

# Mechanisms of Glucose Lowering of Dipeptidyl Peptidase-4 Inhibitor Sitagliptin When Used Alone or With Metformin in Type 2 Diabetes

## A double-tracer study

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**OBJECTIVE**—To assess glucose-lowering mechanisms of sitagliptin (S), metformin (M), and the two combined (M+S).

**RESEARCH DESIGN AND METHODS**—We randomized 16 patients with type 2 diabetes mellitus (T2DM) to four 6-week treatments with placebo (P), M, S, and M+S. After each period, subjects received a 6-h meal tolerance test (MTT) with [ $^{14}$ C]glucose to calculate glucose kinetics. Fasting plasma glucose (FPG), fasting plasma insulin, C-peptide (insulin secretory rate [ISR]), fasting plasma glucagon, and bioactive glucagon-like peptide (GLP-1) and gastrointestinal insulinotropic peptide (GIP) was measured.

**RESULTS**—FPG decreased from P,  $160 \pm 4$  to M,  $150 \pm 4$ ; S,  $154 \pm 4$ ; and M+S,  $125 \pm 3$  mg/dL. Mean post-MTT PG decreased from P,  $207 \pm 5$  to M,  $191 \pm 4$ ; S,  $195 \pm 4$ ; and M+S,  $161 \pm 3$  mg/dL ( $P < 0.01$ ). The increase in mean post-MTT plasma insulin and in ISR was similar in P, M, and S and slightly greater in M+S. Fasting plasma glucagon was equal ( $\sim 65$ – $75$  pg/mL) with all treatments, but there was a significant drop during the initial 120 min with S 24% and M+S 34% (both  $P < 0.05$ ) vs. P 17% and M 16%. Fasting and mean post-MTT plasma bioactive GLP-1 were higher ( $P < 0.01$ ) after S and M+S vs. M and P. Basal endogenous glucose production (EGP) fell from P  $2.0 \pm 0.1$  to S  $1.8 \pm 0.1$  mg/kg  $\cdot$  min, M  $1.8 \pm 0.2$  mg/kg  $\cdot$  min [both  $P < 0.05$  vs. P], and M+S  $1.5 \pm 0.1$  mg/kg  $\cdot$  min ( $P < 0.01$  vs. P). Although the EGP slope of decline was faster in M and M+S vs. S, all had comparable greater post-MTT EGP inhibition vs. P ( $P < 0.05$ ).

**CONCLUSIONS**—M+S combined produce additive effects to 1) reduce FPG and postmeal PG, 2) augment GLP-1 secretion and  $\beta$ -cell function, 3) decrease plasma glucagon, and 4) inhibit fasting and postmeal EGP compared with M or S monotherapy.

In diet-treated patients with type 2 diabetes mellitus (T2DM) and HbA<sub>1c</sub> of  $\sim 8.0\%$ , sitagliptin (S) reduces HbA<sub>1c</sub> by 0.6–0.7% over a 6-month period (1). A slightly greater HbA<sub>1c</sub> decline ( $\sim 0.8$ – $0.9\%$ ) is observed when metformin (M) therapy is added to S (2). Dipeptidyl peptidase (DPP)-4 inhibitors predominantly affect the postprandial plasma glucose excursion, but a significant, albeit modest, reduction in fasting plasma glucose (FPG) also is observed (1–3). The

mechanism of action of the DPP-4 inhibitors has been well studied and includes increased plasma glucagon-like peptide (GLP)-1 and gastrointestinal insulinotropic peptide (GIP) levels, resulting in increased insulin and reduced glucagon secretion (4–6). The increase in plasma insulin and the decline in glucagon inhibit basal endogenous glucose production (EGP) and enhance the suppression of EGP without affecting splanchnic (hepatic) glucose uptake or gastric emptying

(6,7). Therapy with S and M combined (M+S) exerts an additive effect to reduce HgA<sub>1c</sub>; the mechanism of action of this combination has yet to be examined. Several studies have demonstrated that M inhibits DPP-4 activity, thus increasing plasma active GLP-1 levels (8–10). There are also reports indicating that the decline in plasma glucose with M therapy can restore the  $\beta$ -cells' sensitivity to the stimulatory effect of incretins on insulin secretion (11,12). Despite these reports, we still do not fully understand the reasons why the use of M in diabetic patients is not accompanied by changes in insulin release. In the current study, we used the double-tracer technique (7) to examine the mechanism(s) via which M+S and each agent alone reduce the fasting and postmeal plasma glucose concentration in T2DM.

