Differential Antidiabetic Efficacy of Incretin Agonists Versus DPP-4 Inhibition in High Fat–Fed Mice

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OBJECTIVE—We examined whether chronic administration of a glucagon-like peptide 1 (GLP-1) receptor agonist exendin-4 (Ex-4), a glucose-dependent insulinotropic polypeptide (GIP) receptor agonist D-Ala$_2$-GIP (DA-GIP), or a dipeptidyl peptidase-4 (DPP-4) inhibitor (DPP-4i) des-fluoro-sitagliptin produced comparable antidiabetic actions in high fat–fed mice.

RESEARCH DESIGN AND METHODS—High fat–fed mice were administered twice-daily injections of Ex-4, DA-GIP, vehicle (saline), or vehicle with the addition of des-fluoro-sitagliptin (DPP-4i) in food to produce sustained inhibition of DPP-4 activity.

RESULTS AND CONCLUSIONS—Mice treated with vehicle alone or DA-GIP exhibited progressive weight gain, whereas treatment with Ex-4 or DPP-4i prevented weight gain. Although Ex-4 improved oral glucose tolerance and insulin-to-glucose ratios after an intraperitoneal glucose tolerance test (IPGTT), DPP-4i had no significant effect after IPGTT but improved glucose excursion and insulin levels after an oral glucose tolerance test. The extent of improvement in glycemic control was more sustained with continuous DPP-4 inhibition, as evidenced by loss of glucose control evident 9 h after peptide administration and a significant reduction in A1C observed with DPP-4i but not with DA-GIP or Ex-4 therapy. DA-GIP, but not Ex-4 or DPP-4i, was associated with impairment in insulin sensitivity and increased levels of plasma leptin and resistin. Although none of the therapies increased β-cell mass, only Ex-4–treated mice exhibited increased pancreatic mRNA transcripts for *Irs2*, *Egfr*, and *Gck*. These findings highlight significant differences between pharmacological administration of incretin receptor agonists and potentiation of endogenous GLP-1 and GIP via DPP-4 inhibition.
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The gastrointestinal tract plays a key role in the control of food digestion, nutrient absorption, and energy assimilation (1). It is increasingly recognized that specialized enteroendocrine cells, distributed along the length of the small and large intestine, contribute to the control of energy homeostasis by secreting hormones important for regulation of satiety, gut motility, and pancreatic islet function. Two of these gut hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), subserve important roles as incretins, gut-derived peptides that augment insulin secretion from the β-cell after enteral nutrient ingestion (2). The interest in the actions of these incretin hormones has increased considerably in recent years following clinical evidence suggesting that incretin-based therapies may be useful for the treatment of human subjects with type 2 diabetes (3,4).

GLP-1 receptor (GLP-1R) agonists control glycemia not only via stimulation of insulin secretion but also through inhibition of glucagon secretion and reduction of the rate of gastric emptying. Moreover, chronic administration of GLP-1R agonists may improve β-cell function in preclinical studies via enhancement of β-cell mass (5,6) and via induction of genes important for differentiated β-cell function (7). In addition, GLP-1R agonists control satiety and may produce weight loss, leading to indirect enhancement of insulin sensitivity (3). Taken together, this favorable spectrum of actions underlies the current enthusiasm for the use of the prototype GLP-1R agonist, exendin-4 (Ex-4), for the treatment of type 2 diabetes.

There is also considerable interest in determining whether the originally described incretin, GIP, may be useful as a therapeutic agent in the treatment of type 2 diabetes. GIP receptor agonists also potentiate glucose-stimulated insulin secretion, but unlike GLP-1, GIP does not appear to have significant effects on glucagon secretion, gastric emptying, or body weight (1). Unexpectedly, either GIP receptor activation or ablation of GIP receptor signaling exerts beneficial metabolic actions in preclinical models of experimental diabetes (8,9). Hence there is considerable ongoing debate as to the extent to which modulation of GIP receptor signaling will be a useful therapeutic option in type 2 diabetes.

A third approach for enhancing incretin action involves the prevention of incretin degradation by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors (DPP-4is) stabilize the postprandial levels of bioactive GLP-1 and GIP (10,11) and have been approved for the treatment of type 2 diabetes. DPP-4is exert their actions predominantly via potentiation of GLP-1 and GIP receptor signaling (12), but the endogenous levels of GLP-1 and GIP achieved using DPP-4i are much lower than that observed after pharmacological administration of GLP-1 or GIPR peptide agonists. Hence, the extent to which incretin receptor agonists and DPP-4i will exhibit overlapping versus contrasting mechanisms of action remains uncertain. Moreover, there have been no published reports comparing therapy with DPP-4i versus GLP-1R agonists in humans, and only limited data are available contrasting the effects of GIPR versus GLP-1R agonists relative to

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AUC, area under the glucose curve; DA-GIP, D-Ala$_2$-glucose-dependent insulinotropic polypeptide; DPP-4, dipeptidyl peptidase-4; DPP-4i, dipeptidyl peptidase-4 inhibitor; Ex-4, exendin-4; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; KIU, kallikrein inhibitor unit; MCP-1, monocyte chemoattractant protein-1; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor 1.

DPP-4i in preclinical studies. Accordingly, we have now examined the relative efficacy and contrasting mechanisms of action of DPP-4i versus Ex-4 versus D-Ala_2-GIP (DA-GIP) in high fat–fed mice with glucose intolerance.

