A Protein Preload Enhances the Glucose-Lowering Efficacy of Vildagliptin in Type 2 Diabetes

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OBJECTIVE

Nutrient "preloads" given before meals can attenuate postprandial glycemic excursions, at least partly by slowing gastric emptying and stimulating secretion of the incretins (i.e., glucagon-like peptide-1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP]). This study was designed to evaluate whether a protein preload could improve the efficacy of the dipeptidyl peptidase-4 (DPP-4) inhibitor vildagliptin to increase incretin concentrations, slow gastric emptying, and lower postprandial glycemia in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Twenty-two patients with type 2 diabetes treated with metformin were studied on four occasions, receiving either 50 mg vildagliptin (VILD) or placebo (PLBO) on both the evening before and the morning of each study day. The latter dose was followed after 60 min by a preload drink containing either 25 g whey protein (WHEY) or control flavoring (CTRL), and after another 30 min by a ^{13}C-octanoate–labeled mashed potato meal. Plasma glucose and hormones, and gastric emptying, were evaluated.

RESULTS

Compared with PLBO/CTRL, PLBO/WHEY reduced postprandial peak glycemia, increased plasma insulin, glucagon, and incretin hormones (total and intact), and slowed gastric emptying, whereas VILD/CTRL reduced both the peak and area under the curve for glucose, increased plasma intact incretins, and slowed gastric emptying but suppressed plasma glucagon and total incretins ($P < 0.05$ each). Compared with both PLBO/WHEY and VILD/CTRL, VILD/WHEY was associated with higher plasma intact GLP-1 and GIP, slower gastric emptying, and lower postprandial glycemia ($P < 0.05$ each).

CONCLUSIONS

In metformin-treated type 2 diabetes, a protein preload has the capacity to enhance the efficacy of vildagliptin to slow gastric emptying, increase plasma intact incretins, and reduce postprandial glycemia.

The "incretin" hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are major determinants of postprandial glycemia (1). The latter is an important target in patients with type 2 diabetes, particularly those with modestly elevated HbA_{1c} (2). In health, both incretins stimulate insulin secretion in a glucose-dependent manner (1). However, the effect of GIP is substantially diminished in type 2 diabetes (3), whereas GLP-1

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retains considerable insulinotropic activity (3) and also slows gastric emptying (4) and suppresses glucagon secretion (5) and energy intake (6).

Dipeptidyl peptidase-4 (DPP-4) inhibitors prevent rapid degradation of endogenous incretins, thereby elevating plasma concentrations of the intact forms. However, the overall secretion of incretin hormones is reduced as a result of negative feedback regulation (1). Given the loss of response to GIP in type 2 diabetes (3), the glycemic effect of DPP-4 inhibitors has been considered largely GLP-1 related. Indeed, the GLP-1 receptor antagonist exendin(9–39) attenuates the glucose-lowering and insulinotropic effects of the DPP-4 inhibitor sitagliptin by ~50% in patients with type 2 diabetes and abolishes any slowing of gastric emptying (7). Therefore, strategies that stimulate endogenous GLP-1 secretion could potentially enhance the efficacy of DPP-4 inhibition.

Our group has developed a novel "preload" concept, which primarily targets the postprandial glycemic excursion in the management of type 2 diabetes. Consumption of a macronutrient preload before the main meal can stimulate release of gut peptides, including GLP-1, slow gastric emptying, and reduce the glycemic response to the meal (8). We recently reported that a preload of xylose (a poorly absorbed pentose) augments the reduction of postprandial glycemia by sitagliptin in type 2 diabetes, associated with sustained elevation of plasma intact GLP-1 concentrations (9). However, diarrhea and flatulence may limit the tolerability of xylose for long-term use. Whey protein preloads also stimulate GLP-1 and reduce postprandial glycemia in type 2 diabetes and are well tolerated (10,11). However, the doses of whey used (~50 g) entail a substantial burden in energy intake and are relatively expensive. A smaller whey preload (25 g) was also reported to slow gastric emptying and reduce postprandial peak blood glucose, without causing significant weight gain with sustained use in patients with type 2 diabetes (12), but whether it is sufficient to increase plasma GLP-1 concentrations and, accordingly, enhance the efficacy of DPP-4 inhibitors has not been established.

