent increase in potency was most evident when comparing the 600 mg Met DR dose to the 1,000 mg Met XR dose (Fig. 2*A* and *B*). The fact that the administration of 1,000 mg Met DR lowered fasting glucose levels by ~50% more than the same dose of 1,000 mg Met XR provides further support of increased potency with Met DR. This leftward shift of the dose-response curve is all the more striking given the ~50% reduction in plasma metformin exposure observed in study 1 (Fig. 1) and the >75% reduction (Fig. 2*C*) in fasting metformin concentrations in study 2 at equally effective doses of Met DR (600 mg) and Met XR (1,000 mg). These data indicate that the gut is the primary site of action for the glucose-lowering effect of metformin and that plasma exposure is less important, at least at these therapeutic doses.

From a mechanistic perspective, a limitation of the current study is that a higher Met DR dose was not included. Since the highest dose of Met DR (1,000 mg) achieved 70% of the glycemic control of 2,000 mg Met XR, it is not possible to determine whether all of the glucose-lowering effect of metformin can be explained by lower-bowel gut-based mechanisms. It is conceivable that the full metformin effect requires a certain threshold of upper-bowel metformin exposure and/or plasma exposure along with the lower-bowel exposure. The duodenum has a low density of gut hormone-secreting L cells, and it has been proposed that the rapid appearance of GLP-1 following a meal is a result of a complex integration of proximal and distal neural and hormonal signaling (31). However, by virtue of its enteric coating, Met DR limits both proximal gut exposure and plasma exposure, so it is not possible to quantitate their potential individual contributions in these studies. Our data support the idea that the distal intestine is responsible for at least 70% of the maximal glucose-lowering effect of metformin, and future studies using higher doses may indicate an even greater contribution.

Importantly, our data are not in conflict with those from a recent report by Madiraju et al. (4) indicating that intravenous metformin administration in rats reduces the conversion of lactate and glycerol to glucose, thus decreasing hepatic gluconeogenesis. Their finding is
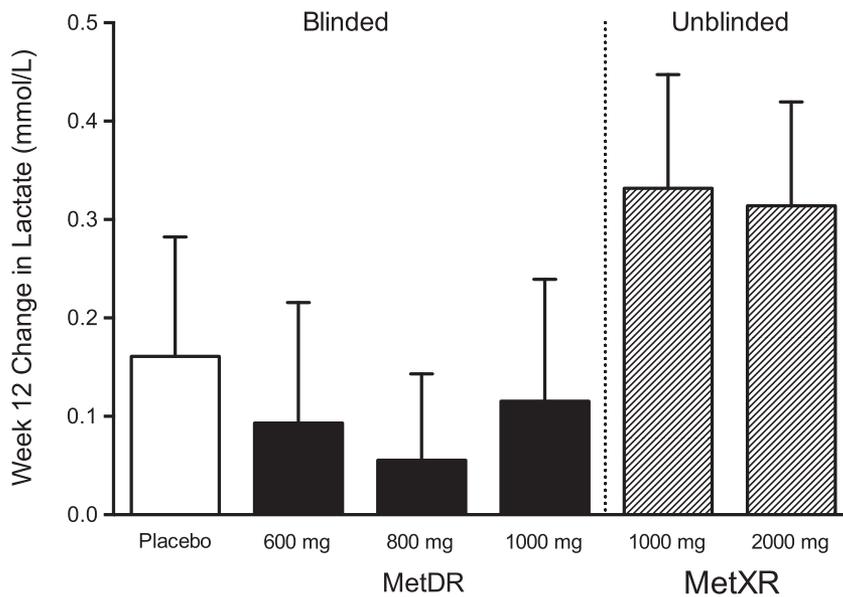
Figure 3—Change from baseline to week 12 in fasting lactate (study 2). Data are reported as the mean (SE) for the ITT population (*N* = 240). Normal lactate range is 0.5–2.2 mmol/L.

entirely consistent with multiple observations in animals and humans (including our data) that high plasma concentrations of the guanide/biguanide class increase plasma lactate levels (14,32–34). While their study used metformin doses equivalent to the clinically therapeutic range (20 and 50 mg/kg), they were administered intravenously or intraperitoneally, which would result in much higher peak metformin plasma concentrations and overall exposures than would be observed with oral administration. Thus, while gut-based mechanisms appear to account for the majority of the glucose-lowering effect of metformin at therapeutic doses, the inhibition of the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase may have important glucose-lowering actions at higher metformin plasma exposures.

Our data show an increase in plasma lactate concentrations with Met XR treatments compared with placebo that was not observed with any of the Met DR groups. Conditions that increase metformin plasma exposure (renal impairment, hepatic insufficiency, or states of circulatory dysfunction) can increase the risk of metformin-associated lactic acidosis (MALA), a rare but life-threatening condition (15). With typical metformin use, the incidence of MALA is very low (<10 cases per 100,000 patient-years) (15,35,36). MALA events

that are reported are usually associated with an elevated metformin dose or plasma exposure and an intercurrent event that further disrupts lactate production or clearance, such as sepsis, reduced tissue perfusion, anoxia, or impaired hepatic metabolism (15,22,36–39). Optimization of the presystemic gut-restricted metformin mechanisms of action may yield a significant treatment advantage by lowering the risk of MALA, particularly in at-risk populations. Of note, simply reducing the dose of currently available metformin formulations to reduce the risk of MALA is not a viable approach because low doses do not provide optimal glycemic control (13,40). Since the bioavailability of metformin increases with decreasing dose, the lower bowel is exposed to disproportionately less metformin when doses of ≤1,000 mg are administered (11), likely reducing or eliminating the glucose-lowering contribution of the gut-based mechanisms.

In summary, the delivery of metformin to the lower bowel with Met DR resulted in a glucose-lowering efficacy comparable to that with Met XR, but with lower doses and significantly lower systemic exposure. These data provide substantial evidence that currently prescribed metformin doses work predominantly in the gut and that the contribution of systemic metformin is small. Based on its gut-restricted

properties, Met DR may allow for the metformin treatment of patients with renal impairment without the risk of lactic acidosis associated with metformin accumulation.

Prior Presentation. Parts of these studies were presented in abstract form at the 50th Annual Meeting of the European Association for the Study of Diabetes, Vienna, Austria, 15–19 September 2014, and at the 75th Scientific Sessions of the American Diabetes Association, Boston, MA, 5–9 June 2015.

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