RESEARCH DESIGN AND METHODS

C57BL/6 mice fed a high-fat diet were used as a model because they have previously been shown to gain excess weight and develop mild hyperglycemia (13). Male C57BL/6 mice were purchased from Charles River Laboratories (Montreal, PQ, Canada) at 8 weeks of age. All mice were housed under specific pathogen-free conditions in microisolator cages and maintained on a 12-h light (7:00 A.M.)/dark (7:00 P.M.) cycle with free access to food and water. They were acclimatized to the Toronto General Hospital animal facility for 4 weeks, during which time they were fed a normal chow diet. At 12 weeks of age, they were placed on a high-fat diet (60% kcal from fat; D12492) from Research Diets (New Brunswick, NJ) until completion of the study. After 4 weeks on the high-fat diet, they were treated with twice-daily (7:00 A.M. and 6:00 P.M.) intraperitoneal injections of 24 nmol/kg Ex-4, 24 nmol/kg DA-GIP, vehicle (PBS), or vehicle with the addition of des-fluoro-sitagliptin (an analog of sitagliptin) in the food at a concentration of 11 g/kg (DPP-4i). This concentration of des-fluoro-sitagliptin produced ~90% inhibition of plasma DPP-4 activity (data not shown). Doses of the peptides were chosen based on literature review and pilot studies in our laboratory. Peptides were purchased from California Peptide Research (Napa, CA), and des-fluoro-sitagliptin (14) was supplied by Merck Research Labs (Rahway, NJ). All experiments were carried out in accordance with the protocols and guidelines approved by the Toronto General Hospital Animal Care Committee.

After the mice were switched to the high-fat diet, body weight and food intake were monitored weekly. The assessment of fat and lean mass was carried out using a mouse whole-body magnetic resonance analyzer (Echo Medical Systems, Houston, TX). After 6 weeks of treatment, blood was collected from the tail vein of mice at different times during the day (1 and 9 h after the morning injections), and blood glucose was measured using a Glucometer Elite blood glucose meter (Ascensia; Bayer, Toronto, ON, Canada).

On completion of the study (after 8 weeks of drug treatment) mice were killed 2 h after their final morning peptide injection. Immediately before euthanasia, blood was collected from the tail vein for assessment of A1C. Cardiac blood (600 μl) was collected for the assessment of circulating hormones and plasma DPP-4 activity. The blood (200 μl) that was to be used for the DPP-4 activity assay was immediately mixed with a 10% volume of a chilled solution containing 5,000 kallikrein inhibitor units (KIU)/ml trasylol and 1.2 mg/ml EDTA. Diprotin A was also added to the remaining volume (400 μl) up to a final concentration of 0.01 mmol/l. Plasma was separated by centrifugation at 4°C and stored at −20°C until assayed. The pancreas was rapidly excised, and samples for RNA and protein extraction were snap-frozen in liquid N_2 and stored at −80°C. Samples for histological analysis were fixed in a 10% neutral buffered formalin solution (Sigma, St. Louis, MO) for 48 h and embedded in paraffin.

Indirect calorimetry and locomotor activity. For the determination of oxygen consumption and locomotor activity, mice were placed into individual metabolic chambers and had free access to food and water. Measurements were carried out 1 week before and 5 weeks after the beginning of treatment. During the treatment period, the mice received the morning injections ~6 h before the measurements began but did not receive their normal evening injection. Oxygen consumption was measured by indirect calorimetry using the Oxymax System (Columbus Instruments, Columbus, OH). Oxygen consumption was measured at 15-min intervals for a total of 20 h and was normalized to body weight (lean body mass). Locomotor activity was determined by the total distance traveled, calculated from the measurement of beam breaks in Opto M3 activity monitors (Columbus Instruments).

Glucose tolerance and plasma insulin levels. Glucose tolerance was assessed by oral (OGTTs) and intraperitoneal glucose tolerance tests (IPGTTs) after 4 and 7 weeks of treatment, respectively. Mice were fasted for 6 h, and the tests were carried out at 1:30 P.M. In preliminary experiments, we observed that after 6 h of fasting, DPP-4 activity was still inhibited by 65% in DPP-4i–treated mice (data not shown). On the days that these experiments were carried out, peptide and saline injections were administered 2 h before the test. Glucose (1.5 mg/g body weight) was administered via either an oral gavage (OGTT) or IP injection (IPGTT). Blood samples were drawn from the tail vein at 0, 10, 20, 30, 60, and 120 min after glucose administration. Blood glucose levels were measured using a Glucometer (Bayer). For plasma insulin determinations, a blood sample (100 μl) was collected from the tail vein during the 10- to 20-min time period. Blood samples were immediately mixed with a 10% volume of a chilled solution containing 5,000 KIU/ml trasylol, 1.2 mg/ml EDTA, and 0.1 mmol/l diprotin A. Plasma was separated by centrifugation at 4°C and stored at −20°C until assayed. Plasma was assayed for insulin using a mouse insulin ELISA kit (Linco Research, St. Charles, MO). For calculation of plasma insulin-to-glucose ratios, the plasma insulin concentration (nanograms per milliliter) was divided by the plasma glucose concentration (millimoles per liter) at the 20-min time point.

Insulin tolerance. The insulin tolerance tests (ITTs) involved a 1.2 units/kg i.p. insulin injection (100 units/ml; Novolin ge Toronto; Novo Nordisk, Mississauga, ON, Canada) and were carried out at 1:30 P.M. Mice were fasted for 6 h, and drug injections were administered 2 h before the test. Blood glucose was measured in tail vein blood samples that were collected at 0, 15, 30, 60, and 120 min after insulin administration.

Analytical techniques. Blood A1C was determined using the DCA 2000+ Analyzer (Bayer). Levels of resistin, monocyte chemoattractant protein (MCP-1), and total plasminogen activator inhibitor 1 (PAI-1) were determined in plasma samples using the Mouse Serum Adipokine Lincoplex assay (Linco Research). The levels of plasma leptin were measured with the Mouse Endocrine Lincoplex assay (Linco Research). Plasma DPP-4 activity was determined by an enzymatic assay as previously described (15).

For the determination of β-cell mass, pancreatic sections were immunostained for insulin using rabbit anti-insulin (1:30 dilution; Dako, Glostrup, Denmark) as primary antibody and biotinylated goat anti-rabbit (1:200 dilution; Vector Laboratories) as secondary antibody. The sections were then incubated with horseradish peroxidase–conjugated Ultra Streptavidin (ID Labs, London, ON, Canada) for 30 min, and color was developed with freshly prepared 3,3′-diaminobenzidine tetrahydrochloride (Dako) solution. The insulin antibody–stained sections were scanned using the Scanscope CS system (Aperio Technologies, Vista, CA) at magnification ×20 (16). The digital images were analyzed with the Scanscope software (Aperio Technologies) using a preset positive pixel count algorithm to quantify the insulin-positive and -negative areas. The β-cell mass for each animal was calculated as the product of the total cross-sectional area of β-cells/total pancreas area and the weight of the pancreas before fixation.

