Original Article

Chronic Inhibition of Dipeptidyl Peptidase-4 With a Sitagliptin Analog Preserves Pancreatic β-Cell Mass and Function in a Rodent Model of Type 2 Diabetes

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Inhibitors of dipeptidyl peptidase-4 (DPP-4), a key regulator of the actions of incretin hormones, exert antihyperglycemic effects in type 2 diabetic patients. A major unanswered question concerns the potential ability of DPP-4 inhibition to have beneficial disease-modifying effects, specifically to attenuate loss of pancreatic β-cell mass and function. Here, we investigated the effects of a potent and selective DPP-4 inhibitor, an analog of sitagliptin (des-fluoro-sitagliptin), on glycemic control and pancreatic β-cell mass and function in a mouse model with defects in insulin sensitivity and secretion, namely high-fat diet (HFD) streptozotocin (STZ)-induced diabetic mice. Significant and dose-dependent correction of postprandial and fasting hyperglycemia, HbA_{1c}, and plasma triglyceride and free fatty acid levels were observed in HFD/STZ mice following 2–3 months of chronic therapy. Treatment with des-fluoro-sitagliptin dose dependently increased the number of insulin-positive β-cells in islets, leading to the normalization of β-cell mass and β-cell–to–α-cell ratio. In addition, treatment of mice with des-fluoro-sitagliptin, but not glipizide, significantly increased islet insulin content and improved glucose-stimulated insulin secretion in isolated islets. These findings suggest that DPP-4 inhibitors may offer long-lasting efficacy in the treatment of type 2 diabetes by modifying the courses of the disease. *Diabetes* 55:1695–1704, 2006

In patients with type 2 diabetes, several key pathogenic abnormalities contribute to increased blood glucose levels, including abnormal insulin secretion caused by impaired β-cell function and insulin resistance in target tissues (1). Insulin resistance appears to be an important early lesion that is accompanied by compensatory increases in pancreatic β-cell insulin release (2). In patients destined to develop type 2 diabetes, however, β-cell function deteriorates progressively and >50% of β-cell function is typically lost by the time hyperglycemia is diagnosed (2–5). This loss of β-cell function and/or mass leads to rising blood glucose levels and to frank diabetes.

The incretin hormone glucagon-like peptide-1 (GLP-1) plays a key role in the regulation of insulin secretion and glucose homeostasis. Administration of GLP-1 and its analogs or the GLP-1 mimetic exendin-4 has shown remarkable glucose-lowering efficacy in diabetic subjects (6–8). Importantly, these agents have demonstrated beneficial effects on increasing islet neogenesis and differentiation as well as modulating β-cell mass in part by reducing apoptosis in animal models of diabetes (9–12). These findings have engendered significant interest in the potential of GLP-1–based therapeutics to enhance β-cell function and thereby modify the course of disease in subjects with type 2 diabetes.

Orally administered dipeptidyl peptidase-4 (DPP-4; or CD26) inhibitors have emerged as a new class of antidiabetic agents owing to their ability to extend the biological effects of incretin hormones (13–15). DPP-4 is a multifunctional glycoprotein that contains NH_2-terminal serine dipeptidase activity and is present both in circulation and on the cell surface (16). DPP-4 has been implicated in pleiotropic cellular processes involving immune, inflammatory, and endocrine functions (16,17) and has been shown to cleave several hormones and chemokines in vitro. The best validated substrates, however, are members of the glucagon family of peptides, including GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), as well as pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide (GRP) (18–20).

Clinical proof of concept for glucose-lowering efficacy in diabetic subjects was first achieved with NVP DPP728 (21). Since then, multiple DPP-4 inhibitors have been characterized extensively in preclinical and clinical studies, including vildagliptin and sitagliptin (22–25). It is, however, not clear whether the prolonged biological effects of incretin hormones by DPP-4 inhibitors will have lasting beneficial effects on β-cell mass and function, as has been observed in rodents with GLP-1 analogs. While previous studies have reported that improved glycemic control in VDF (*fa/fa*) Zucker rats administered the DPP-4 inhibitor isoleucyl thiazolidide was not associated with

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DPP-4, dipeptidyl peptidase-4; FFA, free fatty acid; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GRP, gastrin-releasing peptide; GSIS, glucose-stimulated insulin secretion; HFD, high-fat diet; PACAP, pituitary adenylate cyclase-activating polypeptide; STZ, streptozotocin.

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TABLE 1
Characterization of the HFD/STZ mouse model

	Blood glucose (mg/dl)	Plasma insulin (ng/ml)	Plasma triglyceride (mg/dl)	Plasma FFA (mmol/l)	Body weight (g)
Control	141 ± 6	0.90 ± 0.09	158 ± 18	2.3 ± 0.2	39.3 ± 1.1
STZ alone	191 ± 29	0.58 ± 0.09*	143 ± 13	2.3 ± 0.2	36.8 ± 1.0*
HFD alone	169 ± 20	1.14 ± 0.17	146 ± 18	2.1 ± 0.1	46.7 ± 2.2*
HFD/STZ	310 ± 16*	0.56 ± 0.05*	447 ± 115*	4.2 ± 0.5*	40.6 ± 1.1

Data are means ± SE (n = 8–10). ICR mice were fed with regular diet and injected with saline (control) or STZ (STZ alone) or fed with HFD and injected with saline (HFD alone) or STZ (HFD/STZ). Blood glucose, plasma insulin, triglycerides, FFAs, and body weight were measured under the fed condition. * $P < 0.05$ comparing control and treated group.

improvement in β-cell mass and islet morphology, similar treatment of streptozotocin (STZ)-induced diabetic rats resulted in a significant increase in the number of β-cells and islet neogenesis (26,27).

In the current study, we investigated the role of chronic DPP-4 inhibition by a highly selective DPP-4 inhibitor in a nongenetic rodent model of type 2 diabetes, the high-fat diet (HFD)/STZ mouse. In this model, overt hyperglycemia results from a combination of insulin resistance induced by HFD feeding and defects in insulin secretion induced by single low-dose STZ treatment. We show that DPP-4 inhibition effectively ameliorated hyperglycemia and hyperlipidemia in this mouse model and significantly increased β-cell mass and improvement of islet architecture. Furthermore, treatment of diabetic mice with the selective DPP-4 inhibitor, but not with glipizide, resulted in increased islet insulin content and improved responsiveness of islets to glucose-stimulated insulin secretion (GSIS). These findings suggest that the therapeutic potential of DPP-4 inhibitors in diabetic subjects may extend beyond glycemic control to include increases in β-cell mass and function.

RESEARCH DESIGN AND METHODS

The DPP-4 inhibitor used in this study was a des-fluoro analog of sitagliptin, 7-[(3*R*)-3-amino-1-oxo-4-(2,5-difluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine L-tartaric acid salt (compound 25 in ref. 25), and is referred to herein as des-fluoro-sitagliptin. Des-fluoro-sitagliptin was prepared by Process Research, Merck Research Labs (Rahway, NJ). Glipizide, STZ, and other chemicals were purchased from Sigma Chemical (St. Louis, MO).