## RESEARCH DESIGN AND METHODS

Sixteen patients with T2DM (9 male and 7 female; mean age  $47 \pm 3$  years; body weight,  $92.1 \pm 4.2$  kg; BMI,  $33.5 \pm 2.0$  kg/m<sup>2</sup>; A1C,  $8.8 \pm 1.2\%$ ; FPG,  $165 \pm 12$  mg/dL; and diabetes duration,  $1.5 \pm 0.5$  years) were studied. All subjects were in good health as determined by medical history, physical exam, routine laboratory analysis, and electrocardiogram. T2DM was controlled by diet ( $n = 7$ ) or with a stable dose ( $\geq 6$  months) of sulfonylurea ( $n = 6$ ) or M ( $n = 3$ ). Three patients were on a "statin" stable dose. All studies were carried out at the Clinical Research Center at Texas Diabetes Institute. The study was approved by the institutional review board of University of Texas Health Science Center at San Antonio (UTHSCSA), and informed written consent was obtained from each patient before participation.

The study used a double-blind placebo (P)-controlled crossover design in which each subject randomly underwent four 6-week treatment periods with S alone, M alone, M+S, and P with a 2-week

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washout period between each treatment period. At screening, all subjects met with a dietitian and were instructed to consume a weight-maintaining diet (55% carbohydrate, 20% protein, and 25% fat). Patients who were taking antidiabetes drugs were asked to discontinue the medication for 6 weeks (washout prestudy period) and were monitored weekly to document that the FPG changed by <10% from week 4 to 6 prior to study initiation. Eligible subjects then received P medication for 2 weeks to document compliance, after which they were randomized for "treatment sequence" using a computer-generated schedule to receive P, M (1,000 mg b.i.d.) plus P, S (100 mg) plus P, or M+S (Fig. 1).

### Meal tolerance test with double tracer

Meal tolerance test (MTT) was performed after the completion of each 6-week treatment period for each allocation treatment. After a 10-h overnight fast, subjects were admitted to the Clinical Research Center at 0700 h and catheters were placed in each antecubital vein for blood withdrawal and infusion of test substances. At 0730 h, a prime ( $25 \mu\text{Ci} \times \text{FPG}/100$ ) continuous ( $0.25 \mu\text{Ci}/\text{min}$ ) infusion of [ $3\text{-}^3\text{H}$ ]glucose was started and continued until study end at 1630 h. At 1030 h, patients ingested a standardized meal (one hard-boiled egg, 2 ounces of Munster cheese, and 75 g glucose containing  $100 \mu\text{Ci}$  of  $1\text{-}^{14}\text{C}$ -glucose in 300 mL orange-flavored water). At 1000 h, blood samples were obtained every 10–15 min for determination of plasma [ $14\text{-}^1\text{C}$ ]glucose and [ $3\text{-}^3\text{H}$ ]glucose radioactivity and plasma glucose, insulin, C-peptide, and bioactive GLP-1/GIP concentrations. Urine was collected prior to the meal ingestion and from 1030 to 1630 h for determination of glucose concentration. After completion of each MTT, subjects took no medication for 2 weeks (washout period).

### Analytical determinations

Plasma glucose concentration was determined by glucose oxidase method (Analox Glucose Analyzer, Boston, MA). Plasma insulin, C-peptide, and glucagon concentrations were determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma [ $3\text{-}^3\text{H}$ ]glucose and [ $14\text{-}^1\text{C}$ ]glucose radioactivities were determined on barium hydroxide/zinc sulfate-precipitated plasma extracts as previously described (6,7). Plasma bioactive GLP-1 concentration was measured by ELISA using  $\text{NH}_2$ -terminal-specific antibody (BD

Diagnostics, St Charles, MO) and plasma intact GIP by radioimmunoassay with an antibody specific for the  $\text{NH}_2$ -terminus (BD Diagnostics).

### Calculations

The basal rate of EGP was calculated as the [ $3\text{-}^3\text{H}$ ]glucose infusion (dpm/min) divided by the steady-state plasma [ $3\text{-}^3\text{H}$ ]glucose specific activity (dpm/mg). After glucose ingestion, non-steady state conditions prevail and total  $R_a$  ( $R_{aT}$ ) and  $R_d$  from the systemic circulation were computed from the [ $3\text{-}^3\text{H}$ ]glucose data using the Steele equation as previously reported (7). Total tissue glucose disposal was calculated by  $R_d$  (integrated over the time period 0–360 min) minus the measured urinary glucose loss over the same time period. The [ $1\text{-}^{14}\text{C}$ ]glucose data were used to calculate the  $R_a$  of oral glucose ( $R_{aO}$ ), and after the meal EGP was calculated as the difference between  $R_{aT}$  and  $R_{aO}$  (7). Splanchnic (hepatic) glucose uptake was calculated as follows:  $75 \text{ g} - R_{aO}$  from 0 to 360 min. Insulin secretory rate (ISR) was calculated from deconvolution of plasma C-peptide curve (13) using ISEC (14). Indices of insulin secretion were calculated as  $\Delta I/\Delta G$  and  $\Delta \text{ISR}/\Delta G$  and of  $\beta$ -cell function as  $\Delta I/\Delta G \times \text{MI}$  and  $\Delta \text{ISR}/\Delta G \times \text{MI}$ , where MI is Matsuda index of insulin sensitivity: a composite index of hepatic insulin sensitivity calculated using baseline and changes in plasma insulin and glucose concentrations as previously described (15).