Total RNA was extracted from pancreas samples using Tri Reagent (Sigma), and gene expression was analyzed by quantitative real-time RT-PCR. First-strand cDNA was synthesized from total RNA using the SuperScript II synthesis system (Invitrogen, Carlsbad, CA) and random hexamers. The real-time PCR was carried out with the ABI Prism 7900 Sequence Detection System using TaqMan Gene Expression Assays (*Akt1*, Mm00437443_m1; *Egfr*, Mm00433023_m1; *Gcg*, Mm00801712_m1; *Gck*, Mm00439129_m1; *Iapp*, Mm00439403_m1; *Ins2*, Mm00731595_gH; *Irs2*, Mm03038438_m1; *Kcnj11*, Mm00440050_s1; *Pdx1*, Mm00435565_m1; *Slc2a2*, Mm00446224_m1) and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The relative mRNA transcript levels were quantified with the $2^{-\Delta\Delta CT}$ method (17), using amylin (*Iapp*) as the internal control gene.

Statistical analysis. All results are presented as means ± SE. The Prism software package (version 4; GraphPad Software) was used to complete the statistical analysis. Differences between multiple groups were analyzed by one-way ANOVA. Dunnett's multiple comparison test was used to make comparisons between the multiple treatment groups and the PBS control group. The Newman-Keuls multiple comparison test was used to make comparisons between all groups. A P value of <0.05 was considered significant.

RESULTS

DPP-4 inhibition and Ex-4 reduced body weight gain. Before initiation of the different incretin therapies, there were no differences between groups of mice allocated to the different treatment groups in food intake, oxygen consumption, or locomotor activity (data not shown). High-fat–fed mice treated with Ex-4 or the DPP-4i exhibited significantly reduced body weight gain over time (Fig. 1*A*), a trend that was sustained over the entire treatment period (Fig. 1*C*). In contrast, DA-GIP had no significant effect on body weight gain (Fig. 1). To examine the mechanisms underlying the reduced weight gain in the face of an energy-rich diet, we determined levels of energy intake and energy expenditure. Ex-4–treated mice had a significant reduction in average daily food intake over the 8-week treatment period (Fig. 1*D*). When corrected for lean body mass, Ex-4 treatment was not associated with any change in energy expenditure (Fig. 1*E*) or locomotor activity (Fig. 1*F*). Although there was a persistent trend for