In the current study, we hypothesized that a protein preload would enhance the efficacy of glucose lowering by DPP-4 inhibition and evaluated the acute effect of the DPP-4 inhibitor vildagliptin with and without a 25-g whey preload on postprandial glycemia in patients with type 2 diabetes managed with metformin monotherapy, a group in whom DPP-4 inhibitors are commonly added (13).

RESEARCH DESIGN AND METHODS

Subjects

Twenty-two male subjects with type 2 diabetes, managed by metformin monotherapy (500–2,000 mg/day, stable for ≥3 months), were studied after providing written informed consent. The mean (±SE) age was 64.2 ± 1.4 years, BMI 27.9 ± 1.7 kg/m^2, HbA_{1c} 6.6 ± 0.2% (48.8 ± 2.2 mmol/mol), and duration of known diabetes 5.6 ± 1.2 years. None were smokers or took medications affecting gastrointestinal function. The protocol was approved by the Human Research Ethics Committee of Royal Adelaide Hospital and was conducted in accordance with the principles of the Declaration of Helsinki.

Protocol

Each subject was studied on four occasions, separated by 7 days, in randomized, double-blind fashion. On the evening before each study day (~1900 h), subjects consumed a standardized beef lasagna meal (2,472 kJ; McCain Foods Proprietary Ltd., Victoria, Australia) with a tablet of either 50 mg vildagliptin (Novartis, NSW, Australia) or matching placebo, together with their usual evening dose of metformin. Compliance was reinforced with a phone call and evaluated by pill count.

Subjects then fasted until morning and attended the laboratory at ~0730 h. Any usual morning medications, including metformin, were withheld until the end of the visit. An intravenous cannula was inserted for repeated blood sampling, and subjects remained seated throughout the study. Vildagliptin 50 mg or a matching placebo tablet was administered orally with 30 mL water (at t = −90 min), followed after 60 min (t = −30 min) by a 250-mL preload drink containing either 25 g whey protein isolate (89 kcal; Murray Goulburn, Melbourne, Australia) or 25 g control flavoring (8 kcal; Cottee's, Southbank, Australia), so that the four treatments were vildagliptin + whey preload (VILD/WHEY), vildagliptin + control preload (VILD/CTRL), placebo + whey preload (PLBO/WHEY), and placebo + control preload (PLBO/CTRL) (Fig. 1). Thirty minutes later (between t = 0 and 5 min), subjects ate a semisolid meal comprising 65 g powdered potato (Deb Instant Mashed Potato; Continental, Epping, Australia) and 20 g glucose, reconstituted with 200 mL water and one egg yolk containing 100 μL ^{13}C-octanoate. Breath samples were collected immediately before and every 5 min after meal ingestion in the 1st hour and every 15 min for a further 3 h for the measurement of gastric emptying. Venous blood samples were collected at t = −90, −30, −15, 0, 15, 30, 60, 90, 120, 180, and 240 min, for measurements of plasma glucose, insulin, glucagon, and GIP and GLP-1 (both total and intact forms). Samples were stored on ice in tubes containing EDTA and DPP-4 inhibitor (DPP4-010; Linco Research Inc., St. Charles, MO), before centrifugation at 3,200 rpm for 15 min at 4°C within 15 min of collection. Plasma was separated and stored at −80°C for subsequent analysis.

Measurements

Plasma glucose concentrations were measured by the glucose oxidase technique (2300 STAT Plus; YSI, Yellow Springs, OH). Plasma insulin was measured by ELISA immunoassay (10-1113; Mercodia, Uppsala, Sweden). Plasma glucagon was measured by radioimmunoassay (GL-32K; Millipore, Billerica, MA). Plasma GIP and GLP-1 analyses were performed as previously described (14,15). Intact and total GIP were analyzed with the N-terminally and C-terminally directed antisera 98171 (14) and 80867 (15), respectively. Intact GLP-1 was measured using a two-site ELISA (C-terminally directed GLP-1F5–catching antibody and N-terminally directed Mab26.1-detecting antibody) (14). Total GLP-1 was assayed using antiserum 89390, requiring the intact amidated C terminus of the molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite (14).