### Statistical methods

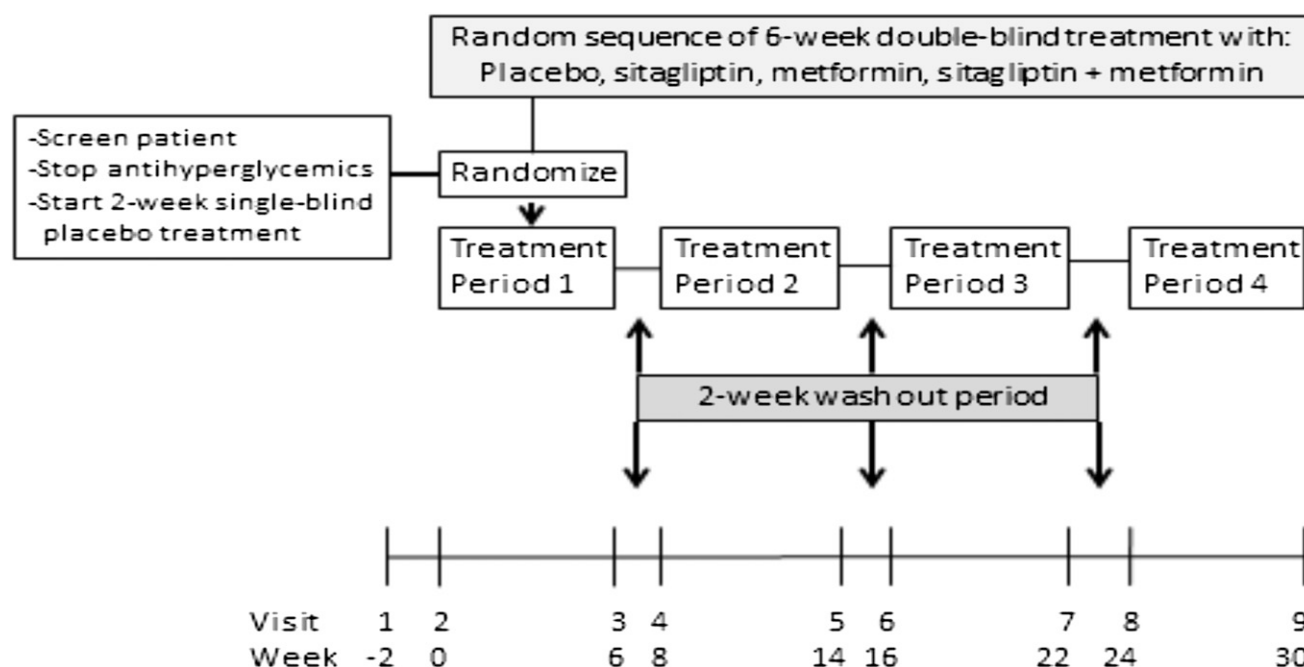
All 16 patients completed all four treatment periods and were included in the analysis. ANOVA with fixed terms for period and treatment and a random term for subject was used to compare treatment groups. A general unstructured variance-covariance matrix was applied for measurements at different periods. Between-group comparisons after 6 weeks of treatment were assessed using the ANOVA model at  $\alpha = 0.05$  (two sided) and an appropriate contrast statement. Power calculations indicated that with 16 patients, the study would provide ~90% power to detect a difference in EGP of  $0.28 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  between treatment groups with a half-width of the 95% CI of  $0.17 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . This calculation used a within-subject SD estimate of  $0.24 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  with  $\alpha = 0.05$  and a two-sided test. The treatment effect of  $0.28 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  was the difference observed between single-dose

vildagliptin and P in a two-period cross-over study (6). Correlation coefficients and multiple regression analyses were derived and used as a measure of association.

**RESULTS**—All subjects tolerated the antidiabetes medications well, and there were no adverse events. Body weight ( $92.1 \pm 4.2$  vs.  $91.8 \pm 3.8$  kg) did not change, and mean  $\text{HbA}_{1c}$  decreased to  $7.8 \pm 1.0\%$  at study end. FPG (Fig. 2A) decreased from  $160 \pm 4$  mg/dL (P) to  $150 \pm 4$  mg/dL (M), to  $154 \pm 4$  (S) (both  $P < 0.05$  vs. P), and to  $125 \pm 3$  mg/dL (M+S) ( $P < 0.01$  vs. M and S). Mean post-MTT plasma glucose decreased from  $207 \pm 5$  mg/dL (P) to  $191 \pm 4$  mg/dL (M), to  $195 \pm 4$  mg/dL (S) (both  $P < 0.01$  vs. P), and to  $161 \pm 3$  mg/dL (M+S) ( $P < 0.01$  vs. M and S).