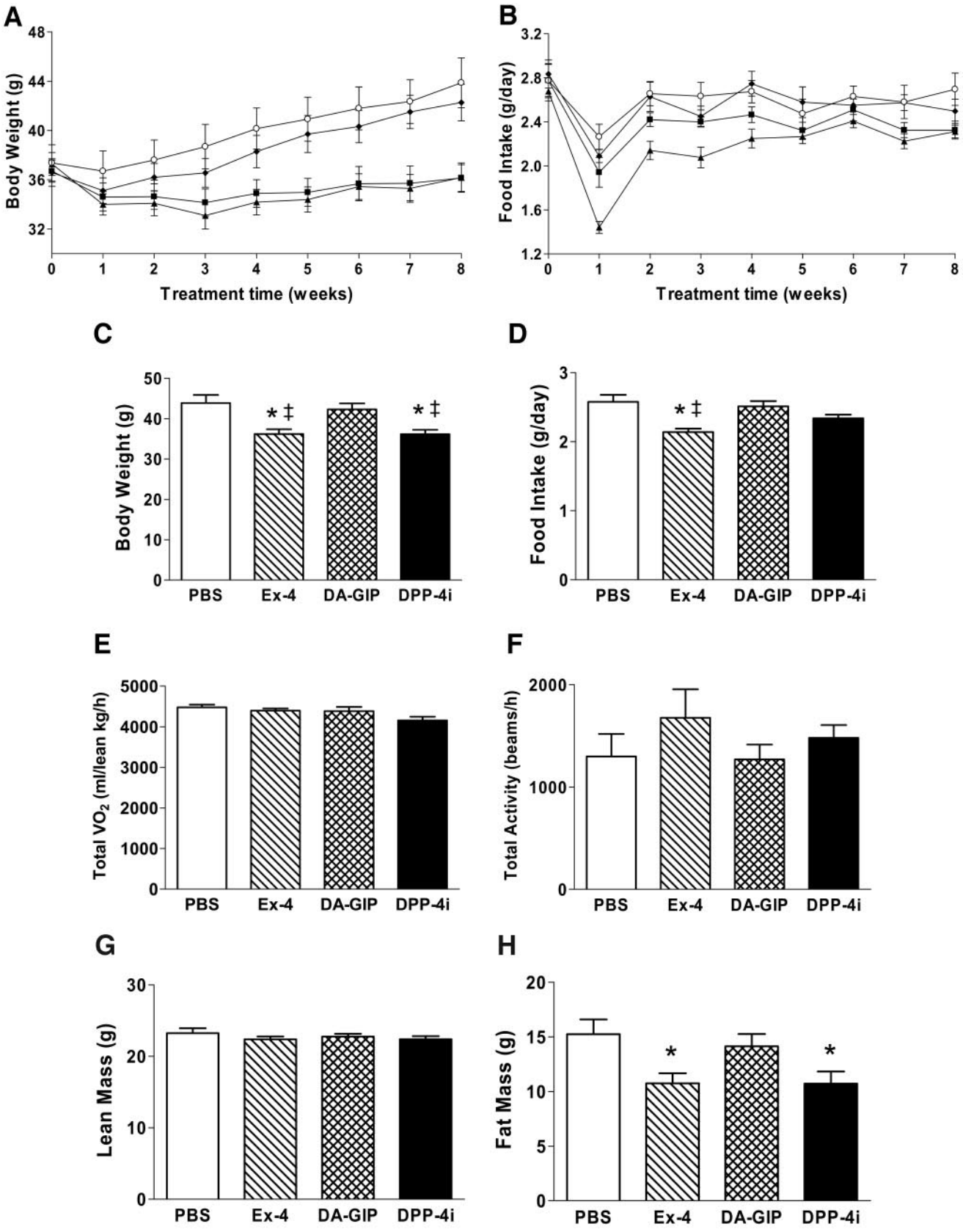

FIG. 1. Body weight and food intake in high fat–fed mice treated with Ex-4, DA-GIP, or a DPP-4i des-fluoro-sitagliptin. Analysis with two-way ANOVA revealed that Ex-4 ($P = 0.0200$) and the DPP-4i ($P = 0.0348$) had significant effects on body weight gain (*A*) and that Ex-4 ($P = 0.0029$) but not DPP-4i ($P = 0.0983$) also had significant effects on food intake (*B*). DA-GIP had no significant effects on either body weight or food intake. ○, PBS; ▲, Ex-4; ♦, DA-GIP; ■, DPP-4i. *C*: Comparison of body weight at the end of the study. *D*: Average daily food intake over the entire 8-week treatment period. *$P < 0.05$ vs. PBS, ‡$P < 0.05$ vs. DA-GIP. Total oxygen consumption (*E*) and physical activity (*F*) measured (during light and dark phases) on 1 day after 5 weeks of treatment. Total lean (*G*) and fat mass (*H*) measured after 5 weeks of treatment. The fat and lean mass were not different between mice before treatment (data not shown). $n = 7–8$.

a reduction in food intake in mice treated with the DPP-4i, this difference did not reach statistical significance (Fig. 1*B* and *D*). Similarly, there were no significant differences in oxygen consumption or locomotor activity in mice after prolonged DPP4 inhibition (Fig. 1*E* and *F*). Moreover, DA-GIP administration was not associated with changes in either food intake or energy expenditure (Fig. 1*D* and *E*). The reduced body weight gain in high fat–fed mice treated with either Ex-4 or the DPP-4i resulted in significant reductions in fat mass but no difference in lean mass compared with saline-treated mice (Fig. 1*G* and *H*). Consistent with an absence of an effect on body weight, DA-GIP administration had no significant effect on either lean mass or fat mass (Fig. 1*G* and *H*).

Comparative actions of incretin receptor agonists versus a DPP-4i on glucose tolerance and insulin sensitivity. Incretin receptor agonists and DPP-4is exhibit both overlapping and distinct mechanisms leading to improved glucose homeostasis (18,19). When glucose was administered via an intraperitoneal injection, both Ex-4 and DA-GIP significantly reduced the area under the glucose curve (AUC) compared with saline-treated mice (Fig. 2*A* and *C*). In this experiment, the effect of Ex-4 on glucose reduction was more potent, and Ex-4 significantly increased the insulin-to-glucose ratio (Fig. 2*C*); DA-GIP treatment was also associated with an increase in the insulin-to-glucose ratio, but this did not achieve statistical significance. In contrast, therapy with the DPP4i did not reduce AUC (glucose) during the IPGTT (Fig. 2*C*). We next compared the glucoregulatory actions of the different treatments during OGTT. Ex-4, DA-GIP, and the DPP-4i all significantly reduced the AUC (glucose) relative to saline-treated mice (Fig. 2*B* and *D*). Ex-4 had the greatest effect on improving oral glucose tolerance (Fig. 2*D*); however, Ex-4 did not increase the insulin-to-glucose ratio during the OGTT (Fig. 2*F*). In contrast the DPP-4i significantly improved the insulin-to-glucose ratio after oral glucose administration (Fig. 2*F*). These observations suggest that the glucose-lowering actions of Ex-4 may be attributable to sites of action independent of the β-cell, likely via inhibition of gastric emptying or glucagon secretion.

To indirectly assess the effects of differential incretin receptor agonism and DPP-4i on insulin sensitivity, we carried out ITTs. Before administration of insulin, the blood glucose levels of mice receiving incretin-based therapies were significantly lower ($P < 0.01$) than those in the saline-treated group (9.1 ± 0.6 mmol/l); the glucose levels of the Ex-4–treated (5.9 ± 0.15 mmol/l) and DA-GIP–treated (6.1 ± 0.15 mmol/l) mice were also significantly lower ($P < 0.01$) than those of the DPP4i-treated mice (7.6 ± 0.21 mmol/l). Hence, the data are presented as a percentage of the basal fasting glucose. There was no difference in the ability of insulin to lower blood glucose in Ex-4– and DPP-4i–treated mice (Fig. 2*G* and *H*); however, the AUC (% glucose) of the DA-GIP–treated mice was significantly greater than that of all of the other groups, consistent with a reduction in insulin sensitivity in DA-GIP–treated mice (Fig. 2*G*).

The insulin resistance induced by DA-GIP treatment was accompanied by elevated plasma levels of leptin and resistin (Fig. 3). These increases in plasma adipokines were detected in the absence of any significant difference in fat mass of DA-GIP–treated versus saline-treated mice (Fig. 1*H*). In contrast, Ex-4– and DPP-4i–treated mice had reduced fat mass and decreased plasma leptin levels (Fig. 3). None of the therapies had any significant effects on circulating levels of total PAI-1 or MCP-1 (Fig. 3).

Ex-4 increases the expression of β-cell genes. To determine whether incretin therapies differentially regulate the expression of genes important for β-cell function, we assessed gene expression profiles using real-time PCR analysis of RNA isolated from mouse pancreas. For all the genes examined (*Irs2*, *Egfr*, *Akt1*, *Pdx1*, *Gck*, *Slc2a2*, *Ins2*, *Gcg*, and *Kcnj11*), only Ex-4 treatment resulted in significant changes in gene expression. Ex-4 increased the levels of mRNA transcripts for the IRS-2 signaling protein (*Irs2*), Glucokinase (*Gck*), and the EGF receptor (*Egfr*) (Fig. 4), genes that are thought to be important downstream transducers and targets of GLP-1R signaling in the β-cell (20–22). There was also a trend for Ex-4 to increase the expression of known GLP-1–regulated genes such as *Akt1* and *Pdx-1*, but these differences did not achieve statistical significance (Fig. 4). Despite the significant induction of *Irs2* and *Egfr* gene expression, Ex-4, DA-GIP, and DPP-4i had no detectable effect on β-cell mass (data not shown).