The $^{13}CO_2$ concentration in breath samples was measured by an isotope ratio mass spectrometer (ABCA 2020; Europa Scientific, Crewe, U.K.) with an

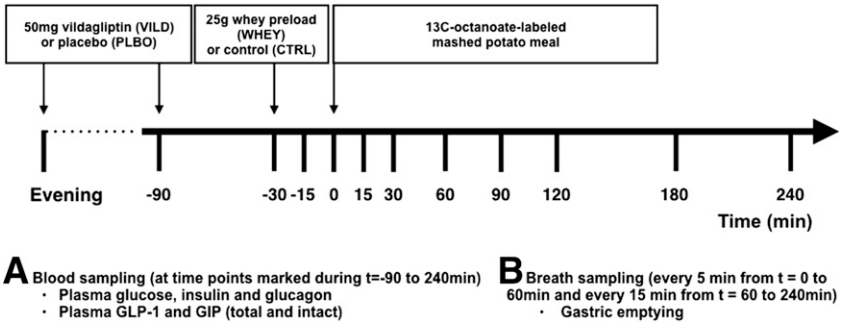

Figure 1—Outline of study protocol. On the preceding evening of each study visit, either 50 mg vildagliptin (VILD) or a placebo (PLBO) tablet was given with a standardized meal. On each study day, at $t = -90$ min, either 50 mg vildagliptin or a placebo tablet was given. At $t = -30$ min, a 250-mL preload drink containing either 25 g whey or control flavoring (CTRL) was consumed, followed by a ^{13}C-octanoate–labeled mashed potato meal (at $t = 0$–5 min). Venous blood was obtained at frequent intervals during the study for measurement of plasma glucose, insulin, glucagon, GLP-1, and GIP. Gastric emptying was determined by breath test.

online gas chromatographic purification system. The half-emptying time (T50) was calculated, using the formula described by Ghoos et al. (16). This method has been validated against scintigraphy for the measurement of gastric emptying (17).

Statistical Analysis

The differences in fasting plasma glucose and hormone levels for vildagliptin versus placebo, and for whey versus control preload days, were evaluated using two-factor repeated-measures ANOVA, with drug and preload as factors. Areas under the curve (AUCs) were calculated using the trapezoidal rule for plasma glucose and hormones, which, together with postprandial peak plasma glucose and T50, were compared using one-factor repeated-measures ANOVA. The variables were also assessed using two-factor repeated-measures ANOVA, with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni-Holm correction, were performed if ANOVAs revealed significant effects. Relationships between variables were assessed using univariate linear regression analysis. Based on our previous work (9,10,12), a sample size of 22 subjects was calculated to have at least 80% power (at $\alpha = 0.008$ to enable correction for multiple post hoc testing) to detect a difference in the AUC for blood glucose of 2.5 mmol/L · h with an SD of 3.3 mmol/L · h between treatments. All analyses were performed with SPSS Statistics (Version 21; IBM, New York, NY). Data are presented as means ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Compliance with medication was complete, and all subjects tolerated the study well.

Plasma Glucose Concentrations

Fasting plasma glucose did not differ between whey and control but was slightly lower with vildagliptin than placebo ($P = 0.003$ at $t = -90$ min and $P = 0.006$ at $t = -30$ min, respectively) (Table 1). Before the meal ($t = -30$ to 0 min), plasma glucose remained unchanged after both control and whey preloads. After the meal, the plasma glucose excursion showed significant treatment effects on both the peak and AUC ($P < 0.001$ for each), such that the peak was lower for PLBO/WHEY and VILD/CTRL versus PLBO/CTRL and was lowest after VILD/WHEY ($P < 0.05$ for each), whereas the AUC was lower for VILD/CTRL versus PLBO/CTRL and lowest after VILD/WHEY ($P < 0.05$ for each), without a significant difference between PLBO/CTRL and PLBO/WHEY (Table 2 and Fig. 2*A*).