All animal procedures were performed in accordance with the guidelines of the institutional animal care and use committee of Merck. Mice were housed eight per cage and allowed access to diet and autoclaved water. Animal housing rooms were maintained at a constant room temperature (25°C) in a 12-h light (7:00 A.M.)/dark (7:00 P.M.) cycle.

Generation of diabetic model and treatment with compounds. Four-week-old male ICR mice were purchased from Taconic Farm (Germantown, NY) and placed on the HFD D12492 (Research Diets, New Brunswick, NJ), in which 60% of kilocalories is from fat. After 3 weeks of HFD feeding, the mice were injected once with low-dose STZ (intraperitoneal at 90–100 mg/kg) to induce partial insulin deficiency. Three weeks after STZ injection, the majority of HFD/STZ-treated mice displayed hyperglycemia, insulin resistance, and glucose intolerance as previously reported (36). At ~10 weeks of age (and 6 weeks of HFD feeding), animals with similar degrees of hyperglycemia and body weight were randomly divided to various vehicle or compound treatment groups. The normal diet–fed mice were used as nondiabetic controls.

Des-fluoro-sitagliptin was administrated orally by premixing with the HFD D12492 at 0.1, 0.4, and 1.1% (wt/wt; the mixing and repelleting was performed by Research Diets). Glipizide was also dosed as admixture to HFD at 0.02%. The target doses were 43, 208, and 576 mg/kg (milligrams drug per kilograms body weight) for des-fluoro-sitagliptin and 20 mg/kg for glipizide.

Postprandial plasma glucose, body weight, and food consumption were monitored weekly. Plasma HbA_{1c} (A1C) level was measured using a Micromat II test kit from Bio-Rad Laboratories. Six-hour fasting glucose levels were also monitored. Hepatic triglyceride levels were measured as described (28). Plasma drug levels were measured by liquid chromatography/tandem mass spectrometry; plasma DPP-4 activities and $GLP\text{-}1_{intact}$ levels were measured as previously described (25).

To study the acute effect of glipizide on glucose lowering, HFD/STZ mice were given oral administration of vehicle (0.5% methylcellulose) or 10 mg/kg of glipizide. Glucose levels were monitored at 0, 1, 2, 3, and 4 h after dosing. Age-matched ICR mice without HFD/STZ treatment were used as controls.

Oral glucose tolerance tests. Mice were fasted for 4 h before glucose tolerance tests. Oral glucose load was administered at 2 g/kg of body weight. Glucose levels were measured from tail bleeds with a glucometer (Lifescan, Milpitas, CA) at specified time points after glucose administration.

Immunolabeling of pancreas sections and quantification of islet cell mass. Measurement of islet cell mass was performed using modifications of previously described methodologies (9,12,29,30). In brief, pancreatic tissue was collected at necropsy and immediately frozen in Tissue Freezing Medium (Electron Microscopy Sciences, Hatfield, PA)–filled tissue molds (for insulin/glucagon labeling). Cryostat sections were immunolabeled with rabbit anti-glucagon followed by guinea pig anti-insulin (or a combination of anti-insulin, anti-glucagon, and anti-somatostatin antibodies). Digital images were captured in a Zeiss Axioplan II microscope equipped with a Slidebook Image analysis workstation (Intelligent Imaging Innovations, Denver, CO).

The insulin-positive β-cell–to–total islet area and glucagon positive α-cell–to–total islet area ratios were calculated from digitized images captured through the 20× objective using the Slidebook software. Images of 10 randomly chosen islets were captured from each section. Immunolabeled sections were prepared from four animals per treatment group.

Analyses of all immunohistochemical images were carried out twice by two separate operators who were blinded to the treatment group.

Islet isolation. Pancreatic islets of Langerhans were isolated from the pancreata by collagenase digestion and discontinuous Ficoll gradient separation, a modification of the original method of Lacy and Kostianovsky (31). The islets were cultured 2 h in RPMI-1640 medium (11 mmol/l glucose) before GSIS assay.

GSIS and islet insulin content. To measure GSIS, islets were incubated with 2, 8, or 16 mmol/l glucose or 2 mmol/l glucose + 30 mmol/l KCl to measure maximum insulin secretion. Insulin was measured in aliquots of the incubation buffer by enzyme-linked immunosorbent assay with a commercial kit (ALPCO Diagnostics, Windham, NH). Insulin content was measured after acid ethanol (0.18 mol/l HCl in 70% ethanol) extraction as described (32).

Pancreatic insulin content. The whole pancreas was dissected free from fat and other nonpancreas tissue immediately after the mouse was killed. After the measurement of the wet weight, the pancreas was extracted with the acid ethanol method, and insulin concentration was determined.

Calculations. All data are expressed as means ± SE. Statistical analysis was conducted by using Student's t test. Statistical significance was defined as $P < 0.05$.

RESULTS

The HFD/STZ mouse model of type 2 diabetes. To generate a nongenetic rodent model mimicking human type 2 diabetes with a combination of insulin resistance and insulin deficiency, ICR mice were fed an HFD for 3 weeks and then injected with a single low dose of STZ followed by continued HFD feeding for an additional 3 weeks (33). As shown in Table 1, HFD or STZ injection alone did not significantly affect blood glucose, whereas the combination of HFD and STZ treatment led to frank hyperglycemia. The low dose of STZ caused an ~40% reduction of plasma insulin levels, which was not sufficient to cause hyperglycemia in mice fed with normal diet. In