DPP-4 inhibition reduced blood glucose levels over a longer period of time relative to Ex-4 and DA-GIP. To ascertain whether the distinct incretin therapies produced differential effects on blood glucose at different time points, we measured random-fed blood glucose levels at 1 and 9 h after the morning injections. Ex-4, DA-GIP, and DPP-4i therapies all significantly reduced blood glucose levels in the morning, 1 h after the morning injections (Fig. 5*A*). Furthermore, Ex-4 and DA-GIP exhibited greater glucose-lowering effects than the DPP-4i at this time point (Fig. 5*A*). In contrast, analysis of blood glucose levels in the afternoon 9 h after the last set of injections demonstrated that glucose levels were significantly lower only in the DPP-4i–treated mice (Fig. 5*B*). Despite the potent acute glucose-lowering actions of Ex-4 and DA-GIP (Fig. 5*A*), only the DPP-4i–treated mice had lower levels of A1C at the end of the 8-week study (Fig. 5*C*).

DISCUSSION

The two principal incretins GIP and GLP-1 share overlapping actions on islet β-cells yet exhibit divergent actions on α-cells, gastric emptying, satiety, and adipose tissue (18). Agents based on enhancement of incretin action, namely Exenatide (Ex-4) and Sitagliptin, have been approved for the treatment of type 2 diabetes, yet no head-to-head clinical studies of these agents have been reported. Moreover, degradation-resistant analogs of GIP exhibit potent actions in preclinical studies in vivo (23); however, there is scant information available about the differential antidiabetic actions of GLP-1R agonists versus GIPR agonists versus DPP-4is. A recent study compared the antidiabetic actions of the GLP-1R agonist liraglutide and the DPP-4i vildagliptin in candy-fed rats (24). Although liraglutide reduced food intake and body weight, no significant differences in levels of glucose or A1C were observed in the different treatment groups. In contrast, we observed lower levels of A1C in high fat–fed mice treated with the sitagliptin analog compared with twice-daily injection of Ex-4. Differences in the administration and potency of either the two DPP-4is or the different GLP-1R agonists (liraglutide vs. Ex-4) may account for the contrasting observations in these studies. Continuous administration of des-fluoro-sitagliptin in the food in our study likely achieves more potent and sustained 24-h inhibition