Fasting plasma insulin did not change significantly with any treatment (Fig. 2B). Mean post-MTT insulin concentration after M ( $55 \pm 2 \mu\text{U}/\text{mL}$ ) and S ( $53 \pm 2 \mu\text{U}/\text{mL}$ ) were similar to P ( $50 \pm 3$  L) but increased to  $64 \pm 4 \mu\text{U}/\text{mL}$  after M+S ( $P < 0.05$  vs. P, M, and S). Fasting ISR was equivalent with all treatments (Fig. 2C). Mean post-MTT ISRs was similar after M ( $7.3 \pm 0.2 \text{ pmol}/\text{kg} \cdot \text{min}$ ), S ( $7.1 \pm 0.3 \text{ pmol}/\text{kg} \cdot \text{min}$ ), and P ( $6.8 \pm 0.4 \text{ pmol}/\text{kg} \cdot \text{min}$ ) but increased significantly to  $7.8 \pm 0.4 \text{ pmol}/\text{kg} \cdot \text{min}$  after M+S ( $P < 0.05$  vs. P, M, and S). Fasting plasma glucagon was comparable with all treatments (Fig. 2D). During the initial 120 min of the MTT, mean plasma glucagon concentration after S ( $71 \pm 5 \text{ pg}/\text{mL}$ ) and M+S ( $72 \pm 6 \text{ pg}/\text{mL}$ ) was reduced compared with both P ( $84 \pm 5 \text{ pg}/\text{mL}$ ) and M ( $88 \pm 7 \text{ pg}/\text{mL}$ ). The baseline plasma bioactive GLP-1 concentration (Fig. 2E) was higher after S ( $10.1 \pm 1.2 \text{ pg}/\text{mL}$ ) and M+S ( $16.9 \pm 2.2 \text{ pg}/\text{mL}$ ) compared with M ( $5.5 \pm 0.5 \text{ pg}/\text{mL}$ ) or P ( $6.9 \pm 1.0 \text{ pg}/\text{mL}$ ). Mean post-MTT plasma bioactive GLP-1 concentration was higher after S ( $26.1 \pm 2.2 \text{ pg}/\text{mL}$ ) and M+S ( $34.9 \pm 2.0 \text{ pg}/\text{mL}$ ) compared with M ( $14.2 \pm 1.3 \text{ pg}/\text{mL}$ ) and P ( $13.1 \pm 1.0 \text{ pg}/\text{mL}$ ) (both  $P < 0.01$ ). Baseline plasma GIP concentrations were similar ( $\sim 44 \text{ pg}/\text{mL}$ ) in all four treatment groups and increased similarly in P ( $186 \pm 8 \text{ pg}/\text{mL}$ ), M ( $171 \pm 7 \text{ pg}/\text{mL}$ ), S ( $173 \pm 6 \text{ pg}/\text{mL}$ ), and M+S ( $183 \pm 9 \text{ pg}/\text{mL}$ ).

Under fasting conditions, EGP (Fig. 3A) in P ( $1.95 \pm 0.06 \text{ mg}/\text{kg} \cdot \text{min}$ ) decreased modestly after M ( $1.84 \pm 0.08 \text{ mg}/\text{kg} \cdot \text{min}$ ) and S ( $1.82 \pm 0.09 \text{ mg}/\text{kg} \cdot \text{min}$ ) (both  $P < 0.05$  vs. P) and decreased further after M+S



**Figure 1**—Schematic representation of the study design. A random sequence was assigned for each subject to receive 6 weeks of treatment with P, M, S, and M+S combination. A 2-week washout period was observed between treatment periods.