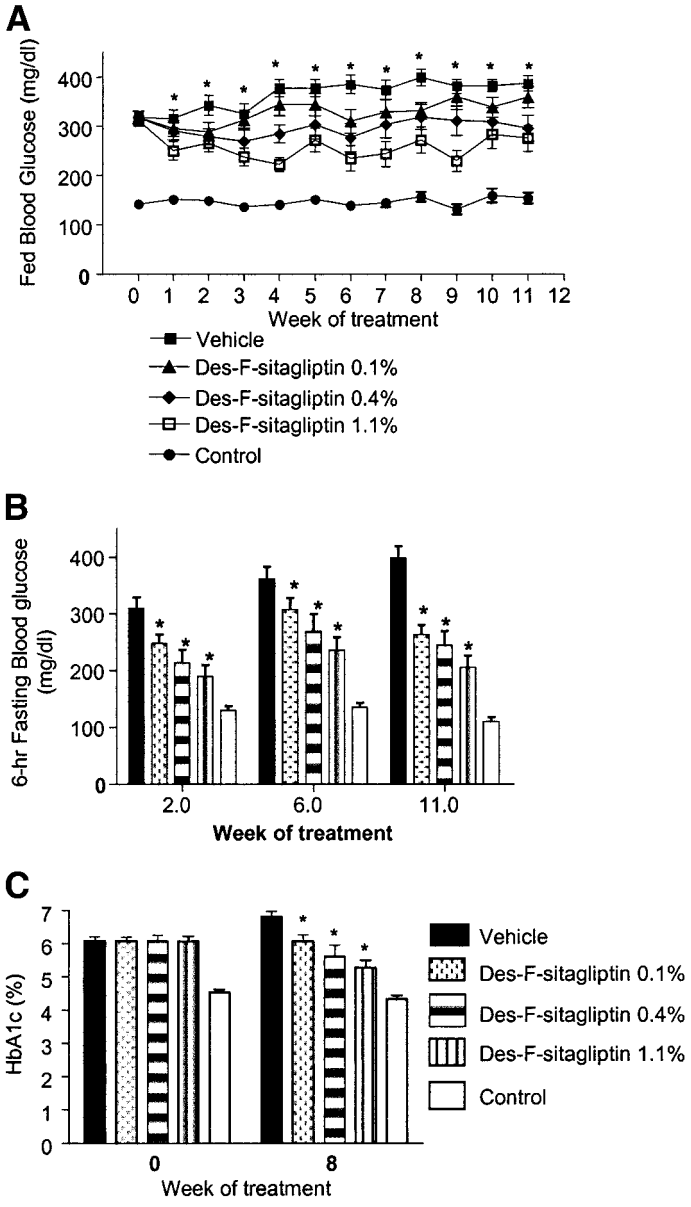

FIG. 1. Effect of des-fluoro-sitagliptin (des-F-sitagliptin) on glycemia control. HFD/STZ mice were treated with des-fluoro-sitagliptin at indicated dosages as admixture to diet for 11 weeks. Postprandial blood glucose levels (*A*), 6-h fasting blood glucose (*B*), and A1C (*C*) were measured at the indicated week during the treatment period. All the HFD/STZ mice had 6-h fasting blood glucose values of 260–270 mg/dl at week 0. Normal diet–fed mice were used as nondiabetic controls. Data are means ± SE of 10–20 mice in each group. *$P < 0.05$ vs. the vehicle-treated group.

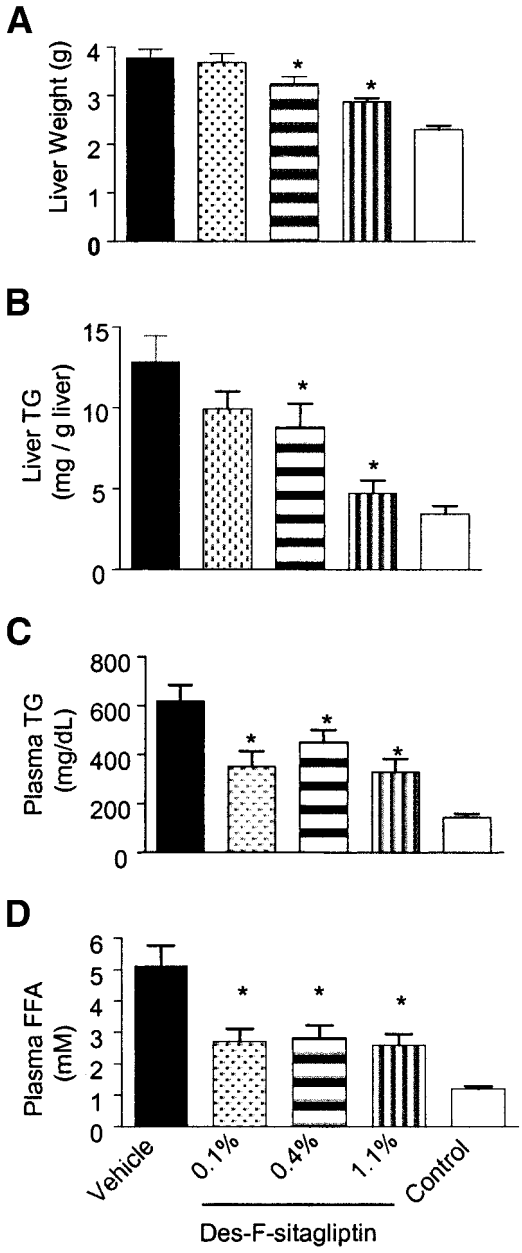

FIG. 2. Effect of des-fluoro-sitagliptin (des-F-sitagliptin) on dyslipidemia. HFD/STZ mice were treated with des-fluoro-sitagliptin at indicated dosages as adimixture to diet for 11 weeks. Liver weights (*A*), liver triglyceride (TG) (*B*), plasma triglyceride (*C*), and plasma FFAs (*D*) were determined at the end of the 11-week study. *$P < 0.05$ vs. the vehicle-treated group; $n = 10–20$ in each group.

contrast, this level of hypoinsulinemia precipitated hyperglycemia in the face of insulin resistance induced by the HFD. The mean body weight of HFD/STZ mice was comparable to that of normal diet–fed control animals, but their plasma triglyceride and free fatty acid (FFA) levels were significantly elevated. The HFD/STZ mouse model thus manifests hyperglycemia and hyperlipidemia associated with insulin resistance and impaired insulin secretion. We therefore choose it as a model of type 2 diabetes to study the effects of chronic DPP-4 inhibition.

Effects of chronic DPP-4 inhibition on metabolic control. DPP-4 inhibitors improve glycemic control in animal models and in type 2 diabetic subjects, but the long-term consequences of DPP-4 inhibition on pancreatic β-cell function and β-cell mass are not fully characterized. We therefore performed an 11-week study to test the effect of the selective DPP-4 inhibitor des-fluoro-sitagliptin on glycemic control and pancreatic islet morphology in the HFD/STZ mouse model. Des-fluoro-sitagliptin is a potent and selective DPP-4 inhibitor with half-maximal inhibitory concentration values of 27 and 97 nmol/l against human and mouse DPP-4, respectively (25). The potency, selectivity, and pharmacokinetic properties of this compound are virtually identical to those of sitagliptin. HFD/STZ mice maintained on a standard light-dark cycle were treated with the compound as admixture to the diet at 0.1, 0.4, and 1.1% (corresponding to 43, 208, and 576 mg/kg). Mean plasma peak/trough drug levels (estimated by measure-