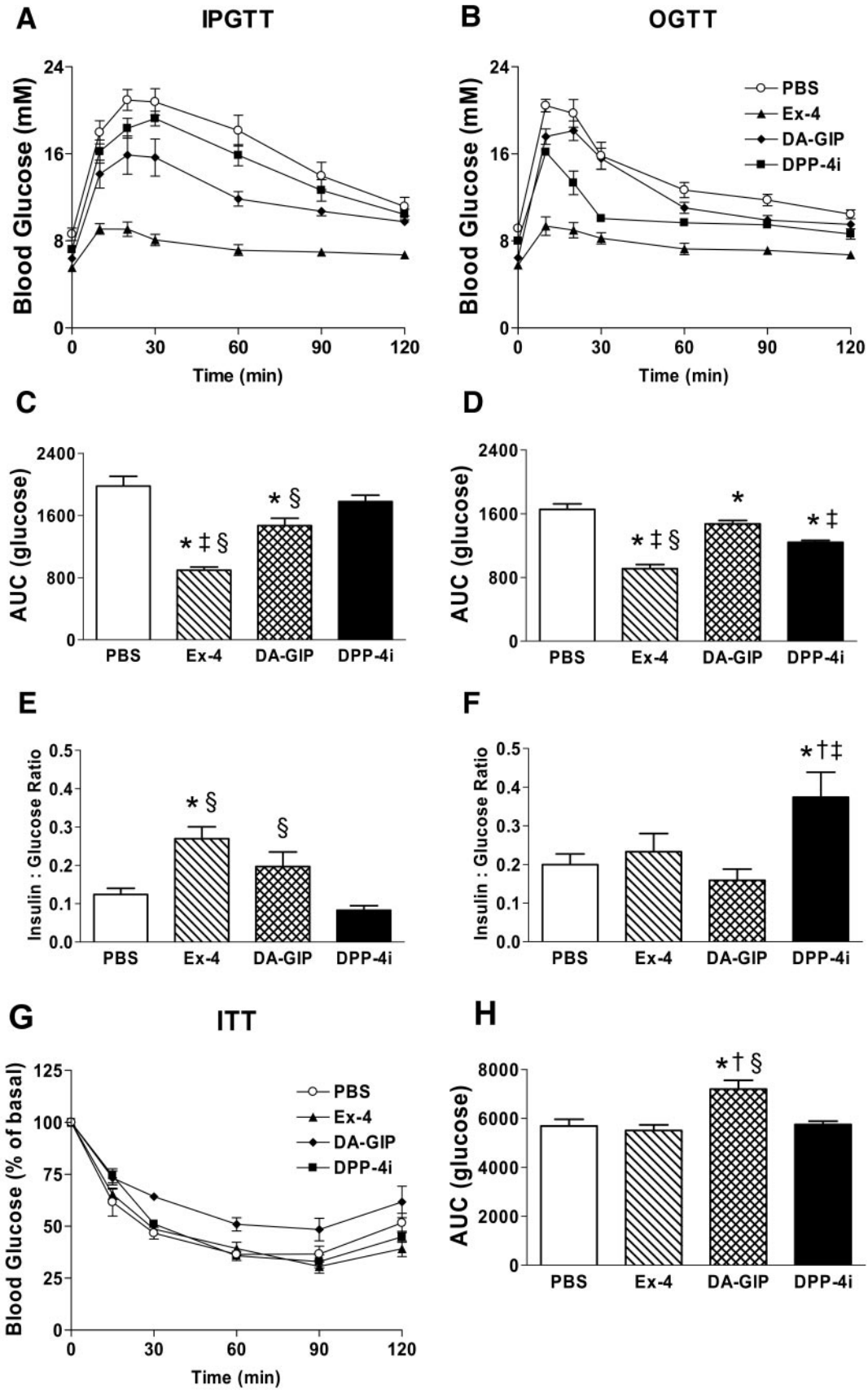

FIG. 2. Effects of Ex-4, DA-GIP, and DPP-4 inhibition on glucose tolerance and insulin sensitivity in high fat–fed mice. IPGTTs (*A*), OGTTs (*B*), and intraperitoneal ITTs (*G*) were completed during the 8-week treatment period. All tests were performed 2 h after injection with drug or vehicle in mice that were fasted for 6 h. AUC for the IPGTTs (*C*), OGTTs (*D*), and ITTs (*H*) was calculated from 0 to 120 min (units are millimoles per liter per minute). Insulin-to-glucose ratios for the IPGTTs (*E*) and OGTTs (*F*) were calculated by dividing the plasma insulin concentration by the blood glucose concentration at 20 min. $n = 7–8$, *$P < 0.05$ vs. PBS, †$P < 0.05$ vs. Ex-4, ‡$P < 0.05$ vs. DA-GIP, and §$P < 0.05$ vs. DPP-4i.

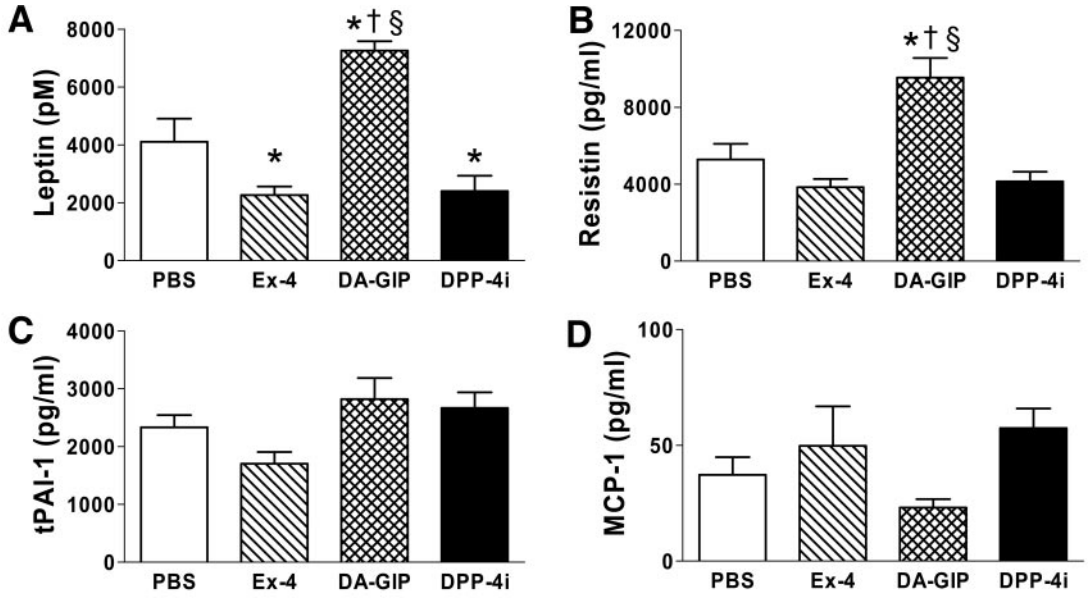

FIG. 3. Levels of plasma adipokines in high fat–fed mice in response to Ex-4, DA-GIP, or DPP-4i treatment. Leptin (*A*), resistin (*B*), total PAI-1 (*C*), and MCP-1 (*D*) were assayed in plasma from cardiac blood samples collected from random-fed mice at the end of the 8-week treatment period. $n = 6–8$, *$P < 0.05$ vs. PBS, †$P < 0.05$ vs. Ex-4, and §$P < 0.05$ vs. DPP-4i.

of DPP-4 activity relative to the twice-daily administration of vildagliptin used in the previous experiments (24).

Consistent with the hypothesis that sustained incretin receptor activation is required for optimal glucose control, we observed that levels of blood glucose 9 h after the last injection of Ex-4 or DA-GIP were actually lower in mice receiving continuous DPP-4i relative to mice receiving the injected incretin peptides (Fig. 5*B*). These observations agree with our data demonstrating that 8 weeks of therapy with des-fluoro-sitagliptin, but not DA-GIP or Ex-4, re-