( $1.49 \pm 0.05$  mg/kg  $\cdot$  min) ( $P < 0.01$  vs. P). During the MTT, the mean  $R_{aT}$  was  $2.78 \pm 0.18$  mg/kg  $\cdot$  min after P, decreased to  $2.59 \pm 0.17$  mg/kg  $\cdot$  min after M and to  $2.56 \pm 0.11$  mg/kg  $\cdot$  min after S (both  $P < 0.05$  vs. P), and decreased further to  $2.23 \pm 0.12$  mg/kg  $\cdot$  min after M+S ( $P < 0.01$  vs. S and M). During the MTT, EGP ( $R_{aT} - R_{aO}$ ) was suppressed to  $0.73 \pm 0.14$  mg/kg  $\cdot$  min after P (Fig. 3B). Treatments with M ( $0.33 \pm 0.12$  mg/kg  $\cdot$  min), S ( $0.51 \pm 0.09$  mg/kg  $\cdot$  min), and M+S combined ( $0.25 \pm 0.10$  mg/kg  $\cdot$  min) further enhanced the suppression of EGP during the MMT (all  $P < 0.05$  vs. P). The mean rate of oral glucose appearance (Fig. 3C) was similar after all four treatment regimens. The total amount of glucose that appeared in the peripheral circulation over the postmeal period of 360 min ranged between 62 and 68 g, i.e., 82–91% of the ingested glucose load of 75 g. Thus, the splanchnic glucose uptake was similar (7–13 g) after all four treatments. The mean tissue  $R_d$  (Fig. 3D) during the MTT were comparable after treatment with P ( $2.56 \pm 0.12$  mg/kg  $\cdot$  min), M ( $2.51 \pm 0.13$  mg/kg  $\cdot$  min), and S ( $2.41 \pm 0.10$  mg/kg  $\cdot$  min) and after M+S ( $2.38 \pm 0.11$  mg/kg  $\cdot$  min).

Insulin sensitivity and insulinogenic indices were calculated from 0 to 180 min for each treatment period, adapted from a previous publication (15). These indices were calculated between 0 and 180 min

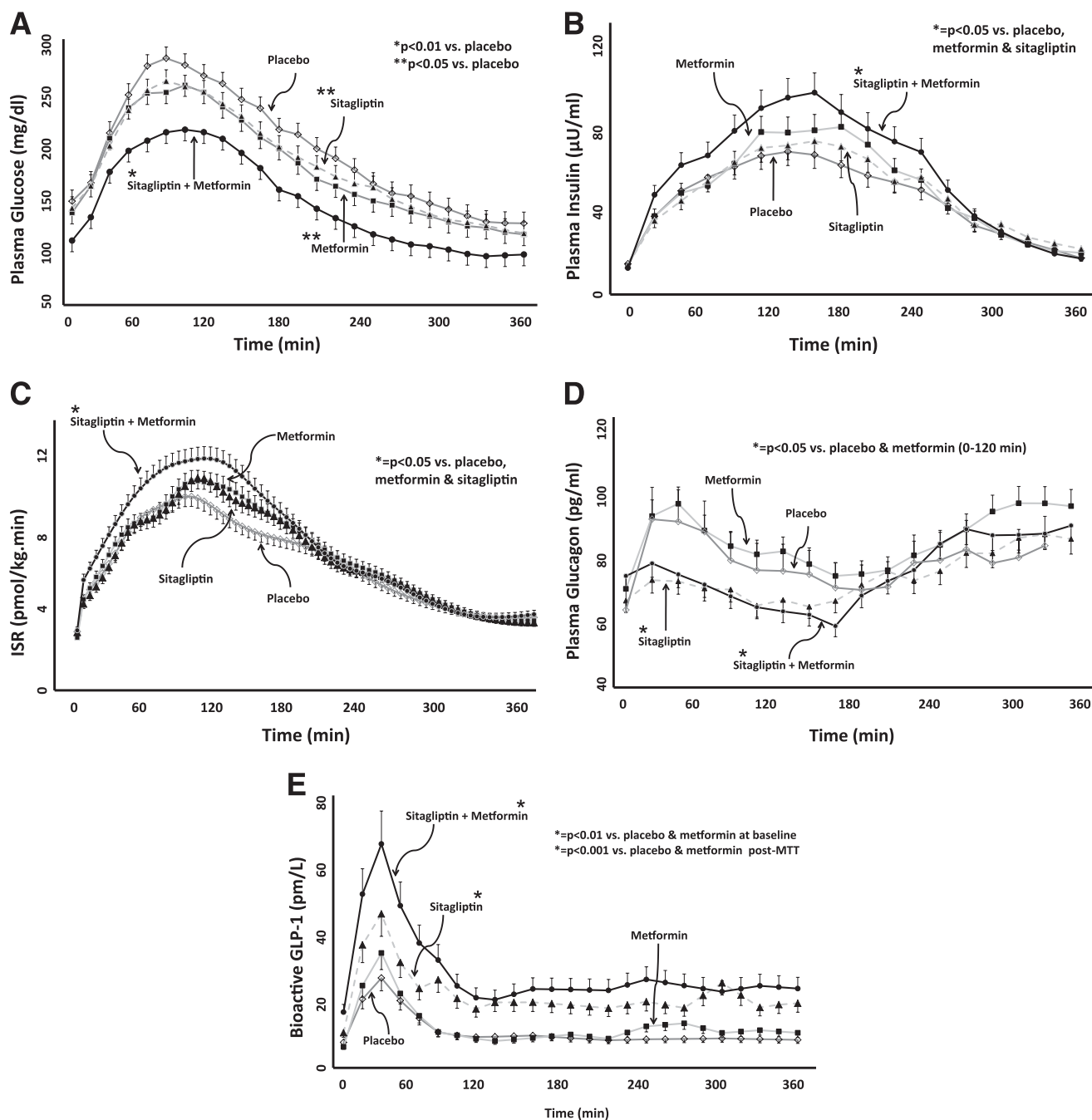
because most hormonal changes occur during this postmeal period. The MI of insulin sensitivity was similar after each therapy, while hepatic insulin sensitivity improved significantly with M and M+S ( $13.2 \pm 2.1$  and  $12.6 \pm 1.5$   $\mu$ U/mL  $\cdot$  mg/kg  $\cdot$  min) but not with S alone ( $18.0 \pm 2.7$   $\mu$ U/mL  $\cdot$  mg/kg  $\cdot$  min) compared with P ( $20.8 \pm 4.2$   $\mu$ U/mL  $\cdot$  mg/kg  $\cdot$  min). Insulin secretion measured both as ( $\Delta I/\Delta G$  and  $\Delta ISR/\Delta G$ ) increased mildly with S alone and M alone (both  $P = \text{NS}$  and further after M+S treatment,  $P < 0.05$ ). The calculated  $\beta$ -cell function index ( $\Delta I/\Delta G \times \text{MI}$ ) paralleled insulin secretion. There was close concordance between the changes in insulinogenic indices calculated over the entire 360-min period of the MTT and the initial 180 min of the MTT for all four treatment periods.