Plasma Insulin Concentrations

Fasting plasma insulin (at $t = -90$ and -30 min) did not differ across study days (Table 1). Before the meal ($t = -30$ to 0 min), plasma insulin remained

Table 1—Fasting plasma glucose, insulin, glucagon, and GIP and GLP-1 (both total and intact forms) concentrations on the control (CTRL) and whey preload days after an acute dose of 50 mg vildagliptin (VILD) or placebo (PLBO) at ~1900 h on the preceding evening in patients with type 2 diabetes treated with metformin ($n = 22$)*

	PLBO (CTRL)	PLBO (WHEY)	VILD (CTRL)	VILD (WHEY)	Difference due to drug	Difference due to preload
At $t = -90$ min						
Plasma glucose (mmol/L)	8.8 ± 0.5	8.8 ± 0.5	8.4 ± 0.5	8.3 ± 0.4	0.003	0.501
Plasma insulin (mU/L)	9.3 ± 1.5	8.4 ± 1.0	8.2 ± 1.1	9.0 ± 1.2	0.534	0.900
Plasma glucagon (pg/mL)	95.5 ± 4.7	93.6 ± 4.0	91.8 ± 4.8	90.2 ± 24.5	0.092	0.249
Plasma total GIP (pmol/L)	11.5 ± 1.1	10.5 ± 0.9	9.8 ± 0.7	9.7 ± 0.9	0.022	0.202
Plasma intact GIP (pmol/L)	6.4 ± 0.4	6.4 ± 0.5	7.9 ± 0.6	8.2 ± 0.7	0.002	0.639
Plasma total GLP-1 (pmol/L)	4.6 ± 0.4	5.2 ± 0.8	5.4 ± 0.7	4.9 ± 0.6	0.494	0.803
Plasma intact GLP-1 (pmol/L)	0.2 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	1.0 ± 0.4	0.003	0.340
At $t = -30$ min						
Plasma glucose (mmol/L)	8.7 ± 0.4	8.6 ± 0.5	8.4 ± 0.5	8.1 ± 0.4	0.006	0.251
Plasma insulin (mU/L)	8.2 ± 1.4	6.7 ± 1.0	7.9 ± 1.3	7.9 ± 1.2	0.216	0.130
Plasma glucagon (pg/mL)	88.0 ± 4.5	86.5 ± 3.7	83.7 ± 4.2	84.2 ± 3.7	0.007	0.776
Plasma total GIP (pmol/L)	9.6 ± 0.8	10.0 ± 1.1	8.2 ± 0.6	8.6 ± 0.9	0.004	0.553
Plasma intact GIP (pmol/L)	5.6 ± 0.5	6.0 ± 0.4	7.3 ± 0.6	8.3 ± 0.9	0.003	0.093
Plasma total GLP-1 (pmol/L)	4.1 ± 0.4	5.0 ± 0.6	4.3 ± 0.6	4.5 ± 0.7	0.378	0.097
Plasma intact GLP-1 (pmol/L)	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.3	0.007	0.905

Data are mean ± SEM. *Two-factor ANOVA, with drug and preload as factors, was used to determine statistical difference.

Table 2—Effects of vildagliptin, with or without a whey preload, on postprandial glycemic peak and the AUC for plasma glucose, insulin, glucagon, and GIP and GLP-1 (both total and intact forms) in response to a carbohydrate meal in patients with type 2 diabetes treated with metformin (n = 22)

	PLBO/CTRL	PLBO/WHEY	VILD/CTRL	VILD/WHEY	P value
Glycemic peak (mmol/L)	15.4 ± 0.7	14.7 ± 0.9§	14.4 ± 0.8#	13.1 ± 0.8α,δ,ε	<0.001
Glucose $AUC_{-30\ to\ 240min}$ (mmol/L · h)	51.6 ± 3.2	51.2 ± 3.6	48.7 ± 3.3#	46.3 ± 3.0α,δ,ε	<0.001
Insulin $AUC_{-30\ to\ 240min}$ (mU/L · h)	130.7 ± 20.4	155.0 ± 21.5§	137.9 ± 23.4	166.3 ± 26.5α,ε	<0.001
Glucagon $AUC_{-30\ to\ 240min}$ (pg/mL · h)	380.5 ± 16.9	496.4 ± 20.8§	358.7 ± 15.8#	468.7 ± 21.1α,δ,ε	<0.001
Total GIP $AUC_{-30\ to\ 240min}$ (pmol/L · h)	163.9 ± 14.9	193.3 ± 21.0§	135.6 ± 12.4#	167.4 ± 24.5δ,ε	<0.001
Intact GIP $AUC_{-30\ to\ 240min}$ (pmol/L · h)	59.0 ± 2.9	72.5 ± 5.5§	114.7 ± 9.8#	144.3 ± 19.6α,δ,ε	<0.001
Total GLP-1 $AUC_{-30\ to\ 240min}$ (pmol/L · h)	30.4 ± 2.0	37.4 ± 2.5§	25.5 ± 2.2#	31.8 ± 3.1δ,ε	<0.001
Intact GLP-1 $AUC_{-30\ to\ 240min}$ (pmol/L · h)	1.7 ± 0.4	3.9 ± 0.8§	6.4 ± 1.0#	10.1 ± 1.5α,δ,ε	<0.001