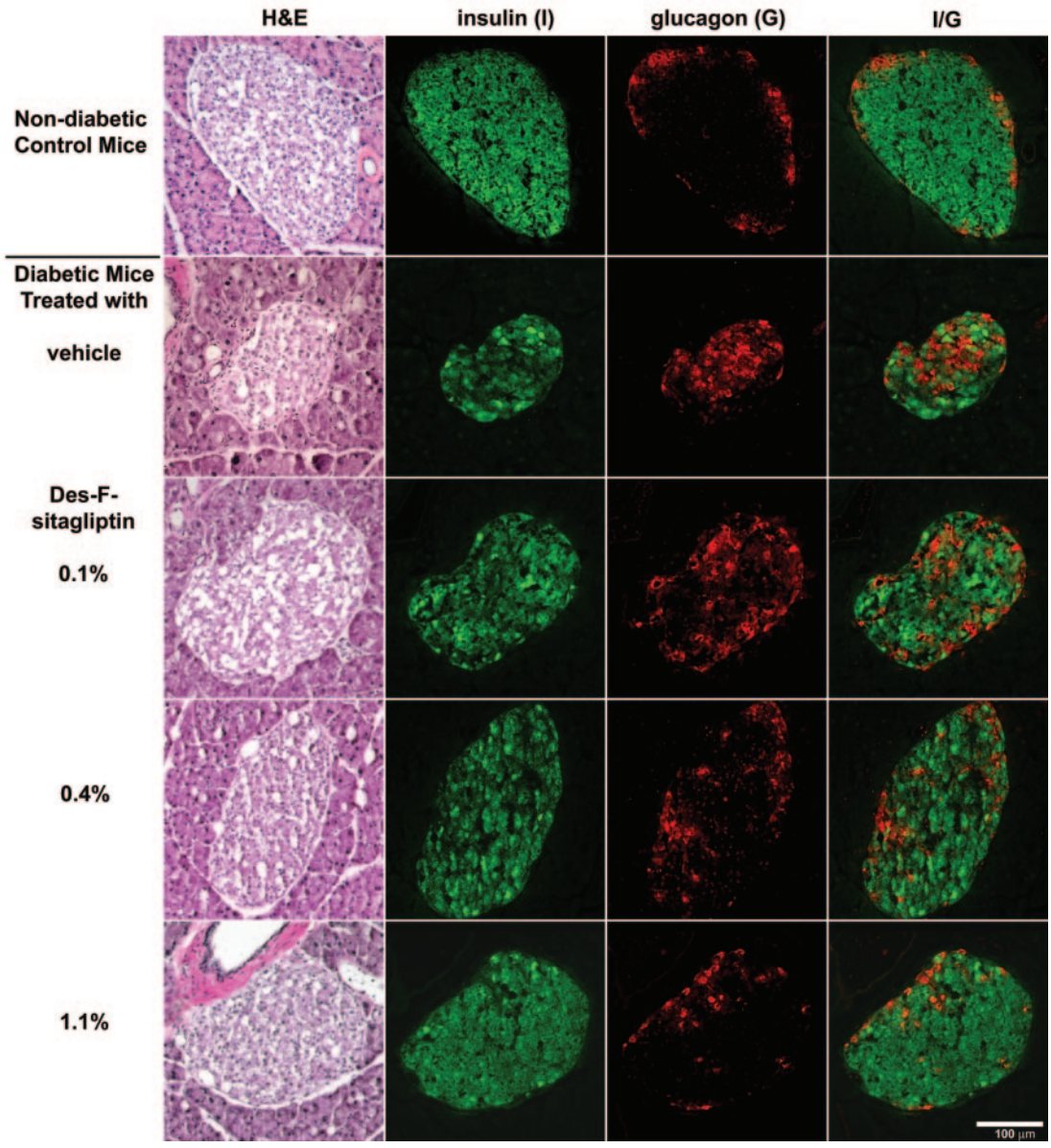

FIG. 3. Immunohistochemical analysis of pancreatic sections. HFD/STZ diabetic mice were treated with vehicle or des-fluoro-sitagliptin (des-F-sitagliptin) at indicated dosages for 11 weeks. Whole pancreas from each was cryopreserved, and consecutive sections were stained with hematoxylin and eosin (H&E), anti-insulin antibody (green), or anti-glucagon antibody (red). Shown are representative islets from each groups with each staining and the overlay of the insulin and glucagon staining (I/G).

ments at 8:00 A.M./4:00 P.M.) were 0.7/0.2, 3.5/1.0, and 17.6/1.3 μmol/l at 0.1, 0.4, and 1.1% des-fluoro-sitagliptin, respectively. The high doses of des-fluoro-sitagliptin needed to achieve these efficacious drug levels reflect the short half-life of the compound in mice (1–2 h). At the high dose used in the study, >95% inhibition of DPP-4 in the plasma was achieved at the trough drug levels (>1 umol/l) measured at 4:00 P.M. The animals ingested the inhibitor while feeding during the dark phase. Postprandial glucose levels were measured 1 h after the beginning of the light phase.

As illustrated in Fig. 1*A–C*, significant and dose-dependent reductions of postprandial and 6-h fasting blood glucose, as well as of A1C, were observed over the course of the study. Body weight and food intake were not significantly different between vehicle- and des-fluoro-sitagliptin–treated groups (data not shown). Vehicle-treated HFD/STZ mice also manifested hepatomegaly and hepatic steatosis relative to normal diet–fed control mice at the termination of the study. Treatment with des-fluoro-sitagliptin mitigated these abnormalities and resulted in significant and dose-dependent reductions in liver weight (Fig. 2*A*), hepatic triglyceride content (Fig. 2*B*), circulating triglycerides (Fig. 2*C*), and FFA levels (Fig. 2*D*) relative to vehicle. These results suggest that chronic efficacy of DPP-4 inhibitors may extend beyond improved glycemic control to include beneficial effects on other metabolic disturbances associated with type 2 diabetes, including hyperlipidemia and hepatic steatosis.

Effects of DPP-4 inhibition on islet morphology and β-cell mass. At termination of the 11-week des-fluoro-sitagliptin treatment, the pancreas of each animal was cryopreserved, and multiple cryostat sections were obtained from each sample for measurement of β-cell mass and characterization of islet morphology.