In each of the four treatment protocols, the decrement in EGP correlated with the decrement in FPG concentration ( $r = 0.54$ ,  $P < 0.03$ ). The increment in plasma insulin concentration and the decrement in plasma glucagon concentration were correlated with the decrement in EGP ( $r = 0.45$ ,  $P < 0.01$ , and  $r = 0.35$ ,  $P < 0.05$ , respectively) after the M+S combination. The decrement in plasma glucagon concentration also correlated with the decrement in EGP ( $r = 0.35$ ,  $P < 0.05$ ) after the S monotherapy. The increment in bioactive GLP-1 plasma

concentration correlated with both the increment in plasma insulin concentration and the decrement in plasma glucagon concentration ( $r = 0.38$ ,  $P < 0.05$ , and  $r = 0.22$ ,  $P < 0.05$ , respectively) after combined M+S therapy. Multiple regression analyses performed for all of these parameters further indicate that FPG concentration is correlated with changes in post-MTT glucose, EGP, insulin, glucagon, and bioactive GLP-1 ( $r^2 = 0.16$ ,  $P = 0.05$ ).

There was no correlation between the increment in plasma GIP concentration and either the increment in plasma insulin concentration or the decrement in plasma glucagon concentration after any of the four treatments.

**CONCLUSIONS**—Combination therapy with M+S is an effective treatment option for patients with T2DM (2). However, the mechanism(s) underlying the “additive” effect of M+S have yet to be established. Our findings confirm that, when used as monotherapy, both S and M reduce postprandial glycemic excursion to a comparable degree, while combination of the two promotes greater attenuation of the postprandial hyperglycemia. The postprandial effect of M monotherapy appears to be secondary to a direct suppression of endogenous (hepatic) glucose production, since neither