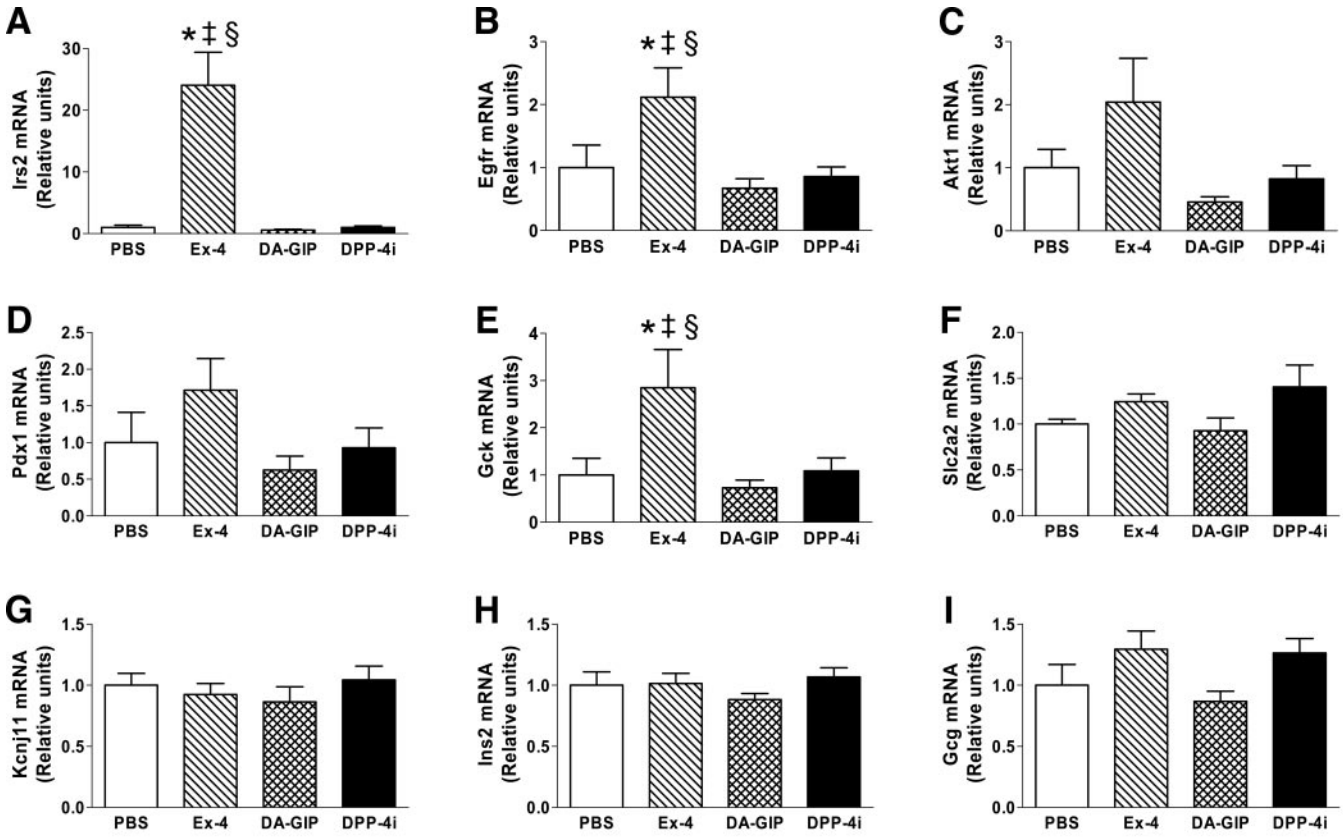

FIG. 4. Analysis of β-cell gene expression in pancreas RNA from high fat–fed mice treated with PBS, Ex-4, DA-GIP, or a DPP-4i. Relative levels of *Irs2* (*A*), *Egfr* (*B*), *Akt1* (*C*), *Pdx1* (*D*), *Gck* (*E*), *Slc2a2* (*F*), *Kcnj11* (*G*), *Ins2* (*H*), and *Gcg* (*I*) mRNA as assessed by quantitative real-time PCR were normalized to levels of *Iapp* mRNA transcripts in the same samples. $n = 7–8$, *$P < 0.05$ vs. PBS, ‡$P < 0.05$ vs. DA-GIP, and §$P < 0.05$ vs. DPP-4i.

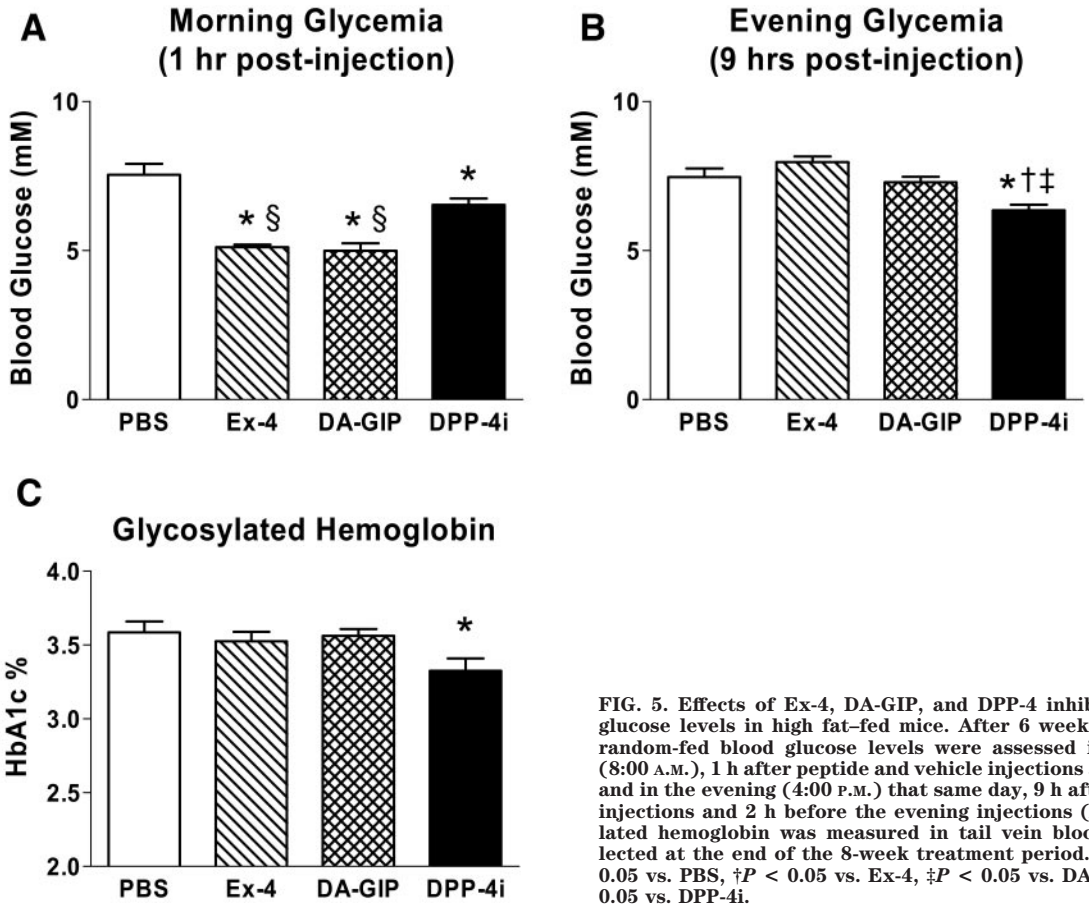

FIG. 5. Effects of Ex-4, DA-GIP, and DPP-4 inhibition on blood glucose levels in high fat–fed mice. After 6 weeks of treatment, random-fed blood glucose levels were assessed in the morning (8:00 A.M.), 1 h after peptide and vehicle injections at 7:00 A.M. (*A*), and in the evening (4:00 P.M.) that same day, 9 h after the morning injections and 2 h before the evening injections (*B*). *C*: Glycosylated hemoglobin was measured in tail vein blood samples collected at the end of the 8-week treatment period. n = 7–8, *P < 0.05 vs. PBS, †P < 0.05 vs. Ex-4, ‡P < 0.05 vs. DA-GIP, and §P < 0.05 vs. DPP-4i.

sulted in a significant reduction of A1C. Hence, continuous rather than intermittent activation of the incretin axis appears critical for optimal blood glucose control (25).