Data are mean ± SEM. Post hoc comparisons were adjusted by Bonferroni-Holm correction. One-factor ANOVA was used to determine statistical difference. §$P < 0.05$, PLBO/WHEY vs. PLBO/CTRL. #$P < 0.05$, VILD/CTRL vs. PLBO/CTRL. α$P < 0.05$, VILD/WHEY vs. PLBO/CTRL. δ$P < 0.05$, VILD/WHEY vs. PLBO/WHEY. ε$P < 0.05$, VILD/WHEY vs. VILD/CTRL.

unchanged on the control days (PLBO/CTRL and VILD/CTRL) and increased after the whey preload (PLBO/WHEY and VILD/WHEY). After the meal, plasma insulin concentrations showed a significant treatment effect for AUC ($P <$ 0.001), such that plasma insulin was higher for PLBO/WHEY versus PLBO/CTRL and for VILD/WHEY versus VILD/CTRL ($P < 0.05$ for each), without significant difference between PLBO/CTRL and VILD/CTRL or between PLBO/WHEY and VILD/WHEY (Table 2 and Fig. 2*C*).

Plasma Glucagon Concentrations

Fasting plasma glucagon did not differ between the whey and control days but was slightly lower with vildagliptin compared with placebo (P = 0.092 at $t = -90$ min and P = 0.007 at $t = -30$ min, respectively) (Table 1). Before the meal ($t = -30$ to 0 min), plasma glucagon concentrations remained unchanged on the control days (PLBO/CTRL and VILD/CTRL) but increased markedly after the whey preload (PLBO/WHEY and VILD/WHEY). After the meal, plasma glucagon showed a significant treatment effect for AUC ($P <$ 0.001), such that plasma glucagon was higher for PLBO/WHEY versus PLBO/CTRL and for VILD/WHEY versus VILD/CTRL but lower for VILD/CTRL versus PLBO/CTRL and for VILD/WHEY versus PLBO/WHEY ($P <$ 0.05 for each) (Table 2 and Fig. 2*D*).

Plasma Total and Intact GIP Concentrations

Fasting plasma total and intact GIP did not differ between the whey and control days, but plasma total GIP was slightly lower (P = 0.022 at $t = -90$ min and P = 0.004 at $t = -30$ min, respectively) whereas intact GIP was higher (P = 0.002 at $t = -90$ min and P = 0.003 at $t = -30$ min, respectively) with vildagliptin compared with placebo (Table 1). Before the meal ($t = -30$ to 0 min), both total and intact GIP concentrations remained unchanged on the control days (PLBO/CTRL and VILD/CTRL) but increased after the whey preload (PLBO/WHEY and VILD/WHEY). After the meal, both total and intact GIP concentrations showed significant treatment effects for the AUC ($P <$ 0.001 for each), such that total GIP was higher for PLBO/WHEY versus PLBO/CTRL and for VILD/WHEY versus VILD/CTRL but lower for VILD/CTRL versus PLBO/CTRL and for VILD/WHEY versus PLBO/WHEY, whereas intact GIP was higher for PLBO/WHEY and VILD/CTRL versus PLBO/CTRL and highest after VILD/WHEY ($P < 0.05$ for each) (Table 2 and Fig. 2*E* and *F*).