Dramatic differences in islet architecture and islet cell

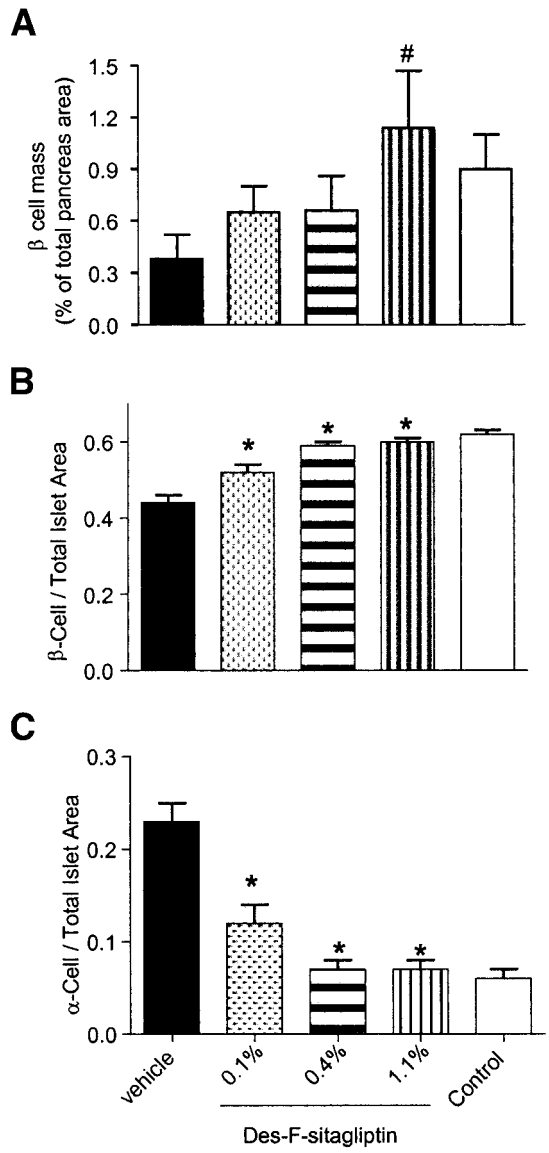

FIG. 4. Morphometric analysis of islet cell composition. Digital images of immunohistochemically stained pancreas sections, exemplified in Fig. 3, were captured to quantify the percentage of insulin-positive area in whole pancreas section as β-cell volume (*A*) (#$P < 0.03$; $n = 4$ animals), ratios of insulin positive β-cell–to–total islet area (*B*), and glucagon positive α-cell–to–total islet area (*C*) (*$P < 0.001$ vs. the vehicle-treated group; $n = 40$ islets).

composition were observed by immunohistochemistry between nondiabetic control mice and various HFD/STZ groups at the end of the study (Fig. 3). As expected, islets of normal diet–fed control mice comprised a large insulin-positive β-cell core surrounded by a mantle of glucagon-positive α-cells. In contrast, islets from vehicle-treated HFD/STZ diabetic mice contained many more glucagon-positive α-cells, which infiltrated the entire islet including the central core. The 11-week treatment with increasing doses of des-fluoro-sitagliptin significantly reduced the number of α-cells in the islet core and restored the normal β-cell/α-cell distribution pattern in islets.

We also examined low-power digital images of whole pancreata sections after staining with a combination of anti-insulin, anti-glucagon, and anti-somatostation antibodies. These sections generally contained 200–300 islets and thus provided a full view of the islet area in each animal. Analysis of these images demonstrated that vehicle-treated HFD/STZ mice had ~50% reduction of total β-cell mass relative to normal diet–fed nondiabetic controls, as measured by percent β-cell area/total pancreas area (Fig. 4*A*). Treatment with the DPP-4 inhibitor restored β-cell mass but did not expand it beyond that of normal mice.

Insulin-positive β-cell–to–total islet area and glucagon-positive α-cell–to–total islet area ratios were calculated from acquired digitized images. As shown in Fig. 4*B*, the ratio of insulin-positive β-cells to total islet area was reduced from 0.62 ± 0.06 in nondiabetic control mice to 0.44 ± 0.10 in diabetic mice treated with vehicle ($P < 0.001$, $n = 40$ islets). Treatment of the diabetic mice with des-fluoro-sitagliptin dose dependently increased the insulin-positive β-cell–to–total islet area ratio with a complete normalization of the ratio in the two higher-dose–treated groups. On the other hand, there was an elevation in the ratio of glucagon-positive α-cell to total islet area in diabetic mice compared with the nondiabetic control mice (Fig. 4*C*). Treatment of the diabetic mice with des-fluoro-sitagliptin reduced the glucagon-positive α-cell–to–total islet area ratio to the normal range. Consistent with these morphological findings, we also observed a slight but significant increase in circulating glucagon levels in vehicle-treated HFD/STZ mice (60.8 ± 5.6 pg/ml, $n = 18$) compared with nornal diet–fed control mice (34.6 ± 3.9 pg/ml, $n = 18$), which was also alleviated by treatment with the DPP-4 inhibitor at the high dose (49.4 ± 5.0 pg/ml, $n = 18$).

Comparison of effects of chronic treatment with a DPP-4 inhibitor versus sulfonylurea on glycemic control in HFD/STZ mice. The sulfonylurea class of ATP-sensitive potassium channel blockers such as glipizide are commonly used in the treatment of type 2 diabetes as monotherapy or in combination with other agents (34–37). Patients on sulfonylurea therapy often convert to insulin therapy progressively due to unsatifactory glycemic control (38,39). Following a single oral dose of glipizide at 10 mg/kg, significant reductions in blood glucose levels were observed in diabetic HFD/STZ mice as well as in control nondiabetic mice (Fig. 5*A*). When islets isolated from control and HFD/STZ mice were incubated with 2 mmol/l glucose in the presence of glipizide, glipizide was effective in stimulating insulin secretion in a dose-dependent manner (Fig. 5*B*). As expected, islets from nondiabetic control mice responded better to glipizide or 16 mmol/l glucose for insulin secretion (Fig. 5*B*). Taken together, these data demonstrate that the HFD/STZ mice are responsive to glipizide following acute treatment.

We then compared the long-term efficacy of des-fluoro-sitagliptin and glipizide on glycemic control in HFD/STZ mice. Diabetic mice were treated with either 576 mg/kg des-fluoro-sitagliptin (administered as 1.1% of diet) or 20 mg/kg glipizide (0.02% of diet) for 10 weeks. Plasma peak/trough drug levels achieved were 20.1/1.4 μmol/l for des-fluoro-sitagliptin and 28.2/13.3 μmol/l for glipizide in this study.

As illustrated in Fig. 5*C*, significant corrections of postprandial hyperglycemia were observed in mice following a 10-week treatment with des-fluoro-sitagliptin. Glipizide administration in the same paradigm resulted in postprandial glucose lowering only in the first 4 weeks, but its efficacy diminished afterward possibly due to exhaustion of insulin-producing capacity of β-cells. Furthermore, elevated A1C levels (assessed at the termination of the 10-week treatment) were significantly alleviated only by