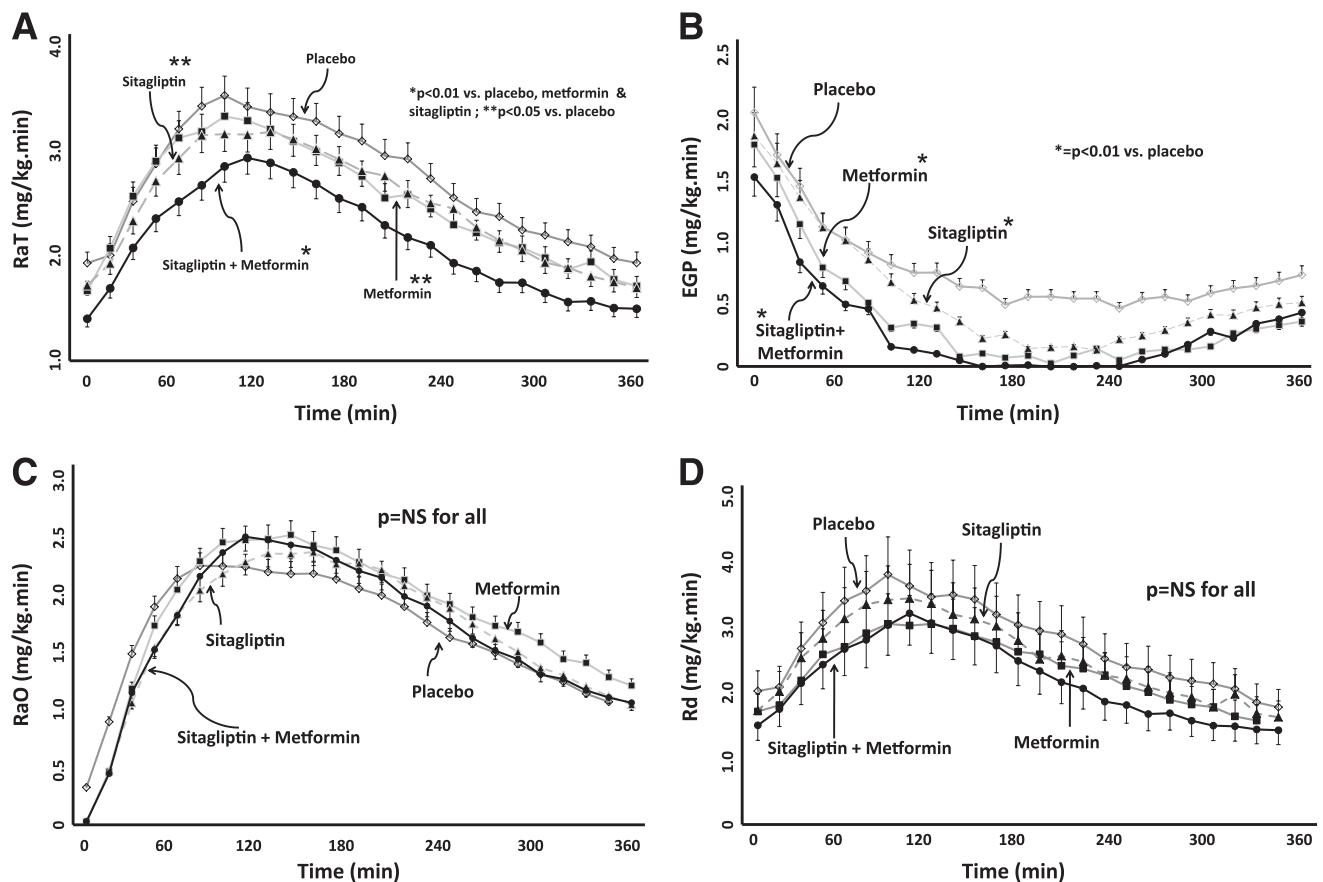
**Figure 2**—A: Changes in plasma glucose concentration during MTT. B: Changes in plasma insulin concentration during MTT. C: Changes in ISR, calculated from the plasma C-peptide deconvolution curve, during MTT. D: Changes in plasma glucagon concentration during MTT. E: Plasma bioactive GLP-1 concentration during MTT.

plasma insulin nor glucagon levels changed significantly, while that of S is mediated primarily by the suppression of glucagon secretion. The direct effect of M on the liver presumably is hormone independent, whereas the reduction in EGP after S therapy most likely is secondary to alterations in the portal insulin-to-glucagon ratio resulting from the increase in plasma GLP-1. The steep slope of decline in EGP after M compared with

the slower decrease in EGP after S monotherapy also suggests that different mechanisms are involved in the enhancement of EGP suppression. The marked decrease in basal EGP and the steep slope with greater suppression of EGP after combination M+S also are consistent with independent and additive effects of the two antidiabetes agents. Comparable EGP suppression has been reported in normal subjects after a glucose challenge, which

also indicated that most of the glucose was disposed peripherally (16).

Neither plasma insulin nor ISRs were significantly enhanced after the meal with either M or S monotherapy. In contrast, M+S combination was accompanied by a clear augmentation of insulin secretion and potentiating of plasma glucagon suppression, resulting in the greatest suppression of EGP. Of note, plasma glucagon suppression after M+S therapy



**Figure 3**—A: Changes in  $R_{aT}$  during MTT. B: Changes in EGP during MTT. C:  $R_{aO}$  during MTT. D:  $R_d$  during MTT.

correlated with the quick surge (initial 120 min) in plasma bioactive GLP-1. We did not observe a significant change in postprandial insulin secretion after 6 weeks of S monotherapy despite the modest increase in plasma bioactive GLP-1 concentration. However, equivalent plasma insulin and C-peptide levels (i.e., increased  $\Delta I/\Delta G$  and  $\Delta ISR/\Delta G$ ) in the face of a lower plasma glucose concentration after S (compared with P) indicates that  $\beta$ -cell function improved. These findings are consistent with a previous report (6) showing that the immediate (minutes to hours) absolute increase in plasma insulin response induced by vildagliptin dissipates overtime. Unlike insulin, suppression of plasma glucagon was detected during the initial 120 min of the MTT after treatment with S alone and the combined M+S. These observations suggest a differential sensitivity of pancreatic  $\alpha$ - and  $\beta$ -cells to GLP-1 (17,18).