Although Ex-4 and DA-GIP reduced glycemic excursion during an IPGTT treatment, des-fluoro-sitagliptin was only effective at improving glucose tolerance during the OGTT, where it resulted in a greater insulin-to-glucose ratio. Previous studies suggest that DPP-4is have little or no effect on gastric emptying (26), and our results imply that potentiation of the physiological levels of GLP-1 and GIP after nutrient ingestion lower blood glucose predominantly through improvement of islet function. Although DPP-4is potently suppress levels of plasma glucagon in diabetic human subjects (27,28), we were unable to obtain sufficient plasma to determine the levels of glucagon in the different groups of high fat–fed mice. Hence, we cannot exclude the possibility that des-fluoro-sitagliptin exerts its antidiabetic actions in part via suppression of the α-cell.

A major focus of interest in the incretin field is the possibility that one or more of the incretin-based agents may favorably modify β-cell function, resulting in sustained and durable improvement in antidiabetic efficacy. Although no clinical data yet exist in support of this concept, substantial amounts of preclinical data have demonstrated that in the appropriate experimental context, GLP-1R agonists such as Ex-4 may expand β-cell mass through stimulation of β-cell proliferation and/or inhibition of apoptosis (29). In contrast, although GIP stimulates β-cell proliferation and inhibits apoptosis in vitro and in short term in vivo studies (30,31), whether GIP receptor agonists or the physiological increases in GLP-1 and GIP achieved with DPP-4 inhibition can result in changes in β-cell mass comparable to that achieved with GLP-1R agonists is less clear (5,6,32). We did not observe significant increases in β-cell mass in high fat–fed mice with any of the incretin therapies; one possibility for this observation is that the mice were not sufficiently hyperglycemic; previous studies have shown that the ambient level of glucose and metabolic control is a potential determinant of the extent to which GLP-1R signaling is coupled to expansion of β-cell mass (33). In contrast, we observed changes in β-cell gene expression, notably increases in levels of *Irs2*, *Egfr*, and *Gck*, in mice treated with Ex-4; however, these changes were not evident in mice treated with either DA-GIP or the DPP-4i (Fig. 4). These observations imply that pharmacological levels of GLP-1R activation may be necessary for induction of a β-cell gene expression program, whereas pharmacological administration of GIP alone or physiological levels of GIP and GLP-1 achieved with DPP-4 inhibition may not be sufficient. In addition, these contrasting observations could also be due in part to different pharmacokinetic properties of the peptides used in our experiments.

Although GIP can have beneficial effects on β-cell function in normoglycemic human subjects, several lines of evidence from preclinical studies suggest that GIP action on adipocytes may be associated with impaired insulin action. Chemical ablation of GIPR signaling using GIPR antagonists results in improved glucose tolerance and enhanced insulin action in diabetic rodents (8,34,35), and genetic ablation of the *Gipr* promotes a lean phenotype and resistance to diet-induced obesity in mice (36–38). Consistent with these findings, we observed that DA-GIP–treated mice had a mildly reduced response to

insulin during an ITT and increased circulating levels of leptin and resistin. We previously demonstrated that DA-GIP acutely increases plasma levels of resistin in mice (38), suggesting that GIPR activation in the adipocyte may be coupled to stimulation of adipokine secretion. Although DPP-4 inhibition enhances the physiological levels of intact GIP (28,39,40), we did not observe impairment of insulin sensitivity or increased levels of plasma adipokines in mice treated with des-fluoro-sitagliptin alone. Hence, although chemical or genetic ablation of GIPR signaling improves insulin sensitivity (8,34–38), sustained increases in physiological levels of GIP induced by DPP-4 inhibition appear insufficient to promote insulin resistance.

A major difference in the actions of various incretin therapies relates to their effects on satiety and weight loss. GLP-1R agonists consistently produce satiety (41,42) and weight loss (3,43,44), whereas chronic administration of GIP has not been shown to reduce appetite or body weight. In agreement with previous findings, we observed that Ex-4 prevented further weight gain in mice that were fed a high-fat diet. Somewhat surprising was the observation that mice treated with des-fluoro-sitagliptin also exhibited attenuation of weight gain on a high-fat diet. Although genetic ablation of the *Dpp4* gene is associated with resistance to diet-induced obesity (45), the majority of human diabetic subjects treated with DPP-4is do not experience significant weight loss (46). These findings may be explained in part by species-specific differences in energy homeostasis arising as a result of loss of DPP-4 activity.

In conclusion, although GIP agonists acutely reduce blood glucose and improve glucose tolerance through enhancement of β-cell function, the actions of DA-GIP to increase plasma adipokines and impair insulin action taken together with defective GIP action in human diabetic subjects (47) make it unclear whether chronic GIPR activation alone represents the most promising strategy for the treatment of type 2 diabetes. It should be noted that the GIP analog (DA-GIP) tested in this study may not represent the best in its class, and additional studies with more potent long-acting GIPR agonists are warranted. Moreover, emerging evidence suggests that amelioration of hyperglycemia may be associated with partial restoration of GIP responsivity in human subjects with type 2 diabetes. Although both Ex-4 and DPP-4is are effective agents for achieving blood glucose control, our data imply that sustained activation of the incretin axis appears essential for optimal glucoregulation. Whether DPP-4is will produce comparable effects on β-cell mass and gene expression profiles relative to those observed in studies with GLP-1R agonists requires further study.

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