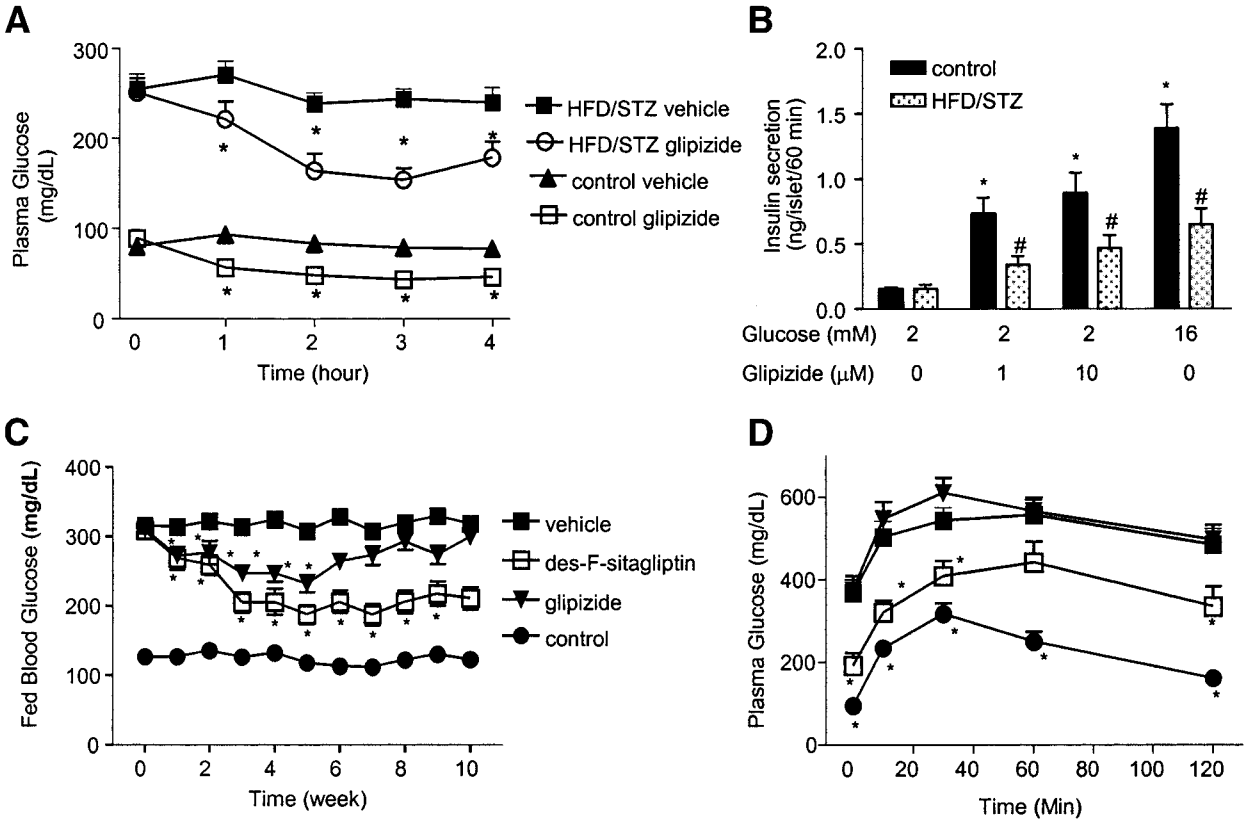

FIG. 5. Comparison of des-fluoro-sitagliptin and glipizide on glycemic control. *A*: Acute glucose-lowering effects of glipizide in HFD/STZ and control mice. Mice were dosed with vehicle or glipizide (10 mg/kg). Blood glucose levels were measured at indicated time points. *$P < 0.05$ vs. the vehicle-treated group; $n = 8$ mice in each group. *B*: Stimulation of insulin secretion by glipizide in isolated islets. Islets were isolated from HFD/STZ or control mice that had not gone through the long-term treatment and incubated with 2 mmol/l glucose in the presence of indicated concentrations of glipizide or with 16 mmol/l glucose. Insulin secretion to the media was measured. *#$P < 0.05$ vs. 2 mmol/l glucose alone for control and HFD/STZ mice, respectively; $n = 7$ mice in each group. *C*: HFD/STZ mice were treated with des-fluoro-sitagliptin (1.1%) or glipizide (0.02%) as admixture to diet for 10 weeks. Postprandial glucose levels were measured at the indicated time points. *D*: An oral glucose tolerance test after 4-h fasting was performed after 8 weeks of treatment. For *C* and *D*, *$P < 0.05$ vs. the vehicle-treated group; $n = 10$–20 mice in each group.

des-fluoro-sitagliptin but not by glipizide treatment (Table 2). To further evaluate glycemic control among the various groups, we performed an oral glucose tolerance test study after 8 weeks of compound treatment. Vehicle-treated diabetic mice manifested significantly elevated glucose excursions following oral glucose challenge compared with nondiabetic control mice. The glucose excursion was significantly reduced in diabetic mice treated with des-fluoro-sitagliptin (Fig. 5*D*) but not with glipizide. Furthermore, treatment with des-fluoro-sitagliptin resulted in an elevation of circulating $GLP\text{-}1_{intact}$ and a reduction in plasma glucagon levels, whereas glipizide treatment had no effects on these hormones following the 10-week treatment (Table 2). Thus, the DPP-4 inhibitor exhibits superior antihyperglycemic efficacy compared with sulfonylurea in this animal model.

Contrasting effects of des-fluoro-sitagliptin and glipizide on plasma triglyceride and FFA levels were also observed in the present study. Elevations in plasma triglyceride and FFA levels in HFD/STZ mice were completely prevented by the 10-week treatment with des-fluoro-sitagliptin, whereas glipizide administration over the same period had no effect on these parameters (Table 2).

Effects of chronic des-fluoro-sitagliptin and glipizide treatment on islet morphology and islet function. Cyrostat pancreata sections of animals from the study were analyzed by anti-insulin and anti-glucagon double immunolabeling to evaluate islet morphology. As illus-

TABLE 2
Effects of des-fluoro-sitagliptin and glipizide on plasma parameters in the HFD/STZ mouse model

	A1C (%)	$GLP\text{-}1_{intact}$ (pmol/l)	Glucagon (pg/ml)	Triglyceride (mg/dl)	FFA (mmol/l)
Vehicle	7.5 ± 0.2	2.1 ± 0.4	92 ± 14	400 ± 35	3.4 ± 0.4
Des-fluoro-sitagliptin	5.6 ± 0.3*	9.9 ± 3.4*	51 ± 6*	196 ± 32*	1.3 ± 0.2*
Glipizide	7.2 ± 0.2	1.8 ± 0.5	100 ± 15	322 ± 43	2.4 ± 0.3
Nondiabetic control	4.5 ± 0.1*	1.9 ± 0.4	52 ± 4*	110 ± 10*	0.6 ± 0.1*

Data are means ± SE ($n = 8$–10). Nondiabetic control mice or diabetic HFD/STZ mice were treated with HFD containing vehicle, des-fluoro-sitagliptin (1.1% admixture), or glipzide (0.02% admixture) for 10 weeks. A1C, plasma GLP-1, glucagon, triglycerides, and FFAs (FFA levels were measured under the fed condition). *$P < 0.05$ compared with the vehicle-treated group.