The present results demonstrate that M, S, and the combination M+S therapy did not have any effects on the  $R_a$  of ingested glucose in the peripheral circulation, on splanchnic glucose utilization, or

on the rate of tissue glucose disposal. However, we cannot entirely rule out small differences among treatments in either splanchnic glucose uptake or tissue glucose disposal, since these could have escaped our detection. These results indicate that the reduction in EGP was the primary factor responsible for the attenuation in the postmeal plasma glucose concentration after each of the three therapeutic interventions. Thus, unlike the GLP-1 analogs (19–21) S has no effect on either gastric emptying or splanchnic glucose utilization. The failure to observe any increase peripheral glucose disposal is not surprising for two reasons: 1) neither M, in the absence of weight loss (22,23), nor S (1–3) is known to enhance muscle insulin sensitivity and 2) despite the increase in plasma insulin response with the M+S treatment, any increment in insulin-stimulated glucose disposal would have been offset by the large reduction in plasma glucose concentration.

In this study, we show a two- to threefold increase in basal plasma bioactive GLP-1 concentration in T2DM patients after 6 weeks of S used in combination

with M. Within 120 min after meal ingestion, bioactive GLP-1 concentration increased with S treatment, and this elevation was much greater with the combined M+S treatment, even though there was no significant rise with M treatment. This observation is consistent with previous reports (8–12) and underscores the clinical relevance of the additive, even synergistic, effect of M when used together with S to increase plasma GLP-1 levels. However, this effect of M apparently relies on simultaneous improvement in plasma glucose concentration, perhaps leading to augmented insulin secretion and enhanced suppression of glucagon secretion. A novel finding was the fourfold increase in plasma GIP levels observed with M monotherapy. However, since basal and postmeal plasma GIP levels were comparable in all four treatment periods, the enhanced stimulation of insulin secretion and suppression of glucagon secretion in T2DM patients treated with S alone or in combination with M must have resulted from the increase in bioactive plasma GLP-1 levels. Our results are consistent with those of others (24,25) and indicate

that GIP does not potentiate the antidiabetes effect of GLP-1 in T2DM patients. Consistent with this, there is no known role for GIP on glucagon suppression and the effect of GIP on insulin stimulation overlap with the GLP-1 intracellular pathways (17).

Whether the magnified GLP-1 response to a standard meal after DPP-4 treatment in combination with M is a consequence of the effect of S plus better glycemic control, superimposed on M stimulation of endogenous GLP-1 secretion, cannot be determined from our study. M, as well as some other antidiabetes agents (8–10,24), has been suggested to directly enhance GLP-1 release from the intestinal L cells. However, the possibility that this is a nonspecific effect related to improved glycemic control has yet to be excluded (26). Because M monotherapy was not accompanied by any significant change in plasma GLP-1 in our studies either at baseline or after the meal, our data support the notion that the magnified GLP-1 response is primarily explained by better glycemic control. These observations are of considerable clinical significance, since the combination of M with a DPP-4 inhibitor provides a unique mechanism of glucose lowering with additive effect to reduce plasma glucose concentration in T2DM patients.

In summary, the current study demonstrates that S and M monotherapy reduces glycemic excursions via independent and additive mechanisms. The inhibition of endogenous (hepatic) glucose production by M most likely resulted from a direct effect on the liver, whereas the effect of S on EGP was indirect and associated with elevated plasma GLP-1 levels with subsequent glucagon suppression. Combination M+S therapy further reduced postmeal plasma glucose concentrations subsequent to a greater increase in fasting and postmeal GLP-1 levels, accompanied by a further enhancement in insulin secretion and glucagon suppression. There were no changes in splanchnic glucose uptake, gastric emptying, or insulin-mediated glucose disposal with any treatment regimens, and the attenuation of the postprandial hyperglycemia with all therapies was entirely attributed to the reduction in EGP.

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C.S.-H. executed the research procedures, sample collection, laboratory analyses, and data interpretation. C.T. designed the study; supervised research procedures, clinical management, and laboratory analyses; interpreted data; contributed to the discussion; and reviewed and edited the manuscript. J.d.J.G.-G. executed the research procedures, sample collection, laboratory analyses, and data interpretation. J.A. provided laboratory analyses, data interpretation, and technical assistance. R.A.D. and E.C. designed the study; supervised research procedures, clinical management, and laboratory analyses; interpreted data; contributed to the discussion; and reviewed and edited the manuscript. E.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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