Plasma Total and Intact GLP-1 Concentrations

Fasting plasma total GLP-1 did not differ between study days, whereas intact GLP-1 concentrations did not differ between the whey and control days but were slightly higher with vildagliptin compared with placebo (P = 0.003 at $t = -90$ min and P = 0.007 at $t = -30$ min, respectively) (Table 1). Before the meal ($t = -30$ to 0 min), both total and intact GLP-1 concentrations remained unchanged on the control days (PLBO/CTRL and VILD/CTRL) but increased after the whey preload (PLBO/WHEY and VILD/WHEY). After the meal, there were significant treatment effects on the AUC for both total and intact GLP-1 ($P <$ 0.001 for each), such that total GLP-1 was higher for PLBO/WHEY versus PLBO/CTRL and for VILD/WHEY versus VILD/CTRL but lower for VILD/CTRL versus PLBO/CTRL and for VILD/WHEY versus PLBO/WHEY, whereas intact GLP-1 was higher for PLBO/WHEY and VILD/CTRL versus PLBO/CTRL and highest after VILD/WHEY ($P < 0.05$ for each) (Table 2 and Fig. 2*G* and *H*).

Gastric Emptying

There was a significant treatment effect on the half-emptying time of the meal (T50) ($P <$ 0.001), such that gastric emptying was slower for PLBO/WHEY (T50: 172.3 ± 5.7 min) and VILD/CTRL (T50: 161.3 ± 5.7 min) versus PLBO/CTRL (T50: 147.7 ± 4.4 min) and was slowest after VILD/WHEY (T50: 193.7 ± 7.3 min) ($P < 0.05$ for each) (Fig. 2*B*).

Relationships of Glycemia With Gastric Emptying, Insulin, GIP, and GLP-1

When data from the four study visits were pooled, postprandial glycemia at t = 30, 60, and 90 min was inversely related to T50 ($r = -0.25$, P = 0.02; $r = -0.40$, $P <$ 0.001; $r = -0.28$, P = 0.008, respectively), and at t = 240 min directly related to T50 (r = 0.25, P = 0.02).

Compared with placebo, the magnitude of reduction in peak postprandial glucose with vildagliptin was related directly to the slowing of gastric emptying (r = 0.42, P = 0.005), while the reduction in AUC for postprandial plasma glucose was related to the increase in AUC for plasma intact GLP-1 (r = 0.31, P = 0.04), but not GIP, insulin, or glucagon.