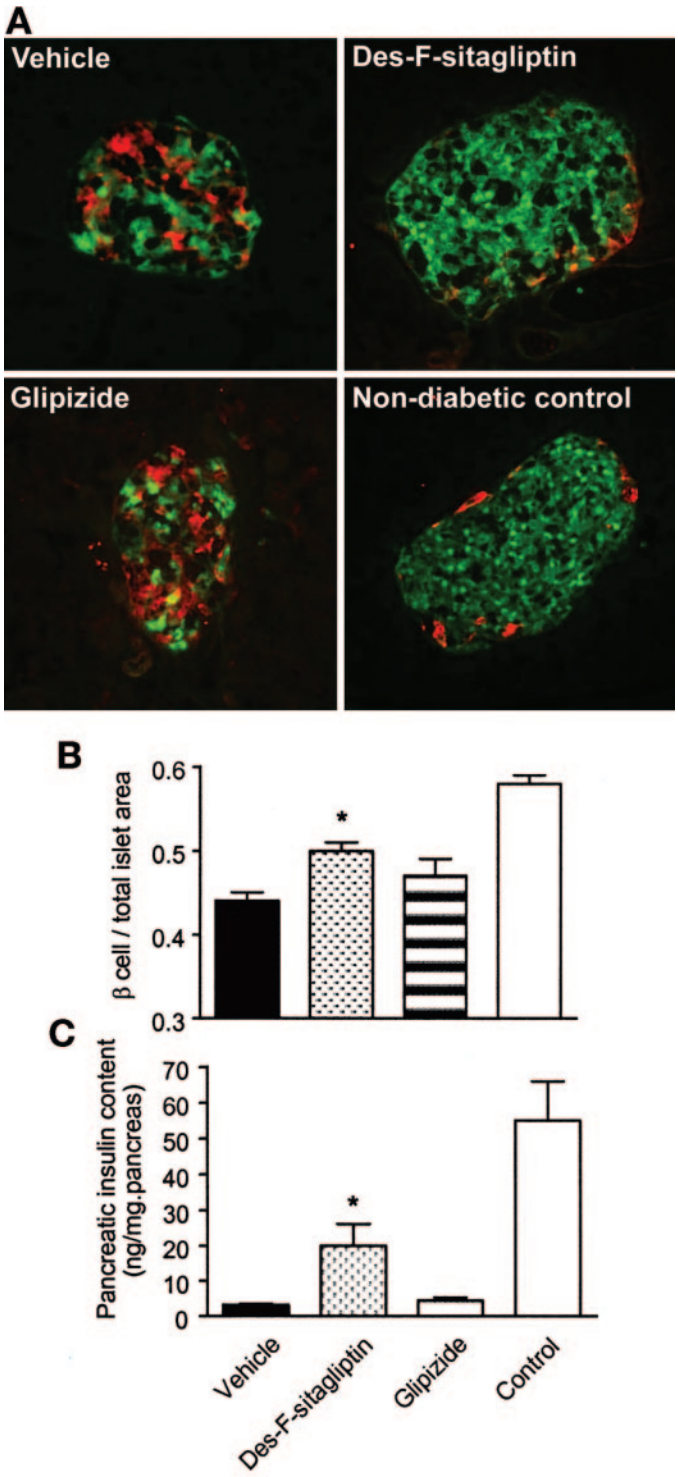

FIG. 6. Immunohistochemical analysis and quantification of islet cells. HFD/STZ diabetic mice were treated with vehicle, des-fluoro-sitagliptin (des-F-sitagliptin), or glipizide. Pancreatic sections from various groups of HFD/STZ mice and nondiabetic control mice were stained with anti-insulin antibody or anti-glucagon antibody. Shown are images with the overlay of the insulin (green) and glucagon (red) staining (*A*). Insulin-positive β-cell–to–total islet area ratio (*B*) was also measured from these images. Pancreata from separated groups of animals of this study were collected, weighed, and used to measure insulin content (*C*) after acid ethanol extraction. **P* < 0.01 vs. the vehicle-treated group, *n* = 40 islets in each group.

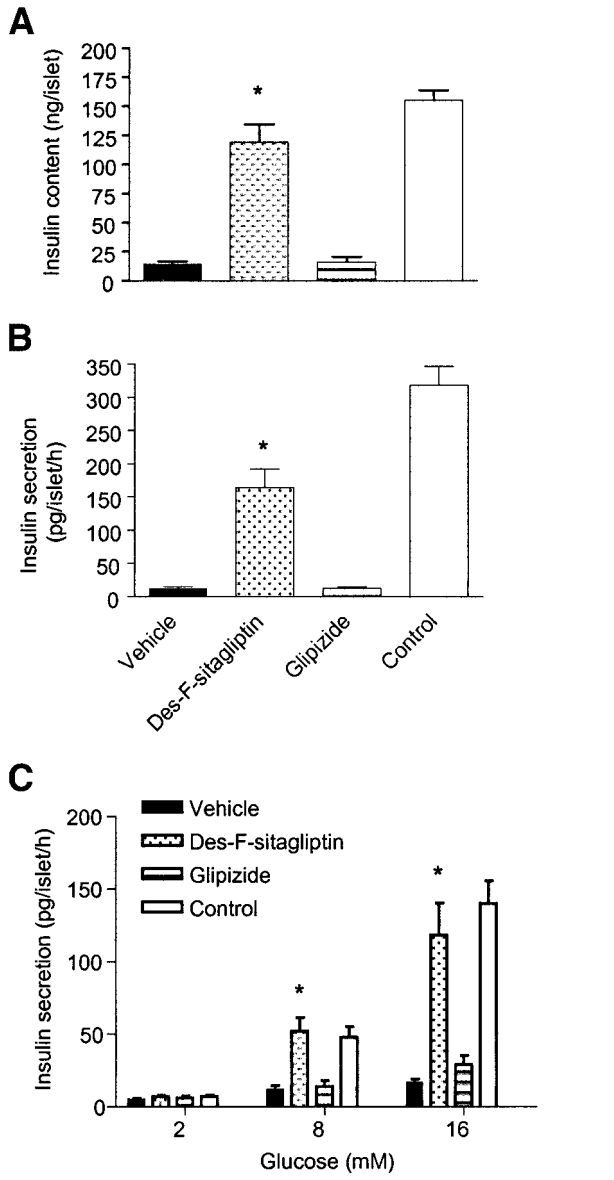

FIG. 7. Ex vivo analyses of pancreatic islets. Islets were isolated from HFD/STZ mice treated with vehicle, des-fluoro-sitagliptin (des-F-sitagliptin), or glipizde and from control mice. Insulin contents (*A*) in islets were determined after acid ethanol extraction. Insulin secretory responses to 30 mmol/l KCl (*B*) and an increasing concentration of glucose (*C*) were determined by the 60-min static incubations in Krebs-Ringer bicarbonate buffer medium. Data are means ± SE of six separate islet preparations from an individual mouse. **P* < 0.05 vs. the vehicle-treated group; *n* = 4 animals in each group.

trated in Fig. 6*A* and *B*, islets from vehicle-treated diabetic mice had markedly reduced β-cell–to–islet area ratio compared with islets from nondiabetic control mice. Consistent with the study shown in Fig. 3, treatment with des-fluoro-sitagliptin corrected these abnormalities in islet β-cell composition and distribution in the HFD/STZ mice. In contrast, glipizide failed to correct the reduced β-cell–to–islet area ratio in HFD/STZ mice. The contrasting effects of des-fluoro-sitagliptin and glipizide on β-cell mass were also clearly reflected in the measurement of pancreatic insulin content. As shown in Fig. 6*C*, vehicle-treated HFD/STZ diabetic mice suffered severe depletion of pancreatic insulin content compared with normal diet–fed normal control mice. Treatment of the diabetic animals with des-fluoro-sitagliptin, but not glipizide, partially restored pancreatic insulin content.