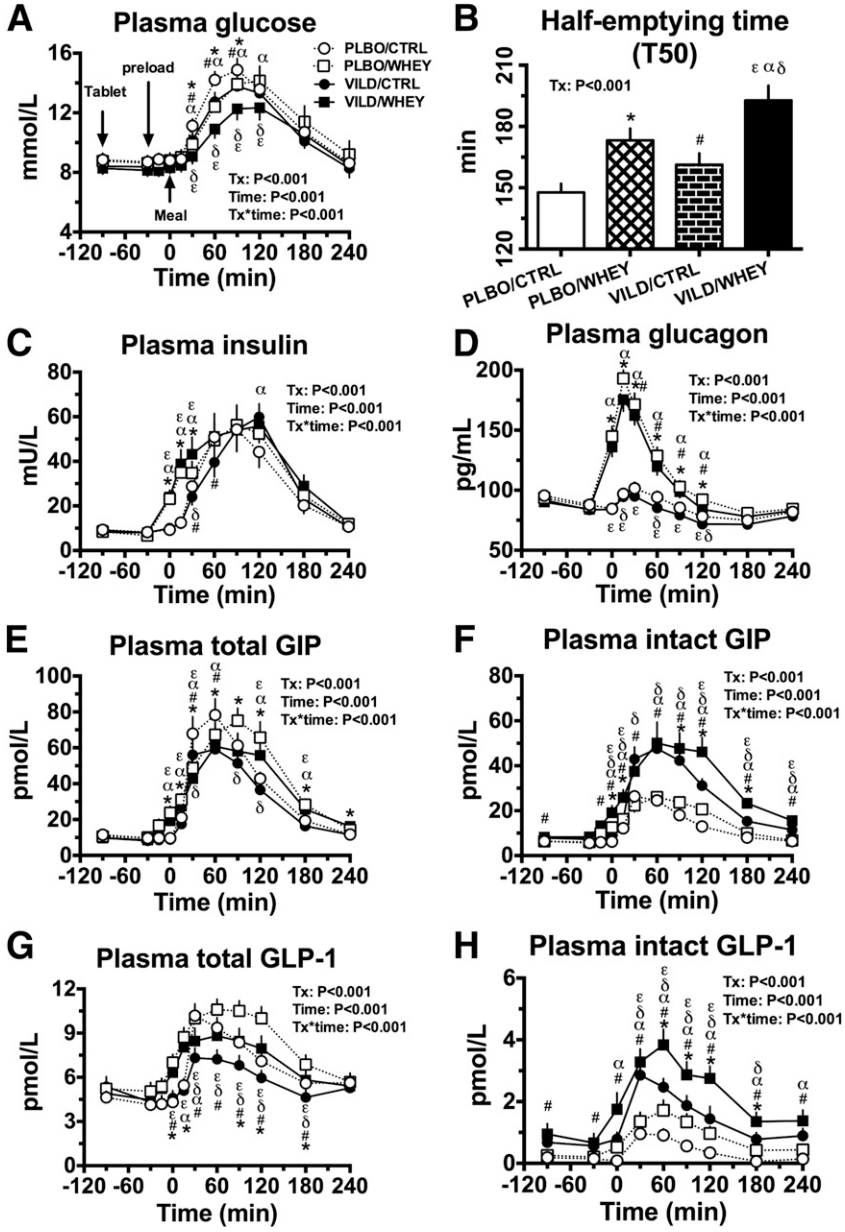

Figure 2—Effects of vildagliptin, with or without a whey preload, on plasma glucose (*A*), gastric half-emptying time (*B*), plasma insulin (*C*), plasma glucagon (*D*), and plasma GIP (*E* and *F* for total and intact forms, respectively) and GLP-1 (*G* and *H* for total and intact forms, respectively) in response to a high-carbohydrate meal in patients with type 2 diabetes managed by metformin monotherapy (n = 22). The four treatments were VILD/WHEY, VILD/CTRL, PLBO/WHEY, and PLBO/CTRL. Repeated-measures ANOVA was used to determine statistical difference. Results of ANOVA were reported as P values for differences by experiment (Tx), differences over time (Time), and differences due to interaction of experiment and time (Tx*time). Post hoc comparisons were adjusted by Bonferroni-Holm correction. *$P < 0.05$, PLBO/WHEY vs. PLBO/CTRL; #$P < 0.05$, VILD/CTRL vs. PLBO/CTRL; $\alpha P < 0.05$, VILD/WHEY vs. PLBO/CTRL; $\delta P < 0.05$, VILD/WHEY vs. PLBO/WHEY; $\varepsilon P < 0.05$, VILD/WHEY vs. VILD/CTRL. Data are mean ± SEM.

CONCLUSIONS

In this study of patients with type 2 diabetes who were relatively well controlled on metformin alone, we observed that *1*) a low-dose whey preload reduces postprandial glycemic excursions in association with slowing of gastric emptying and stimulation of incretin hormone, insulin, and glucagon secretion; *2*) acute dosing with vildagliptin increases plasma intact GLP-1 and GIP, suppresses plasma glucagon and total GLP-1 and GIP, slows gastric emptying, and attenuates postprandial glycemia independent of enhancement of insulin secretion; and *3*) combining vildagliptin with a whey preload is more effective for increasing plasma intact GLP-1 and GIP, slowing gastric emptying, and reducing postprandial glycemia, compared with either treatment alone. Remarkably, the addition of the whey preload to treatment with vildagliptin approximately doubled the reduction in peak postprandial blood glucose.

The dose of whey, although lower than used previously (10,11), proved sufficient to stimulate GLP-1 and GIP secretion and slow gastric emptying. Whey also induced early increases in plasma insulin and glucagon, probably due to direct β- and α-cell stimulation by absorbed amino acids, since the modest elevation in intact GLP-1 and GIP occurred later. However, the effects of insulin and glucagon on blood glucose appeared to be counterbalanced. Although the modest increase in intact GLP-1 and GIP in the absence of vildagliptin was not associated with significant lowering of the AUC for postprandial glycemia, the "early" phase of the postprandial glycemic excursion was attenuated, in line with slowing of gastric emptying after whey (18). The latter would also favor a reduction in the meal-induced insulin response (19). In the presence of vildagliptin, both the early rise and overall AUC for postprandial glycemia were attenuated after whey compared with control, highlighting the complementary actions of a dietary strategy combined with DPP-4 inhibition.

As anticipated, vildagliptin increased intact GLP-1 and GIP concentrations, an effect that was enhanced by the whey preload. The reduction in total GLP-1 and GIP concentrations is consistent with the concept of negative feedback on incretin hormone secretion (1). Although there is recent evidence that about half of the insulinotropic and glucose-lowering effects of sitagliptin are GLP-1 independent (7), we showed that the magnitude of reduction in postprandial glycemia was related directly to the increase in plasma intact GLP-1, consistent with complementary glucose-lowering actions of GLP-1 in type 2 diabetes (1). Elevated intact GIP concentrations are likely to contribute less (3,20,21), and GIP administration can even antagonize the glucagonostatic effect of GLP-1 (20). In the current study, the glucagonostatic effect of intact GLP-1 prevailed, and may, to some extent, have contributed

to glucose lowering by vildagliptin. However, relative to the increase of glucagon after whey, this effect was modest. Therefore, additional mechanisms are likely to be important in accounting for the glycemic effect after combined treatment with whey preload and vildagliptin. Despite augmentation of intact GLP-1 after vildagliptin, particularly when combined with whey, there was minimal increase in insulin, probably because the insulinotropic activity of GLP-1 is glucose dependent (1). The glycemic effect of vildagliptin observed in the current study was also unlikely to be related to improvement in insulin sensitivity; administration of 50 mg vildagliptin twice daily for 9 days was previously reported not to alter insulin action in patients with type 2 diabetes (22). Nevertheless, during pancreatic clamp studies where insulin and glucagon secretion are inhibited, GLP-1 remains effective to suppress endogenous glucose production (23) and enhance peripheral glucose uptake (24), suggesting that GLP-1 has the capacity to lower blood glucose independent of changes in islet hormones.

Vildagliptin slowed gastric emptying modestly both in the absence and presence of the whey preload, associated with the reduction in peak blood glucose. This has been reported previously in patients with type 2 diabetes receiving a single dose of vildagliptin (25) and is consistent with evidence that endogenous GLP-1 slows gastric emptying (4,7). Several studies failed to show any effect of DPP-4 inhibitors on gastric emptying in health or type 2 diabetes (9,26–28), probably related to changes in other peptides that regulate gastric emptying (e.g., reduced conversion of peptide YY[1-36] to [3-36]) (29), and differences in test meal, subject characteristics, or duration of DPP-4 inhibition. Sustained exposure to elevated GLP-1 during prolonged DPP-4 inhibition may potentially cause tachyphylaxis for its effect on gastric emptying (30). Furthermore, the magnitude of GLP-1 secretion is dependent on the load and nutrient composition of the meal (1) and may be influenced by concurrent medications (e.g., metformin) (31).

We studied only one dose of whey on the basis of its established effect on gastric emptying (12); testing of different doses at various intervals before the meal may further optimize the interaction with DPP-4 inhibitors. Alternative preloads could be evaluated; Tricò et al. (32) recently reported that a preload of protein (egg) and fat (cheese) reduced the glycemic response to oral glucose substantially, but the effect on the secretion of incretin hormones was rather modest, despite higher caloric value of the preload compared with our whey preload. We studied only male patients with relatively good glycemic control on metformin in order to minimize heterogeneity in this "proof of concept" study. Therefore, there should be caution in generalizing our findings to the broader community of patients with type 2 diabetes.

In summary, in metformin-treated patients with type 2 diabetes, the efficacy of vildagliptin in reducing postprandial glycemia is substantially improved by combination with a small whey preload, associated with augmentation of plasma intact GLP-1 concentrations and slowing of gastric emptying. The strategy of combining a dietary and pharmacological approach warrants evaluation in longer-term clinical trials.

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