Ex vivo evalution of islet insulin content and function. The effects of chronic inhibition of DPP-4 on the function of pancreatic islets were also evaluated at the end of the chronic study using isolated islets from animals under different treatments. Significant reductions in insulin content (Fig. 7*A*) and maximum releasable insulin (stimulated by 30 mmol/l KCl) in islets (Fig. 7*B*) from vehicle- and glipizide-treated diabetic mice were observed compared with nondiabetic control mice. Treatment with des-fluoro-sitagliptin led to significant increases in islet insulin content and the maximum insulin secretion capacity in HFD/STZ mice. GSIS was also assessed by incubating the islets in media containing 2, 8, or 16 mmol/l glucose. As shown in Fig. 7*C*, islets from vehicle-treated diabetic mice exhibited significantly impaired GSIS relative to islets from normal diet–fed control mice. Islets from des-fluoro-sitagliptin–treated mice exhibited significantly improved insulin secretory responses to 8 and 16 mmol/l glucose, whereas no improvement in GSIS was observed in islets from glipizide-treated mice. These results indicate that chronic treatment with the DPP-4 inhibitor, but not with glipizide, significantly improves islet morphology and function in this model.

DISCUSSION

A key challenge in the management of type 2 diabetes is the gradual deterioration of glycemic control over time, which is believed to be linked to the progressive loss of β-cell mass and function (2,4). A critical, unmet medical need is therefore to alter the course of the disease and to achieve durability of efficacy. Therapies that maintain the capacity of the β-cell to synthesize and release insulin are most likely to fulfill this unmet need. An agent that increases β-cell mass and function over time could inhibit the natural progression of type 2 diabetes, leading to delayed deterioration of glycemic control, and a decrease in the need for combination therapy to lower blood glucose. DPP-4 inhibitors are a new therapeutic approach to diabetes that act, at least in part, by increasing GSIS (13–15). This effect is believed to be mediated primarily via stabilization of the incretin hormones including GLP-1.

In the current study, we report that chronic treatment in a nongenetic mouse model of type 2 diabetes with a potent and selective DPP-4 inhibitor resulted in significant improvement in glycemic control and metabolic profile. The improved glucose homeostasis was associated with dose-dependent increases in β-cell mass, increased ratios of insulin-positive β-cell to total islet area, and the restoration of normal islet architecture (β-/α-cell distribution pattern) (Figs. 3 and 4). These findings highlight the potential utility of DPP-4 inhibitors in tackling the underlying pathogenic cause of type 2 diabetes, namely the progressive loss of pancreatic β-cell mass. The therapeutic potential of DPP-4 inhibitors versus conventional insulin secretagogues like the sulfonylureas is highlighted by our comparison of chronic DPP-4 inhibitor administration with glipizide treatment in the same model. Glipizide is a sulfonylurea that is in current clinical use for the treatment of type 2 diabetes (34–36). In the present study, glipizide was equally effective in reducing blood glucose levels in HFD/STZ mice as des-fluoro-sitagliptin during the first few weeks of treatment, but it gradually lost its efficacy on blood glucose late in the study. Thus, while des-fluoro-sitagliptin was efficacious in decreasing A1C in diabetic mice following treatment for 10 weeks, glipizide was ineffective in lowering A1C (Fig. 5). With long-term use, there is a progressive decrease in the effectiveness of sulfonylureas resulting from a gradual reduction in and eventual exhaustion of insulin-producing capacity of the pancreatic β-cell. When islets were isolated from control and treated animals and evaluated ex vivo, it was apparent that only des-fluoro-sitagliptin, but not glipizide, was effective in replenishing severely depleted islet insulin content and improving islet β-cell insulin secretory function measured by glucose- and KCl-stimulated insulin secretion (Fig. 7).

It is possible that the observed beneficial effects of the DPP-4 inhibitor on β-cells could simply be a result of improvements in glycemia control. However, it is highly plausible that the beneficial effects of des-fluoro-sitagliptin on glycemic regulation and β-cell mass are at least partially mediated via increased GLP-1 and GIP signaling. This hypothesis would be consistent with previous reports that treatment with DPP-4 inhibitors increases circulating GLP-1 levels and stimulates β-cell survival and proliferation in STZ-induced diabetic rats (26,27). In addition, we and others have shown that mice deficient in DPP-4 ($CD26^{-/-}$) have elevated levels of circulating GLP-1 and GIP and are resistant to STZ-induced β-cell destruction (40,41). Furthermore, GLP-1 treatment has been shown to prevent STZ-induced apoptosis of a β-cell line (INS-1) in vitro in a dose-dependent manner (37). Similarly, GIP has also been reported to stimulate β-cell proliferation (37,42). Results from a recent study using mice lacking both GLP-1 and GIP receptors clearly indicate that both of these incretins are mediators of the acute effects of DPP-4 inhibitors on glycemic control (43). A recent study reported by Ahren and Hughes (44) indicated that inhibition of DPP-4 by valine pyrrolidide in mice resulted in augmentation of the bioactivity of exogenously administered peptide hormones, including not only GLP-1 and GIP but also PACAP and GRP. The potential effects of PACAP and GRP on β-cell apoptosis and proliferation may warrant further investigation. Detailed studies on the mechanisms of β- and α-cell mass regulation and the regulation of insulin production and insulin secretion in this regard will be the subject of follow-up studies.

While both glipizide and des-fluoro-sitagliptin were effective in reducing postprandial hyperglycemia, only the DPP-4 inhibitor corrected disturbances in lipid metabolism in this study. Similar lipid-lowering effects have also been observed with another DPP-4 inhibitor in STZ-induced diabetic rats (26) and ZDF rats (45). It is yet to be determined whether the lipid-lowering effects of DPP-4 inhibitors in preclinical studies are mediated by the enhanced action of incretin hormones. Nevertheless, elevated FFA levels have been demonstrated to be detrimental to the pancreatic β-cell (the so-called lipotoxicity) (46–48). The normalization of plasma lipids may contribute in part to the beneficial effects of des-fluoro-sitagliptin on β-cell function and islet morphology observed herein.

In conclusion, results from the current study show that des-fluoro-sitagliptin is efficacious in improving glucose homeostasis and metabolic profile in a mouse model of type 2 diabetes with defects in insulin sensitivity and secretion. The beneficial effect of the DPP-4 inhibitor on glycemia control is clearly associated with significantly increased β-cell mass and function. Furthermore, chronic DPP-4 inhibition with des-fluoro-sitagliptin demonstrated superior glucose-lowering efficacy and β-cell–preserving

effects compared with glipizide. These findings suggest that the beneficial effects of DPP-4 inhibitors could extend beyond glycemic regulation via modulation of insulin secretion to include a potential reduction in β-cell failure commonly observed in diabetic patients. The potential for DPP-4 inhibitors to alter the course of diabetes as a progressive disease warrants further studies in the clinic.

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